Borlaug Global Rust Initiative

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Proceedings
Oral Papers

Edited by Robert McIntosh

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Oral Papers
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1. The Agricultural Research and Experimentation Board of the State of Sonora "A model for productive farmer-researcher partnerships"

A. Gandara[1]

History of Agricultural Research in Sonora

The development of agricultural research in the Yaqui Valley was marked by series of significant events:

1. Agricultural research in Sonora began in 1910, when the Richardson Company, concessionaire of the Mexican Government for land surveying and development in the Yaqui Valley, established field trials at "Campo Ontagota".

2. In the early thirties, the "El Yaqui" Experiment Station was established at the initiative of Rodolfo Elias Calles, Governor of the State of Sonora, who passed a law assigning the land in block 611 of the Yaqui Valley to this purpose. It was agreed that the experiment station was to be operated by the Ministry of Agriculture, through the Agricultural Research Institute (Instituto de Investigaciones Agricolas, IIA).

3. In 1943, the Office of Special Studies (OSS), a Rockefeller Foundation/Ministry of Agriculture cooperative program, initiated research in Mexico with the participation of American and Mexican scientists. Dr. Borlaug arrived in Mexico in September 1944 and he his colleagues, members of the research team, established the first field trials for selecting wheat lines with improved rust resistance at the "El Yaqui" Experiment Station.

4. In 1955, with the initiative and support of the Yaqui Valley farmers, the land in block 910 was appropriated to establish the Northwest Agricultural Research Center (CIANO), within the structure of OSS; its mission was to solve problems limiting production in the region.

5. In 1960, OSS and IIA merged, to form the National Agricultural Research Institute (INIA), today INIFAP.

6. In 1964, Yaqui Valley farmers decided to create their own organization to provide continuous and systematic financial support for agricultural research. This initiative led to the creation of The Agricultural Research and Experimentation Board of the State of Sonora (PIEAES), known simply as Patronato.

Objectives

Patronato's main objective is to provide moral and financial support to agricultural research activities conduced by INIFAP-CIMMYT in Sonora, as well as to other research Institutions whose work is relevant to farmers. Patronato also coordinates, jointly with CIANO and CIMMYT, agricultural research and development and technology transfer programs within the state. In addition, it grants fellowships to researchers, promotes scientific meetings, and provides services to farmers, such as the production of certified seed of new, improved varieties developed by INIFAP-CIMMYT, or other research institutions.

Organizational structure

Patronato's governing body is made up of a General Assembly, a Board of Directors, and four Regional Technical Committees. The Assembly is the supreme authority, made up of 38 farmers' unions and associations - private and communal farmers - formally established in the state.

Financial resources

Patronato's main funding source is the farmers' voluntary contributions in the form of a quota based on crop production per hectare, which is collected at planting time. Others sources of income include; marketing registered seed to farmers' unions and associations, collaborative agreements with research institutions, and private and public companies, donations by philanthropic foundations and public and private institutions interested in promoting agricultural development in the region.

Since 1996, all federal and state government financial support for agricultural research is managed by Fundacion Produce Sonora.

The Laboratory for Phytosanitary Diagnosis of the PIEAES is another important funding source.

Importance of Patronato's research participation

In northwestern Mexico, the most outstanding achievement over 45 years of Patronato-CIANO-CIMMYT association has been the development of the model for a fruitful working partnership. This partnership has strengthened research programs and has led to the development of advanced technologies to solve real problems and enhance agricultural productivity and sustainability in the state of Sonora. An example for this association was the detection of a widespread epidemic of leaf rust on all commercially grown durum wheat varieties in the Yaqui and Mayo Valleys in March 2001.

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Response to the leaf rust epidemic in 2001 accelerated the release of Jupare 2001

In response to the epidemic CIMMYT and INFIAP researchers, jointly developed an emergency plan, using late sowings to select in a rapid way lines with resistance to the new race of leaf rust. As a result of the late sowing in May of 2001, a resistant line with high yield potential, STOT//ALTAR84/ALONDRA, was identified; however this line had low grain pigment concentration. In the summer of 2001, 2.5 ha were sown at CIMMYT headquarters, El Batán, Edo. de México, using low seeding rates.. This resulted in the production of 7.5 tonnes of basic seed by Patronato/INIFAP/CIMMYT. The variety description was developed and this line was registered by INIFAP as the wheat variety Júpare C2001. During the crop cycle 2001-2002, 75 ha of JÚPARE C2001 were sown in the Yaqui Valley, again using low seeding rates. This planting produced 450 tonnes of registered seed. With this seed 3,214 ha were sown during the 2002-2003 cycle, in the Yaqui and Mayo Valleys. By the 2003-2004 crop cycle 137,743 ha were sown with certified seed in northwestern México.

Based on the experience gained during the multiplication of JUPARE C2001, in spring 2005 researchers accelerated the seed multiplication process of the following durum varieties which have export quality: Patronato ORO C2008, Sawali ORO C2008 and CEVY ORO C2008. From 2005 to 2008 these lines were advanced using shuttle breeding between the experimental stations of CIMMYT, El Batán, and INIFAP in the Yaqui Valley. These línes were tested in replicated yield trials at CIMMYT and INIFAP, under different environmental conditions, such as sowing dates and different moisture regimes in 2006-2007. In September 2007, INIFAP, PIEAES, Fundacion Produce Sonora, and industry bodies concerned with exportability of grain, met to discuss the types of varieties needed to access export markets for grain produced in Sonora. In this way, INIFAP identified an initial group of promising lines, of which, three with effective resistance to leaf rust, competitive yield potential, and high grain pigment values that were demanded by the export markets. In 2007 - 2008 these three lines were multiplied, on an area of one ha each at the INIFAP station. In the summer of 2008, basic seed was multiplied in an accelerated way by PIEAES, INIFAP and CIMMYT, at El Batán. The descriptions and registrations were made by INIFAP:

- SAWALI ORO C2008, with 16 tonnes of basic seed.
- CEVY ORO C2008, with 15 tonnes of basic seed.
- PATRONATO ORO C2008, with 10 tonnes of basic seed.

In southern Sonora during the 2008-2009, 363.2 ha of the three varieties were sown with registered seed, the statistics were as follows:

Variety	Areas			Total
	Pieaes	Yaqui Valley	Mayo Valley	
Patronato Oro C2008	10 ha	39.23 ha	38 ha	87.23 ha
Sawali Oro C2008	10 ha	68.35 ha	61 ha	139.35 ha
Cevy Oro C2008	10 ha	77.61 ha	47 ha	134.61 ha

Impacts of agricultural research in Sonora

The financial and moral support given to CIANO and CIMMYT by Patronato since 1964 has facilitated the development of advanced technologies that farmers have adopted. More importantly, it has improved agricultural competitiveness and profitability for the entire region.

The Patronato model

Patronato's success depends mainly on the active participation of its Board of Directors, whose vision, experience, and dedication are largely responsible for maintaining farmers' voluntary participation and enthusiastic team spirit. Members of the General Assembly recognize the need to support and promote agricultural research and to focus on solving real production constraints of its associates. For this reason, farmers view their financial contributions as investments. They have left technical decisions to the researchers, but financial reports are regularly reviewed by members of Assembly. As Dr. Norman E. Borlaug stated, "what's important is that farmers get involved to protect research from the vagaries of political pressure".

In the opinions of many farmers, Patronato's support to agricultural research in Sonora is a proven model that should be replicated in other regions of Mexico, and abroad.

2. History and status of the wheat rusts

Robert A. McIntosh

Abstract

The rusts have been ongoing problems for wheat production probably since domestication of the crop about 8,000 years ago. Epidemics vary in size and frequency with host genotype and environment, wet years being 'rust' years. Although partial control in modern agriculture was achieved with resistant varieties, conditions favoring epidemics were made worse with the intensification of production and greater resistance gene uniformity in the host. The current Ug99 incident illustrates the situation of very widely adapted successful genotypes grown across huge areas in the presence of an ongoing threat from a recently emerged widely virulent and obviously highly aggressive pathotype of the stem rust pathogen. This paper addresses some of the history of cereal rusts and reviews underlying principles of host pathogen genetics, some of which are being neglected in the period of modern genetics.

Keywords

Cereal rusts • host : pathogen genetics • resistance • pathogenicity

Introduction

The cereal rusts can be serious diseases of the small grain winter cereals, including wheat, rye, triticale, oats and barley. The rusts of wheat attract the most attention because wheat is one of the two most important food crops for mankind. While all the cereal rust pathogens can be grouped as 'rust pathogens' because the different (*Puccinia*) species have many similarities, there are also clear differences in terms of life cycles, alternate hosts, host range and genetics.

The rusts and powdery mildews have been constant, irregular curses for farmers throughout the history of agriculture, but it was not until the 19th century that it was known that they were caused by fungi – the rust pathogens being Basidiomycetes. The discovery by de Bary that *Puccinia graminis* was a heteroecious species explained the much earlier observations that stem rust was often more serious when wheat was grown adjacent to hedgerows of barberry (*Berberis vulgaris*). The next important step in understanding cereal rusts came in the late 19th Century when Eriksson and co-workers demonstrated the existence of *formae speciales* as variants of a single fungal species varying in ability to parasitize different host groups. For example, the wheat-attacking form of *P. graminis*, *P. graminis* f. sp. *tritici* (*PGT*), the rye-attacking form *P. g.* f. sp. *secalis* (*PGS*), and the oat-attacking form, *P. g.* f. sp. *avenae* (*PGA*). All of these forms are capable of completing their life cycles on the alternate (sexual stage) host, but differ widely in crossability. For example, *PGT* and *PGS* hybridize readily, but neither is crossable with *PGA*. By far the most attention has been given to these forms, and relationships with, and among, other forms are not so well known, but likely some are more closely related to *PGA* than to *PGT* or *PGS*.

During the 1910s, Stakman and co-workers in the USA showed that *PGT* was comprised of pathotypes (phenotypes, races, strains) with the ability to attack only certain combinations of *Triticum* genotypes, treating diploids, tetraploids and hexaploids as a group. Initially such pathotypes were given the status of genetically fixed entities more or less like species. Subsequently, pathotype variability was demonstrated in all cereal rust pathogen species and the number of pathotypes simply depended upon the number of host lines (later, differential genes or gene combinations), and the number of variations in infection type considered significant for any one host line, keeping in mind that the genetic bases of the various resistances were unknown. Thus, to some extent, the numbers of pathotypes depended on the amount of effort that investigators wished to invest in defining them. Nevertheless, pathotype identification and surveys became, and remain, routine activities in rust research laboratories worldwide.

Although de Bary described the various spore stages of *P. graminis, P. coronata* and *P. triticina* (*PT*) and identified their alternate hosts in *Berberis* spp., *Rhamnus* spp. and *Thalictrum* spp., respectively, it was Craigie in Canada who in 1927 demonstrated heterothallism and sexual reproduction in *P. graminis*, soon followed by Waterhouse (1929) in Australia with similar work with *P. triticina*.

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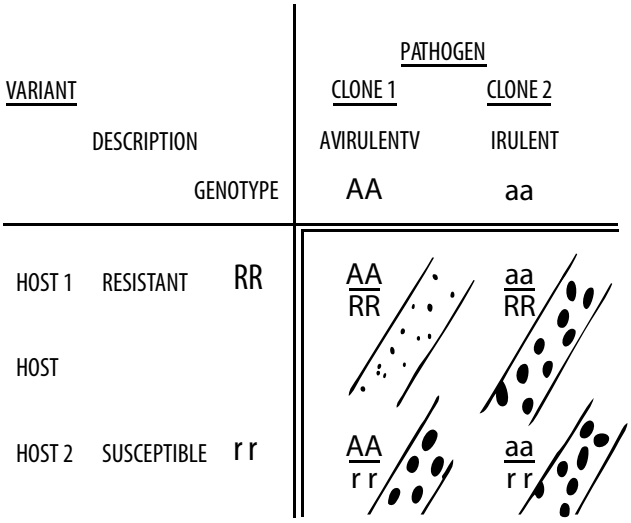
Fig. 1 ***The interaction of a corresponding gene pair (CGP) in host and pathogen assuming homozygosity in both organisms. LIT = LP/LR (Low infection type = low pathogenicity : low reaction)***



In the 1940s and 1950s, host : pathogen genetics was put on a sound footing with the work on flax rust (an autoecious rust system) by Flor who, from genetic studies in both host and pathogen, showed that the expression of resistance in a host plant was specifically dependent upon the presence of a corresponding gene for avirulence in the pathogen. Any genetic or environmental (e.g. temperature) factor that prevented the presumed direct or indirect interaction of the gene products of the corresponding gene pairs resulted in a compatible disease response. I visualize a single corresponding gene pair (CGP) (Fig. 1) as the first law of host : pathogen genetics. The interactions shown in Fig. 1 assume homozygosity (or complete dominance) of both host resistance and pathogen avirulence alleles (cereal rust pathogens on cereals are dikaryotic, but behave as diploids). Differential phenotypic responses (infection types) can occur with incomplete dominance. For example, Samborski (1963) showed that a rare intermediate response produced by a *P. triticina* isolate on line Transfer with *Lr9* was due to heterozygosity of the corresponding *P9* locus in the pathogen.

If the above model is extended to a second CGP, the matrix rapidly becomes very complicated (Fig. 2) even when assuming homozygosity. However, this is the basis for the second law of host : pathogen genetics which is about the interaction of CGPs. When more than a single CGP is involved, the outcome is a phenotype that is as incompatible, or more incompatible, than the most incompatible of the individual participating CGPs. The use of these laws leads to the four basic experimental designs in host : pathogen genetics outlined by Browder (1971) (Table 1). One of the outcomes of Flor's work was that the pathology community was very slow in appreciating its value; rather they saw it a phenomenon that had to be proved for each host : pathogen system before it could be applied experimentally. This was caused largely by a lack of awareness of genetics among pathologists. However, a reflective view of the gene-for-gene relationship is that it is the simplest logical explanation for genetic interactions between organisms, be they parasitic or otherwise. Such thinking led W.Q. Loegering (1985) to propose a sub-discipline of inter-organism genetics. Once the significance of the gene-for-gene interaction became known, there was an overuse of host genotype predictions based on multi-pathotype testing. People around the world could always postulate additional genes that did not fit the analysis, especially when constrained by the amount of variability among the pathotypes used. The necessary host genetic analyses required to validate the postulations were rarely performed.

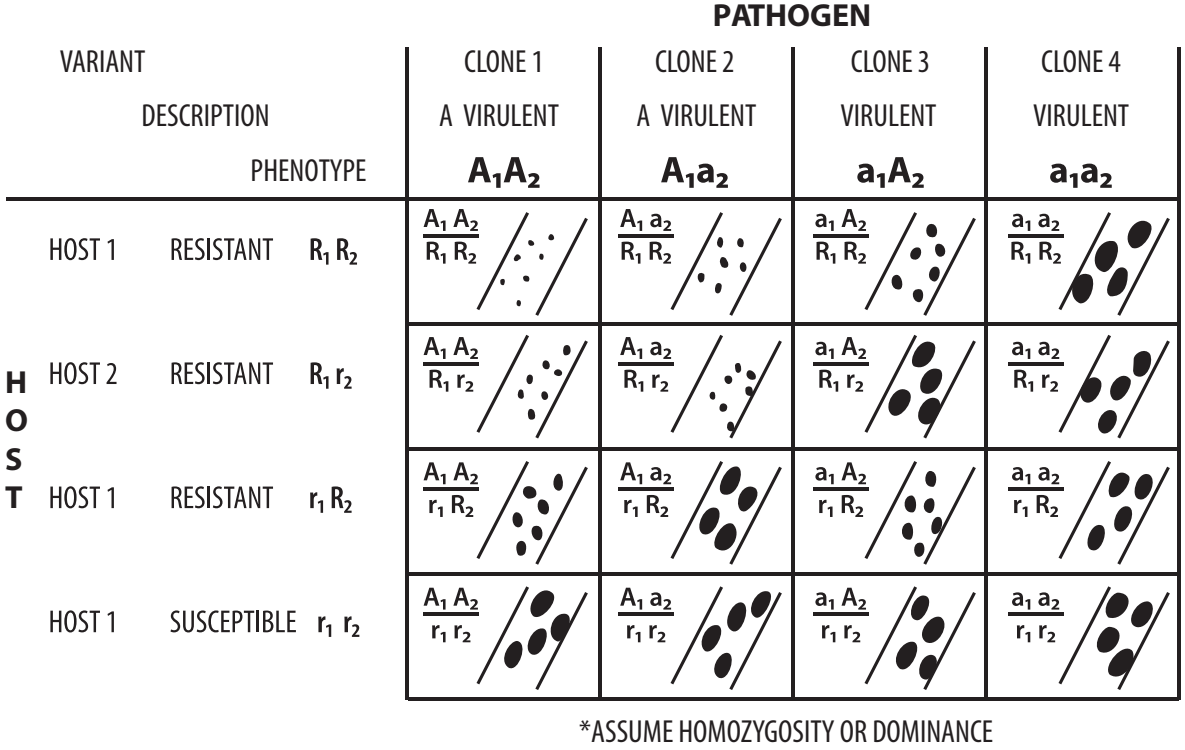
Table 1 ***Experimental designs used in host : pathogen genetics (after Browder 1971)***

Genetics of		Use
Host	Pathogen	
Unknown	Unknown	Postulation of CGPs
Known (fixed)	Unknown	Pathotype analysis
Unknown	Known (fixed)	Host gene postulation
Known (fixed)	Known (fixed)	Physiologic and environmental studies

Centers of origin and global spread of wheat rust pathogens

There is general agreement that the centers of origin of pathogens are usually the same as the centers of origin of the host species (Karnal bunt may be an exception). In the case of heteroecious pathogens those areas should overlap with the alternate hosts. The pathogens (or at least components of their populations) then move from those areas along with the host species. To much of Europe, northern Africa and Asia, urediniospores could have been wind-borne from the Fertile Crescent. To more distal areas of southern Africa, the Americas and Australia, pathogens may have been wind-borne (at very low frequencies because

Fig. 2 ***Interaction of two corresponding gene pairs. Note the orthogonality of the matrix. LIT I,2 = or < LIT 1, where LIT 1 < LIT 2. This is the basis of pathotyping and of gene postulation***



the populations are quite different), but the original founding populations were more likely to have been transported in cereal hay (along with weed seed) used to feed animals. It seems highly unlikely that suitably adapted or adaptable pathogen populations would have been present on grasses (or alternate hosts) prior to colonization. Moreover, since the diseases usually appeared soon after colonization, the target areas of cultivated cereals would have been far too small to intercept what would have been extremely low levels of wind-borne viable spores.

The initial occurrences of stripe rust in South America (Chile) and North America were suggested to have arisen from native *P. striiformis* populations on indigenous grass communities. The South American pathotypes were very similar to European forms, but no exotic source was suggested for North American pathotypes.

In contrast to North America, Australian cereal rust researchers were always cognizant of their geographical isolation, and proposed the likelihood of introduced inoculum for a number of significant pathotype changes. For example, Watson and De Sousa (1983) presented both pathotype and meteorological evidence for the possibility of wind-borne spores from southern Africa, but origins of other instances of putative introductions of *PGT* and *PT* were never identified. While it has been generally assumed that there is no exchange of inoculum between northern and eastern Africa and southern Africa, a recent report (Visser et al. 2009) indicates that pathotypes closely related to Ug99 are not only present in South Africa, but are evolving in a parallel manner, and are current predominant pathotypes in that country.

The introductions of *P. striiformis* to eastern Australia in 1979 and 1998 (barley grass stripe rust), to Western Australia in 2002 and on to eastern Australia in 2003, South Africa in 1996, and to the USA in 2000 are likely examples of man-borne introductions. The 1979 introduction to Australia was probably from western Europe (based on pathotype identity), the barley grass rust and Western Australian introductions were likely from the USA (based on visitor frequencies), and the introduction to South Africa was probably from Turkey or neighboring areas (based on pathotype similarity). No origin has been proposed for the recent new group in the USA, but eastern Asia may be a possibility. The lack of commonality between differentials used in Europe, Australia and South Africa with those used in India, China and in the USA confounds the problem of pathotype comparisons, especially when we know that many of the genotypes used in each area carry combinations of (seedling) resistance genes rather than single genes. Whereas many will argue the need for molecular markers

to solve problems of identity, we should not lose sight of our very poor genetic understanding of variability in stripe rust resistance globally.

Australian researchers have proposed several recent introductions of *PT*, but in only one instance, was a probable geographical origin proposed. That one instance was pathotype 53-1,(6),(7),10,11, with virulence for *Lr13*, likely introduced from New Zealand; notably, it was a previously recent introduction to that country. Park (pers. comm.) identified pathotype 10-1,3,9,10,11,12 that was virulent to cv. Mackeller with *Lr13*. This pathotype was avirulent on seedlings of Morocco, a genotype that many of us know to be highly susceptible in the greenhouse and field, and perhaps look upon as a 'universal suscept'. One might have expected it would be easy to trace the source of a *PT* population avirulent on seedlings of Morocco. Another example of this type is from personal experience in Japan. The island of Honshu cultivates only about 2,000 to 3,000 hectares of a single wheat cultivar, Norin 63. In 2000 McIntosh, Katto and Endo (unpublished) collected several samples of leaf rust from different locations on Honshu. All isolates were avirulent on seedlings of Thatcher, and a single gene for resistance was located on chromosome 2B using chromosome substitution lines. Such a pathotype had not been reported from other areas. Interestingly, very different pathotypes of *PT* were being reported from nearby Hokkaido where different varieties were grown and some breeding for leaf rust resistance had been undertaken. Obviously, in laboratories where Morocco and/or Thatcher are being used as susceptible hosts for initial increases of rust isolates, such pathotypes might not be detected even when present. There is probably no such thing as a 'universal suscept'.

Somatic hybridization

Evidence has been presented for a possible role of somatic hybrization in the evolution of the cereal rust pathogens. The best evidence probably comes from the studies of Watson and others, initially at the University of Minnesota, and later in Australia using colored and rare pathotypes as sources of markers. Watson (1981) discussed the role of somatic hybridization in producing one group of *PGT* pathotypes in Australia. Following the introduction of *PGS* to Australia in about 1950, pathotypes that appeared to be somatic hybrids of *PGS* and *PGT* were isolated from grasses, especially *Agropyron scabrum* and barley. These isolates were very similar to sexual and somatic hybrids produced in the laboratory.

The distinction of putative hybrids derived from *PGT* and *PGS* was based on comparisons with the parents using key differential testers of both wheat and rye, and lots of experience.

Park et al. (1995) presented pathogenic, isozymic and RAPD data to propose that one pathotype of *PT* uniquely pathogenic to certain hybrid wheats, that carried *Lr1* and were heterozygous for *Lr13*, was a somatic hybrid between two contemporary pathotypes.

Somatic hybrids were also reported in *PST*, but were not implicated in any significant role at the agricultural level.

Life cycles and disease cycles of the wheat rust pathogens

The stem rust and leaf rust pathogens are macrocyclic although the sexual stages probably no longer play significant roles in any major wheat-producing region. The alternate hosts of *PG* are *Berberis* and *Mahonia* spp. Historically, the telial/aecial cycles had important roles in that the telia are a resting (over-seasoning) stage, and were particularly important in areas with a long break between wheat crops, or with harsh environments that prevent over-seasoning of the uredinial stage. In the presence of barberry initial wheat-infecting aeciospores were obtained from barberry before incoming wind-borne urediniospores arrived from areas with milder climates. The second aspect is that sexual reproduction occurs on the alternate host, leading to new pathotypes; that is, generation of (homozygous) virulent genotypes from avirulent genotypes, and new combinations of virulence and avirulence alleles.

Leaf rust on wheat could be caused by more than one fungal species and there could be up to three different leaf rust diseases with each attacking three very different alternate host species, viz. *Thalictrum* spp. (Ranunculaceae), *Anchusa* spp. (Boraginaceae), *Isopyrum fumaroides* and *Clematis* spp. One form on tetraploid wheat in northern Africa and infecting *Anchusa* is considered to be *P. recondita*, usually recognized as the leaf rust pathogen of cereal rye, but the pathogenicty of wheat isolates on rye has not been reported. The rye leaf rust pathogen, *P. recondita* f. sp. *recondita* is highly avirulent on wheat, and *P. triticina* is likewise highly avirulent on rye, thus the relationships are quite different from the stem rust system. Relatively little seems to be known about the form infecting *Isopyrum*. Again, in contrast to stem rust, *P. triticina* does not attack barley and *P. hordei*, the barley leaf rust pathogen, does not attack wheat. An interesting question is whether genes in wheat conferring resistance to *Anchusa*-infecting pathotypes are effective in conferring resistance to *P. triticina* and vice versa. These relationships are important to discussions on host and non-host resistances.

The wheat stripe rust pathogen is microcylic, having no known alternate host and only a uredinial cycle. Nevertheless, the species is equally variable in pathogenicity to other cereal rust pathogens. *Puccinia striiformis* f. sp. *tritici* (*PST*) was a relatively recent introduction to Victoria, Australia, in 1979, but there were two other notable incursions of *P. striiformis* with a form attacking barley grass (*Hordeum murinum* complex) occurring in eastern Australia in 1998, and a very different pathotype of *PST* appearing in Western Australia in 2002. It is of interest that although stripe rust had survived and spread in eastern Australia and New Zealand after 1979, it was not found in WA until 2002, largely paralleling what had been observed with the various cereal rust pathogens over many years of surveys by Waterhouse, Watson and co-workers.

Our interest in stripe rust on barley grass started from 1979 when we were interested in establishing if ancillary (uredinial) hosts might be important in the survival of *PST*. Although infected barley grass could be found in, and near, wheat fields it was never strongly implicated in over-season survival. However, Wellings (2007) isolated clones of *PST* that showed pathogenic differences on different isolates of barley grass. The *PS* identified in 1998 was clearly apparent by its moderate virulence on wheat differential Chinese 166 (*Yr1*) and its avirulence on the other *PST* differentials and most wheat lines, including Morocco. This form of *PS* subsequently became widely established on barley grass in eastern Australia and its frequencies of occurrence have waxed and waned with seasonal conditions. My particular interest was the origin of this pathogen, which can be considered a different *forma specialis*. Following earlier reports and discussions I formed the opinion that it came from the Americas. On a visit to Chile in 1999 I noted widespread stripe rusting of barley grass in the streets of Santiago and along roadsides to the south of the country. The infections were often long distances from wheat and barley suggesting that the pathogen was neither *PST* nor *PSH*. No laboratory work was possible. In 2003 I visited California with the specific purpose of finding barley grass rust. Widespread infections were found in non-cereal areas of western California and in the vineyard areas of the Napa valley where the understories included stripe rusted barley grass in situations where occasional wheat and barley plants were not rusted. Clearly the pathogen involved was not *PST* or *PSH*.

Many of the inheritance studies in wheat of wheat stripe rust resistance undertaken by Dr. XM Chen and colleagues at Washington State University, Pullman, include race PST-21, which is virulent on seedlings of Chinese 166 and no other differential. Such studies invariably detect new genes for resistance, some of which have been designated as regular wheat resistance genes. I contend that PST-21 is an isolate of 'barley grass stripe rust'. It was originally collected from triticale in California in 1978, and despite subsequent collections in California on 'wheat and/or triticale' no actual sources were named. In the laboratory, it is avirulent on seedlings of Lemhi and all PNW *PST* differentials except Chinese 166. In genetic and molecular comparisons, PST-21 was always an extreme outlier, just as expected for a different *forma specialis*.

Whereas there is considerable knowledge about pathogenic variability of the individual pathogen species on their cereal hosts, comparatively little is known about the specialization that might occur in the same species with respect to the alternate hosts, either at the intra- or interspecies levels, and likewise, relatively little is known about the genetics of interaction with ancillary hosts.

Ancillary hosts

Rust pathogen species vary in regard to the hosts they infect, a feature that cannot be ignored in relation to epidemiology and survival of inoculum. To be an effective contributor in the rust cycle of a particular host (or genotype), the pathogen must not only be capable of infection in a laboratory test, but also be capable of producing a significant and timely amount of urediniospores to be a significant source of inoculum to a cereal crop.

Barley is a host of *PGT*, but under Australian conditions it is seldom affected by stem rust in the absence of stem rust on wheat during the cropping season. Yet, out of season, stem rust can often be found on self-sown or regrowth barley. Thus, while barley is usually not affected by stem rust, it can be a significant carrier of inoculum through the summer for transfer to the wheat crop of the following season. During the cropping season, stem rust appears on barley crops later than on wheat, but as barley usually matures before wheat, the likelihood of losses is extremely low. Thus stem rust will be a problem in barley only when it is a problem in wheat or triticale. In North America, however, the situation appears to be at least partly different – pathotype QCC can sustain damaging epidemics on barley.

The presence of stem rust on off-season barley (and on *Agropyron scabrum*) in Australia always requires further investigation because the forms involved could be *PGT, PGS* or a more commonly encountered putative *PGT* x *PGS* somatic hybrid group, with only the first being a threat to wheat. Indeed, it seems that the hybrid forms are preferentially virulent on barley, leading Dr NH Luig to often refer to them as *PG hordei*.

Crop losses

The dynamics of rust epidemics parallel the dynamics of wildfires – the degree of fire damage is very much determined by fuel types and amounts (susceptible tissue), temperature and humidity (environment) and wind (weather patterns). In the case of an epidemic the timing and frequency of initial infection determine the initiation of the log phase of the epidemic, and influence the eventual crop loss. Like fire, epidemics feed on themselves in the sense that when the intensity is sufficiently great, even resistant materials (like green vegetation) will be affected to some degree. This effect was very clear in descriptions of the race 15B epidemics in North America in the 1950s, when various Hope (*Sr2*) and Thatcher derivatives were affected, only to be later used as some of our most important and sustainable sources of resistance.

Stem rust is usually considered to be the most damaging of the cereal rusts, but given extremely susceptible varieties covering large areas, early initial infection and favorable conditions, all three diseases can be extremely damaging with yield losses exceeding 70%. The history of significant stripe rust epidemics in China is quite instructive, because of the recurrent events based on overuse of single resistance sources. In the 1950s there was an epidemic on varieties with the Bima 1 (*Yr1*) source of resistance (estimated losses 6 mt), in 1964 the Mentana (*Yr?*) source was affected (losses 3.2 mt), in 1992 the 1BL.1RS (*Yr9*) source failed (losses 1.8 mt), and in 2002 in Sichuan it was the Fan 6 (*Yr*) source (losses 1.3 mt) (Wan et al. 2007). My prediction is that China is on the way to the next epidemic that will occur on wheats with *Yr24/Yr26*, an unfortunate circumstance because the two sources of resistance in the Nanjing-produced *Haynaldia* derivatives and CIMMYT synthetics were initially thought to represent genetic diversity. Such events are not unique as illustrated by the use of *Lr24/Sr24* in Australia after 1980 when white seeded lines were first developed. By 2002 when virulence for *Lr24* appeared, these genes were present in more than forty released and potential cultivars, despite warnings of genetic vulnerability. Why? Because those genes were highly effective, protected against two diseases, easy to use, and were being used by competing breeders.

Saari and Prescott (1985) summarized global losses to wheat rusts for the post-World War II period. Of particular interest is a map showing the migration of a *PST* pathotyoe virulent on '8156 cultivars' (Kalyansona, Siete Cerros, PV18A, Indus 66, Laketch, Mivhor 77) from the Anatolian plateau to India during 1967 – 1970. To this day we do not know what gene was involved, but presumably it was *Yr2*.

Strategies to reduce or prevent losses include prevention of widespread over-season survival of inoculum by cultivation and grazing, targeting both the primary and ancillary host species. Forty years ago in Australia, we regularly collected stem and leaf rusted wheat plants on roadsides, railway lines and around public and farm sites protected from animals, but such findings are now quite rare in eastern Australia. The wheat plants surviving in such areas can be out-of-date genotypes. Resistant varieties are just as important in preventing local survival of inoculum as they are in preventing crop loss. Singh et al. (2007) described the potential benefit of *Lr34* in preventing over-summer/early autumn survival of leaf rust. Clearly, chemicals can be used for crop protection, but are economically prohibitive until a potential crop yield is assured.

Control strategies must target the degree of susceptibility and the magnitude of use of susceptible genotypes through the availability of competitive resistant varieties. In many regions the removal of the more extremely susceptible genotypes (VS category) will have significant effects on both inoculum loads and crop loss. Any suggestion of legislative measures to control varieties is not acceptable.

Epidemiology of the rusts

Van der Plank (1963; 1968) and others illustrated the basic principles of epidemiology – widespread damaging epidemics occur as a result of large areas of susceptible hosts, high levels of initial inoculum and a continuing favorable environment. In some agricultural areas such conditions are difficult to avoid. For example, in Kenya wheat is continually planted and harvested, even in adjacent fields. As a consequence, inoculum passes from the maturing crop to the emerging crop. In China wheat can be continuously cropped at variable altitudes such that inoculum passes up and down the slopes in a single region. In both examples inoculum is available for wind-borne dispersal at any time of the year, and it is well known that urediniospores of *Puccinia* spp. can be transported over long distances. Wheat stripe rust very likely arrived in Australia about May or June 1979 (when it could have been present in wheat crops in Europe), when receptive crops in Victoria had emerged. Within two weeks of its initial discovery, we established by survey that it was already on a 600 Km front, and by the end of the 1979 crop season, it had been reported in central Queensland, in excess of 2,000 Km from its presumed initial focus of establishment. And this was a rust pathogen that was, at the time, considered to be relatively slow moving in Europe and North America.

It is useful to remind ourselves that stripe rust did not appear in the wheat belt of Western Australia until 2002, some 23 years after its occurrence in eastern Australia, but appeared in New Zealand only one year later. Interestingly, the pathotype in WA was a new exotic. It appeared in eastern Australia one year later. Many of the events reported for stripe rust were repeats of the patterns that Waterhouse and Watson and colleagues had established earlier with other cereal rust pathogens.

It is often considered that rust survival and spread follows certain (*Puccinia*) pathways as has been described not only in Australia and New Zealand, but also in North America, China and India, and probably being repeated with Ug99 in Asia, with over-season survival either in milder latitudes or at higher altitudes in mountain areas. The over-season areas were considered as part of continuous cycles of inoculum or as inoculum exporting areas. Australia has no significant mountain areas where cereal rust pathogens would have an advantageous survival rate, and experience there suggests more or less random survival on regrowth and self-sown cereal hosts throughout the agricultural regions. If that is true in a dry country such as Australia, it is also more likely than usually acknowledged in many other areas. The long term survival of distinct pathotypic groups of *P. triticina* in North America would also support inoculum survival in distinct regions extending at least to the Canadian border. This, of course, does not exclude wider exchanges of inoculum over longer time periods.

Host : pathogen interaction

In the early days of host : pathogen interaction studies people (mainly pathologists) observed that genotypes resistant in the field usually produced low responses in seedling tests performed with the same pathogen clones in a greenhouse. Thus the seedling test became an **assay** for resistance (or avirulence) under field and farm conditions. Moreover, they noted a range of highly repeatable and characteristic phenotypes varying from 'immunity' or no visible symptoms to large pustules that were characteristic of many genotypes that were susceptible in the field. These varying symptoms were described by Stakman and co-workers on a descriptive 0, ; (hypersensitive fleck), 1 to 4 scale that many of us continue to use at the present time, a scale that some people have now converted to 0 – 9 and increasingly used as a quantitative scale. Based on correlated observations between seedling responses at about 20°C in the greenhouse and reactions under field conditions they decided that IT 3 and 4 represented compatibility and those below IT 3 represented incompatibility. These decisions were based completely on observation and involved no genetics. Unfortunately, this distinction is still used in many laboratories at the present time and many recorded IT 3 responses continue to be incorrectly interpreted, and examples will be discussed below.

The rusts are generally not serious diseases of seedlings and any research on seedlings is based on an assumption that the results will be highly correlated with responses in the field. In cases where seedlings at the first or second leaf stage are susceptible, but then become increasingly resistant as they develop we refer to such resistance as adult plant resistance or post-seedling resistance in contrast to seedling or whole of life resistance. These distinctions are not clear-cut. For example, *Lr13* was originally (and unfortunately still is!!) described by some researchers as an APR, but we have no problem with scoring and interpreting it as a seedling resistance by using higher temperatures and interpreting certain IT 3 responses as low. On the other hand *Lr18* was described as a seedling resistance. Under Australian conditions we learnt that this gene conferred a high seedling resistance only at low temperatures (<18°C), and that it reversed its dominance over a range of temperatures, finally becoming ineffective at about 26°C. All along the way, however, this gene was highly effective under the field conditions through which we worked. Thus in both examples we learnt how to conduct our greenhouse testing system (our laboratory assay systems) to maximize opportunities for identifying genes conferring resistance in our breeding nurseries.

Greenhouse studies on leaf rust and stem rust in North America were usually conducted as close to 20°C as possible, obviously to control one parameter of the disease triangle. Indeed during my 1969-70 post-doctoral experience at the University of Missouri, I noted how rust work in the greenhouse largely ceased during the summer (too hot) and during the winter (too dark for dependence on natural light). The consequence was a relatively stable greenhouse environment. In Australia, we strived for a 12 month working cycle, and despite a less extreme ambient weather cycle, our greenhouse environments were in fact more variable. This allowed us to detect and interpret environmental effects that later became key to gene identification and manipulation.

After Flor placed host pathogen interactions on a genetic footing, and Sears provided the aneuploids stocks that permitted genes to be associated with chromosomes, we had the tools to permit a systematic cataloguing of resistance genes. In contrast to the various genes with which Flor worked where the high and low infection types were very distinctive, those produced in the cereal rust systems were often much more intermediate generating problems with decisions on effectiveness, but at the same time providing

distinctive phenotypes that often enabled or hastened gene identification – indeed this was a prime reason for producing the atlas of rust resistance genes in wheat (McIntosh et al. 1995).

Genetics is the study of inheritance of variation, and distinctions between high and low responses based on a century-old judgement that IT 3 was a cut-off between incompatibility and compatibility is not genetically based. That cut-off should be based on contrasting phenotypes confirmed by progeny testing. An extreme example of this is the gene *Sr23* which has no value *per se* in breeding but is an excellent 'probe' for the presence of *Lr16*. Against one *PGT* culture in the Sydney University collection, seedlings with this gene alone confer a necrotic low reaction (IT 1NN); with all other cultures, and the appropriate high light conditions the same host lines produce IT 3+N, very large (compatible?) pustules but with a characteristic and repeatable brown necrotic center, that is predictive of *Sr23* and *Lr16*. Should we regard IT 3+N as high or low? Obviously, the decision is circumstantial and will vary with the purpose of the test.

Having thus used seedling response data as a probe for identifying variation, the relevance of that variation must be established using adult plants or a field plot situation. One recent study analysed 'seedling resistance' as a unique trait, and on the basis of separate QTL studies, concluded that the underlying genetic basis of variation was different from the genes controlling variation in response under field conditions. What is the practical use of a gene(s) that confers 'seedling resistance' to wheat breeding?

Pathogenicity studies

Why conduct a pathogenicity survey?

Reasons include: everybody else does it, we want a set of markers to track different clones in a complex asexually propagating population, we want to relate variation among clones to the likely responses of local cultivars, we want to isolate and identify clones for use in breeding nurseries, we want to relate clones internationally, and/or we want to identify and intercept clones that might be moving globally.

Conducting pathogenicity surveys

Having decided that a pathogenicity survey should be undertaken, decisions then have to be made as to what differentials should be used and that depends on which of the above questions are being asked. The number and type (isogenic lines vs cultivars) of differentials must be considered in the context of the area of the survey, keeping in mind that laboratory/greenhouse space (more samples or more differentials) and budgets will always be limiting. Should known genes for APR be used in pathogenicity surveys? It is interesting that all *PT* isolates virulent on *Lr27* + *Lr31* in seedling tests are virulent for *Lr12* which is apparently identical to *Lr31* but behaves as an independent APR gene. But we do not know how frequent *Lr12* is in the host population and therefore whether using an *Lr27* + *Lr31* seedling tester is justifiable for that purpose.

The use of an international gene nomenclature system depends on the acceptance and (implicit) use of common differentials, but this is not acceptable to all researchers for a range of reasons, including personal, economic and historic ones. If *PGT* is taken as an example, genes *Sr9a* and *Sr9d* have never been effective in Australia, so it would be a wasted resource to include them in local routine surveys; similarly in North America *Sr9g* and perhaps *Sr21*, although it is used, might be considered of little use. On a global monitoring scale; however, such differentials are important because their various responses could be indicative of isolates from specific regions. Obviously, the detection of differences at this level is a current target for molecular markers capable of detecting clonal groups, but not of pathogenic variation within those groups.

Most modern race nomenclature systems are binary-based and place no value on differing low ITs for any particular differential or CGP. The original (see Stakman et al. 1962) system was based on actual infection types, but the variation on them was caused by the possibility of multiple resistance genes in some differentials as well as variation relating to single CGPs. This problem is another major reason why the Australian group has not adopted the North American nomenclature binary-based nomenclature system. Many of the *PGT* clonal groupings identified by Watson and co-workers were based on distinguishable differences in phenotypes produced by single CGPs including *Sr6* and *Sr15* (Watson and Luig 1968).

To overcome economic and space constraints, leaf rust surveys in Europe were based on inoculated leaf segments. For some differentials, mainly those giving very low seedling responses the method worked very well; for others giving intermediate and mesothetic responses on entire seedlings, there were problems and the genes involved, including *Lr13* and *Lr14a* were not monitored despite being present (and possibly effective) at significant frequencies in European wheats.

Can pathogenicity studies be field-based?

Host genotype-based pathogenicity studies are possible and could be related to individual gene responses provided we included the single gene lines and various combination stocks to enable the identification of individual pathotypes; that is, the

gene combinations are necessary for the resolution of pathotypic mixtures. In an attempt to generate suitable gene combinations based on the Avocet S background I quickly came to realize that the use of such a set of lines on a large geographical scale will be confronted with problems of line purity and identification. Ideal surveys of this type also assume that only historically identified resistance genes are monitoring the variation, and that if those genes are seedling-effective factors, there are no additional genes for adult plant resistance. We already know that some of the Avocet S NILs (AvS+Yr1, AvS+Yr5, AvS+Yr10, AvS+Yr15, AvS+YrSp) carry *Yr18*. There are published reports on using the traditional stripe rust differentials in field nurseries, but as the majority of such differentials have APR genes additional to the seedling resistance factors, and the data obtained cannot easily be related to the current base of genetic knowledge or to the wheat genotypes being produced in the local area.

Virulence surveillance projects are special types of field-based surveys and are important sub-programs of the BGRI and DRRW. Here, care must be exercised to ensure that all participants are using correctly identified sets of tester genotypes if field data are to be collected across wide geographic areas. A problem with field-based surveys is that they are dependent on natural infection, many will not be infected, most will be sparsely infected, and therefore capable of providing inoculum for processing in laboratories, and only a few will generate sufficient host response differences for reliable recording. Whereas frequent lack of infection will be great for the local farmers, scientific institutions and scientists may soon be looking for cost-cutting and other ways to use their time in a more rewarding manner.

Interpretations from pathogenicity survey data

In recent years there has been an increasing tendency to interpret survey data simply from seedling infection type records without confirming what is being concluded. An example of this is interpretation of data for *Lr21*. A significant number of (particularly European) papers have reported virulence for *Lr21* based on IT 3 or 3-. In no case has a validation test of mature plants with this gene been carried out. I have discussed this issue with Dr J Kolmer on a number of occasions, such that he now emphatically states in his survey reports that *Lr21* continues to be effective. Given my contention that the seedling test is an assay, validation experiments must be undertaken, especially where there are no indicative field data to support such reports. Thus, as a challenge to the rust community I will state that *Lr21* is currently universally effective.

Resistance

Concepts of resistance

People from different backgrounds have different ways of conceiving resistance, and often the fact that what we see and interpret is based on host genotype, pathogen genotype and environment is ignored. Perhaps we should reflect on how we might think about resistance:

- as a pathologist: Stakman and co-workers made many decisions in the absence of genetics, yet we often cite their papers as a basis for our current phenotyping, especially in deciding what is high and what is low.
- as an epidemiologist: Here we are interested in delaying the increase and spread of rust at the field level or at the national level. Preventing over-season survival using *Lr34* could be important.
- as a geneticist: Genetics is about phenotypes and differences – IT 3 can be high or low depending on circumstances. A 'super' gene for a geneticist may not be so for a breeder.
- as a molecular geneticist: With extrapolations to genomics and function.
- as a breeder: Necessity? Aesthetics? Amount of protection? Novelty? Cost?
- as an economist: How much is a resistance gene or source worth?
- as a farmer: Availability of resistance at critical times, effectiveness of the resistance, cost of using it as part of risk analysis, knowing that rust is a 'compound interest disease' and that public risk/protection issues may be involved.
- as an agricultural scientist: An integration of the above.

Types of resistance

Resistances are divided into 'seedling', or 'all stage resistance', and APR, or post-seedling resistance, for convenience. Any resistance that cannot be characterized as a seedling resistance is designated APR; its time of onset depends on genotype and environment. Chen and co-workers at Washington State University describe a special type of APR that they designate HTAPR (high temperature APR), but my personal observations are that all APR to stripe rust is temperature-sensitive, and increases and decreases in sporulation on post-flowering plants occur with changing weather patterns.

Not all sources of APR are non-specific. *Lr12* is an excellent example of a genotype-specific (often called race-specific) APR to leaf rust. *Lr35*, an alien APR source transferred to wheat from *Aegilops speltoides*, might be predicted to be genotype-specific based on its hypersensitive response. Although not stated as such,

components of stripe rust APR, inadequately defined as *Yr11*, *Yr12*, *Yr13* and *Yr14*, are almost certainly genotype-specific. Pathotypes allegedly virulent for these genes were isolated on the basis that they conferred increased rust levels, but the respective source hosts were not scored as highly susceptible to the new pathotypes, and genetic stocks with those genes individually were never produced or identified. Zadoks referred to 'field races' in a similar context. There are now emerging hints that certain QTLs for stripe rust resistance are genotype-specific (Rosewarne et al. 2008; Bansal and Bariana, pers comm).

Comparing the three rusts of wheat, stripe rust APRs are much more commonly encountered and reported, but increasing numbers of examples of leaf rust APRs are emerging in both hexaploid and tetraploid wheats. The reporting of effective APR to *PGT* pathotype Ug99 in the breeding program of RP Singh and others is most encouraging, but further genetic studies are urgently needed to determine if those resistances actually involve genes that are new.

New sources of resistance and resistance to race Ug99

Despite our increasing awareness of the characteristics of Ug99 and its potential as a significant global threat, the discovery of new sources of resistance has been rather slow. Only one new gene for stem rust resistance (Lagudah et al. pers. comm.) has been documented in the last 10 years and the one documented before that (*Sr45*, Marais et al. 1998) is a duplicate of *Sr21*.

Some examples and problems of stem rust resistance

The gene *Sr13* originally transferred to common wheat from *T. dicoccum* is easily recognized and widely effective in seedling tests (IT 2 to 3-), but common wheat lines with this gene alone respond with relatively high MS responses in the field. Grain weight losses in rusted plots of lines with *Sr13* can be as high as 50% relative to rust-protected controls indicating that this gene might have limited value. However, Australian cultivars Machete and Madden, which combine this gene with *Sr2*, are highly stem rust resistant. Although it has been used in stem rust resistance breeding for almost a century, *Sr2* in some genetic backgrounds again confers only limited protection in rust nursery situations. However, its continued use and apparent durability over such a long period dictates that breeders should continue to use it in the future despite its limited field protection under experimental conditions, its sometimes excessive association with pseudo-black chaff symptoms, and its possible close repulsion linkage with *Fhb1*, an important gene for resistance to Fusarium head blight. *Sr13* might be usefully utilized as part of a resistance package including *Sr2*. *Sr13*, or alleles at the same locus, is a very common gene for resistance in tetraploid wheats. Any attempt to isolate and characterize resistance genes in tetraploid wheat should focus on genes that are not *Sr13, Sr8b* or identified *Sr9* alleles.

A significant problem with resistance genes that are moved from lower levels of ploidy to hexaploid wheat is a loss in level of effectiveness with increasing ploidy. For example, the infection types expressed in hexaploid lines with the *T. monococcum*-derived genes *Sr21* and *Sr22* are significantly higher than in the diploid sources. This was particularly true of *Sr21* and is probably the reason that Ug99 was scored virulent for this gene. Apparently the decision to assess Ug99 as virulent for *Sr21*, for which the differential was a Sydney University line, was somewhat arbitrary, but the consequences can be significant for genetic research. According to the North American pathotype designation system, Ug99 was described as race TTKSK, the first 'T' indicating virulence for *Sr21*, or in host-talk, *Sr21* is not effective. Two factors alerted me that there was a problem. Firstly, it was stated in the DRRW document that *Sr21* was ineffective, whereas *Sr45* was effective. Work in our laboratory had earlier shown that PGT pathotypes had the same specificities for these two genes indicating the genes were the same, although they derived from different species and were located in different homoeologous groups (chromosomes 2A and 1D, respectively). Secondly, hexaploid lines with *Sr21* were resistant in the Ug99 nursery in Kenya in 2008. I have not been able to get actual data for the response of lines with *Sr45* to Ug99 from the DRRW research document or elsewhere. Rouse and Jin (2008) reported a summary of a survey of accessions of *T. monococcum* tested with Ug99. Based on work conducted by The in Australia (The 1973, 1976), it was clear that the only gene that could give the frequency of resistance reported by Rouse and Jin was *Sr21*. Rouse and Jin also reported two other genes, both of which were previously identified, reported, and transferred to hexaploid wheat by us (McIntosh et al. 1984). Subsequent discussions with Tom Fetch (AFFRC, Canada) indicated that different isolates of Ug99 may vary in pathogenicity on seedlings of lines with *Sr21* in which case I also predict they would correspondingly vary on seedlings with *Sr45*.

To some observers the above discussion may appear trivial. However, if we are to use the principles of host : pathogen genetics in a genetically meaningful and predictive way our phenotyping must be genetically based and correct. I am sure the resources used to test 1,062 accessions of einkorn wheat could have been used more effectively. Furthermore, the likely reason for the original mis-classification of Ug99 was based on an empirical interpretation of IT 3 as high.

Near–isogenic and single gene reference stocks

Near-isogenic lines are important resources for studies involving all traits, and partial sets of NILs are available for all three rust systems, viz., the Marquis and LMPG sets (Knott), Chinese Spring (Loegering) and W2691 and Line E sets (Watson and Luig) for stem rust, the Thatcher series (Dyck) for leaf rust and the Avocet S (Wellings) and Chinese sets for stripe rust. Unfortunately none of the sets are being extended for newly identified genes because production and conservation of such lines in the public domain is not seen as high profile science. Yet these are the very genetic resources that are required by basic researchers.

I therefore make a plea for international collaboration in the continuing and future development and conservation of appropriate NIL sets for all three wheat rusts. Perhaps the BGRI would be an ideal vehicle to promote and foster the development of such materials. In my role as the co-ordinator of the wheat gene catalogue I raised the issue of public availability of genetic stocks as a condition of naming genes. Although this was agreed in principle, my recent experience is that the collection of seed and getting it available in approved collections through barriers of import permits, export permits, and phytosanitary regulations at both ends is no longer an easy or cheap exercise.

One of the greatest hurdles to agreement on NIL development is agreement on genetic backgrounds – winter or spring wheat? Popular variety? Chinese Spring? Obviously that decision is based on the intended use of such lines. In encouraging the development of the Avocet S NILs for stripe rust work, I saw not only a very susceptible line at all growth stages, but also a widely adapted, easily handled pot plant.

Obviously, if markers are available, MAS can be used to generate the NILs – a good test of validation for both major genes and worthwhile QTLs – and a rust laboratory would not be required to complete the exercise.

Do we need to change the scientific method?

The classic and conservative approach to host: pathogen genetics and gene postulation was that when multi-pathotype test data arrays were identical we assumed that the resistance genes involved were the same until proven different. Given that there is a large volume of data from various laboratories on gene postulation, the likelihood of a rapid discovery of many new genes for stem rust resistance is relatively low. In the DRRW document the statements '*Markers are also essential for determining genetic relationships of different varieties and sources of resistance. Two closely related varieties that possess unknown resistance alleles could have unknowingly derived their resistance from the same source*' are made to justify large-scale haplotyping of wheat genetic resources. Markers are not essential, although very helpful, and to me, the second sentence implies that we must prove genes to be the same, rather than to show they are different – a rather dangerous and hardly justifiable approach to the present problem.

Can we analyse by QTL and forget the pathogen population?

There is no doubt there are many situations where QTL analyses of disease data are justified. However, in situations where the analyses indicate one or two major QTLs accounting for most, say, 45% or greater, of the phenotypic variation across environments it might be worth considering a qualitative analysis and the likelihood that individual genes can be characterized. In two studies in Europe, one in common wheat and the other in durum, major QTLs were co-incident with the known position of *Lr14a* in chromosome 7B. As mentioned earlier pathogenicity for *Lr14a* cannot be determined using the leaf segment testing regime, but is known to occur from whole seedling tests. Swiss workers reported a QTL for leaf rust resistance in cultivar Forno that was co-incident with the position of *Lr14a* in chromosome 7BL. Cultivar Forno carries *Lr14a* (Pathan and Park 2006) thus suggesting the gene is *Lr14a*. A recent publication by Maccaferri et al. (2008) identified a major QTL ($R^2 = 0.73$) in the *Lr14a* region of durum cultivar Creso considered to have durable leaf rust resistance. Although specificity among pathogen isolates was demonstrated, and the seedling resistance was correlated with field response, the authors dismiss the likelihood that resistance was based on *Lr14a* because a virulent isolate was one of the 16 pooled to create the field epidemic. Unfortunately there was no sampling from the field to ensure the presence of that variant. The possibility of the gene being *Lr14a* was apparently considered not important, even though workers in Mexico had recently reported the presence of the gene in some of their durum populations – perhaps not completely surprising since it was originally transferred to hexaploid wheat from cultivated emmer. A reputation of durable resistance and a QTL analysis can easily lead to complacent attitudes.

Another study (Naz et al. 2008) involved a QTL analysis of seedling and adult plant (field) resistance in a backcross-derived population of a resistant synthetic/susceptible wheat cross using a single isolate of the pathogen in the greenhouse, and natural infection over several sites and seasons in the field. One wonders about the biological meaning of a mean seedling IT score and its standard deviation. A total of 11 QTL was identified, six at the seedling stage and seven for APR. One QTL

associated with *Xbarc149-1D* and having the largest effect was indicative of *Lr21* which would likely come from *Aegilops tauschii*. However, it was suggested that the gene would have to be a new allele of *Lr21* because the Tc+Lr21 NIL gave IT 2 (Thatcher IT 3) which was interpreted as a high reaction. As stated earlier, there is no evidence for virulence for *Lr21* anywhere in the world. A suggestion for another QTL, on chromosome 1B, was *Lr26*. Whereas the single test culture used in the greenhouse was clearly avirulent for *Lr26*, presumably there would have been pathogenic variation or total virulence across the many field sites because of the wide use of 1BL.1RS cultivars in Europe over many years. In any case, a synthetic wheat involving *T. dicoccoides* as parent could not carry *Lr26*. Other naive comparisons are made. It seems that some research laboratories have become addicted to the power of the QTL approach, and the widespread assumption that QTLs represent non-specificity. The biological methodologies and ways of interpreting host : pathogen data, as well as the knowledge accumulated over the last 90 years seems to be forgotten.

Alien segments in wheat usually retain their integrity

A considerable number of wheat lines carry alien segments that have either contributed, or have potential of contributing, to agriculture. Those segments should generally not recombine with wheat chromosomes and genetic mapping should locate the translocation break-points. Yet I see published (and even more unpublished) genetic maps that seem to ignore cytogenetic realities. A recent paper by Mebrate et al. (2008) can be taken as an example because it provides the F_3 phenotypic data used to allegedly map *Agropyron intermedium*-derived gene *Lr38* in chromosome 6DL. Apart from the fact that a result of 16:0 was treated as genotype RR and 15:1 was assumed to be Rr, the data for segregating lines are highly heterogeneous and should not have been treated as a uniform group of samples. If markers *Xwmc773*, *Xcfd5* and *Xcfd60* were dominant instead of co-dominant because the alien segment lacks amplifiable alleles, they should have co-segregated with *Lr38*. The authors proposed 'a massive screen for polymorphic markers' in close proximity to *Lr38*, but maybe a microscope and some cytology will be more biologically rewarding. The basic principles of Mendelian genetics and statistics should not be over-ridden with MAPMAKER.

Non-host resistance

The rally-call to the BGRI was the question of why rice has no rust disease raised by Dr Borlaug. I think Dr Borlaug can recall his days at the University of Minnesota when people were asking why cereal rye and oats were resistant to *PGT* and why wheat was resistant to *PGS* and *PGA*. It seems to me we can address the question of non-host resistance in two ways – try to understand the closer relationships just exampled, or search for effectors and receptors that might work at more distant levels.

Barley is a non-host (or maybe a near non-host in the terms used by Niks and co-workers) of wheat leaf rust. When we add the individual chromosomes of barley to wheat, no single addition line confers leaf rust resistance. Cereal rye is a non-host of wheat leaf rust; if we add rye chromosomes to wheat, some addition lines do have resistance (e.g. Petkus 1R lines with *Lr26*). By extension we might speculate the consequences of adding single chromosomes of oats, maize or rice to wheat and clearly we cannot predict what the consequences might be. Thus even if we can characterize the components of non-host resistance there is no assurance that they will function if placed in a wheat background. A search will then be needed to find the molecular tools that enable the expression of potential R genes in the recipient background. Various researchers have documented instances where rust and mildew resistances obviously expressed in potential donor species are not expressed in the amphiploids derived from them. An understanding of what is required for the expression of those resistances in wheat backgrounds would seem to be also relevant to understanding and perhaps utilizing some components of non-host resistance.

Working at the *formae speciales* level Australian rust workers showed that wheat carries resistance genes that are effective against *PGS*. The gene *Sr18* in wheat is not only widely effective against *PGS*, but is present in most common wheat genotypes. Thus *PGT* must have virulence for this gene. The presence of this gene and a low number of others is adequate to largely protect wheat against *PGS*. At the same time it can be shown that rye carries a set of genes that protect it against *PGT*. All *PGS* and *PGT* x *PGS* isolates in Australia are virulent for *Sr11* and polymorphic in pathogenicity for *Sr5*. Likewise, *PGT* clones vary in pathogenicity on certain cereal rye genotypes. The wheat lines W2691 and Line E were developed as genetic platforms allowing studies using isolates of both *PGT* and *PGS* and their hybrids.

Returning to the rice example one approach might follow the wheat : rye example by screening a wide representation of rice genetic resources, including Chinese wild rice, searching for unrelated accessions allowing some symptoms of infection. These could be intercrossed to permit a search for transgressive segregation, not forgetting the disease triangle, and thus the use of a geographic array of pathogen cultures and manipulation of the environment to enhance differences.

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3. Using race survey outputs to protect wheat from rust

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Abstract

Race (pathotype) surveys of cereal rust pathogens have been conducted in many parts of the world since the early 1900s. The only way to identify rust pathotypes remains virulence testing in greenhouse tests using genotypes ("differentials") carrying different resistance genes. Virulence determinations have rarely targeted genes conferring adult plant resistance because of the technical difficulties of working with adult plants under controlled conditions. Where pathotype surveys have been conducted in a robust and relevant way, they have provided both information and pathogen isolates that underpinned rust control efforts, from gene discovery to post-release management of resistance resources. Information generated by pathotype surveys has been used to: devise breeding strategies; indicate the most relevant isolates for use in screening and breeding; define the distribution of virulence and virulence combinations; allow predictions of the effectiveness/ineffectiveness of resistance genes; and issue advance warning to growers by identifying new pathotypes (both locally evolved and introduced) before they reach levels likely to cause significant economic damage. To be most effective, pathotype surveys should also provide fully characterized isolates (defined pathotypes) for use in identifying new sources of resistance and screening breeding material. Although constrained to some extent by a lack of markers, particularly those not subject to natural selection, surveys have also provided considerable insight into the dynamics of rust pathogen populations, including the evolution and maintenance of virulence, and migration pathways, including periodic long-distance migration events.

Keywords

Avena, genetics, *Hordeum*, leaf rust, *Puccinia*, stem rust, stripe rust, *Triticum*, yellow rust

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Introduction

Formal genetic analysis of disease resistance in plants began over 100 years ago when Biffin (1905) demonstrated that resistance to stripe rust in wheat (caused by *Puccinia striiformis* f. sp. *tritici*) was inherited as a single recessive Mendelian trait. At the time of those studies, Biffin was unaware of pathogenic variability in the stripe rust pathogen. The discovery of physiologic races in the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) by Stakman and Piemeisel (1917), and later in other rust pathogens of cereals, was important in demonstrating the need to monitor pathogenic variability and to use this information and the most relevant rust isolates in genetic research and resistance breeding. Unfortunately, publications that ignore the importance of pathogenic variability in rust pathogens continue to appear in the scientific literature.

While the discovery of heritable resistance to rust led to great optimism that a solution to rust diseases had been found (Watson and Butler 1984), early attempts to develop genetically resistant wheats involved the development of cultivars with single genes for resistance. Almost invariably, matching virulence in the rust pathogen followed with the cultivar being rendered susceptible in what became known as the "boom and bust" cycle. For example, the first stem rust resistant wheat released in Australia was cv. Eureka (1938), protected by the single resistance gene *Sr6*. It increased in popularity and by 1945 occupied about 18% of the wheat area in northern New South Wales and Queensland (Watson and Luig 1963). Virulence for *Sr6* was first detected in 1942, and its frequency in the *P. graminis* f. sp. *tritici* population in this region increased as the area sown to Eureka increased (Watson and Luig 1963). Many examples of such "boom and bust" cycles were documented in the years since. Similarly, the breakdown of genes such as *Sr24*, *Sr27*, *Sr36*, *SrSatu*, *Yr25* and *YrA* has had a significant impact on wheat production in South Africa (Pretorius et al. 2007a; Pretorius ZA unpublished).

Race (pathotype) Surveys

Pathotype surveys of cereal rust pathogens are conducted in many parts of the world, and typically involve identifying pathotypes present in rust samples collected from crops, volunteer (self sown) cereals, rust susceptible grass species, and experimental plots (including breeders' plots and rust trap nurseries). The only way to identify pathotypes is virulence testing in greenhouse tests using genotypes carrying different resistance genes ("differentials").

The sources of rust samples used to inoculate differential sets differ between laboratories. In some cases, single pustule isolates are established from rust samples before inoculating differential sets (e.g. North America), whereas in others, a spore suspension derived from the original field sample is used (e.g. Australia, India). The latter approach often allows detection of pathotypes present in samples at low levels, but can also confound determinations if pathotypic diversity is high and mixtures are encountered. In these instances, it is necessary to establish single pustule sub-cultures from specific differential genotypes. These are then applied individually to differential sets to determine the component pathotypes. In situations where single pustules are sampled for pathotyping, bulked spore samples from residues are used to inoculate key universally resistant genotypes as a means of detecting rare but potentially important variants.

While some studies have examined virulence for genes conferring adult plant resistance (e.g. Park and McIntosh, 1994), this has not been done on a routine basis because of the technical difficulties of working with adult plants under controlled conditions. However, Pretorius et al. (2000; 2007b) successfully tested mini-adult plants for resistance to leaf and stripe rust in controlled environments, showing that analysis of pathogenicity for adult plant resistance genes is possible.

Race surveys and pre-breeding for rust resistance

Genetic studies of host resistance

An important, but inadequately acknowledged contribution to resistance breeding made by pathotype surveys is the provision of characterized pathogen isolates for use in identifying resistance in germplasm. A comprehensive collection of well characterized rust isolates, coupled with a basic understanding of the genetics of host : pathogen interactions, are powerful tools to resolve the identities and relationships between resistance genes, and to assess the potential value of new resistance sources.

Knowing the virulence attributes of rust isolates used in genetic studies, whether they be greenhouse-based studies of seedling resistance, or field-based studies of resistance expressed at adult plant growth stages, is vital if the results obtained are to be interpreted in a meaningful way. A wheat genotype that displays resistance at adult plant growth stages in the field could carry either seedling resistance, adult plant resistance (APR), or both, and the only way to discriminate between the two types of resistance is to have some understanding of the seedling resistance genes present in the genotype and of the virulence(s) of the isolate(s) present in the field nursery. For example, an assessment of APR to stem rust in a wheat genotype carrying the seedling resistance genes *Sr24* and *Sr31* can only be made using a stem rust isolate carrying virulences for both genes (e.g. TTKST or "Ug99 +Sr24") and to which the wheat genotype is seedling-susceptible. If such an isolate is not available, a mapping population could be developed, and lines lacking either resistance gene can be identified and assessed for adult plant rust response. In the absence of such information it is impossible to relate mapping data to known rust resistance genes.

Using characterized rust isolates to identify resistance genes.

Valuable preliminary information on the genetic basis of rust resistance in cereal germplasm can be obtained using multipathotype tests in which an array of rust cultures with known pathogenicity is used for gene postulation (Loegering et al. 1971). Australian and South African pathogenicity surveys of the wheat rust pathogens have identified groups of pathotypes considered to represent closely related clonal lineages comprising step-wise mutants that differ in virulence/ avirulence for single resistance genes. These are the pathogen equivalent of near-isogenic host series carrying individual rust resistance genes in a common genetic background, and are invaluable in multipathotype testing aimed at postulating the identities of resistance genes and in recognizing potentially new resistance genes.

At least 96 loci confer resistance to *Puccinia coronata* f. sp . *avenae* (*P. c. avenae*) in oats (http://www.cdl.umn.edu/res_gene/ocr.html). A lack of single gene reference stocks for many of these genes, plus high levels of genetic diversity in the pathogen, make it very difficult to identify *Pc* genes in germplasm by multipathotype testing. In Australia, the seedling resistances of many Australian oat cultivars, all of which have been overcome by matching virulence in *P. c. avenae*, are unknown. Detailed studies of pathotypes virulent on 10 oat cultivars that were regarded as seedling resistant to *P.c. avenae* when released between 1991 and 2003 (Barcoo, Bettong, Cleanleaf, Culgoa, Graza 68, Gwydir, Moola, Nugene, Taipan and Warrego) demonstrated that they are pathogenically very similar and were likely derived via single-step mutations (Park RF unpublished). The pathotypes were characterized

extensively on host stocks, and in turn were used to resolve the identities of the resistance genes present in the cultivars. Whilst some of the 10 resistant oat cultivars were regarded as having "new" uncharacterized seedling resistances, it is now clear from the detailed comparative multipathotype studies that most possess combinations of previously characterized genes. For example, cv Cleanleaf was previously reported to carry *Pc38*, *Pc39* and an uncharacterized resistance gene (Bonnett 1996) that on the basis of multipathotype testing is now considered likely to be *Pc52* (Park RF unpublished).

Pathotypes virulent on the 10 oat cultivars have also been invaluable in identifying seedling resistance genes present in other oat germplasm. Detailed multipathotype tests of 166 lines from the 1998 and 1999 Quaker oat nurseries indicated a range of resistance genes, and it was clear that some lines carried the resistance genes present in cultivars Bettong (42 entries), Gwydir (six entries), Nugene (one entry) and Warrego (four entries) (Haque S and Park RF unpublished). These studies also permitted the field identification of 12 nursery entries lacking effective seedling resistance genes, but possessing very high levels of APR to crown rust (Haque 2004).

It is hoped that these related pathotypes will also assist in resolving the confusion surrounding many of the *Pc* genes described so far. Recently, seedling tests of known genetic stocks using a pathotype virulent for *Pc94* and a series of isolates that included the putative parent of this pathotype implicated the presence of this resistance gene in *Avena strigosa* accession CI 3815. This line was originally reported to carry *Pc19* and *Pc30* (Simons et al. 1959; Marshall and Myers 1961), and more recently, to carry five tightly linked genes, designated *Pc81–85* (Yu and Wise 2000). The genetic relationships between *Pc19*, *Pc30* and the *Pc81–85* complex are not known; however, the evidence from our tests suggests that one of these genes and *Pc94* are synonymous. Gene *Pc94* was introgressed into hexaploid oats by Aung et al. (1996) from *A. strigosa* accession RL1697. Tests of RL1697 with the *Pc94*-virulent and -avirulent *P. c. avenae* pathotypes would be a simple means of testing this hypothesis further.

Virulence associations can provide insight into the genetic basis of rust resistance. Australian isolates of *P. triticina* virulent for APR gene *Lr12* are also virulent for the complementary seedling resistance genes *Lr27* and *Lr31* (Park and McIntosh 1994). Similar associations were communicated to these authors by colleagues in South Africa, Argentina and Mexico. Because of this virulence association, and the location of both *Lr12* and *Lr31* on chromosome 4B, Park and McIntosh (1994) predicted the genes were either linked or at the same locus. Subsequent genetic analyses established that the two genes were either completely linked or the same (Singh et al. 1999). If the latter is correct, then *Lr27* acts in a complementary manner with *Lr12* in seedlings to confer resistance, but its presence is not necessary for the adult plant expression of resistance conferred by *Lr12*. This interesting genetic model was developed based on an original observation of an association between virulences for the genes *Lr12* and *Lr27*+*Lr31*, and demonstrates clearly the insight that can be gained from detailed knowledge of pathogen virulence. Furthermore, it also established that virulence on the APR gene *Lr12* can most likely be monitored in seedling-based pathogenicity assays using a differential genotype carrying *Lr27*+*Lr31*.

Pre-breeding

A comprehensive set of well characterized rust isolates permits the identification of potentially new sources of resistance (see example in preceding section on crown rust resistance in oat genotype CI 3815), and also allows an assessment of the effectiveness of new rust resistance genes to local pathotypes prior to their use. For example, although the resistance genes *Lr3ka*, *Lr15* and *Lr41* (leaf rust resistance in wheat), *Sr8b* and *Sr35* (stem rust resistance in wheat), *Yr8* (stripe rust resistance in wheat), and *Rph5*, *Rph6*, *Rph10* and *Rph13* (leaf rust resistance in barley), and *Pc92* and *Pc94* (crown rust resistance in oats) have never been deployed in Australia, virulence to all existed in at least one isolate maintained in an historical cereal rust collection compiled over the past 80 years of pathotype surveys. While this suggests that such resistance genes may not be durable if deployed, where the frequency of virulence is low (e.g. *Lr41*, *Sr35*, *Rph13* and *Pc94*), the existence of such pathogen isolates provides a means of selecting lines carrying these genes with other effective resistance genes.

The Australian Cereal Rust Control Program (ACRCP) at the University of Sydney undertakes "parent building", in which key wheat genotypes (selected to represent the current range of maturity and quality classes) are used as recurrent parents into which new rust resistance genes are backcrossed and then distributed to breeding groups as locally adapted donor sources. In these cases, choice of pathotype to select individual backcross plants from the BC_2 generation onwards is crucial in ensuring the target gene is selected.

Race surveys and breeding for rust resistance

Disease resistance breeding strategies

The recognition of mutation as a major source of variability in wheat rust pathogens led to the development of both gene combinations (Watson and Singh 1952; aka "gene stacking" or "gene pyramiding") and pre-emptive or anticipatory breeding (McIntosh and Brown 1997).

The former strategy assumes that mutation events are independent, and therefore that the frequency of simultaneous mutations for virulence to more than one resistance gene will be extremely low. In a dikaryotic organism in which mutation to virulence may have to occur in two nuclei, the probability is even lower. Following a series of "boom and bust" cycles in northern NSW and Queensland, Luig and Watson (1970) stated that "During the past 15 years, it has become abundantly clear that cultivars with single genes for resistance to stem rust are of limited value in Region 1". Whereas good molecular markers add precision to selection and make it easier to combine resistance genes, breeders had success in the past in combining multiple effective rust resistance genes without such markers. For example, the genes *Sr24* and *Sr26* were combined in the Australian wheat cultivar Sunelg, released over 20 years ago, and *Sr24* and *Sr38* were combined to produce cultivars QAL2000 and QALBis. In both cases, the combinations were produced in the absence of cultures virulent for either gene and of linked molecular markers. In the latter example, seedling stem rust tests using a culture avirulent for both genes allowed reliable identification of lines combining the genes because the genes interact to confer an infection type lower than that produced by the two genes individually (Brown GN unpublished). Similarly, many combinations involving the durable but recessive adult plant resistance gene *Sr2* have been assembled by utilising the linked traits pseudo black chaff and seedling chlorosis (Brown 1997; McIntosh et al. 1995). The linkages of *Lr24* with *Sr24*, and of *Lr37* and *Yr17* with *Sr38* (McIntosh et al. 1995) allowed selection of the stem rust resistance component in the presence of other stem rust genes using the completely linked leaf rust or stripe rust genes as "markers".

Anticipatory breeding is based on the premise that future mutations in a pathogen can be predicted (McIntosh and Brown 1997). Monitoring virulence in rust pathogen populations allows predictions of the effectiveness/ ineffectiveness of resistance genes, and in so doing, provides direction for breeders. For example, the resistance gene *Lr24* remained effective in Australia from 1983 until virulence was detected in a single pathotype in South Australia in 2000 (Park et al. 2002). In contrast, *Lr24* was either ineffective or overcome not long after its deployment in the USA (Long et al. 2000), Canada (Kolmer 1998), and South Africa (Pretorius et al. 1987). The rapid development of *Lr24* virulence in these countries was a clear indication of the potential for this to occur in Australia. Pathotype surveys of *P. triticina* during this period monitored virulence for *Lr24* and the frequency of this gene in breeding populations and wheat cultivars. At least 28 cultivars with *Lr24* were released in Australia following the release of Torres in 1983, and by 1993, the area sown to cultivars with *Lr24* was about 45% in Queensland and 35% in New South Wales (Brown 1994). The reason(s) why virulence for *Lr24* was not detected in Australia for 17 years after the gene was first deployed, although not entirely clear, likely relate in part to its initial deployment in northern NSW and Queensland where leaf rust inoculum levels were low, the result of strict adherence to rust resistance standards in this region from the 1970s onwards (Platz and Sheppard 2007; Wallwork 2007). In this region, fewer mutational events would be expected in situations of small pathogen population size. The initial detection of virulence for *Lr24* in southern regions is consistent with this theory, because leaf rust was at relatively high levels in this region from the 1990s (Park et al. 2002). The presence of *Lr34* in addition to *Lr24* in some cultivars deployed in the north (Singh et al. 2007) also likely contributed to its effectiveness in this region.

Rust resistance screening

Developing germplasm with resistance to rust usually involves screening with rust isolates in either field nurseries and/or the greenhouse. This is most effective, and the resistances selected are most useful when isolates of greatest relevance to the target agricultural production system are used. Pathotype surveys play a central role in this process by providing information on the frequencies and distributions of pathotypes, and the isolates for use in germplasm screening. However, the effectiveness of pathotype surveys in achieving this is determined largely by the differential genotypes used to identify pathotypes. Resistance genes deployed in commercial cultivars should be represented in the differential sets used for pathotype determinations to ensure the relevance of information to breeding programs. Because the resistance genes in use differ between geographical regions, the composition of differential sets will also differ if pathotype surveys are to be relevant to local conditions.

The ACRCP provides greenhouse seedling and field adult plant screening services to all cereal breeding groups in Australia. The pathotypes used to screen breeding material are selected based on their

current relevance and their virulence combinations - knowledge gained from pathotype surveys. Field rust nurseries typically use from 1 to 3 key pathotypes of each species, which are selectively encouraged using cereal genotypes specific to each (e.g. a mixture of wheat genotypes Worrakatta (*Lr24*), Sunstar (*Lr13*) and Marombi (*Lr37*) to promote pathotypes with virulence for each gene individually). A similar service is provided in South Africa where the University of the Free State tests commercial varieties and elite breeding lines with stategic pathotypes on an annual basis.

Long term pathotype surveys have shown that the composition of rust pathogen populations vary enormously with time (see section below). This has at times included situations where new or previously rare genes for avirulence have been introduced and increased in frequency. The "Mackellar" *P. triticina* pathotype, first detected in Victoria in 2004, was considered to be of exotic origin because of more than 5 pathogenic differences from local *P. triticina* pathotypes (Park RF unpublished). Of interest was avirulence for the wheat genotype Morocco (since found to carry a resistance gene located on chromosome 2BS) and a range of Australian wheat cultivars including Halberd, Avocet, Tarsa and Tincurrin (Park RF and Singh D unpublished). Knowledge of this avirulence is very important to avoid inadvertent selection for the gene, which is of limited or no use in resistance breeding because of virulence in virtually all other Australian *P. triticina* pathotypes. Similarly, from 1979 to 2002, virtually all pathotypes of the wheat stripe rust pathogen identified in race surveys were virulent for the gene *Yr3* (Wellings 2007). However, the introduction of a new pathotype in 2002, avirulent for *Yr3* was followed by a rapid decline in virulence for these genes (Wellings 2007). Despite this, selection and deployment of *Yr3* would not be advisable, and its presence in breeding populations is now being monitored, based on knowledge and rust isolates generated by pathogenicity surveys.

Post-breeding management of rust resistance

Whereas most emphasis is placed on identifying, characterizing, and incorporating rust resistance into cereal cultivars, the attention given to post-release management of rust resistant cultivars is unfortunately often much less. An understanding of the resistance genes present in commercial cereal cultivars is important in allowing the risk and implications of resistance gene breakdown to be assessed and managed.

Predicting cultivar vulnerability to mutational change

The wheat cultivar Oxley carrying the stem rust resistance genes *Sr5*, *Sr6*, *Sr8a* and *Sr12*, occupied between 7 and 9% of the area in northern New South Wales and Queensland from 1976 to 1985, despite being susceptible to stem rust pathotype 343-1,2,3,5,6 (Zwer et al. 1992). At that time, a significant proportion of the wheat crop in this region comprised the cultivars Cook (*Sr5*, *Sr6*, *Sr8a*, *Sr36*), Songlen (*Sr2*, *Sr5*, *Sr6*, *Sr8a*, *Sr36*) and Timgalen (*Sr5*, *Sr6*, *Sr8a*, *Sr36*). Watson (1981) warned of the vulnerability of the latter cultivars to mutational change in the Oxley-attacking pathotype, which only had to acquire virulence for *Sr36* to render all sufficiently susceptible to suffer yield losses. Pathotype 343-1,2,3,4,5,6, regarded as a single-step mutational derivative of the Oxley attacking pathotype with virulence for *Sr36*, was duly detected in 1984, after which the area sown to these cultivars declined (Zwer et al. 1992).

Monitoring rust pathogen populations

Rust samples used for pathotype surveys can come from a range of sources, including experimental plots and commercial cereal crops. Where resources do not exist to undertake structured sampling from commercial crops over large areas (e.g. transport), important information on virulence and virulence combinations in rust pathogen populations can nonetheless be gained from undertaking pathotype analyses on samples collected from plots at experimental field sites.

Structured race surveys in which samples are collected across a large area from commercial crops or in areas where cereals are grown should include the collection of information on rust incidence (i.e. surveillance). Such information is important in identifying potential build-up of inoculum. In Australia and South Africa, monitoring the incidence of rust diseases between cropping cycles during the summer period is an important component of rust management. During wet summers, volunteer (self sown) cereals can establish and give rise to "green bridges" that provide opportunities for rusts to build up. In such situations, extension is important to notify the agricultural community of the importance of green bridge destruction in minimising opportunities for oversummering of rust pathogens. This knowledge also provides some advance warning of potential rust build-up, which can be used to advise growers of the need to consider seed treatment and/or advance purchase of fungicides. It is also of relevance to chemical suppliers, who may need to make decisions on whether to stockpile chemicals well in advance of a given production cycle.

Surveillance and pathotype information combined provide an understanding of migration pathways and the distribution of rust pathotypes, which in turn allow assessment of the regional effectiveness of resistance genes and the resistance status of cereal cultivars. In Australia, the eastern and western cereal belts are separated by ca. 1,500 km of desert and long-term race surveys have provided clear evidence of inoculum exchange between the two regions. These surveys have however indicated that this exchange occurs mainly west-to-east on prevailing winds; for example, since 1988 there have been seven incidences of inoculum exchange from west to east, but only one instance where this has occurred in the opposite direction. The appearance of new pathotypes of *P. striiformis* f. sp. *tritici* and *P. triticina* in eastern wheat areas over the past 20 to 30 years in particular, via exotic incursion or local mutation, has resulted in the presence of many virulences that were not recorded in the west (e.g. *Lr13*, *Lr24*, *Yr3*, *Yr4*, *Yr17, Yr27*), and these genes therefore remain important in protecting the Western Australian wheat crop from the respective diseases.

Early warning

The early detection of a new pathotype allows advance warnings to be issued to growers, especially when such pathotypes are identified and characterized before they reach levels likely to cause significant economic damage. To be most effective, early warnings should include an assessment of the risk of a new pathotype to all currently grown cultivars. This can be achieved by initial characterization of the virulence attributes of the pathotype, followed by seedling and eventually adult plant field based tests of cereal cultivars with the new pathotype. For example, virulence for the resistance gene *Yr27* in eastern Australia was detected in late 2008, and at that time, it was known that three wheat cultivars carried this resistance gene. Detailed comparative greenhouse seedling tests of all Australian wheat cultivars with the new pathotype provided not only confirmation of the occurrence of *Yr27* in these three cultivars, but also an indication of a lack of additional effective seedling resistance in all three (Wellings CR unpublished). Since then, extension bulletins have been prepared and distributed to the farming community. The next step will be to establish the new pathotype in field rust nurseries in 2009, to assess whether these cultivars carry residual APR and also to determine the impact of this mutational change on breeding populations.

Rust pathogen population dynamics

Although constrained to some extent by a lack of markers, particularly those not subject to natural selection, surveys have also provided considerable insight into the dynamics of rust pathogen populations, including the evolution and maintenance of virulence, and migration pathways, including periodic long-distance migration events. Because of the isolation of the Australian continent from other cereal-growing regions of the world, the long term surveys of pathogenic variability in *P. graminis* f. sp. *tritici* and *P. triticina* in particular have provided rare insights into rust population dynamics and the processes that generate variability in asexually reproducing pathogen populations. Combined, the survey data strongly implicate periodic introduction of exotic pathotypes, single-step mutation, and more rarely, somatic hybridization, as the major determinants of cereal rust population structure in Australia. All three processes were observed in pathogenicity surveys of *P. triticina* between 1980 and 2005 (Park et al. 1995, 1999). Exotic rust incursions are equally important in South Africa as exemplified in recent years by the introduction of *P. striiformis tritici* pathotype 6E16A- in 1996 (Pretorius et al. 1997) and *P. graminis* f. sp. *tritici* pathotype TTKSF in 2000 (Visser et al. 2009).

Rust pathogen populations vary enormously over time with wide shifts in virulence occurring, often for no apparent reason. In Australia, there have been four instances in which an exotic incursion led to a complete change in the local wheat rust pathogen population: viz. *P. graminis* f. sp. *tritici* race 126 in 1925; *P. graminis* f. sp. *tritici* race 21 in 1954; *P.triticina* race 104 in 1984; and *P. striiformis* f. sp. *tritici* race 134 in 2002. Presumably in each case, the new pathogen genotype was more aggressive and better able to compete, survive and build up, features that also appear to characterize "Ug99".

Future directions in pathogenicity surveys

Australian pathotype surveys of wheat rust pathogens over the past 80 years have clearly shown an increase in the frequency of exotic incursions with time, possibly a consequence of increased international movement of people. The origins of most of these incursions are unknown. The advent of remote sensing and of GIS technologies have provided additional tools for rust surveillance, that in conjunction with pathotype analysis, should provide increased understanding of intercontinental long-distance movement of rust pathogens. The application of new DNA-based marker

systems to study rust pathogen variability will allow more critical appraisals of the role of mutation in generating variability, and also provide insight into global variability in these pathogens. Central to all of this work, and fundamental to sustained genetic control of rust pathogens, will be the need for relevant and informed pathogenicity surveys. Any future increase in restrictions on the movement of biological material will further highlight the need to develop in-country pathotype analysis capabilities, both in terms of infrastructure and skilled personnel.

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4. Using trap plot outputs to protect wheat from rust

K Nazari[1], AH Yahyaoui[1], R Singh[2], T Fetch[3], D Hodson[2], R Park[4]

Stem rust of wheat, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), historically causes severe epidemics, and was once the most feared wheat disease worldwide. Complete crop failure can occur when susceptible cultivars are grown in favorable conditions. Stem rust has been controlled partially or successfully in many regions through the eradication of alternate hosts, deployment of stem rust resistance genes, and by growing wheat cultivars with earlier maturity. The widely used gene *Sr31*, located on the 1BL/1RS translocation, has contributed significantly to the control of stem rust for almost four decades. *Sr31* is common in European winter wheat cultivars and spring wheat cultivars developed by CIMMYT and ICARDA. It was also widely used in China. *Pgt* race TTKSK (commonly referred to as Ug99), and two mutational derivatives carrying separate virulences for *Sr24* and *Sr36*, are the only known races that possess virulence for *Sr31*. Ug99 was first detected in Uganda in 1998, then spread to Kenya, Ethiopia, Yemen and Sudan. In 2007 it was detected in Iran. It is expected to spread to North Africa, the Middle East, Europe, and beyond with prevailing winds.

The analysis of pathogenic variation in rust pathogens is fundamental to understanding pathogen population structure, host-pathogen co-evolution and breeding for durable resistance. Over the last 70 years, rust workers have conducted pathogenicity surveys and race analysis using differential host genotypes for seedling and adult-plant resistance genes. Currently, the threat of long distance migration of rust pathogens, the use of similar wheat genotypes in many wheat growing areas, and a lack of basic facilities and expertise in many developing countries have increased the importance of international collaboration in monitoring cereal rusts. National, regional and international biological rust trap nurseries comprising differential genotypes, known cultivars with widely used rust resistance genes and local commercial cultivars, have been established in almost all wheat growing areas worldwide. For example the 2nd Ug99 International Stem Rust Trap Nursery (2nd Ug99 ISRTN) and 4th International Stem Rust Trap Nursery (4th ISRTN) were distributed by ICARDA in 2009 to 144 locations in 32 countries. Surveys worldwide have provided ample evidence of the usefulness of these nurseries in epidemiology and pathogen evolutionary studies, but they may have been of only limited benefit to breeding programs. Several factors have influenced the value of trap nurseries to wheat breeders.

In this paper, achievements, present status and future plans for the use of stem rust trap nurseries in disease surveillance, early warning and breeding for durable resistance will be discussed.

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5. The global cereal rust monitoring system

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Abstract

Cereal rusts have long been the scourge of wheat farmers worldwide. Three fungal rusts are capable of inflicting serious economic damage to wheat; namely, leaf rust, stripe rust, and stem rust. Historically, stem rust was the most feared disease of wheat, but since the 1950s, effective resistance has protected crops and livelihoods. By the mid 1990s stem rust had been reduced to negligible levels worldwide. The detection of the Ug99 lineage of stem rust in Uganda in 1998 has challenged the assumption that stem rust was a conquered disease, and up to 80% of the world's wheat is now considered stem rust susceptible. Ug99 has sparked a global effort by wheat scientists to counter the threat and has highlighted the need for effective surveillance and monitoring systems. Outside of a few developed countries, monitoring efforts are often irregular or even non-existent and no coordinated global surveillance effort currently exists. Ug99 has provided the impetus to implement a global surveillance and monitoring system that provides relevant and timely information as a global public good. Key components, current status and future plans for this emerging cereal rust monitoring system are described. The immediate concern regarding Ug99 makes it an initial priority focus, but the other cereal rusts cannot nor should be excluded.

Lessons can be learned and parallels drawn from existing successful trans-boundary monitoring schemes such as the Desert Locust monitoring and early warning system implemented by the UN Food and Agriculture Organization (FAO). Successful networking, expanded capacity of partners, efficient field surveys and data handling, plus regular timely targeted information products are all components of the Desert Locust scheme that need to be transferred to a cereal rust monitoring system. Through a consortium of partners several advances have already been made targeting the Ug99 lineage of stem rust. GIS technology is forming the backbone of an emerging rust monitoring and surveillance system being developed collaboratively by international agricultural research centers, UN agencies and advanced research institutes. The system already incorporates a rapidly expanding volume of standardized geo-referenced field survey data, routine use of wind models and public domain web tools delivering information in near-real time. Several challenges still remain before a fully operational system is created, and these are outlined.

The need for vigilance and a lack of complacency regarding unexpected events are highlighted. These might include; accidental assisted movements, natural long distance dispersal and the threat of rust pathogens occurring in "non-traditional" areas as a result of climate change.

Keywords

Ug99, GIS, trans-boundary, networking, surveillance

Introduction

Globally, wheat is grown on over 200 million hectares from the equator to latitudes of 60°N to 44°S and elevations ranging from sea level to over 3,000 m (e.g. Hodson and White 2007). Total global production amounts to approximately 600 million tonnes, with developing countries accounting for nearly half of this total. Wheat is a global staple cereal and accounts for a significant proportion of total caloric intake in several countries, notably in North Africa / Mediterranean, the Middle East, and parts of Central Asia where annual per capita consumption rates can reach over 200 kg (FAOSTAT 2003). Any economically important pathogen of wheat is obviously a high priority for research and control. The three fungal rusts that infect wheat have long been the focus of intense study.

In recent years, leaf (or brown) rust (caused by *Puccinia recondita*) and stripe (or yellow) rust (*Puccinia striiformis* f. sp. *tritici*) have been the most damaging fungal diseases of wheat, causing considerable losses worldwide (e.g. Singh et al. 2008). As a result, most of the recent research and breeding efforts have focused on these diseases. However, historically stem (or black) rust (*Puccinia graminis* f. sp. *tritici*) was the most feared disease of wheat, capable of causing periodic severe devastation across all continents and in all areas where wheat is grown. There is a solid foundation behind this fear as an apparently healthy crop only 3 weeks from harvest could be reduced to nothing more than a tangle of black stems and shriveled grain by harvest. Under favorable conditions, yield losses of 70% or more are possible (Roelfs et al. 1992).

The last major epidemics from stem rust occurred in the mid 1950s when over 40% of the North American spring wheat crop was lost to devastating epidemics

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(Leonard 2001). These devastating losses resulted from the emergence of a new stem rust race named 15B, which overcome the resistances in widely grown cultivars at the time. As a consequence of the devastating stem rust epidemics in North America, Nobel laureate Dr N.E. Borlaug initiated his wheat improvement program in Mexico, with the development of stem rust resistant varieties being a primary goal. The resulting semi-dwarf wheat varieties that he developed, which underpinned the "Green Revolution" in the 1960/70s, and subsequently became adopted on millions of hectares worldwide, had very effective resistance to stem rust (Saari and Prescott 1985).

Since the epidemics of the 1950s, widespread use of resistant wheat cultivars worldwide reduced the threat of stem rust to the extent that it was not a significant factor in wheat production losses. By the mid 1990s stem rust was largely considered to be a disease under control (e.g. Roelfs et al. 1992). This resulted in a dramatic shift away from stem rust research and a lack of direct experience with the disease in the field for many pathologists and breeders. Stem rust was almost considered a vanquished disease. However, with the detection of a new virulent stem rust race lineage - popularly named Ug99, after being first identified in Uganda during 1999 (Pretorius et al. 2000) - that perspective has now changed. Up to 80% of world's wheat is now considered stem rust susceptible. As a result, stem rust is now very firmly back on the agenda of wheat scientists worldwide. Detection of Ug99 has highlighted the need for effective global surveillance and monitoring systems.

Outside of a few developed countries, monitoring efforts are often irregular or even non-existent and no coordinated global surveillance effort currently exists. Ug99 has provided the impetus to implement a global surveillance and monitoring system that provides relevant and timely information as a global public good. International collaborative efforts are now underway to develop a global cereal rust monitoring and surveillance system underpinned by GIS technology. Key components, current status, future plans and the challenges for this emerging cereal rust monitoring system are described. The immediate concern regarding Ug99 makes it an initial priority focus, but the other cereal rusts cannot nor should be excluded.

Challenges in establishing a global cereal rust monitoring system

The basic biology of rust fungi makes them a monitoring challenge. They are highly mobile, air-borne pathogens capable of moving hundreds or even thousands of kilometers within a short period of time. They cross international boundaries with impunity and can survive in a wide range of environments and on a wide range of hosts.

Taking stem rust as an example, billions of urediniospores are produced from a moderately rusted field of wheat (e.g. Stakman 1957). Although the vast majority of the spores will be deposited close to the source (Roelfs and Martell 1984), huge numbers remain with the potential to infect more distant wheat fields. The robust nature of these spores ensures protection against environmental damage and permits them to remain viable for journeys of 100s or even 1000s of kilometers. Medium to long distance dispersal occurs primarily though air-borne means, but accidental long-distance transmission on infected clothing or plant material is also possible (e.g. Brown and Hovmøller 2002).

The stem rust pathogen is an obligate biotroph so needs a live primary host. Wheat, barley and triticale are the hosts of primary economic concern, but a wide range of grass species can also act as hosts. In terms of environment, stem rust favors humid conditions and thrives in warmer temperatures (optimal range 15-35 °C) than the other wheat rusts (Roelfs et al. 1992).

The high mobility of the pathogen, coupled to the enormous areas grown to potential hosts and the relatively wide range of suitable environmental conditions all combine to make effective global monitoring a serious challenge. For an effective and useful global monitoring system to be put in place, several challenges have to be overcome. Surveillance efforts must be undertaken over vast areas, surveys must be timely, there have to be efficient data flows from the field, value-adding analysis and interpretation must be undertaken and targeted information must be delivered to decision-makers in a timely manner. Additional mitigating factors are that disease epidemics do not occur on a regular or predictable basis, and that the pathogen population can change as new variants occur. The Ug99 lineage of stem rust is a good example; in less than five years two new variants of the original race have been identified showing virulence to additional stem rust resistance (*Sr*) genes, namely; *Sr24* and *Sr36*. Ideally, the monitoring system has to provide information on which pathotypes are present in which areas. Additionally, detection of the pathogen in specific areas does not guarantee appearance of

epidemics – a complex series of factors all have to occur simultaneously in time and space for an epidemic to occur; to date for the Ug99 lineage, this has only occurred during 2007 in Kenya.

Given all of the above factors, the task of establishing a global cereal rust monitoring system might be considered a daunting task. However, assistance can be found in the form of established model monitoring systems and also by building on efforts that have already been initiated in response to the threat of the Ug99 lineage of stem rust.

Model monitoring systems – FAO Desert Locust monitoring

At first glance the parallels between an insect pest such as the desert locust and fungal diseases of cereals might not appear obvious. However, many parallels do exist and there are several opportunities for technology and systems transfer.

The Desert Locust monitoring and early warning system has a very long history; initiated originally in 1930, it has been running successfully under the auspices of FAO since 1978. Desert locusts occur in more than 50 countries with a range that covers approximately 20% of the world's land mass. This, like cereal rusts, implies the need for monitoring and surveillance activities to be undertaken and coordinated over vast areas. Desert locusts are highly mobile, undertaking long-distance migratory movements that are wind assisted. These movements are trans-boundary, crossing political borders at will. Problematic outbreaks, i.e. plagues, are highly irregular with intervals of several years often occurring between plague events. All of these factors have strong parallels to cereal rusts.

A well established, efficient system of monitoring, early warning and information dissemination has been created for desert locusts. The system relies on field surveys undertaken by an extensive network of national surveillance teams. All field data is collected electronically using standardized survey forms incorporated into a GPS-enabled data logger. Data is automatically transmitted from the field via satellite links directly into central databases. A national desert locust focal point in each country compiles the data, ensures quality control and transmits data to an international focal point based at FAO headquarters in Rome. Integrated analysis of all country data is undertaken by the international focal point using GIS technology and information is disseminated in the form of monthly bulletins, forecasts, alerts and status maps. The international focal point also provides technical support, training and capacity building to all the national focal points in the network.

The desert locust system is well established and represents a good example of a fully operational, trans-boundary pest monitoring and early warning system. It is envisaged that the emerging global cereal rust monitoring system will be developed using similar technologies and operating procedures as this functional model system.

Foundation activities for a global cereal rust monitoring system

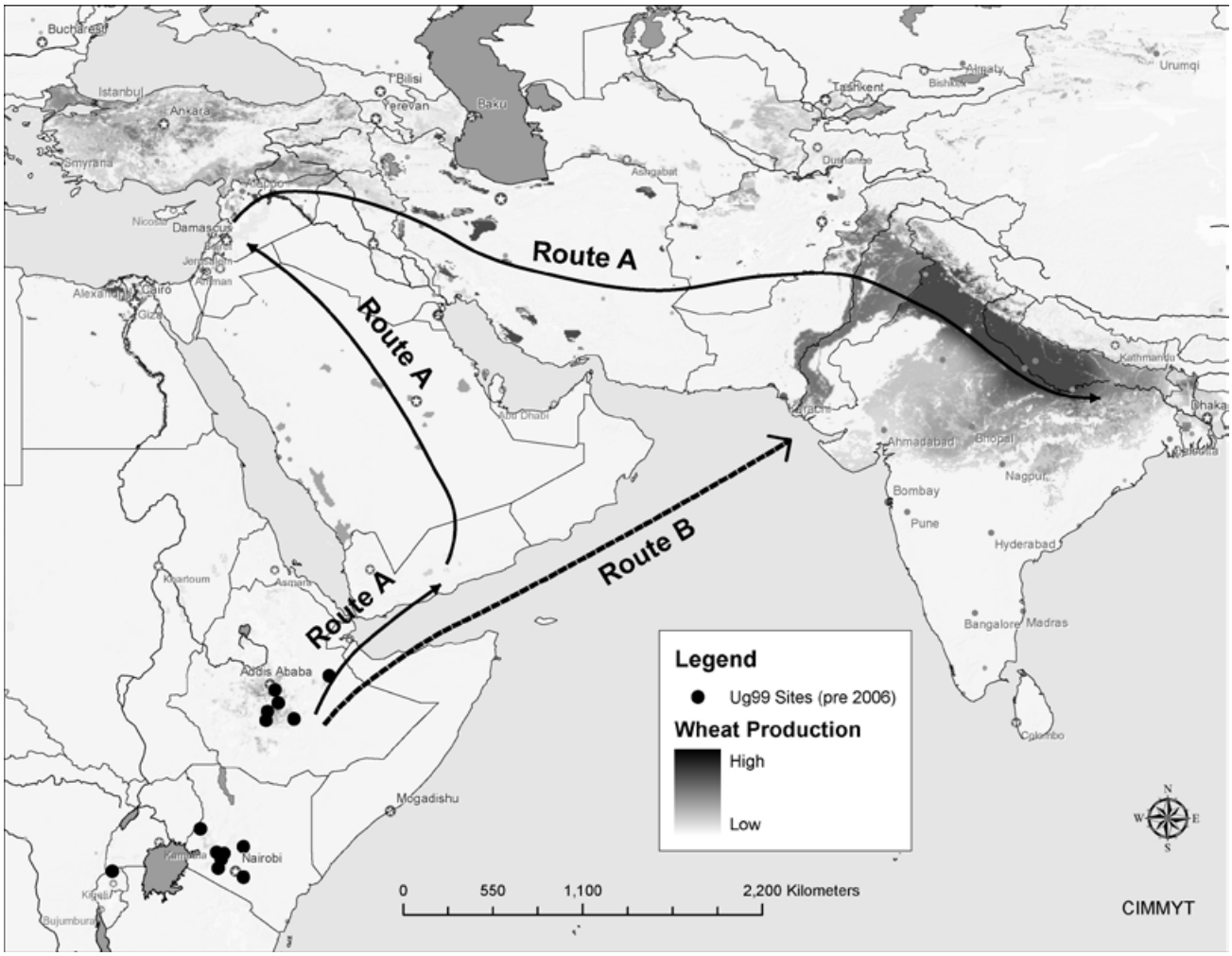
In 2005, Nobel Laureate Dr N.E. Borlaug brought the emerging threat of stem rust race Ug99 to the world's attention. Publication of an expert panel report (CIMMYT 2005) and the formation of an international coalition – the "Global Rust Initiative" (which subsequently became the "Borlaug Global Rust Initiative" BGRI) - put stem rust, and Ug99 in particular, back onto the agenda of wheat scientists worldwide.

The 2005 expert panel recognized the clear need for a global monitoring system and given the inherently spatial nature of many key factors, flagged GIS technology as being a useful tool. The expert panel made the following recommendation:

"Recommendation #1. Because the stem rust pathogen is airborne and genetically variable, the Panel **recommends** (1) population monitoring by means of trap nurseries and limited sampling for race analysis for the Kenya - Ethiopia region, adjacent areas, and beyond; (2) the establishment of a warning system based on the above data and modeling, using GIS and other appropriate tools."

Increasing awareness of a potential re-emerging threat from stem rust prompted the need for information to address key questions. What was the current status of Ug99? Where will it move next? How many wheat varieties currently grown are susceptible? Which are the areas at greatest risk? These were all very valid questions being posed by scientists, policy-makers, and decision-makers at the time. In response to these questions initial assessments were made using GIS technology to integrate important factors such as; known locations, wheat areas, susceptibility, wind movements, documented historical movements, and climatic factors (Hodson et al. 2005). As a result, the first predicted potential migration routes were postulated (Singh et al. 2006), i.e. pathogen movement out of Africa, crossing the Red Sea into the Middle East and onwards to South Asia (Fig. 1). At the time, confirmed locations for Ug99 were known only from Uganda, and across the wheat areas of Kenya and Ethiopia.

Fig. 1 *Initial predicted migration routes for Ug99 (after Singh et al. 2006)*



Actual confirmed observations of Ug99, obtained after the first postulated migration routes were produced have been supportive, and not contradictory, of the initial GIS-based predictions. In 2006, reports of stem rust were received from a site close to New Halfa in eastern Sudan, and subsequently from at least two sites in Western Yemen in the same year. Analysis of rust samples collected from these locations subsequently confirmed the presence of Ug99 (race TTKSK). The observed range of expansion of Ug99 indicated step-wise movements following regional winds. Crossing of the Red Sea into Yemen was considered particularly significant as several lines of evidence indicated that this might prove to be a gateway for onward movement into important wheat areas of the Middle East and Asia. In 2008, confirmatory race analysis data were obtained from stem rust samples that had been collected at two sites in Iran – Borujerd and Hamadan – at the end (July) of the 2007 wheat season (Nazari et al. 2008). Step-wise expansion of the Ug99 range had continued and the pathogen had now penetrated the major wheat producing areas of the Middle East. Fig. 2 shows current known locations.

Recorded known locations of Ug99 over time illustrate the mobility of the pathogen and highlight the possibilities for long-distance air-borne transmission. Obviously, understanding, and if possible predicting, likely air-borne movements is going to be a critical component of any cereal rust monitoring system. This is a non-trivial task, as predicting air-borne particle movement is a challenge due to the inherent complexity and variability of the underlying system. Hence it must be borne in mind that there will always be considerable uncertainties associated with any such pathogen prediction studies. These uncertainties also have implications regarding any assumptions of "fixed repeatable pathways"; such hypotheses must be approached with the utmost caution as significant deviations may well occur. It is not a given that the new

variants of Ug99 identified in Kenya will follow exactly the same pathway as the original race TTKSK.

The initial determination of potential migration routes for Ug99 relied solely on generalized monthly "long-term normal" wind trajectories (NOAA 2005). Despite obvious limitations, these data have provided some useful initial insights. However, given the crucial nature of wind movements, it was seen as high priority to improve the spatial, and more critically, the temporal resolution of the air-flow predictions. This resulted in a search for suitable, accessible models and final implementation of the HYSPLIT (Hybrid Single-Particle Lagrangian Integrated Trajectory) model developed by the Air Resources Laboratory at NOAA and the Australian Bureau of Meteorology (Draxler and Rolph 2003).

Routine implementation of the HYSPLIT model using Ug99 data has resulted in an improved understanding of observed movements. An analysis of wind trajectories from confirmed Ug99 sites in Sudan and Yemen during the main wheat growing season was undertaken using wind data from Dec. 2005-Apr. 2006 and Dec. 2006-Apr. 2007. Results from both seasons were nearly identical, implying some consistency in seasonal wind patterns. Trajectories from Yemen indicated the potential for spore movements in a predominantly north-easterly direction, across the Arabian Peninsula and towards Iran and Iraq. Conversely, trajectory data from the New Halfa site in eastern Sudan indicated a predominantly south-westerly direction for potential spore movements (Fig. 3). These results added more weight to the hypothesis that Yemen could serve as a gateway into the important wheat areas of the Middle East and Asia. It should be noted that the trajectory model results were obtained prior to the confirmed reports of Ug99 from Iran, indicating that trajectory models might have some predictive value for movement patterns.

Fig. 2 ***Current known locations of Ug99***

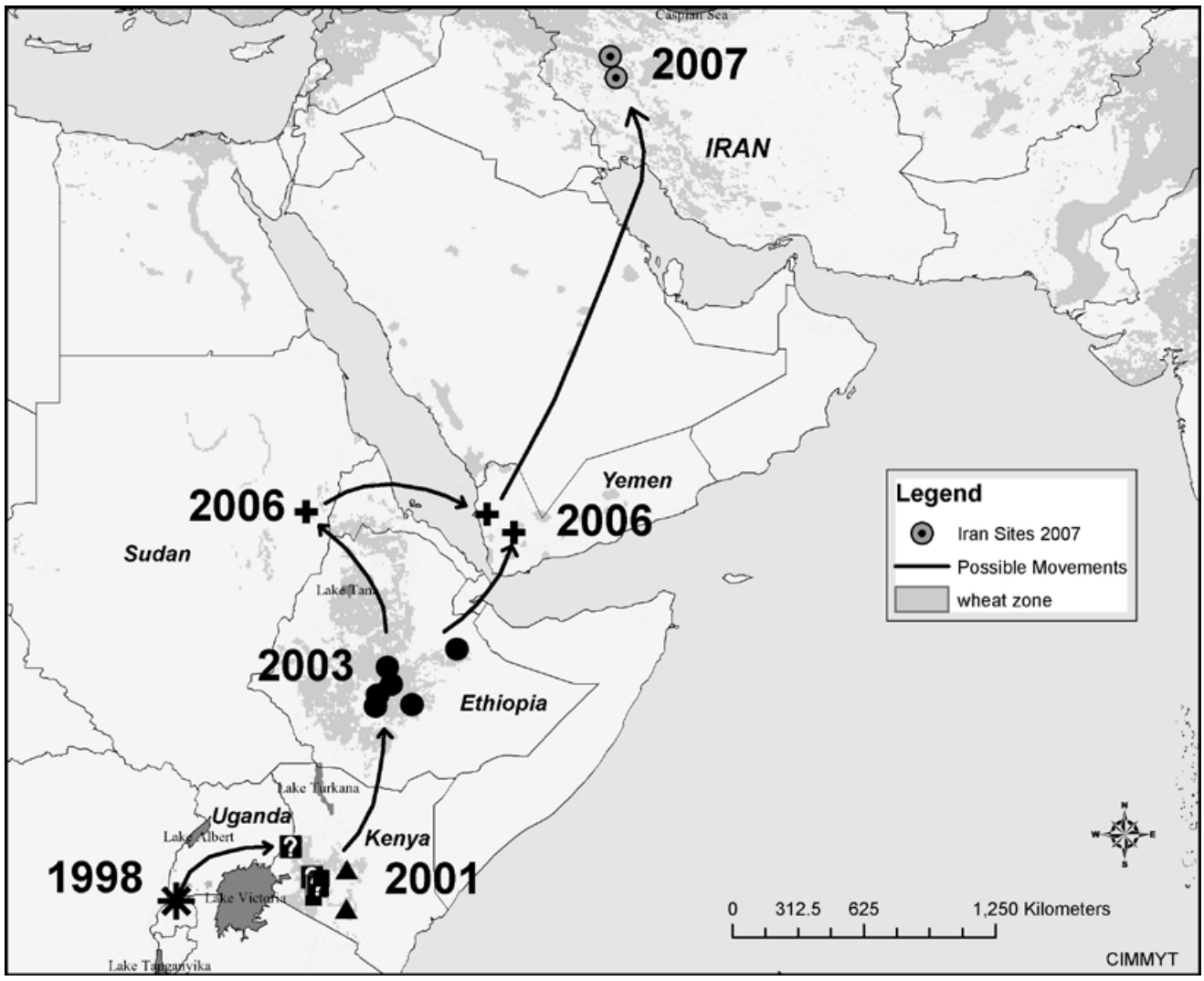
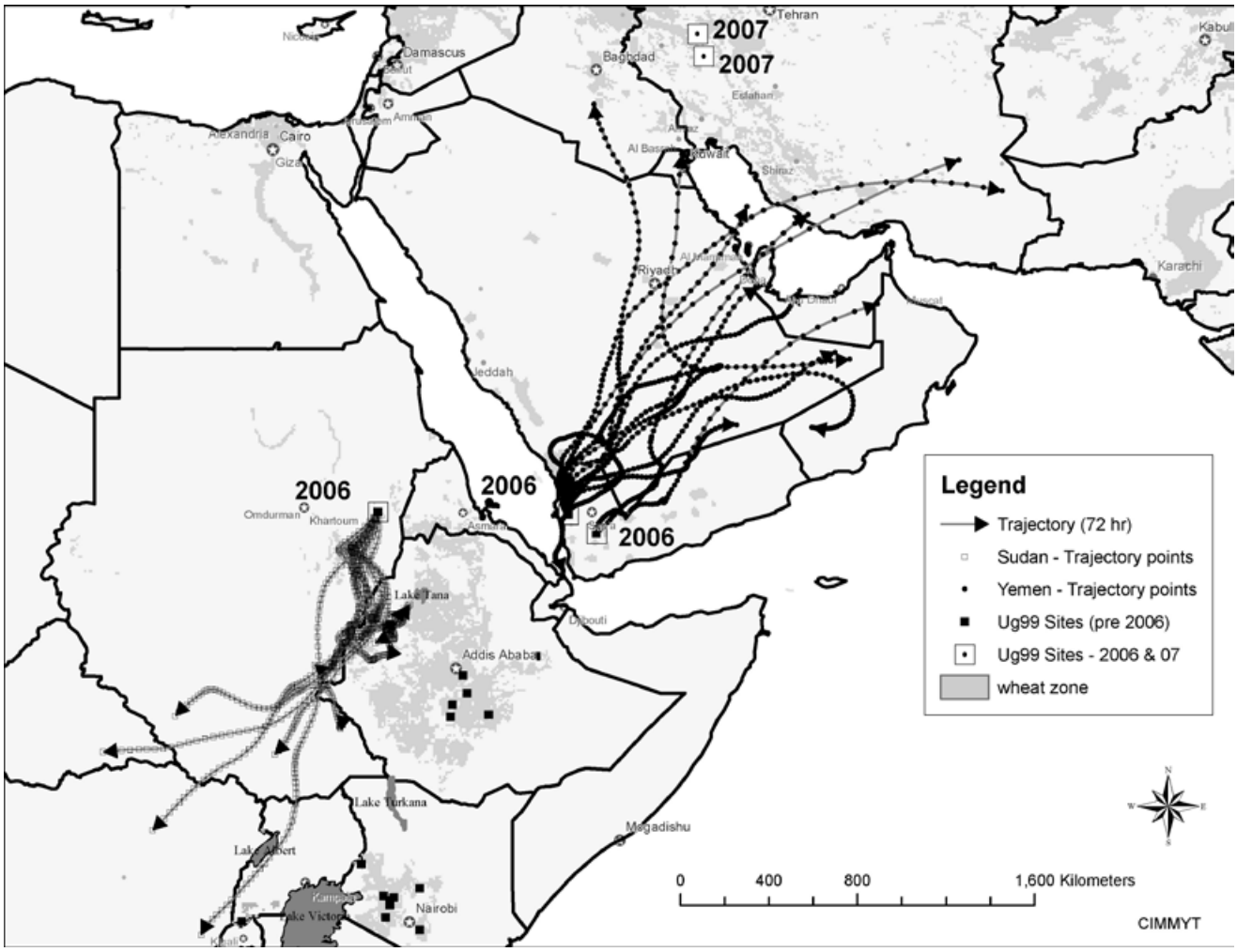


Fig. 3 *Selected wind trajectories (72 hours) from the HYSPLIT model during Dec. 2006 to Mar. 2007 originating at confirmed Ug99 sites in Yemen and Sudan*



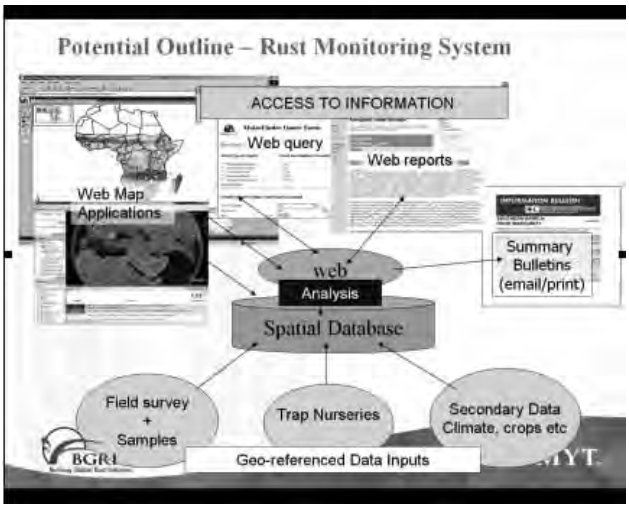
The two confirmed Ug99 (race TTKSK) sites in western Iran - Borujerd and Hamadan – as reported by Nazari et al. (2008) were not exactly on the trajectory paths predicted by the HYSPLIT model, but were in close proximity. However further analysis, using daily rainfall estimates in combination with the trajectory models, has provided circumstantial evidence of an intermediate staging location either in southern Iran or southern Iraq. Rainfall events are important as they are the principle means by which spore deposition occurs (Rowell and Romig 1966). On two occasions during the period Dec. 2006 to Jan. 2007 significant rainfall events were exactly coincident in time and space with wind trajectories originating from confirmed Ug99 sites in Yemen. It is feasible that these rainfall events had the potential to deposit rust spores in either southern Iraq or around the gulf coast of Iran. Backwards trajectory data (i.e. using wind data models to find potential sources for known final destinations) also highlighted these same areas as potential zones of origin for onward spore movements that terminated in Borujerd and Hamadan. Finally, unconfirmed but credible reports of Ug99 were obtained from a site near Shiraz in Iran earlier in the wheat season of 2007. This location is coincident with one of the postulated intermediate staging zones derived from the combined model outputs.

All of these foundation activities, i.e. the compilation of key datasets, the mapping of known Ug99 sites, and the use of wind and rain models to understand potential movements, provide a basis upon which a fully operational monitoring system can be developed. Obviously these activities do not constitute a fully functional monitoring system and several additional key components are required.

The emerging global cereal rust monitoring system

In parallel to the already existing UN monitoring system for desert locusts, the cereal rust monitoring system is being built using GIS technology as a backbone. Few, if any, other technologies have the capacity for multi-thematic data integration and analysis that is vital to the success of the entire system. This means there is a fundamental requirement for geo-referenced data inputs to drive the entire system. To ensure a global overview there is also a need for a centralized database from which integrated analysis can be undertaken. Finally, for the system to have utility it must deliver timely and targeted information to a range of end-users, i.e. scientists, decision-makers and policy-makers. A simplified schematic overview of the cereal rust monitoring system is given in Fig. 4.

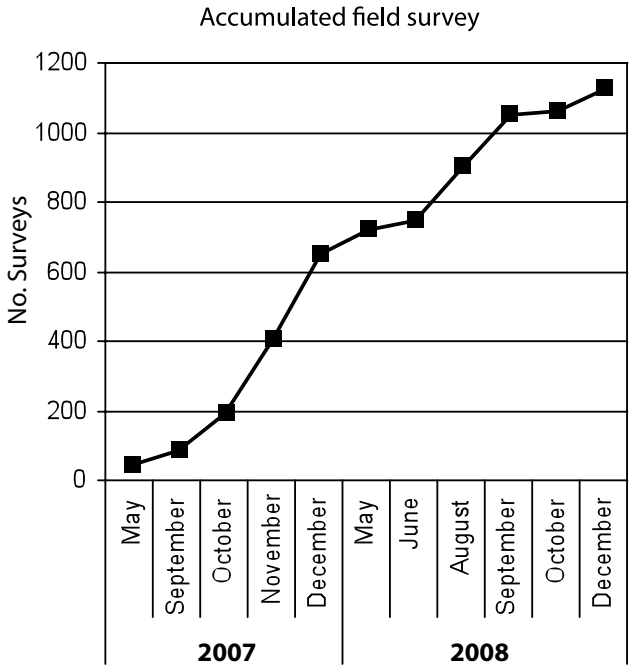
Fig. 4 ***Schematic outline of the proposed global cereal rust monitoring system***



Some progress has already been made against several key elements.

Collection of geo-referenced field survey data is fundamental to the entire system. To facilitate this, a set of standardized protocols were developed under the auspices of the BGRI. These protocols include standardized field survey forms, instructions on the use of GPS to record locations, protocols for pathogen sampling and simple cost-effective race analysis. A series of regional training workshops have been undertaken for national partners using the BGRI Protocols Manual as a base. In addition GPS units have been distributed to partners in 29 countries in Africa, Asia and the Middle East. Provision of this survey "toolkit" with associated capacity building is already paying dividends, as a rapidly increasing amount of geo-referenced field survey data is being received (Fig. 5).

Fig. 5 ***Cumulative field survey records for stem rust***



Data management is another critical issue. A centralized database has been created and this is being populated with the increasing amounts of field data collected by national partners. The centralized database is directly connected to a GIS system, permitting routine mapping of survey data, plus integration with other relevant data, e.g. climate, wheat production zones. In addition, wind trajectory models using HYSPLIT are routinely run at five day intervals based on positive stem rust locations recorded in the database.

Provision of information in a timely manner is a critical aspect of the monitoring system. The vision is to provide a range of information products that are similar to those already being delivered by the UN Desert Locust monitoring and early warning system, i.e. web-based status reports, alerts, monthly bulletins distributed to targeted users, dynamic web maps. Not all of the desired information products are currently in place for cereal rusts, but several tools have been developed presenting synthesized information on-line in respect to stem rust race Ug99. Other information products are planned in the near future. Publicly available tools are briefly described in the following sections.

RustMapper

Google Earth is one of the most widely known and used virtual globes, with over 350 million downloads claimed by Google (http://google-latlong.blogspot.com/2008/02/truly-global.html). This widespread use made Google Earth the platform of choice for an initial

information tool named RustMapper developed by the GIS unit at CIMMYT. RustMapper is publicly available at http://www.cimmyt.org/gis/RustMapper/index.htm as a downloadable Google Earth networked link which automatically updates after download. Key information incorporated into RustMapper includes; all known sites for Ug99 or variants, recent survey sites and collected data, near real time wind trajectories from the HYSPLIT model originating from known Ug99 sites or sites recording stem rust (these trajectories are run for 24, 48, 72 hour durations and updated every 5 days), and major wheat growing areas in Africa and Asia. In addition, country level summary information is provided on susceptibility estimates to Ug99 and basic wheat production statistics. A complete archive of wind trajectories back to April 2007 is also included. Any new information that is obtained and cleared for public release, e.g. sites, wind trajectories, etc. is automatically incorporated into RustMapper.

RustMapper Web

RustMapper Web is a derived "lite" version of the original RustMapper running within a browser environment (see Fig. 6). A free downloadable plug-in from Google is all that is required to implement RustMapper Web. Key components of the original RustMapper were migrated into a browser-based tool to increase the options for data access. RustMapper Web is publicly available and updated every five days (see http://www.cimmyt.org/gis/rustmapper/RustMapper_Web.html). All of the primary components of RustMapper are included in the web version, although only the most recent wind trajectories are presented. RustMapper Web now functions on all major browsers on both Windows and Mac.

Fig. 6 ***RustMapper Web – a Google Earth based information tool for Ug99***

The above examples illustrate how geography-based visualization platforms are being used to present timely, integrated information relating to stem rust. The initial focus has been on the Ug99 lineage, but future expansion to incorporate other rusts of concern is entirely feasible. Despite the progress that has been made, further improvements and advances are still needed if the goal of a fully operational monitoring and surveillance system for cereal rusts is to be achieved.

Future activities

Some areas of the envisaged global cereal rust monitoring system that are lacking or requiring substantial improvement have already been eluded to in previous sections. The survey "tool kit" that has already been developed, coupled to the regional training workshops, has resulted in a significant increase in available field data. However, these efforts need to be greatly expanded both in terms of geographic scope and data volumes. In order for this to be achieved, national surveillance teams need to be empowered and supported to create a fully functional and sustainable network.

Efficient and timely flows of data from the field into centralized databases will be critical to the entire system. Several national partners have made outstanding efforts to ensure that this flow of data has been initiated. Expansion of these efforts and facilitation to make the process as simple and rapid as possible should be undertaken. One future option might be the implementation of GPS-enabled data logger technology, which would permit electronic data capture in the field with automatic upload and transfer to central databases. Efforts are already underway to mirror the UN Desert Locust networking system to facilitate data exchange by creating a network of national focal points with responsibility for national data compilation and quality control. To provide global oversight, an international focal point based at FAO Headquarters in Rome has just been appointed.

Increased effort needs to be undertaken in the analytical assessments of risk for disease occurrence. Some fundamental layers of information need improvement, e.g. quality of the wheat area distribution information, distribution of varieties and estimates of areas currently planted in farmers' fields, timing of crop growth stage in different geographical areas. In addition, routine integration of key climatic factors at appropriate temporal and spatial scales should be undertaken. However, it is important to realize that even with improved data inputs the possibility of precise prediction of future epidemics is an unrealistic goal. Given the complexity of interacting factors to trigger

an outbreak, identification of all the required factors in time and space is unlikely. Hence, seemingly favorable conditions for disease occurrence might be identified, but no disease occurs, or conversely, seemingly unfavorable conditions might see a disease outbreak.

Another major missing component at present is the integration of race analysis data into the centralized spatial database. For the Ug99 lineage, two new variants of Ug99 are now recognized from Kenya, both very closely related and thought to have arisen through single-step mutations (Jin et al. 2008). These new variants have rendered additional important stem rust resistance genes ineffective, namely *Sr24* (race TTKST) and *Sr36* (race TTTSK). At present, available information indicates that both variants are only present in Kenya, but on-ward migration to other areas as has been observed for the original TTKSK race is considered to be virtually inevitable. There is a clear need for the global monitoring system to provide information about the location and status of emerging races. The appearance of the *Sr24* variant of Ug99 is considered particularly significant as the additional breakdown of this *Sr* gene halved the estimated number of current varieties previously considered resistant to Ug99 (Singh et al. 2008). This race, TTKST, was responsible for epidemics in Kenya during 2007, only one year after it was initially identified.

Recent work from South Africa (Visser et al. 2009), using molecular markers to compare the genetic diversity of South African stem rust races with the original isolates of Ug99, has identified yet another variant in the Ug99 lineage. This race, termed UVPgt55 or TTKSF in North American notation, is postulated to be the original progenitor of Ug99. It exhibits very similar virulence/ avirulence profiles to Ug99 race TTKSK with the notable exception that it is avirulent for *Sr*31 and genetically it is nearly identical to Ug99. Visser et al (2009) proposed that TTKSF may have originated in East Africa, where it gave rise to Ug99, and subsequently appeared in South Africa as an exotic introduction. If this hypothesis is correct then it implies southerly movement from Eastern to Southern Africa either by air-borne transmission or accidental transfer. Since 2000, race TTKSF has become the most prevalent pathotype in South Africa (Komen 2007). In 2007, an additional race in the Ug99 lineage, TTKSP, was detected in South Africa. TTKSP shows virulence to *Sr*24, but is avirulent to *Sr*31 and is believed to be a mutation of TTKSF (Z. Pretorius pers comm).

Incorporation of specific pathotype information is a non-trivial task. Race analysis of rust pathogens requires bioassays performed on collected rust samples under controlled conditions, using sets of differential testers, i.e. specific wheat varieties that have known stem rust resistance genes. At present, not all countries have the capacity to undertake such analysis and transfer of samples to advanced research laboratories in North America is required. Strict quarantine regulations introduce a considerable time lag (up to 6 months or more) between sample collection in the field and final race identification. This time lag, combined with the multi-institutional and cross-continental elements of race analysis, implies the requirement for a very stringent procedure to track samples and trace race analysis results back to source collection sites. Strict sample coding, or even bar-coding, will be required to effectively connect pathotype data to field collection sites. The development of facilities and expertise to allow race analysis in countries already having Ug99 should allow more rapid provision of survey results in the future.

The final area of improvement surrounds the need for effective information dissemination. Activities have been initiated through the development of RustMapper, but a broader range of information tools which are carefully targeted to specific user-groups need to be developed. It is envisaged that a web-based status report and alert system will be established, plus monthly status bulletins distributed electronically or in printed form to targeted end-users. Additional simple web mapping tools are also planned to complement RustMapper.

At present, monitoring efforts have focused largely on the Ug99 lineage of stem rust. Given the immediate nature of the threat posed by this lineage, such an approach is entirely justified. However, the other rusts, i.e. leaf rust and stripe rust, cannot, nor should, be excluded from a Global Cereal Rust Monitoring System. In recent years both of these rusts have been more economically damaging to wheat production worldwide In both cases specific lineages are under investigation, e.g. leaf rust specifically adapted to durum wheats, and a new lineage of the stripe rust pathogen that has undergone rapid global expansion (see Hovmøller, these proceedings). The current strategy is to develop the functional aspects of surveillance networks, data flows, data management and information tools using stem rust, and Ug99 specifically, as a platform for the Global Cereal Rust Monitoring System and then expand this platform to incorporate the other rusts. Field data being received for Ug99 deliberately incorporates observations on other rust species, so the foundation stones for future expansion are already being put in place.

Expecting the unexpected

The previous sections have concentrated on actual status and attempts to track and understand the known movements of Ug99. However, consideration should

also be given to lower probability, but highly realistic, scenarios that could have a very major influence on future movements and distribution. At least three major factors are considered important and should be borne in mind in the context of rust monitoring and surveillance.

(i) Accidental long distance transfer

It is important to note that rust spores can also travel long-distances by assisted means – either on travelers clothing or on infected plant material. The world is an ever-connected place. Global air-travel has increased exponentially in the last decades. Despite strict phytosanitory regulations, there is an increasing risk of pathogen spread. As an example, a race of wheat stripe rust was accidently transferred from Europe to Australia in 1979, almost certainly on a traveler's clothing (Steele et al. 2001). No computer-based models are going to predict these accidental movements. Hence, continual vigilance and regular field surveys in wheat areas that might be considered "low risk" are the best tools to provide early detection of any such transfer.

(ii) Natural long distance dispersal – "rare events"

Single events of extremely long distance (up to several 1,000 km) movements are not common, but the literature contains several examples that provide good evidence of such natural wind-borne movements. Many of these documented movements involve rust species, due to the robust nature and long viability of spores. Brown and Hovmøller (2002) present a very good review of long distance dispersal that includes at least one wheat stem rust windborne introduction into Australia from southern / eastern Africa. Watson and de Sousa (1983) consider another two such movements involving stem rust from Africa to Australia. An area of real concern is the potential status of Ug99 or variants in areas to the south of Kenya or Uganda in Africa. The recent work of Visser et al. (2009) documenting the probable progenitor of Ug99 (race TTKSF) in South Africa, and the subsequent identification of a mutant of TTKSF, i.e. race TTKSP with virulence to *Sr24*, highlights this issue. Race TTKSF has a postulated origin in East Africa, indicating that southerly movements from Eastern to Southern Africa can occur. Although wheat is a minor crop in much of this region, with the notable exception of South Africa, the basis of concern is the region's potential as a source for on-ward movement (albeit at very low probability) to either Australia or the Americas. Documented historical evidence indicates the potential threat is real. Nagarajan and Singh (1990), Brown and Hovmøller (2002), Isard et al. (2004) and Prospero et al. (2005) all cite convincing examples supported by high altitude balloon data, wind trajectory model data and sample analysis, of cross-continental rust or bacteria movements originating in southern or western Africa and moving to South America or Australia. The possibility of a similar "rare event" type movement involving Ug99 cannot be totally excluded; hence vigilance and continued surveillance activities in southern Africa appear warranted.

(iii) Climate change and the potential for pathogen distribution changes

Stem rust favors humid conditions and thrives in warmer temperatures (optimal range 15-35C) than the other wheat rusts (Roelfs et al. 1992). Historically, known traditional "hot-spots" for stem rust were present in many countries and these represented the warmer, often lowland, wheat growing areas. Examples of these might include the Rift valley areas of Kenya and Ethiopia, the Caspian Sea area of Iran, and the southern hills of India. Global warming is resulting in increasing numbers of species distributional changes being reported, with changes often occurring up the elevation gradient as warming temperatures permit expansion into areas previously too cool. At present nothing more than very circumstantial evidence exists for stem rust and more questions remain than answers. However, with global warming the potential does exist for stem rust to move into non-traditional areas. Some potential indicators of change are that stem rust is now being recorded at highland sites (>3,000m) in Kenya; historically, these were stem rust free. In Iran, the confirmed locations for Ug99 are in cool facultative wheat areas, again not the traditional warmer, lowland areas traditionally associated with stem rust. Future climate models, such as those which provide a basis of the IPCC climate change reports, predict a substantial warming in many important wheat areas of the Middle East and Asia by 2020. Whether such predicted changes will affect future stem rust distributions remains unknown at present, but it is a factor that should be considered and researched further.

Conclusion

Historically, wheat stem rust was the most feared plant disease capable of devastating epidemics and crop losses. By the mid 1990s widespread use of resistant cultivars had reduced disease incidence to non-significant levels worldwide. Stem rust research and resistance breeding ceased to be a priority activity. The identification of a new virulent stem rust race lineage in Uganda in 1998/99, popularly named Ug99, and subsequent variants have rendered 80% or more of global wheat varieties stem rust susceptible. Detection and spread of the Ug99 lineage has put stem rust firmly

back on the agenda of wheat scientists worldwide. Ug99 has clearly highlighted the need for global rust monitoring and surveillance.

In line with the recommendations of an expert panel convened to assess the threat of Ug99, GIS technology forms the backbone of an emerging monitoring and surveillance system for cereal rusts. This embryonic system is initially focused on the emerging stem rust threat, but longer-term plans to incorporate the other cereal rusts is being developed collaboratively by international agricultural research centers, UN agencies and advanced research institutions. The existing FAO Desert Locust monitoring and early warning system is seen as a useful model system upon which a Global Cereal Rust Monitoring System can be based.

Through successful international collaboration, several of the required elements of the monitoring and surveillance system are already starting to be addressed. Standardized field protocols have been developed; provision of, and training in the use of GPS has been initiated capitalizing on existing and expanded national wheat partner networks. These efforts have already resulted in a substantial increase in the amount of geo-referenced survey data incorporated into a centralized spatial database. As a result, regularly updated known distribution maps for Ug99 are now being produced. Routine incorporation of wind trajectory models is providing improved information on potential movements, with results to date corresponding closely to actual confirmed observations in the field. Results obtained indicate movements and range expansion of Ug99 in line with predicted regional air-flows, and generally following previously reported movements of other rust races that originated in East Africa (see Singh et al. 2004). However, there is neither room for complacency regarding future movements nor any substitute for regular, timely field surveys of the key wheat areas.

Good progress has been made in the development and release of initial information tools that draw upon existing centralized data and provide near real-time information on the current status of Ug99. The ready availability of powerful geographic-based visualization options, such as provided by Google Earth, has been a key factor in the successful presentation of information in a clear and flexible way. In the future, an expansion of the type and range of information products is planned. These will be targeted very closely to wheat scientists, and decision- and policy-makers in at-risk countries. Using the successful FAO desert locust monitoring system as a model (see http://www.fao.org/ag/locusts/en/info/info/index.html), an improved and expanded web presence will be created issuing status reports and alerts, along with a lightweight rapid mapping capacity, plus regular summary bulletins.

Challenges remain in several areas before a fully functional Global Cereal Rust Monitoring System is operational. These include strengthened networks for data flows, improved dissemination of information, integration of race analysis data, and expansion to include other rusts. In addition, several lower probability factors, e.g. accidental transfer, natural long-distance dispersal, and shifting distributions as a result of climate change, must be given consideration. All of these elements have implications in respect to survey and monitoring activities in areas that might be considered "low risk" for the Ug99 lineage. However, progress against all of these challenges is considered entirely feasible if effective partnerships and networks are created within the global wheat community.

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6. Sequencing Ug99 and other stem rust races: progress and results

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Over the last decade a number of different molecular methods have been used to characterize genetic diversity in *Puccinia graminis*. Multilocus DNA fingerprinting methods (AFLPs, RAPDs, SAMs and S-SAPs) have proven to be useful, but limited to phenotypic analysis due to the dikaryotic nature of rust fungi. More recently, locus-specific markers (SSRs) were developed and used to characterize different races of *P. graminis* f. sp. *tritici* including Ug99. SSR analysis has demonstrated that Ug99 is distinct from common North American races as well as a select set of worldwide races. SSR analysis indicated that races TTKST and TTTSK from North Africa represent variants of TTKSK (Ug99), but was unable to distinguish between members of this lineage. The recent genome sequencing of a North American isolate of *P. graminis* f. sp. *tritici* provides a major opportunity for genetic analysis and development of rapid molecular methods for race analysis. The current assembly contains 392 scaffolds spanning 88.64 Mb and represents a consensus of the two haploid genomes (www.broad.mit.edu/annotation/genome/puccinia_graminis.3). Thirteen pairs of scaffolds have been identified that correspond to homologous regions of the two haploid genomes. Ug99 (05KEN156/04: 05KEN) was sequenced using Illumina technology as well as the isolate used for the original sequencing project (CRL 75-36-799-3: reference). Approximately 21 million reads (1.6 gb) representing an average of 20X coverage were used in the analysis. Ninety seven percent of the genome was covered when reads from the reference isolate was aligned back against itself. In contrast, 79.6% of the reference genome was covered when the 05KEN reads were aligned indicating that approximately 18% of the reference genome is unique compared to the 05KEN isolate. In addition, only 41% of the 05KEN reads aligned to the reference genome as compared to 69.6% percent of the reference reads. The frequency of single nucleotide polymorphisms (SNPs) of the reference isolate was one SNP per 959 bases indicating a moderate level of polymorphism between the two haploid genomes. Comparing the 05KEN with the reference genome, the frequency of SNPs was 1 for every 212 bases indicating a high level of genetic diversity between these two isolates. Currently, the distribution of SNPs across the genome is being analyzed. The high level of genetic diversity between Ug99 and the North American reference genome is currently being exploited for the development of a diagnostic assay for the rapid identification of the Ug99 lineage.

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7. Advances in host-pathogen molecular interactions: rust effectors as targets for recognition

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Abstract

Rust fungi can cause devastating diseases in agriculture and are particularly important pathogens of wheat. We have been using the flax (*Linum usitatissimum*) and flax rust (*Melampsora lini*) model system to study disease resistance mechanisms to this important class of pathogens. Rust resistance in flax and other plants is mediated by the plant innate immunity system in which highly polymorphic resistance (R) proteins act as receptors that recognize specific avirulence (Avr) proteins produced by the pathogen. This race-specific resistance is characterised by Flor's "gene-for-gene" model, first proposed based on the flax rust system. In gene-for-gene resistance, recognition between the R and Avr proteins initiates defense responses leading to host resistance to infection, including a localised necrosis or hypersensitive response. Nineteen different rust resistance genes have been cloned from flax, including 11 allelic variants of the *L* locus, which all encode cytosolic proteins with conserved nucleotide-binding (NB) and Leucine-rich repeat (LRR) domains. Four families of Avr genes, *AvrL567*, *AvrM*, *AvrP123* and *AvrP4*, have been identified in the flax rust pathogen and all encode small secreted proteins. Rust Avr proteins are secreted from haustoria, specialized infection structures that penetrate the host cell wall, and are translocated across the host plasma membrane and into the host cytoplasm. These proteins are probably members of a suite of disease 'effectors' involved in manipulating host cell biology to facilitate infection, but have become targeted for recognition by the host immune system. As yet the mechanism of Avr protein transport is unknown, but could prove to be a useful target for novel disease control strategies. Recognition of at least two of these Avr proteins is based on direct interaction with the cytoplasmic NB-LRR R proteins. One interesting observation from the flax rust system is that all of the virulent rust strains retain intact copies of the Avr genes, but have altered their sequences sufficiently to escape recognition. Thus it may be possible to re-engineer R genes to recognise new Avr gene variants. We are currently identifying haustorially expressed secreted proteins from wheat stem rust as candidate Avr/effector proteins.

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haustoria, avirulence, resistance, secreted proteins, effectors

Introduction

In order to successfully establish disease, plant pathogens such as the wheat rusts, must first overcome the natural defences of the plant. These include preformed barriers, such as the waxy cuticle, as well as inducible responses triggered by the plant innate immunity system (Takemoto and Jones 2004). The first layer of the immune system involves recognition of pathogen associated molecular patterns (PAMPs) such as chitin or flagellin (Jones and Dangl 2006). Recognition of these factors by cell surface receptors leads to PAMP-triggered immunity (PTI) which is effective in preventing infection by non-adapted pathogens. Bacterial pathogens of plants are known to overcome these defences through the use of effector proteins that are delivered into host cells by the Type III secretion system. However, many of these effectors are recognized by a second layer of the plant defense system that involves intracellular receptors that are the products of the classically defined resistance (R) genes of the gene-for-gene system, first defined in the flax rust disease system (Flor 1971). In this context pathogen effectors are known as *avirulence (Avr) proteins* and their recognition leads to rapid activation of a localized cell death termed the hypersensitive response (HR), which is thought to limit the spread of the pathogen from the infection site (Chisholm et al. 2006). This layer of defense has been termed effector triggered immunity (ETI), and involves direct or indirect recognition of pathogen effector proteins by plant R proteins. Recent advances in the study of biotrophic oomycete and fungal pathogens, including rusts, indicate that this general picture of pathogen effector/host immunity interactions also holds true for these eukaryotic pathogens (Ellis et al. 2007; Tyler 2009).

Among studies of biotrophic fungi, work on the flax (*Linum usitatissimum*) and flax rust (*Melampsora lini*) disease system has so far yielded the most information (Lawrence et al. 2007). Rust fungi are obligate biotrophs, meaning that they are completely dependent on nutritional resources obtained from living host cells

for their growth and reproduction. During infection of host plants, fungal hyphae grow in the intercellular spaces of the leaf, but form a close association with host mesophyll cells through haustoria. These specialized infection structures penetrate the plant cell wall and invaginate the plant cell plasma membrane, and are thought to be the primary sites of nutrient acquisition from the plant (Voegele and Mendgen 2003). The flax rust system has been an enduring model in plant disease resistance, having been the basis for Flor's "gene-for-gene" model. In gene-for-gene resistance, the products of host resistance (R) genes determine recognition of pathogen "avirulence" (Avr) gene products to initiate defense responses leading to resistance. One of the hallmarks of this system is the high degree of specificity between corresponding R and Avr genes. Previous isolation of flax resistance genes, including 11 alleles of the *L* locus and representatives of the M, N and P loci, has provided insights into resistance gene specificity. The recent identification of flax rust Avr proteins has now allowed more detailed analysis of the recognition events that trigger rust resistance.

Flax R genes and their products

Genetic studies of the interaction between the flax plant and flax rust have identified about 30 flax resistance (R) genes, which occur as series of closely linked or allelic genes at 5 loci, and about 30 corresponding flax rust avirulence (Avr) genes that are mostly dispersed in the flax rust genome. Nineteen different rust resistance genes have now been cloned from flax, including 11 allelic variants of the *L* locus and representatives of the *M*, *N* and *P* loci (Ellis et al. 1999; Anderson et al. 1997; Dodds et al. 2001a, b). These genes all encode predicted cytosolic resistance proteins containing nucleotide binding (NB) and leucine rich repeat (LRR) domains, as found for the majority of known *R* genes in plants. The flax R proteins belong to a major subclass of this family which contain an N-terminal domain related to the *Drosophila* Toll and human interleukin-like receptor intracellular signaling domains (TIR domain). The precise roles of these domains and the mechanism by which recognition is linked to the activation of defense signaling is not well understood. However, the LRR domain appears to be the major determinant of recognition specificity, since most amino acid variation occurs in this domain as a result of strong positive selection (Dodds et al. 2000), and domain swaps between alleles of either the *L* or *P* loci of flax, show that this region controls recognition specificity (Ellis et al. 1999; Dodds et al. 2001a). LRR domains occur in a wide range of proteins and are generally implicated in protein-protein interactions (Kobe and Kajava 2001). The TIR domain is likely to be involved in signaling, as suggested by the functions of mammalian homologues and the observation that deletion and point mutations in this region of the *N* gene disrupt signaling events that lead to tobacco mosaic virus resistance (Dinesh-Kumar et al. 2000). Indeed we have found that overexpression of the L6 TIR domain leads to activation of the HR (Frost et al. 2004). However, the TIR may also play a role in pathogen recognition (Ellis et al. 1999; Luck et al. 2000; Burch-Smith et al. 2007). The NB domain is presumed to bind and hydrolyse ATP, as has now been shown for two tomato R proteins (Tameling et al. 2002, 2006), and it is likely that ATP/ADP exchange plays a role in controlling R protein activation. Studies on several NB-LRR proteins demonstrated that domains within the proteins interact (Bendahmane et al. 2002; Moffett et al. 2002; Ueda et al. 2006) and some constitutive gain-of-function mutations have been identified in the NB domain. These data support the notion that R proteins may be held in an inactive state through intramolecular interactions that are released by the presence of the Avr protein.

Flax rust Avr genes and their products

Until recently isolation of avirulence (Avr) genes from biotrophic fungi and oomycetes has been difficult because these organisms cannot be readily cultured or transformed. However, four families of Avr genes, *AvrL567*, *AvrM*, *AvrP123* and *AvrP4* have now been identified in flax rust (Table 1; Dodds et al. 2004, Catanzariti et al. 2006). The first of these (*AvrL567*) was

Table 1 ***Cloned Avr gene families from flax rust***

Avirulence locus	Product size (aa)	Cys rich	# gene family members	Cognate R genes
AvrL567	150	no	12	L5, L6, L7
AvrM	260-384	no	6	M
AvrP4	95	yes	3	P4
AvrP123	117	yes	>2	P, P1, P2, P3

isolated by a subtractive hybridisation screen for rust genes expressed during infection followed by genetic mapping in a rust family segregating for multiple Avr specificities. The subsequent three were isolated by screening a cDNA library from rust haustoria by ConA-affinity chromatography (Hahn and Mendgen 1992) for genes encoding secreted proteins. All four Avr gene families encode small secreted proteins that are expressed in haustoria and are apparently translocated into host cells during infection. Evidence for this translocation comes from the observation that transient expression of these Avr proteins as cytoplasmic proteins (i.e. lacking the signal peptide) in plants can trigger a defense response dependent on the corresponding R genes. This shows that Avr protein recognition occurs inside plant cells, implying that these proteins are translocated during infection. Indeed, our recent work has detected the flax rust AvrM protein inside infected host cells by immunolocalisation. Kemen et al. (2005) also showed that a protein (UfRTP1) secreted from broad-bean rust (*Uromyces fabae*) haustoria is translocated into host cells. It seems likely that these proteins are part of a larger suite of proteins (probably including the other 20 or so flax rust Avr gene products) that are secreted from rust haustoria and translocated into the plant cytoplasm. These proteins represent a set of host-targeted effector proteins that are presumed to play roles in promoting the infection process.

Biotrophic and hemibiotrophic oomycetes also secrete large arrays of effector proteins that are directed into the host cytoplasm during infection (Kamoun et al. 2006). These oomycete proteins are characterised by a conserved RxLR motif that is related to a transport signal responsible for uptake of secreted proteins of the malaria parasite (*Plasmodium falciparum*) across the erythrocyte vacuolar membrane (Hiller et al. 2004; Marti et al. 2004, Bhattarcharjee et al. 2006). Thus there appears to be a conserved translocation mechanism used by these distantly related plant and animal pathogens. These motifs apparently direct uptake of the oomycete effectors into host cells in the absence of the pathogen, implicating a transport mechanism involving host plant components (Dou et al. 2008). Although the flax rust Avr proteins do not contain such a highly conserved motif, initial experimental data indicate that their likely route of uptake is also via a host encoded system rather than a specialized rust secretory system. For example, transient expression of the AvrM protein with or without the signal peptide (SP) induces an *M* gene specific HR, but addition of the HDEL endoplasmic retention signal prevents recognition of the secreted, but not the cytoplasmic, version (Catanzariti et al. 2006). This is consistent with recognition of the secreted form by the cytoplasmic M protein *after* secretion and re-entry into the plant cell.

More recently we have shown that full length AvrL567- and AvrM-GFP fusion proteins, including the signal peptide, accumulate inside host cells after transient expression in plants. However, fusion of just the SPs of these proteins to GFP results in GFP accumulation outside the plant cell confirming that these signals do correctly direct secretion of the fusion protein in plants. Furthermore, addition of an HDEL endoplasmic reticulum retention signal to the full length Avr-GFP fusion results in accumulation of GFP in the endoplasmic reticulum, confirming that these proteins do enter the secretory pathway. Thus, these results indicate that uptake of the rust effectors into host cells from the apoplast can occur in the absence of the pathogen, implicating a host-derived transport mechanism. We have tested several truncation constructs using smaller regions of AvrL567 and AvrM, and narrowed down the uptake signal to the N-terminal regions of AvrL567 and AvrM. These regions do not contain an obviously conserved motif such as the RxLR motif in oomycete effector proteins, but are rich in the positively charged amino acids arginine and lysine which are common to several proteins known to transport across membranes.

The molecular basis of Avr protein recognition and gene-for-gene specificity

The co-localization of Avr and R proteins in the flax cytoplasm and the genetics of gene-for-gene interactions are consistent with direct interaction between these proteins. This hypothesis has been investigated experimentally using the yeast two hybrid system to detect R-Avr protein interactions. In these experiments the full length L5, L6 alleles and a chimeric construct L6L11RV that differs from L6 by 11 amino acid differences derived from L11 in the 3 C-terminal LRR units, and 12 AvrL567 variants (AvrL567-A to AvrL567-L) were co-expressed in yeast two hybrid assays. Protein-protein interactions were detected in yeast for the same combinations of L and AvrL567 genes as induced HR in transient expression assays in planta (Dodds et al. 2006). The close correspondence between the detection of a protein interaction in yeast and the induction of HR in planta indicates that direct R-Avr protein interaction is the basis for recognition specificity. For example, L6 but not L5, interacts with AvrL567-D in yeast, and co-expression of L6, but not L5, with AvrL567-D induces HR in planta. Furthermore, the L6L11RV chimera interacts with only AvrL567-J in yeast and again induces HR with only this Avr gene in planta. The observation that L6L11RV and L6 differ only

in the last 3 LRR units indicates that both the resistance and interaction specificities are controlled by the LRR domain. No interactions were detected in yeast between the resistance proteins and the proteins encoded by the virulence alleles that do not induce HR in flax lines.

In the flax rust system, the observation of direct interaction between L5 and L6 proteins and corresponding Avr proteins has now been extended to M and AvrM (PN Dodds, unpublished results). However, whereas M is approximately 80% identical to L5 and L6, the AvrL567 and AvrM proteins are unrelated. Similarly, while L6 and L11 differ by only 32 LRR polymorphisms, their corresponding Avr proteins are also apparently unrelated. In addition, all the other distinct L alleles interact with genetically independent avirulence genes and these are not sufficiently related in DNA sequence to be detected by AvrL567 DNA probes. If as seems likely, all these R proteins directly interact with their corresponding Avr proteins, the picture that is emerging is that NBS-LRR proteins can interact with diverse ligands and that the LRR region is highly flexible in an evolutionary sense with the capacity to recognize by direct interaction diverse pathogen ligands when coupled with the NBS domain.

Co-evolution of *Avr* and *R* genes in the flax rust system

Recognition by direct interaction has led to a high level of sequence diversity in rust Avr genes as a consequence of strong diversifying selection to escape recognition and host resistance. The AvrL567 genes are highly variable, with 12 different sequence variants (A-L) found in six rust strains of diverse origin. The 127 amino acid sequence of the mature AvrL567 protein contains 35 polymorphic sites, with nine sites showing multiple polymorphisms. These variants have arisen through positive selection, as indicated by the excess of non-synonymous nucleotide substitutions over synonymous changes in their coding sequences. This suggests that there has been a co-evolutionary "arms race" between the corresponding Avr and R genes in this system. Evidence that the diversification of the AvrL567 genes is driven by R gene-mediated selection comes from the observation that the sequence differences between the AvrL567 proteins lead to differences in recognition specificity by the corresponding L5, L6 and L7 resistance proteins.

The structures of AvrL567-A and –D have been determined by X-ray crystallography (Wang et al. 2007) and structural modeling indicates that avirulence and virulence variants of this protein have very similar structures and physical properties. The polymorphic residues map to the surface of the protein and polymorphisms in residues associated with recognition differences for the R proteins lead to significant changes in surface chemical properties. Analysis of single and multiple amino acid substitutions in AvrL567 proteins has confirmed the role of individual residues in conferring differences in recognition, but also suggest that the specificity results from the cumulative effects of multiple amino acid contacts.

The fact that naturally occurring virulence forms are expressed and encode products highly related to the avirulence variants suggests that there has been selection for Avr variants that escape detection by R proteins but retain a selective value for the pathogen, most likely through a virulence effector function. AvrL567 proteins show no similarity to any known or predicted proteins in current data bases and do not contain any known functional motifs, so the identification of their postulated virulence function is an important target of continuing research. Transgenic flax expressing the rust avirulence genes show no obvious phenotype in the absence of the corresponding resistance gene, and are not compromised in their expression of resistance to otherwise avirulent rust strains, which could have indicated a suppression of defense activity.

Diversifying selection is also evident in the other flax rust Avr genes, and most particularly the *AvrP123* gene, which like *AvrL567*, encodes an array of allelic variants with diverse recognition specificities for the corresponding *P*, *P1*, *P2* and *P3* resistance genes. Co-expression of the AvrP123 alleles with the P or P2 resistance genes in tobacco shows a conservation of the recognition and HR induction in this heterologous host, which is also consistent with a direct recognition event that does not require conservation of other host recognition factors. Thus it seems likely that direct R-Avr protein recognition prevails in this disease system, which contrasts with Arabidopsis-Pseudomonas disease resistance interactions. Part of the evolutionary explanation for this discrepancy may lie in the obligate parasitic and narrow host range characteristics of flax rust compared to bacterial pathogens. Mechanistically, the rust effectors may influence host target proteins through binding interactions rather than enzymatic modifications that can be detected indirectly.

Towards isolating stem rust effectors and Avr proteins

Wheat stem rust (caused by *Puccinia graminis* f.sp. *tricini* – Pgt) is one of the most destructive diseases of wheat, but has until recently been recalcitrant to molecular analysis. However, the recent advances in rust effector identification as well as the increased power and

availability of genome sequencing technologies have provided new approaches to the previously intractable system. A draft genome sequence for Pgt has now been developed (http://www.broad.mit.edu/annotation/genome/puccinia_graminis/Home.html) which will serve as a scaffold for understanding variation between rust fungal strains and identifying genes associated with virulence differences. We have now extended the haustorial secreted protein screen that was successful in isolating flax rust avirulence proteins to this system. We used the ConA affinitiy binding approach to isolate haustoria from wheat leaves infected with the Australian stem rust race 21-0. This strain was chosen because it is avirulent on a large number of wheat stem rust resistance (*Sr*) genes. It also represents the founder line of a lineage of Australian field isolates that were derived by sequential mutation to overcome specific *Sr* genes employed in agriculture. These isolates will provide a powerful genetic resource to identify genes that were altered during evolution to overcome these resistances. A total of about 13,000 ESTs have been sequenced from this Pgt haustorial library, representing about 4,800 unique genes. Bioinformatic analysis has predicted 360 secreted proteins in this set, which are now candidates to encode avirulence and effector activities. Identifying these functions will involve genetic screens for association with avirulence and virulence phenotypes and functional screens based on candidate gene expression in wheat

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8. Global stem rust surveillance in practice

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Abstract

An assessment was made of stem rust race analysis on a global scale. Responses were obtained from 23 rust workers representing 21 countries. Five laboratories have an institutional history in stem rust race analysis of more than 60 years, whereas personal experience in this field ranged from 0 to 35 years. The number of stem rust samples processed from 2006 to 2008 varied greatly between countries. For the three year period most collections were characterized in Canada, followed by Georgia, USA, South Africa and Australia. Most laboratories use the North American differential set and nomenclature system. However, these entries are often supplemented by additional tester lines from the Stakman set, other single gene lines or local cultivars. Differential sets varied between eight and 50 entries. More than half of the respondents indicated that they often encounter seed mixtures amongst their differentiating lines. In recent surveys most races were detected in Ethiopia, followed by Georgia and China. One race dominated the USA and Canadian stem rust population. In South America and Australia stem rust has been rare in commercial wheat for many years. Races within the Ug99 cluster were frequently identified in stem rust collections from Kenya and Ethiopia. Two races related to Ug99, but avirulent on *Sr31*, occur in South Africa. Several laboratories are in the process of purifying and bulking differential seed, which appears to be one of the major limiting factors in reliable stem rust race analysis. Improvement of infrastructure and training of individuals inexperienced with stem rust should improve global surveillance efforts. In addition, countries doing race analysis should keep viable culture collections in long-term storage.

Key words

Pathotype, *Puccinia graminis* f. sp. *tritici*, race analysis, surveys

Introduction

Studies on pathogenic variation in *Puccinia graminis* f. sp. *tritici* were initiated almost 100 years ago (Stakman and Piemeisel 1917; Stakman et al. 1918; Stakman and Levine 1922). Since the original descriptions, similar studies were conducted in many countries to monitor the occurrence and distribution of stem rust races (McIntosh et al. 1995). Knowledge of the virulence profile of prevailing races and the availability of representative cultures are important in screening and breeding wheat for resistance to stem rust. In addition, pathogen and host analyses over time provide information on evolutionary relationships and dispersal patterns.

The detection of stem rust race Ug99 (Pretorius et al. 2000) has renewed a global interest in the threat posed by stem rust and in research to combat this disease. Regular stem rust surveys and race analyses are thus considered important in monitoring the anticipated spread of Ug99 and its variants (Singh et al. 2008). The objective of this study was to determine the extent of stem rust surveys currently undertaken on a global scale. The paper is not intended as a detailed inventory of avirulence/virulence profiles detected worldwide, but rather a summary of country activities in terms of race analysis in *P. graminis* f. sp. *tritici*.

Data acquisition

A questionnaire covering biographical details of the respondent, institutional and personal experience in stem rust race analysis, countries/regions surveyed, frequency of surveys and collection methods, differential set used and its original source, frequency of differential seed multiplication and purity of entries, number of samples processed from 2006-2008, number of races identified during the last survey, greenhouse and inoculation facilities, and general comments, was compiled.

The questionnaire was distributed by the Borlaug Global Rust Initiative (BGRI) secretariat to 31 scientists known to be working on wheat rusts.

Results and discussion

Completed questionnaires were received from 23 respondents representing 21 countries. Some reports were comprehensive and provided information on all sections whereas others were incomplete or only indicated a desire to do stem rust analysis. Accurate records for institutional experience were not always available. This summary is only based on the information supplied and acknowledges the fact that other institutions doing similar work may have been omitted from the distribution list. Respondents are listed in Table 1.

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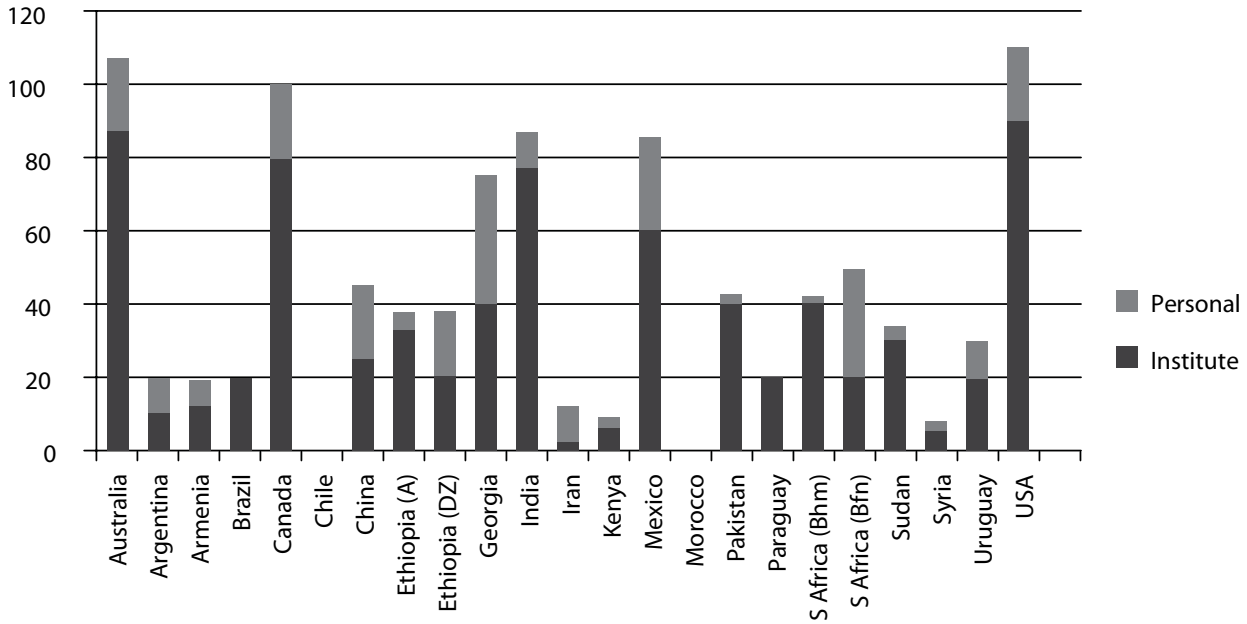
Table 1 Countries responding in the global summary of stem rust race analysis activities

Country	Institution	Contact person
Australia	PBI Cobbitty, University of Sydney, Camden	Prof Robert Park
Argentina	Instituto Nacional de Tecnología, Bordenave	Dr Pablo Campos
Armenia	Scientific Center of Agriculture and Plant Protection, Echmiadzin	Dr Hrant Terlemezyan
Brazil	Empresa Brasileira de Pesquisa Agropecuária, Passo Fundo	Dr Marcia Soares Chaves
Canada	Agriculture and Agri-Food Canada, Winnipeg	Dr Thomas Fetch
Chile	Instituto de Investigaciones Agropecuarias, Chillan	Dr Ricarda Madariaga
China	Institute of Plant Protection, Chinese Academy of Agricultural Science, Beijing	Dr Wanquan Chen
Ethiopia (A)*	Ethiopian Institute of Agricultural Research, Ambo	Dr Getaneh Woldeab Wolderufael
Ethiopia (DZ)*	Ethiopian Institute of Agricultural Research, Debre Zeit	Dr Ayele Badebo Huluka
Georgia	Instsitute of Plant Immunity, Kobuleti, Adjara	Dr Zola Sikharulidze
India	Wheat Rust Laboratory, Directorate of Wheat Research, Flowerdale, Shimla	Dr Mohinder Prashar
Iran	Seed and Plant Improvement Institute, Karaj	Dr Farzad Afshari
Kenya	Kenya Agricultural Research Institute, Njoro	Dr Ruth Wanyera
Mexico	CIMMYT	Dr Ravi Singh / Dr Julio Huerta
Morocco	Institut National de la Recherche Agronomique, Meknès	Dr Ramdani Abdelhamid
Pakistan	Institute of Plant and Enviromental Protection, Islamabad	Dr Javed Iqbal Mirza
Paraguay	Centro Regional de Investigación Agrícola, Capitán Miranda	Dr Lidia de Viedma
South Africa (Bfn)*	University of the Free State, Bloemfontein	Prof ZA Pretorius
South Africa (Bhm)*	ARC-Small Grain Institute, Bethlehem	Dr Tarekegn Geleta Terefe
Sudan	Agricultural Research Corporation, New Halfa	Dr Abdalla Kurmut
Syria	ICARDA	Dr Kumarse Nazari
Uruguay	Insituto Nacional de Investigación Agropecuaria, Colonia	Dr Silvia German
USA	USDA-ARS Cereal Disease Laboratory, St. Paul	Dr Yue Jin

*A and DZ refer to Ambo and Debre Zeit; Bfn and Bhm refer to Bloemfontein and Bethlehem

Fig. 1 *A summary of institutional and individual experience (years) in stem rust race analysis. In Ethiopia (A) refers to Ambo and (DZ) to Debre Zeit. In South Africa (Bhm) indicates the ARC Small Grain Institute at Bethlehem and (Bfn) the University of the Free State at Bloemfontein*



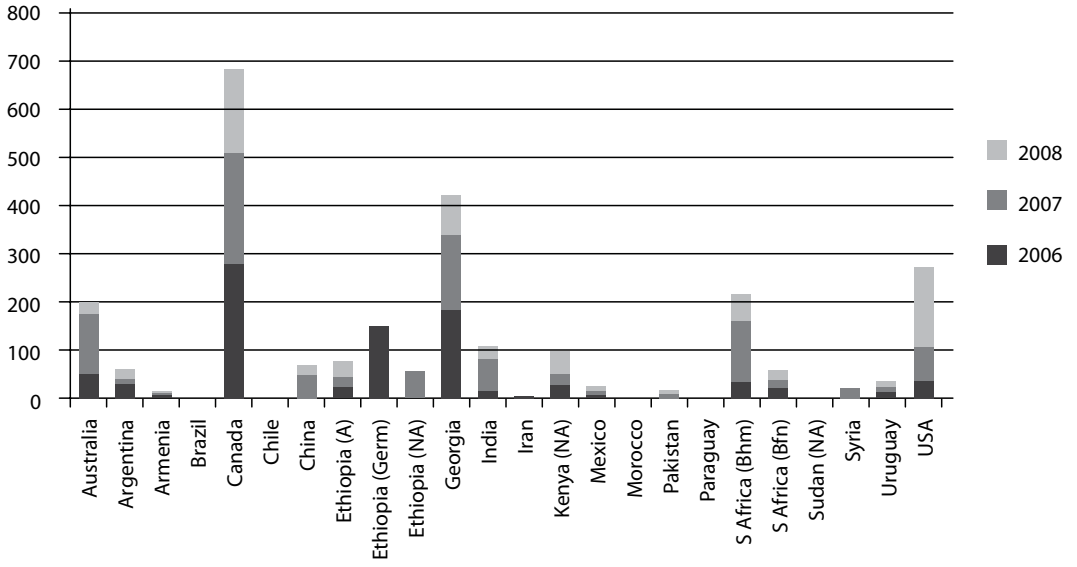
Five countries, viz. Australia (PBI Cobbitty, University of Sydney), Canada (Agriculture and Agri-Food, Winnipeg), India (DWR, Flowerdale, Shimla), Mexico (CIMMYT) and USA (USDA-ARS CDL, St. Paul) have institutional histories of more than 60 years in stem rust race analysis. Personal experience ranged from 0 to 35 years (Fig. 1). Some respondents mentioned that they lack stem rust experience, but have been trained in leaf or stripe rust analysis. Respondents from Pakistan and South Africa (ARC Bethlehem) indicated that their institutions have a significant history of stem rust work, but that they personally lacked experience in this field. Considering both personal and institutional experience, countries such as Australia, Canada, China, Georgia, Mexico, South Africa and USA have had good continuity in stem rust race surveys.

In terms of the number of stem rust samples processed during 2006 to 2008, the disease was most prevalent in Canada (684 isolates), Georgia (422), USA (273) South Africa (218) and Australia (200) (Fig. 2). Although no response was received from Yemen, 32 isolates of *P. graminis* f. sp. *tritici* were pathotyped at the CDL in St. Paul and Agriculture and Agri-Food Canada in Winnipeg. In a study completed in Germany, 152 Ethiopian wheat stem rust isolates were characterized (Admassu et al. 2009). Similarly, due to a lack of infrastructure, race analyses of stem rust samples collected in Kenya (99 isolates), Ethiopia (56) and Sudan (1) were done in St. Paul and Winnipeg. Despite valuable information being obtained from these collections, the mortality of East African wheat stem rust samples sent to the USA was unacceptably high (Y. Jin pers comm).

The numbers of entries in differential sets ranged from eight (Iran) to 50 (CIMMYT). Cereal rust laboratories in Argentina, Brazil, Canada, China, Mexico, Pakistan, South Africa, Uruguay and USA use, or plan to use, the North American (NA) (Roelfs and Martens 1988; Roelfs et al. 1993; Jin et al. 2008) stem rust set. Other countries such as Armenia, Ethiopia (Ambo), Morocco and Syria use the ICARDA set which includes the NA differential lines. Not all countries using the NA system have the same number of entries or the same genotype per *Sr* gene and it is possible that the expression of resistance genes in dissimilar backgrounds may influence race designation. Of some concern is the fact that more than one version of the NA race code exists in the literature. Jin et al. (2008) proposed an official fifth set (*Sr24*, *31*, *38* and McNair 701) to the NA differential series (Roelfs et al. 1993). However, a fifth set consisting of *Sr7a, 8b, 13* and McNair, previously added by Canadian rust workers (Fetch and Dunsmore 2004), is still being used (Admassu et al. 2009). Papers using the NA nomenclature thus have to be clear on which *Sr* gene set was used.

Australia and Georgia use the Stakman system plus additional tester lines, which differ between the two countries, and Iran and India have their own differential sets. However, data obtained from the Georgian and Indian sets can be converted to the NA code. The ARC

Fig. 2 ***Numbers of stem rust isolates pathotyped during 2006-2008. Ethiopian isolates were characterized at Ambo (A), Germany (Germ) and North America (NA). Kenyan and Sudanese collections were processed in North America. In South Africa the official stem rust survey is conducted by the ARC Small Grain Institute at Bethlehem (Bhm)***



Small Grain Institute in South Africa allocates a '2SA' race number, but the current differential set also allows conversion to a NA code.

Most respondents indicated that they reselect or increase their differential entries annually and 64% indicated that they often observe mixtures within differentials. Even laboratories starting with seed multiplication of 'new' stocks may be at risk of impurities because most likely their lines were obtained from established institutions where these entries have been increased many times. An international effort to purify differential lines and establishment of a single stock for each entry from which subsets can be distributed, will greatly enhance the accuracy of global data.

Respondents from Australia, Argentina, Brazil, Canada, China, India, Iran, Mexico, Morocco, Pakistan, South Africa, Uruguay and USA mentioned that they have access to air-conditioned greenhouses, an isolated facility for raising seedlings uncontaminated by rust spores, efficient inoculation and incubation facilities, and the capacity to sub-culture stem rust isolates. In general these facilities are sufficient for conducting reliable cereal rust research. Furthermore, efforts are currently underway in Kenya and Ethiopia to improve their rust research facilities. Together with trained personnel and appropriate seed stocks and rust isolates, stem rust race data, in addition to those from established laboratories, should be available from several countries in future.

Based on recent survey data, most diversity in stem rust populations appears to occur in China, Ethiopia and Georgia. Despite being a historically important disease in China, stem rust has been controlled through resistance breeding and now occurs mainly on spring wheat in some regions. In a study conducted in Germany, Admassu et al. (2009) reported 22 stem rust races from 152 collections made in Ethiopia in 2006. No virulence for *Sr24* was detected but TTKS was commonly found. At Ambo problems were encountered with poor seed germination and mixed differentials resulting in incomplete data sets. However, virulence for *Sr31* was common. The respondent from Georgia mentioned that barberry occurs in certain regions, but did not provide information indicating its potential contribution to racial diversity in stem rust.

In Australia, stem rust has been rare in commercial wheat in recent years. Most samples come from experimental plots and only three races were detected in the most recent survey. This low incidence is attributed to resistance breeding using *Sr* genes which remain effective, individually, or in combinations. The South American countries reported that, in general, stem rust has not been a problem for many years. Several institutes are currently increasing differential lines and should start with race analysis in 2010. In Argentina, representative isolates from 2000 onwards are available for testing once the system has been optimized.

In North America, one race (QFCSC) has dominated the Great Plains, Eastern USA and Canada in recent years. Unique races were found in the Northwest US, most likely from a sexual population (Y. Jin pers. comm.). In Mexico, diversity in wheat stem rust is uncommon and only one race has lately been identified.

Races within the TTKS cluster were commonly found in Kenyan and Ethiopian collections. Interestingly, some of these appeared to be avirulent for *Sr21* (T. Fetch pers. comm.). Nazari et al. (2009) confirmed the presence of race TTKSK in Iran, emphasizing the importance of continued stem rust surveys in countries at risk to Ug99. Race TTKSF (2SA88), which is believed to be an introduction (Visser et al. 2009), has been predominant in South Africa for several years. A *Sr24*-virulent variant, TTKSP, was detected in the Western Cape in 2007. A further variant virulent for *SrSatu* was discovered in South Africa in 2005.

The value of global race data will be significantly increased once there is agreement on a standard set of differentials of common origin, and standardized protocols and interpretation of infection types. Progress has been made in this regard with the BGRI Manual on handling stem rust samples and current discussions on the composition of a basic international set of differential entries. Training of rust pathologists and improved visual aids of infection types, particularly as to what is considered low, intermediate and high for each *Sr* gene included in the set, will be of value. A validation of predominant global stem rust races at an accredited laboratory, equipped with containment facilities, will greatly add to accuracy of data. However, biosecurity and phytosanitary issues, as well as material transfer agreements and capacity to do this work at a central facility, will have to be negotiated.

The use of surveillance technology and detailed record keeping should be emphasized at all laboratories doing stem rust surveys. Furthermore, culture collections are extremely important for rust research and resistance breeding. Institutions collecting wheat stem rust and identifying races should invest in reliable storage facilities with the necessary back-up systems. Such collections provide the materials for molecular and phenotypic comparisons of *P. graminis* f. sp. *tritici* isolates to improve our perspectives of evolutionary relationships within this variable pathogen.

It will be to the advantage of the cereal rust community if global stem rust survey data are more freely available. *The Rustopedia website* (www.rustopedia.org) *provides an excellent opportunity for pointing rust workers to published articles, institutional reports and websites, books, manuals and other relevant sources on the topic*.

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9. Race nomenclature systems: Can we speak the same language?

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Abstract

The first system describing physiologic specialization in the cereal rust fungi was that by Stakman and Levine (1922) for the wheat stem rust pathogen. Thirty seven biologic forms or "races" were identified using 12 differential wheat lines. Since then, additional variability in physiologic specialization was found and several systems evolved to describe this variation using numbers, letters, or combinations of both. This led to difficulties in comparing races, most often because of differences in the system that is used and the differential lines employed. A system that describes virulence succinctly and allows easily-made comparisons between races is highly desirable. Additionally, differential lines should be monogenic or near-isogenic so that virulence is classified on a genetic basis. Wherever near-isogenic stocks are used, it is vital that the recurrent parent is included. The systems that appear to be best suited to describing virulence with the above parameters are the letter-code and octal nomenclature. Of these, the letter-code system is the most commonly used based on a survey of research scientists working on stem rust. Thus, the letter-code system that uses 20 differential host lines is proposed to describe the nomenclature of *Puccinia graminis* f. sp. *tritici* on a worldwide basis. In addition, the source seedstock line for each differential gene is provided.

Keywords

Wheat, rust, pathogens, pathotypes

Introduction

Several nomenclature systems describing virulence in *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. have been developed, but to date, there is no worldwide consensus on a single system. Physiologic races of *P. graminis* f. sp. *tritici* were first described by Stakman (1914), and the first key to describe 37 races of wheat stem rust was published by Stakman and Levine (1922) using 12 differential wheat host lines. This key was updated by Stakman et al. (1962) to describe 297 races. This key used the differential host lines Little Club (*SrLC*), Marquis (*Sr7b, 18, 19, 20*), Reliance (*Sr5, 16, 18, 20*), Kota (*Sr7b, 18, 19, 28, Kt2*), Arnautka (*Sr9d, a*), Mindum (*Sr9d, a*), Spelmar (*Sr9d, a, b*), Kubanka (*Sr9g, c*), Acme (*Sr9g, d*), Einkorn (*Sr21*), Vernal emmer (*Sr9e*), and Khapli (*Sr7a, 13, 14*) (Roelfs and Martens 1988). This sequential number system set the standard on which the naming of cereal rust races was founded.

With an understanding of the gene-for-gene relationship in the 1950s and the development of lines with single stem rust resistance genes, new systems of nomenclature were developed (Roelfs and Martens 1988). Watson and Luig (1963) in Australia modified the Stakman system by adding 6 additional lines (e.g. race 98-1,2,3,5,6, which denoted equivalency to Stakman race 98 with virulence on additional lines numbered 1, 2, 3, 5, and 6). The current system in Australia is similar, but with 13 additional lines (McIntosh et. al. 1995). In Canada, a "C-race" formula system was developed by Green using 8 single *Sr* gene differential lines, and later amended to 16 lines (Martens et al. 1989). Green et al. (1970) also devised an East Africa (EA) nomenclature to describe races in Kenya, Ethiopia and Tanzania using nine differential cultivars, similar to the Stakman number system. In South Africa, a formula system similar to the "C-race" system is currently used, in which a prefix (2=stem rust, SA=South Africa) is used in tandem with a number (Pretorius et al. 2007) and uses 16 *Sr* genes. In China, a combination of the Stakman system, five Chinese supplemental lines, and a letter-code is currently used to describe races of wheat stem rust (C. Yuanyin pers comm).

In 1988, a new hexadecimal system using letters to describe virulence in *P. graminis* f. sp. *tritici* was proposed by Roelfs and Martens (1988) for international use. This system uses consonants from "B" to "T" to describe virulence patterns (Low or High for each line) across sets of 4 lines (Table 1). Initially, three sets were proposed, but currently five sets are used to describe virulence using the letter code system (Jin et al. 2008); the fifth set being added recently to differentiate strains within the race TTKS (Ug99) cluster. TTKSK (Ug99) is a highly virulent race that recently emerged in Africa (Pretorius et al. 2000) and is unique by being the only strain with virulence for gene *Sr31*, which is widely deployed in CIMMYT, European, and Chinese wheat varieties.

Another system for nomenclature of plant pathogens was proposed by Limpert et al. (1994) and uses a mathematical code. This uses coded triplets and is known commonly as the octal nomenclature system. Differential lines are grouped in ordered sets of three,

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and scores for each line are assigned based on low or high responses. If the response is low, the score for the line is zero. If the response is high, the score is a one for line one, two for line two, and four for line three. Scores are added across the three lines to obtain the octal number for that set of three lines, e.g. if high on lines one and three, the score is 1+0+4=5. Thus, an octal code of 3.1.4.5 on 12 differential lines indicates virulence on differential lines 1, 2, 4, 9, 10, and 12. The octal code is currently used to describe virulence in *Puccinia hordei* and *Blumeria graminis*.

Table 1 ***Letter code system for P. graminis f. sp. tritici***

	Four gene differential sets			
	Sr5	Sr21	Sr9e	Sr7b
	Sr11	Sr6	Sr8a	Sr9g
	Sr36	Sr9b	Sr30	Sr17
	Sr9a	Sr9d	Sr10	SrTmp
Pgt letter	Sr24	Sr31	Sr38	SrMcN
B	L	L	L	L
C	L	L	L	H
D	L	L	H	L
F	L	L	H	H
G	L	H	L	L
H	L	H	L	H
J	L	H	H	L
K	L	H	H	H
L	H	L	L	L
M	H	L	L	H
N	H	L	H	L
P	H	L	H	H
Q	H	H	L	L
R	H	H	L	H
S	H	H	H	L
T	H	H	H	H

This system assigns a letter for each four-gene set of differential lines based on the pattern of low (L) and high (H) seedling responses, where L = avirulent and H = virulent on each specific Sr gene line.

Previous attempts (Roelfs and Martens 1988) to establish a unified system to describe rust nomenclature were not successful, but with the efforts to track the movement of Ug99 and to identify dangerous new variants, it is clear that a single system would be preferred to communicate virulence information on a worldwide basis. The desired attributes of any nomenclature system are that it should be concise, relatively easy to use, and easily discern relationships among races. The problems with some current nomenclature systems are: 1) some differential lines are not monogenic; 2) some genes are not useful on a worldwide scale and are difficult to score unambiguously into low or high categories; 3) comparisons to other systems are very difficult; and 4) regional virulence determines the selection of differential lines used. In order to address these issues, discussion among scientists at several institutions where rust race pathotyping is performed resolved the following: 1) since the host-pathogen genetics follows a gene-for-gene system, differential lines should be selected on a single-gene basis and not as a line; 2) since there are several backgrounds in which single-gene lines have been developed, the source line for each gene needs to be agreed upon; and 3) a single system communicating avirulence/virulence information for international comparison needs to be decided.

In order to decide which system is most appropriate, comparisons were made across four currently-used nomenclature systems for wheat stem rust. These systems were: 1) Stakman or modifications thereof; 2) formula or "C" system; 3) octal system; and 4) letter-code system. Of these, it was clear that the numerical systems used to name races simply in order of discovery (Stakman and "C" race) do not provide information that allows comparisons of easily seen phenotypic similarities and differences. Thus, mathematical systems (octal, letter-code) were seen as clearly superior in quickly and easily showing comparative information across races. A survey conducted by Z. Pretorius (these proceedings) indicated that the letter-code using five sets of differential lines (20 *Sr* genes) is being used by many institutions conducting race analysis in order to track movement of Ug99. Thus, this system was chosen for use in international descriptions of virulence in *Puccinia graminis* f. sp. *tritici*, but local reports could continue to use current systems (and additional lines) as they chose. If additional lines are found to be important to more fully describe the avirulence/virulence phenotype of an isolate, then the description should use the five-letter code followed by a dash and then indicate any additional virulences using the corresponding *Sr* gene (not the line). For example, if an isolate of TTKSK was found with virulence for *Sr13* and *Sr26*, then the code would be TTKSK-*Sr13*, *Sr26*. Additional letters can be appended if more sets of four differential lines are needed.

In addition to selection of the letter-code system for use in international nomenclature for *Puccinia graminis* f. sp. *tritici*, specific single-gene lines for each differential

gene were agreed upon (Table 2). This was done using the known reactions of the different lines currently used, and recommending the line with the clearest low infection type. Development of a near-isogenic series was also discussed, and the Avocet background will be used as a starting point (R. Park, University of Sydney) in attempting to develop a new series of single-gene lines.

The agreement on the letter-code system, selection of specific *Sr* genes, and source seedstocks is a step forward in being able to undertake race pathotyping in a unified manner on a worldwide basis, enabling use of a common language in terms of avirulence/virulence phenotyping in *Puccinia graminis* f. sp. *tritici*.

Table 2 ***Differential genotypes recommended for use in pathotyping*** *Puccinia graminis* ***f. sp.*** *tritici*

Gene	LIT	Accession	Description
Sr5	0	CI 14159	ISr5-Ra; Thatcher/Chinese Spring
Sr21	1^-	Unassigned	*T. monococcum*/8*LMPG-6
Sr9e	1^- to 2^-	PI 442914	Vernstein
Sr7b	2	CI 14165	ISr7b-Ra; Hope/Chinese Spring
Sr11	; to 2^-	PI 155433	Yalta
Sr6	0;	CI 14163	ISr6-Ra; Red Egyptian/Chinese Spring
Sr8a	2^- to 2	PI 221154	Mentana
Sr9g	2^-	CI 5284	Acme
Sr36	0;	CI 17385	W2691/SrTt-1 (CI 12632)
Sr9b	2	Unassigned	Prelude*4/2/Marquis*6/Kenya 117A
Sr30	1^+ to 2	PI 330957	Festiguay
Sr17	;1	Unassigned	Prelude/8*Marquis*2/2/Esp 518/9
Sr9a	1^- to 2^-	CI 14169	ISr9a-Ra Red Egyptian/Chinese Spring
Sr9d	1^- to 1	CI 14177	ISr9d-Ra Hope/Chinese Spring
Sr10	;1N to 3C	CI 17388	W2691/2/2*Marquis*4/Egypt NA95
SrTmp	2^-	Unassigned	Triumph 64 (CI 13679)/Chinese Spring
Sr24	1^- to 2^-	Unassigned	Little Club/Agent (CI 13523)
Sr31	1^- to 2	Unassigned	Federation*4/ Kavkaz
Sr38	X=	AUS 99172	Trident = Spear*4/VPM (PI 519303)
SrMcN	2^-	CI 15288	McNair 701

LIT = Low infection type commonly displayed in an incompatible response

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10. Are rust pathogens under control in the Southern Cone of South America?

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Abstract

Approximately nine million ha of wheat (*Triticum aestivum* and *T. durum*) were sown annually in the Southern Cone of South America (Argentina, Brazil, Chile, Paraguay and Uruguay) during 2003-2007. Presently, leaf rust (caused by *Puccinia triticina*) is the most important rust of wheat throughout the region. The pathogen population is extremely dynamic leading to short-lived resistance in commercial cultivars. Leaf rust management relies on the use of resistant cultivars and fungicides. Sources of adult plant resistance conferred by minor additive genes have been increasingly used in breeding programs to obtain cultivars with more durable resistance. Stripe rust (*P. striiformis* f. sp. *tritici*) is endemic in central and southern Chile, where fungicides are required to control the disease on susceptible cultivars. Stem rust (*P. graminis* f. sp. t*ritici*) has not caused widespread epidemics in the last 25 years due to the use of resistant cultivars. Virulence to *Sr24* and *Sr31*, the most important genes conferring resistance to local races, has not been reported in the region. The areas sown with cultivars susceptible to local races in Argentina and Uruguay have increased in recent years. Since most varieties sown in the region are susceptible to Ug99 or derived races, testing and selection for resistance in Kenya, facilitated by the Borlaug Global Rust Initiative, is highly relevant for research aimed at preventing epidemics, which may occur if these races migrate, or are accidentally introduced to our region. The resistances identified in east Africa will also contribute to increasing the levels of resistance to current local races.

Keywords:

Wheat, *Triticum aestivum*, *Puccinia triticina*, *Puccinia striiformis* f. sp. *tritici*, *Puccinia graminis* f. sp. *tritici,* pathogen variability, breeding for resistance

Introduction

During 2003-2007 wheat (*Triticum aestivum* L. and *T. durum* Desf., syn. *T. turgidum* L.) was planted annually on approximately nine million ha in the Southern Cone of America (Argentina, Brazil, Chile, Paraguay and Uruguay) (FAOSTAT 2009). Argentina was the largest wheat producer (14 million tonnes(mt)) followed by Brazil (4.6 mt) and production in the smaller countries ranged from 0.5 to 1.6 mt, totaling 21.7 mt for the region. High yields of 4.5 t/ha are obtained in Chile where wheat is planted at higher latitudes (33° to 41°S) contrasting with lower yields of 1.9 to 2.9 t/ha obtained in the other Southern Cone countries.

Most of the area is sown to common wheat cultivars. Durum wheats are cultivated only in Chile and in the south of the Argentinean wheat area, representing a low percentage of the production (Germán et al. 2007). Spring wheats are used in most of the region, but alternative wheats are also used in Chile, Uruguay and the southern Argentinean wheat areas. Winter wheats are used in a smaller proportion of the regional wheat area, mostly in southern Chile and to a lesser extent in Argentina. All wheat types are sown during the fall and winter and harvested in spring or early summer. At lower latitudes of the Atlantic area, spring cultivars are mostly sown under zero tillage in double cropping with soybeans or other summer crops. Most of the pacific coastal area rotates dryland cereals with rapeseed, lupins and other crops under irrigation, such as potatoes, sugar beet, beans, sunflower, chicory or vegetables.

Presently, leaf rust (caused by *Puccinia triticina* Eriks.) is the most important rust of wheat throughout the region (Germán et al. 2007; Singh et al. 2009). Stripe rust is endemic in southern Chile. Control of leaf rust and stripe rust on susceptible cultivars relies on the use of fungicides. Due to the high economic importance of the rusts, resistance to leaf rust in all five countries, and to stripe rust in Chile, are long-term objectives for regional breeding programs. Although stem rust has not caused epidemics for over 25 years, awareness of the potential risk represented by the unlikely migration, or accidental introduction, of race Ug99 and derivatives has increased in recent years.

The prevalence of bread wheat rust diseases in the Southern Cone of South America, use of chemical control, variability of the pathogen populations, and breeding for resistance will be described.

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Wheat rusts - prevalence and geographical distribution

Climatic conditions in the Southern Cone are favorable for the development of rusts. Weather patterns in southern Chile are favorable for the development stripe rust (*P. striiformis* f. sp. *tritici* West.) while leaf rust and stem rust (*P. graminis* f. sp. *tritici* Pers.) epidemics may occur throughout the entire Southern Cone.

Leaf rust is currently the most prevalent and severe wheat disease in the region. It is present every year, causing widespread epidemics on susceptible or moderately susceptible cultivars, which are grown in a high proportion of the wheat area (Germán et al. 2007; Singh et al. 2009). Leaf rust causes severe epidemics in Brazil, Paraguay and Uruguay, and has an increasing importance in Chile (Mellado 2007, Hacke 2007). Leaf rust is present in all Argentinean wheat areas, varying in importance between sub-regions according to weather conditions (Campos 2008). Approximately 40% of the total area under wheat in Argentina is affected by severe leaf rust epidemics, whereas cool temperatures and/or dry conditions limit disease development in the rest of the Argentinean wheat area (Germán et al. 2004). Leaf rust can cause grain yield losses higher than 50% under severe epidemics if fungicides are not applied.

Stripe rust is endemic in central and southern Chile. In the past it was present every year mostly on winter and facultative wheats. Stripe rust infections have not caused major concerns since a severe epidemic in 2001 affected several spring cultivars, including some carrying *Yr9*. During 1929 and 1930, stripe rust caused very extensive and severe epidemics in Chile, Argentina, Uruguay and in Río Grande do Sul in Brazil. After these epidemic years, and in contrast with the increasing importance of this disease in other parts of the world, only sporadic localized outbreaks of stripe rust occurred on highly susceptible cultivars in Argentina, Brazil and Uruguay. Stripe rust was last detected in experimental fields in Argentina during 2004. A high proportion of commercial cultivars and advanced breeding lines from Argentina, Brazil, Paraguay and Uruguay routinely tested in cooperative nurseries in Chile are susceptible to stripe rust.

Although stem rust was the most damaging rust in the past, it has not caused widespread epidemics in the last 25 years due to the use of resistant cultivars in most wheat areas (Germán et al. 2007). The release of stem rust susceptible cultivars led to the reappearance of the disease in commercial fields in northern Argentina in 2001 and 2003. After 2003, the pathogen has frequently been observed in experimental fields in Argentina and Uruguay. Although not observed in the rest of the region for many years, some infections were detected in experimental fields in Passo Fundo (Brazil) in 2007 and in Chillan (Chile) in early 2009.

Rust epidemiology – over summering and development of epidemics during the crop season

Based on differences in rust race populations, two epidemiological zones separated by the Andean Mountains were suggested for South America (Rajaram and Campos 1974). Some similarities between the rust populations of Chile (representing the western zone), and the eastern zone (Argentina, Brazil, Paraguay and Uruguay) indicate that migration between zones occurs (Germán et al. 2007). No barriers for urediniospores transported by wind currents exist in the larger eastern zone, where wheat is planted in lowlands in Argentina, Paraguay and Uruguay and higher plateaus (600-800 masl) in Brazil.

The presence of alternate hosts of *P. triticina* and *P. graminis* f. sp. *tritici* has not been reported in the region. Therefore rust fungi survive the critical season (summer) on volunteer plants of wheat or secondary hosts. Over summering of leaf rust on volunteer wheat has been observed in most of the region. Stripe rust in Chile probably survives locally, on volunteer wheat or on secondary hosts at cooler higher altitudes in the mountains close to cropping areas. Over summering of stem rust has been observed on volunteer wheat in the south of Buenos Aires province (Argentina) and on volunteer wheat and barley plants in Uruguay (Germán et al. 2007).

The high proportion of susceptible cultivars in the cropping area allows *P. triticina* to oversummer across large areas and hence to develop severe epidemics during the subsequent cropping season. Leaf rust infections in commercial fields are first observed in the north and develop with the crop towards the south (Barcellos et al. 1982). However, early sown alternative cultivars used in Uruguay and the south of Buenos Aires Province (Argentina) may be infected early under the favorable conditions that occur during the fall. Lower temperatures during the winter slow the development of epidemics, but the presence of inoculum in the crops allows fast epidemic development during spring. Earlier and more severe epidemics have occurred during years when winter temperatures have been above average for long periods, as occurred in 2001 and 2005.

Variation in the pathogen populations - evolution of races and associated epidemics

The *P. triticina* population in the Southern Cone is extremely dynamic, leading to short-lived resistance in commercial cultivars (Germán et al. 2007). A large

number of races are generally present every year. The prevalent races change dramatically over time, in accordance with the area sown to cultivars susceptible to different pathotypes. After short periods of time, with few exceptions, resistance of new cultivars is overcome by new virulent races of the pathogen.

Leaf rust surveys and race identifications are performed annually in Argentina, Brazil and Uruguay. Samples from Chile and Paraguay are also analyzed for race identification. During 2004-2007 races MCP (Long and Kolmer 1989) with additional virulence on *Lr10* (MCP-10), MDR with additional virulence on *Lr10* and *Lr20* (MDR-10,20) and MFP, also virulent on *Lr20* (MFP-20), were present in high frequencies in Argentina (Campos 2008) and Uruguay (Table 1). MCP-10 was associated with severe epidemics on Klein Don Enrique (*Lr26* and additional resistance +; Antonelli 2003) in Argentina and Uruguay, MDR-10,20 was associated with epidemics on INIA Torcaza (*Lr10, Lr24* +; Germán et al. 2005; Demichellis et al. 2008) and INIA Churrinche (*Lr10, Lr24*; Demichelis et al. 2008) in Uruguay, and MFP-20 was associated with epidemics on INIA Tero (*Lr17, Lr24*; Germán et al. 2005) in Uruguay. MFP-20 (Brazilian designation B56) was the first race virulent on BRS 194, widely grown in Brazil from 1988 to 1994 (Chaves et al. 2009). MDT-10,20 and related race MFT-10,20 (Brazilian designation B55, Chaves 2007) have been prevalent in Brazil since 2005 and since first detected in Argentina and Uruguay in 2007. These races are also present in Paraguay. Related races MDT-10,20 and MFT-10,20 are virulent on a wide range of commercial cultivars, and have caused severe epidemics on the most popular cultivars in Brazil and Paraguay, as well as other popular cultivars from Argentina, Brazil and Uruguay. MCT-10/MHT-10 was frequently found in Argentina during 2004-2006. Race MCD-10,20 (previously prevalent across the region) and MCP-10,20 were also identified in samples from Chile and Paraguay. Race MCD-10,20, was isolated from samples collected during 2007 and 2008 in Chile and analyzed in Uruguay and the USDA-Cereal Disease Laboratory (J. Kolmer pers comm). Some races isolated from durum wheats were different from races previously identified in the region (J. Kolmer, pers. comm.). The most significant changes in the leaf rust population during 1996-2003 affecting 10 cultivars represented an estimated loss of US$172 million to Southern Cone wheat farmers (Germán et al. 2004).

Table 1 Race frequencies of Puccinia triticina isolates collected in Argentina, Brazil and Uruguay during 2004-2007

Argentina							Brazil					Uruguay				
Code	Brazil. code	First detect.	2004	2005	2006	2007	First detect.	2004	2005	2006	2007	First detect.	2004	2005	2006	2007
CHT			10.0	2.6	1.0							1997			0.7	
MCD-10			4.0	5.8								2001	15.0	8.0		
MCP-10		2000	46.0	16.9	29.0	3.0						2000	28.0	15.4	1.5	
MCP-10,19		2005		10.4	4.0	4.0						2006			2.2	1.7
MCT-10	B34		4.0	17.5	25.0	2.0	1989	2.0	3.7	3.1	2.9	1992		1.7	2.2	
MDP-10,20	B58	2005		1.9	7.0	9.0	2007				2.5	2004	4.0	10.9	18.7	19.0
MDR-10,20		2004	1.0	16.9	8.0	8.0						2003	17.0	32.0	20.1	3.3
MFP-20	B56	2004		3.9	11.0	10.0	2005		0.7	5.2	2.5	2005		1.7	28.4	4.1
MFR-10,20		2007				10.0						2004	4.0	4.0	3.7	2.5
MDT-10,20	B55	2007				21.0	2005		26.4	66.5	73.0	2007				24.0
MFT-10,20	B55	2007				8.0	2004	5.1	49.2			2007				14.0
MFT-20	B51	2004	1.0				2002	16.2	3.3							
MHT-10		2003	12.0	1.9												
SPJ-10	B50						2002	4.6		12.0	1.6					
TFT-20	B54						2004	21.8								
No isolates					96	138		197	299	191	244		100	175	134	121

Virulence frequencies have generally been high on lines with *Lr1, Lr3a, Lr3ka, Lr10, Lr14a, Lr14b, Lr11, Lr26* and *Lr30*, intermediate on lines with *Lr20*, and low on those with *Lr2a, Lr2c, Lr9* and *Lr19* (Campos 2008; Germán et al. 2007). Virulence frequencies on lines with *Lr17a* and *Lr24* have increased in Argentina (Campos 2008) and Uruguay. Isolates with intermediate infection types on the *Lr16* differential have also increased in frequency in Argentina (Campos 2008). Low infection on Thatcher lines with *Lr2a*, *Lr9* and *Lr18* in Chile over recent years indicates that the pathogen has low or no virulence to these genes.

Historically, frequent race changes in the *Puccinia striiformis* population have occurred in Chile as evidenced by changes in severities of initially resistant cultivars. However, no change in the field reactions of cultivars and single *Yr* gene lines has occurred since 2001, indicating no recent significant change in race structure. Genes *Yr5, Yr10* and *Yr15* reported as effective by Madariaga et al. (2004) remain highly effective.

Thirty *P. graminis* races combining virulence for four to 14 *Sr* genes (*Sr5, Sr6, Sr7a, Sr8a, Sr9a, Sr9b, Sr9e, Sr10, Sr11, Sr12, Sr13, Sr14, Sr17, Sr29, Sr30, Sr36, Sr37*) were identified in Brazil during 1949-1994. During the beginning of the 2000s one race (RTTTR, Brazilian designation, G30) was prevalent in Brazil and Uruguay. Since 2002 some changes in the pathogen population have been observed in Uruguay (Germán et al. 2007) and Argentina where eight pathothypes were differentiated on 74 wheat cultivars and available stem rust differentials (Campos and López 2008). No virulence has been detected for the important resistance genes *Sr24* and *Sr31*.

Use of fungicides to control wheat rusts

The management of leaf rust on susceptible cultivars relies on the use of fungicides. Fungicides are widely used to control leaf rust in Brazil, Paraguay and Uruguay, where the disease is more economically important. During early and severe epidemics two or three applications of fungicides are required to control the disease on highly susceptible cultivars. However in Brazil and Paraguay, a fourth application may be required. In Argentina rarely more than one fungicide application is used on about 25% of the wheat crops. In Chile at least one fungicide application is used by farmers to control stripe rust and leaf rust. The yields of susceptible cultivars, such as Otto, increased from 8.6 t/ha to 10.1 t/ha after one application of the strobilurin fungicide Juwel Top (Madariaga 2008). Due to the large areas sown to cultivars that require chemical control, the regional annual cost of fungicide applications to control leaf rust in an average epidemic was estimated at US$50 million (Germán et al. 2004).

Mixtures of triazols and strobilurins control leaf rust more efficiently than triazols used alone. Triazols efficiently control the disease when used at the proper dose and with adequate application technology (Maciel and Chaves 2008) in average epidemics. Singer (2008) reported that isolates of some new prevalent Brazilian *P. triticina* races are less sensitive to tebuconazole than old races. These new races include MFT-10,20, MDT-10,20 (B55), MFP-20 (B56) and MDP-10,20, also present or prevalent in Argentina, Uruguay and Paraguay.

Breeding for resistance - the best strategy to control the wheat rusts

Most of the effective resistance to leaf rust present in current cultivars is conferred by combinations of major resistance genes present in the regional germplasm. *Lr34* is also frequently present (Germán et al. 2007). Resistance genes *Lr10, Lr23, Lr24* and *Lr26*, most common in Brazilian germplasm tested during 1996-1997 (Zoldan and Barcellos 2002), continued to be present in the most important cultivars used in 2004. Adult plant resistance (APR) genes *Lr13* and *Lr34* are also common in Brazilian germplasm (Zoldan et al. 2000). Other APR genes have been described in Brazilian cultivars, such as *Trp1* and *Trp2* present in Toropí (Barcellos et al. 2000) and an undesignated APR gene present in BR35 (Brammer et al. 2004). Using molecular markers, the presence of *Lr9, Lr10, Lr19, Lr20, Lr24, Lr26, Lr34, Lr37* and *Lr47* was detected, either singly or in combinations of two or three genes, in 98 modern Argentinean cultivars (Demichelis et al. 2008). *Lr47* was introduced into INTA germplasm (Argentina) using molecular markers, leading to the release of Biointa 2004. Seedling resistance genes *Lr3, Lr10, Lr14b, Lr16, Lr17a, Lr24, Lr26* and APR genes *Lr13* and *Lr34* were found or postulated in Uruguayan cultivars released since 1995 (Germán et al. 2005; unpublished data).

Sources of APR to leaf rust conferred by minor additive genes have been increasingly used in regional programs to introduce more durable resistance in breeding materials (Singh et al. 2009). Old characterized sources of APR distributed by CIMMYT, such as Parula (*Lr34, Lr46* + one or two minor genes), Chapio (*Lr34* + three or four minor genes), Amadina (four minor genes), and other lines with this type of resistance (Singh et al. 2003) have been used as sources of resistance in crosses with adapted materials. Other materials with APR to leaf rust, such as old cultivars, new breeding lines and cultivars developed by local programs, have also been used to introduce this type or resistance in recently developed germplasm.

Field resistance is the most important criterion for selection in populations derived from crosses with adapted materials. Molecular markers for *Lr34* (Lagudah et al. 2006), used in Argentina to confirm the presence of this gene in advanced lines, will also be used in Brazil and Uruguay. *Trp1* and *Trp2* markers for the APR present in Toropí (S. Brammer et al. unpublished) are under validation and will be used to screen breeding lines in Brazil. In contrast with APR sources from CIMMYT, Toropí has intermediate resistance to Fusarium head blight (caused by *Fusarium* spp.), which can be severe in Argentina, Brazil, Paraguay and Uruguay when favorable weather conditions occur. Molecular markers for minor genes conferring APR to leaf rust will be particularly useful to screen populations or lines from crosses involving one parent with intermediate or high resistance to leaf rust conferred by major genes. Homogeneous adapted advanced lines have been selected from crosses involving sources of APR to leaf rust and adapted high yielding materials and are being used to start a second cycle of selection for APR to leaf rust. Selected lines will eventually be released as commercial cultivars.

Although both the economic importance of stripe rust and pathogen variation have decreased in the last decade, breeding for resistance has remained a long term objective of the INIA-Chile wheat breeding program. Since susceptible materials are annually completely destroyed by the disease, indicating that favorable environment and compatible pathogen/host combinations prevail, the use of sources of durable resistance to stripe rust is a priority. Due to the association between APR to leaf rust and APR to stripe rust (Singh 1992; Singh et al. 2003), selection for resistance to leaf rust will indirectly increase the level of resistance to stripe rust in the germplasm developed in eastern countries of the Southern Cone.

Stem rust resistance was a major wheat breeding objective when the disease was prevalent. The absence of the disease for many years not only decreased opportunities for selection, but also led to changed priorities for breeding programs. As a result, susceptible cultivars were released in Argentina and Uruguay. Stem rust susceptible cultivars were grown on 35% and 15% of the wheat areas in Argentina and Uruguay, respectively, during 2007, and this figure increased to almost 30% in Uruguay during 2008. Except for a few Brazilian cultivars, which are also grown in Argentina and Uruguay, there is no available information on the stem rust reactions of cultivars used in Brazil, Chile and Paraguay to races present in the region. The increasing areas of susceptible cultivars may result in inoculum increase and development of stem rust epidemics

In Argentina, the frequent presence of natural stem rust infections in summer breeding nurseries at Balcarce allows selection and characterization of stem rust reactions to local races. In Uruguay, an artificially inoculated late sown nursery is used to characterize the stem rust field reactions of new breeding lines and commercial cultivars. Information of seedling reactions to local races is also available in Argentina and Uruguay. Selection for resistance is performed in breeding materials where segregation for resistance is present, but no efforts are directed to identify and systematically use sources of resistance to the local pathogen population.

The most important genes conferring resistance in the regional germplasm are *Sr24* and *Sr31* (Campos and López 2008; Germán et al. 2007). A high proportion of the Uruguayan, Argentinean and Brazilian wheat germplasm carries the associated genes *Lr24* and *Lr26* (Campos 2008; Germán et al. 2007). Only limited information of other *Sr* genes present in modern germplasm is available.

Facing the threat of Ug99 and derived races

Widespread severe stem rust epidemics associated with new pathogen races virulent to most commercially grown cultivars have occurred in the Southern Cone (Germán et al. 2007). Likewise, since most varieties used by farmers are susceptible in Kenya, the region is facing a potential threat of significant epidemics if Ug99 or derived races were accidentally introduced (Campos and López 2008; Germán and Verges 2005; Germán et al. 2007).

Table 2 ***Numbers and percentages of stem rust resistant and moderately resistant lines identified in Kenya, 2005-2008***

	Argentina		Brazil		Uruguay		Chile		Total	
Year	Nº	% R&MR	Nº	% R&MR	Nº	% R&MR	Nº	% R&MR	Nº	% R&MR
2005	39	20.5	92	27.2	13	53.8	10	0.0	154	26.0
2006			92	10.9	100	14.0	88	10.2	280	11.8
2007	38	21.1	93	9.7	199	8.5			330	10.3
2008	68	30.9			225	15.6			293	19.1

Table 3 *Pedigrees and stem rust reactions of resistant cultivars identified in Kenya*

Material	Cross	Stem rust
Argentina		02/10/2008
BIOINTA 1003	S check	70 MSS[a]
BIOINTA 1000	DTE/PTS/4/SOTY//TZC*3/SKA/3/PPAI/5/JAR`s/ CHRIS/3/NO/CON//MJI S	5 MR
BIOINTA 1002	HAIL/5/PIDAL/4/CNO67/MED//MON `S	5 MR
BIOINTA 2004	PPTAL*4/PVN t	T MR-MS[a]
BIOINTA 2002	BPONCHO/CCTP-F7-7792-122(87)	30 M
Brazil		30/03/07
BRS 179	S check	70 S
BRS 120	PF 83899/PF 813//F 27141	10 R
BRS 176	HLN/CNT 7//AMIGO/CNT 7	5 R
BRS 177	PF 9293 = PF 83899/PF 813//F 27141	5 S
BRS 192	PF 869114/PF8722	10 MS
BRS 194	CEP 14/BR23//CEP19	5 M
BRS 209	JUP73/EMB16	5 RMR
BRS FIGUEIRA	COKER 762*2/CNT 8	5 MSS
BRS TIMBAUVA	BR32/PF 869120	5 RMR
BRS 229	WT 96168 =EMB27*3//BR 35/BUCK PONCHO	5 M
BRS CAMBOATA	PF 970151 = PF 93232 -- SEL 14 bem 16 = PF 86238	5 R
BRS CAMBOIM	PF 980144 =EMB 27*4/KLEN CARTUCHO//PF 869114/BR 23	5 MSS
BRC CANELA	PF 979064 =PF 91205//PF 91204*2/ANA 75	5 R
BRS GUATAMBU	AMIGO/2*BR 23	5 R
BRS LOURO	PF 970128 = PF 869114/BR 23	5 MSS
CD 111	EMBRAPA 27/OCEPAR 18//ANAHUAC 75	5 MSS
CE P27	CEP 8057/BUTUI//CEP 8324	5 RMR
EMBRAPA 16	HULHA NEGRA/CNT 7//AMIGO/CNT 7	1 R
FEPAGRO-RS15	PF 82250/RS1	10 MSS
IPR 90	OSTE 'S'//CTA 'S'/YAV 'S'	5 MS
SAFIRA	PF 9099/OR 1//GRANITO	10 MS
VANGUARDA	OR 1/3/ORL9217/EMB 16/OR 1	5 R
Paraguay		2007
Itapúa 65	S check	100 S
IAN 15	PAT10/ALD"S"/VEE"S".	5 MR
Uruguay		28/10/2008
F6-CL-06-14629	S check	100 S
LE 2303 (INIA TERO)	LI107/C-CH-91-1642	0

[a] Severity: modified Cobb scale (Peterson et al. 1948); Reaction: (Stakman et al. (1982)

International testing in Kenya and Ethiopia made possible by the Borlaug Global Rust Initiative has allowed highly relevant testing and selection for resistance in South American breeding materials. An increased number of wheat materials from the region have been tested at the Kenyan Agricultural Research Institute-Njoro Plant Breeding Resarch Center since 2005 (Table 2). The reduced proportion of resistant materials in 2006 and 2007 relative to 2005 was due to the new variant virulent for *Sr24* (Jin et al. 2008). However, a number of cultivars and lines continued to be resistant (Table 3). The genetic bases of the resistances are unknown.

Several sources of resistance to stem rust identified in North East Africa, including APR sources such as Pavon 76 and Parula (Singh et al. 2008) have been used in crosses with locally adapted cultivars in Argentina, Brazil, Paraguay and Uruguay. Other materials with APR to leaf rust distributed through collaborative research projects among programs in the Southern Cone were also resistant in Kenya (e.g. Suz6/Opata (Table 4), BR23//CEP19/PF85490).

Homogeneous adapted F_6 lines combining resistance to Ug99 and derived races and resistance to leaf rust were selected in Uruguay (Table 4). LE2304 is a sister line of INIA Tero, and likely carries the same seedling resistance to stem rust. Parula and Genaro*3/Parula posses APR to leaf rust and stripe rust, and Parula also has high levels of APR to stem rust. Lines R07 F5-3027 and R07 F5-3037 derived from the cross Genaro*3/Parula//LE 2252 were more resistant to stem rust in Kenya than Genaro*3/Parula.

Sources of APR to stem rust identified in east Africa were distributed in the International Stem Rust Resistance Screening Nurseries (ISRRSN). Entries that do not carry *Sr31* or *Sr24*, but were resistant in Kenya, were also resistant or moderately resistant when tested in Uruguay. These sources of resistance are probably also effective against other races present in the region. *Therefore research efforts to introduce stem rust resistance to Ug99 and derived races into the regional germplasm will also increase the level of resistance to current local races of the pathogen.*

Table 4 *Field stem rust (Kenya) and leaf rust reactions (Uruguay) of F_6 Uruguayan breeding lines*

Line	Cross		Stem rust Kenya	Leaf rust Uruguay
			28/10/2008	07/11/2009
INIA Mirlo	CAR853/COC//VEE S/3/URES	Check for *Sr31*	50-80MSS[a]	
INIA Caburé	EFED/BUCK 6//MR74507	Check for *Sr24*	60-70MSS	
LE 2304	LI107/C-CH-91-1642	Seedling R to SR	--	90S[a]
PARULA	FKN/3/2*FCR//KAD/GB54/4/BB/CHA	APR to LR and SR	5M	TR
R07 F4-21322	LE 2304*2/PARULA		0	5MRMS
R07 F4-21356	LE 2304*2/PARULA		0	5MS
INIA TIJERETA	LE 2132/ECALANDRIA		S[b]	MS[b]
	SUZ6/OPATA	APR to LR and SR	5M	0
R07 F5-3737	I.TIJERETA*2/SUZ6/OPATA		20MR	0-10MS
R07 F5-3738	I.TIJERETA*2/SUZ6/OPATA		30M	0-5MSS
LE 2252	LE 2120/BUCK 12		--	60MS
	GENARO*3/PARULA	APR to LR, SR?	40MSS	5MR
R07 F5-3027	GENARO*3/PARULA//LE 2252		10M	0
R07 F5-3037	GENARO*3/PARULA//LE 2252		5M	TRMR

[a] Severity: modified Cobb scale (Peterson et al. 1948); Reaction: (Stakman et al (1982)

[b] 2007

Conclusions

The control of wheat rusts continues to be a challenge for breeders and rust pathologists in the Southern Cone of South America. Breeding wheat cultivars with APR to all three rusts is being undertaken as the best strategy to obtain cultivars with effective and more durable resistances.

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11. Recent experiences with global surveillance of wheat stripe rust

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Wheat rust fungi can overcome the effect of host resistance genes rapidly, and spores can disperse over long distance by wind. Here, we report rapid intercontinental spread of two closely related strains of *Puccinia striiformis* f. sp. *tritici*, which were characterised by short generation time and high spore production capacity. These conclusions are based on selected isolates from past race survey collections in Europe, America, Australia and South Africa, recent sampling from northern Europe, eastern USA, east Africa, and west-, central and south Asia, as well as epidemiological observations of yellow rust epidemics in these areas. One strain defined by identity at 15 virulence loci and 130 AFLP fragments was exclusive to North America (present since 2000) and Australia (since 2002). This strain became rapidly widespread across these two continents. Another strain of the same virulence phenotype, but differing in two AFLP fragments, was exclusive to Europe (present since 2000-01) as well as western and central Asia and east Africa (first appearance unknown). While the new strain gave rise to severe epidemics in many areas in Africa and Asia, the use of resistant wheat varieties has so far prevented widespread epidemics in Europe. The limited divergence between the two strains and their derivatives, and the temporal-spatial occurrence pattern confirmed a recent spread. The data gave evidence for additional intercontinental dispersal events in the past, i.e. from many isolates sampled prior to 2000 from Europe, North America and Australia had similar AFLP fingerprints, and isolates from South Africa, which showed no divergence in AFLP, differed by only two fragments from particular isolates from central Asia, west Asia and southern Europe, respectively. Previous research demonstrated that isolates of the two new strains produced up to two-to-threefold more spores per day than strains found in USA and Europe prior to 2000, with the greatest differences at high temperature. These results suggest that increased aggressiveness at this level may accelerate global spread of crop pathogens.

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12. The development and application of near-isogenic lines for monitoring cereal rust pathogens

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Summary

The purpose of monitoring cereal rust pathogens is to provide a basis for disease control strategies that include breeding for resistance, predicting disease response in commercial cultivars and responding to the dynamics of pathogen change. The means of achieving this vary from regular collection surveys based on assessments of sample collections in greenhouse tests, to monitoring and recording static trap plots. Factors governing the method of approach include the size of the target region, the available research resources and the experience of staff involved.

This paper is a brief review of the development of near-isogenic lines as a means of monitoring cereal rust pathogens. Emphasis will be given to wheat stripe/yellow rust and the development and application of a near-isogenic set of materials based on the spring wheat cultivar Avocet. This parent was selected because of its high degree of susceptibility to the disease, agronomic adaptability (semidwarf, spring habit, moderate vernalisation and day-length requirements), and resistance to stem rust. The relative benefits of using these materials will be discussed in the context of available data.

Keywords

Pathogen monitoring, stripe rust, *P. striiformis*, race surveys

Introduction

Wheat stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a recurrent disease problem in the majority of cereal-producing regions of the world, causing yield losses and increasing fungicide usage. These regions tend to be cool, temperate regions including the Americas, Europe, Asia, the Middle East, central and Southeast Asia, Russia, China and east Africa. These environments encompass an array of latitude and elevation combinations that are generally characterised by varying periods of cool temperature (0 to 15^{0}C) and high humidity that are ideal for infection and disease development. Where these environmental conditions coincide with available pathogen inoculum and susceptible hosts, including specific cereal and grass genera (Wellings 2007), stripe rust epidemics of varying intensity and duration may develop. Recurrent epidemics occurred in the Middle East (Yemen, Egypt, Syria, Lebanon, Turkey) and west Asia (Iran, Iraq) during the mid-1990s. Although these regions were generally considered too hot for pathogen survival and spread, it is clear that conducive seasonal temperatures combined with susceptible cultivars provided ideal conditions for epidemic development and crop losses (Wellings et al. 2000a). These circumstances prevailed in Central Asia (Azerbaijan, Turkmenistan, Uzbekistan, Tajikistan, Kazakhstan) in 1999, and in China in 2002, resulting in historic stripe rust epidemics and widespread crop losses.

The vulnerability of such regions to stripe rust epidemics confirms the need for the deployment of resistant cultivars. A major contributing factor in the Middle East epidemics during the mid-1990s was the widespread adoption of 'Veery' wheats, which were released and named by several national programs throughout the region (e.g. Falat in Iran; Seri 82 in Syria, Lebanon and Turkey; Dashen in Ethiopia and Yemen). These cultivars, which carry *Yr9*, were resistant to *Pst* when released. However, pathogenic change in the *Pst* population resulted in a new pathotype that was virulent for *Yr9*. Like *P. graminis* f. sp. *tritici* pathotype Ug99, the *Yr9*-virulent pathotype likely evolved in the highlands of east Africa and migrated to the Middle East and the Indian subcontinent (Singh et al. 2004). The pathotype multiplied rapidly on the vulnerable 'Veery' wheats in years where climatic conditions and pathogen survival coincided with their wide scale cultivation.

Unpredicted changes in the pathogenic capabilities of *Pst* populations were important factors, among others, for epidemics. However, pathogenic variation in *Pst* has received variable attention throughout wheat-growing areas worldwide. Regional and national surveys in North America, India, China, Australasia and western Europe were both intensive and extensive, whereas significantly large epidemiologic zones, especially in developing countries, have received comparatively little attention. International surveys of variability in the stripe rust pathogen conducted by RW Stubbs and colleagues at the Research Institute for Plant Protection (IPO) Wageningen, The Netherlands, provided valuable data for determining variability in *Pst* in developing countries from the 1970s through to the early 1990s. However, samples tended to be intermittent and in low frequencies over locations and years, and the data were largely unrelated to variation in resistance gene deployment in commercial wheats.

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A limiting factor in the implementation of pathogenicity surveys for *Pst* has been the need for environmental control for pathotype determinations, especially temperature and light intensity, and the essential experience required in the interpretation of results. It became evident that a simple but reliable, field-based methodology was required to monitor pathogenic variation. The objective of a project funded by the Australian Center for International Agricultural Research (ACIAR) was to develop, and assess as widely as possible, a set of near-isogenic lines (NILs) as differential testers of pathogenic variability in *Pst* in both greenhouse and field experiments. The NILs were based on the Australian cultivar Avocet and represented a set of wheat lines that were similar except for the presence of single genes for resistance.

Materials and methods

The recurrent parent

Avocet (WW119/WW15 (=Anza)//Egret; AUS 20601, Australian Winter Cereals Collection, Tamworth, NSW) is a bearded, white grained semidwarf (*Rht1*) soft textured Australian wheat cultivar. It is near day-length insensitive and has a moderate vernalization requirement. The cultivar had a mixed response to the original clone of *Pst* introduced to Australia in 1979. The resistant component of Avocet (selection Avocet 'R'; AUS 90660) was overcome by a mutant variant of the pathogen first detected in 1981 (Wellings et al. 1988). The susceptible selection Avocet 'S' (AUS 90661) was highly susceptible to both pathotypes, at the seedling and adult plant stages, and was selected as a candidate recurrent parent in NIL development. Several alternative Australian genotypes, including M2435 (Teal derivative with *Sr26*), Harrier and Spear, were also considered in the early developmental stages of the project but were rejected because of moderate levels of adult plant resistance or were ill-adapted for widespread international application.

Multipathotype tests undertaken at IPO, Wageningen (Wellings and Stubbs unpublished), indicated that Avocet 'S' was susceptible to a wide range of isolates collected world-wide and selected for avirulence on the standard differential set, i.e. Avocet S showed no evidence of a resistance gene/s present in the differentials. In contrast, the alternative candidate genotypes were resistant to a majority of these pathogen isolates and hence were eliminated from further NIL development. Subsequent testing of Avocet in India (Nayar pers comm) and Denmark (Hovmoller pers comm) identified certain isolates avirulent on Avocet S and hence the NIL set may not be useful in all geographic locations.

Avocet carries *Lr13* for resistance to leaf rust (*P. triticina*) and is highly susceptible to *Lr13*-virulent pathotypes that are now likely to be common in most geographic areas. Avocet also carries *Sr5, Sr8a* and *Sr26* for resistance to stem rust (*P. graminis* f. sp. *tritici*), and should be at least moderately resistant to stem rust at all sites since *Sr26* is considered to be effective globally.

Near-isogenic lines in Avocet S

Despite some reservations in the expectation that the genotype would be susceptible to *Pst* in all environments, Avocet S was used to introduce single gene resistances to *Pst* using a conventional backcross method. Backcross F_1 seed was derived using Avocet S as the male parent. In the majority of cases, the BCF_1 segregated in seedling tests into even proportions of susceptible and resistant phenotypes; the resistant plants were retained for further backcrossing. The exception was *Yr6* which was inherited as a recessive gene; in this case every second generation was self-pollinated to identify *Yr6Yr6* homozygotes for the next cycle of backcrossing. After several cycles of back-crossing, homozygous resistant lines were selected and multiplied in field experiments. Three cohorts of NILs (Table 1) were developed including some BC_3 lines to allow early evaluation in international trap plots. NILs were finalized at BC_6.

Confirmation of the target gene in each NIL was undertaken using various methods, including the characteristic low infection type. When appropriate pathotypes with matching virulence were available, these were used in seedling tests to confirm the presence of the specific genes. Tests conducted at PBI Cobbitty confirmed the presence of *Yr1, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr17, Yr27,* and *Yr32*. Molecular assays were conducted to confirm the presence of *Yr17* (Ventriup-LN2: Helguera et al. 2003) and *Yr18* (csLV34: Lagudah et al. 2006). The latter test revealed the presence of *Yr18* in several Avocet S NILs. Some of these were predicted (Yr18NIL), and others were explainable based on pedigree and gene source (Yr8NIL, Yr17NIL). However the origin of *Yr18* in Yr1NIL, Yr5NIL, Yr10NIL and YrSPNIL remains unclear. The csLV34 marker is currently being used to purify these sources and identify appropriate gene combinations involving *Yr18*.

Data collection and interpretation

The NIL sets were distributed for field nursery sowings at many locations where co-operators monitored the development of stripe rust on individual lines and variously reported results as infection type, leaf area affected according to the modified Cobb scale (Peterson et al. 1948) and combinations of both.

Table 1 Avocet S Near-isogenic Lines developed and released in three cohort

Gene	Source	NILs97	NILs 98	NILs 99
Yr1	Chinese 166	Yr1/6* Avocet S	Yr1/6* Avocet S	Yr1/6* Avocet S
Yr5	*T. spelta album*	Yr5/6* Avocet S	Yr5/6* Avocet S	Yr5/6* Avocet S
Yr6	Oxley		Yr6/6* Avocet S	Yr6/6* Avocet S
Yr7	Lee	Yr7/6* Avocet S	Yr7/6* Avocet S	Yr7/6* Avocet S
Yr8	Compair	Yr8/6* Avocet S	Yr8/6* Avocet S	Yr8/6* Avocet S
Yr9	Clement	Yr9/6* Avocet S	Yr9/6* Avocet S	Yr9/6* Avocet S
Yr10	Moro	Yr10/6* Avocet S	Yr10/6* Avocet S	Yr10/6* Avocet S
Yr15	*T. dicoccoides* (V763-251-wb)	Yr15/6* Avocet S	Yr15/6* Avocet S	Yr15/6* Avocet S
Yr17	Shortim/VPM1	Yr17/3* Avocet S	Yr17/6* Avocet S	Yr17/6* Avocet S
Yr18	Jupateco R		Yr18/3* Avocet S	Yr18/3* Avocet S
Yr24	Meering2*//K733/*T. tauschii* (CPI 18911)			Yr24/3* Avocet S
Yr26	*Haynaldia villosa* derivative (C94.153)			Yr26/3* Avocet S
Yr27	Opata 85		YrSk/3* Avocet S	YrSk/3* Avocet S
YrSP	Spaldings Prolific		YrSp/6* Avocet S	YrSp/6* Avocet S
		Avocet S	Avocet S	Avocet S
Supplemental Stocks				
Yr2		Kalyansona		
Yr18		Jupateco R	Jupateco R	Jupateco R
		Jupateco S	Jupateco S	Jupateco S

Results

Data returns were intermittent, depending on the nature of the sites chosen in any particular year. This was compounded by variation in environmental conditions and the presence of adequate inoculum. Communication difficulties also resulted in seed arriving too late for planting in some years, and occasionally failure to retain seed for the following season. Despite these circumstances, data was collected and reported from 21 countries in the five year period from 1998 to 2002.

The data collected for three NIL cohorts are presented in Tables 2, 3, and 4. The data across sites was examined in comparison to the response of Avocet S. Those lines showing evidence of virulence in the pathogen at a particular site (i.e. showing a disease response similar to Avocet S) are highlighted. Sites where Avocet S was poorly infected were not included in the analysis, resulting in 72 data sets. Pathogenic variation for specific genes or gene groups are considered below:

***Variation for** Yr1*

Virulence for *Yr1* was common in central Asia and China, and was considered to be characteristic of *Pst* populations east of the Caspian Sea during this period. These observations agreed with earlier data presented by Stubbs (1985). In contrast, virulence for *Yr1* was rare in the Middle East, and absent in west Asia and east Africa. The data showed some variation for *Yr1* virulence in the Indian subcontinent and in South America.

***Variation for** Yr2*

In the absence of a *Yr2* NIL, Kalyansona was used in some field plots in the 1997 NIL set (Table 1). Virulence was common in the Middle East, variable in China, and not recorded in east Africa and central America. Historically, the occurrence of virulence for *Yr2* in the 8156 cultivars (Kalyansona in India, Mexipak in Pakistan) traced from a reputed origin in Turkey in 1967 through to the Indian subcontinent by 1970 (Saari and Prescott 1985) aided by a weather system referred to as the 'western disturbance' (Nagarajan and Joshi 1978).

Table 2 *Responses of the 1997 Avocet S NIL set to Pst at various locations*

NIL	Turkey					Lebanon	Syria		India	China				Kenya	Uganda	Ecuador	Chile	Mexico
	1998	1998	1998	1999	1999	1999	1998	1999	1998	1998	1999	1999	1999	1998	1998	1998	1998	1999
	Adana	Izmir	Ankara	Haymana	Gissar	Terbol	T. Hadya	T. Hadya	Ludhiana	Sichuan	Xindu Sichuan	Chengdu Sichuan	Yaan Sichuan	Njoro	Kalingree	Santa Catalina	Carillanca	Toluca
Yr1 / 6* Avocet S	R	R	R	R	-	5MR	tR	R	60S	40S	65S	40S	100S	R	R	R	30MS	1S
Yr5 / 6* Avocet S	60	R,20	R	10R	10S	5MR	tR	R	R	R	R	R	R	10MR	1MR	R	R	R
Yr7 / 6* Avocet S	80	60	80S	80S	-	90S	95S	80S	40S	100S	20S	50S	40S	40MS	40S	70S	50MS	40MR-100S
Yr8 / 6* Avocet S	5	R	R	30MS	90	30S	30M	60MS	30S	-	20R	5R	5R	10M	5MS	R	R	R
Yr9 / 6* Avocet S	80	60	70S	70S	-	95S	95S	90S	R	R	100S	100S	100S	40S	40S	80S	90S	100S
Yr10 / 6* Avocet S	R	R	R	R	-	5MR	5R	R	R	R	R	R	R	10MR	1MR	R	R	R
Yr15 / 6* Avocet S	R	R	R	R	R	5MR	tR	R	-	R	R	R	R	R	1MR	R	R	R
Yr17 / 6* Avocet S	R	R	R	R		90S	30MS-S	70S	-	R	40S	10R	90S	10MR	1MS	R	40MS	1MR
Avocet S	100	100	90S	90S	80S	95S	95S	90S	80S	80S	-	100S	-	90S	30MS	90S	100S	100S
Avocet R (YrA)	100	100	90S	90S	R	95S	90S	80S	30S	100S	100S	100S	100S	80S	40S	90S	90S	100S
Jupateco R (Yr18)	40	20	50MS	60S	90	80S	80S	70MR	-	R	20S	R	5R	40S	R	30	10R	30M
Jupateco S	60	60	70S	80S	90	90S	85S	80MS	-	R	20S	5R	90S	50MSS	R	50	50MR	90M
Kalyansona	10	100	10MS	80S	90	90S	70S	80S	-	R	5R	10R	65S	30M	5MR	40M	20MS	40M

virulent avirulent

Table 3 ***Responses of the 1998 Avocet S NIL set to*** *Pst* ***at various locations***

NIL	Turkey	Lebanon	Syria		Iraq	Iran									
	1999	1999	1999	2000	1999	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
	Eskisehir	Terbol	Tel Hadya	Tel Hadya	Tuwaitha	Neishabur Khorasan	Torogh Khorasan	Jolgeh Rokh Korasan	Kalaleh Golestan	Agh Ghala Golestan	Gorgan Golestan	Ultan Ardabil	Jafar Abad Ardabil	Gharakhil Mazandaran	Alarogh Ardabil
Yr1 / 6* Avocet S	R	5MR	R	R	R	80S	60S	90S	R	R	R	tR	tR	R	R
Yr5 / 6* Avocet S	70S	5MR	R	R	R	R	R	R	R	R	R	tR	tR	5R	R
Yr6 / 6* Avocet S	-	-	80S	85S	100S	-	90S	-	-	-	80S	100S	-	-	-
Yr7 / 6* Avocet S	60S	90S	90S	80S	100S	70S	80S	80S	80S	20S	80S	90S	60S	20S	40S
Yr8 / 6* Avocet S	30S	30S	TMR	10MS	5R	R	R	70S	60S	R	R	10S	40S	R	30S
Yr9 / 6* Avocet S	50S	95S	90S	75S	100S	80S	50S	50S	100S	30S	70S	80S	100S	100S	70S
Yr10 / 6* Avocet S	R	5MR	R	R	R	R	R	R	R	R	R	20MR	20MR	R	10MS
Yr15 / 6* Avocet S	R	5MR	R	R	R	R	R	R	R	R	R	tR	t MR	5R	R
Yr17 / 6* Avocet S	10MR	90S	R	5MR	5R	30S	80S	40S	R	R	20S	tR	20S	10S	30S
Yr18 / 3* Avocet S	-	-	20MR	20MR	65S	-	-	-	-	-	-	-	-	-	-
YrSp / 6* Avocet S	-	-	R	R	R	-	R	-	-	-	R	tR	-	-	-
Yr27 / 3* Avocet S	-	-	20MR/MS	20MS	25MR	-	25S	-	-	-	R	20MR	-	-	-
Jupateco R (Yr18)	20MS	80S	-	30MS	_	20S	R	60S	50S	5R	5MS	30MR	30MR	40MS	30S
Jupateco S	20S	90S	-	50S	_	50S	60S	60S	100S	15S	40S	80S	100S	100S	20S
Avocet R (YrA)	90S	95S	90S	90S	100S	90S	70S	80S	100S	80S	60S	100S	100S	100S	70S
Avocet S	80S	95S	60S	80S	100S	80S	80S	90S	100S	20S	100S	100S	100S	100S	80S

virulent avirulent

Table 3 *(cont)*

NIL	Iran							Azerbaijan			Tajikistan	Kazakhstan	
	2001	2001	2001	2001	2001	2001	2001	2000	2000	2000	2000	1999	1999
	Golestan Gorgan	Gharakhil Mazandaran	Baye Kola Mazandaran	Pars Abad Ardabil	Aslandos Ardabil	Alarogh Ardabil	Mioandoab West Azar.	Absheron	Tartar	Jalilabad	Tursun	Almaty	Almaty 2
Yr1 / 6* Avocet S	tR	5MS	5MS	tR	tR	0-TMS	tR	R	R	R	50S	R	80
Yr5 / 6* Avocet S	tR	tR	tR	tR	tR	tR	tR	R	R	-	70S	R	R
Yr6 / 6* Avocet S	tR	90S	80S	70S	90S	20-50S	40S	80S	20S	60MS	5MR	40	80
Yr7 / 6* Avocet S	tR	90S	80S	80S	90S	30S	50S	90S	20S	100S	5R	40	80
Yr8 / 6* Avocet S	tR	tR	tR	5S	30MS	10S	tR	10MR	R	10R	100S	60	40
Yr9 / 6* Avocet S	20S	70S	60S	70S	70S	0-TMS	20S	60S	40S	5R	100S	10	40
Yr10 / 6* Avocet S	tR	tR	tR	tR	tR	tR	tR	10MR	R	5R	3MR	R	R
Yr15 / 6* Avocet S	tR	tR	5R	tR	5R	tR	tR	R	20S	-	5MR	tR	R
Yr17 / 6* Avocet S	tR	tR	tR	tR	20MS	tR	tR	20MS	R	20R	0	R	60
Yr18 / 3* Avocet S	tR	20MS	20MRS	tR	20MR	5MS-5S	30S	20MS	40S	-	0	5	60
YrSp / 6* Avocet S	tR	tR	tR	tR	tR	tR	tR	R	R	10R	0	R	R
Yr27 / 3* Avocet S	tR	tR	tR	tMS	20MR	tR	20S	10MS	R	10R	R	10	40
Jupateco R (Yr18)	5MR	tMR	tR	tMR	20MR	5-10MS	tR	20MS	60S	90S	R	_	_
Jupateco S	5MR	5MR	tRMR	40MS	60S	5MS	30S	50S	70S	100S	5MS	_	_
Avocet R (YrA)	100S	100S	80S	80S	100S	90S	80S	80S	60S	100S	5	80S	80S
Avocet S	50S	90S	60S	50MS	50S	20-50S	60S	70S	70S	20MR	40	60S	80S

virulent avirulent

Table 4 ***Responses of the 1999 NIL set to*** *Pst* ***at various locations***

NIL	UK	Spain			Romania	India		South Africa	New Zealand		Ecuador
	2001	2000	2001	2001	2001	2002	2002	2001	2000	2001	2001
	NIAB Cambridge	Cordoba	Jerez	Cordoba	Fundulea	Ludhiana	Bajaura	Greytown	Lincoln	Lincoln	Santa Catalina
Yr1 / 6* Avocet S	7S/MS	0	0	0	R	50S	0	R	60S	15SMS	60S
Yr5 / 6* Avocet S	R	0	0	0	R	R	0	tR	R	0	R
Yr6 / 6* Avocet S	15 MSS	10	10	87	30MSS	60S	60s	100S	50S	30S	90S
Yr7 / 6* Avocet S	12 MSS	65	60	87	70S	70S	70s	100S	90S	60S	90S
Yr8 / 6* Avocet S	R	20	0	30	10MR/MS	tR/20S	0	30R	60S	15MSS	R
Yr9 / 6* Avocet S	20 MSS	0	0	0	R	70S	70s	tR,60MS,90S	90S	15SMS	90S
Yr10 / 6* Avocet S	R	0	0	0	R	R	0	R	R	0	R
Yr15 / 6* Avocet S	R	0	0	0	R	R	0	R	R	0	R
Yr17 / 6* Avocet S	15 MSS	1	tr	40	R	tR/10S	0	90MR	40S	tS	10MS
Yr18 / 3* Avocet S	10 MSS	35	40	40	tR MSS	40S	30s	60MRMS	70S	60RMR	90S
Yr24 / 3* Avocet S	3 MSS	10	0	53	R	20S	20s	90MR	60S	20RMR	90S
Yr26 / 3* Avocet S	1 MSS	5	0	33	R	tR/20S	Ts-10s	tR, 70RMR	15MR	15RMR	70S
YrSp / 6* Avocet S	0.1 MSS	0	0	0	tR MR	R	0	R	20S	2S	10MS
Yr27 / 3* Avocet S	1 MSS	0	0	0	R	30S	Tr	15R	25MS	20SMS	50S
Jupateco R (Yr18)	10 MSS	10	20	47	5MR	40S	40S	15R	40S	20RMR	60S
Jupateco S	35 MSS	10	40	60	5MR/MS	80S	70S	90MR	90S	40RMR	90S
Avocet R (YrA)	7 MSS	0	0	7	5R/MR	80S	70S	10R	100S	20SMS	90S
Avocet S	20 MSS	60	80	86	60S	70S	80S	100S	100S	70S	90S

virulent avirulent

***Variation for** Yr5*

The resistance gene *Yr5* has not been deployed in commercial wheat varieties. Trap plot data suggests virulence for *Yr5* was rare, and recorded only from Turkey (two locations in two years), and single locations in China and Tajikistan. Virulence has also been recorded at very low frequency in Australia (Wellings and McIntosh 1990).

***Variation for** Yr6, Yr7 **and** YrA*

The data suggested that virulence for all three genes was very high across all sites and years. This agrees with earlier observations for *Yr6* and *Yr7* (Stubbs 1985) and in situations where the occurrence of virulence was detected shortly after commercial deployment of varieties carrying these three genes alone or in combination (Wellings and McIntosh 1990).

***Variation for** Yr8*

Stubbs (1985) reported that virulence for *Yr8* was common in the center of origin of *Aegilops comosa*, the original source of the gene. This region was considered to be the Aegean region of Greece and Turkey (Riley et al. 1968), and Stubbs proposed that virulence migrated from these regions to Europe and the Mediterranean. The data presented in Tables 2, 3 and 4 suggest that virulence occurred in Turkey and Syria, consistent with the proposal of Stubbs (1985), and further west including locations in Iran, central Asia (Tajikistan, Kazakhstan) and India. One location in New Zealand (Table 4) showed evidence of virulence for *Yr8* in 2000. Similar features for the occurrence of *Yr8* virulence were more recently reported in China (Wan et al. 2004), Syria (Yahyaoui et al. 2002) and South Africa (Boshoff et al. 2002).

***Variation for** Yr9*

Virulence for *Yr9* was widespread throughout the 1990s, encompassing regions from the Middle East to the Indian subcontinent (Singh et al. 2004). *Yr9* virulence remained a consistent feature of the *Pst* population in the Middle East (Turkey, Syria, Iraq, Iran, Azerbaijan), central Asia (Tajikistan, Kazakhstan), Indian subcontinent (India, Pakistan) and China (Tables 2,3 and 4). The majority of regions recorded virulence, with the only exception being the Republic of South Africa where the gene remained effective, consistent with the observations of Boshoff et al. (2002).

***Variation for** Yr17*

Yr17 resistance, derived from *Aegilops ventricosa,* was effective in areas such as Turkey, east Africa (Kenya, Uganda), Iraq and Ecuador. However, virulence was evidently present for this gene in the Middle East (Lebanon, Syria, Iran), central Asia (Kazakhstan), China and South Africa. This is consistent with reports from China (Wan et al. 2004) and South Africa (Boshoff et al. 2002); however, the occurrence of virulence for *Yr17* in the Middle East and central Asia was not reported previously.

***Variation for** Yr24 **and** Yr26*

The resistance genes *Yr24* and *Yr26* were initially designated as originating from different wheat relatives. Subsequent work has shown that these genes are identical and originated from durum wheat in the development of the respective lines (Li et al. 2006). Although these genes have not been deployed in commercial cultivars, other than in China, the first evidence of virulence for *Yr24* and *Yr26* was at the Ecuador site in 2001 (Table 4). Virulence was detected in a single isolate from Australia in 2006 (Wellings and McIntosh unpublished).

***Variation for** Yr27*

The *Yr27* resistance was effective in all locations during the period of evaluation, except Gansu (China) (Table 3) and Ecuador in 2001 (Table 4). This resistance is frequent in CIMMYT derived spring wheats (Wellings 1992) with virulence reported from the early 1970s in Near East, east Africa and the Indian subcontinent (Stubbs et al. 1974). Virulence for *Yr27* was reported in several pathotypes in China (Wan et al. 2004). Widely cultivated varieties in Iran based on Atilla (Cahmran *Yr27*; Shiroudi *Yr9, Yr27*) became susceptible in 2006-07 (Afshari and Nazari pers comm). In the same time period reports indicate virulence for *Yr27*, present in Inqualab 91 and PBW343 in India and Pakistan, with the suggestion that this pathotype was also detected in Central Asian (Kyrgyzstan, Tajikistan, Afghanistan) *Pst* populations (Duvellier et al. 2007).

***Variation for** Yr10, Yr15, Yr18, YrSP*

Avirulence for *Yr10*, *Yr15, YrSP* and *Yr18* was observed at all sites. However, the conclusion of avirulence in respect to *Yr18* has been controversial. The Bajaura (India, 1999; Table 3) and Ecuador sites (Table 4) indicated very high responses for Jupateco R in comparison with Jupateco S, although only the latter showed a correspondingly high response for the Yr18NIL. The data were collected late in the season, and in the case of the Ecuador site, may have reflected severe terminal disease reactions in a highly Pst-conducive environment.

Discussion

Relevant and effective pathogenicity surveys are important components of breeding programs aimed at incorporating resistance to obligate plant pathogens. Surveys monitor the distribution of current pathotypes, are directed at the early detection of new avirulence/virulence combinations of importance to agriculture, and if results can be related genetically to cultivar genotypes, contribute to decisions on cultivar recommendation. Surveys also allow the selection of isolates of known pathogenic profile for use in screening activities, and the accumulated historical data provide valuable insights into pathogen epidemiology and disease management.

The requirements for pathogenicity assessments that are reliable, low cost and less dependent on experienced observers with environmentally controlled facilities provided the momentum to re-examine the materials and methods of field-sown differential nurseries. Although this concept was introduced for *Pst* studies in western Europe by Zadoks in the 1960s, there has been limited practical success due to difficulties associated with the availability of suitable materials of known genotype for disease and pathogenicity monitoring. Phenological variation among the traditional differential sets used in seedling- based pathotype assays, and the confounding influence of additional uncharacterized adult plant resistances, resulted in contentious results that could not easily be reconciled with seedling tests. The Avocet S NILs overcame some of these constraints and provided helpful, and in some instances unique, insights into the nature of the *Pst* population across a broad range of locations. The value of this information can be assessed according to the following criteria.

Predicting disease response

Efforts aimed at monitoring pathogenicity characteristics in the *Pst* population in major regions vulnerable to epidemics are able to assist in the development of control strategies through predicting and recommending cultivars with resistances known to be effective against current *Pst* pathotypes. For example, Inqualab 91 (*Yr27*) was predicted on the basis of the data from this project to protect against the advancing *Yr9*-virulent pathotype in Pakistan, although it subsequently became ineffective with the occurrence of *Yr27* virulence in 2002 (Duvellier et al. 2007).

Breeding for resistance

Pathogen survey data should assume an integral role in the research and selection methods adopted within regional breeding programs, i.e. the survey should retain the most appropriate pathotypes for use in screening and for detection of new sources of resistance. Where pathotype isolates cannot be maintained, the project should identify the locations most likely to represent a conducive environment combined with a regionally appropriate spectrum of *Pst* avirulence/virulence. An example encountered was the evident use of relatively avirulent pathotypes in selection nurseries in the main breeding center in Iran during the 2000 season, when pathotypes virulent for *Yr9* were evidently causing problems in other areas of the country. This situation subsequently changed and Iranian breeders and pathologists now select for resistance in the presence of the most virulent pathotypes.

Recognition of new pathotype introductions

The occurrence of *Pst* in regions clearly conducive to epidemic development, but previously free of the disease, has resulted in significant national issues over the past 20 years. This progressive extension of the geographical distribution of *P. striiformis* has served to highlight the increased potential for foreign pathotype incursions. Notable examples include:

(i) Barley stripe rust (*P. striiformis* f. sp. *hordei*) in Central America in 1975 (Dubin and Stubbs 1986) and subsequently North America in 1991 (Marshall and Sutton 1995); it was considered an introduction from Europe.

(ii) Wheat stripe rust in Australia in 1979 was attributed to a single pathotype introduced from Europe (Wellings 2007).

(iii) Wheat stripe rust in South Africa was first reported in 1996 (Pretorius et al. 1997).

(iv) *P. striiformis* isolates from barley grasses (*Hordeum* spp.) in 1998 were shown to be highly avirulent on *Pst* differentials and concluded to be a new introduction to Australia (Wellings et al. 2000b).

(v) New pathotypes of *Pst* in North America from 2000 (Chen 2005); origins remain unknown.

(vi) The occurrence of *Pst* in Western Australia for the first time in 2002 was concluded to be due to a foreign pathotype incursion, and suspected to have originated from the *Pst* population introduced to North America in 2000 (Wellings et al. 2003).

It has been argued that pathogenicity surveys will be less effective in situations where the resistance genes being monitored do not include those deployed in commercial agriculture. However the incorporation of certain genes such as *Yr8*, which has never been deployed in wheat cultivars, may be useful in the broader aims of pathogenicity surveys. In this case, virulence for *Yr8* has been a feature of certain west Asian and Middle Eastern pathotypes and it was this character that provided some evidence for the suspected origin of the South African incursion in 1996 (Pretorius pers comm).

The presence of exotic pathotypes in new geographical regions can only be determined on the basis of a detailed knowledge of pathogen populations contemporary to those particular regions. The availability of this background data can be critical in allowing a basis for predicting the impact of such introductions, and to determine the best response strategies, such as importing cultivars with appropriate resistances and introducing diverse resistance sources into breeding programs.

Despite the advantages of the Avocet S NILs, caution must also be noted in the inherent difficulties associated with these materials. The opportunities for error in seed maintenance will be increased with the expected uniformity among closely related NILs. However, certain diagnostic characters such as brown chaff associated with *Yr10*, leaf tip necrosis with *Yr18* and the late maturity of the Yr8NIL will be useful in distinguishing among lines. These difficulties will continue to occur, but will be minimized through awareness of the possible problems and continued international testing. An additional limitation is their inability to detect pathotype mixtures at a single nursery location. Current efforts are being directed to establish appropriate two-gene combinations that will allow partial resolution of pathotype mixtures.

The current data successfully established the value and role of a NIL set of materials in monitoring pathogenicity characteristics of regional *Pst* populations. Several attempts have been made to develop NIL sets incorporating genes for resistance to *Pst*. Partial sets have been constructed in the susceptible genotypes Lemhi (facultative USA cultivar used in UK studies – Johnson pers comm) and Taichung 29 (spring type originating in China and developed in The Netherlands – van Silfhout pers comm). However, these particular attempts were abandoned due to difficulties with certain key pathotypes that were avirulent on the recurrent parents. NIL sets have also been developed in China, based on the susceptible winter type Mingxian 169, and the susceptible spring wheat Taichung 29 (SC Xu and WQ Chen pers comm). The Avocet NILs represent a modest but useful development in extending the methodology of pathogen monitoring. Certain virulence factors in the pathogen population detected in this work were not previously reported.

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13. Progress and prospects in discovery and use of novel sources of stem rust resistance

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Abstract

A number of stem rust resistance genes derived from wild relatives of wheat appeared to be more effective against race TTKSK (Ug99) of *Puccinia graminis* f. sp. *tritici* than *Sr* genes of wheat origin. In an attempt to identify sources of stem rust resistance genes effective against TTKSK, we evaluated several cultivated and wild relatives of wheat for resistance to TTKSK and other stem rust races with broad virulence in seedling tests. Preliminary results indicated that TTKSK resistance could readily be found, but frequencies of resistance varied among the species. *Aegilops speltoides* had the highest frequency of resistance (nearly 100%). Other species having high frequencies of TTKSK resistance included triticale (77.7% of 567 accessions), *Triticum urartu* (96.8% of 205 accessions), and *T. monococcum* (61% of 1020 accessions). Frequencies of TTKSK resistance in other species were: 14.7% in *Ae. tauschii* (456 accessions), 15% in *T. timopheevii* (298 accessions), and 17% in *T. turgidum* ssp. *dicoccoides* (157 accessions). Based on specific infection types to several races, we postulated that novel genes for resistance to TTKSK are present in some of these species. Accessions with putatively new resistance genes were selected to develop crosses for introgressing resistance into wheat and for developing mapping populations.

Keywords

Puccinia graminis f. sp. *tritici, Triticum urartu, T. monococcum, T. timopheevii, T. turgidum* ssp. *dicoccoides, Aegilops speltoides, Ae. tauschii, X Triticosecale*

Introduction

A number of stem rust resistance genes derived from wild relatives of wheat appeared to be more effective against race TTKSK (or Ug99) of *Puccinia graminis* f. sp. *tritici* than *Sr* genes of wheat origin (Jin et al. 2007). In an attempt to identify novel sources of stem rust resistance genes that are effective against TTKSK, we evaluated several cultivated and wild relatives of wheat for resistance to TTKSK and other stem rust races with broad virulence in seedling tests.

Materials and methods

Accessions of several cultivated and wild relatives of wheat were evaluated for reactions to isolates of races TTKSK, TTTTF and TRTTF of *P. graminis* f. sp. *tritici* in seedling tests. Races QFCSC and MCCFC were also used in testing selected species. Race identities, origins, and avirulence/virulence formulae of the isolates used in the study are given in Table 1. Experimental procedures in inoculation and disease assessment were reported previously (Jin et al. 2007).

Results and discussion

Triticum urartu Nearly all of the *T. urartu* accessions (96.8% of the 205 accessions) screened from the USDA National Small Grains Collection (NSGC) were resistant to race TTKSK, giving low infection types (IT) 2- to 2. Twenty-eight percent of the accessions were resistant to race MCCFC. Resistance to races TTTTF, TRTTF or QFCSC was not observed. Resistance to TTKSK was specific; thus, the gene(s) from this species may have limited value for offering broad resistance to other stem rust races.

T. monococcum A total of 1,020 accessions of *T. monococcum* from the USDA NSGC were evaluated against races TTKSK, TTTTF, TRTTF, MCCFC and QFCSC. Sixty-one percent of the accessions were resistant to TTKSK with low ITs ranging from 0 to 2+, and 6.6% were

Table 1. Isolates, race identities, origin, and avirulence/virulence formulae of Puccinia graminis f. sp. tritici isolates used in seedling tests

Isolate	Race	Origin	Avirulence/ virulence formula
04KEN156/04	TTKSK	Kenya	*Sr24 36 Tmp/ 5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 31 38 McN*
01MN89A-1-2	TTTTF	USA	*Sr24 31/ 5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 Tmp McN*
06YEM34-1	TRTTF	Yemen	*Sr8a 24 31/ 5 6 7b 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 Tmp McN*
06ND76C	QFCSC	USA	Sr*6 7b 9b 9e 11 24 30 31 36 38 Tmp/ 5 8a 9a 9d 9g 10 17 21 McN*
59KS19	MCCFC	USA	Sr6 8a 9a 9b 9d 9e 11 21 24 30 31 36 38/ 5 7b 9g 10 17 Tmp McN

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Table 2. Frequencies of triticale accessions resistant, susceptible, and heterogeneous for reaction to Puccinia graminis f. sp. tritici races TTKSK, TRTTF and TTTTF

	TTKSK no. access.	%	TRTT no. access.	%	TTTTF no. access.	%
Resistant	440	77.7	405	71.8	434	76.8
Susceptible	75	13.3	97	17.2	85	15.0
Heterogeneous[1]	51	9.0	62	11.0	46	8.2

[1]Accessions including both resistant and susceptible plants

resistant to all five races. Based on the reactions to the five races, there appeared to be novel resistance to TTKSK in *T. monococcum*. Additional studies are needed to confirm gene postulations and to determine the number and allelic relationships of the potentially new genes.

Aegilops tauschii A total of 456 accessions of *Ae. tauschii* from the USDA NSGC and Wheat Genetic and Genomic Resources Center (Manhattan, KS) were evaluated for resistance to races TTKSK, TRTTF, and TTTTF. Sixty-five accessions (14.7%) were resistant to TTKSK with ITs ranging from ; to 2+, and eight accessions (1.8%) were resistant to all races. Based upon the different infection types to various races, we postulated that one or more novel genes for resistance to race TTKSK is present in this species. Selected accessions are being backcrossed to hexaploid wheat to introgress the resistances to race TTKSK.

Ae. speltoides Ninety-two accessions from the USDA NSGC were evaluated and all accessions were resistant to race TTKSK. One plant from a heterogeneous accession was susceptible with IT 3. Most of the accessions exhibited ITs from 0 to ;1. The frequencies for resistance to races TTTTF (96%) and TRTTF (98%) also were high. Although this species offers a high frequency of TTKSK resistance, it will be difficult to differentiate novel resistance from resistance genes already transferred to common wheat, such as *Sr32, Sr39* and *Sr47*.

T. timopheevii A collection of 298 accessions from the USDA NSGC were evaluated, and 15% were resistant to race TTKSK with ITs ranging from 0 to ;12+. Resistance to races TTTTF and TRTTF was not detected among these accessions. Accessions resistant to TTKSK were mostly from *T. timopheevii* ssp. *timopheevii*, with infection types predominantly 0 to 0;. The low ITs (0 or 0;) to race TTKSK in combination with susceptibility to races TRTTF and TTTTF suggested that the majority of resistant accessions have *Sr36*. Accession CI 11802, which was regarded as the standard for *Sr37* (McIntosh et al. 1985), produced IT ;1 to TTKSK, and 3 and 4 to TRTTF and TTTTF, respectively. Five accessions of *T. timopheevii* ssp. *timopheevii* produced a similar pattern, with ITs ranging from ;1 to ;12+. Three accessions of *T. timopheevii* ssp. *armeniacum* showed resistance to TTKSK, with ITs ;1 and ;12. The relationship between resistance in this germplasm and *Sr40* is uncertain at this time.

T. turgidum* ssp. *dicoccoides A collection of 157 accessions from the USDA NSGC was evaluated for resistance to races TTKSK, TRTTF and TTTTF. There was a low frequency of resistance to the three races: 16.6% to TTKSK, 8.9% to TRTTF, and 11.5% to TTTTF. Low ITs were variable, ranging from 0; to X with the majority being 2 to 2+. Three accessions were resistant to both TTKSK and TRTTF, one accession was resistant to TTKSK and TTTTF, and two accessions were resistant to all three races.

X Triticosecale A collection of 567 triticale accessions from the USDA NSGC was evaluated for resistance to races TTKSK, TRTTF and TTTTF. There was a high frequency of resistance to race TTKSK; 440 accessions (77.7%) exhibited low ITs ranging from 0; to 2+ (Table 2). Based on the ITs we postulated a number of resistance genes, viz. *Sr27* (IT ;2= to ;12), *SrSatu* (IT ;CN), *SrVen* (IT ;13) or *SrNin* (IT 22-) (McIntosh et al. 1985). ITs different from those conferred by known genes were also observed, suggesting the presence of novel resistance genes or unique combinations of known genes. Screening of the resistant lines with *P. graminis* f. sp. *tritici* races virulent on known triticale genes available in Australia and South Africa will help to facilitate the identification of any new resistance genes. Resistance to the three races was highly associated as the majority (87%) of the accessions, that were resistant to TTKSK, were also resistant to races TTTTF and TRTTF.

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14. Cytogenetic manipulation to enhance the utility of alien resistance genes

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Abstract

Although many wild relatives in the Triticeae tribe have been exploited to transfer stem rust resistance genes to wheat, the derived germplasms have often not been immediately useful in wheat breeding programs. Too frequently, large chromosome segments surrounding desirable genes also harbor deleterious genes that result in unacceptable yield or quality. Recombination between chromosomes of wheat and chromosomes of distant relatives is very rare due to genetic restrictions on chromosome pairing in polyploid wheat. However, chromosome pairing can be manipulated by utilizing mutant stocks that relax this tight genetic control. The *ph1b* mutant produced by E.R. Sears over 30 years ago is an invaluable chromosome engineering tool, readily employed in the age of high-throughput molecular genetics. Shortened translocations have already been produced for stem rust resistance genes *Sr26* and *SrR* using *ph1b*-induced homoeologous recombination. We are currently using induced-homoeologous recombination to reduce the sizes of alien chromosome segments surrounding TTKSK-effective genes *Sr32*, *Sr37*, *Sr39*, *Sr40*, *Sr43*, *Sr47*, *SrTt3, Sr2S#1* and *SrAeg5* to eliminate linkage drag putatively associated with these genes. Additional TTKSK-effective genes *Sr44, SrHv6, SrAsp5,* and *SrAse3* were first targeted for development of compensating translocation stocks and then for shortening the size of each alien segment. Population development is also underway to characterize several potentially new sources of resistance.

Keywords

Stem rust, wheat, Ug99, translocations

Introduction

The Triticeae relatives of cultivated wheat are valuable sources of genes for wheat improvement. Genes from close relatives, or homologous genomes, are readily deployed in elite germplasm due to more "normal" recombination rates, which provide a mechanism to recombine chromosomes and select superior germplasm. However, a delicate balance must be achieved between desirable target traits and undesirable "wild" genes or chromosome regions when transferring genes from more diverged, or homoeologous genomes. Only a handful of large translocation chromosomes have been successfully used in global wheat production, such as T1BL·1RS or T1AL·1RS (Villareal et al. 1995). Despite persistent breeding attempts, the majority of large translocations have not been exploited in agriculture.

Chromosome pairing in wheat is restricted to strict homologs by genetic control, preventing recombination among homoeologous chromosomes in the polyploid nucleus. This control maintains the stability of the polyploid genome, resulting in diploid-like pairing of 21 bivalents in metaphase I of meiosis for hexaploid wheat, and 14 bivalents in tetraploid wheat. Pairing homoeologous loci *Ph1* and *Ph2* (Riley and Chapman 1958; Mello-Sampayo 1971; Sears 1977), located on the long arm of chromosome 5B and the short arm of chromosome 3D, respectively, are primarily responsible for suppressing homoeologous recombination. While crucial to maintaining stability of the wheat genome, the *Ph* genes present an obstacle when transferring agronomically important traits from diverged genomes. Sears (1977) produced a deletion mutant of the *Ph1* locus, *ph1b*, in a Chinese Spring (CS) background that enables straightforward transfer of desirable genes from homoeologous genomes. In the absence of *Ph1*, chromosome pairing is enhanced between wheat chromosomes and alien homoeologs and recombinants may be recovered.

Even with *ph1b* stocks, the limiting steps in alien gene transfer have long been the ability to screen and detect desired genotypes for population development and to select recombinant progeny, constrained by the laborious and highly-technical nature of the required cytogenetic techniques. Fortunately, advances in DNA marker development and application have allowed a shift from cytogenetic observations to molecular genetic screening. Homozygous *ph1b* genotypes can be selected by DNA markers (Roberts et al. 1999), simplifying population development. Although many alien donor species have poor to non-existent molecular marker resources, and wheat microsatellite (SSR) markers are rarely transferable, PCR-based markers sufficient for detecting target alien translocations can be routinely developed from mapped expressed sequence tags (ESTs) (Qi et al. 2007).

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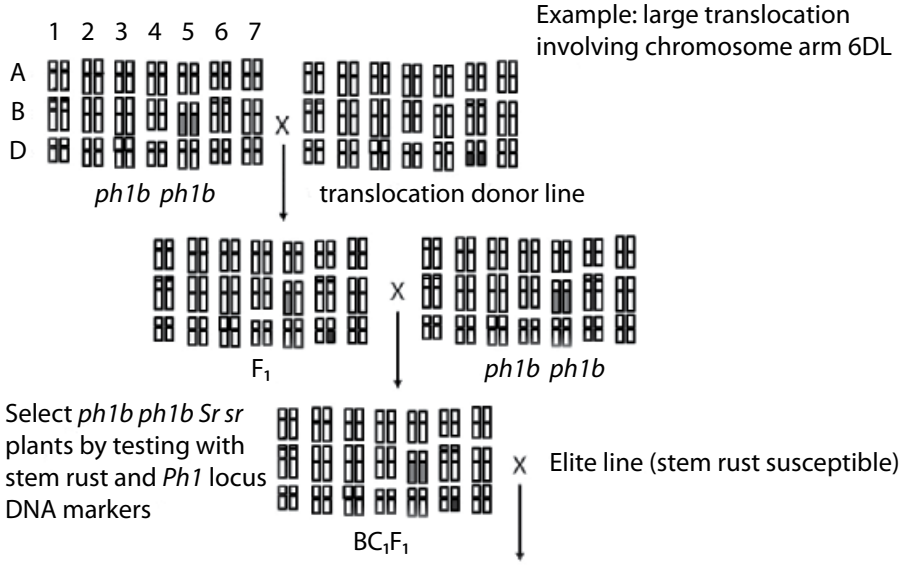
Fig. 1 Population development and screening strategy to reduce the size of an alien translocation with a desirable stem rust resistance gene. A hypothetical whole-chromosome arm translocation replacing 6DL is presented (shaded by dark gray). After crossing and backcrossing, a large translocation donor line with the *ph1b* mutant stocks (*ph1b* mutation indicated by light gray shading), progeny are selected that are homozygous *ph1b ph1b* and hemizygous for the target translocation and homoeologous wheat chromosome. Recombination between wheat chromosomes and homoeologous translocations is enhanced in these selected plants and they are crossed to an elite wheat line. The resulting progeny are then screened for the resistance gene by rust phenotyping and for shortened alien segments using one or more DNA markers that tag the translocation chromosome. Plants with shorter alien segments should lack specific alien markers while retaining rust resistance.



Screen 100s-1000s of progeny with stem rust and translocation-specific DNA markers to identify recombinants

Our goal is to make useful to variety development programs, effective stem rust resistance genes derived from wild species. Numerous stem rust resistance genes (*Sr)* from wheat relatives, such as *Aegilops speltoides* Tausch*, Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey*, Triticum timopheevii* (Zhuk.) Zhuk., and *Secale cereale* L., have been incorporated into wheat genomes in the form of chromosome translocations. More than a dozen of these genes are effective against race TTKSK (Ug99) and related derivatives (Singh et al. 2006; Jin et al. 2007). Unfortunately, most of them are associated with deleterious linkage drag. Reducing the amount of alien chromatin increases the likelihood of a translocation having commercial value. Their manipulation and use is important given the overarching goal of long-lasting rust protection in wheat crops worldwide, particularly when two or more broadly effective genes are pyramided and/or combined with minor-gene resistance in a single cultivar.

The specific gene targets of this project are *Sr32*, *Sr37*, *Sr39*, *Sr40*, *Sr43*, *Sr44, Sr47*, *SrAeg5, SrAsp5, SrAse3, SrHv6, SrTt3,* and *Sr2S#1*. Traditional hybridization and chromosome manipulation methods are coupled with DNA marker development, stem rust phenotyping, and genotyping of large populations to identify recombinant progeny with smaller translocations. After reducing the amount of alien chromatin, the *Sr* genes are transferred to elite wheat germplasm adapted to Africa and/or Asia. The following summaries document progress in our ongoing chromosome engineering efforts on each alien-derived stem rust resistance gene.

Approaches to induce homoeologous recombination

The preferred method for reducing the size of alien segments is to employ the *ph1b* mutant of hexaploid wheat. In this approach, either F_2 or BC_1F_1 populations are firstly produced from crosses between the translocation lines and *ph1b* mutant stocks. DNA markers that detect the *ph1b* mutation, and stem rust phenotypic screening, are applied to progeny to identify individual plants homozygous *ph1b ph1b* and hemizygous for the target translocation and homoeologous wheat chromosomes (Fig. 1). Populations developed from the selected plants are then screened for stem rust resistance and genotyped for translocation-specific marker alleles to identify putative recombinant progeny. Fluorescent genomic *in situ* hybridization (GISH) is then applied on putative recombinants to confirm shorter translocation lines carrying each *Sr* gene.

The crossing and screening procedure for tetraploid wheat is similar to that for hexaploid wheat, but homoeologous pairing is induced in durum 5D(5B) substitution lines lacking *Ph1*, rather than using *ph1b* or *ph1c* mutants. Durum lines 'Rusty' and '47-1' are widely susceptible to stem rust races and provide good backgrounds to investigate genetics of stem rust resistance (Klindworth et al. 2006).

Chromosome engineering of cataloged *Sr* genes

Sr26. The University of Adelaide has developed several lines with shortened alien segments carrying *Sr26* (Dundas et al. 2007). Dundas and Shepherd (1994; 1996; 1998) described the isolation of nine plants identified with 6Ae#1 chromosome segments of reduced size compared to that in Australian cv. Eagle. Seven carried *Sr26* (viz. WA1, WA2, WA5, WA6, WA8, WA9 and WA12) and two (WA7 and WA11) did not (Dundas et al. 2007). *Sr26* was localized to the extreme distal portion of chromosome 6Ae#1 and was closely linked to loci *Xmwg573-6Ae#1*, *Xmwg798-6Ae#1*, and *Xmwg2053-6Ae#1*. Lines WA1, WA5, WA6 and WA9 were provided to wheat breeding programs in Australia and the USA, and are currently in advanced stages of backcrossing to Australian cultivars. A simple PCR-based marker is available for selection of *Sr26* on shortened segments (Mago et al. 2005).

Sr32. Fifteen lines carrying modified segments of the *Ae. speltoides* 2S#1 chromosome were selected from 97 putative recombinants showing dissociation of chromosome 2S#1-specific markers in a *ph1b ph1b* genotype (Dundas et al. 2007). These were derived from the original T2DL-2S#1L·2S#1S translocation produced by E.R. Sears. Eleven were stem rust resistant; two lines carry *Sr32* on the short arm of the 2S#1 chromosome (lines 2S#1/ 102 and 2S#1/ 122a). In situ hybridization studies on these lines confirmed that the original 2S#1 segment was altered in structure. Lines carrying *Sr32* were resistant to the east African pathotypes TTKSK (Ug99), TTKST, and TTTSK and Yemani pathotypes TRTT at the USDA-ARS Cereal Disease Laboratory (Table 1). Both lines have a largely cv. Angas background. All resistant lines were initially backcrossed to Angas, and are currently undergoing backcrossing with Australian cv. Westonia and Indian cv. HUW234. Crosses with Westonia are at the BC_2 stage.

Sr37. The translocation line W3563 carrying *Sr37* on chromosome 4G from *T. timopheevi* (2n=4x=28, A^tA^tGG) was originally developed by McIntosh and Gyarfas (1971). W3563 has a 4B/4G chromosome translocation (Friebe et al. 1996) and is resistant to TTKSK (Jin et al. 2007) and certain North American races. A total of 17 representative lines with confirmed shortened *T. timopheevii* chromosome 4G#1 segments were selected from about 50 initial lines after screening for dissociation of *Sr37* and 4G#1 markers (Dundas et al. 2007) in a *ph1b ph1b* genotype. Three lines showing modified *T. timopheevii* 4G#1 chromosome segments derived from the 4B-4G#1 chromosome were backcrossed to cv. Angas and are currently undergoing backcrossing to Westonia and HUW234. Lines 4G#1/ 327, 4G#1/ 361 and 4G#1/ 376 were selected from others on the basis of fertility and growth habit.

Sr39. Stem rust resistance gene *Sr39* was transferred to hexaploid wheat cv. Thatcher (Tc) from *Ae. speltoides* by Kerber and Dyck (1990). Seven lines with shortened *Ae. speltoides* chromosome segments were produced after screening for dissociation of 2S#2 RFLP markers in chromosome T2BL-2S#2L·2S#2S-2BS (Kerber and Dyck 1990) in a *ph1b ph1b* genotype. Stem rust resistant lines (+*Sr39*) 2S#2/ 163, 2S#2/ 220 and 2S#2/ 247 showed obvious structural alterations relative to the *Ae. speltoides* chromosome after in situ hybridization. All of these lines and 2S#2/ 151 (+*Sr39*) were backcrossed to cv. Angas and are currently being backcrossed to Westonia and HUW234. Lines 2S#2/ 151 and 2S#2/ 247 are at the BC_2 stage with Westonia.

Sr40. Eleven plants were confirmed by progeny testing to carry shortened segments of the *T. timopheevii* 2G#2 chromosome derived from the T2BL-2G#2S translocation chromosome (Dyck 1992). Six of these are resistant to stem rust, but only lines 2G#2/ 286, 2G#2/ 300, 2G#2/ 301 and 2G#2/ 305 showed adequate fertility and vigor in field plots. These four lines are undergoing backcrossing with Westonia and HUW234.

Sr43. *Sr43* was transferred from *Th. elongatum* (Host) D.R. Dewey to wheat chromosome 7D using *ph1b*-induced homoeologous recombination (Knott et al.1977; Kibirige-Sebunya and Knott 1983). Three stocks with *Sr43*, viz. KS10-2, KS24-1, and LMq6-28-1a, were obtained from D.R. Knott, Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Two lines, KS10-2 and KS24-1, were chosen for this study. The translocation chromosomes in KS10-2 and KS24-1 were identified as T7DL-7Ae#2L·7Ae#2S and T7DS·7Ae#2L, respectively (Kim et al. 1993; McIntosh et al. 2008). The results from FGISH analysis showed that the long arm and about 50% of short arm of the interchanged chromosome in KS10-2 came from *Th. elongatum*, and only the short arm of the interchanged chromosome in KS24-1 was from *Th. elongatum*. The TTKSK infection type for KS10-2 was fleck, but for KS24-1 was ;1 in our testing (Xu et al. 2009). These two lines were crossed and backcrossed to the CS *ph1b* mutant. Over 2,500 hybrid seeds for each translocation line were produced from crosses of resistant BC_1F_1 plants with

CS. Eighteen specific SSR markers associated with alien chromatin were developed. Hybrid seeds consisting of *ph1b*-induced homoeologous recombinants are ready to be screened with molecular markers and stem rust inoculations with TTKSK.

Sr44. *Sr44* is currently available on a non-compensating translocation chromosome (T7DS-7Ai#1L·7Ai#1S) that is not useful for wheat breeding or amendable to directed chromosome manipulations (Friebe et al. 1996). We are first producing new compensating translocation stocks. The original 7Ai#1 disomic addition line (Vilmorin 27-DA 7Ai#1; Cauderon et al. 1973) was crossed to a CS plant monosomic for chromosome 7D (CS M7D). Double monosomic progeny of a CS M7D/Vilmorin 27-DA 7Ai#1 population are expected to produce compensating centromeric translocation lines among the F_2 progeny. A combination of molecular marker screening, cytology, and stem rust response screening was applied to approximately 300 F_2 plants to identify compensating T7DL·7Ai#1S Robertsonian translocations with *Sr44*. Four progeny are potential candidates at this time, based on the presence of *Sr44* and 7Ai#1S DNA marker alleles, and the absence of 7Ai#1L DNA marker alleles. These progeny will be characterized by GISH to rule out the possibility of telosomic chromosomes. Once produced, the compensating translocation stock(s) will be crossed and backcrossed to *ph1b ph1b* genotypes to initiate reduction in the size of this alien segment.

Sr47. *Sr47* was described by Faris et al. (2008). The gene is carried in the tetraploid stock DAS15, which has a background of line 47-1, a stem rust susceptible durum. The gene originated from *Ae. speltoides* and is carried in a T2BL-2SL·2SS translocation chromosome. The translocation in DAS15 was characterized using FGISH by Faris et al. (2008). Line DAS15 was reported as having IT 2= to Ug99, and in subsequent tests was shown to be resistant to Ug99 variants. To reduce linkage drag associated with *Sr47*, DAS15 was crossed to Rusty 5D(5B) and the F_1 crossed to 47-1 5D(5B). These F_1 plants will be crossed to Rusty in 2009. DAS15 was crossed to CS *ph1b*, and two backcrosses to CS *ph1b* have been completed in an attempt to transfer *Sr47* to hexaploid wheat. SSR markers additional to those identified by Faris et al. (2008) are presently being sought for use in shortening the alien segment in this line.

Chromosome engineering of tentatively designated Sr genes

2S#1. Of 11 stem rust resistant recombinants derived from the original T2DL-2S#1L·2S#1S stock with *Sr32*, nine had a second gene (temporarily named *Sr2S#1*) on the long arm of the 2S#1 chromosome (lines 2S#1/ 44, 2S#1/ 45, 2S#1/ 52, 2S#1/ 70, 2S#1/ 122b, 2S#1/ 122c, 2S#1/ 142, 2S#1/ 145b and 2S#1/ 287). Line 2S#1/ 45 is stem rust resistant but carries a telocentric chromosome of the long arm of the original T2DL-2S#1L·2S#1S chromosome. This evidence confirms that the *Sr2S#1* gene is located on the long arm of the original translocation chromosome. Because it has a telocentric chromosome, line 2S#1/ 45 will not be suitable for agricultural use.

SrR. The University of Adelaide has developed several new lines with shortened alien segments carrying *SrR* derived from Imperial rye. Koebner and Shepherd (1986a, b) induced homoeologous recombination between rye chromosome arm 1RS of the translocation line 1DL·1RS and the short arm of homoeologous wheat chromosome 1D in an attempt to break the linkage between *SrR* and the secalin gene. Intercrossing two of these primary recombinants resulted in the secondary recombinant DRA-1 with an interstitial rye segment carrying *SrR* and *Sec-1* (Koebner and Shepherd 1988; Rogowsky et al. 1991, 1993). Anugrahwati et al. (2008) produced the tertiary recombinant T6-1 derived from DRA-1 with *SrR* and lacking *Sec-1*. T6-1 is currently undergoing backcrossing in Australian wheat breeding programs.

SrTt3. This *T. timopheevii*-derived *Sr* gene, linked to *Sr36*, is located on chromosome 2G#3, (McIntosh et al. 1995). The translocation line AH (McIntosh et al. 2005) was crossed with Angas *ph1b ph1b*. A population of 100 F_3 plants derived from F_2 *ph1b ph1b* genotypes heterozygous for chromosomes 2B and 2B-2G#3 was screened for dissociation of 2G#3 markers generated with probes ABC252 and ABG58. Putative recombinants are now undergoing progeny-testing to confirm the marker patterns.

SrAge5. Screening a set of *Ae. geniculata* Roth addition lines in a CS background (*Ae. geniculata* donor accession TA2899) revealed that TA7659, a disomic addition line with $5M^g$ (21"+1" $5M^g$#1), was resistant to a composite stem rust infection; TA7670, a ditelosomic addition line with the short arm of $5M^g$ (21"+t"$5M^g$#1S), was susceptible; a long arm ditelosomic addition line was not available. These results prompted us to test existing $5M^g$ translocation stocks for stem rust resistance (Kuraparthy et al. 2007). Although the *Ae. geniculata* donor accession, TA10437, used to develop the *Lr57/ Yr40* transfers (Kuraparthy et al. 2007) was a different accession, the line TA5599 (T$5M^g$S·$5M^g$L-5DL) in a WL711 background was resistant, whereas TA5602 [T5DL·5DS-$5M^g$S (0.95)] and WL711 were susceptible. The infection types were similar between the TA7659 and TA5599 and both showed low infection types against TTKSK. To further characterize these materials, a population

Table 1 Infection types of wheat lines carrying SrR (rye), Sr32 or Sr2S#1 (Ae. speltoides), chromosome segments of 2S#3 (Ae. speltoides AEG357-4), 2S#4 (CS/ Ae. speltoides TA8026) or 2S#5 (CS/ Ae. speltoides TS01) against four exotic pathotypes (Yue Jin unpublished 2009). The SrR line and some 2S#4 lines were known to be segregating for the alien chromosome

	TTKSK	TTKSK	TTKST	TTTSK	TRTT
Line	04KEN156/04	04KEN156/04	06KEN19v3	07KEN24-4	06YEM34-1
SrR^{+} Sec^{-}	2-,4	;2-	2	;2-	;
2S#1/ 102 (+*Sr32*)	2-	;2-	;2-	;2-	2-
2S#1/ 122a (+*Sr32*)	2-;,3+	;2-,4	;2-	;2-,3	2
2S#1/ 122b (+*Sr2S#1*)	2-;	;2-,2	;2-	;,4	2-
2S#1/ 122c (*Sr2S#1*)	12+	23-	23-,3+	23-,;	-
2S#3 recomb #3	2+	22+	2	2	2
2S#3 recomb #16	;2--	2-	;2-	;2-	2-
2S#3 recomb #20	;2-	-	-	-	-
2S#3 recomb #27	2	2	2-	2-	2
2S#3 recomb #79	2	;2-	2	;2-	2
2S#4	;2,2+,3	2;,3	;2-,22+,23	;,23,4	22+
2S#4	2-;	;2-	;2-	0	2
2S#4 recomb 25	;,;N	;1	;1	;1	22+
2S#5	;,2-,2+	;	;	;	2-
2S#5	;1	;	;,2-	;,;2-	2
Westonia	4	4	4	4	4
Angas	2++3	3	3+	3+	2+

was developed by crossing TA5599 and TA5602. The F_3 families were evaluated for stem rust resistance by inoculation with race RKQQ at the two-leaf stage. Transmission of T5M^{g}S·5M^{g}L-5DL (23 homozygous resistant: 80 segregating: 44 homozygous susceptible; $chi^2_{1:2:1} = 7.12$; $P<0.05$) was significantly reduced, but *SrAge5* appeared to segregate as a single gene because DNA markers tagging 5M^{g}L co-segregated with resistance.

SrAsp5. TA7693, a disomic addition line with chromosome 5S of *Ae. speltoides* (21″+1″ [5S#3]) in CS background, is resistant to North American races and race TTKSK. TA7693 was crossed with CS M5D. Selected double monosomic F_1 plants were self-pollinated and ~250 F_2 progeny were screened with race RKQQ and characterized by molecular markers to identify putative Robertsonian translocation progeny. Based on marker results, resistant progeny had 5SL, whereas susceptible progeny lacked 5SL. Putative Robertsonian events were identified as progeny lacking 5SS marker alleles and having 5SL marker alleles. F_3 families of putative Robertsonian progeny were screened by C-banding. Family U5909-2-166 was identified as a Robertsonian translocation T5DS·5S#3L; the others were telosomic lines. U5909-2-166 was crossed to CS *ph1b* to develop populations for reducing the size of this translocation.

SrHv6. TA7682, a disomic addition line with chromosome 6V of *Haynaldia villosa* in CS background (21″+1″ [6V#3]), is resistant to North American stem rust races and TTKSK. Addition lines involving the other six chromosomes from the same donor were susceptible. A population was produced from the cross CS M6A (20″ + 6A′) / DA 6V in an effort to derive Robertsonian translocation chromosomes involving the 6V short and

long arms. Selected double monosomic F_1 plants were self-pollinated and their F_2 progeny were characterized by molecular markers and FGISH. C-banding analysis and molecular markers were then used to characterize and verify these Robertsonian translocation events and T6AS·6V#3L translocations were discovered. The T6AS·6V#3L translocation has been crossed to Chinese Spring *ph1b* to develop populations for reducing the size of this translocation.

SrAse3. TA3852 is a disomic addition line with chromosome $3S^S$ of *Ae. searsii* Feldman & Kislev ex Hammer in CS background (21″+1″[$3S^S$#1]) and is resistant to North American stem rust races and TTKSK. A ditelosomic addition line with the short arm of $3S^S$ (TA7533; 21″+t″[$3S^S$#1S]) was resistant, whereas the $3S^S$#1L ditelosomic addition line (TA7534; 21″+t″) was susceptible. Thus, this gene is located on the short arm of $3S^S$#1. In order to produce compensating translocations and begin reducing the amount of alien chromatin, disomic substitution lines involving all three homoeologous wheat chromosomes (TA6555, 20″+1″ [$3S^S$#1 (3A CS)]; TA6556, 20″+1″[$3S^S$#1 (3B CS)]; TA6557, 20″+1″[$3S^S$#1 (3D CS)]) were crossed to CS *ph1b*. F_2 populations of each cross were screened for putative Robertsonian translocations by DNA markers, whereas BC_1F_1 populations were developed by crossing to CS *ph1b* to induce homoeologous recombination. Confirmation of Roberstonian translocations and identification of recombinants are underway.

Discovery of new *Sr* genes requiring cytogenetic manipulation

A number of wheat-alien species derivatives with resistance to multiple stem rust races including TTKSK were indentified from *Ae. caudata* L., *Ae. speltoides*, *Th. intermedium* , *Th. Junceum* (L.) Á. Löve, and *Th. ponticum*. Population development, molecular marker testing, and phenotypic screening are underway to further characterize these potentially new sources of resistance:

Ae. speltoides. Five *Ae. speltoides* accessions, including AEG357-4, AEG363-5, AEG818-4, AEG874-60, and AEG2106-38, were obtained courtesy of The Harold and Adele Lieberman Germplasm Bank, Tel Aviv University, Israel. Each of these accessions showed high levels of resistance to Australian pathotypes (Dundas et al. 2008) and Ug99 pathotype TTKSK, and have been targeted for introgression into hexaploid wheat. Angas*7/AEG357-4 plants with resistance to Australian races *Pgt* 34-1,2,3,4,5,6,7 and *Pgt* 343-1,2,3,5,6 were identified. *Ae. speltoides* group 2 markers for probes ABG358, ABC454 and BCD111 were found in these plants whereas markers for other homoeologous groups were not found. The *Ae. speltoides* chromosome suspected of carrying the stem rust resistance gene was named 2S#3 (Dundas et al. 2008). Crosses between a resistant wheat plant carrying the 2S#3 chromosome from AEG357-4 were made with Angas *ph1b ph1b*. A total of 214 plants were screened for dissociation of 2S#3 markers for ABG358 and BCD111; seven confirmed dissociations were found. Five of these were resistant to *Pgt* 343-1,2,3,5,6; three had the marker pattern ABG-2S#3 (+ve), BCD111-2S#3 (-ve), whereas the other two showed the reverse pattern. This indicated that *Sr2S#3* may be located between the two RFLP marker loci.

Lines carrying the 2S chromosomes (and apparently no other 'S' genome chromosome) in a cv. Angas background were derived from CS/ *Ae. speltoides* amphiploids TA8026 and TS01. The amphiploids originated from the Wheat Genetics Resource Center (Kansas) and Dr M. Feldman (Israel), respectively. Both 2S addition lines have resistance to Australian pathotypes (*Pgt* 343-1,2,3,5,6; and 34-1,2,3,4,5,6,7). Crosses were made between lines carrying the 2S chromosome from TA8026 (named 2S#4) and Angas *ph1bph1b*. A total of 51 seedlings (F_3) were screened for dissociation of RFLP probes BCD111 and ABC252 and 10 confirmed dissociation plants were identified. Only one (recombinant # 25) (BCD-2S#4, +ve; ABC252-2S#4, -ve) of eight lines tested with *Pgt* 343-1,2,3,5,6 was resistant. Recombinant #25 also shows resistance to Ug99 and derivatives.

Accessions TA1776-1, TA1783-1, TA1793-1, TA1955-1, TA1935-1, TA1936-2, TA2099-1, TA2771-1, TA2779-2, and TA2780-2 represent the initial set of Wheat Genetic and Genomic Resources Center *Ae. speltoides* accessions targeted for direct introgression into hexaploid wheat. Each accession is nearly immune to North American races and TTKSK, but come from dispersed geographic locations. BC_1F_1 populations have been produced for each accession by crossing with WL711, CS *ph1b*, and other stem rust susceptible backgrounds. Three durum Langdon-*Ae. speltoides* amphiploids, and two Langdon 5D(5B)-*Ae. speltoides* amphiploids showing near-immunity or high levels of resistance to North American stem rust races and Ug99 are also undergoing genetic characterization.

Ae. caudata. Stem rust resistance was found in two stocks with *Ae. caudata* chromosomes. Alcedo-*Ae. caudata* disomic addition line 'AIII' has seedling resistance to TTKSK, whereas Alcedo is susceptible, and likely has a new gene(s) for stem rust resistance because no *Sr* gene currently available is from *Ae. caudata*. The characterization of addition line 'AIII' and introgression of the stem rust resistance gene from this addition line are currently in progress. An amphiploid of wheat- *Ae. caudata* (TA3368) has resistance to stem rust

and populations derived from this line are being used to associate the rust resistance gene with molecular markers at the University of Adelaide.

***Thinopyrum* spp**. Stem rust tests have been conducted on BC_2 populations of partial amphiploid *Thinopyrum ponticum* OK7211542 (provided by Dr R. Conner, Canada). Resistance to *Pgt* 34-1,2,3,4,5,6,7 was found in these populations and characterizations are underway. A series of wheat-*Th. intermedium* partial amphiploids (Zhong 4, Zhong 5, Zhong 6, Zhong 7, Zhong 8, and 78829) and one wheat- *Th. ponticum* partial amphiploid (SS5) have high levels of seedling resistance to Ug99 and populations are under development for potentially new resistance genes.

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15. Cloned rust resistance genes and gene based molecular markers in wheat: Current status and future prospects.

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Abstract

Two broad categories of resistance genes in wheat have been described. One group represents the so called seedling resistance or the 'gene for gene' class that often provides strong resistance to some but not all strains of a rust species. The other category referred to as adult plant resistance provide partial resistance that is expressed in adult plants during the critical grain filling stage of wheat development. A few seedling rust resistance genes have been cloned in wheat and other cereals and are predominantly from the nucleotide binding site/leucine rich repeat class which is associated with localized cell death at the pathogen entry site. Until recently, the molecular basis of race non-specific, partial and slow rusting adult plant resistance genes were unknown. Gene products that differ from known plant resistance genes were revealed from the recent cloning of the *Yr18*, *Yr36* and *Lr34* adult plant genes in wheat. The available range of diverse resistance gene sequences provide entry points for developing gene-based markers and will facilitate selection of germplasm containing unique resistance gene combinations.

Keywords

seedling resistance, adult plant resistance, cloned resistance genes

Resistance to wheat rusts caused by *Puccinia graminis* (stem rust), *P. triticina* (leaf rust) and *P. striiformis* (stripe rust) has relied on genes from cultivated wheat as well as those introgressed from close and distant relatives. The majority of genes belong to the so called seedling resistance group, sometimes referred to as major R genes, and a few are of the adult plant resistance category. Six rust resistance genes in wheat have been cloned. Three of these genes, *Lr1*, *Lr10* and *Lr21* (Huang et al. 2003; Feuillet et al. 2003; Cloutier et al. 2007) confer seedling resistance to leaf rust of which *Lr1* and *Lr10* provide resistance to a limited range of pathotypes. No virulence for *Lr21* has been confirmed (see McIntosh, these proceedings). All three genes encode for proteins with nucleotide binding sites and leucine rich repeats (NB-LRR), which represent the largest class of known resistance genes in plants to date.

The other three cloned genes, *Yr18*, *Yr36* and *Lr34* are of the adult plant rust resistance class; *Yr18* and *Yr36* confer stripe rust resistance and *Lr34* leaf rust resistance. These genes provide partial resistance and are race non-specific. *Yr36* encodes a protein with a kinase and 'START'(lipid binding) domain and the resistance is expressed at high temperatures (Fu et al 2009). A single gene confers *Yr18* and *Lr34* resistance and encodes an ATP Binding Cassette (ABC) Transporter (Krattinger et al. 2009).

No stem rust resistance gene in wheat has been cloned. However, in barley two stem rust resistance genes, *Rpg1* and *Rpg5* that encode a protein kinase and NB-LRR fused with a kinase, respectively, have been cloned (Brueggemann et al 2002; 2008). The expectation is that many more of the wheat R genes encode NB-LRR genes. A number of candidate NB-LRR genes have been reported for wheat R gene loci and these include genes for resistance to stem rust. By virtue of the fact that the aforementioned genes have been cloned, their DNA sequences provide ideal templates for deriving gene-based markers for marker assisted selection. Given the abundant representation of NB-LRR sequences in plant genomes, primer combinations targeting conserved domains in these gene sequences have been used to derive resistance gene analogs (RGA). Such RGAs are located on all wheat chromosomes (McFadden et al. 2006), and serve as candidate gene markers in attempts to identify R genes that co-localize with the RGAs. Progress in the RGA mapping approach is reliant on the level of precision mapping of R genes in wheat segregating families.

Of particular significance is the observation that the molecular bases of the APR genes identified so far differ from the seedling R genes. This lends support to a different mechanistic process for broad spectrum disease resistance associated with some APR genes. Cloned APR gene sequences could be used as probes to characterize the large gene pool of wheat as part of allele mining strategies. Furthermore, completely different APR genes are likely to be revealed as related sequences or gene

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family members of *Yr18/Lr34* and *Yr36* are non-existent at some of the other APR gene loci currently under investigation.

Ultimately combining multiple resistance genes with an additive effect and preferably with different defense mechanisms will ensure more durable resistance. As progress is made in cloning additional genes the questions that need to be addressed are: What gene combinations provide optimal effects? What strategies can best ensure the rapid transfer of 4-5 rust resistance genes into a single cultivar bearing in mind that breeders have other additional traits to select for? Will developing gene cassettes with multiple genes, e.g. APR genes, on a single T-DNA inherited as a single locus be helpful?

Finally, nothing is known about the molecular basis of suppressors of rust resistance present in the wheat genome which includes suppression of resistance to race Ug99 and its derivatives. Additional research investment is needed in this area.

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16. Molecular-genetic dissection of rice nonhost resistance to wheat stem rust

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Abstract

Rust diseases remain a significant threat to the production of most cereals including wheat. New sources of resistance are continually sought by breeders to combat the emergence of new pathogen races. Rice is atypical in that it is an intensively grown cereal with no known rust pathogen. The resistance of rice to cereal rust diseases is referred to as nonhost resistance (NHR), a resistance mechanism that has only recently become genetically tractable. In this report, the mechanisms of rice NHR to wheat stem rust and other cereal rust diseases are explored and the potential for transferring this durable disease resistance to wheat is considered. Approaches being undertaken for the molecular-genetic dissection of rice NHR to rust are described.

Keywords:

Puccinia, *Oryza*, *Triticum*, effector, immunity

Introduction

Rust diseases caused by fungal pathogens in the *Puccinia* genus remain a constant threat to cereal production. Wheat, one of the world's most important agricultural crops, accounting for 30% of global calorific intake, is subject to three rust diseases. These diseases are wheat leaf rust, wheat stripe rust and wheat stem rust caused by the fungal pathogens *Puccinia triticina*, *Puccinia striiformis* f. sp *tritici* and *Puccinia graminis* f. sp. *tritici*, respectively.

New sources of resistance to these three wheat pathogens are continually sought by breeders due to the ability of the pathogens to overcome host resistance by a combination of mutation, parasexuality and sexual recombination. Effective, durable resistance to rust diseases has been coveted by wheat agricultural scientists (Ayliffe et al. 2008). A case in point is the emergence of a new stem rust isolate from Uganda (race Ug99) in 1999 that can overcome *Sr31*, a highly effective stem rust resistance gene that had been effective for 30 years (Pretorius et al. 2000; Singh et al. 2006; 2007; Stokstad 2007). Subsequent mutation of this isolate has led to the breakdown of further resistance genes (e.g. *Sr24*, *Sr36*) making many of the world's commercial cultivars vulnerable to stem rust epidemics (Singh et al. 2006; 2007; Stockstad 2007).

In contrast, rice, which is an equally important cereal in terms of food production, is apparently immune to all known rust diseases. This species is unique compared with all other cereals and cultivated grasses, which are parasitized by at least one rust pathogen (e.g. wheat, barley, rye, triticale, maize, sorghum, millet, oats, sugarcane). The immunity of rice to rust disease is presumably mediated by nonhost resistance (NHR), a resistance mechanism that has recently become tractable using molecular-genetic approaches.

Given the apparent durability of NHR, an attractive proposition is to transfer this rust resistance from rice into wheat and other agricultural cereals. This paper describes approaches being undertaken to characterize the nonhost resistance of rice to cereal rusts at a molecular genetic level and explores the possibility of transferring this resistance to other cereals.

Host and nonhost resistance – mechanistically distinct or overlapping processes?

The current model of plant disease resistance (Jones and Dangl 2006) proposes that microbes produce an unknown number of conserved molecules that plant cells can recognize with membrane spanning receptor kinases (reviewed by Zipfel 2008). These microbial molecules, called MAMPs or PAMPs (microbe or pathogen associated molecular patterns) include diverse molecules such as flagellin, chitin, lipopolysacharide and translation elongation factors. Upon recognition of these molecules a basal defense response, or PAMP-triggered immunity, is activated that prevents further microbial colonization (Zipfel 2008). Pathogens of a given plant species have the capacity to circumvent this basal defense response by introducing a suite of molecules termed effectors into plant cells which suppress host cell defenses by interacting with specific host target molecules (reviewed by Hogenhout et al. 2009). In turn plants have evolved a large number of genes that encode resistance proteins (R proteins), each of which recognizes a specific pathogen effector. Upon R protein recognition of an effector, previously known as an avirulence product, a resistance response, or effector-triggered immunity, is activated that frequently involves hypersensitive cell death. This effector triggered

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response is the underlying molecular basis of the "gene-for-gene" hypothesis (Jones and Dangl 2006).

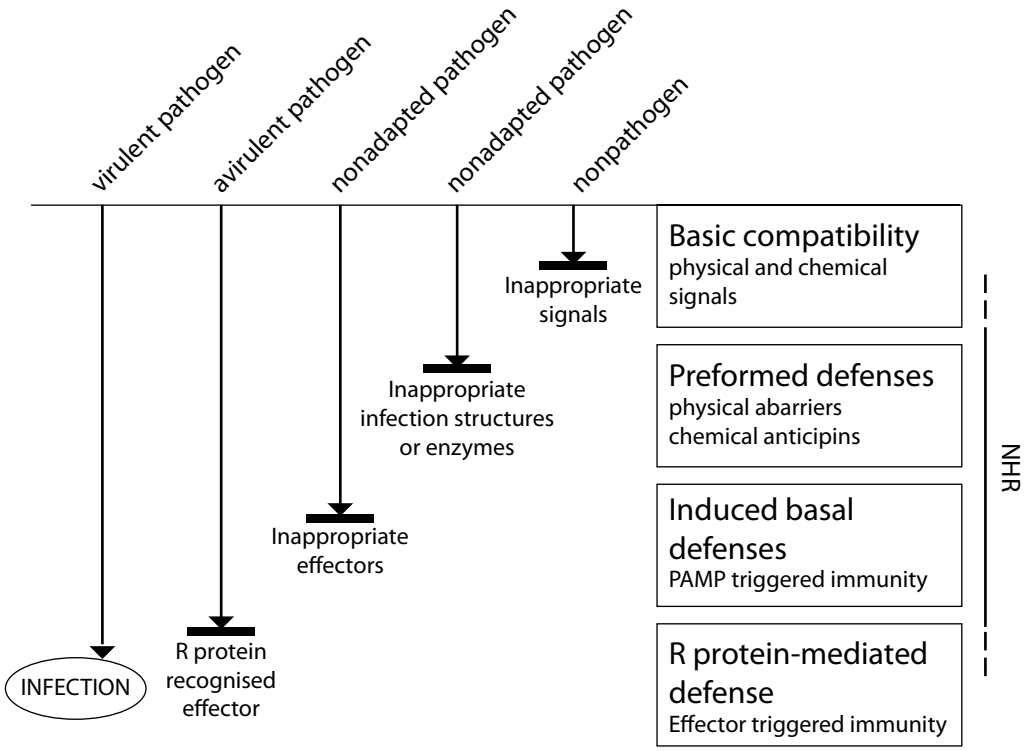
Less well defined are the molecular mechanisms that constitute NHR, although clearly NHR and basal resistance are intimately related. The extent of R protein mediated defenses in NHR is yet to be determined, but in some cases these molecules appear to have a role (described below). NHR can range from a basic incompatibility between a plant and microbial species (i.e. inappropriate physical or chemical signals for microbial recognition of a potential host), passive defense (i.e. preformed physical or chemical barriers) to active defenses involving chemical synthesis, production of reactive oxygen species and in some instances hypersensitive cell death.

The first isolation of genes conferring NHR was achieved in Arabidopsis by the cloning of three *PEN* genes (*PEN1-3*) (reviewed by Lipka et al. 2008). Initially a microscopic mutation screen was undertaken on EMS-mutagenized plants that had been infected with barley powdery mildew (*Blumeria graminis* f. sp *tritici*) for which Arabidopsis is a nonhost species. Cytological analysis of 12,000 mutagenized plants identified nine which showed a greater penetration of the leaf epidermis by this non-adapted pathogen (Stein et al. 2006). Three mutant loci (*pen1-3*) were identified in these nine plants and were subsequently isolated by map-based cloning.

The *PEN1* gene was shown to encode a syntaxin protein which is involved in targeting vesicles to the site of attempted mildew penetration (Collins et al. 2003; Kwon et al. 2008). *PEN2* encodes a peroxisome-localized glycoside hydrolase with 4-methoxy-indol-3-ylmethylglucosinolate the likely substrate (Lipka et al. 2005; Benarek et al. 2009; Clay et al. 2009). The *PEN3* gene encodes a plasma-membrane localised ATP-binding cassette transporter involved in cadmium extrusion (Stein et al. 2006; Kim et al. 2007). Both the *PEN2* and *PEN3* genes contribute to a signaling pathway leading to callose formation following PAMP recognition (Clay et al. 2009).

The *PEN* mutations did not enable colonization of the nonhost plant, rather they enabled enhanced fungal penetration and growth before pathogen restriction by other components of NHR. The *pen2* and *pen3* genes were combined with mutations in two genes, *pad4* and *eds1* that encode proteins with key regulatory roles in basal defense, salicylic acid induced defense and R protein defense (Wiermer et al. 2005). These double mutant plants showed increased permissiveness for growth of non-adapted mildew pathogens, allowing infection and occasional sporulation. In a triple mutant background (*pen2pad4sag101*) this nonhost permissiveness was exacerbated to virtual susceptibility (Lipka et al. 2005; Stein et al. 2006; Lipka et al. 2008).

Fig. 1 Plant defense mechanisms that must be circumvented for successful infection by a plant pathogen



The *PEN* genes indicate that NHR is genetically tractable if appropriate mutation screens are undertaken. In addition they demonstrate that NHR is not necessarily as genetically complex as previously considered. Finally they suggest that NHR, basal defense and R protein mediated resistance mechanisms overlap, given the central role of EDS1 and PAD4 in all these defense mechanisms. Plant resistance can be envisaged as layers of defense that must be circumvented by a pathogen beginning with passive physical and chemical barriers, induced basal defense and/or NHR mechanisms and finally avoidance of R protein recognition (Fig. 1).

Studies on NHR to rust pathogens

A number of studies have been undertaken in both dicot and monocotyledonous plants to elucidate the molecular basis of NHR to rust pathogens. Microscopic analyses identified genetic variation amongst Arabidopsis accessions for the extent of fungal development of the cowpea rust pathogen *Uromyces vignae*, on this nonhost species (Mellersch and Heath 2003). Arabidopsis plants deficient in R protein signalling pathways mediated by *EDS1* and *NDR1* did not show an altered response to cowpea rust infection but plants deficient in salicyclic acid (*sid2* and *NahG*) showed increased growth by this non-adapted pathogen (Mellersch and Heath 2003).

Most barley accessions, but not all, are immune to wheat leaf rust (*P. triticina*) and several other fungal rust species (*P. hordei-murini, P. hordei-secalini, P. persistens*). In an analysis of this "near-nonhost resistance" mapping families were produced between immune and rare susceptible parents (Jafary et al. 2008). Resistance to these rust pathogens was shown to be polygenically inherited, with substantial overlap between QTL's for resistance to each rust species. Interestingly a number of these same QTL's coincided with QTLs for partial resistance to the adapted barley leaf rust pathogen *Puccinia hordei*. No association with known R genes to barley leaf rust was observed (Jafary et al. 2008).

Analysis of the infection of Arabidopsis with wheat leaf rust (*P. triticina*) demonstrated that the non-adapted pathogen rarely entered the leaf (Shafiei et al. 2007). Only 12% of germinated urediniospores successfully located a stomate while just 0.2% produced a haustorium in a mesophyll cell. Infection of a series of well characterized defense mutants showed no altered infection phenotype. However, attempted infection by the cereal pathogen induced the formation of reactive oxygen species and salicylic acid, in addition to the induction of several defense related genes. Variation in the frequency of substomatal vesicle formation was observed between several Arabidopsis accessions and a number of QTLs identified that contributed small to medium effects on the frequency of substomatal vesicle formation and guard cell death (Shafiei et al. 2007).

Prats et al (2007) concluded that the nonhost reaction between wheat and barley leaf rust resulted in a rapid programmed cell death in the early stages of infection. Treatment with inhibitors of the phenylpropanoid biosynthetic enzymes phenylalanine ammonia lyase and cinnamyl alcohol dehydrogenase had no effect on this NHR. However, treatment of plants with D-mannose, which reduces energy availability, reduced the frequency of host cell death associated with infection sites three days post inoculation (Prats et al. 2007).

Asian soybean rust (*Phakopsora pachyrizi*) has a different asexual infection process compared with that of cereal rust pathogens, in that rather than entering the leaf through stomata, the pathogen directly penetrates the leaf epidermis in a strategy similar to that of mildew pathogens. To this end, mutations in the *PEN* genes (*pen1-3*) increased fungal growth on the nonhost plant Arabidopsis. Fungal growth was further exacerbated in a *pen* background by mutations in jasmonic acid (*jar1-1*), salicylic acid (*sid2-1*) and *eds1* signaling pathways (Loehrer et al. 2008). Epidermis based, penetration resistance mechanisms are unlikely to be applicable to the asexual urediniospore phase of the wheat stem rust pathogen, as it enters the plant leaf directly through stomatal pores. However, the sexual cycle of the wheat stem rust fungus involves the production of basidiospores, the germlings of which actually penetrate the epidermis of *Berberis* species (the alternate host of *P. graminis*) (Schafer et al. 1984). Epidermal based defense mechanisms may therefore play a role in NHR of plants to sexual cycle infection structures of cereal rust pathogens.

These data indicate that an active defense response involving salicylic acid signaling and the production of reactive oxygen species is common to a number of non-adapted rust pathogen/nonhost plant interactions.

Infection of rice with cereal rusts

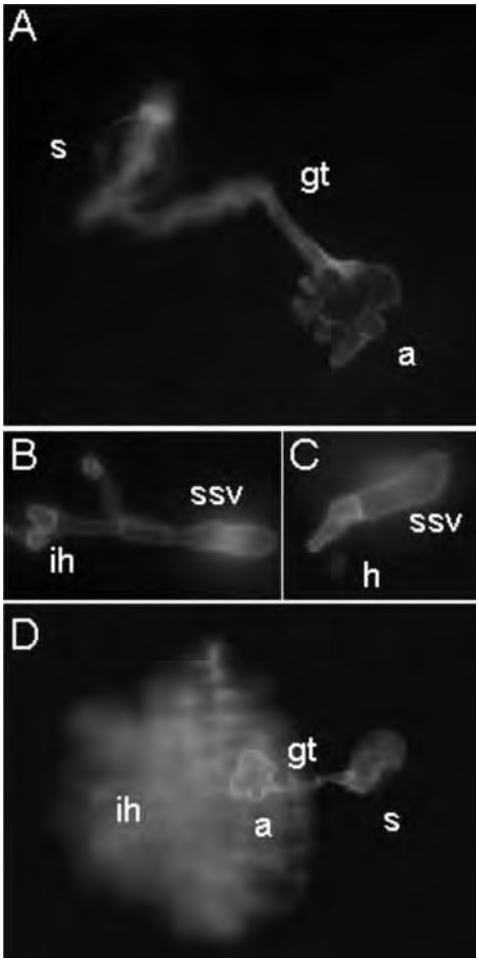
A prerequisite for elucidating the NHR response of rice to cereal rusts was a demonstration that these pathogens are capable of initiating infection upon this species. Plant pathogens perceive potential hosts by a combination of physical and chemical stimuli. A prolonged separation of plant and parasite could result in absence of appropriate host signals leading to a basic incompatibility rather than resistance *per se* (Fig. 1).

Microscopic analysis of rice infected with a number of cereal rusts [viz. *P. graminis* f. sp *tritici* (wheat stem rust pathogen), *P. striiformis* f. sp. *tritici* (wheat stripe rust), *P. recondita* (wheat leaf rust), *P. hordei* (barley leaf rust) and *P. sorghi* (common maize rust] showed that all

these rust pathogens were capable of producing the necessary infection structures for parasitism.

Fig. 2 Formation of wheat stem rust infection structures on rice

A) A wheat stem rust urediniospore (s) germinated on the surface of a rice leaf showing a germ tube (gt) and an appressorium (a) over a rice stomate (not shown).
B) Substomatal vesicle (ssv) and infection hyphae (ih) of wheat stem rust within the rice apoplast.
C) Stem rust infection site in which a haustorium (h) has been produced within a rice leaf mesophyll cell (not shown).
D) A large stem rust infection site on rice. Shown on the leaf surface is a germinated urediniospore that has produced an appressorium. Immediately beneath the appressorium (in a different focal plane) are infection hyphae ramifying throughout the intercellular spaces, with haustoria inserted within mesophyll cells (not visible)



These include germ tubes, appressoria, infection pegs, substomatal vesicles, infection hyphae, haustorial mother cells and haustoria (Fig. 2).

In some instances cereal rust infection sites on rice were large and encompassed several hundred mesophyll cells (Fig. 2). The size of these larger infection sites argues that nutrients are being acquired by the non-adapted pathogen during the early phases of infection, given the substantial fungal biomass produced and presumably finite energy resources contained within a single urediniospore.

Further cytological analysis of the nonhost interaction identified the production of callose deposition, reactive oxygen species and autofluorescence surrounding some infection sites. These observations are indicative of an active host response with features similar to typical R gene mediated resistance. The NHR of rice to cereal rusts is therefore also not a consequence of either basic incompatibility or solely due to passive physical and chemical defenses. In addition, unlike the situation for non-adapted powdery mildew pathogens grown on Arabidopsis, *cereal rusts can effectively enter the rice leaf at high frequency and produce all of the necessary infection structures required for parasitism*.

Strategies for isolating rice genes conferring NHR to wheat stem rust

To elucidate the molecular-genetic basis of rice NHR to wheat stem rust, several lines of investigation are being undertaken as part of the Borlaug Global Rust Initiative. A high-throughput macroscopic screen is being undertaken at the Cereal Rust Diseases Laboratory and Department of Plant Pathology, University of Minnesota, (USA), and Huazhong Agricultural University and Northwest Agriculture and Forestry University (China). Thousands of rice lines and landraces are being screened for altered macroscopic responses to infection with wheat stem rust isolates. Potential phenotypes include macroscopic lesions or possibly evidence of sporulation or pustule formation. Included amongst the germplasm to be screened are 20,000 chemical and irradiation-mutagenised lines from IRRI, which include several hundred mutant lines that have been identified as having impaired basal resistance to the rice blast pathogen (*Magnaportha oryzae*).

A second complementary mutation screen is being undertaken at CSIRO Plant Industry (Australia), which is similar to the Arabidopsis/non-adapted powdery mildew screen used to identify the *PEN1-3* genes. Rice mutant lines from IRRI, including those having reduced basal resistance to rice blast, are being microscopically assessed for increased amounts of wheat stem rust

growth prior to pathogen restriction by the NHR response. Although not as high-throughput as the macroscopic screening being undertaken, it is envisaged that several thousand lines will be analyzed for an altered rust infection phenotype.

In addition to these mutagenised lines, rice plants containing T-DNA insertions in homologues of known defense signaling genes are being analyzed microscopically for altered wheat stem rust infection phenotypes. Finally, preliminary analyses of several different rice cultivars suggest that reproducible genetic variation in NHR efficacy to wheat stem rust exists. This observation opens the possibility of genetically mapping QTL loci involved in the efficacy of this NHR response. F_2 mapping families have been constructed and are currently being screened for segregation of this response.

Potential mechanisms of rice NHR to wheat stem rust

As stated above, *P. graminis* f. sp. *tritici* and a number of other cereal rust pathogens, can successfully infect rice, and this nonhost plant responds with an active defense response. This observation suggests that this NHR to cereal rusts is not a consequence of basic incompatibility, and that preformed physical or chemical defenses alone are not sufficient to prevent initial infection. *Prima face* it would appear that induced defenses are likely to play a significant role in restricting growth of non-adapted rust pathogens on rice.

One possible explanation for the immunity of rice to wheat stem rust is that the non-adapted pathogen and nonhost plant have diverged sufficiently, such that stem rust no longer has the appropriate suite of effector molecules to suppress the basal/NHR response of rice. This could be a consequence of divergence of rice proteins that are equivalent to the targets of stem rust effectors in wheat. The identification of these host effector targets may enable the corresponding homologues to be isolated from rice. Alternatively, mutation in the rice equivalent of an essential host effector target may cause increased "susceptibility" that is detectable in mutation screens. If these rice proteins are capable of performing an equivalent function in wheat, their immunity to effector-mediated suppression may render wheat a nonhost to wheat stem rust, whereby the pathogen is suppressed by a basal defense response.

Typically induced plant defenses include the synthesis of antimicrobial compounds such as phytoalexins and degradative enzymes like chitinases. If the molecular suite of these compounds produced by rice is sufficiently different from that produced by wheat during rust infection, it may explain rice immunity to cereal rusts and the suppression of pathogen infection by the basal defense response. The observation that the PEN2 and 3 genes are involved in glucinosylate biosynthesis and callose deposition and that PEN1 targets vesicles potentially containing antimicrobial compounds to the site of pathogen infection, is consistent with phytoalexins playing a role in NHR to non-adpated mildew pathogens. The mapping studies in barley by Jafary et al. (2008) suggesting an overlap between adult plant resistance/ partial resistance to adapted pathogens and resistance to "near nonpathogens" is of interest. Partial resistance mechanisms to adapted pathogens are poorly understood, but the recent demonstration that the wheat leaf rust *Lr34* adult plant resistance gene encodes an ABC transporter suggests a role for antimicrobial compounds in this resistance (Krattinger et al. 2009). Mutations in rice genes encoding transporters, synthesis enzymes or regulators of antimicrobial compounds may allow increased growth of wheat stem rust on rice.

The production of reactive oxygen species and cell death are often hallmarks of programmed cell death evoked by R gene mediated resistance. The observation that a double Arabidopsis mutant (*pen3 eds1*), deficient for both penetration resistance and signalling by some R proteins, is a virtual host of non-adapted mildew pathogens suggests a possible role for R gene mediated resistance in NHR. Similarly, the transfer of the maize *Rxo1* gene, which confers resistance to the adapted bacterial pathogen *Burkholderia andropogonis*, into rice where it confers resistance to *Xanthomonas oryzae* pv. *oryzicola*, a nonpathogen of maize, implicates a role for R genes in NHR (Zhao et al. 2005). This is the first example of successful transfer of NHR between species to address a practical problem in rice, where no specific resistance to *Xanthomonas oryzae* pv. *oryzicola* has yet been found in rice germplasm. A hypothetical mechanism for the NHR of rice to wheat stem rust is a suite of conserved R genes in rice that recognise conserved effectors, or effector domains, found in all wheat stem rust isolates. This natural R gene pyramid could potentially provide durable resistance to all wheat stem rust isolates and make wheat a nonhost of this pathogen.

The NHR of rice to cereal rusts is almost certainly a polygenic trait. The ability to dissect this process using mutagenesis is dependent upon at least some of these genes providing additive effects that are not entirely functionally redundant. A large suite of co-dominant R genes in rice that are effective against all wheat stem rust isolates would be very difficult to resolve by conventional mutation approaches. Mutations in rice homologs, of known defense signaling molecules, could provide evidence for the existence of such a pyramid, but it would not enable the actual R genes involved to be identified.

An alternative strategy for identifying such genes may be to indentify wheat stem rust molecules that function as elicitors in rice and identify each individual elicitor receptor in rice by mutation and map based cloning. Recent advances in high-throughput identification of pathogen elicitors using microbial delivery systems makes this approach more feasible (Sohn et al. 2007; Rentel et al. 2008). An alternative, but extremely ambitious approach would be to introduce all 600 rice NBS-LRR genes (Goff et al. 2002) into wheat and determine if subsequent transgenics had resistance to wheat stem rust.

The question then arises as to how frequently rice proteins, be they R proteins or homologues of effector targets or involved in antimicrobial production, can function in a heterologous wheat recipient. Numerous examples exist amongst Solanaceous species whereby NBS-LRR genes have been transferred between species and shown to function, albeit with recognition of the same effector ligand in both the donor and recipient species. However, the transfer of R genes between dicot species from different families was often unsuccessful, leading to the postulation of "restricted taxonomic functionality" of R genes (Tai et al. 1999). Amongst cereal species, all of which are members of the Poaceae, two examples of R gene transfer between species have been reported, one of which was successful (Zhao et al. 2005) and one of which was unsuccessful (Ayliffe et al. 2004). The general applicability of R gene transfer between cereal species is therefore poorly defined. The ability of rice homologs of stem rust effector targets to function in wheat is unknown. However, the engineering of phytoalexin pathways in wheat is a distinct possibility.

A final point of concern is that if the NHR of rice to wheat stem rust is due to an R gene pyramid, haphazard deployment of individual rice genes into wheat could potentially result in multiple, single-step selections for a rust isolate that could actually parasitize rice. The possibility of compromising rice NHR to cereal rusts by the deployment of rice homologs of rust effector targets seems less likely. The large number of effectors introduced into host cells by plant pathogens (see Tyler 2009) argues that multiple basal defense pathways need to be overcome for true pathogenicity. This would demand multiple rice genes to be introduced into wheat to circumvent entirely effector suppression of basal defense.

In summary, the feasibility of exploiting the NHR mechanisms of rice to cereal rusts, in other cereals like wheat, remains to be determined. Demonstrating heritable phenotypes in rice in response to rust infection will be a critical first step. Our preliminary observations suggest that measurable phenotypic differences exist among rice genotypes. The large store of natural and induced variation available in rice greatly increases the likelihood of detecting genetic differences in NHR characteristics amongst germplasm and mutants. Regardless of the outcome, the underlying molecular basis of this atypical cereal NHR to rust diseases is a question of great scientific interest.

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17. Screening for stem rust resistance in East Africa

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Abstract

The East Africa program of the Borlaug Global Rust Initiative (BGRI) was launched to reduce the scale and scope of wheat stem rust epidemics in Kenya and Ethiopia, and to mitigate the global threat of virulent and dangerous rust races originating from this region. Since the launch in 2005, the screening facilities in Kenya and Ethiopia have helped to determine the extent of the world's vulnerability to stem rust race Ug99 and its variants, identify diverse sources of resistance including adult plant resistance based on minor genes, and catalyze a comprehensive global response, leading to expanded awareness, expanded research and breeding activities, and resource mobilization. This paper reviews the role and achievements of the eastern African screening facilities along with the opportunities and challenges faced by the facilities during the ongoing global response to the emergence of Ug99 and its variants.

Keywords

wheat, *Puccinia graminis tritici*, screening, facilities, Ug99

Introduction

Cereal rusts are among the world's most destructive plant diseases and can cause substantial yield losses or even destroy entire cereal crops. In addition, rust pathogens continue to pose high bio-security risks because they can spread quickly over large distances, easily adapt to the new areas and reach epidemic levels in a short period of time. The rust pathogens are hard to eradicate once introduced because of the continuous and rapid evolution of new races through, mutation, recombination (asexual, or sexual on the alternate host) and selection. Ample examples could be cited on the evolution of new races and corresponding loss of race-specific resistance genes soon after their deployment (a cycle generally referred to as boom-and-bust). Deployment of a single resistance gene on a large scale eventually leads to the selection and increased frequency of new, or previously rare, virulent races that may be a prelude to epidemics.

Environments, such as the east African highlands, further aggravate the problem as these 'hot-spots' enable large populations of rust pathogens to persist year round, contributing to speedy evolution and spread of new physiological races. The discovery of stem rust (caused by *Puccinia graminis* f. sp. *tritici*) race Ug99 in Uganda in 1998 (Pretorius et al. 2000) and its spread/establishment in Kenya and Ethiopia by 2003, Sudan and Yemen in 2006, and more recently in Iran in 2007, supplemented by two independent Ug99 mutations detected in Kenya with added virulence for gene *Sr24* or *Sr36* in 2006 and 2007, respectively, is a classical example of swift pathogen migration and evolution, and the danger it poses on global wheat production. Based on the areas planted to known varieties, pedigrees, and corresponding disease ratings to Ug99 from field screening in Kenya between 2005 and 2006, such variants pose an increased threat to the majority of cultivars grown on at least 90% of the area in the potential risk zones (Singh et al. 2006). It was estimated that the wheat area under risk to Ug99 is around 50 million hectares, which translates to about 25% of world's wheat area (Joshi et al. 2008).

The East Africa program of the Borlaug Global Rust Initiative (BGRI) was recently launched to reduce the scale and scope of stem rust epidemics in Kenya and Ethiopia, and to improve the likelihood that new virulent and dangerous races originating in this region are confined to east Africa. The East African component of the program was therefore designed to also monitor further migration of Ug99 and its variants, facilitate field screening of international wheat germplasm, identify new sources of resistance and understand the genetic basis of resistance (in particular, the durable types), carry out a targeted breeding program to incorporate resistance genes into germplasm of interest, and to enhance the capacity of national programs in breeding for rust resistance.

This paper reports on the operations of screening facilities in Kenya and Ethiopia, and discusses the opportunities and challenges of managing them.

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East African screening facilities and operational logistics

Screening locations

The facilities operate from two sites – Njoro Plant Breeding Research Center (NPBRC), Kenya Agricultural Research Institute (KARI); and Debra Zeit Research Center (DZRC), Ethiopian Institute of Agricultural Research (EIAR).

The KARI NPBRC is 200 Km west of Nairobi in Nakuru district, Rift Valley province. The center is located 2,185m above sea level at latitude $0^0$20'S, longitude $35^0$56'E. The average rainfall is 933 mm per annum with average daily minimum and maximum temperatures of 10^0 (night) and 23^0 (day), respectively. The station screening activities are mainly for bread wheat (limited durums and barley) and are coordinated across two seasons ('Main' = June to October, and 'Off-season' = November to April). Twelve hectares of irrigated land has been dedicated to field screening (4 ha available per season to accommodate a 3-season rotation) involving more than 20,000 entries per season. The germplasm evaluated includes both spring wheat and winter wheat representing advanced breeding materials, landraces, local cultivars, mapping populations and historical germplasm. The facilities also cater for shuttle breeding by CIMMYT, ICARDA, and other NARS and organizations.

The EIAR DZRC is based 40 Km south-east of Addis Ababa. The center is at an elevation of 1,850 m above sea level at latitude of $8^0$44'N, longitude 38^0 85'E. The average rainfall is 851 mm annually with average daily minimum and maximum temperatures of 9^0 (night) and 24^0 (noon), respectively. DZRC's screening activities are also co-ordinated over 12 ha of land (6 ha/rotation) during two seasons ('Main' = June to November, and 'Off-season' = January to April). The station co-ordinates screening of international wheat materials and shuttle breeding (primarily durum wheat) and can handle more than 15,000 entries per season. In addition, there are facilities at Ambo Plant Protection Research Center which focuses on rust surveillance and race analysis; and Kulumsa Research Center with emphases on wheat breeding, rust screening of bread wheat and seed multiplication.

Screening methodology

For phenotyping, the spring wheat materials are planted as double 1-m-rows. Winter wheat is vernalized for 6-8 weeks at 4^0C in vernalization chambers before transplanting to the field as hill plots. To facilitate inoculum increase and uniform spread within the nursery, clumps of selected spreaders (mixture of cultivars susceptible to Ug99 and variants) are planted adjacent to entries. The spreader rows are inoculated either by dusting them with a mixture of talcum powder and urediniospores, or by syringe inoculations with water suspensions, as outlined in McIntosh et al. (1995). In 2008, the predominant field races were typed as TTKSK (Ug99) + TTKST (Ug99+*Sr24*) in Kenya, and TTKSK in Ethiopia. Infection responses are categorized into four discrete classes: viz. resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). Infection responses overlapping between any two categories are denoted using a dash (e.g. MR-MS to represent overlapping between MR and MS responses). Stem rust severity is assessed using the modified Cobb scale (Peterson et al. 1948). Entries are evaluated for infection response and stem rust severity 2-3 times between heading and maturity. In addition, notes are taken (although not routinely) on growth habit, stripe rust and leaf rust response. The rust data include responses to natural stripe rust and leaf rust infections occurring in the Njoro plots.

Operational logistics

Staff of KARI, EIAR and CIMMYT work very closely with national and international collaborators, stakeholders and donors. Collaborators liaise with the National focal points (KARI and EIAR) via the International focal point (CIMMYT-Nairobi) three months in advance of each growing season. Both facilities cater for two cycles per year so planting dates are crucial and it is imperative that deadlines be achieved. If material does not arrive by the specified deadlines, it is held over until the following season. All seed-lots imported into Kenya and Ethiopia require valid import permits and must meet phytosanitary requirements. Import permits are organized and sent to collaborators on request. An ordinary seed import permit normally takes 2-5 days to be granted and is valid for six months after issue. Instructions outlining importation procedures must be followed strictly to ensure successful importation. On customs and quarantine clearance, the material is taken to the research testing sites of NPBRC and DZRC, where it may be subjected to further plant health checks to prevent the possibility of exotic pests and diseases from outside east Africa. The material will be planted only after final clearances.

In 2009, all germplasm will be exchanged under a standard material transfer agreement (SMTA) for the purpose of research and breeding. KARI and EIAR will hold the germplasm in trust for the international community in accordance with the terms of the SMTA of the International Treaty for Plant Genetic Resources for Food and Agriculture. For commercialization purposes and other potential uses, germplasm exchange can take place on a case-by-case basis, with mutually

agreed arrangements between the collaborators. The information generated will be open-access unless there is a special MTA with the supplier. Kenyan and Ethiopian NARS will have direct access to all materials and information for research and breeding purposes because of their contribution/services to the screening facilities.

Almost all developing country programs and International Agricultural Research Centers are currently exempt from the payment of screening fees. However, a fee may be applicable to some industrialized country programs and private companies. For further information on the operations of rust screening facilities in east Africa, visit www.globalrust.org

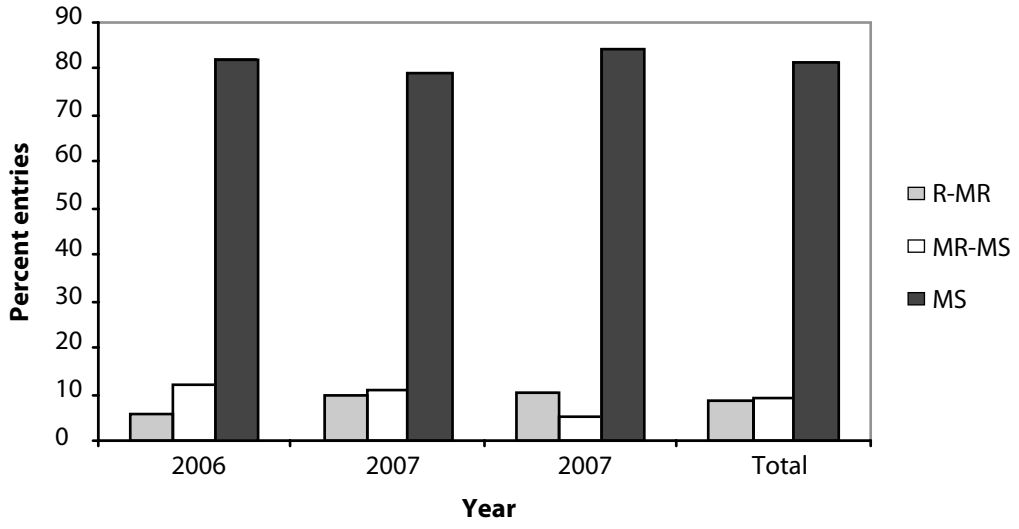
Achievements

Wheat-producing nations throughout the world (more than 25 collaborators from 20 countries) have participated in stem rust response tests of wheat (over 80,000 research plots) in both main and off-season nurseries in Kenya and Ethiopia since 2005. The resistance status of these lines is available and has been disseminated to collaborators. There has been a high demand for international screening over the last three years, and screening requests have almost tripled since 2005.

In 2008, more than 20,000 lines from 20 countries were screened in Kenya. Rust infection was excellent and disease pressure was very high. The responses of controls/differentials showed virulence for genes *Sr31* and *Sr24* in the screening nursery, indicating the likely presence of Ug99 and its variant, Ug99+*Sr24*. *Sr36* was partially effective probably because of a low frequency of *Sr36*-virulence in the pathogen population. A low frequency of resistant entries was a common feature among wheat materials from many countries with more than 80% of screened germplasm susceptible, a trend not much different from the previous two years (Fig. 1). Among the resistant materials (classified in the R-MR and lower MR-MS categories), the highest frequencies of resistant entries were in Canadian germplasm (30%), followed by CIMMYT (25%), ICARDA (13%), USDA (9%), Australia (5%), India (5%), Egypt (4%), Uruguay (3%), Argentina (3%), and all others 3% (Fig. 2). Lines with notable resistance included *Sr25* derivatives, several tall Giza lines from Egypt, derivatives of the Chinese wheat cultivar Shanghai#7, Canadian materials (Thatcher background plus leaf rust resistance gene *Lr34*), some ICARDA and CIMMYT lines, and several Egyptian and CIMMYT durums. Varied responses of materials with *Sr2* were also evident.

Good progress was made in identifying diverse sources of resistance to Ug99 and its variants in international germplasm including minor gene adult plant resistance (APR), which in cereal rust systems has a reputation of durability. More than 300 germplasm sets in the form of three stem rust resistance screening nurseries (1st SRRSN, 2nd SRRSN and 3rd SRRSN) were distributed, or are under the process of being distributed. A high proportion of lines (44%) in these three nurseries have shown good to moderate levels of resistance in at least two seasons of evaluation in Kenya. Some promising lines with very good agronomic traits and resistance to Ug99 and its variants have been

Fig. 1 Comparative field response (R-MR, up to 20% disease severity with small uredinia; MR-MS, up to 40% disease severity with medium uredinia; MS-S, 50-100% disease severity with medium to large uredinia) of germplasm screened at KARI during 2006-2008



identified in both the Ethiopian and Kenyan breeding programs, and are under further evaluation or testing for use in breeding programs worldwide, or for direct release and registration in Kenya and Ethiopia.

Opportunities and Challenges

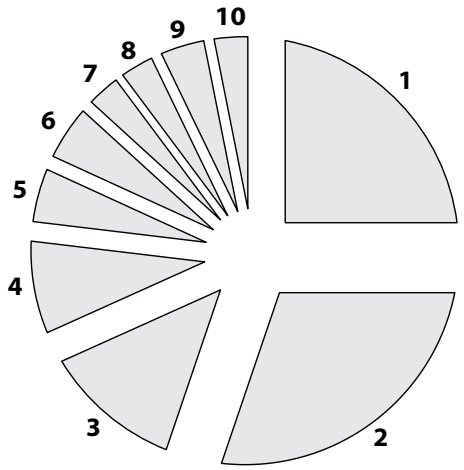
The screening work in east Africa has confirmed the vulnerability of the global wheat industry to race Ug99 (and its variants), and has assessed the effectiveness of known stem rust resistance genes against the Ug99 lineage. East Africa remains the only region where, currently, field screening for responses to the Ug99 lineage can be conducted with a reasonable capacity and with international support. However, fully operational 'Critical Facilities in East Africa' require further investments in field, irrigation, greenhouse, and laboratory facilities and equipment, as well as operational support, for mission-dedicated teams of national and international scientists. Currently, the facilities are being supported by the BGRI/DRRW Project, but long-term funding needs to be secured for sustainability and commitment to a global effort for minimizing yield losses by breeding wheat cultivars resistant to Ug99. Facilities for screening at the hot-spot locations in Kenya and Ethiopia should be expanded and strengthened to cater for future international needs. Protection of the world wheat crop through development of varieties durably resistant to Ug99 cannot be achieved without the continued expansion of the recently initiated collaborative research conducted in east Africa. At the same time, international and regional co-operation should be enhanced to facilitate human resource development in testing, surveillance and pathology, breeding, information sharing, and data management and access.

The objectives of the BGRI/DRRW Project and collaborations with NARS public sector elements are, in principle, not subject to screening fees. However, we have developed a fee-for-service model for private companies and the industrialized countries for screening germplasm. In return, this will support and sustain the activities of the facilities and will provide quality assurance of the data generated. This fee-for-service will be implemented from main season 2009.

Kenya and Ethiopia are free from certain diseases and the frequent importation of seed from many locations poses a risk of exotic pests/diseases that may accompany seed imported from outside east Africa. Because a breach in quarantine could place the entire testing and screening program in jeopardy, it is important that a seed health/ containment facility be established at each station, in addition to normal national phytosanitary and quarantine protocols. Procedures and policies for such laboratories are currently being discussed.

If current activities can be sustained, with all opportunities exploited and challenges met, the critical facilities in Kenya and Ethiopia will be high caliber components of an integrated world effort to minimize the destabilizing effects of rusts on world wheat production and food security.

Fig. 2 Percentages of resistant (R-MR and MR-MS) spring wheat entries from different countries/institutions during 2008 screening at Njoro (1, CIMMYT; 2, Canada; 3, ICARDA; 4, USDA; 5, Australia; 6, India; 7, Uruguay; 8, Argentina; 9, Egypt; 10, Others)



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18. Developing and optimizing markers for stem rust resistance in wheat

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Abstract

High quality molecular markers that are closely linked, codominant, and high throughput are critical for developing varieties with durable rust resistance. We are using a combination of microsatellite, sequence tagged site, and Diversity Array Technology markers for haplotyping, pyramiding, and mapping stem rust resistance genes. The primary goal of our research team is to identify and optimize markers for previously characterized and novel stem rust resistance genes in wheat. The specific objectives are to: 1) optimize markers for previously characterized stem rust resistance genes to maximize efficiency of the breeding programs, 2) haplotype uncharacterized rust resistant genotypes to infer novelty and to plan new mapping experiments, 3) pyramid novel sources of rust resistance, and 4) map novel sources of rust resistance, including adult plant resistance. To date, we have evaluated 58 markers associated with 21 stem rust resistance genes and used 20 for haplotyping 318 wheat lines and varieties for 15 Ug99 effective resistance genes. This germplasm panel is also being DArT genotyped. For tetraploids, the pyramiding includes *Sr2*, *Sr13* and *Sr25* in the breeding line UC1113 which is a high yielding semi-dwarf durum variety with the high-grain protein content gene *Gpc-B1* and the non-race specific stripe rust resistance gene *Yr36*. The Australian group is developing markers for the stem rust resistance genes *Sr33* and *Sr45* that come from *Aegilops tauschii* and are located on wheat chromosomes 1DS. Diagnostic, codominant markers for *Sr25* and *Sr26* have been developed and are being pyramided into CIMMYT breeding lines. Three new sources of race-specific resistance in CIMMYT-derived spring wheat have been mapped and are designated *SrA*, *SrB*, and *SrC*. *SrA* mapped on 3DL, *SrB* on 3BS and *SrC* on 5DL. These genes provided moderate levels of resistance to stem rust at the seedling stage and acceptable to moderate levels at the adult plant stage.

Key words

Haplotype, Sr gene, Ug99

Introduction

Stem rust (caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.) is one of the most serious diseases of wheat worldwide. The recent emergence of wheat stem rust race Ug99 (TTKSK) threatens global wheat production. The high priority of developing durable and effective disease resistant wheat varieties is our primary goal. Resistance to wheat stem rust is mediated by R genes. To date, about 50 stem rust resistance (*Sr*) genes have been identified and mapped to specific chromosome positions (McIntosh et al. 2008; see also summarized table for stem rust resistance genes and markers at http://rustopedia.get-traction.com/traction. Among *Sr* genes identified, however, less than half are effective to Ug99 (See Singh et al. 2006 for review). Genes effective against Ug99 include *Sr28, 29* and *Tmp* from *Triticum aestivum*, *Sr2* and *13* from *T. turgidum*, *Sr22* and *35* from *T. monococcum*, *Sr36* and *37* from *T. timopheevii*, *Sr32* and *39* from *Aegilops speltoides*, *Sr33* and *45* from *Ae. tauschi*, *Sr40* from *T. araraticum*, *Sr24, 25, 26* and *43* from *Thinopyrum elongatum*, *Sr44* from *Th. intermedium*, and *Sr27 R* and *1A/1R* from *Secale cereale*.

Host resistance is more effective and durable when several stem rust resistance genes are pyramided into a single genotype (Pederson and Leath 1988), a process that can be facilitated by marker-assisted selection. Our objectives are to develop and optimize molecular markers for stem rust resistance for use in breeding programs, and to provide resources necessary for developing varieties with durable rust resistance. A large number of inexpensive markers are necessary for mapping and cloning genes linked to economically important traits and for implementation of efficient breeding methods for rapid development of rust resistant varieties. Wheat has one of the largest collections of ESTs for a major crop species, but the least amount of genome sequence information and the fewest molecular markers. This is largely because marker development for wheat is complicated by polyploidy and low polymorphism, especially in cultivated germplasm. Single-nucleotide polymorphism (SNP) markers have become the technology of choice for most organisms because of their high frequency, wide distribution in genomes, and adaptation to highly multiplex detection systems. However, there are not enough SNP markers available for wheat. Consequently,

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this project will use a combination of microsatellite (SSR), sequence tagged site (STS), and Diversity Array Technology (DArT) markers for haplotyping, pyramiding, and mapping stem rust resistance genes until a SNP marker platform is available for cultivated wheat.

The primary goal of this objective is to identify robust markers for previously characterized and novel sources of stem rust resistance in wheat. The specific objectives are to:

1) optimize markers for previously characterized stem rust resistance genes from the primary gene pool providing effective stem rust resistance to maximize efficiency of breeding programs. The outcome is to develop materials and information necessary for marker-assisted breeding and to provide resources necessary for pyramiding genes for durable resistance.
2) haplotype uncharacterized rust resistant genotypes to infer novelty and to plan new mapping experiments, including identification of haplotypes for major resistance loci of uncharacterized sources of stem rust resistance. The outcome will provide information required for cross-referencing sources of rust resistance and develop a catalog of all known sources of marker alleles linked to rust resistance genes.
3) combine sources of rust resistance and develop several different breeding populations homozygous for 3 or more stem rust resistance genes. The outcome will be availability of molecular markers for new sources of stem rust resistance for use in breeding programs and resources necessary for developing varieties with durable quantitative rust resistance.
4) map sources of rust resistance, including QTLs for adult plant resistance (APR) in 4 to 6 different mapping populations. The outcome for this objective will provide new markers for stem rust resistance for use in the breeding programs to develop varieties with durable quantitative rust resistance.

The Durable Rust Resistance Wheat Project was funded by The Bill and Melinda Gates Foundation initially for three years. We are responsible for Objective 6. The specific milestones and timelines for Objective 6 are as follows:

Year 1: Optimization of markers for previously characterized rust resistance gene markers

1.1 Compile & prioritize a comprehensive list of major genes, their markers, and genome locations
1.2 Optimize high priority markers for major known genes in the first 18 months.
1.3 Optimize high priority markers for newly discovered major known genes.

Year 2. Haplotyping uncharacterized rust resistant genotypes to infer novelty and to plan new mapping experiments

2.1 Acquire seed of genetic stocks, germplasm and varieties for haplotyping
2.2 Haplotype known sources of rust resistance and susceptible controls at known loci.
2.3 Haplotype novel sources of rust resistance not characterized at major resistance gene loci.
2.4 Assess stem rust resistance gene marker diversity.

Year 3. Pyramiding novel sources of rust resistance

3.1 Make first round of crosses among high priority known sources of major genes for pyramiding in an adapted background
3.2 Select plants that are homozygous for two genes, bulk harvest and deliver to breeding programs.
3.3 Make second round of crosses among high priority known sources of effective major genes for pyramiding in an adapted background.
3.4 Select plants that are homozygous for three or more genes, bulk harvest and deliver to breeding programs.

Years 3 and 4. Mapping novel sources of rust resistance

4.1 Identify new sources of rust resistance and develop mapping populations.
4.2 Select APR mapping populations based on phenotype.
4.3 Genotype 4-6 selected APR mapping populations.
4.4 Analyze rust resistance QTL data for APR, complete the mapping of novel sources of rust resistance.

The research team for Objective 6 consists of six research groups including James Anderson's group at University of Minnesota, Michael Baum's group at ICARDA, Jorge Dubcovsky's group at UC Davis, Evans Lagudah's group at CSIRO, Ravi Singh's group at CIMMYT, and Mark Sorrells' group at Cornell University.

We report here the recent advances for developing and optimizing molecular markers for stem rust resistance in wheat.

Section 1. Haplotying uncharacterized sources for stem rust resistance in wheat using available markers (*Contributed by Long-Xi Yu and Mark E. Sorrells*)

Gene selection: To evaluate the quality of the markers for stem rust resistance, a survey of stem rust resistance genes, including those conferring resistance to Ug99, was completed by our group. All mapped major stem rust resistance genes were characterized

for source, markers available, current research activities, and prioritized for this project (http://rustopedia.gettraction.com/traction)**.** Haplotyping was initiated for stem rust resistance genes, including *Sr1A1R, Sr2, Sr9a, Sr13, Sr15, Sr17, Sr19, Sr22, Sr24, Sr25, Sr26, Sr31, Sr32, Sr33, Sr35, Sr36, Sr38, Sr40, Sr45, Sr46,* and *SrR*. Table 1 lists the major stem rust resistance genes and linked markers, including SSR, STS, BARC, and DArT markers.

Genetic resources: We started with a diverse collection of wheat accessions selected by CIMMYT and ICARDA wheat breeders. This collection consists of more than 300 lines from CYMMIT and ICARDA programs in Africa, China, Turkey and Mexico. In the present study, we will report on 260 wheat lines of diverse origins including 115 lines from CIMMYT, 43 lines from China, and 102 lines of miscellaneous origins. To estimate their genetic relationships to known stem rust resistance gene sources, we also included wheat lines with known *Sr* genes when available.

Marker validation: To evaluate the functionality and polymorphism for the available markers, we first screened 58 markers associated with 21 stem rust resistance genes among 16 randomly selected wheat lines. Using DNA from leaf tissue, we tested the primers for each marker (Table 1) and analyzed the polymerase chain reaction (PCR) products using agarose gels. Forty six (80%) of the markers amplified clear fragments and, of those, 35 (75%) showed polymorphism.

Haplotyping diverse wheat germplasm: We then extracted DNA from the other 260 wheat lines and analyzed the haplotypes by comparison with known sources of stem rust resistance genes. PCR amplification was carried out using primers of molecular markers associated with major stem rust resistance genes as shown in Table 1. PCR products were analyzed using both PAGE and ABI 3730. To date, 20 markers associated with major genes *Sr1A1R, Sr2, Sr9a, Sr13, Sr17, Sr19, Sr22, Sr24, Sr25, Sr31, Sr32, Sr35, Sr36, Sr40* and *Sr44* were analyzed. The sizes of PCR amplicons were recorded.

To analyze the distribution of alleles linked with the stem rust resistance QTLs, we grouped PCR amplicons based on their fragment sizes (in bp) among wheat lines analyzed. PCR amplicons of the same size were grouped together and color coded to help interpretation of haplotype structure. Fig. 1 shows haplotype groups for 10 loci in our panel of accessions. Of those with known reactions to stem rust, a group of susceptible and moderately susceptible lines were grouped together as a susceptible haplotype (blue color). Genotypes containing the same gene such as *Sr25* or with resistant phenotypes were sorted together as resistant haplotypes (red color). These preliminary results were from a small number of markers and we will use more markers to improve the resolution of the relationships. DNA of each line was also sent to Triticarte, Australia (http://www.triticarte.com.au) for DArT analysis. The identified DArT markers will be used for haplotyping and association analysis in combination with the phenotypic data. Our goal is to be able to predict the stem rust resistance genotype.

***Progress in pyramiding stem rust resistance*:** Our pyramiding work is a collaboraton with Dr. Gina Brown-Guidera, director of the USDA genotyping laboratory in Raleigh, NC and Dr. Michael Pumphrey, ARS-Manhattan, KS. As the initial targets for pyramiding, we made F1 hybrids by intercrossing wheat lines with combinations of *Sr22, 24, 32, 36 and Amigo*, and also between the sources of these genes and local lines with high yield and quality in fall 2008. More crosses will be made between these sources of major genes and CIMMYT, Chinese and African wheat germplasm. Our goal is to develop wheat germplasm with durable resistance to Ug99 for the high risk wheat growing regions, especially Africa.

Acknowledgements

We acknowledge the germplasm and information provided by our co-authors, as well as collaborators Drs. Gina Brown-Guidera, Michael Pumphrey, Alexi Morgounov, Zhonghu He, Steven Xu, and Yue Jin, in the preparation of this report.

Section 2. Developing and optimizing markers for *Sr13* and pyramiding *Sr2*, *Sr13* and *Sr25* in tetraploid what (*Contributed by Zewdie Abate and Jorge Dubcovsky*)

Specific objectives assigned to the UC Davis program

During the March 12, 2008 meeting of the stem rust marker group at UC Davis, it was decided that the UC Davis program would focus on stem rust resistance genes from diploid and tetraploid wheats. It was also agreed that the pyramiding activities at UCD would incorporate multiple stem rust resistance genes effective against Ug99 into high yielding tetraploid backgrounds that already have been targeted for the incorporation of stripe rust resistance genes. These tetraploid lines will be excellent parental lines to deliver multiple *Sr* and *Yr* genes in simple crosses with germplasm in the SEWANA durum production regions. This activity will also include the transfer of resistance genes from hexaploid to tetraploid wheat. In the area of mapping, the UC Davis group will focus on the precise mapping of genes *Sr13* and *Sr25* and on the discovery of new sources of resistance against Ug99 in tetraploid wheat and *T. monococcum* mapping populations.

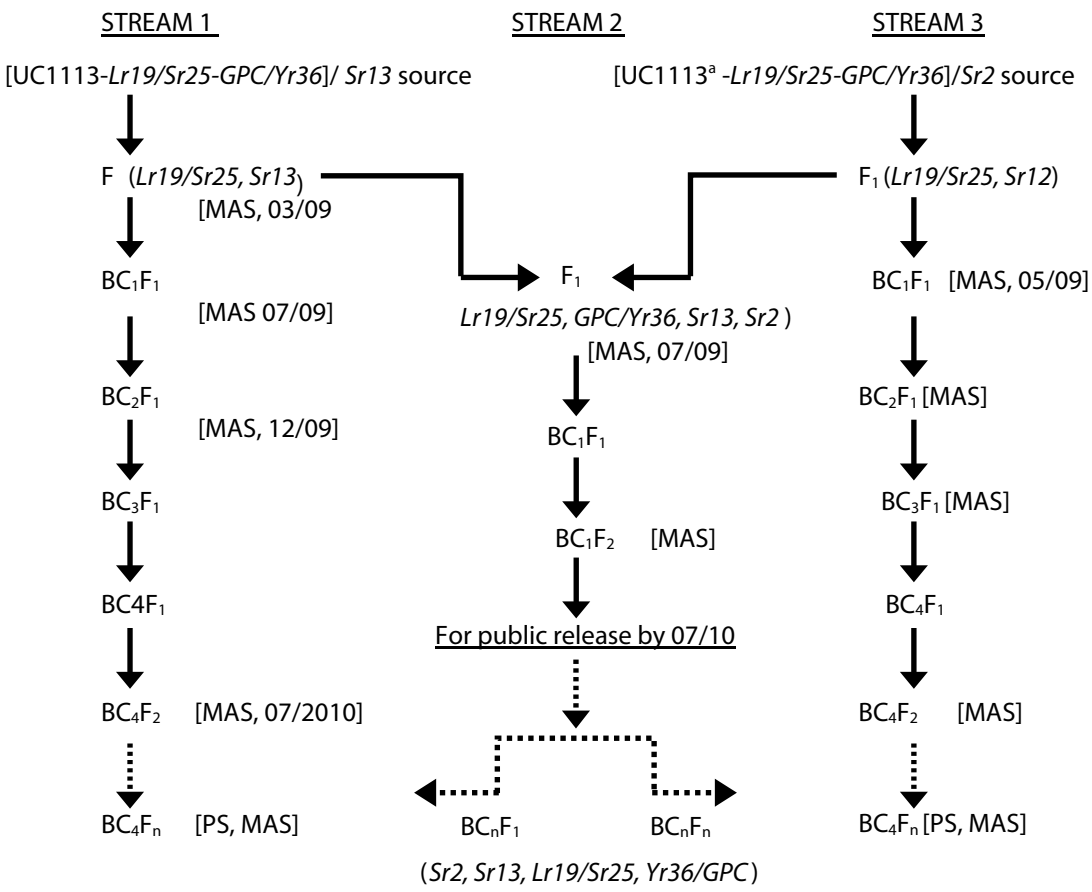
Table 1 Stem rust resistance genes and linked markers and their primers used for genotyping

Major Sr gene	Chr. location	Marker name	Forward primer	Reverse primer
Sr1A1R	1AL1RS	barc1048	5'-acgtggtaattagttgggagtctgta	5'-gcgaagtcaagaagtgggcttttcaagag
		scm9	5'-tgacaacccccttccctcgt	5'-tcatcgacgctaaggaggaccc
		barc028	5'-ctccccggctagtgaccaca	5'-gcggcatctttcattaacgagctagt
Sr2	3BS	gwm533	5'-aaggcgaatcaaacggaata	5'-gttgctttaggggaaaagcc
		barc133	5'-agcgctcgaaaagtcag	5'-ggcaggtccaactccag
		gwm389	5'-atcatgtcgatctccttgacg	5'-tgccatgcacattagcagat
		wpt5716	5'-tgcctagatttcagaagatg	5'-ctgtcaagagggagctgaag
Sr6	2DS	wmc453	5'-acttgtgtccataaccgacctt	5'-atcttttgaggttacaacccga
		cfd43	5'-aacaaaagtcggtgcagtcc	5'-ccaaaaacatggttaaagggg
Sr9a	2BL	gwm47	5'-ttgctaccatgcatgaccat	5'-ttcacctcgattgaggtcct
		wmc175	5'-gctcagtcaaaccgctacttct	5'-cactactccaatctatcgccgt
		gwm120	5'-gatccaccttcctctctctc	5'-gattatactggtgccgaaac
		barc101	5'-gctcctctcacgatcacgcaaag	5'-gcgagtcgatcacactatgagccaatg
Sr13	6A	wmc580	5'-AAggcgcacaacacaatgac	5'-ggtcttttgtgcagtgaactgaag
Sr15	7AL	STS638	5'-gcggtgactacacagcgatgaagcaatgaaa	5'-gcggtgactagtccagttggttgatggaat
Sr17	7BL	wpt5343,	5'-tattctacaacgctccatcc	5'-cgcatgcaanccatacctttt
		wpt0600	5'-agctcgtacaatggtgg	5'-catgaaataagctgccactt
Sr19	2BS	wpt9402	5'-attttatattgccgtgccag	5'-atggccagcacgatagagag
Sr22	7AL	Cfa2019	5'-gacgagctaactgcagaccc	5'-ctcaatcctgatgcggagat
		Cfa2123	5'-cggtctttgtttgctctaaacc	5'-accggccatctatgatgaag
		barc121	5' actgatcagcaatgtcaactgaa	5'-ccggtgtctttcctaacgctatg
Sr24	3DL	Sr24#12	5'-cacccgtgacatgctcgta	5'-aacaggaaatgagcaacgatgt
		Sr24#50	5'-cccagcatcggtgaaagaa	5'-atgcggagccttcacatttt
		Barc71	5'-gcgcttgttcctcacctgctcata	5'-gcgtatattctctcgtcttcttgttggtt
Sr25	7DL	Gb	5'-catccttggggacctc	5'-ccagctcgcatacatcca
	7BL	STSLr19-130	5'-catccttggggacctc	5'-ccagctcgcatacatcca
Sr26	6AL	Sr26#43	5'-aatcgtccacattggcttct	5'-cgcaacaaaatcatgcacta
		Umn6A-5	5'-tcgtttgggaacacagctta	5'-ccggcgaaatataatgcaaa
Sr31	1BS	1B-159	5'-agcgcagataatgtttgaacc-3'	5'-aagtcgaaaccacagttatc
		lag95	5'-ctctgtggatagttacttgatcga	5'-cctagaacatgcatggctgttaca
		wpt8949	5'- tgggatgcgagaatatccgg	5'- tgcgatgcctaaagcctctc
		wpt1328	5'- gcgccggtcggacagaccgg	5'- gaactactaattactgtaca
Sr32	2AS	stm773	5'-aaacgccccaaccacctctctc	5'-atggtttgttgtgttgtgtgtagg
		barc55	5'-gcggtcaacacactccactcctctctc	5'-cgctgctcccattgctcgccgtta
Sr33	1DS	ABC156	5'-ttacgggatcaaagctgaggc	5'-gacaagcaacaccaaccaagc
Sr35	3AL	cfa2076	5'-cgaaaaaccatgatcgacag	5'-acctgtccagctagcctcca
		cfa2193	5'-acatgtgatgtgcggtcatt	5' tcctcagaaccccattcttg
		barc51	5' cgcatgagcaaacaagccaacaact	5' cggccacagcatcggttctccaaa
Sr36	2BS	stm773-2	5'-atggtttgttgtgttgtgtgtagg	5'-aaacgccccaaccacctctctc
		gwm271	5'-caagatcgtggagccagc 3'	5'-agctgctagcttttgggaca 3'
		wmc477	5'-cgtcgaaaaccgtacactctcc	5'-gcgaaacagaatagccctgatg
		gwm319	5'-ggttgctgtacaagtgttcacg	5'-cgggtgctgtgtgtaatgac
Sr39	2B	Sr39F/Sr39R	5'-agagagagtagaagagctgc	5'-agagagagagcatccacc
Sr40	2BS	gwm344,	5'-caaggaaataggcggtaact	5'-atttgagtctgaagtttgca
		wmc661	5'-ccaccatggtgctaatagtgtc	5'-agctcgtaacgtaatgcaactg
		Xgwm374	5'-atagtgtgttgcatgctgtgtg	5'-tctaattagcgttggctgcc
		Xwmc474	5'-atgctattaaactagcatgtgtcg	5'-agtggaaacatcattcctggta
Sr44	7DS	cdo475	5'-gacacattgaccgcatctta	5'-ccttcacctcgctccctacc
		wpt2565	5- tactttgatttggtcagttg	5'-tcgcgaccaagctctacaat
Sr45	1DS	Xwmc222	5'-aaaggtgcgttcatagaaaattaga	5'-agaggtgtttgagactaatttggta
		Xcfa2158	5'-tttcgtcttcaaaatgcactg	5'-tggtagcttacaaaggtgcg
SrR	1DL	AW2-5	5'-gaatcccattgttcagcaagt	5'-tagcactccagcagactccac
		CI2F	5'-agggtcacacaggcaatctaa	5'-cattctggttttccgcagcaac
		1B-159	5'-agcgcagataatgtttgaacc'	5'-aagtcgaaaccacagttatc
		1B-267	5'-gcaagtaagcagcttgatttagc	5'-aatggatgtcccggtgagtgg'
		Xmwg060	5'-caacgatacaacaggctcaa	5'-ctggatagagaagccatgga

Fig. 1 Haplotype analysis for stem rust resistance for wheat lines of known or unknown resistance. PCR amplicon fragment sizes are shown for each accession along with the stem rust reaction or gene if known. R, resistant; S, susceptible; MR and MS, moderately resistant and susceptible, respectively. Color codes: pink for resistance (bold), blue for susceptible (bold italic) and yellow for unknown phenotypes (underlined)

Genotype	Sr gene	*1A 1R*	*Sr2*	*Sr2*	*Sr22*	*Sr22*	*Sr24*	*Sr32,36*	*Sr40*	*Sr40*	*Sr40*
	Marker	BARC28	GWM533	BARC133	BARC121	CFA2123	BARC71	STM773	WMC474	GWM344	WMC661
	Resistance										
LcSr25Ars	***Sr25***	250	N	N	215	245	126	180	N	132	225
CIMMYT11	***Sr25***	250	117	114	215	N	126	188	140	127	230
CIMMYT12	***Sr25***	250	117	114	215	N	126	185	130	127	230
CIMMYT69	***Sr25***	250	117	114	230	N	126	188	135	125	230
CIMMYT13	***MR***	250	117	114	215	N	126	188	130	127	230
CIMMYT26	***MR***	250	117	114	230	N	126	185	130	127	230
CIMMYT28	***Sr-Sharp***	250	117	114	230	N	130	188	132	127	230
CIMMYT82	***R***	250	117	117	230	N	126	188	135	127	230
CIMMYT67	***SrHUW234***	250	117	117	215	245	126	188	135	125	230
CIMMYT50	***MR***	250	117	117	215	245	126	188	135	125	230
CIMMYT77	***NA***	250	117	117	215	250	126	188	135	127	230
CIMMYT90	***R-MR***	250	117	117	215	250	126	188	135	125	225
CIMMYT46	***MR***	250	117	117	215	250	126	188	135	127	225
CIMMYT36	***NA***	250	117	114	215	250	126	188	135	127	225
CIMMYT84	***MR***	250	117	114	215	N	126	188	135	127	230
CIMMYT92	***R***	250	117	114	217	250	135	188	135	127	230
CIMMYT71	***SrSha7***	250	117	119	215	250	126	188	135	132	230
CIMMYT72	***SrSha7***	250	117	117	215	250	135	188	N	127	230
CIMMYT79	***MR***	245	117	119	215	250	126	188	130	N	230
CIMMYT63	***R***	250	117	119	215	250	126	188	135	N	230
CIMMYT91	***NA***	250	117	90	230	250	126	188	140	127	230
CIMMYT19	***S***	250	143	90	215	250	126	185	137	127	230
CIMMYT2	***NA***	250	118	117	230	N	126	185	130	132	230
CIMMYT96	***NA***	200	95	90	215	245	135	188	130	N	230
CIMMYT95	***NA***	200	95	119	215	245	135	188	132	N	230
CIMMYT7	***Sr-HUW234***	250	117	113	230	N	126	185	135	N	230
CIMMYT14	***MR***	250	117	114	215	245	126	188	140	N	230
CIMMYT15	***Sr33?***	250	117	112	230	245	126	188	135	N	250
PW327	***Sr26***	250	207	126	215	245	126	185	207	125	250
Thatcher	***SrThatch***	N	N	114	215	250	126	180	N	125	N
73.214.3-1	***Sr27***	N	N	121	215	245	N	N	N	125	N
CnsSr32	***Sr32***	195	209	117	215	250	135	190	137	132	160
C82	***Sr32***	250	237	117	215	N	113	190	267	N	160
CIMMYT94	***SrSha***	250	117	117	230	245	135	190	132	125	230
CombiNtion	***Sr13***	250	127	120	215	250	113	180	203	132	210
NY6432-18	***NA***	250	N	N	230	N	126	185	N	127	225
CHINA28	***NA***	285	115	N	215	250	135	185	N	127	225
ARS05-0456	***Sr36?***	250	N	N	215	250	126	160	N	132	225
CIMMYT113	***NA***	N	142	N	230	N	N	N	132	N	250
CIMMYT51	***S***	N	N	N	215	245	N	N	N	125	N
ARS04-1267	***Amigo***	260	145	117	215	N	126	188	116	127	N
ARS05-0146	***NA***	260	145	117	230	N	126	160	148	N	210
CIMMYT102	***NA***	260	122	117	230	250	135	188	130	127	250
CIMMYT88	***SrSyNAthetic***	260	117	114	230	250	135	188	135	127	250
CIMMYT80	***MR***	260	117	117	215	N	135	185	130	N	230
ARS05-0005	***S***	N	N	118	215	N	108	190	116	127	N
CHINA10	***NA***	N	N	N	215	N	N	N	129	127	N
CIMMYT4	***Sr-NAD643***	250	120	121	215	N	126	185	130	127	N
CIMMYT24	***MS***	*250*	*117*	*114*	*215*	*250*	*126*	*188*	*135*	*127*	*N*
CIMMYT44	***S***	*250*	*117*	*114*	*215*	*250*	*126*	*188*	*135*	*127*	*N*
CIMMYT59	***S***	*250*	*117*	*117*	*215*	*250*	*126*	*188*	*135*	*127*	*N*
CIMMYT1	***MS***	*250*	*117*	*114*	*215*	*N*	*126*	*188*	*130*	*132*	*230*
CIMMYT34	***MS***	*250*	*117*	*114*	*230*	245	*126*	*188*	*135*	*132*	*225*
CIMMYT21	***MS***	*250*	*117*	*114*	*230*	*N*	*N*	*188*	140	*132*	*225*
CIMMYT53	***S***	*250*	*117*	*114*	*230*	*N*	*126*	185	*130*	*127*	*N*
CIMMYT64	***S***	*250*	*117*	*117*	*215*	*250*	*126*	*188*	*130*	N	*230*
CIMMYT60	***MS***	*250*	*117*	*117*	*215*	*250*	*126*	*188*	*130*	*127*	*N*
CIMMYT58	***MS***	*250*	*117*	*117*	*215*	*250*	135	*188*	*130*	*127*	*N*
CIMMYT39	***S***	*250*	*117*	*117*	*215*	*250*	*N*	*188*	*130*	*127*	*N*
CIMMYT61	***MS***	*250*	*117*	119	*215*	*250*	*126*	*188*	*135*	*127*	*230*

Fig. 2 ***Outline for pyramiding Sr2, Sr13, and Sr25 in durum background using three streams (1, 2, and 3) of crosses. Marker assisted selection will be used for selecting recombinant plants along with selective phenotypic selection (PS) at later generations and to combine the different genes in stream 3.***



Gene selection: We completed a survey of available stem rust resistance genes in tetraploid wheat. We prioritized genes that conferred resistance to Ug99, were mapped, and for which molecular markers were available. Based on these criteria we selected resistance genes *Sr2*, *Sr13*, and *Sr25* as the initial targets for pyramiding.

Sr13 originated from *T. turgidum* var. *dicoccum* cv. Khapli and was transferred to several wheat cultivars grown worldwide (McIntosh et al.1995).

Sr2 was introgressed from *T. turgidum* var. *dicoccum* cv. Yaroslav and transferred into several wheat backgrounds. It provides partial resistance to Ug99 and this resistance has been effective for the last 80 years (McIntosh et al. 1995; Singh et al. 2006). The resistance conferred by *Sr2* alone is not sufficient and therefore, it is necessary to combine this gene with other *Sr* genes for full protection (Ayliffe et al. 2008).

Sr25 is present in a distal 7EL translocation from *Th. ponticum*. The 7EL translocation also carries leaf rust resistance gene *Lr19* and yellow flour pigment gene *Y* which is desirable in durum wheat (*T. turgidum* var. *durum*) (Zhang et al. 2005).

Pyramiding of stem rust resistance genes in tetraploid wheat

Recurrent parent: Breeding line UC1113 was selected as a recurrent parent for initial pyramiding. This line was selected for several reasons. Firstly, UC1113 is a high yielding semi-dwarf durum variety with a very broad adaptability in California. Secondly, we have already introgressed in the high-grain protein content gene *Gpc-B1* from wild wheat (Uauy et al. 2006) and the non-race-specific stripe rust resistance gene *Yr36* (Uauy et al. 2005) in this line. The incorporation of the *Gpc-B1* gene increased grain protein, iron and zinc by approximately 10%. Finally, UC1113 is a public breeding line that can be distributed without intellectual property constraints. The UC1113 line with *Gpc-B1* and *Yr36* genes has been already deposited in the USDA-ARS National Small Grain Collection (PI 638741) (Chicaiza et al. 2006). We will incorporate additional recurrent durum parents from Ethiopia when we receive the seeds.

Table 2 *Reaction of UC1113 and Kofa for 9 stem rust races*

Race	QTHJ	RCRS	RKQQ	TPMK	TTTT	TTKSK	TTKSK	TTKST	TTTSK
UC1113	;	;1N	;	3+;1	4	4	4	4	4
Kofa	;	;N	;	;1	;1	0;/2/4	2	2	2

Progress on pyramiding Sr2, Sr13 and Sr25 in durum wheat: The initial objective of the pyramiding work in tetraploid wheat is to combine the stem rust resistance genes *Sr2*, *Sr13* and *Sr25* in a common genetic background. The presence of multiple resistance genes is expected to extend the durability of the individual genes. *Th. elongatum* is the source of *Sr25,* Yaroslav the donor of *Sr2* and Khapli for *Sr13*. The *Th. elongatum* chromosome segment also carries the leaf rust resistance gene *Lr19*. We have completed the incorporation of the *Sr25/Lr19* genes together with the *Yr36/Gpc-B1* genes into UC1113 by six backcrosses.

The pyramiding will be carried out in three streams (Fig. 2). Stream 1 will be used to combine *Sr13* and *Sr25/Lr19*, and Stream 3 to combine *Sr2* and *Sr25/Lr19*. Backcrossing 1 and 3 will converge into Stream 2 to put the three genes together. The initial cross to combine the linked *Sr25/Lr19* genes in UC1113 background with *Sr2* (Yaroslav) was completed in July 2008. We are currently growing the F_1s to generate the first backcross generation to combine different genes in Stream 3.

For stream 1, parents are currently growing F_1s carrying the *Sr25/Lr19* and *Sr13* resistance genes (in addition to *Gpc-B1/Yr36*). A line homozygous for the three genes (BC_1F_2) should be available by mid 2010 (assuming 4 months per generation). Although this line would not be fixed for the rest of the genome, it will be a useful parental line, and will be distributed to all interested wheat breeding programs. We will continue the backcrossing program to obtain more stable lines for different gene combinations.

Mapping and marker development in tetraploid wheat

Sr13 is located on wheat chromosome 6AL (Klindworth et al. 2007; McIntosh et al. 1995). Unpublished results at USDA-ARS Biosciences Research Lab showed that *Sr13* is closely linked (≈2cM) to marker *Xwmc580* (Drs. Shiaoman Chao, North Dakota; and Evans Lagudah, CSIRO). The UCD group is collaborating with Drs. Chao and Lagudah to define precisely the location of *Sr13*.

The UCD group used a segregating population from the cross between tetraploid lines Kofa (resistant to TTTTF and TTKSK) and UC1113 (susceptible to TTTTF and TTKSK) to map *Sr13* (Table 2). The 93 SSD lines were scored against races TTKSK and TTTTF by Dr. Yue Jin and a single resistance genes, *Sr13*, was mapped at the end of chromosome 6AL between microsatellite markers *wmc580* and *dupw167* (Fig. 3). The complete molecular map has 269 markers (including 23 SNP) and a total length of 2,140 cM.

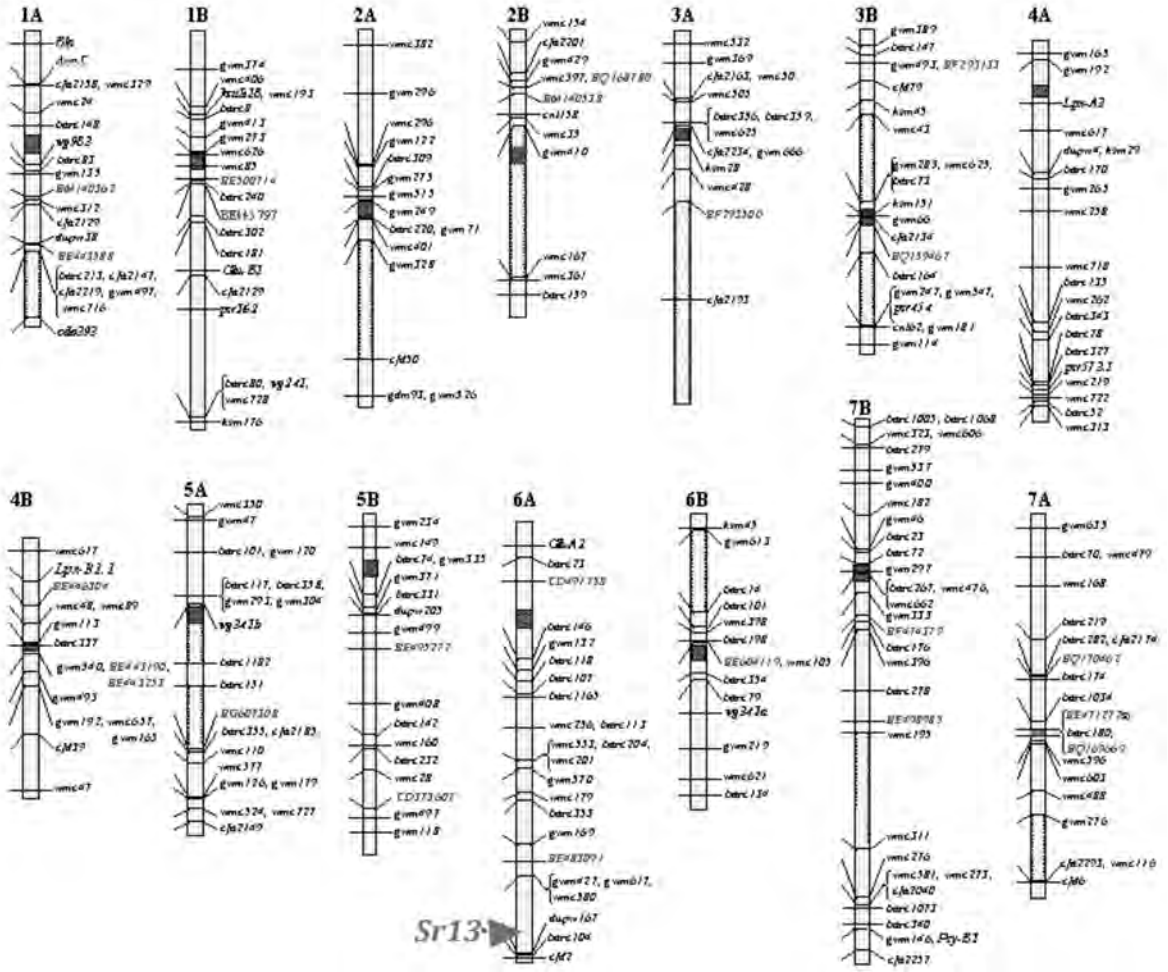
To refine the mapping position of *Sr13* we are backcrossing the *Sr13* region into UC1113 and generating BC_2F_2 segregating seed for high-density mapping. The F_2 plants will be screened to identify recombination events between *Sr13* flanking markers (Fig. 3). Seed will be saved from plants heterozygous at both flanking markers to generate seeds for a high density genetic map in order to identify a tightly linked diagnostic marker.

Finally, two additional populations are being developed for validation of the identity of *Sr13* using Khapli (CItr4013) as one of the parental lines. The crosses Rusty/Khapli and 47-1/Khapli have been completed and the F_1 plants are currently growing to produce F_2 seeds for validation. Once closer markers become available for *Sr13* we will check Kofa, Khapli, and Khapstein (the germplasm used to name *Sr13*) to confirm if they all have the same haplotype.

Sr2 is located on the short arm of wheat chromosome 3B (Hare and Mcintosh 1979) closely linked to microsatellite marker *Xgwm533* (Spielmeyer et al. 2003). Its recessive inheritance of resistance expressed at the adult plant stage complicates selection for this gene (Knott 1968). Drs. W. Spielmeyer and E. Lagudah developed a high-density genetic map of *Sr2* and designed a diagnostic marker for the gene. They have provided us access to this unpublished marker, and we have confirmed that it is diagnostic for *Sr2* in our germplasm. Using this marker we have confirmed the presence of *Sr2* in our seed source of Yaroslav (Fig. 4), which was essential for our pyramiding work.

Sr25 was mapped on the 7EL chromosome segment from *Th. ponticum* distal to the *Lr19* locus and close to the yellow pigment gene *Y* (*Lr19-Sr25/Y*). We developed a tetraploid line carrying *Sr25, Lr19*, and *Y* in the UC1113 genetic background (line 1-23, Fig. 5) (Zhang et al. 2005). This 7EL segment does not recombine with wheat chromosomes in the presence of *Ph1* so any marker for the 7EL segment works as a perfect marker for *Sr25* (Zhang et al. 2005; Zhang et al. 2008).

Fig. 3 *Genetic map based on a population of 93 single seed descent lines from Kofa (a Ug99 resistant line) / UC1113 (a susceptible high yielding advanced durum breeding line)*



To better define the map location of *Sr25* within the 7EL segment we sent the recombinant lines to Kenya and based on the resistance scores against Ug99 we mapped *Sr25* linked to the *Phytoene Synthetase 1* gene (*PSY1*) at the distal end of chromosome 7EL (Fig. 5).

Precise mapping of *Sr25* is important for the engineering of a shorter segment of 7EL carrying *Sr25* and also for the engineering of a chromosome segment combining *Lr19/Sr25* and barley yellow dwarf resistance gene *Bdv2*. *Bdv2* was mapped on the distal region of chromosome 7JL from *T. intermedium,* which is co-linear with the 7EL region where *Sr25* is located. In collaboration with Dr. Adam Lukaszewski (University of California, Riverside) we developed several lines carrying recombinant 7EL/7JL chromosomes and we started their characterization with molecular markers. We sent those lines to Kenya and to Dr. Yue Jin to test their reaction to Ug99.

We are also developing markers distal to *PSY1* to better define the position of *Sr25.* We have selected the most distal ESTs (BF483039, BG262960, and BF484041) and we are testing them for polymorphism between the 7E and 7J alleles.

Mapping and marker development in diploid wheat T. monococcum

In collaboration with Dr. Yue Jin we screened *T. monococcum* lines DV92 and G3116 (parents of the mapping population DV92 x G3116 (Dubcovsky et al. 1996) with seven different stem rust races including Ug99 (Table 3).

We developed a single seed descent mapping population from the cross DV92/G3116 (150 lines). Results from the evaluation of these lines with races RKQQC and TRTTF (Dr. Yue Jin) suggest segregation for 2 resistance genes (one of them probably *Sr21*).

Dr. Yue Jin has completed the screening of a large number of *T. monococcum* accessions and new crosses are being made. Once we clarify the genes segregating in the available populations we will use these new populations to identify new sources of resistance.

Fig. 4 ***The unpublished diagnostic marker for*** *Sr2* ***amplifies a ~400 bp band in Yaroslav (PI 2789) and Opata 85, but not in Langdon, confirming the presence of*** *Sr2* ***in Yaroslav (*** *Sr2* ***source of our pyramiding work).***

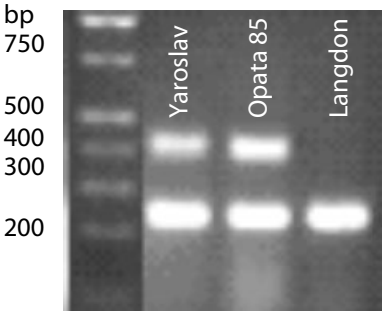


Table 3 ***Reaction of three*** *T. monococcum* ***accessions to different races of stem rust (Yue Jin 06/08, 01/08, 12/08)***

Race	G1777	G2528	G3116	DV92
TTTT	3	3-	3	-
TPMK	4	4	4	; 3, 4, very LIF
RKQQ	4	4	4	0
TTKSK	1/1 2Z	;12-	22+	0;
TTKST	; 1	; 1 2-	22 +	0 ;
TTTSK	; 1 2-	; 1	12	0 ;
TRTT	33+	33+	3+4	0

TTKSK= Ug99; **TTKST** = Ug99 + Sr24 virulence; **TTTSK** = Ug99 + Sr36 virulence; **TRTT**: race from Yemen with virulence on lines with 1AL.1RS. **LIF**, Low Infection Frequency; many leaves with IT 0, 1, or 2

Acknowledgements

We acknowledge the support and information provided by our colleagues Dr. Shiaoman Chao, Dr. Yue Jin, Dr. Adam Lukaszewski and Dr. Evans Lagudah for the preparation of this report

Section 3. Developing markers for the stem rust resistance genes *Sr33* and *Sr45*

(Contributed by P.K. Sambasivam, U.K. Bansal, H.S. Bariana and E.S. Lagudah)

Background

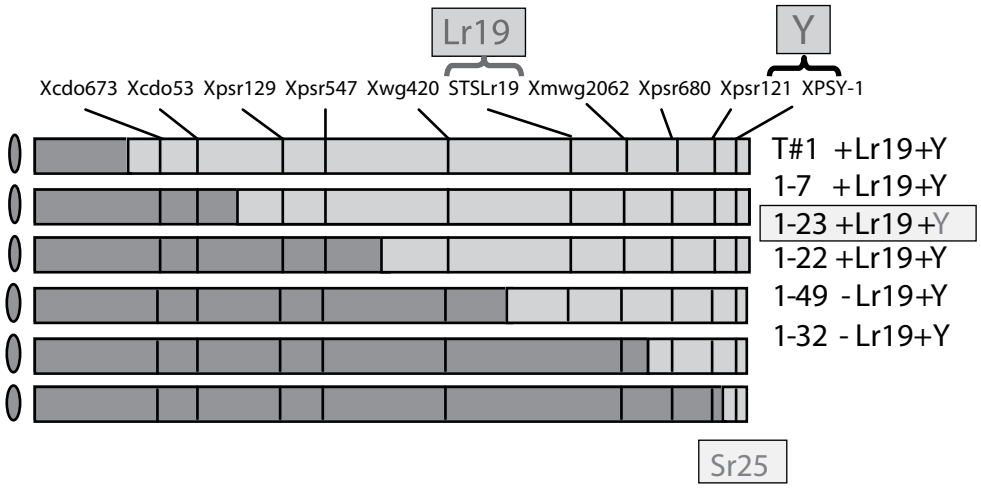
Diverse species from the Triticeae have been explored in attempts to identify new sources of resistance to stem rust. Resistance sources from the diploid D genome progenitor, *Aegilops tauschii*, are among species containing stem rust resistance genes that have been transferred to bread wheat. Three named genes derived from *A. tauschii* are *Sr33*, *Sr45*, *Sr46*; the first two genes are located on wheat chromosome 1DS and the third on 2DS. One of the advantages of genes derived from *A. tauschii* when compared with other wheat relatives, is the ease of gene transfer by homologous recombination with the corresponding D genome of hexaploid bread wheat. The vast germplasm resource of so-called synthetic hexaploids obtained from crossing tetraploid wheat with *A. tauschii* provides a potentially rich source of genetic variability for rust resistance. However, to ensure that unique resistance genes can be identified, they need to be differentiated from the known genes. A variety of methods that can be used include response of a given genotype to a range of pathogen isolates, chromosomal location / genetic analysis and the use of tightly linked or diagnostic markers. Similar infection types produced by resistance genes present in the tetraploid wheats used in creating the synthetic hexaploids can often confound the identification of new sources of resistance from *A. tauschii* in the absence of discriminating pathotypes. The availability of diagnostic markers for the known or named stem rust resistance genes provides a useful tool to establish the unique identities of putatively new sources of resistance. A diagnostic marker for the *Sr46* gene was recently developed (Lagudah unpublished).

Efforts to develop markers for *Sr33* and *Sr45* are in progress. *Sr33* has shown resistance to a wide range of stem rust isolates, and often shows an intermediate response. In tests conducted with Ug99, *Sr33* provided a moderate level of resistance under field conditions in Kenya (Jin et al. 2007). Stem rust isolates virulent for *Sr45* have previously been reported. In comparisons between *Sr33* and *Sr45* against isolates where both genes are effective, the latter often shows a stronger resistance response (McIntosh et al. 1995; Sambasivam et al. 2008). Seedling tests conducted using Ug99 (TTKS) have shown *Sr45* to be effective.

Genetic stocks and marker development

An important variable in the progress and success of marker development is the reliability of the phenotypic assessment. To ensure accurate phenotyping for *Sr33* and *Sr45*, we made use of genetic stocks where the respective genes were introduced into the wheat variety Chinese Spring (CS) as a single chromosome substitution line. The parental stocks for *Sr33* designated CS(1D5405) and *Sr45* designated as CS(1D5406) were tested using *Puccinia graminis* f. sp. *tritici* (Pgt) pathotype 34-1,2,3,4,5,6,7,11 (Plant Breeding Institute[PBI] culture no. 171) at the seedling stage, and pathotype 98-

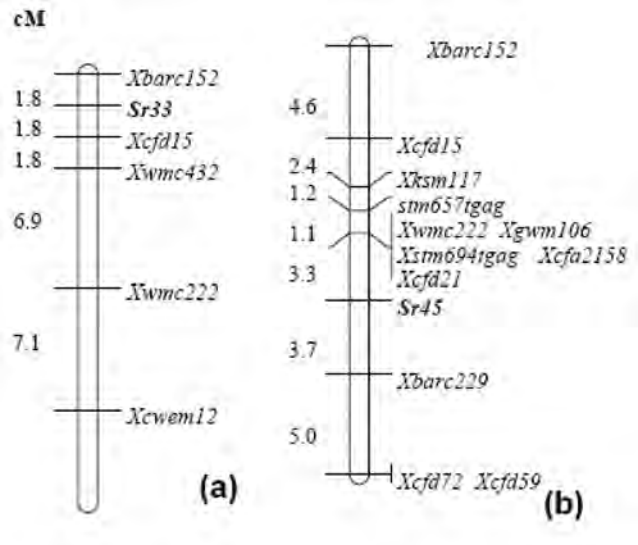
***Fig. 5 Mapping of** Sr25 **using recombinant lines carrying** Lr19 **and** Y*



1,2,3,5,6 (PBI culture no. 279) was used for adult plant stem rust assessment in Australia. Seedling infection types were scored on a 0 – 4 scale (McIntosh et al. 1995) and adult plant assessments were made on 1 – 9 scale (Bariana et al. 2007). CS1D5405 (*Sr33*) and CS1D5406 (*Sr45*) produced low infection types (IT) IT2= and IT;;1-, respectively, when tested against Pgt pathotype 34-1,2,3,4,5,6,7,11 at the seedling stage. The susceptible parent Chinese Spring displayed a susceptible response (IT3+). At the adult plant stage, resistant parents produced a rust response of 4 (CS1D5406) and 5 to 6 (CS1D5405), whereas the susceptible parent produced a high field response of 8. A low resolution mapping family based on recombinant inbred lines (<100) from the crosses CS(1D5405)/CS and CS(1D5406)/CS were phenotyped at the seedling and adult stages. A perfect correlation between the seedling and adult rust responses was obtained. Using microsatellite markers from 1DS, framework maps around the *Sr33* and *Sr45* regions were developed (Fig. 6). It was apparent that the genetic map resolution was very low as several markers immediately distal to *Sr45* could not be separated by recombination.

To increase the resolution, additional mapping families from CS ditelo 1DS/CS(1D5405) and CS ditelo1DS/CS(1D5406) (kindly supplied by J. Dvorak, UC Davis) were used to recover recombination events that separated the cluster of markers (Fig. 6b). We also developed large F_2 populations (>2,000) from CS(1D5405)/CS and CS(1D5406)/CS for *Sr33* and *Sr45*, respectively, and those will be used in constructing high resolution mapping families. The ditelocentric families will not be used in the high resolution mapping because of the anomalous chromosome transmission of aneuploidy. Additional markers were developed by exploring the co-linearity of ESTs on wheat group 1 with rice chromosome 5. None of the ESTs mapped in the vicinity of *Sr33* or *Sr45,* and all were located in more proximal regions. Thus, the targeted *Sr33* and *Sr45* regions show negligible co-linearity with the predicted syntenic region of rice chromosome 5. Comparative maps of wheat and its relatives in the group1S chromosomal region were examined for additional markers; however, there are very few markers in the *Sr33* and *Sr45* regions. We have since isolated more wheat ESTs and through RFLP analysis have identified markers closely linked to *Sr33* and *Sr45*. These markers will be tested on the high resolution mapping family, and if shown to be tightly linked, will provide entry points towards developing markers suitable for breeding applications.

Fig. 6 ***Low resolution genetic maps of the** Sr33 **and** Sr45 **regions in wheat chromosome 1DS (Sambasivam et al. 2008)***



Section 4. Developing diagnostic markers for *Sr25* and *Sr26* and pyramiding *Sr25* and *Sr26* (*Contributed by Sixin Liu and James A. Anderson*)

Materials and Methods

Eight wheat lines including two lines with gene *Sr25*, 'Wheatear' and CIMMYT line C80.1/3*Batavia//2*Weebil, gene *Sr26* line Eagle, and five lines without gene *Sr25* or *Sr26*, 'Cranbrook', 'Weebil', MN02072-7, MN03130-1-62 and MN03148, were used to validate published markers for *Sr25* and *Sr26* (Table 4). The PCR protocols were the same as described by Liu and Anderson (2003) with the exception of 400 nM instead of 100 nM for each primer. The PCR products were separated on 3% agarose gels and visualized with ethidium bromide under UV light.

Wheat ESTs mapped to deletion bin 6AL8-0.90-1.00 (Qi et al. 2004) were used to design STS (sequence tagged site) markers with software Primer 3. Chromosome 6AL-specific markers were identified with aneuploid analysis.

Results

Marker Gb (Prins et al. 2001) for gene *Sr25* and *Sr26*#43 (Mago et al. 2005) for gene *Sr26* were validated with eight wheat lines. As expected, a faint 130 bp fragment was amplified with marker Gb in the two lines with *Sr25*, Wheatear and C80.1/3*Batavia//2*Weebil. The other six lines without *Sr25* did not amplify any detectable fragment with these primers. Similarly, only cultivar Eagle was positive for marker *Sr26*#43 and no PCR product was observed for the other seven lines. Since co-dominant markers are needed to distinguish homozygous plants from heterozygous plants, we decided to develop and test co-dominant markers for genes *Sr25* and *Sr26*.

Ayala-Navarrete et al. (2007) developed STS markers from wheat ESTs mapped to chromosome 7DL that is homoeologous to the translocated segment of *Th. elongatum* containing *Sr25* and *Lr19*. Among the six markers tested on the eight wheat lines, BE404744 and BF145935 were co-dominant in marking *Sr25*. We tested both markers with additional lines used to pyramid *Sr25* and *Sr26*. Both markers were diagnostic for *Sr25*. However, marker BF145935 consistently worked well and was easier to score. We successfully genotyped five F_2 populations segregating for *Sr25* with marker BF145935. Therefore, marker BF145935 is a robust co-dominant marker for *Sr25*.

Dundas et al. (2007) reported that *Sr26* is located in the extreme distal portion of the 6Ae#1 chromosome. Sixteen wheat ESTs mapped to deletion bin 6AL8-0.90-1.00 were selected to design STS markers. None of the 16 STS markers were co-dominant between lines with or without *Sr26*, but six markers specific to chromosome 6AL amplified no PCR product from Eagle. We reasoned that multiplex PCR with the combination of one 6AL-specific marker and *Sr26*-specific marker *Sr26*#43 could be used to distinguish *Sr26* homozygotes from heterozygotes. Because the 6AL-specific marker UMN6A-5 (Table 4) consistently worked well and amplified a 320 bp fragment from lines without *Sr26*, we combined equal amounts of primer for marker UMN6A-5 and *Sr26*#43 to genotype an F_2 population segregating for *Sr26*. For the heterozygous plants, the 320 bp allele was weaker than the 207 bp allele amplified with primer *Sr26*#43. After doubling the primer concentration for marker UMN6A-5, the 6A-specific allele and the *Sr26*-specific allele were amplified with similar intensities. Two F_2 populations segregating for *Sr26* were successfully genotyped with this optimized, multiplex PCR.

One of the goals of this project is to combine *Sr25* and *Sr26* in the genetic background of four high yielding CIMMYT lines that are well-adapted to Ug99-affected and surrounding regions and already possess at least moderate adult plant resistance to Ug99. In fall 2008, we made F_1 hybrids between each of the CIMMYT lines and lines with *Sr25* or *Sr26*. Complex F_1's will be made this season and lines containing *Sr2*, *Sr25* and *Sr26* will be selected using co-dominant markers.

Section 5. Mapping of new sources of race-specific resistance in CIMMYT-derived spring wheat

(Contributed by Sridhar Bhavani and Ravi P. Singh)

Summary: Genomic regions for three putative, novel race-specific resistance genes, temporarily designated as *SrA, SrB* and *SrC*, present in three CIMMYT derived spring wheats were determined. Gene *SrA* mapped on 3DL (linked markers *Xgwm52, Xgwm341*), *SrB* on 3BS (*Xgwm566, Wmc231*) and *SrC* on 5DL (*Xgwm292, Xgwm212)*.

Mapping of new sources of race-specific resistance in CIMMYT-derived spring wheats

About 150 F_3 or F_4 lines derived from the crosses of susceptible parent PBW343 with three resistant parents, viz. 'Milan/Sha7/3/Thb/CEP7780//Sha4/Lira/4/Sha4/Chil', 'Ning9415/3/Ures/Bow//Opata/4/Ningmai 7' and 'Chen/Ae.Sq//2*Weaver/3/Oasis /5*Borl95' suspected to carry novel race-specific resistance genes, were characterized for stem rust responses in the field at Njoro during 2008 and in the USDA-ARS greenhouse by Dr. Y. Jin. The three resistant parents displayed infection types 2- or 2 in seedling tests and MR to MR-MS (moderately

Table 4 *DNA markers for stem rust resistance gene Sr25 and Sr26*

Marker	Forward primer	Reverse primer	Reference
Sr25			
Gb	CAT CCT TGG GGA CCT C	CCA GCT CGC ATA CAT CCA	Prins et al. 2001
BF145935	CTTCACCTCCAAGGAGTTCCAC	GCGTACCTGATCACCACCTTGAAGG	Ayala-Navarrete et al. 2007
Sr26			
Sr26#43	AATCGTCCACATTGGCTTCT	CGCAACAAAATCATGCACTA	Mago et al. 2005
UMN6A-5	TCGTTTGGGAACACAGCTTA	CCGGCGAAATATAATGCAAA	This study

resistant-moderately susceptible) reactions in the field. Lines clearly identified as homozygous resistant (HR) or homozygous susceptible (HS) were used in mapping and results obtained so far are summarized below.

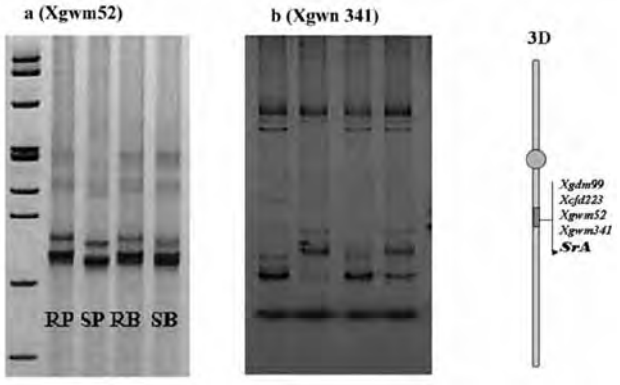
PBW343 X Milan/Sha7/3/Thb/CEP7780//Sha4/Lira/4/Sha4/Chil – F_3 Population

Bulk segregant analysis (BSA) identified SSR markers *Xgwm52* and *Xgwm341* showing linked polymorphisms between the parents and bulks (Fig. 7a, b). These markers were mapped on 96 F_3 families (HR & HS). Markers *Xgwm341* and *Xgwm52* mapped 8.6cM and 15.6cM, respectively, from the resistance locus on the long arm of chromosome 3D. The gene was temporarily designated *SrA*.

PBW343 X Ning9415/3/Ures/Bow//Opata/4/Ningmai 7 - F_4 Population

SSR markers *Xgwm566* and *Wmc231* showed linked polymorphisms between the parents and bulks (Fig. 8a, b). These linked markers were mapped on 72 F3 families (HR & HS). Markers *Xgwm566* and *Xwmc231* were mapped 12.5cM and 18cM, respectively, from the resistance gene on the short arm of chromosome 3B. The gene was temporarily designated as *SrB*.

Fig. 7 ***Molecular markers linked to stem rust resistance gene SrA located on chromosome 3DL***



PBW343 X Chen/Ae.Sq//2*Weaver/3/Oasis/5*Borl 95 - F_3 Population

Chromosome 5DL-located markers *Xgwm292* and *Xgwm212* showed linked polymorphism between parents and bulks (Fig. 9a, b). These linked markers were mapped on 47 F4 families (HR & HS). SSR markers *Xgwm292* and X*gwm212* mapped 10.2cM and 16.1cM away from the resistance locus, respectively, on the long arm of chromosome 5D. The gene was designated *SrC*. This population also showed segregation for stem rust resistance gene *Sr2*. SSR markers *Xgwm493* and *Xgwm533* (Fig. 9c) showed linked polymorphisms between the parents and bulks. It is likely that the HR lines we used carried both *Sr2* and *SrC* and that HS lines lacked them.

The majority of the stem rust resistance genes characterized so far produce intermediate infection types 2 to 2+ at the seedling stage (*Sr7a, 7b, 8a, 9a ,9b, 9d, 9f, 9g, 13, 16, 20, 22, 23, 24, 25, 26, 29, 30, 31, 32, 33, 34 and 39*). The genes characterized during this study also provide moderate levels of resistance to stem

Fig. 8 ***Molecular markers linked to stem rust resistance gene SrB located on chromosome 3BS***

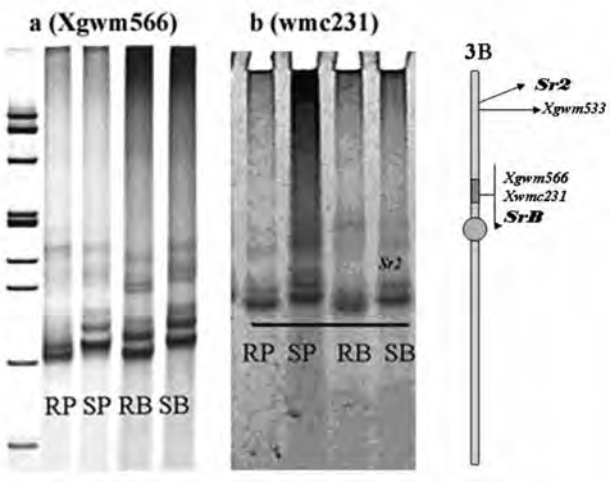
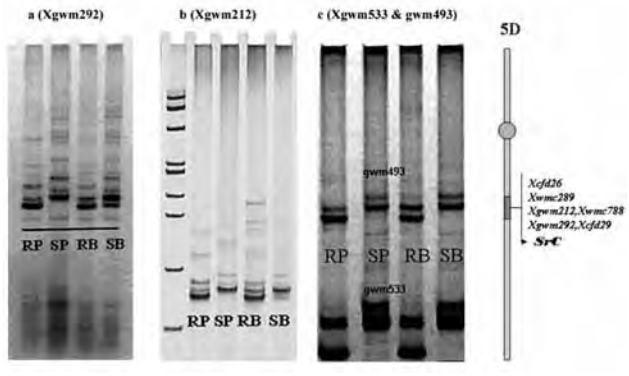


Fig. 9 ***Molecular markers linked to stem rust resistance gene*** *SrC* ***located on chromosome 5DL***



rust at the seedling stage and acceptable to moderate levels or resistance at the adult stage. The genomic regions identified in this study (3DL, 3BS and 5DL) likely represent new genes and sources of resistance to Ug99. Selected F_3 or F_4 lines that were segregating for the above genes are being used in developing new mapping populations in an attempt to remove maturity effects that caused difficulties in phenotyping stem rust responses in the field in Kenya. These populations will also be used for identifying tightly linked molecular markers for use in marker-assisted breeding.

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19. Breeding for minor gene-based adult plant resistance to stem rust in wheat

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Abstract

An attractive strategy for a long-term control of Ug99 and other races of the wheat stem rust pathogen is to develop and deploy varieties that have durable, or race-nonspecific, adult plant resistance (APR) conferred by multiple minor genes. Screening of spring wheat materials in Kenya under high stem rust pressure, and characterization of seedling reactions to infection in the greenhouse, resulted in the identification of a low frequency of high-yielding lines with high to adequate levels of APR. Slow rusting APR gene *Sr2* is present in these materials and is therefore an important component of the complex adult-plant resistance, commonly known as the "*Sr2*-complex". Simple, single-backcross and top (3-way) crosses followed by a selected-bulk selection scheme are being used at CIMMYT to develop new high-yielding wheat materials with high levels of APR to stem and other rusts, as well as additional necessary traits. We have adopted a shuttle breeding strategy using the Ciudad Obregon and Toluca breeding sites in Mexico and Njoro in Kenya. The segregating populations are selected at Njoro for two generations before making final plant selections at Cd. Obregon. Advanced lines retained in Mexico are then phenotyped for stem rust response at Njoro, and grain yield performance, end-use quality and other traits are determined in Mexico. The first products of the breeding scheme are currently (2008-09 crop season) being characterized for yield potential and stem rust resistance at Cd. Obregon and Njoro, respectively.

Keywords

Triticum aestivum, *Puccinia graminis tritici*, Ug99, shuttle breeding, durable resistance**.**

Introduction

Stem, or black, rust of wheat, caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., periodically causes severe devastation of wheat crops, and is the most feared disease in many countries where wheat is grown. Strong emphases on the identification of sources of resistance to stem rust and on breeding resistant varieties were earlier adopted in the USA, Canada, Australia and parts of Europe. Although the major epidemic of 1916 in the USA and Canada had already triggered extensive research on stem rust, efforts in the US, Canada and Australia were intensified further with epidemics during the following decades. Initially, resistances present in some hexaploid wheats were used, but the most successful control was achieved when H.K. Hayes at the University of Minnesota, and E.S. McFadden at South Dakota State University, transferred stem rust resistance from the tetraploid 'Iumillo' durum and 'Yaroslav' emmer, respectively, to bread wheat resulting in the hexaploid varieties 'Thatcher' and 'Hope' (Kolmer 2001). Although some race-specific resistance genes are present in Hope and Thatcher, the most effective components of resistance in both varieties were genes conferring adult plant resistance (APR). Thatcher and Hope (and Hope sib 'H44-24a') and other varieties derived from them, such as 'Selkirk' and 'Chris' that combined resistance genes from other sources including *Sr6* found in a single plant selection made by J. McMurachy in 1930. 'Kenya 58' and other Kenyan varieties carrying *Sr6* were also used extensively in Australia by S.L. Macindoe, and in Mexico by N.E. Borlaug in addition to Hope and Thatcher derivatives. Efforts to find a solution to the stem rust problems facilitated global collaboration amongst wheat scientists via the International Rust Nurseries to share, grow and evaluate wheat germplasm in the quest of finding different sources of resistance to stem rust. Resistant wheat germplasm developed at Njoro, Kenya, through support from Canadian scientists in the 1960s and 1970s, contributed substantially to international breeding efforts.

The APR gene *Sr2*, transferred to Hope and H44-24a from Yaroslav and possibly to 'Khapstein' from 'Khapli' emmer by W.L. Waterhouse in Australia, conferred slow rusting. Combinations of *Sr2* with other unknown slow rusting resistance genes possibly originating from Thatcher and the Thatcher-derived Chris, commonly known as the "*Sr2*-complex", provided the foundation of durable resistance to stem rust in germplasm from the University of Minnesota, in the U.S.A, Sydney University in Australia, and spring wheat germplasm developed by N.E. Borlaug (McIntosh 1988; Rajaram et al. 1988). *Sr2* can be detected through its complete linkage with the pseudo-black chaff (PBC) phenotype; however, excessive expression of PBC in certain environments sometimes leads to its elimination in breeding programs. Under the same environmental conditions, negligible to

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high expression of PBC is often observed in advanced breeding materials indicating that selection of lines with *Sr2* and negligible PBC is possible. Knott (1982, 1988) showed that adequate levels of multigenic resistance to stem rust could be achieved by accumulating approximately five minor resistance genes.

With the exception of *Sr2*, unfortunately, not much is known about the other genes involved in the *Sr2* complex and their interactions. However earlier work by Knott (1982, 1988), knowledge on durable resistances to leaf and yellow rusts (Singh et al. 2004), and observations made on breeding materials and a F_6 mapping population involving 'Pavon 76' indicate that the rate of rust progress is a function of both the individual and accumulative effects of minor genes (Fig. 1). The accumulation of between 4 and 5 minor genes is likely to delay disease progress to negligible disease levels at maturity under conditions of high disease pressure. This phenotype was described as "near-immunity" by Singh et al. (2000).

Although some of the old tall varieties from Kenya, Canada and US continue to be resistant to stem rust in Kenya under Ug99 pressure, it is important to utilize improved semidwarf wheat materials with APR to continue making breeding progress and to develop new wheat materials that have potential to replace current popular varieties in the shortest possible timeframe. To achieve this objective, the following two approaches are being used:

1. Characterizing the existing semidwarf wheat materials for resistance to stem rust in the field at Njoro, Kenya, and subsequently characterizing the identified resistant materials for seedling reaction to Ug99 (TTKSK) and/or the *Sr24*-virulent variant of Ug99 (TTKST) in the greenhouse. Wheat materials with resistance in the field, but with susceptible seedling reactions, are considered to possess APR.

2. Directed transfer of APR to adapted varieties and high yielding wheat backgrounds through a single-backcross approach, intercrossing (simple and three-way crosses) of high-yielding materials with intermediate or high levels of APR, and a Mexico-Kenya shuttle breeding of selected-bulk populations in segregating generations. Both types of crosses are expected to result in new wheat germplasm with enhanced yield potential and high to adequate levels of APR to stem and other rusts in combination with other essential traits.

Adult plant resistance to Ug99 in improved semidwarf wheats

Organized testing to identify sources of resistance to stem rust was initiated in Kenya and Ethiopia in 2005. The Njoro research station of the Kenyan Agricultural Research Institute is currently being used for screening bread wheat due to the presence of *Sr24* virulent variant TTKST. Two selection cycles can be achieved annually. Information on resistant spring wheat germplasm distributed worldwide by CIMMYT during 2006, 2007 and 2008, through the 1st, 2nd and 3rd Stem Rust Resistance Screening Nurseries (SRRSN) is summarized in Table 1. Totals of 29 (28%), 48 (37%) and 67 (65%) lines in these three nurseries, respectively, have shown from high to moderate levels (up to 30% stem rust severity) of resistance when susceptible entries have been annihilated (100% severity) over two seasons of evaluation. Entries included in the 2nd and 3rd SRRSN have high yield potential in combination with other desirable traits. These improved wheat materials have the potential to be released directly, or to be used by breeding programs worldwide.

Some of the best materials with APR included in the 1st, 2nd and 3rd SRRSNs are listed in Tables 2, 3 and 4, respectively, along with their field stem rust ratings. Wheat lines such as 'Kingbird', 'Pavon F76' and 'Kiritati' have maintained high to adequate levels of APR. Kingbird and several other advanced lines with APR have Pavon F76 and Kiritati in their pedigrees and carry slow rusting gene *Sr2*. Kingbird has shown a high level of APR in field tests conducted during the last six crop seasons at Njoro, including 2008 main-season, which was characterized by very high stem rust pressure. The genetic basis of the 'near-immune' APR in Kingbird is not known; however, a mapping population has been developed to identify genomic regions contributing to it. Like Pavon 76, 'Parula', another *Rht2*- carrying wheat bred in the late 1970s, has shown a high level of APR to Ug99 in evaluations conducted during 2007 and 2008. Kingbird and Kiritati are more recent wheats carrying *Rht1* and *Rht2*, respectively.

Fig. 1 Graphical representation of the additive effects from estimated numbers of minor genes in retarding rust progress in the field

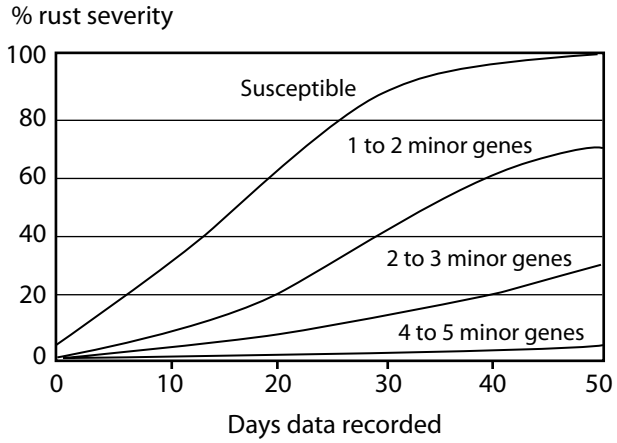

Table 1 *Stem rust resistance of entries included in 1st, 2nd and 3rd SRRSN (Stem Rust Resistance Screening Nursery)*

Resistance category	1st SRRSN		2nd SRRSN		3rd SRRSN	
	Number	%	Number	%	Number	%
Adult-plant[1]:						
R (5-10% severity)	4	4	0	0	3	3
R-MR (15-20% severity)	19	18	26	20	33	32
MR (30% severity)	6	6	22	17	21	20
MR-MS (40% severity)	2	2	15	12	0	0
MS (50-60% severity)	0	0	17	13	0	0
S (70-100% severity)	0	0	4	3	0	0
Race-specific:						
Sr24	39	38	0	0	0	0
Sr25	17	17	0	0	11	11
Sr36 (+*Sr24*)	0	0	0	0	4	4
Sr1A.1R (+*Sr24*)	2	2				
SrTmp	0	0	25	20	6	6
SrSynt	4	4	8	6	5	5
SrSha7	9	9	8	6	4	5
SrND643	0	0	0	0	12	12
SrUnknown	1	1	3	2	5	5
Total	103		128		104	

[1] Adult plant resistance categories include lines that were susceptible in seedling greenhouse tests and with the highest rating during multiple years/seasons (2005-07) when susceptible entries were dead. Ratings based on the modified Cobb Scale as described in Roelfs et al. (1992)

Shuttle breeding to develop high-yielding wheat with APR to stem rust

Because a large proportion of high-yielding spring wheat varieties and germplasms do not carry effective race-specific stem rust resistances to race Ug99, the availability of genotypes with moderate to high levels of APR provide opportunities to reconstitute high levels of adult plant resistance in newer hybrid populations. In the absence of molecular markers for adult plant resistance genes and the absence of Ug99 in Mexico, a shuttle breeding scheme between Mexican field sites (Ciudad Obregon in northwestern Mexico during winter, and Toluca or El Batan in the highlands near Mexico City during summer) and Njoro, Kenya, was initiated in 2006 to build adult-plant resistances in modern semidwarf wheats. Two crop seasons per year in both Mexico and Kenya halve the number of years required to generate and test advanced breeding lines. The "single-backcross, selected-bulk" breeding approach (Singh and Trethowan 2007) is being applied for transferring multiple minor genes to adapted backgrounds. Simple and three-way crosses, where one or more parents carry adult-plant resistance, are being used to breed new high-yielding, near-immune wheat materials. The flow of breeding materials in the "Mexico-Kenya Shuttle" is described in Table 5.

In the single-backcross approach, we cross the resistance sources with adapted high yielding wheats. A single backcross is made with the recurrent parent to obtain 350-400 BC_1 seeds. The BC_1 plants are selected for desired agronomic features and resistance to leaf rust and stripe rust, and harvested as bulk in Mexico.

Table 2 *Some wheat lines with adult plant resistance (APR) to stem rust in the 1st Stem Rust Resistance Screening Nursery distributed by CIMMYT during 2006-07 with field ratings to stem rust in three crop seasons at Njoro, Kenya*

Entry No.	Cross	Stem rust rating[1]		
		2005	2006	2007
6032	Babax/3/Oasis/SKauz//4*Bcn/4/Pastor	15 MR	30 MSS	10 MSS
6034	Cndo/R143//Ente/Mexi2/3/Ae. Sq./4/Weaver/5/2*Kauz/6/Fret2	20 S	20 M	20 MSS
6036	Fret2*2/Kukuna	20 MSS	20 M	30 M
6039	Hpo/Tan//Vee/3/2*Pgo/4/Milan/5/SSeri1	5 MR	40 MSS	10 MSS
6041	Kamb1*2/Khvaki	15 MS	20 M	10 MSS
6042	Kiritati	10 MR	15 M	10 MS
6044	Pastor/Milan	30 MSS	15 S	5 S
6045	Pavon F76	5 MR	15 M	5 M
6050	Pfau/Weaver*2//Kiritati	10 MR	10 M	5 MSS
6056	Pgo//Croc1/Ae.Sq. (224)/3/2*Borlaug F95/4/Circus	5 M	10 M	10 MS
6057	Pgo/Seri//Bav92	15 MR	15 M	10 MSS
6058	Pvn//Car422/Ana/5/Bow/Crow//Buc/Pvn/3/Yr/4/Trap#1	20 MS	30 M	10 MS
6061	Kingbird	5 MR	5 M	5 MSS
	Susceptible entries	100 S	100 S	100 S(N)

[1] The stem rust rating has two components: disease severity based on the modified Cobb Scale and host reaction, both described in Roelfs et al. (1992)

F_2 plants derived from the BC_1, simple, and top (3-way) crosses with desired agronomic features and resistance to leaf rust and stripe rust are selected for agronomic traits and resistance to other diseases at Cd. Obregon or Toluca and harvested as bulks. If the F_2 populations were grown at Cd. Obregon, where the quarantine disease "Karnal bunt" may occur, the F_3 populations are grown at Toluca for another round of selection. About 1,000 seeds of each F_3 and F_4 population obtained from the Toluca harvest are grown densely at Njoro for selection under high stem rust pressure during the off-season. Populations not carrying sufficient resistant plants are discarded. In populations that segregate for height, tall plants are removed by cutting their spikes. The remaining plants are bulk-harvested and plump grains are selected for establishing F_4 and F_5 populations of about 400 plants during the main season at Njoro under high stem rust pressure. Because stem rust affects grain filling, we expect plants with insufficient resistance to have shriveled grains. About 400 plump seeds harvested from the selected plants are returned to Mexico and grown at Cd. Obregon for final selection as individual plants in the F_5 and F_6 generations. Small plots of advanced lines obtained by selecting individual plants in Cd. Obregon are grown at the El Batan and Toluca field sites to select for agronomic characteristics and resistance to leaf rust and stripe rust.

Products of the 1st set of shuttle breeding materials, about one thousand advanced lines, were planted at Cd. Obregon during the 2008-09 crop season to determine yield performance and other traits. These lines are also planted at Njoro and Santa Catalina, Ecuador, for further assessment of stem rust and stripe rust responses, respectively. In addition, over 800 lines from the same crosses, but without selection at Njoro, are included in the yield trials and in stem and stripe rust phenotyping to determine the progress gained by the Mexico-Kenya shuttle. The second set of 290 F_5 and F_6 populations generated through Mexico-Kenya shuttle breeding are also planted at Cd. Obregon and the 3rd set of 1,195 F_3 and F_4 populations are planted at Njoro during the current crop season. We expect that the frequency of advanced lines with high yield potential, wide adaptation, end-use quality characteristics and high or adequate levels of resistance to all three rusts will increase over time through the use of the Mexico-Kenya shuttle program. This approach is expected to rebuild durable APR in modern semidwarf wheat germplasm.

Table 3 *Some wheat lines with adult plant resistance (APR) to stem rust in the 2nd Stem Rust Resistance Screening Nursery distributed by CIMMYT during 2007-08 with field ratings to stem rust in three crop seasons at Njoro, Kenya*

Entry No.	Cross	Stem rust rating[1]		
		2007 Off-season	2007 Main-season	2008 Main-season
6026	Barbet1*2/Kiritati	30 MSS	30 MSS	30 M
6036	Galvez/Weebill1	10 M	15 M	30 M
6044	Kiritati//PBW65/2*Seri.1B	20 M	20 M	40 M
6048	Kiritati//Seri/Rayon	20 M	15 M	30 M
6049	Kiritati/4/Seri.1B*2/3/Kauz*2/Bow//Kauz	30 MSS	10 M	30 M
6057	Pfau/Weaver*2//Chapio	20 M	20 M	30 MSS
6059	Pfau/Weaver*2//Pavon 7S3	20 M	30 MSS	20 MSS
6070	Thelin/2*Waxwing	15 M	10 MS	30 M
6077	Pauraque	20 M	30 MSS	40 M
6085	Grackle	30 M	30 MSS	40 M
60101	Becard	20 M	15 M	30 M
6113	Inqualab 91*2/Kukuna	-	-	30 M
6114	Kiritati	15 M	-	15 M
6118	Pavon F76	10 M	-	15 M
6119	PBW343*2/Kukuna	-	-	30 M
6122	Pfau/Weaver//Kiritati	10 MSS	-	30 M
6123	Pvn//Car422/Ana/5/Bow/Crow//Buc/Pvn/3/Yr/4/Trap#1	5 MS	-	30 M
6125	Kingbird	5 MSS	-	5 M
6126	Temporalers M87*2/Chos	-	-	5 M
6130	Tepoca+LR34/2*Borlaug F95	-	-	20 MSS
6137	Cacuke (Susceptible Check)	100 S	100 S(N)	100 S(N)

[1] The stem rust rating has two components: disease severity based on the modified Cobb Scale and host reaction, both described in Roelfs et al. (1992)

Performance of high-yielding wheats with APR to stem rust and seed multiplication

The scheme presented in Table 5 delivers material for CIMMYT international yield trials and screening nurseries to co-operators in the 7th year. However, the best Ug99 resistant advanced lines were delivered to high risk countries during the current and past two cropping seasons for growing in the 6th year after crosses were made, through a nursery called the Elite Bread Wheat Trial (EBWYT). To achieve this we used one year of grain yield data combined with agronomic characteristics, quality and disease resistance information and seed was multiplied at El Batan for distribution. The EBWYT trial has space for 30 entries (including checks) with 3 replicates arranged in an alpha-lattice design. The 1stEBWYT was used initially to distribute new lines with APR to leaf rust and stripe rust, but 2nd and 3rd EBWYT also include lines with Ug99 resistance.

Eleven entries in the 2nd EBWYT had APR in the following categories (based on at least two seasons of data from Njoro and final scores recorded when the susceptible check was dead (100% severity): R-MR (15-20% severity) = 4, MR (30% severity) = 4, MR-MS (40% severity) = 3. The resistance of two entries each

Table 4 *Some wheat lines with adult plant resistance (APR) to stem rust in the 3rd Stem Rust Resistance Screening Nursery distributed by CIMMYT during 2008-2009 with field ratings to stem rust in three crop seasons at Njoro, Kenya*

Entry No.	Cross	Stem rust rating[1]			
		2007 Off-season	2007 Main-season	2008 Off-season	2008 Main-season
6006	Pauraque	20 M	10MS	30M	40 M
6015	Picaflor	20 M	20M	20S	30 MSS
6027	Danphe	10 MS	20MSS	15MSS	20 MSS
6030	Kiritati/4/2*Seri.1B*2/3/Kauz*2/Bow//Kauz	20 MSS	20MSS	30MSS	30 M
6032	Kiritati//2*Seri/Rayon	15 M	10M	30S	20 MSS
6053	Seri.1B*2/3/Kauz*2/Bow//Kauz/4/Varis	20 MSS	5MSS	30S	40 M
6059	Kinde	10 MSS	10MSS	30S	40 MSS
6061	Inqualab 91*2/Kukuna//Kiritati	15 M	5M	10M	30 MR
6066	Jup/Zp//Coc/3/Pvn/4/Tnmu/5/Tnmu/6/Site/7/Tnmu	30 MSS	20MSS	20MSS	20 MSS
6078	Galvez/Fret2	20 MSS	20 MSS	30S	40 S
6083	NS732/Her/3/Prl/Sara//Tsi/Vee#5/4/Fret2	30 MSS	20 MSS	20S	40 MSS
	Cacuke (Susceptible Check)	100 S	100 S(N)	100S	100S(N)

[1] The stem rust rating has two components: disease severity based on the modified Cobb Scale and host reaction, both described in Roelfs et al. (1992)

was based on *Sr25* and *SrTmp*, a temporary designation for an undesignated gene derived from 'Triumph'. Similarly, 21 entries in the 3rd EBWYT had APR in the following categories: R (<10% severity) = 1, R-MR = 15, MR = 5. Resistance in four entries was based on *Sr25*, two on *SrTmp* and one entry possesses a resistance gene from 'HUW234+*Lr34*', temporarily designated as *SrHuw234*. Using the 2008 stem rust data from Njoro, a season marked by the most severe stem rust seen since 2005, the 21 entries with APR in the 4th EBWYT were classified: R-MR (30% severity) = 6, MR = 40% severity, and MR-MS (50% severity) = 4. In addition, four entries carry *Sr25* and one possesses *SrHuw234*. The two best performing entries from the 2nd and 3rd EBWYT were included as CIMMYT checks in the 4th EBWYT and both carry APR to stem rust.

Eleven wheat lines (Table 6) were selected based on grain yield performance data in the 2nd and/or 3rd EBWYT trials from various countries, and were included in a seed production program implemented during the 2008-09 crop season. Seed was provided from Mexico. Eight of the 11 entries carry APR to stem rust. Additional Ug99-resistant entries were also chosen by Egypt and Afghanistan. Iran has initiated multiplication of three entries from the 2nd EBWYT on its own initiative. Countries participating in the seed multiplication program will conduct further yield performance evaluations while simultaneously multiplying the seed. This approach is likely to result in the release of at least one variety in each of a number of countries that should have sufficient seed for rapid seed multiplication after official release.

Conclusions

Significant progress has been made in identifying and utilizing APR to stem rust in CIMMYT-derived spring bread wheats since the launch of Borlaug Global Rust Initiative and initiation of a screening program at Njoro, Kenya, in 2005. The Mexico-Kenya shuttle breeding strategy currently used should result in the accumulation of adequate APR to stem rust in modern high-yielding wheat germplasm with higher yield potential than current popular varieties. The initiation of multiplication and release of Ug99-resistant materials, especially those with APR, in various countries should reduce the Ug99

threat and help to achieve durable resistance. The identification of molecular markers, tightly linked to the minor genes contributing to APR, should improve breeding efficiency especially when it is not possible to shuttle segregating materials to field sites exposed to Ug99 epidemics.

Acknowledgements

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Table 5 ***Flow of breeding materials in the Mexico-Kenya shuttle scheme, utilizing two crop seasons per year, for developing high-yielding wheat germplasm combining adult plant resistance to stem rust with other traits***

Year	Locations[1]	Activities
1	Cd. Obregon	New crosses made.
	El Batan	F_1 grown, BC_1 & F_1-Top made on selected F_1.
2	Cd. Obregon	BC_1 & F_1-Top (350 plants), F_2 (1000 plants from simple crosses) grown & selected for agronomic traits and leaf rust resistance. Spikes from selected plants harvested as bulk & plump grains retained.
	Toluca	F_2 (1000 plants from BC_1 and F_1-Top) and F_3 (350 plants from F_2 simple) grown and selected for agronomic traits, resistance to stripe rust, Septoria tritici blotch. Spikes from selected plants harvested as bulk & plump grain retained.
3	Njoro	F_3 and F_4 (800 plants) grown densely under stem & stripe rust pressures, tall plants removed by cutting spikes. Remaining plants harvested as bulk and plump grains selected.
	Njoro	F_4 and F_5 (400 plants) grown, spikes from short plants resistant to stem and stripe rust selected & harvested as bulk. Plump grains retained.
4	Cd. Obregon	F_5 and F_6 (350 plants) grown & selected for agronomic traits and resistance to leaf rust. Plants harvested individually and those with plump grains retained.
	El Batan & Toluca	Advanced lines grown as small plots, selected for agronomic traits and resistance to stripe rust and Septoria tritici blotch at Toluca and leaf rust at El Batan. Best lines harvested in El Batan and those with plump grains promoted to yield trials.
5	Cd. Obregon, Njoro and Santa Catalina	Advanced lines grown as replicated yield trials at Cd. Obregon and as small plots at all three sites, & phenotyped for leaf rust, stem rust and stripe rust at Cd. Obregon, Njoro and Santa Catalina, respectively. Best lines retained.
	El Batan, Toluca, & Njoro	Seed of International Nurseries Candidates multiplied at El Batan. Lines also grown at all sites and phenotyped for leaf rust, stripe rust, stem rust, Septoria tritici blotch, Fusarium head blight, etc. Quality analysis conducted using Obregon grain.
6	Cd. Obregon, Mexicali & Njoro	2nd year yield trials conducted in 5 environments at Obregon, seed multiplication for international distribution at Mexicali & phenotyped for stem rust resistance at Njoro.
	El Batan	International Yield Trials and Screening Nurseries prepared and distributed.
7	International	Countries with wheat seasons between April-December.
8	International	Countries with wheat seasons between October-June.

[1] Cd. Obregon, Toluca, El Batan and Mexicali are in Mexico, Njoro is in Kenya and Santa Catalina is in Ecuador

Table 6 *Ug99-resistant entries included in seed multiplication in seven countries*

CIMMYT		Resistance	Maturity	Country and seed quantity (Kg)						
name	Cross	category[1]	type	Bangla-desh	Nepal	Pakistan	Turkey	Afghanistan	Egypt	Ethiopia
Danphe#1	Kiritati//2*PBW65/2*Seri.1B	APR: R-MR	Normal		100					100
Chonte#1	Seri.1B*2/3/Kauz*2/Bow//Kauz/4/PBW343*2/Kukuna	APR: R-MR	Normal			300	100	50	25	100
Chewink#1	Whear/Tukuru//Whear	Sr25	Normal						25	
Picaflor#1	Kiritati//Seri/Rayon	APR: R-MR	Early	100	100			50		100
Quaiu#1	Babax/Lr42//Babax*2/3/Vivitsi	SrTmp	Normal			300		50		
Quaiu#2	Babax/Lr42//Babax*2/3/Vivitsi	SrTmp	Normal	100	100		100			100
Pauraque#1	Waxwing*2/4/Sni/Trap#1/3/Kauz*2/Trap//Kauz	APR: R-MR	Early	100	100					
Grackle#1	Waxwing*2/Kukuna	APR: MR	Normal						25	
Becard#1	Weebill1*2/Kiritati	APR: MR	Normal		100					
Munal#1	Waxwing*2/Kiritati	APR: MR	Normal		100	300	100	50		100
Francolin#1	Waxwing*2/Vivitsi	APR: MR	Early	100	100					

[1] Resistance category is based on Ug99 response data obtained for at least two seasons (2006 and 2007) at Njoro, Kenya. APR (adult-plant resistance) categories are R-MR = resistant to moderately resistant (15-20% stem rust severity) and MR = moderately resistant (30% disease) when susceptible checks were dead following 100% severity

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20. Breeding strategies in the use and deployment of major genes for rust resistance

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Effective strategic use of resistance genes is predicated upon having an ample quantity of genes together with the genes affecting different processes in the development of the pathogen. This elusive goal is typically not met because usable, effective genes are often in short supply, and they often target similar stages in pathogen development. In addition, deployment of resistance genes often becomes a somewhat hollow term in that; the resistance gene is part of the entire genotype of a variety, which is typically delineated by agronomic adaptation. Nevertheless, defining breeding strategies for resistance gene deployment can bring into focus necessary lines of research that will lead to durability of resistance.

Combining resistance genes into stacks or pyramids may be an effective method of controlling rusts over time. Molecular markers have greatly increased our ability to combine major resistance genes into single plant genotypes. Probability theory indicates that varieties possessing multiple, race-specific resistance genes owe their collective durability to a low probability of the pathogen mutating to virulence independently at *avr* loci corresponding to those resistance genes. However, not all resistance genes are equal in their effects on the pathogen, and not all virulence mutations at different loci are independent. So, it could be that the specific resistance gene combination is at least as important as the number of genes that are combined.

An effective deployment strategy for utilizing different resistance genes is the use of mixtures or blends of varieties, breeding lines, or near-isogenic lines. This strategy is often practiced by combining different types of resistance (for example, major gene resistance, partial resistance, and susceptibility) in two or three component combinations. Yield achieved is often near the mid-value of the individual component yield; however, disease severity is often reduced, year-to-year fluctuations in performance are typically minimized, and the effects of unforeseen stress on the crop may be reduced due to differential responses of the components.

Perhaps, strategies for using disease resistance genes will expand as greater molecular characterization of disease resistance may blur the existing dogmas of major resistance (characterized by single, race-specific genes, lack of durability, not developmentally or environmentally influenced, and complete in its effect) and non-major gene or partial resistance (characterized by multigenic, race non-specific genes, durable, often developmentally and environmentally influenced, and incomplete in effect).

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21. Overview of durum wheat research in Ethiopia: Challenges and prospects

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Abstract

In East Africa, durum wheat (*Triticum turgidum* subsp. *durum* Desf.) is predominantly grown in Ethiopia, and to some extent, also in Eritrea. It is a traditional crop grown by resource-poor small-scale farmers on over 500,000 ha of land under rain-fed conditions. The grain is used mainly for local food recipes. Landraces account for more than 85% of the durum area. Despite the huge genetic diversity and potential environments for wheat production, large amounts of durum are imported annually to meet the requirements of local pasta factories. This paper gives an overview of the past and present research outputs and further attempts to predict future research directions. Aspects of durum production and its constraints, research achievements, and challenges to develop varieties with high yield, wide adaptability and resistance to stem rust race Ug99 are discussed. We also discuss various opportunities to deal with these challenges, such as the availability of genetic diversity, the presence of strong public research support, and the longstanding collaboration with international partners like CIMMYT, ICARDA, and more recently BGRI. The development of durum varieties with high yield and quality, wide adaptability, and combining resistance to prevalent stem rust races, including Ug99, will be a formidable task for the national durum wheat improvement program in Ethiopia

Keywords

Landraces, stem rust resistance, tetraploid wheats, *Triticum aestivum*, *T. turgidum*

Durum wheat production and utilization

Ethiopia is the largest wheat producer in sub-Saharan Africa with annual grain harvests of about 2.9 million tonnes from about 1.5 million ha (FAOSTAT 2008). Both hexaploid (bread) (*Triticum aestivum* L) and tetraploid wheats (*T. turgidum* L) are grown. The latter are indigenous whereas the former was probably introduced by the Portuguese or the Italians during the early 1920s (Anonymous 1995). Durum wheat is the predominant tetraploid type and its production statistics are often confounded with bread wheat. Consequently, reliable separate statistics for the different species are meager. Durum wheat covered a larger proportion of wheat area in the early 1980s, but has gradually been replaced by widely adapted high yielding semi-dwarf bread wheat cultivars. Currently, it covers about 40% of the national wheat area.

It is not known when and how durum wheat was introduced to east Africa. It might have reached the northern highlands of Ethiopia and Eritrea around 3000 BC (Belay 2006). About 85% of the durum cultivars are landraces adapted to specific areas. Despite their endurance to many of the prevailing biotic and abiotic stresses, they have tall and weak plant stature, and are not particularly responsive to modern agricultural inputs such as fertilizers. Consequently, their productivity has remained extremely low (Tesemma 1988). Durum is often grown under rain-fed conditions by resource-poor farmers on heavy black clay soils (vertisols) in the highlands at altitudes of 1,800-2,700 masl. Vertisols account for 24% of all cropped highland soils in Ethiopia (Abebe et al. 1992). These soils crack extensively on drying and rapidly become waterlogged during the rainy season. Due to the water-logging, planting is delayed until late in the season, and as a result, plant stands are poor and yields are reduced due to terminal moisture stress.

In spite of the availability of suitable environmental conditions for the cultivation of durum wheat, age-old traditional husbandry, huge genetic diversity, several decades of research efforts, and technical and financial support from international partners, the average durum production and productivity has remained extremely low due to various technical, physical and socio-economic constraints. The major technical constraints are the low inherent yield potential of landraces and unavailability of high yielding, widely adapted, disease resistant varieties meeting international quality standards. High yielding semi-dwarf durum cultivars meeting the yield and quality parameters are available, but they often succumb to diseases, such as rusts, Fusarium head blight, and Septoria leaf blotch. Furthermore, low soil fertility, water-logging, low moisture stress and frost in the mountain areas also affect the production and productivity of durum. In addition, shortages of improved seed, lack of linkages to the local and international markets and poor infrastructure are continuing impediments for the durum industry.

For centuries, landraces have been selected for use in recipes that are different from pasta products, such as macaroni, spaghetti and noodles. The local food

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recipes include *kitta* (unleavened bread), *injera (*spongy flat unleavened bread), *kinche* (boiled coarse-ground wheat), *nifro* (boiled whole grain), *kollo* (roasted grain), *dabo-kollo* (ground and seasoned dough), and many others. Improved durum cultivars are also used for local consumption. Due its vitreous and amber grain color and large seed size, durum often commands higher prices, even in the local market, than bread wheat. However, due to irregular supply and/or lack of market linkages, the local pasta factories annually import more than 0.2 million tonnes of durum from abroad (UN DATA 2008).

Durum wheat improvement milestones

Wheat research in Ethiopia began in 1949 at the Paradiso Experimental Station, near Asmara, Eritrea. Since then improved durum varieties have been developed through selection from indigenous landraces, introduction and selection, and hybridization (Tesemma 1988).

Early improvement work based on selection of superior lines from landraces, led to four local selections, viz. A10, R18, P20 and H23, released in 1952 to farmers in Eritrea (Bechere et al. 1994). In 1953, wheat research was moved to Debre Zeit, and two local selections, Arendeto (DZ04-118) and Marou (DZ04-688), were released in 1966 and 1967, respectively (Tesemma and Belay 1991).

Acquisition of germplasm from CIMMYT, ALDA (now ICARDA), FAO and USDA in the form of nurseries and segregating populations has occurred since the early 1970s (Anonymous 1995). From 1976 to 1993, four varieties, viz. Cocorit 71, Gerardo, Ld 357 and Boohai, were released (Bechere et al. 1994). Except for Ld 357, they were introductions from CIMMYT (Table 1). These improved varieties had yield potentials of 2.5 - 4 t/ha on farmers' fields under good management conditions, whereas the local landraces produced 1.5 - 2.5 t/ha under similar conditions (Tesemma 1988). Germplasm introduction has continued to be one of the strategies of the national durum improvement program; consequently, from 1993 to 2007, nine improved varieties originating from CIMMYT and two from ICARDA materials were released to Ethiopian farmers (Table 1).

Hybridization of durum wheat was initiated at Debre Zeit in 1974 (Anonymous 1995). Several advanced lines were used as parents in the crossing program. The objective was to combine desirable traits into single genotypes. Two-way crosses were common in the early stage, then, top crossing was identified to be quite useful. Initially, the pedigree selection method was used; however, a modified bulk method has been in use since 1986. From 1994 to 2007, 13 durum varieties were released from crosses made at Debre Zeit (DZARC) (Table 1).

Early research focused mainly on productivity, adaptability and disease resistance. Since 1966, 30 durum wheat varieties were released from research centers (Table 1). In addition, agronomic research has targeted the use of appropriate drainage systems in waterlogged vertisols and fertilizer applications to enhance both yield and quality of durum wheat. In recent work at the experimental field level, durum planted on a broad bed and furrow system and traditional ridge and furrow, had increased yields by 65 and 35%, respectively, compared to flat seedbeds (Abebe et al. 1992).

Although quality-based improvement work started late in the 1980's, some of the varieties have good pasta quality parameters meeting the quality standards of the local processing industries (Table 2). In general, the identification of varieties with wide adaptation and high stable yields has been rather difficult under the Ethiopian conditions (Tesemma et al. 1992). Some recently introduced or locally bred semi-dwarf durums yielded to 5 t/ha, but are susceptible to major diseases in hot spot areas (Table 3). In recent years, a number of leading farmers in various places of the country involved in the production of improved durum wheat varieties have managed to increase their incomes substantially along with improving their livelihoods. At present, the demand for durum wheat is increasing. Local pasta industries that import many thousands of tonnes of durum worth tens of millions of dollars in foreign exchange are also showing increased interest in locally produced durum.

Challenges of durum improvement

The pasta-making quality (protein quality) is often affected by environmental conditions. Low soil fertility, excessive rainfall and cool temperatures reduce pasta quality (Belay 2006). Good pasta quality is obtained from durum grown in well drained and fertilized soils where the crop can be harvested under high temperature and low humidity conditions. Potential durum wheat growing areas are located in the warmer rain-fed areas (1800-2300 masl) and irrigated lowlands both of which are favorable for the development of stem rust and leaf rust. Durum improvement in Ethiopia depends heavily on introductions, but the high yielding semi-dwarf varieties often succumb to stem rust particularly in such hot spot areas like Debre Zeit (Table 3, Fig. 1). Stem rust race Ug99 has become a further threat. Some of the 'durum' stem rust races in Ethiopia could be different from races in Kenya which focuses more on bread wheat. There are reports indicating that some durum varieties selected for resistance to race Ug99 in Kenya, are susceptible when grown at Debre Zeit (Abdalla unpublished data). It is possible, therefore, that specialized and divergent 'durum' races have evolved on local landraces in Ethiopia over a long period of time.

Table 1 *Commercial durum wheat cultivars released in Ethiopia*

No.	Local name	Pedigree	Origin	Year of release
1	Flakit	?	CIMMYT	2007
2	Obsa	Altar 84 ALTO-1/AJAYA	CIMMYT	2006
3	Ejersa	CD 98206	CIMMYT	2005
4	BAKALCHA	GEDIFRA/GWEROU	ICARDA	2005
5	KOKATE (DZ2016)	DZ 04-1167// DZ 04-129/Yemen Cit 's'//Plc 's'/3/Taganrog B.B/4/DZ 04-1167/5/Hora/3/Gdoz-46661-130//Gll's'/4/Im//Cit 71/CII/5/GBH	DZARC	2005
6	MALEFIA	ALTAR84/STN..	ICARDA	2005
7	ODA (DZ2227)	DZ 04-688/Imlo//Cit 71/3/RCHI/Ld	DZARC	2004
8	ILANI (DZ2234)	Imlo/Rahum//A4# 72/3/Gerardo	DZARC	2004
9	MEGENAGNA (DZ2023)	DZ 04-1167/Dz-129/Yemen/Cit's'/Pls's'/3/Taganrog B.B/4/5/ Chen's'/RCHI//Hui's'/BHA	DZARC	2004
10	MOSSOBO (DZ-2178)	BHA/Ulnv//2* E# 24	DZARC	2004
11	METTAYA (DZ 2212)	Yemen/Cit 's'//plc 's'/3/Taganrog/4/Hui 's'//Cit 71/CII/5/ Shenkora 25	DZARC	2004
12	SELAM (DZ-1666-2)	61-130/Lds//Gll 's'/3/Cit 's'/4/Hora/3/Megrbce 's'	DZARC	2004
13	LASTE	TOB-2 (Dgo /II /3/ Rutt s// rg s / mexi s)	CIMMYT	2002
14	LELISSO (DZ-1605)	Cocorit71/3/Gerardo//61-130/Gll 's'/4/Boohai/Hora// Gerardo/3/Boohai	DZARC	2002
15	YERER	CD 94026-4Y (chen / Tez /3/ Guil // cII CD 94026- 4y – 040m-030y – pAp -0y)	CIMMYT	2002
16	UDE	CD 95294-2Y (Chen / ALTAR 84// Ald CD 95294-2y)	CIMMYT	2002
17	GINCHI (DZ-1050)	Boohai /ULNU –DZ 1050	DZARC	1999/00
18	ROBE (DZ-1640)	Hora/ cit s // Jo s/ Gs s /3/ some s /4/ hora Respinegroll	DZARC	1998/99
19	ASASA (DZ-2085)	CHo S T arus// yav s /3/ Fg s /4/ Fg s /cr s /5/	DZARC	1997
20	ARSI-ROBE	TOB 66	CIMMYT	1996
21	QUAMI	CD 75533-A (Fg s /Crs/5/ 5/ Fg s/ Dom s/6/ Huj S, CD75533-a)	CIMMYT	1996
22	BICHENA	DZ393-4 (Illumilo/ cocorit 71 ,DZ 393-2)	DZARC	1995
23	KILINTO	DZ 918	DZARC	1994
24	FOKA	Cit 71/candela II, CD 3369	CIMMYT	1993
25	Boohai	Coo's Cndeal II, CD 3862	CIMMYT	1982
26	LD 357	CI 8188 No. 58-40	USDA	1979
27	Gerardo (Jorro)	VZ 466/61-130 x LdsxGII'S" CM 9605	CIMMYT	1976
28	Cocorit 71	RAE/4* TC 6011 STW 63 \3/AA S, DZ 27617- 18-64-0M	CIMMYT	1976
29	Marou	DZ04-688	landrace	1967
30	Arendeto	DZ 04-118	landrace	1966

Table 2 *Yield potential and physical and chemical characteristics of some durum wheat cultivars grown in Ethiopia*

Variety	Year released	Yield (t/ha)		Physical and chemical characteristics		
		Research field	On-farm	Extra Hard/ soft	Wet gluten (%)	Protein %
Arendeto	1966	2.5	2.0	30/70	23.05	10.84
Cocorit 71	1976	4.0	3.0	5/95	12.06	10.20
Gerardo	1976	4.0	3.0	90/10	33.77	15.01
Ld 357	1979	3.5	3.0	15/85	15.40	11.86
Boohai	1982	4.5	4.0	94/6	28.90	13.90
Foka	1993	5.5	4.0	95/5	18.04	13.04
Kilinto	1994	5.5	4.5	94/6	14.40	13.90
Bichena	1995	3.0	2.5	75/25	19.90	11.86
Tob 66	1996	5.0	3.5	97/3	35.80	15.47
Quamy	1996	4.0	3.0	99/1	28.90	13.90
Asassa	1997	4.0	3.0	100/0	32.70	14.77
Robe	1999	5.0	4.0	71/29	18.04	11.52
Ude	2002	5.0	4.0	96/4	31.03	14.39
Yerer	2002	5.0	3.6	99/1	32.12	14.63
Standard				90/10	27-36	13-15

Fig. 1 *Frequencies (%) of durum wheat entries (from international nurseries) in stem rust severity groups for 2005 - 2007*

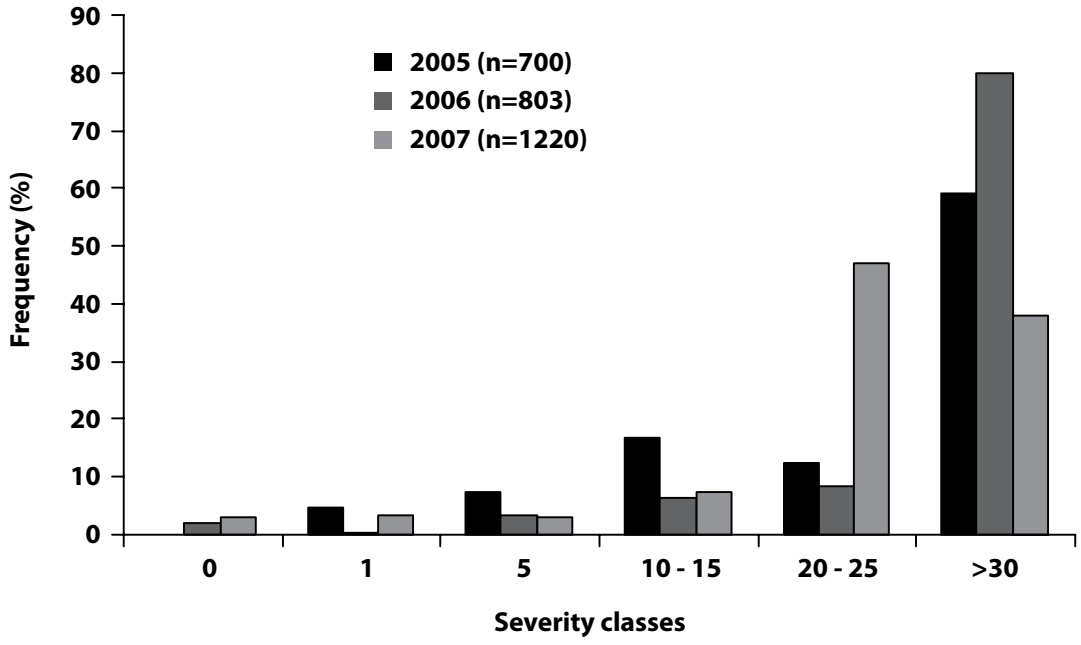

Table 3 ***The reaction of commercial durum wheat cultivars to different diseases tested across years and locations in Ethiopia (source: Badebo et al. unpublished data*****)**

Cultivar	Leaf rust (%)	Stem rust (%)	Stripe rust		Septoria	Scab
			Leaf (%)	Spike (0-5)	(00-99)	(0-5)
Arendeto	40S	60S	10MS	0	73	0
Cocorit 71	15MS	60S	20MS	1	76	4
Gerardo	20MS	30MSS	20MS	1	73	0
Ld 357	30MS	30MSS	60MS	4	62	0
Boohai	20MS	40MSS	25MS	2	76	1
Foka	15MS	50S	25MS	2	75	0
Kilinto	30MSS	50S	20MS	1	72	2
Bichena	15MS	40MSS	25MS	3	76	0
Quamy	20MS	40MSS	30MS	0	76	0
Tob 66	20MS	30MSS	25MS	2	72	2
Asassa	15MS	60S	25MS	3	76	0
Robe	40MSS	30MSS	40MS	2	75	0
Ginchi	30MS	40MSS	30MS	1	76	0
Yerer	20MS	20MS	15MS	0	76	2
Ude	20MS	30MSS	15MS)	0	41	2
Check	-	80S	90S	5	79	3

Opportunities

The availability of immense genetic resources, diverse environments, and a well organized national agricultural research system (NARS), and international support through CIMMYT, ICARDA, BGRI and other partners should be taken as a good opportunity to address emerging research challenges in Ethiopia.

Ethiopia is considered as a center of diversity for tetraploid wheat. All six wheat species observed by Vavilov in the mid-1920s, viz. *Triticum durum* subsp. a*byssinicum*, *T. turgidum* subsp. *abyssinicum, T. dicoccum*, *T. aestivum*, *T. polonicum* and *T. compactum* are still grown by the Ethiopian farmers as landraces (IBC 2007). The tetraploid wheat landraces are the results of many years of natural and human selection under a wide range of varying environments throughout the country. The majority of tetraploid wheat populations consist of mixtures of different genotypes that vary in botanical form and agronomic traits (Belay 2006). The high level of diversity in these landraces offers ample opportunities and scope for selection for different traits including resistance to diseases (Tessema and Belay 1991). Ethiopian tetraploid landraces were identified as potential sources of stripe and stem rust resistance in a number of studies (Anonymous 1989; Belay et al. 1997; Betessilassie et al. 2007; Bonman et al. 2007).

Durum wheat research in Ethiopia is coordinated from Debre Zeit Agricultural Research Center. In collaboration with five federal and six regional research centers and Haramaya University, durum wheat research is conducted at 21 testing sites addressing four different environments (potential areas, waterlogged vertisols, low moisture stress and irrigated lowlands).

The international centers support the durum wheat research through germplasm exchange and capacity building including short and long-term training. The durum wheat research has strong collaboration with CIMMYT and ICARDA. Since 1982, 13 durum varieties were released from materials obtained from CIMMYT and two from ICARDA (Table 1), and several others are in the advanced testing stages.

The BGRI, previously known as GRI, started in 2005 has contributed to awareness creation and training in response to the threat by stem rust race Ug99. The initiative has assisted in establishing irrigation facilities for screening against Ug99 and other local stem rust

races at Debre Zeit. The BGRI through the DRRW project coordinated by Cornell University is currently supporting the field screening activities against Ug99 in Ethiopia and is expected to establish high quality rust screening facilities at the Debre Zeit, Ambo and Kulumsa Research Centers.

Future research directions

The ever-increasing demand for durum wheat in both the global and domestic markets, and the availability of varieties developed from local and introduced germplasm meeting required quality standards for Ethiopian processors offer excellent opportunities for greater commercialization of the crop, thereby contributing towards reducing foreign currency expenditures required for durum imports. This implies that the durum market in Ethiopia must be based on competitiveness in terms of high yield, disease resistance and industrial quality.

Ethiopia is a center of genetic diversity for durum wheat, with a tremendous wealth of genetic variability existing in landraces. However, relatively little effort has been made to utilize or improve the local germplasm. Improvement of landraces and their utilization in breeding programs should receive greater emphasis. Crossing programs should focus on resistance to diseases, especially to stem rust race Ug99 and other variants. This long-standing commitment will be accomplished in collaboration with international partners, especially in the areas of grain quality and utilization of novel resistance genes.

Ethiopia has a huge potential for irrigated wheat, although no irrigated wheat is currently produced. Preliminary studies in the Middle Awash region indicate the possibility of irrigated durum in the cool season followed by rice or cotton in rotation. Currently, about 40,000 ha of land are under cotton, and the potential for irrigation in this region alone is about 175,000 ha. To take advantage of this potential we will require the the many-years of experience of CIMMYT and ICARDA in the exchange of germplasm, knowledge, and capacity building.

Other research and development activities need to give due attention to defining and characterizing suitable durum growing areas, more integrated systems of agronomic practices, improved seed distribution systems (both the formal and informal seed systems), and a coordinated durum marketing, storage and transport system.

The development of durum wheat varieties with high yield, stem rust resistance and quality parameters is a formidable task for the national durum wheat improvement program. However, to be successful the durum industry has to be integrated within a well developed value chain encompassing all aspects of grain production, marketing and utilization before real benefits will flow to Ethiopian farmers.

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22. Strategies to combat race Ug99 and control other wheat rusts in India

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Abstract

Wheat rusts continue to be very important diseases worldwide through their ability to generate new variation. The emergence of Ug99 is therefore a very big challenge for the world wheat community since most presently cultivated wheats are susceptible. Although the world wheat community reacted in different ways, India recognized the threat, and adopted a series of initiatives aimed at addressing the challenge. Surveillance of wheat crops in target areas was strengthened in order to detect any incursions of Ug99 as early as possible. With India joining the international community, it will be possible to keep track of the migration of Ug99 and its variants. Pre-breeding efforts were started with the aim of producing wheat lines with resistance to rusts. Seed multiplication and further distribution of Ug99-resistant wheat cultivars is expected to ensure gradual replacement of susceptible genotypes. These initiatives should enable us to move towards effective resistance and to avoid this threat to food security.

Keywords

Stem rust, surveillance, resistance, pre-breeding

Introduction

Wheat rusts continue to pose serious challenges to wheat production throughout the world. The pathogens continually evolve to new forms that nullify the resistances of varieties in cultivation. Among the wheat rusts, stem rust is the most destructive, severely impeding wheat yields in many areas. Intensive efforts to address stem rust began in the 1960s leading to substantial resistance. This resistance was effective over a long time period and the pathogen population declined all over the world. However, the incidences of leaf rust and stripe rust increased, and the challenges they posed gained priority. Apparently much of the stem rust control was due to one gene, namely *Sr31*. The situation remained stable until race Ug99 was reported in Uganda in 1999 (Pretorius et al. 2000).

The emergence of race Ug99 meant different things to different countries. For some, it brought back memories of past catastrophes caused by stem rust; for others, it was only a distant threat. Still others ignored the threat as more propaganda than science. However, India recognized the event as a future threat that needed to be addressed. It was particularly crucial at a time when India was poised to increase its wheat production in order to feed its ever-growing population.

With urgency in mind, it was decided to initiate strategic measures to address the serious challenge. In the past, new rust variants led to distinct decisions in meeting such challenges. For example, on two occasions when virulences for *Yr9* and *Yr27* were first reported (Prashar et al. 2007), the Wheat Rust Laboratory at Flowerdale began to screen breeders' lines with them before economic losses occurred. This enabled new sources of resistance to be identified and new resistant varieties to be distributed to farmers in a timely way. However, in the case of Ug99, the threat is recognized, but the particular pathotypes do not exist in the country. Awareness of the threat urged the nation to intensify surveillance activities to enable early detection if there were an incursion. International participation permitted screening of Indian breeding materials and germplasm in east Africa. To achieve these ends, the following initiatives were adopted:

1. Screening Indian lines in Kenya and Ethiopia for resistance to race Ug99 and its variants
2. Further strengthening of surveillance activities
3. Identification of resistance sources and their use in breeding
4. International cooperation to track ongoing movement of race of Ug99 and its variants.

Screening Indian lines in Kenya and Ethiopia against race Ug99 and its variants

Upon the emergence of race Ug99, the Indian Council of Agricultural Research (ICAR) recognized the threat and immediately sent a set of 22 advanced lines for testing in Kenya. This enabled identification the resistance sources FLW2, FLW6 and FLW8 carrying *Sr24* and *Sr25*, genetic stocks that had previously been developed at Flowerdale. These stocks were multipled and provided as germplasm to wheat breeders throughout the country.

Strengthening surveillance activities

Surveys in expected target areas were intensified. This should enable us to detect race Ug99 or a variant before it spreads to large areas and causes crop losses.

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Experience indicates that it takes a few seasons for a new race to build up and cause economic losses. This time lag can be utilized to multiply resistant varieties and to aggressively distribute seed of resistant genotypes to farmers. As a precautionary measure, ICAR constituted a special survey team to monitor summer wheat crops in August, 2008. This survey team did not find stem rust, although other rusts were found. In addition, the Wheat Rust Laboratory organized trap plots in hotspot areas to detect the first occurrences of Ug99. All three rusts are also being monitored as stripe rust remains our first concern. Our national survey efforts revealed that *Sr24* and *Sr25*, two sources of resistance identified initially against Ug99, are no longer completely effective against the local pathogen population in India. Efforts are now being directed at introgression of *Sr26* and other sources of adult plant resistance that could provide longer lasting resistance. Another rust trap nursery, named the South Asian Association of Regional Cooperation (SAARC) trap nursery is also being coordinated by India as a pre-emptive strategy with the aim of ascertaining rust variation in the region.

Identification of resistant sources and their incorporation

As part of pre-breeding activities, ICAR and the Directorate of Wheat Research (DWR) have embarked on a project to strengthen the resistance of PBW343 through the addition of resistance genes by marker assisted selection. At The Indian Agricultural Research Institute (IARI), one of the authors (KVP) and colleagues developed PBW343 derivatives with *Lr24* and *Lr48*. The aim was to have seedling protection with *Lr24*, and post-seedling resistance with *Lr48*. Another author (RT) at DWR has developed high yielding lines carrying resistance genes *Yr5* and *Yr10*. Both seedling and adult plant resistances are being used to obtain resistance to Ug99.

Screening of Indian wheats against race Ug99 and its derivatives in Kenya has identified 35 resistant lines, which were multiplied and provided to wheat breeders throughout the country. During 2007-08, more than 400 tonnes of breeder seed of 11 Ug99 resistant varieties were produced; these included GW 273, GW 322, HI 1500, HD 2781, MP 4010, HUW 510, MACS 2846 (durum), HI 8498 (durum), UP 2338, DL 153-2 and HW 1085. At the August, 2008 National Wheat Workshop, a new Ug99-resistant variety, Raj 4120, was approved for release.

International cooperation to track the movement of Ug99 and its variants

India became a core member of the Global Rust Initiative immediately upon its launching, and has since actively participated in international efforts to track the movement of Ug99. It has also been engaged in building defenses against this serious threat to wheat production. We strongly support the international effort to create awareness of the threat in all countries in the path of Ug99, and encourage them to release resistant varieties as soon as possible. Indian wheat materials will continue to be screened in Kenya on a regular basis.

Conclusion

India is clearly aware of the Ug99 threat, and is already moving towards having effective resistances in place, not only to Ug99 and its derivatives, but also the other wheat rusts.

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23. Strategies and progress towards the development of rust resistant wheat varieties in China

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Yellow (stripe) rust is a major wheat disease in northwestern and southwestern China. Resistance based on both major and minor genes is employed at present. Understanding resistance genes in current varieties, and development of molecular markers is crucial for improving breeding efficiency. Gene postulation and adult stage testing indicated that resistance genes *Yr2*, *Yr3a*, *Yr4a*, *Yr6*, *Yr7*, *Yr9*, *Yr26*, *Yr27*, and *YrSD*, either singly or in combination were identified, with *Yr9*, *Yr26* and *YrZH84* being the most predominant genes. However, only *Yr5*, *Yr10*, *Yr15*, *Yr26* (or *Yr24*), and *YrZH84* are still effective and can be used in developing new varieties. Thirty-three varieties or advanced lines showed slow rusting resistance and they could be used as crossing parents in breeding programs. Various markers for *Yr5*, *Yr10* and *Yr26* have been developed, and molecular mapping of *YrCH42* and *YrZH84* in Chinese wheat varieties has also been undertaken. *Yr24*, *Yr26* and *YrCH42* are the same gene, whereas *YrZH84* is new. Pyramiding of *YrZH84* and *YrCH42*, each present in varieties with outstanding agronomic characters, can be used to develop new varieties. A shuttle breeding program has been established between CIMMYT Mexico, and Sichuan province to integrate minor gene-based slow rusting into Chinese varieties. Three way crosses (CIMMYT/Sichuan//Sichuan genotypes), and selections made in Mexico from F_1 to F_4, and continued after F_4 in Sichuan, proved to be effective in combining yield potential, slow rusting, and adaptation to the Sichuan environment. More than 10 varieties were released and Chuanmai 42, developed from synthetic wheat, is a leading variety in Sichuan.

Stem rust was basically controlled in China after the 1960s, however, race Ug99 could be a potential threat to Chinese wheat production. Therefore, a joint China-CIMMYT collaborative program has been established. The major activities include pathogen monitoring, introduction and evaluation of new varieties with rust resistance, testing Chinese varieties in Kenya, development and application of molecular markers, breeding of stem rust resistant varieties, and training. The ten most representative varieties or promising new lines from five provinces (Hebei, Henan, Shandong, Sichuan, and Heilongjiang) will be used for marker-assisted incorporation of race-specific resistance through limited backcrossing and field selection. More than 700 Chinese varieties and lines were tested in Kenya, and 10 conferred medium or high levels of resistance. They included Jimai 20 from Shandong, six lines (ELT 102, ZL-21, KD-9, XKD-21-1, XKD27-4, and Nei 2836 from Sichuan, Yunxuan 11-12 and Jingmai 8 from Yunnan, 656 and 12-1 from Xinjiang, Nongpin 5 from Inner Mongolia, Longfu 02-0667 from Heilongjiang, and Ningchun 18 and Ningchun 37 from Ningxia. Jimai 20 is a leading variety in north China, grown on more than 1 million hectares in 2008.

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24. Status of wheat and wheat rusts in Iran

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Abstract

Wheat is the major field crop in Iran. The total wheat area exceeds more than 6.95 million ha from which 2.63 million ha (38%) are irrigated, with an average grain yield of about 3,870 kg ha^{-1}, and about 4.32 million ha (62%) are rainfed with an average grain yield of 1,100 kg ha^{-1}. In normal years about 70% of wheat production is produced under irrigation. Winter, facultative and spring types are grown in different agro-climatic regions. The temperate agro-climatic zone is the most favorable area for wheat production with the highest grain yields recorded at Kangavar in Kermanshah province (about 14 tonnes ha^{-1}) and in Daryoun in Fars Province (>12 tonnes ha^{-1}). Biotic (e.g. YR, LR, SR, Septoria, FHB) and abiotic (drought, heat, cold, salinity) stresses are among the major limiting factors for wheat production. Yellow (stripe) rust, leaf rust and stem rust occur in different parts of the country; however, yellow rust remains the major wheat disease in more favorable years. In 1993, about 1.5 million tonnes of wheat were lost due to yellow rust. Fusarium head blight and Septoria leaf blotch are becoming more serious in the Caspian Sea regions as well as in south-west Iran. Leaf rust and stem rust appear late in the season, but there are no reports on crop losses due to these diseases over the last three decades. In 2007, stem rust race Ug99 was reported from Broujerd and Hamadan in western Iran. However, this race was not found in 2008.

Keywords

Triticum aestivum, *Puccinia striiformis, Puccinia. graminis*, *Puccinia. triticina*, grain yield, irrigated wheat, dryland wheat

Introduction

Iran is a vast and diverse country. The total land area of Iran is 164.8 million hectares (m ha), much of which is mountainous. About 18.5 m ha are used for agriculture as follows:

- 6.0 m ha = 32.4%, Irrigated
- 6.0 m ha = 32.4%, Dryland
- 4.5 m ha = 24.4%, Fallow
- 2.0 m ha = 10.8%, Horticultural crops

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The total harvested wheat area is more than 6.95 m ha from which 2.63 m ha (38%) are irrigated and 4.32 m ha (62%) are rainfed (Table 1).

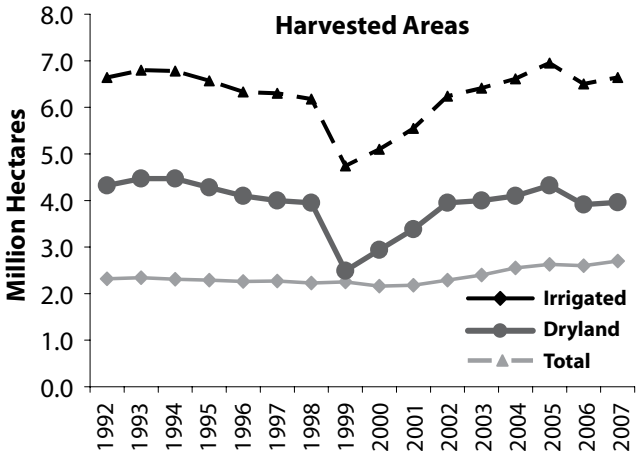
Table 1 *Harvested small grain cereals areas (000 ha) in the 2005-2006 cropping season*

	Wheat	Barley	Total
Irrigated	2634	607	3241
Rainfed	4317	1052	5369
Total	6951	1659	8610

Source: Statistics and Information Technology Office, Ministry of Jihad-e-Agriculture, Iran, 2007

The total harvested area in Iran has not changed significantly over the period 1992-2007; however, it dropped to its lowest level of about 5.0 m ha in 1998-1999 due mainly to severe drought conditions and consequently, there was a large decrease in the harvested areas of dryland wheat (Fig. 1).

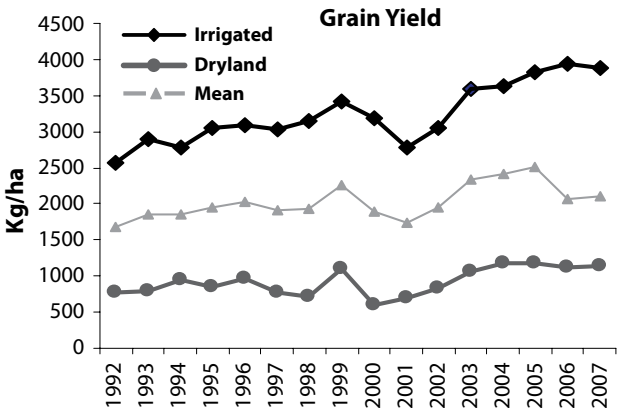
Fig.1 *Harvested wheat areas in Iran for the period of 1992-2007*



(After Jalal Kamali et al. 2007)

The average wheat yield peaked to about 2,500 kg ha^{-1} in 2005, but decreased to about 2,150 kg ha^{-1} in 2007 due to drought conditions (Fig. 2). The average yield of irrigated wheat was 3,870 kg ha^{-1} whereas the average grain yield for dryland wheat was 1,100 kg ha^{-1}. Clearly, the average grain yields in the country are more influenced by the average yield of the irrigated areas, which increased over the period 1992-2007, particularly 2003-2007 (Fig. 2).

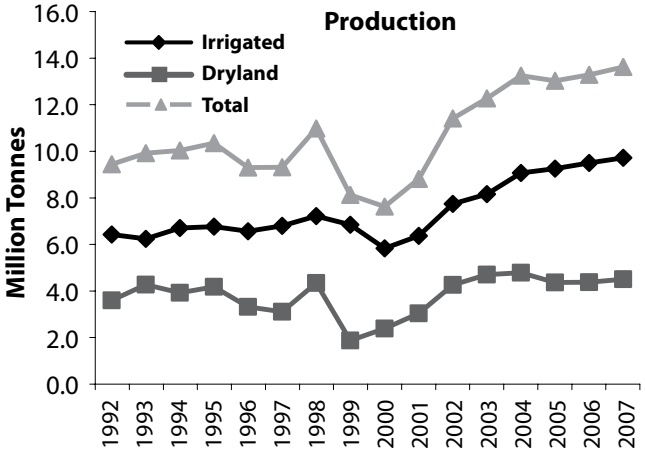
Fig. 2 *Wheat grain yields in Iran in the period 1992-2007*



(After Jalal Kamali et al. 2007)

The total wheat production reached about 15.0 mt in 2007 (Fig. 3). In normal years about 70% of wheat production is produced in irrigated wheat areas. The 2000 harvest was the lowest (about 8.0 mt) recorded for the period 1992-2007 (Fig. 3). Reductions are also evident for 1999 and 2001, again reflecting the effects of drought. Total wheat production increased over the period 2002-2007 because of more favorable growing conditions and a steady increase in irrigated wheat yields (Fig. 3).

Fig. 3 *Wheat production in Iran in the period of 1992-2007*



(After Jalal Kamali et al. 2007)

All three wheat types, viz. winter, facultative and spring, are grown in different agro-climatic zones under irrigated and dryland conditions. The temperate zone is the most favorable area for wheat production; high grain yields have been recorded at Kangavar in Kermanshah province (about 14 t ha^{-1}), and at Daryoun in Fars Province (12 t ha^{-1}) which are part of this zone.

Biotic (e.g. yellow rust, leaf rust, stem rust, Septorias, Fusarium head blight, powdery mildew, Sunn pest) and abiotic (drought, heat, cold, salinity) stresses are major limiting factors for wheat production. Yellow rust, leaf rust and stem rust have been reported from all parts of the country, but yellow rust remains the major disease in more favorable years. In 1993, about 1.5 mt of wheat were lost due to an epidemic of yellow rust. Fusarium head blight and Septoria are becoming increasingly serious diseases in the Caspian Sea region as well as in southwest Iran. Leaf rust and stem rust appear late in the season and there are no recent reports on crop losses due to these diseases.

The stem rust situation in Iran

Stem rust was reported for the first time in Iran by Esfandiari (1947). Epidemics of stem rust in the Caspian Sea region in the northern and southern regions of Iran in 1975 and 1976 were reported by Bamdadian and Torabi (1978). In 1976, an epidemic of stem rust in the southern part of Iran caused 100% crop losses in landraces (Bamdadian and Torabi 1978). The first stem rust race analysis in Iran was reported by Sharif et al. (1970). Race analyses and responses of cultivars and advanced breeding lines were carried out in 1994 and 1995 by Nasrollahi et al. (2001). Stem rust was controlled since 1976 by growing CIMMYT germplasm.

In 2007, the presence of race Ug99 was officially reported from Broujerd and Hamadan. Race analyses of samples collected from Borujerd, Hamedan, Poldokhtar and Kelardasht in 2007 and a race collected from Borujerd in 1997 were conducted using differentials carrying stem rust (*Sr*) resistance genes plus several additional wheat genotypes. Isolates from samples collected at Borujerd and Hamedan in 2007 produced high infection types (IT 33+ - 4) on differential lines (Nazari et al. 2008). These are facultative and winter wheat growing areas. The results were later confirmed using differential lines. There was no evidence of this race in 2008.

International monitoring has implicated the progressive migration of race Ug99 from Africa to Iran. Field evaluations of the responses of Iranian wheat germplasm to Ug99 in Kenya in 2006 showed that 98% of the entries were highly susceptible. Seedling evaluations of Iranian wheat cultivars and advanced lines to isolates of TTKSK from Iran also confirmed high susceptibility. These results reinforce the serious threat of race Ug99 to wheat production in Iran (Nazari et al. 2008a, b). In 2007, 468 Iranian wheat cultivars/lines were evaluated in Kenya and 8.5% of them (40 genotypes) showed acceptable levels of resistance. Evaluation of germplasm continued in 2008 with 550 cultivars and

Table 2 **Reactions of some resistant genotypes to race Ug99 in the glasshouse, at Cereal Research Department, SPII, Karaj** ***(Afshari et al. unpublished 2009). R = resistant; S = susceptible***

Line/ cultivar	Reaction to Ug99	Line/ cultivar	Reaction to Ug99
Bam*	R	C-84-4	R
Kavir	R	C-83-7	R
Arya (durum)	R	C-81-14	R
C-85-13	R	C-81-4*	R
MS-85-12	R	C-85-12*	R
MS-85-15	R	WS-M85-9	R
D-82-1	R	WS-M85-8*	R
D-84-9	R	M-84-17*	R
D-81-15	R	M-84-18*	R
D-79-2	R	M-83-3	R
D-79-18	R	E/Kvz **(*Sr31*)**	S
N-86-7	R	Atrak **(*Sr31*)**	S
C-84-11	R	Morocco (susc. check)	S

lines; 74 (13.5%) were either resistant (10 genotypes) or 50% rusted (62 genotypes). Among the resistant genotypes, two promising resistant lines, M-84-17 and M-84-18, from the national breeding program are approaching release. These lines are also resistant to stripe rust. Commercial cultivars, such as Kavir, Bam, the durum, Arya, and some landraces, such as Sorkhtokhom, also showed variable levels of resistance.

To reconfirm the reported resistances in Kenya, the resistant lines were re-examined in 2008 and 2009 in the glasshouse at Cereal Research Department, SPII, using the Ug99 isolate from Broujerd. Their resistances were reconfirmed (Table 2).

*Resistant in the Kenyan stem rust nursery

Since most of the commercial wheat cultivars in Iran are susceptible to race Ug99 the significance of the threat has been fully recognized. It is estimated that under epidemic conditions with 80% disease severity, about 2.5 m ha of spring wheat would be severely damaged. Assuming an average yield of 2.2 t ha^{-1} in rust-prone areas (irrigated and dryland), the loss would be about 3.5 mt of wheat, amounting to about USD$1,400,000,000. This is more than threefold the damage caused by the 1993 stripe rust epidemic.

Conclusions

In addition to biotic and abiotic stresses, lack of good agronomy is a major limiting factor to the achievement of the genetic potential of improved cultivars in Iran. Tillage practices, rotation, and crop residue management are necessary agronomic practices that need to be researched and improved at the farm level. Drought is always a limiting factor. In the 2007-2008 season, about 80% of dryland wheat and 20-50% of irrigated wheat were lost mainly due to the severe prevailing drought. Cold is also an environmental constraint to production in the winter and facultative wheat areas, and sometimes late frosts cause head frosting in temperate areas. Sunn pest is now a major problem in all wheat-growing areas, having spread to areas where it was previously absent (>1.5 m ha are sprayed). Russian wheat aphid is becoming a growing problem in cold areas, and common cereal aphid (*Schizaphis graminearum*) has become a problem in the temperate and warm areas.

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25. Breeding strategies for developing wheat cultivars resistant to rust diseases in Egypt and progress in breeding for resistance to stem rust race UG99

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Abstract

Wheat breeders in Egypt are attempting to develop high yielding cultivars to decrease the gap between production and consumption. Rust diseases are considered the main constraints in developing such cultivars. Stem rust caused big losses in the early 1930s and resistant sources were introduced from Kenya. A series of resistant cultivars was released in the 1940s. Following the introduction of semi-dwarf cultivars from Mexico in the mid-1960s, wheat breeders started screening for leaf rust resistance in the north and west delta regions, for yellow (stripe) rust in the east Delta and for stem rust in middle Egypt, as these were the local hotspots for each rust. Screening in these hotspots and selection for earliness helped in releasing high yielding rust resistant cultivars. Since 1979, hotspot screening with all three rusts was extended to Ethiopia, Sudan and Yemen. Recently, stem rust race Ug99 became a threat to Egypt. The wheat research program in Egypt in cooperation with the BGRI tested many wheat cultivars and lines in Kenya, Ethiopia and Yemen, and found some sources of resistance to Ug99. In addition, introduced materials from CIMMYT helped to develop seven promising high yielding UG99 resistant lines that will be released at the end of the 2008/09 season. Seed multiplication for these lines has been accelerated to enable rapid dissemination to farmers.

Keywords

Triticum aestivum, Puccinia graminis tritici, durable resistance, rust epidemics

Introduction

Wheat has been the first strategic food crop in Egypt for more than 7,000 years. It has maintained its position as the basic staple food in urban and rural areas for bread making. Ancient Egyptians used to grow the tetraploid wheat species *Triticum pyramidale* until hexaploid bread wheat, *Triticum aestivum*, was introduced from India in the early 20th century. Mass selection was practiced to develop new cultivars with high yielding capacity. However, stem rust caused by *Puccinia graminis tritici* caused big losses in grain yield at the end of the 1930s and early 1940s. Resistance sources were introduced from Kenya and crossed to Egyptian cultivars resulting in resistant cultivars. The first stem rust resistant cultivar, released in 1947, was Giza 139 (Hindi 90/Kenya B256), followed by Giza 144, 145, 147, 148 and 150. In the mid 1960s yellow (stripe) rust caused by *P. striiformis* f. sp. *tritici* threatened wheat fields and caused losses to grain yields. The wheat research program introduced resistant germplasm and succeeded in producing cultivar Giza 155 (Regent/ 2*Giza 139// Mida Cadet /Hindi 162) resistant to yellow rust in 1968. This cultivar was grown on more than 50% of the wheat area until semidwarf genotypes were introduced from Mexico during the Green Revolution initiated by Norman Borloug at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. During the early 1970s a new era of wheat breeding started and the resulting large improvements in grain yield enabled the country to gain in self-sufficiency from 25% in the 1980s to 60% in 2007 even with large increases in the population. The statistics of these increases are shown in Table 1 for the period 1981 to 2007.

Table 1 ***Wheat areas, productivity, and total production, 1981 – 2007***

Year	Area 000 ha	Inc. %	Productivity t/ha	Inc. %	Total production m. tonnes	Inc. %
1981	588	-	3.30	-	1.94	-
1986	507	-14	3.81	15	1.93	-1
1991	931	58	4.82	46	4.48	131
1996	1017	73	5.64	71	5.74	196
2001	984	67	6.35	92	6.26	223
2007	1250	114	6.50	97	8.00	313

The gains in production were not limited to grain yield and its components, but also resulted from improved resistance to rust diseases, which reduced grain losses and stabilized yields over years.

Breeding strategy for developing wheat cultivars resistant to rusts

Rust fungi (*Puccinia* spp.) are historically the most destructive diseases causing drastic yield losses. Wheat stem rust caused losses ranging from 18-25% in the

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1930s. Yellow rust caused losses of 11-25% depending on infection severity and pathogen races. However, leaf rust (caused by *P. triticina*) is the most frequent rust in Egypt especially in the north Delta, but losses generally are not as large as those caused by yellow rust and stem rust. Even with an epidemic, losses to leaf rust seldom exceed 20%.

Resistant cultivars are the best choice to control the spread of rust diseases. Therefore, Egyptian wheat breeders are continually striving for resistance genes and screening for rust resistance in hotspots. Local hotspots in Egypt are not sufficiently reliable to develop durable resistance. Therefore, in 1979 Egypt and the International Center for Agricultural Research in the Dry Areas (ICARDA) initiated a regional project operated through the ICARDA Regional office in Cairo, Egypt, to monitor rust diseases in cereals regionally, including Egypt, Sudan and Ethiopia. The project, named the Nile Valley Project (NVP), was expanded in 1995 to include Eritrea and Yemen and the name was changed to the Nile Valley and Red Sea Regional Program (NVRSRP). The major activities implemented by this network over the region were:

1. Regional rust trap nurseries.
2. Physiological race identification and virulence analysis.
3. Evaluation of effective genes for resistance.
4. Searching for alternate hosts of the different rusts.
5. Identification of resistance sources.
6. Incorporating identified sources of resistance into susceptible commercial cultivars.
7. Development of resistant wheat cultivars.
8. Testing of promising wheat lines and released cultivars in regional hotspots for rusts.
9. Verifying and demonstrating the high yield potential of resistant cultivars.
10. Studying disease incidence and development in relation to weather.

Many cultivars were released with high levels of resistance to rust diseases, and the program continues.

Breeding for resistance to leaf rust

Environmental conditions in the north and west Delta regions are suitable for the development of leaf rust. The leaf rust fungus is particularly variable in the west Delta region and new pathotypes are often first found there. Semi dwarf cultivars introduced from CIMMYT in the early 1970s, e.g. Mexipak 65, Super X, and Chenab 70, were highly susceptible to leaf rust and suffered losses in grain yield of 11-23% in the north and west Delta regions. Wheat breeders sought sources of resistance and were able to produce resistant derivatives.

The multi-location system of screening wheat materials for resistance to leaf rust allowed local cultivars to be classified according to levels of resistance, and losses under infection conditions. Wheat cultivars with high levels of resistance are specified for the west and north Delta, whereas high yielding cultivars with moderate resistance or susceptibility can be recommended for middle and southern Egypt. Thus, a site-specific cultivar recommendation procedure is followed to avoid losses caused by leaf rust, e.g. wheat cultivar Sids 1 is susceptible to leaf rust, but is high yielding and farmers prefer it rather than other cultivars; therefore, it is recommended for middle and southern Egypt where the dry environmental conditions are not conducive to the development of leaf rust. Highly resistant cultivars, such as Giza 168, Sakha 94 and Gemmiza 9, are recommended for the west and north Delta. Gemmiza 7 is a high yielding cultivar with slow leaf rusting genes, and is therefore grown in the north and west Delta. Cultivar Sakha 93 is widely adapted to all regions in the country and is moderately susceptible to leaf rust, but tolerant with no or minor losses. It is therefore recommended for all ecological zones. This strategy in breeding and evaluating wheat genotypes for resistance to leaf rust has reduced yield losses to minimum levels.

Pathotyping studies over the last 15 years show that races 77, 12, 57 and 144 are the prevailing pathotypes, with very high frequencies of 94.6, 85.5, 84.4, and 32.5 %, respectively. Frequencies of avirulence for known genes were variable, viz. *Lr21* (57.9%), *Lr1* (51.8%), *Lr22b* (43%), *Lr18* (42.1%) and *Lr26* (41.5%).

Breeding for yellow rust resistance

Yellow rust has been the most destructive wheat disease in the north and east Delta where the largest wheat areas are located. Old cultivars were considered resistant to yellow rust until virulent races occurred in the 1960s. The first epiphytotic of yellow rust was in 1968, and led to complete destruction of the wheat cultivar Giza 144 in the north Delta (Abdel-Hak et al. 1972). Wheat breeders introduced resistant materials and succeeded in developing two high yielding cultivars resistant to yellow rust. Giza155 (Regent/2*Giza 139 // Mida Cadet/ 2*Hindi 162) was released in 1968 and Giza 156 (Rio-Negro/2*Mentana//Kenya/3/*2Giza 135/ Line 950) in 1972.

In the early 1970s when semidwarf materials were introduced from CIMMYT, new genetic sources of resistance to yellow rust were available and wheat breeders started crossing local cultivars with Mexican cultivars to incorporate resistance genes to develop new cultivars resistant to yellow rust. At the same time, the

breeding program selected adapted promising lines with high yielding ability and resistance to yellow rust. Three semidwarf cultivars resistant to yellow rust were released in 1972 and 1973 (Mexipak 69, Super X, and Chenab 70). However, these cultivars were not accepted by farmers because of their susceptibility to leaf rust, short stature and low straw yield, and red grain color. Breeders crossed resistant lines together to develop new cultivars with more genes for resistance and succeeded in releasing new cultivars resistant to yellow rust from 1976 - 1986, i.e. Sakha 8, Giza 157, Giza 158, Giza 160, Giza 162, Giza 163, Giza 164, Sakha 61, and Sakha 69. The last two cultivars are sister lines and had a high level of resistance to yellow rust. By 1989 all had become susceptible to yellow rust except Sakha 61, Sakha 69, and Giza 164. Wheat cultivar Sakha 69 was widely adapted all over Egypt and occupied 70% of the planted wheat area. Giza 164 (Veery 5) was a heat tolerant cultivar and was preferred by farmers in southern Egypt, while Sakha 61 was limited to the north Delta. The Wheat Research Program had to release new cultivars with new sources of resistance. Screening for resistance was practiced in the north, east and west Delta as hotspots for yellow rust. In addition, new cultivars and promising lines were tested for resistance to yellow rust in Ethiopia and Yemen (NVRSRP project). Although a group of cultivars resistant to yellow rust was released from 1991 to 1998, the new *Yr9*-virulent race infected all wheat cultivars in Egypt in 1995, except Sakha 61 (Inia RL 4220// 7C/ Yr "s"), which showed a very high level of resistance.

The dramatic epiphytotic of 1995 saw most of the commercial cultivars, especially Giza 163, suffering high losses in the north Delta (El-Daoudi et al. 1996). This became a dangerous time in Egypt, and maybe in the world, because it included wheat-growing areas in the middle, near and far eastern zones, and led to high losses in all areas (Abu-Naga et al. 1997). Infection was very severe especially in the north and east Delta and losses were up to 50% in some regions. Many promising lines in the breeding program showed high levels of resistance in 1997 and 1998. Screening under high levels of infection resulted in the release of four resistant cultivars in 1999. These cultivars were Gemmiza 7 (CMH74A.630/5X//Seri 82/3/Agent), Gemmiza 9 (Ald "S" Huac "S"//CMH74A.630/ 5X), Giza 168 (Mil/ Buc// Seri) and Sakha 93 (Sakha 92 TR 810328). Many promising lines were selected under epidemic situations and released in 2004 (Sakha 94, Gemmiza 10, Sids 12). All susceptible cultivars were eliminated, especially Sakha 69, which occupied more than 70% of the wheat area, and resistant cultivars now dominate the entire region.

Mutli-location screening in hotspots locally and regionally accelerated the development resistant cultivars, but selection under epidemic situations identified sources of resistance to new pathotypes.

Pathotyping studies and rust surveillance in the last 15 years indicated that the most frequent *P. striiformis* pathotypes in Egypt were OEO, OE64, 2EO, 4E2, 4E148, 6E134, 70E20, 70E134, and 230E150. The most effective genes for resistance to yellow rust at the seedling stage are *Yr1, 4, SP, SD* and *9*, whereas effective genes for resistance at the adult stage are *Yr5, 8, 10, 15, 1* and *SP*.

Breeding for stem rust resistance

Stem rust is one of the most destructive diseases of wheat. During the first half of the 20th century, stem rust was the major disease (1930s – 1940s) and most wheat cultivars were susceptible until a source of resistance was introduced from Kenya. The first stem rust resistant cultivar, released in 1947, was Giza 139 (Hindi 90/ Kenya B 256) followed by many others. In the early 1970s, semidwarf cultivars and lines introduced from CIMMYT had different genes for resistance. The new cultivars were resistant, and stem rust was successfully controlled for 50 years due to following reasons:

1. Widespread use of resistant cultivars.
2. Early maturing cultivars.
3. Pathogen stability and no aggressive races.
4. Absence of the alternate host.

Some semidwarf cultivars became susceptible to stem rust and were replaced by resistant cultivars. The most frequent races in Egypt are 11, 15, 9, 17, 39, 24, 34, 19, and 53; and the most effective genes are *Sr5, 26, 27, 36, 9e, Gt+, 29*, and *30*.

Singh et al. (2006) stated that the importance of stem rust was declining worldwide with the deployment of various alien resistance genes such as *Sr24, 26, 31* and *38*. Translocations carrying these genes also carried additional genes that conferred resistance to other diseases such as leaf rust, yellow rust or powdery mildew. However, a new stem rust race virulent to resistance gene *Sr31* was detected in Uganda in 1999 (Pretorius et al. 2000) and named Ug99. Later, Wanyera et al. (2006) designated this race as TTKS using the North American nomenclature system. This race was virulent not only to *Sr31*, but also for most of the genes of wheat origin, as well as *Sr38* (Singh et al. 2006). Wanyera et al. (2006) reported that during 2003 and 2004, the majority of current Kenyan cultivars and a large portion of CIMMYT wheat germplasm with gene *Sr31* planted in Kenya were susceptible. They found also that the virulence pattern of the isolate collected in 1999 from Uganda was identical to those in Kenya. Their results

indicated that virulence for *Sr31* was widespread in the eastern Africa highlands and posed a threat to wheat production in the entire region, as well as to other production areas where *Sr31* resistance was important. CIMMYT (2005) reported that Ug99 was likely to spread beyond the borders of the three east African countries and it was a matter of time before it spread to the Arabian Peninsula and beyond.

According to previous investigations, Egypt was considered one of the countries at risk to the spread of Ug99, especially given that most wheat genotypes in Egypt were CIMMYT germplasm.

Because of the possibility of race Ug99 spreading globally, world awareness was raised by Nobel Laureate Norman E. Borlaug who encouraged international centers to work together in combating the challenge. This resulted in establishment of the Borlaug Global Rust Initiative (BGRI). Cornell University obtained funding to establish the "The Durable Rust Resistance in Wheat" project to work globally with the BGRI.

In Egypt, the National Wheat Research Program addressed the problem and initiated research strategies to avoid losses in wheat grain yields caused by race Ug99. The strategies focus on the following activities:

1. Screening Egyptian germplasm for resistance to Ug99, including old cultivars, recent cultivars and promising lines, in hotspots in Kenya and Ethiopia. Yemen also became a hotspot testing site for Ug99 in 2007.
2. Identify resistance genes effective against Ug99 and local races as well as resistance to yellow rust and leaf rust in Egypt.
3. Look for other sources of resistance under hotspot conditions.
4. Molecular studies to identify resistance genes and identify wheat germplasm with durable resistance.
5. Incorporate diverse resistance genes into adapted cultivars and evaluate the derivatives in hotspots (shuttle breeding).
6. Seed multiplication of promising lines resistant to Ug99, and other stem rust, leaf rust, and yellow rust pathotypes in Egypt. New cultivars must be higher yielding than existing cultivars.
7. Demonstration plots on framers' fields of the new cultivars and technology transfer to both extension staff and farmers.
8. Seed dissemination of new high yielding cultivars resistant to Ug99 and other rusts in Egypt.

Progress and achievements of breeding for resistance to Ug99

Screening in hotspots

Screening in hotspots is the most useful method to identify resistant genotypes. Table 2 presents the numbers of genotypes tested in Ethiopia, Kenya and Yemen from 2005 to 2008. In 2005, stem rust development in Ethiopia failed because of the dry season. However, screening 149 genotypes in Kenya in 2006 showed only three genotypes resistant to Ug99; these genotypes were included in the crossing block. Reactions of wheat genotypes in Ethiopia in 2006 showed that 10 durum wheat genotypes were resistant whereas all bread wheat genotypes (117) were susceptible. Screening 156 bread wheat genotypes in Kenya in 2008 resulted in 25 resistant lines being identified; these are included in 2008/09 yield trials and superior lines will be reselected. Wheat materials sent in 2008 to Kenya, Ethiopia and Yemen are currently being tested.

Screening in hotspots is a good tool to identify resistant genotypes and should also help in breeding for durable resistance.

Sources of resistances from exotic materials

Growing season 2006/07 The National Wheat Research Program in 2006/07 received from CIMMYT two nurseries of resistant wheat genotypes tested in

Table 2 ***Egyptian materials tested for resistance to stem rust in hotspot locations. B, bread wheat; D; durum wheat***

No.	Year	Country	No. lines	No. resistant entries
1	June 2005	Ethiopia	96	Escape
2	September 2006	Kenya	149	3 "B"
3	October 2006	Ethiopia	189	10 "D"
4	March 2008	Kenya	72D +156B	25 (BW)
5	March 2008	Ethiopia	180	-
6	October 2008	Kenya	163	-
7	November 2008	Yemen	163	

Table 3 *Average grain yield of eight genotypes selected over six sites in 2007/08*

Entry	Origin	Pedigree	Grain yield t/ha
1	2nd EBWYT 514	Oasis/Skauz//4*BCN/3/2*Pastor	7.93
2	2nd EBWYT 517	WBLL1*/Brambling	7.94
3	2nd EBWYT 521	Waxing*2/ Kritati	8.52
4	2nd EBWYT 530	Skauz/Bav 92	7.88
5	1st SRRSN 6028	SSERI 1/ Milan	8.31
6	1st SRRSN 6029	Takuru/Pastor	8.54
7	1st SRRSN 6067	Ning Mai 50	8.28
8	Local check		7.67

Kenya for three years. The first, the 2nd Elite Bread Wheat Yield Trial (2nd EBWYT) consisting of 29 genotypes and a local check, was sown in a yield trial at four sites (north, west, middle, and south Delta). The growing season in 2006/07 was characterized by high infection with all three rusts, enabling selection on the basis of resistance and grain yield. Five genotypes higher yielding by 500 – 1,290 kg/ha than the local check, and resistant to all three rusts, were selected.

The second nursery, the 1st Stem Rust Resistance Screening Nursery (1st SRRSN), contained 108 lines. Thirteen lines resistant to Ug99 in Kenya and to all three rusts in Egypt were higher yielding than the local check and were selected for further assessment.

In the 2007/08 growing season the selected wheat lines (five from 2nd EBWYT and 13 from 1st SRRSN) were evaluated with four local cultivars in two yield trails at six sites representing different ecological zones. Eight were selected for superiority in grain yield of 179 – 1,680 kg/ha across the six sites (Table 3).

Exotic materials 2007/ 2008 Two yield trials, received from CIMMYT in 2007/2008, included bread wheat genotypes tested for resistance to Ug99 in Kenya for three years. The 29 entries in the Third Elite Bread Wheat Yield Trial (3rd EBWYT) resistant to Ug99 were planted in two sites at north and west Delta, together with the 2nd Stem Rust Resistance Screening Nursery (2nd SRRSN) including 137 genotypes. Thirteen genotypes were selected from the 3rd EBWYT for superiority in grain yield over the local check. However, the selections were based on yielding ability only, because environmental conditions did not allow development of rusts. Similarly, 12 promising lines from the 3rd SRRSN were selected for high yielding ability. Selected lines are being evaluated at six sites for yield potential in 2008/09. Results for the 3rd EBWYT were collected by CIMMYT from different countries and four of these lines proved to be high yielding in Mexico, Egypt, India and Iran. In addition, these four lines were highly resistant to stem rust in Kenya. Three of the lines are under seed multiplication in Egypt along with selected lines from the previous season.

Molecular markers to identify resistance genes

Identifying genes with markers is the key to durable resistance whereby breeders can combine more genes into a single genotype. One of the objectives of the Wheat Research Program is to identify or validate markers in Egyptian wheat populations. A cooperation protocol between the Agricultural Genetic Engineering Research Institute and Wheat Research Program was initiated for that purpose. In addition, two researchers from wheat breeding and pathology worked in ICARDA's biotechnology laboratory to identify stem rust resistance genes in 30 wheat genotypes from Egypt during February, 2009. The materials included nine local bread wheat cultivars, 15 promising bread wheat lines and six local durum cultivars. They used ten specific primers linked to stem rust genes (SSR) and the results indicated that all tested materials do not have *Sr36, Sr46, Sr25, Sr39, Sr26* or *Sr24*. All, except five promising lines did not have *Sr31*. The results suggested that *Sr22* might be present in two promising lines and two durum cultivars. *Sr2* was found in all local cultivars, except Gemmiza 9, Sids 12 and two promising lines. This work will be continued in the biotechnology laboratory in the Wheat Research Program using other specific primers to detect more *Sr* genes.

Doubled Haploids

The biotechnology laboratory in the Wheat Research Program started anther culture research to produce doubled haploid genotypes resistant to Ug99. Nine crosses between five CIMMYT genotypes resistant Ug99 and two Egyptian cultivars were made in 2007/08.

Anthers were obtained from F1 plants and used to develop haploid plants. Anther culture is now in its initiation stage and will be followed by regeneration and chromosome doubling using colchicine. Another twelve crosses are planned this season to follow the same technique.

Using molecular markers for gene identification will help the planning of specific combinations of different genes for resistance, and the anther culture techniques will accelerate development of new genotypes resistant to stem races, including Ug99.

Technology transfer and training

The following training activities were undertaken:

- After the breeding program initiated research to develop cultivars resistant to Ug99, researchers started training extension staff and farmers through an extensive program to identify wheat rusts, with a focus on stem rust. A greater focus on chemical control will occur if needed.
- Demonstration plots were planted at twelve sites covering all wheat areas of Egypt, each site having four promising lines resistant to Ug99 and four local cultivars.
- Two training courses for young scientists in the Wheat Research Program and Wheat Pathology Department were conducted in March, 2007, including lectures, field training, and greenhouse and laboratory training.
- A two-day workshop was held in January, 2008, for researchers and extension staff at government headquarters. ICARDA and CIMMYT scientists contributed in lecturing and training.
- Three Egyptian researchers participated in a training course in breeding for rust resistance in Ethiopia for two weeks, from 23 September – 5 October, 2007.
- A training course in wheat improvement and breeding for rust resistance at CIMMYT for three months (March to May, 2007) for one researcher.
- A two weeks course in rust epidemiology, rust surveillance, and screening for resistance for three pathologists at ICARDA in May, 2008.
- Four weeks training in using molecular markers in gene identification at ICARDA's biotechnology laboratory for two researchers (1-28 February, 2009).
- A one week training course in pathotyping at ICARDA for four researchers (1-7 March, 2009).

Seed multiplication

Ten wheat genotypes resistant to Ug99 are planted for seed multiplication on about 22 ha at three research stations, i.e. Sakha Agricultural Research Station (north Delta), Gemmiza (middle Delta) and Sids (middle Egypt). The current season is producing breeder seed.

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26. Combating stem rust to protect wheat crops in Kenya

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Abstract

Wheat is the second most important cereal in Kenya after maize. Both biotic (stem rust, yellow rust, Septoria tritici blotch) and abiotic (drought, acidic soils) factors are important production constraints faced by wheat producers in Kenya. Stem rust race Ug99 has become number one problem during the last six years. Kenya Agricultural Research Institute (KARI) - Njoro is involved in the screening of international wheat germplasm in the search for new sources of resistance. In addition to the international screening, KARI identified three wheat lines that are high yielding and resistant to race Ug99 and its derivatives. These lines are at the final year of testing before official release to farmers. This report discusses the performance of Kenyan varieties against Ug99 and its variants, progress on identification of new sources of resistance, results of national advanced yield trials, and proposed breeding strategies for incorporating resistance genes into commercial varieties.

Keywords

Triticum aestivum, *Puccinia graminis tritici*, durable resistance

Introduction

In Kenya, wheat (*Triticum aestivum)* is the second most important cereal after maize. It is grown in the highlands of the Rift Valley and on the slopes of Mount Kenya. Annual production stands at 350,000 tonnes compared to an annual consumption of 850,000 tonnes making Kenya a net importer of wheat. The total area under wheat is 150,000 hectares (ha) with an average yield of 2.5 t/ha. Wheat is grown by both small-scale producers (less than 10 ha) and large-scale farmers (50 to 10,000 ha). Large scale farmers use high-input improved technologies, whereas small scale farmers apply minimal inputs resulting in low returns. There is a large production gap (over 70%) between the returns for both groups.

Wheat production in Kenya is not constraint-free and wheat is challenged by both biotic and abiotic constraints. The biotic constraints are diseases especially stem rust and stripe (yellow) rust whereas the abiotic constraints include drought stress and soil acidity. Over the last six years, new races of the stem rust pathogen (*Puccinia graminis tritici)* have been the most serious production problem. These races include Ug99 (Pretorius et al. 2000), or TTKSK based on North American nomenclature system, and its *Sr24*-virulent variant TTKST (Jin et al. 2007). They are able to overcome resistances of all the commercial varieties grown in the country, and yield losses of up to 90% occurred on some farms in the Narok district.

Breeding for resistance is cumbersome due to the rapid evolution, survival and build-up of new variant races. Use of fungicides as a control measure is being practiced to fight the disease. Farmers have been trained during field days, with shows and demonstrations on how to identify the disease at early stages of an epidemic, and on which fungicides to apply. Farmers have also been encouraged to grow varieties such as Njoro BW2 which are moderately resistant to Ug99. Breeding for resistance is the most cost-effective strategy for control of stem rust. KARI is involved in the screening of international wheat germplasm in the search for, and breeding with, new sources of resistance at Njoro. The center is 200 km west of the Kenyan capital, Nairobi, in Nakuru district, of Rift valley province. It is located 2,185m above sea level at latitude $0^0$20'S and longitude $35^0$56'E. The average rainfall is 933 mm with average daily minimum and maximum temperatures of 10^0 (night) and 23^0 (day). The center has a national mandate for small grain cereal research which includes wheat, barley and triticale.

Evaluation of Kenyan varieties for resistance to stem rust

Kenyan varieties display seedling resistance to races of the stem rust pathogen found in the USA and probably other parts of the world (Table 1). However, when tested with race TTKSK (Ug99) all the Kenyan varieties showed susceptible responses with the exception of Bonny which was highly resistant, and Tama and Gem which showed intermediate responses (Table 1). Given that the races have been evolving over time, it is clear that most Kenyan varieties were able to hold resistance against new races until the emergence of Ug99. This can be explained because Ug99 possesses a unique combination of virulences for resistance genes that were commonly used in breeding, especially *Sr31* which is present in some of the more recent varieties derived from CIMMYT germplasm (Singh et al. 2006). This degree of stem rust susceptibility to a single race in Kenyan bread wheat has not been observed previously

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Table 1. ***Seedling infection types[1] produced by Kenyan wheat varieties when tested with USA (6 races) and Kenyan (TTKSK)*** *P. graminis tritici* ***races***

Race	QFCS	QTHJ	RCRS	RKQQ	TPMK	TTTT	TTKSK
Line	06ND76C	75ND717C	77ND82A	99KS76A-1	74MN1407	01MN89A-1-2	04KEN156/04
K-POPO	0	23-	0;	0;	;1	;1	3
K-KUDU	0	2/3	;	4	;	;1+/3(2 pl)	3
K-KULUNGU	0;	;2/3	4	;	;	;1	3
K-FAHARI	0;	2+/;	;1	0;/;2	;1	;	3-
MBEGA	;/2	2-	1-	;1	2	2-	3+
K-NYANGUMI	0	;1--	;	0;	;1	3	3+
K-PAKA	0	;1	0;	0;	;1/2	;	3+
TAMA	0	;	2=	;3	;	3-	;13
K-TEMBO	;	-	0;	-	-	3	3+
NGAMIA	1	23	;	22+	3	3	3+
KWALE	0	0/1	2-	2-	2	2-	4
KIPAPU	0;/1	2	;	2+	;	2	2/4
K-CHIRIKU	-	;1	2-	;	2/3	;/3/2	4
BONNY	0/;1+	0	;	3	;	3;/;	-
PASA	;	2	;1	2-	2	1	4
SWARA	0;	1-	;/2-	0;/2+	;1	3	3
ROMANY	0	;/3	0;	;/3	;1	;C	4
A-MAYO	;1	2	2	0;	;1	3	3-/4
CATCHER	0;	2-	2-	2	2	2	3+
CHOZI	0;	;2-	2-	2-	2-	2-	3+
R-SABANERO	;1	;1+	2+	2C	2	2	3+
DUMA	2-	3-	;2-	2	3-	2/3	4
BOUNTY	0	-	-	-	0	1	3+
K-YOMBI	0/;	2-	1;	2-	2-	1;	3+
K-HEROE	0;	2	;1-	;1	2;	;2-	3+
K-MBWEHA	;2	2-	2-	2-	2-	2-;	3
REGENT	2	2	2+3	3	3+	3+	3
K-ZABADI	2;	2	2	1;	;1	3-	3+
GEM	0;	;1	;/1	23-	3	;	;2+
NGIRI	0;	;	0	0;	0;	0;	3

[1]Based on the infection type scale given by Stakman et al. (1962)

and indicates an erosion of the resistance sources in the wheat germplasm that has provided stable stem rust protection in the Kenyan wheats for over 50 years.

Among the 30 Kenyan wheat varieties evaluated for adult plant resistance in Kenya in 2006 and 2007, 20% were moderately resistant (Table 2). These were mainly the old varieties which included K. Nyangumi, K. Swara, Bonny and Bounty. These varieties possess the *Sr2* resistance gene complex, which is associated with pseudo black chaff (PBC). All these varieties showing adult plant resistance were released in the 1960s and 1070s when the *Sr2* complex was used as the main source of stem rust resistance. The varieties released in the 1980s and 1990s were the most susceptible with average disease severities of 40% and above. These include varieties like Kwale released in 1987, Chozi released in 1999, and Duma released in 1994 (Table 2). However, a few recent semidwarf varieties such as NGamia (the CIMMYT line Buckbuck), released in 1994 and possessing the *Sr2*-complex, also remained resistant in the field. Erosion of resistance in more recent spring wheat germplasm is likely because breeding was done in the absence of stem rust.

Identification of new sources of resistance

Good progress has been made in identifying diverse sources of resistance to Ug99 and its variants in international germplasm including minor gene-based adult plant resistance (APR) having a reputation of durability (Singh et al. 2006, 2007; Jin et al 2007). More than 300 lines in three stem rust resistance screening nurseries (1st SRRSN, 2nd SRRSN and 3rd SRRSN) have been screened and tested in Kenya. A high proportion of lines (44%) in these three nurseries showed good to moderate levels of resistance in at least two seasons of evaluation under high disease pressure. Of 300 lines, 104 were selected in Kenya and their sources for resistance postulated using molecular markers at the University of Sydney, Australia. The main sources for resistance in the selected lines were postulated as *Sr2*, *Sr24* and *Sr38* in various combinations. It is noteworthy that neither *Sr24* nor *Sr38* are effective against TTKST. Some promising lines with desirable agronomic traits and resistance to TTKSK and TTKST were identified in Kenya, and are under further evaluation/tests for use in breeding programs worldwide or for direct release and registration.

Advanced yield trials to identify adapted varieties

After the initial stages of testing for disease resistance at Njoro, 33 lines and 2 checks were selected and evaluated further in national multi-location trials in 2007 and 2008. The lines were planted as plots of 8-rows x 6m in randomized complete block designs (RCBD), with three replicates. Data were collected on yield, disease and maturity period. Table 3 shows the performances of the lines and average yields across sites, Njoro, Eldoret and Timau. Lines R1101, R1107, R1111, R1115, R1116, R1117 and R1120 yielded more than 2 t/ha compared to the best check, Njoro BW2, which yielded less than 2 t/ha.

Based on the results, three lines (R1112, R1115 and R1130) were promoted to the National Performance Trial (NPT) and tested at 7 diverse agro-ecological wheat growing sites distributed across the predominant wheat growing areas of Kenya. The trials were conducted under the guidelines of the Kenya Plant Health Inspectorate Service (KEPHIS), the authorized body for national variety testing and release. The first years data (Table 4) showed that the three lines out performed the check varieties at almost all sites. In addition, these lines were early maturing and produced high quality grains. Line R115 outperformed the best check by over 27%. On average, it out-performed the mean of the checks by 81%. These lines are undergoing their second evaluation in 2009 and are likely to be released in November, 2009.

In 2009, KARI submitted four additional lines (R1120, R1122, R1121, and R1113) selected from CIMMYT germplasm for NPT. These lines are mainly red grained, which is considered more desirable in Kenya because of greater sprouting tolerance.

The breeding program

The overall objective of the Kenyan wheat breeding program is to develop wheat varieties that are widely adapted, high yielding, resistant to abiotic and biotic stresses, and have acceptable end-use qualities. However, over the last 8 years, wheat stem rust has become the most important constraint in wheat production due to the emergence of Ug99 and breeding for stem rust resistance has become imperative. The aim of the new breeding program in Kenya is to identify new sources of durable stem rust resistance and to deploy them in Kenyan commercial wheat varieties.

The breeding strategy used in Kenya is as follows: F_1 plants and parents are grown in the crossing block to develop top-, back- and three-way crosses. The resulting progenies will be space-planted in plots. Resistant ears will be selected and planted as hill plots. This will be repeated up to F_5 when single ear rows will be planted for uniformity. Observation nurseries will be conducted on the 7th generation and the resulting lines will be entered into preliminary yield trials at Njoro. The elite lines with disease resistance will be included in multi-location advanced yield trials in at least six sites. Selected elite lines based on yield and quality will

Table 2 *Kenyan varieties showing pedigree, year of release, seedling infection types to race Ug99 (TTKSK) and field response in 2007*

Variety	Pedigree	Year of release	Seedling reaction to TTKS[1]	Field response[2]
K.KONGONI	CI8154/2*Fr//3*ROM/3/Wis.245-II-50-17/CI8154//2*Fr	1981	-	40S
MBUNI	Za75/Ld357E//Tc3/GU	1989	-	10MS
K.HEROE	MBUNI/SRPC64//YRPC1	1999	3+	40S
CHOZI	F12.71/COC//GEN	1999	3+	30MSS
DUMA	AU/UP301//GLL/SX/3/PEW"S"/4/MAI"S"//PEW"S"	1994	4	60S
MBEGA	Fink "S"	1994	3+	10MSS
K.YOMBI	MBUNI/SRPC64//YRPC1	1999	3+	20MSS
PASA	Buc "S"/Chat "S"	1989	4	30MSS
K.CHIRIKU	KTB/Carpintero "s"	1987	3+	40MSS
KWALE	Kinglet,CM33089-W	1987	3+;	40MSS
K.NYANGUMI	TZPP//SK^{E6}/LR64HDM/3/AFM/4/KSW/K4500-6	1979	3+	5MSS
K.TEMBO	WIS.245/II-50-17//C.I 8154/2*Fr/3/2*Tob.66	1975	3+	5MSS
K.FAHARI	TOBARI66/SRPC527//CI8154/3/2*FROCOR	1977	3-	10MS
K.PAKA	Wis245/II-50-17//CI.8154/2*Fr/3/2*Tob.66	1975	3+	10M
K.POPO	KL. Atl/Tob66//cfn/3/Bb/4/648-2	1982	3	10MSS
NGAMIA	BUCKBUCK "S"	1994	3+	10MSS
NJORO BW I	KM14(PASA MUTANT)	2001	4	60MSS
NJORO BWII	TNMU	2001	-	40MSS
DH4	KWALE/DUMA	2008	-	40MSS
R960	PASTOR	-	-	40MSS
K.KULUNGU	On/Tr. 207/3/cno//Son64/4/6661-53	1982	3	10MSS
K.SWARA	CI8254/Fr2 //T-K^{2}/Y.59.2.B	1970	3+	1MSS
K.KUDU	K131xK184.P.2.A.I.F K1008.K.7.K.2	1966	3	5MSS
BONNY	YF3xBza^{2} VI-116-2-4B-1T-2B-1T	1967	0;	1MSS
BOUNTY	T-Kenya^{2}x Bonza^{2} VI-106-2t-3b-3t-1b-2t	1967	3+	5RMR
REGENT	H44xReward RL975.6	1946	4	5RMR
K.MAMBA	Africa Mayo48x/4/ Wis.245/sup51/3/Fr//Fn/YA	1972	3+	5MSS
GEM	Frontana /Cajeme 54	1963	3-	5MS
CATCHER	Santa CatalinaxFrocor	1960	3	20MSS
R.SABANERO	-	1934	3+	5MSS
K.NGIRI	CI8154/2*Fr//5*WRT.TC/3*MIT/3/2*Tob66	1979	3	10M
TAMA	Yaktana54/Lerma 52	1965	;,1,3	5MS
K.ZABADI	Son64/450^{5E}//Gto/3/Inia/4/K4500-2/5/Ksw/ Tob66//CIANO	1979	3+	10MS
K.MBWEHA	CI8154/2*F/3/2*GB54/36896//II-53-526	1974	3+	10MS

[1]Tested in the CDL greenhouse, University of Minnesota, 2007 (infection types based on Stakman et al. (1962) scale

[2]Tested in the field at Njoro, Kenya, 2007 (field response based on modified Cobb scale (Peterson et al. 1948)

Table 3 *Selected lines from the Advanced Yield Trial, 2007, showing disease scores and average yields at three evaluation sites in Kenya*

PEDIGREE	Line	Disease severity and plant reaction			Average yield (t/ha)
		Njoro	Eldoret	Timau	
BABAX/LR42//BABAX*2/3/TUKURU	R1098	20S	-	15M	1.523
BABAX/LR42//BABAX*2/3/KURUKU	R1099	30S	5S	10MR	1.665
BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	R1100	15S	10S	5M	1.778
SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PBW343*2/KUKUNA	R1101	5R	40S	20MSS	2.058
ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/MILAN/SHA7/4/FRET2	R1102	10R	40S	5MS	1.670
SUNCO/2*PASTOR	R1103	20S	60S	30S	1.629
WAXWING*2/VIVITSI	R1104	30MS	40S	30S	1.976
WBLL1*2/BRAMBLING	R1105	30MS	20S	15M	1.866
THELIN/2*WAXWING	R1106	20S	30MR	15M	1.617
BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	R1107	10R	0	15M	1.373
BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	R1108	10MR	TMS	5MR	2.000
BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	R1109	15S	20S	5M	1.784
ND643/2*WBLL1	R1110	10MR	30S	10MR	1.722
ND643/2*WBLL1	R1111	20MR	40S	20S	2.334
ND643//2*PRL/2*PASTOR **(KSSR 1)**	R1112	5R	5MS	5M	1.992
THELIN#2/TUKURU	R1113	10R	TMS	10M	1.697
BABAX/LR42//BABAX*2/3/BRAMBLING	R1114	10MR	TMS	10MR	1.717
BABAX/LR42//BABAX*2/3/TUKURU **(KSSR II)**	R1115	20MS	TMS	5M	2.828
THELIN/2*WAXWING	R1116	15MR	30S	20MSS	2.249
WAXWING*2/KUKUNA	R1117	15MR	20MS	30MSS	2.373
PFAU/WEAVER*2//JARU	R1118	10MR	20MS	5M	1.138
WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1	R1119	10MR	30MS	20S	1.790
ND643/2*WBLL1	R1120	15R	TMS	15MSS	2.208
PYN/BAU/3/MON/IMU//ALD/PVN/4/VEE#5/SARA//DUCULA **(KSSRIII)**	R1121	10MR	20MR	10MR	1.914
ND643/2*WBLL1	R1122	10MR	30S	15MS	1.900
EMB16/CBRD//CBRD	R1123	5R	10S	10MSS	1.529
EMB16/CBRD//CBRD	R1124	1R	30S	5MR	1.645
MON/IMU//ALD/PVN/3/BORL95/4/OASIS/2*BORL95	R1125	30MS	10S	10M	1.074
PRL/SARA//TSI/VEE#5/3/HUITES	R1126	15S	TMS	30S	0.931
VEE/PJN//2*TUI/3/SKAUZ*2/SRMA	R1127	5R	10MR	30S	1.673
EMB16/CBRD//CBRD	R1128	5R	20S	5MR	1.734
EMB27/OC18//ANA	R1129	30MS	20MR	10MR	0.667
EMB16/CBRD//CBRD **(KSSR IV)**	R1130	10R	40S	5MR	2.269
NJORO BW II		5R	20MS	20S	1.998
KWALE		5R	20S	30S	1.523

Table 4 *Average yields of the selected lines across seven sites in 2008*

Line	Yield (t/ha)							Average yield (T/ha)	%.> best check	%> the mean of the checks
	Njoro	Eldoret	Naivasha	Narok	Rongai	Kitale	Lanet			
R1115*	1.09	1.94	5.37	1.36	1.83	2.95	1.58	2.50	27.65	81.89
R1112*	0.98	1.73	3.85	1.11	1.58	2.52	1.30	2.02	2.94	46.68
R1130*	0.9	2.43	3.51	1.21	1.70	1.11	1.82	1.80	-8.36	30.58
Njoro BW2	1.08	2.13	2.53	0.88	1.13	2.06	2.11	1.82		
Kwale	1.22	1.14	1.60	0.76	1.43	1.46	1.67	1.34		
KS. Mwamba	1.46	0.49	0.76	.76	0.32	0.65	1.64	0.72		
KS. Simba	0.95	2.12	3.03	0.30	0.94	2.44	2.42	1.96		
Mean	1.09	1.67	2.67	0.88	1.18	1.85	1.77	1.6		
CV	24.44	21.96	23.55	15.18	33.19	23.69	12.56			
Lsd (5%)	0.46	0.76	1.08	0.23	0.68	0.76	0.38			

*Lines that have undergone one year of National Performance Trial

be submitted to KEPHIS for NPT and DUS (Distictness, Uniformity and Stability) tests and subsequent variety release. So far, 104 parents have been selected for establishing a crossing block. In collaboration with CIMMYT and the initiation of shuttle breeding, advances have been made for incorporating APR genes into suitable backgrounds. These are now currently represented in advanced segregating populations.

Seed multiplication and distribution

KARI in collaboration with other stakeholders has initiated the process of multiplying seeds of resistant lines that have already undergone the first year of evaluation. In 2008/09, 100 ear rows were planted for each of the three lines likely to be released. The lines will be multiplied in Njoro in small plots in April and in October when the lines are expected to be released as varieties. At that time at least 0.5 tonnes of each line should be available for distribution.

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27. Sources of resistance to stem rust race Ug99 and its variants in Canadian wheat germplasm

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Abstract

Canada produces about 25 million tonnes of wheat annually, of which about 71% is spring hexaploid wheat (*Triticum aestivum* L.), 22% is durum wheat (*T. turgidum* L.), and 7% is winter wheat. Canadian wheat is segregated into market classes based on end-use suitability parameters of protein concentration, gluten strength, and kernel color. Modern and older cultivars representative of all market classes of wheat and key parental founders have been assessed for response to stem rust race Ug99 and its variants in Njoro, Kenya, for one or more years since 2005. Several Canadian cultivars and germplasm lines consistently expressed effective resistance. Notably, resistance in AC Cadillac and Peace, eligible for grades of Canada Western Hard Red Spring, appears to be due to a combination of an unidentified gene on chromosome 6DS associated with *Bt10* bunt resistance in combination with *Lr34*. Based on gene postulation, *Sr2STM559* marker, pedigree, and phenotype *Sr2* may be a contributing factor for resistance in the Canadian spring wheat gene pool. The Canadian durum cultivars Napoleon and Commander also expressed very good resistance that may be associated with a pyramid of genes (*Sr9e*, *Sr13*, *Sr14* and *Sr22*) and to their lack of the "D" genome, which has been associated with suppressors of rust resistance. Efforts to genetically map these genes are underway. Sources of resistance are being evaluated based on molecular marker, pedigree, and race phenotypic information. The application of marker assisted selection is considered when planning crosses, with the intent to incorporate resistance to Ug99 in the absence of diagnostic races. Control of rust diseases in Canada is currently achieved through strategic gene deployment in the hexaploid and tetraploid market classes.

Keywords

Puccinia graminis, *Triticum aestivum*, Ug99, pyramid, marker assisted selection

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Introduction

Canada produces about 25 million tonnes of wheat annually, of which about 71% is spring hexaploid wheat, 22% is durum wheat, and 7% is winter wheat (Table 1). About 70% of hexaploid wheat and about 80% of durum wheat is exported to over 70 countries, generating $3.5 to $5 billion in annual sales. Canadian wheat is segregated into market classes based on end-use suitability parameters of protein concentration, gluten strength, and kernel color. The eastern portion of the Canadian prairies has suffered stem rust (caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.) epidemics that caused major crop losses (McCallum and DePauw 2008). However, the deployment of cultivars with pyramids of genes conferring resistance to stem rust has controlled crop losses since 1954 (McCallum and DePauw 2008). Strain Ug99 (TTKSK) and its variants (TTKST and TTTSK) with virulence on major and widely deployed genes *Sr31*, *Sr24*, and *Sr36* have caused considerable concern for the vulnerability of the Canadian wheat crop. Modern and older cultivars representative of all market classes of wheat and key parental founders have been assessed for response to Ug99 and its variants at Njoro, Kenya, for one or more years since 2005. Of the global genetic materials tested to Ug99 and its variants in Njoro, Kenya, the Canadian genepool had the highest frequency of resistant and moderately resistant responses (Singh et al 2009). Resistance has been detected in some cultivars and germplasm lines which may have unique resistance genes, or combination of genes. The genetic analysis and utilization of these sources of effective resistance will be reported.

Materials and methods

Since 2005 we assessed cultivars, advanced breeding lines, mapping populations, and key parents from spring wheat and durum wheat programs in Canada for response to stem rust at Njoro, Kenya. About 1-2 g seed per entry were planted in 2 m rows and spaced 30 cm apart. Urediniospores collected from lines containing the *Sr31* gene, and thus representative of "Ug99-type" races, were inoculated onto susceptible spreader rows. Rust inoculum was augmented by natural inoculum from surrounding producer fields of wheat. Response to stem rust was rated at least once and some materials were rated a second time. The plant phenological stage varied depending on the photoperiod response. Germplasm lines and cultivars which expressed some resistance were re-tested the following year.

For genetic studies, the highly resistant cultivar AC Cadillac was crossed to the susceptible line LMPG-6, and F_2 and F_3 progeny were assessed for resistance at the seedling stage. DNA was sampled from the F_2 plants for subsequent molecular analyses. Additionally, the highly

Table 1 *Percentage of total wheat area seeded to market classes of spring and winter hexaploid wheat and durum wheat in Canada from 2005 to 2008*

Market class	2005	2006	2007	2008	Mean
Canada Western Red Spring (CWRS)	63.2	71.0	63.3	59.5	64.2
Canada Western Amber Durum (CWAD)	23.6	16.6	22.8	25.5	22.1
Canada Western Red Winter (CWRW)	1.9	2.9	4.8	6.3	4.0
Canada Prairie Spring Red (CPSR)	2.4	2.4	2.4	2.5	2.4
Canada Western Soft White Spring (CWSWS)	0.2	0.4	1.3	1.5	0.9
Canada Western Hard White Spring (CWHWS)	4.5	2.6	1.2	0.6	2.2
Canada Prairie Spring White (CPSW)	0.2	0.2	0.3	0.2	0.2
Canada Western Extra Strong (CWES)	0.1	0.1	0.1	0.1	0.1
Canada Eastern Winter Wheat	3.8	3.8	3.8	3.8	3.8
Total	100	100	100	100	100

resistant cultivar Peace was crossed to susceptible line RL6071 and a doubled haploid (DH) population was derived using the maize pollen technique. Progeny were assessed for both seedling and field responses to Ug99, and DNA sampled from seedling tissue for subsequent molecular analyses. Simultaneously, new genetic combinations were made to transfer the stem resistance into competitive cultivar backgrounds.

In durum wheat, an association mapping (AM) approach was used in conjunction with genetic mapping to identify genomic regions associated with resistance to Ug99 and its variants. For AM, ninety-six diverse durum wheat cultivars and breeding lines collected from breeding programs in Canada, Argentina, Australia, France, Italy, Germany, Mexico, Morocco, United States, New Zealand, Russia, Iran and Spain formed the AM population. A total of 241 microsatellite (SSR) markers were used to amplify 245 loci. Prior to AM analysis, population structure was assessed using the program STRUCTURE v.2 as outlined previously (Reimer et al. 2008). Marker-trait associations were determined using a general linear model in TASSEL version 2.0.1 with the population structure Q-matrix as covariates. For structure and marker-trait associations, rare alleles (frequency <5%) were either combined into a single genotypic class if their combined frequency was greater than 5%, or scored as missing data. Significance of associations between loci and field and seedling responses to Ug99 was based on an F-test, at a significance level of $P \leq 0.01$, corrected for by performing 10,000 permutations.

Current situation

Spring hexaploid wheat accounts for about 71% of production (Table 1) and is marketed in six classes. The most widely grown CWRS cultivar, Lillian (DePauw et al. 2005), was susceptible and the next three most widely grown cultivars were moderately susceptible (Table 2). AC Cadillac (DePauw et al. 1998) and Peace (Humphreys et al. 2002) expressed resistance to Ug99 and its variants for four consecutive years. However, AC Cadillac and Peace yield significantly less grain than the other cultivars, are more prone to lodging, and are generally not adapted to the eastern prairie rust area. Cultivars in the minor classes were primarily susceptible or moderately susceptible (Table 2). AC Taber (Knox et al. 1992) was the exception expressing moderate resistance. Because these cultivars are susceptible to Fusarium head blight (mostly caused by *Fusarium graminearum*), they are not recommended for production in the rust-prone area. Durum wheat is grown on about 22% of the wheat area and preferentially in the drier parts of the Canadian prairies (DePauw and Hunt 2001). The cultivars Commander (Clarke et al. 2005b) and Napoleon expressed resistance to Ug99 and variants; however, these cultivars are also susceptible to Fusarium head blight. Because Canadian winter wheat cultivars require a vernalization period, they have not been grown in field nurseries in Kenya to assess their response to Ug99 and its variants. The first stem rust resistant winter wheat cultivars in western Canada were registered in the early- to mid-1990s, but the resistance gene has not been identified. More recent stem and leaf rust resistant cultivars incorporated *Sr24* linked with *Lr24.* Resistance

Table 2 *Summary of responses of Canadian wheat cultivars to stem rust race Ug99 and variants at Njoro, Kenya, from 2005 to 2008*

Class & cultivar	Field reaction of most widely grown	Field reaction of most resistant	% in 2008[1]	% of 2008 Canadian wheat crop	Reference
CWRS[2]					
Lillian	S[3]		16.9	11.0	DePauw et al. 2005
AC Barrie	MS		10.0	6.5	McCaig et al. 1996
Harvest	S		13.2	8.6	CRC, AAFC
Superb	S		11.5	7.5	CRC, AAFC
AC Cadillac		R	1.6	1.0	DePauw et al. 1998
Peace		R	trace	trace	Humphreys et al. 2002
Minor spring wheat classes					
5700PR	MS		35.3	0.9	Syngenta
AC Taber		MR	2.4	0.1	Knox et al. 1992
Snowbird	S		99	2.2	Humphreys et al. 2007
CDC Rama	S		28.2	trace	CDC, Uni of Sask
AC Andrew	S		96	0.8	Sadasivaiah et al. 2004
CWAD[4]					
Strongfield	MS		51.8	11.6	Clarke et al. 2005a
AC Avonlea	S		18.7	4.2	Clarke et al. 1998
Kyle	I		16.1	3.6	Townley-Smith et al. 1987
AC Navigator	MS		9.1	2.0	Clarke et al. 2000
Commander		R	1.9	0.4	Clarke et al. 2005b
Napoleon		R	0.6	0.1	CRC, AAFC

[1] Canadian Wheat Board variety survey: http://www.cwb.ca/public/en/farmers/surveys/variety/08-09/

[2] CWRS, Canada Western Red Spring wheat

[3] R – resistant, MR – moderately resistant, I – intermediate resistance, MS – moderately susceptible, S – susceptible

[4] CWAD, Canada Western Amber Durum wheat

from VPM *(Sr38)* has also been incorporated into cultivars, but neither gene provides effective resistance against Ug99 or its variants.

Because the majority of cultivars lack resistance to Ug99 and its variants, other control strategies are advised. In the short term until resistant cultivars adapted to the rust area are released, fungicides and / or plant height growth regulators could be used to mitigate losses. Risk could be reduced by shifting to other crops and planting winter wheat which often escapes significant damage from rust and Fusarium. Although these control strategies would mitigate crop losses due to Ug99 and its variants, wheat production losses would still be sustained.

Breeding for resistance

Wheat researchers have agreed to use different sources of resistance in the CWAD class from all other market classes as a gene deployment strategy (Table 3). The cultivars Commander and Napoleon are reasonably well adapted to the durum production area. Consequently, it will be relatively easy to transfer the resistance into a cultivar such as Strongfield (Clarke et al. 2005a) which has high grain yield, low cadmium uptake, high grain protein concentration, and very good semolina milling and pasta making characteristics. The genetic basis of the resistances in Napoleon and Commander may be associated with a pyramid of genes (*Sr9e*, *Sr13*, *Sr14*, and others) and to their lack of the "D" genome, which has been associated with suppressors of rust resistance (Kerber and Green 1980). Three gene maps have been developed to localize genes for resistance in Commander and Napoleon and preliminary data suggests they are different (Pozniak, unpublished data).

All of the breeding programs for the various hexaploid wheat market classes are using AC Cadillac and Peace as sources of resistance to Ug99 and its variants. Other sources of resistance include the DH lines B0071D&01AC08 and B0371AC41&AD007 expressed resistance. They derive from a project to transfer the solid stem trait to the Canadian bread wheat cultivar AC Elsa (Clarke et al. 1997) from a synthetic hexaploid, *T. turgidum* L. var. durum cv. Golden Ball/*T. tauschii* (Clarke et al. 2003). The DH line B0371AC41&AD007 derives from the cross between a backcross-derived synthetic hexaploid (P89-77-1F4/8*AC Elsa-[B1-L02]) and a solid stem line from the cross Chiroca 'S'//3Ag14/4*Condor received from Australia. Breeding programs are also using other sources of resistance received from CIMMYT, Australia, and the USA (Table 3). Two CIMMYT lines from the 2nd Stem Rust Nursery, CID394092 (*SrSha7*) and CID428593 (*SrSynth*), expressed a high level of resistance in the field nursery in Kenya, 2007. Australian cultivar Lang, which has *Sr12*, *Sr24*, and *Sr36* (Park et al. 2007), was also resistant to field races in Kenya, 2008. These genes in combination with *Sr2* and *SrCad* are being pyramided using MAS.

Genetics of resistance

AC Cadillac and Peace are resistant to all stem rust races from North America and Kenya tested to date. AC Cadillac, BW711, and BW165 derive from the backcross BW90*3/BW553 to incorporate gene *Bt10*, which confers resistance to prevalent races of common bunt (*Tilletia laevis* Kühn in Rabenh. and *T. tritici* (Bjerk.) G. Wint. in Rabenh.) (DePauw et al. 1998) (Fig. 1). BW553 derives from a project to transfer *Bt10* from winter wheat into Neepawa (Campbell 1970), a cultivar eligible for grades of CWRS. The parentage of BW553 is Red Bobs*2/ PI 178383//8*Neepawa. However, not all genotypes possessing *Bt10* express resistance in the field to race Ug99 and variants (Table 4).

An F_3 population of LMPG/AC Cadillac fitted a 1:2:1 ratio (χ^2=3.87, P=0.144; Fetch et al. 2009), indicating that a single dominant gene conditioned resistance at the seedling stage. This gene mapped to 6DS, on which genes *Sr5*, *Sr29*, and *Sr42* also have been localized. Genes *Sr5* and *Sr29* are not effective against race TTKSK, but *Sr42* did express seedling resistance in a single unreplicated test. The gene in AC Cadillac, tentatively designated *SrCad*, is 10 cM proximal to marker *cfd49* and 26 cM distal from marker *Xgwm469*; this marker is also about 20 cM proximal to *Bt10*. Thus, *SrCad* was mapped to the same genetic interval as *Bt10*.

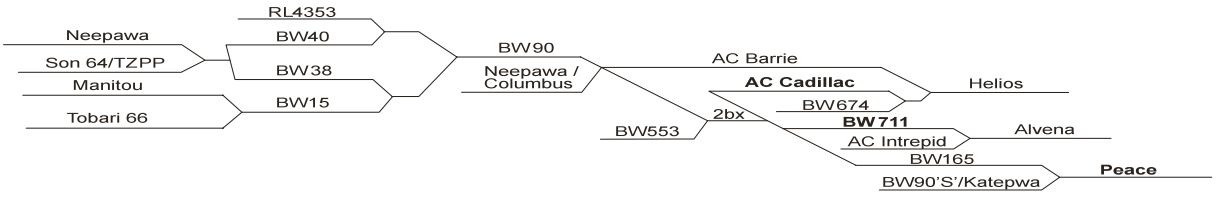
A DH population from the cross of RL6071/Peace consisting of 167 lines was generated to study the genetics of the resistance to Ug99 found in Peace. The DH population was inoculated at the seedling stage with TTKSK (Ug99). Segregation for a single seedling *Sr* gene was observed (1:1, p>0.05). The *Sr* gene was mapped to chromosome 6DS with microsatellite markers and is located in the same genetic interval as S*rCad*. The *Bt10* marker, *ALFSD_RSA*, showed tight linkage with the *Sr* gene on 6DS (<2 cM) (Humphreys et al. 2009). Peace also has the leaf rust resistance gene *Lr34*, which is known to provide or enhance resistance to all three wheat rusts. The DH population was tested with *csLVMS1*, a DNA marker that detects the presence of *Lr34* (reported linkage 0.06 cM, Krattinger et al. 2009). DH lines were classified for the presence of the seedling resistance gene on 6DS using seedling infection types and the presence of *Lr34* using *csLVMS1*. Thus, DH lines were classified as having *Lr34* + the 6DS *Sr* gene, only the 6DS *Sr* gene, only *Lr34*, or neither gene (Hiebert and Fetch, unpublished data). The population fitted a 1:1:1:1 ratio (p>0.05). The DH population was tested for field

Table 3 ***Sources of resistance to Ug99 and variants currently used in Canadian wheat breeding programs and the generation of the experimental lines resulting from hybridization***

Market class	Sources of resistance	Gene postulation and / or DNA markers	Generations
CW[1] Amber Durum	Commander	*Sr9e*, *Sr13*, and *Sr14* and other unknown genes	F_2 to F_{10}
	Napoleon	*Sr9e*, *Sr13*, and *Sr14* and other unknown genes	F_2 to F_{10}
CW Red Spring	AC Cadillac	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_6, DH
	Peace	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	DH3, F_1 to F_8
	B0371AC41&AD007	*Sr2, Sr24*, *Lr34*, Syn Hex, others	F_1
	B0071D&01AC08	*Sr2, Lr34*, others, Syn Hex?	F_1, DH
CW Hard White	AC Cadillac	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_2
	HW341	*Sr2, Sr12*, *Sr13*, *Sr30*, *Lr34* in parents	F_1 to F_{10}
	Peace	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	DH3, F_1 to F_8
CW Soft White Spring	Peace	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_4, DH
	Lang	*Sr24*, *Sr36*	F_1 to F_3
	CID394092	SrSha7	F_1
	CID428593	Sr-synth	F_1
	Hartog	*Sr2, Sr9*, *Sr30*	F_1 to F_2
Canada Prairie Spring_Red	AC Cadillac	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_5
	Peace	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_5
	HY696	*SrCad* linked to *Bt10*, *Lr34*	
	HY697	*SrCad* linked to *Bt10*, *Lr34*	
CW Red Winter	Peace	*SrCad* linked to *Bt10*, *Lr34*	F_1 to F_3, DH
	others	*Sr2, Sr22*, *Sr24*, *Sr29*, *Sr33*, *Sr35*, *Sr36*, *SrTmp*	F_1 to F_8, DH

[1] CW Canada Western

Fig. 1 *Genealogy of cultivars AC Cadillac and Peace which have resistance to Ug99 and its variants*



resistance to stem rust (including Ug99 and its variants) in Kenya in 2008. The mean stem rust severities for each of the four genotypic classes (above) show that the seedling *Sr* gene on 6DS in combination with *Lr34* confers a high level of stem rust resistance, while the 6DS gene alone only provides only moderate resistance in the absence of *Lr34* (Fig. 2). All DH lines that expressed the high level of resistance found in the Peace parent carried both the 6DS *Sr* gene and *Lr34*.

In durum, AM studies identified four regions on chromosomes 1B, 2A, 6A, and 7A (Table 5; Pozniak et al. 2008) significant for both field and seedling data and these chromosomes house known *Sr* genes. Two regions were identified on chromosome 7A, one distal to the centromere, and a second at *gwm276* (Table 5). *Sr22* is linked to *gwm276*, and that gene is effective against Ug99. *Sr13*, derived from cultivated emmer wheat cultivar Khapli, resides on chromosome 6A, is effective against Ug99. Marker *gwm617* was significantly associated with Ug99 resistance (Table 5) and is likely marking *Sr13* as they are both located on the distal region of 6AL. *Sr14*, which is also derived from Khapli and localized distally to the centromere on 1BL, provides intermediate resistance to Ug99 (Jin et al. 2007). Marker *cfd48* (Table 5) is likely detecting variation at *Sr14* (Table 5).

Conclusions

Although all current major cultivars grown in Canada are vulnerable to African races such as Ug99 and its variants, CWRS cultivars AC Cadillac and Peace and durum cultivars Napoleon and Commander are resistant in the two predominant market classes. These resistant cultivars, although lower yielding and with other deficiencies, can serve as a stopgap in the short term should Ug99 virulence appear in North America. Genetic analysis is underway to develop DNA markers which are more tightly linked for resistance in hexaploid spring wheat cultivars AC Cadillac and Peace, and to develop markers for the resistance in durum wheat cultivars Napoleon and Commander. Newly developed markers will be incorporated into the current marker assisted selection strategies of enhancing the gene frequencies of *Lr34* and *Sr2*, which appear to confer resistance to Ug99 and related races. However, assessment of inbred lines with putative resistance based on marker assisted selection will require bioassays under field conditions in the presence of Ug99 and variants.

Acknowlegements

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Fig. 2 *Mean rust severity of four genotypic classes of DH lines from the cross of RL6071/Peace. Number of DH lines in each group is shown in brackets*

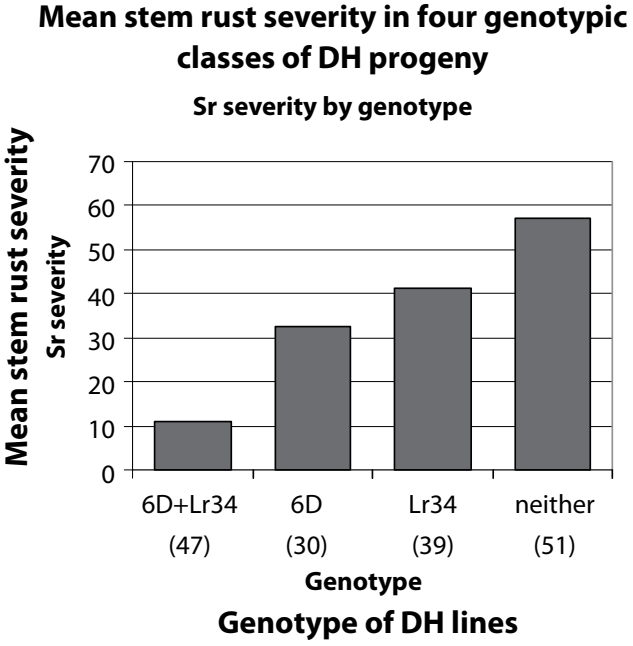

Table 4 ***Response of genotypes to Ug99 and variants in rust nurseries near Njoro, Kenya, postulation of genes based on parentage, molecular markers, and/or phenotype***

	Ug99 +variants	Bt10	Lr34	Sr2 STM559	Other *Sr* genes
AC Barrie	MS	no	no	yes	yes
AC Intrepid	MS	no	no	yes	yes
Alvena	S	no	no	no	yes
Helios	MS	no	no	yes	yes
Katepwa	MS	no	no	no	yes
Manitou	MS	no	no	NT	yes
Neepawa	MS	no	no	no	yes
BW553	MR	yes	no	no	yes
BW90	MS	no	yes	yes	yes
AC Cadillac	R	yes	yes	yes	yes
Peace	R	yes	yes	yes[2]	yes
BW711	R	yes	yes	No, but PBC[3]	yes
AC Taber	MR	yes	NT	NT	yes
AC Foremost	I	yes	no	NT	yes
AC Crystal	MS	yes	NT	NT	yes
AC Karma	MS	yes	no	NT	yes
5700PR	MS	yes	Hetero[4]	NT	yes

[1]NT – Not Tested

[2]Based on marker *X3B028F08*

[3]PBC pseudo black chaff symptoms expressed under high humidity

[4] hetero – heterogeneous

Table 5 ***Chromosome regions and significance of markers (p-value of F-tests) associated with Ug99 field severity (Sev), and infection response (IR) and seedling infection type (IT) determined in greenhouse trials. Marker position is based on the hexaploid wheat consensus map***

Chrom.	Position (cM)	Marker	Sev	IR	IT
1B	40	cfd48	0.019	0.011	0.005
2A	60	gwm372	0.031	0.018	0.043
6A	95	gwm617	0.031	0.019	0.008
7A	40	wmc283	0.018	0.011	0.035
7A	83	gmw276	0.016	0.012	0.043

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28. Principles for rapid variety release, seed multiplication and distribution in developing countries to counter the threat of wheat rust

Thomas Osborn[1], Zewdie Bishaw[2]

Abstract

Stem rust race of 'Ug99', first detected in Uganda (1999), has already reached Kenya (2002), Ethiopia (2003), Yemen (2006) and Iran (2007). Ug99 is a serious threat to global wheat production with potentially serious consequences for global food security. The development of resistant varieties, surveillance systems and effective plant protection strategies are important elements to control Ug99. However, to effectively counteract the threat of Ug99, a range of resistant varieties and production practices need to quickly get into the hands of farmers in areas at risk. In order to do this, national contingency planning for seed production needs to be implemented with a multi-stakeholders and multidisciplinary approach. It should include immediate actions such as fast tracking variety evaluation and release, accelerated seed multiplication, and distribution to farmers, in order to replace existing susceptible wheat varieties in high risk areas. This global threat to food security requires a coordinated rapid response with international, national, local and donor support.

Keywords

Variety release, seed multiplication, contingency planning, wheat, stem rust

Introduction

A virulent race of wheat stem rust known as 'Ug99' was first detected in Uganda (1999) and has spread to Kenya (2002), Ethiopia (2003), Yemen (2006) and Iran (2007). There is a potential threat for the spread of the disease to the most important wheat growing areas of the developing world with serious consequences for global food security. From empirical evidence, it is certain that Ug99 could spread to South Asia and East Asia. Preliminary screening of widely grown commercial wheat varieties from 18 African and Asian countries against 'Ug99' revealed that about 85% of them are susceptible to Ug99. Accordingly, an estimated 52% of the total wheat area of 74.6 million ha planted with wheat in these countries, collectively representing 40% of global wheat production, is planted with susceptible varieties. Furthermore, many wheat varieties grown in major producing and exporting countries, such as Australia, Canada and USA, are also susceptible to race Ug 99 and derivatives. It is therefore a serious threat to global wheat production with potentially catastrophic consequences which could trigger a global food crisis.

The development of resistant varieties, surveillance systems and effective plant protection strategies are important elements of a wheat rust control strategy that need to be urgently implemented. It is critical that current, widely grown, stem rust susceptible varieties are replaced with resistant ones to ensure global food security. Under the Borlaug Global Rust Initiative, CIMMYT, ICARDA and a number of Agricultural Research Institutes (ARIs) and National Agricultural Research Systems (NARS) in developed and developing countries have tested thousands of accessions in Kenya and Ethiopia. A number of elite lines have been identified with adequate resistance against Ug99 and up to 15% yield increase. Some of these materials are part of the Elite Bread Wheat Yield Trial (EBWYT) and Stem Rust Resistance Screening Nurseries (SRRSN) distributed by CIMMYT and/or ICARDA consisting high-yielding wheat lines with adequate levels of resistance to Ug99.

Seed is a means for delivering crop-based innovation to farmers to realize the impacts of investments in agricultural research. Availability of, and access to, wheat seed of resistant varieties is key to counter the threat of stem rust and ensure global food security. The key elements for contingency planning for the rapid dissemination of wheat rust resistant varieties to farmers include:

1. Rapid variety evaluation and release
2. Streamlining regulatory and phytosanitary protocols for movement of seed across international boundaries
3. Creating variety awareness and promotions
4. Rapid seed multiplication
5. Strengthening infrastructure for seed delivery
6. Human resource development for seed production.

Status of the national seed sectors

National seed sectors consist of formal (organized) system and informal (traditional) systems. The formal seed system includes seed production and seed supply mechanisms operated by the public and private sectors under some measure of supervision and regulation in a commercial or quasi-commercial mode within the framework of national seed policy and legislation. Improved varieties are the results of formal plant breeding and variety development that are tested and released in the country if they are proved superior to the

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existing varieties. It is the role of the public or private sector to make the seed of these varieties available to farmers. The formal seed system is governed by regulations intended to maintain varietal identity and purity and guarantee the physical, physiological and health of the seed. The informal seed system is basically what the formal seed is not. It is farmer and community based with a focus on traditional varieties and recycling of previously released modern varieties. The informal system includes the ways by which farmers produce, disseminate and procure seed directly from their own harvest, through barter in the community or purchase at local markets. In developing countries the informal seed system provides 80-90% of the food grains that farmers plant so its significance cannot be overlooked.

Advances in research and plant breeding, efficiency in identifying farmer-preferred varieties, effective seed production and delivery coupled with appropriate agricultural extension backed by appropriate seed campaign and rural development policies can ensure rapid adoption and diffusion of seeds of new varieties. The national seed sector in the country at risk, or affected by wheat rust, operates under a range of conditions in terms of agro-ecology, farming systems, crop varieties and markets. National seed sectors face a number of challenges, including ineffective policies, regulatory and institutional frameworks, lack of adapted new varieties, deficient production, processing and marketing infrastructures for seed, limited technical and managerial capacities, and poor socio-economic conditions. Such inherent weaknesses in the national seed industries hinder farmers' rapid access to new resistant wheat varieties in nearly all countries at risk of wheat rust. Consequently, this situation inhibits rapid responses to the impending threat of wheat rust. At present the formal seed sector meets less than 10% of the national wheat seed requirements (ranging from 4% in Ethiopia to 14% in Pakistan). The majority of farmers plant their own farm-saved seed, increasing the vulnerability of existing wheat varieties to impending rust epidemics. The situation is far worse for small-scale subsistence farmers living in less favorable dry areas and remote regions who directly depend on farming for their livelihoods. Subsistence farmers are more vulnerable to the threats of rust epidemics and risks to food security.

The most pressing challenge is not only developing stem rust resistant varieties, but to find innovative and flexible approaches to accelerate variety release and optimize seed delivery to ensure wide scale adoption and diffusion of rust resistant varieties both at national and regional levels. Taking into account the strength of the seed sector and infrastructure for seed production, both formal (public/private sector) and informal (farmer/community-based) approaches need to be considered to cater for the needs of each country. These can only be achieved by having effective contingency planning for organized fast-track release of new varieties and accelerated seed multiplication underpinned by flexible policies, and commitments by national and/or the international community of stakeholders.

Key elements in rapid seed delivery to farmers

1. Contingency planning for seed production

Contingency planning for seed production is part of the larger contingency planning that is necessary to counter the threat of wheat rust. Effective planning will require engaging all the stakeholders under the umbrella of a national task force at the highest level of government. In many countries the Minister of Agriculture would lead such a task force that would include the national plant protection services, agricultural research institutes, seed sector (both public and private), extension services, farmer organizations, NGOs and donors. It is anticipated that there will be substantial involvement of private sector seed companies and farmer organizations in the planning process. Consultation will be done at the national level, province/state level, and district level to engage the stakeholders to ensure their full commitment. The results of the planning process are to raise awareness and develop action plans that define roles and responsibilities, staffing requirements, supplies and equipment, land requirements with suitable infrastructure for effective seed multiplication (e.g. irrigation) and strategies for diffusion of Ug9 resistant varieties to farmers. The contingency planning would also include key recommendations to the government on policy and legislative issues to enable a rapid response to the threat of wheat rust. The following key elements will be part of the contingency planning.

2. Rapid variety evaluation and release

The lack of new varieties and a long time lag between variety identification and release often results in low variety replacement rates. In addition wheat is a self pollinated crop which the farmer often replants from the harvest of the previous year. The average age of wheat varieties in farmers' fields varies within and between countries and may reach over 14 years in some developing countries. Despite considerable efforts in releasing new varieties, large areas of wheat are still planted with relatively few and sometimes 'obsolete' varieties that are no longer recommended and susceptible to newly emerging diseases. For example, recent reports revealed that in India, 4.5 million ha in

eastern states are planted with three 'obsolete' varieties released between 1971 and 1986, and one variety (released in 1996) alone covers about 7 million ha in the north western states of the country (Ferrara et al. 2007). Lack of wheat varietal diversity and dominance by a few varieties over large areas is also a widespread phenomenon in other countries and regions (e.g. Ethiopia, Pakistan) predisposing farmers to vulnerability to diseases and risks of food insecurity. There is an urgent need to speed up varietal replacement in order to counteract the threats of wheat rust epidemics and ensure the food security.

For promising new wheat varieties, there are standard procedures in most countries for variety testing and release before they can be multiplied and used by farmers. Variety release can require simultaneous testing of promising new lines for registration concerning Distinctness, Uniformity and Stability (DUS), and performance testing of Value for Cultivation and Use (VCU). Some countries have well defined compulsory variety testing and release procedures (both DUS and VCU) in place (e.g. Egypt, Pakistan, India, Turkey), whereas in others, the release system is purely dependent on performance testing conducted by the agricultural research authorities and approved by ad hoc release committees (e.g. Ethiopia, Kyrgyzstan). The established procedures for national variety trials sometimes require many years. This means that even with countries that share similar agro ecologies, most countries require compulsory registration in a national varietal catalog before a variety released in another country could be authorized for cultivation.

Lengthy and slow variety testing and release remains a critical bottleneck to speedy release of new varieties. As part of contingency planning, national authorities should allow NARS to adopt a fast-track release for wheat rust resistant varieties with superior field performance and acceptable organoleptic characteristics. This fast-track approach could introduce varieties with known resistance and test them for two growing seasons in multi-location adaptation trials and release them promptly for large-scale use. The serious threat of wheat rust means that variety evaluation and release may need to be streamlined so that it is efficient and effective, but at the same time carried out in as short a timeframe as possible. Variety evaluation and release, or even compulsory registration, in the national varietal catalog should not delay the process of getting resistant varieties to farmers.

2.1. Regional variety release International Agricultural Research Centers (IARCs) in partnership with NARS are at the forefront of a breeding program to develop stem rust resistant varieties through a coordinated international nurseries network distributing EBWYT and SRRSN. It is anticipated that countries will be able to share promising Ug99 resistant lines in order to accelerate the process of testing and releasing a wide range of resistant wheat varieties. It will be important for national varietal testing systems to be strongly linked with international information sources and sharing varietal data and performance under a wide range of agro-ecologies.

Given the global scope of the threat and the need for a global response, efforts should be made to consider a policy for joint release within regions. Since many NARS are evaluating similar breeding materials across regions, with potential for both wide and specific agro-ecological zones of adaptation, opportunities must be explored for joint or regional release of varieties. Harmonized regional variety release schemes should be considered**.** Streamlining regulatory and phytosanitary protocols for movement of varieties and seeds across international boundaries are also needed as part of regional harmonization of seed rules and regulations. Attention should be given in diversifying the portfolio of varieties released across the countries. In the absence of a regional release system, national authorities should consider a clause for exemption from compulsory registration for wheat rust resistant varieties coming from similar agro-ecological conditions outside of the country.

2.2. Varietal choice It is anticipated there will be many wheat rust resistant lines adapted to a wide range of agro-ecological zones and with farmer preferred traits available in the near future from international and/or national breeding programs. National programs with responsibility to screen wheat rust resistant varieties will be able to source the most promising lines for evaluation on release and to use them in national breeding programs. New wheat varieties must combine not only wheat rust resistance, but also resistance to other major diseases and yield superiority compared to existing varieties as well as adaptation to the use of wheat by farmers, consumers and industries. National breeding programs should aim at developing and releasing a wide range of varieties with diverse genetic backgrounds for wheat rust resistance to reduce vulnerability and risk of disease epidemics. It is important to maintain varietal diversity and overcome varietal dominance by identifying and releasing those with comparable agronomic performance and preferred traits at national and/or regional levels.

2.3. Access to public varieties Wheat breeding in many developing countries is dominated by IARC breeding materials distributed to the NARS. In many countries public seed companies have sole access to new varieties. Access to new publicly bred varieties

and adequate quantities of basic seed remain a major constraint for emerging private sectors which rely on NARS for improved varieties. Given the expected role of private sector seed companies, it is important that access to varieties and sufficient quantities of early generation seed be given priority in the contingency planning. It is clear that the private sector is more effective at marketing seed. In countries where PVP is in place adequate protection should also be provided for stem rust resistant varieties (e.g. through licensing arrangements).

2.4. Variety awareness and promotion In a developed seed industry there is a high degree of commercial-orientation from the outset where variety development is client-oriented and integrated with creating awareness and promotion. Generally, plant breeders have a good idea of what farmers need and through effective marketing farmers are aware of what is available and they have the capacity to use new technologies. By contrast in many developing countries variety development is much more complex because of the range of agro ecological zones and crops grown. In addition, agricultural extension is often cited as the weakest link between research and farmers. Given the threat of wheat rust, any campaign should take into account these shortcomings and design innovative strategies (e.g. subsidized seed prices, paying premiums for production) to promote the use of rust resistant varieties.

Farmers need to become aware of the threat of wheat rust and get to know the resistant varieties. For varieties that will be developed through formal breeding schemes, it will be necessary to establish demonstration plots to create farmer-awareness of resistant varieties and management practices through the NARS, national extension services, farmer organization and NGOs. The organization of field days in seed production plots would also popularize and promote varieties which could help to create seed demand. Initial targeting should be in zones that have a high potential risk of wheat rust. Demonstrations should be linked to training in monitoring and detecting rust and to production practices and other strategies to limit the spread. The distribution of small seed-packs (e.g. 25 kg) of new rust resistant wheat varieties in selected target areas, for farmers to plant alongside currently used varieties would also contribute to adoption of the new genotypes. Farmers should also be encouraged to share seed with other farmers to facilitate farmer to farmer seed diffusion.

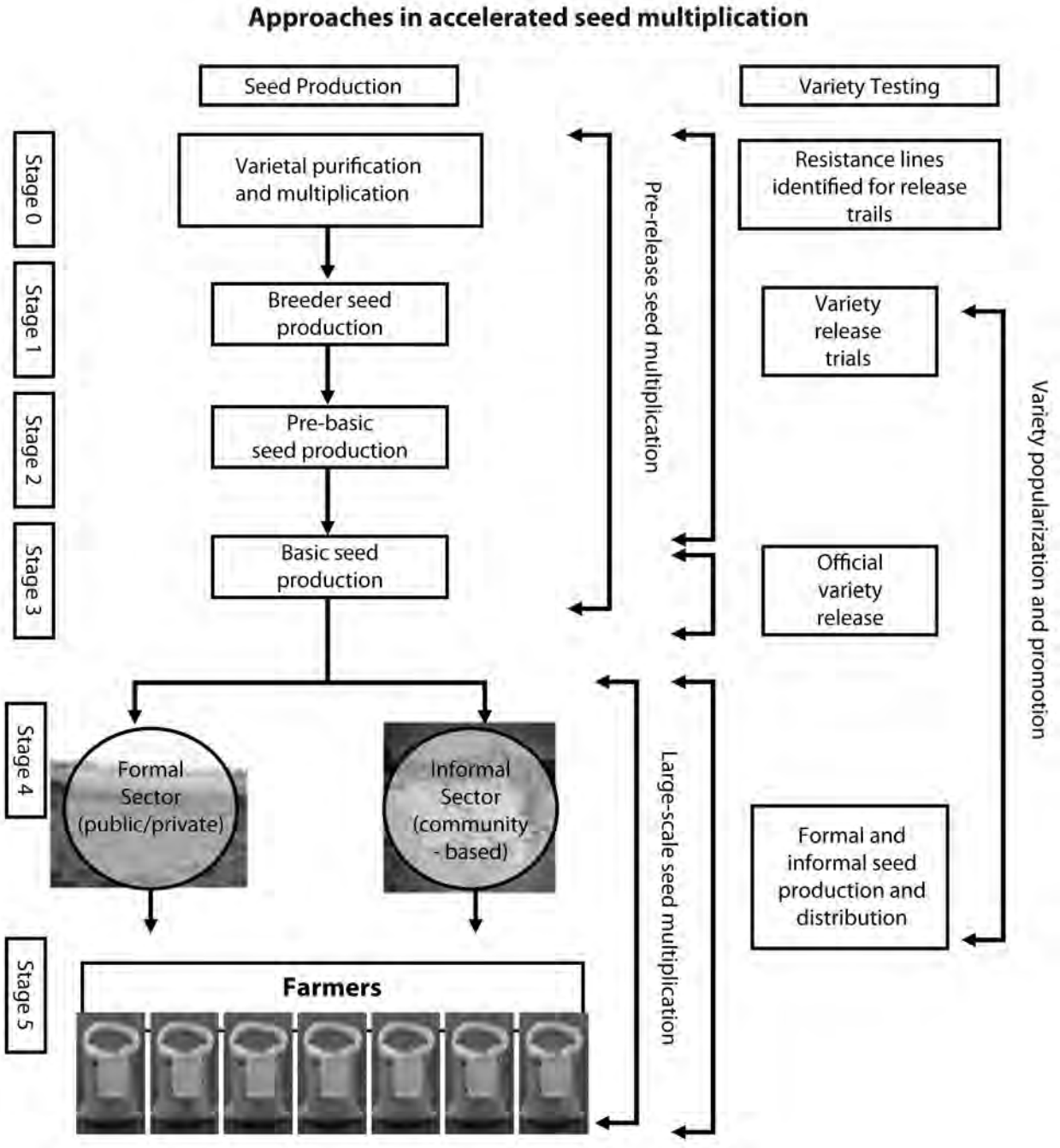
Participatory approaches such Participatory Varietal Selection (PVS), are also proven approaches to facilitate communication between plant breeders and farmers and to speed up the adoption of improved varieties particularly among small-scale farmers with limited access to the formal sector seed supply. Using participatory approaches in the development of resistant varieties could be another way to create awareness on wheat rust issues among farmers and to foster the diffusion of resistant varieties.

3. Rapid seed multiplication

The formal seed sector follows a systematic procedure for seed production in preparing a new variety for commercial distribution. Rapid seed multiplication should be linked with a fast track-variety evaluation and release system to get resistant varieties into the hands of farmers as quickly as possible. There are two critical stages in the pathway between variety development and getting the new varieties to farmers (Fig. 1): (i) pre-release seed multiplication of early generation seed (breeder seed to basic seed); and (ii) large-scale seed multiplication (basic to certified). Van Gastel et al. (2003) described the procedure for production of quality wheat seed.

3.1. Accelerating pre-release seed multiplication The time lag between variety release and availability of sufficient quantities of early generation basic seed can be lengthy because seed multiplication often will not start until the official release of a new variety. However, this is not the approach of the seed industry in many developed countries where pre-release seed multiplication is normally undertaken to bring the new variety more rapidly to the market. Contingency planning should recommend that national authorities undertake pre-release seed multiplication of newly identified potential resistant varieties in order to accelerate the availability of early generation seed while the new variety is undergoing final variety testing and evaluation for release (Fig. 1). Ear rows (spikes) will be collected for varietal purification and breeder seed production from promising lines identified and submitted for variety evaluation and release. Alternatively, the seed of promising lines will be planted as bulks and intensively rogued to maintain the varietal purity. A concerted effort should be made with NARS and public/private sector in target countries to multiply seed of these newly identified promising varieties. It is also possible to use both main and off-season seed production to shorten the time and maximize the availability of sufficient stocks of basic seed. This will allow initiating immediately accelerated large-scale certified seed production by the time a variety is officially released. ICARDA and CIMMYT have already launched pre-release seed multiplications of some identified rust resistant promising lines in selected countries. These activities could be handled in

Fig. 1 *Accelerated seed multiplication scheme for stem rust resistant wheat varieties*



collaboration with NARS, or as joint activities with the public/private seed producers and suppliers.

Despite huge investments in variety development, most NARS pay limited attention to early generation seed production due to lack of funding and incentives coupled with absence of functioning seed units due to insufficient land and facilities (e.g. machinery, irrigation). These tasks require specialized field equipment (e.g. plot planters, plot harvesters, small cleaners/treaters) for timely operations and appropriate facilities (e.g. irrigation, storage, cold storage) for main or off-season seed production. Creating and strengthening such units would institutionalize early generation seed production on a sustainable basis and enable countries to adequately respond to any future emergency situations. In many countries, the benefits generated by selling early generation seed go to government treasury instead of being directly used by the institutions that produce the seed. This financial arrangement is a disincentive and could be partly responsible for lack

of motivation of NARS to invest in early generation seed multiplication. Adopting procedures whereby the income from early generation seed sales are directly returned to the NARS' budgets would increase their motivation to invest in this area. Another additional incentive for plant breeding is the need for establishing royalty systems for public-bred varieties (e.g. Egypt) in the absence of PVP.

3.2. Accelerating large-scale certified seed multiplication Once wheat rust resistant varieties are officially released and registered nationally or regionally and there is a sufficient quantity of early generation seed, the contingency plan should outline a national strategy for the seed multiplication and distribution of certified seed of resistant varieties to replace susceptible varieties in high risk areas or hotspots. The basic seed produced will be made available to: (i) public and private seed sector; and (ii) farmer/community-based seed initiatives for further multiplication to produce quality seed under accelerated conditions both during main/off-season production.

Many countries will want to consider how to speed up the seed multiplication process. Strategies for consideration include:

- Intensive management of the initial seed multiplication to raise the multiplication factor from 30 to 50 or more. This will require excellent crop management, i.e. seed-bed preparation, precision planting, excellent weed control, high levels of soil fertility, irrigation, pest control as well as timely harvesting.
- Producing two crops per year as in some countries and this is a major advantage in accelerating seed multiplication. There could also be cooperative agreements between countries to produce more than one crop a year.
- Importation of large quantities of seed from reliable sources to kick-start seed multiplication. Alternatively, large quantities of seed of adapted varieties can be imported for direct distribution and use. Any seed importation would require compliance with seed import rules and regulations including pest risk assessments.
- Establish a regional approach to accessing varieties in the quantities needed. Specific countries could specialize in producing specific resistant varieties. This would require the establishment of regional agreements to simplify seed import and export procedures.

3.3. Achieving higher seed multiplication rates The multiplication factor (MF), the ratio of the amount of seed planted to the amount of seed produced, determines how quickly the seed can be multiplied and eventually made available to farming communities. The MF differs between crops/varieties and is also influenced by the production environment and crop management. With wheat there is a high yielding capacity at low seeding rates because of the high tillering capacity. When this factor is coupled with its relatively small seed size, it is possible to very rapidly produce seed. At ICARDA, wheat seeding rate studies on multiplication factors show that by reducing the seed rate from 186 kg to 17 kg ha^{-1}, the multiplication rate increased from 20 to 204 under dryland conditions, although little variation existed among five varieties (Srivastava and Simarski 1986). Rapid seed multiplication was also reported from elsewhere, where 1.36 tonnes of seed was multiplied to 15,000 tonnes within a period of two years with nearly a multiplication factor of 105. To facilitate rapid multiplication particularly for early generation seed, it is suggested to increase the space between rows and plant at wider spacing within the rows to double the amount of nucleus seed, and to use seeding rates of 25, 50 and 100 kg ha^{-1} for breeder, basic and certified seed multiplication. This approach needs to be undertaken with superior crop management including soil fertility management, irrigation and excellent weed control.

3.4. Production planning Production of rust resistant varieties is not a one off or short-term effort. It is anticipated that a range of wheat rust resistant varieties will be released over time from both international and national breeding programs given the expected short longevity of rust resistance in wheat[1]. As a starting point in contingency planning, the initial target for rapid seed multiplication could be to cover 10% of the wheat production area. In most countries this can be accomplished within three to four generations (see Table 2). The actual targets for rapid seed multiplication will depend on the actual and potential threat of rust elaborated by the contingency planning and surveillance system. Vulnerability to rust may vary in major wheat production areas within and between countries. For example, south eastern and central Ethiopia are major wheat producing regions and hotspots for rust epidemics. In contrast northwest Ethiopia is at less potential risk to wheat rust epidemics. Admassu et al. (2008) reported that Ug99 was present throughout the country and dominated in all regions except northwest Ethiopia.

National rapid seed multiplication strategies must also include the province/state and district levels since much of the seed multiplication will be undertaken at district level. Although some of the countries at risk to

[1] It is expected that wheat rust strains will adapt rapidly to resistant varieties and that new resistances will have to be continually developed in the future.

Table 1 Estimated seed multiplication with varying yield levels (3, 4 and 6 tonnes ha^{-1})

Generation	Quantity of seed produced in tonnes with different multiplication factors		
	1:30	1:40	1:60
Initial seed quantity (t)	0.05	0.05	0.05
First	1.5 (*0.5 ha*)	2.0 (*0.5 ha*)	3.0 (*0.5 ha*)
Second	45 (*15 ha*)	80 (*20 ha*)	180 (*30 ha*)
Third	1,350 (*450 ha*)	3,200 (*800 ha*)	10,800 (*1800 ha*)
Fourth	40,500 (*13,500 ha*)	128,000 (*32,000 ha*)	648,000 (*108,000 ha*)
Fifth	1,215,000 (*405,000 ha*)	5,120,000 (*1,280,000 ha*)	38,880,000 (*6,480,000 ha*)

Figures in parentheses indicate the areas required to produce the seed of the concerned generation

wheat rust already have systems for seed multiplication, modifications may be needed to cope with the urgency of rapid large-scale multiplication and distribution of resistant varieties, especially to the most vulnerable small farmers.

Partnerships with private sector seed companies may be the quickest and most cost-effective strategy for seed multiplication in some countries. Contingency planning will include a well coordinated system for rapid seed multiplication with an effective partnership and high level of coordination in order to be successful.

Table 1 presents the theoretical basis of seed multiplication assuming an initial 50 kg of nucleus seed of a new variety at a planting rate of 100 kg ha^{-1} and anticipated yields of 3, 4 and 6 tonnes ha^{-1}, i.e. multiplication factors of 30, 40 and 60, respectively. The table provides an idea of the numbers of generations, areas needed for seed multiplication, and areas the seed can cover at various multiplication factors.

The importance of intensive production of seed through optimum crop management practices (e.g. weed control, water) to achieve higher multiplication factors is highlighted in the differences in the MF and the resulting total production after five generations. In addition, the large areas required for seed multiplication provide an idea of the scope of the seed multiplication needed in order to address the wheat rust threat.

Table 2 provides an idea of the quantities of seed that may be needed in each country. A tentative target of seed to cover 10% of the total area in wheat is included. The figures demonstrate the need to undertake intensive wheat seed production in order to reduce the time needed to produce the target quantities of wheat seed.

National seed programs in risk-prone areas will need to have contingency plans for maintaining and managing carry-over certified seed stocks to overcome shortfalls in seed supply due to emergencies or crop failures. The main purpose is to ensure a reliable seed supply to the farming community through the activities of the formal sector. The excellent storability of wheat seed provides the option of rapid seed multiplication of rust resistant varieties and establishing a strategic reserve of early generation seed even before rust has threatened production. With this strategy resistant wheat varieties can be quickly released when needed.

3.5. Seed import and distribution Another potential strategy for the urgent provision of seed of rust resistant varieties may be the direct import and distribution of seed from neighboring countries in areas where agro-climatic conditions are similar and the variety is adapted, tested and released in the importing country. There are clear practical examples of importing seed of improved varieties from neighboring countries for distribution through public/private seed sector and NGOs both under normal and emergency situations.

In order to anticipate such a situation, an effort to undertake regional harmonization of seed rules and regulations, especially in the area of seed certification and plant quarantine would facilitate seed trade between countries of the same region.

Table 2 *Area under wheat cultivation, 10% target area and seed requirement for 10 % of the total area (FAO Statistics)*

Country	Area under cultivation in 2007 (ha)	10% of the area (ha)	Seed required for 10% of wheat area (mt)
Afghanistan	2,190,000	219,000	21,900
Algeria	2,000,000	200,000	20,000
Armenia	113,300	11,330	1,133
Azerbaijan	486,990	48,699	4,870
Bangladesh	372,000	37,200	3,720
China	23,000,000	2,300,000	230,000
Egypt	1,139,000	113,900	11,390
Ethiopia	1,351,000	135,100	13,510
Georgia	61,000	6,100	610
India	28,035,000	2,803,500	280,350
Iran	6,400,000	640,000	64,000
Iraq	2,750,000	275,000	27,500
Jordan	30,000	3,000	300
Kazakhstan	12,876,700	1,287,670	128,767
Kenya	150,000	15,000	1,500
Kyrgyzstan	354,500	35,450	3,545
Lebanon	48,000	4,800	480
Libya	132,000	13,200	1,320
Morocco	2,571,900	257,100	25,710
Nepal	702,664	70,266	7,026
Oman	275	27.5	3
Pakistan	8,494,000	849,400	84,940
Saudi Arabia	462,000	46,200	4,620
Sudan	250,000	25,000	2,500
Syria	1,850,000	185,000	18,500
Tajikistan	330,000	33,000	3,300
Tunisia	856,000	85,600	8,560
Turkey	8,600,000	860,000	86,000
Uganda	11,000	1,100	110
Uzbekistan	1,400,000	140,000	14,000
Yemen	114,030	11,403	1,140

Regional harmonization of seed rules and regulations is underway in several regional areas of Africa. In addition, a seed association has been established in the central Asia and west Asia region to create a forum for dialogs amongst stakeholders concerned with the seed trade/industry development. This association will also play a crucial role in the establishment of procedures and processes for the harmonization of seed rules and regulations aimed at facilitating cross boundary movement of seed. Given the importance of wheat in this region this initiative will be a very important strategy to counter the threat of wheat rust.

3.6. Quality assurance The production of quality certified seeds will require critical roles for the national seed services and related seed certification agencies to ensure the inspection, testing and certification of the seed. Some countries may need to strengthen their seed certification services to respond to this demand through training of additional technical officers to handle the increased workload and transportation support. In addition, related facilities such as seed testing laboratories may need to be established or upgraded to cope with the higher demand for services and possibly to implement regionally harmonized seed rules and regulations for variety release, phytosanitary standards and the seed trade.

The databases of the National Seed Certification Agencies and national variety registries should also be strengthened to include information on attributes such as responses to rust races to which wheat varieties are resistant, and lists of plant quarantine pests to facilitate exchange of tolerant varieties among countries.

4. Strengthening seed multiplication capacity

Rapid seed multiplication of large quantities of seed will require capacity building beyond the ongoing normal seed activities at country or regional levels. This emergency situation brings more work to already limited infrastructures to undertake a huge task of both pre-release and large-scale seed multiplication where time is an essence of all operations. Apart from efficient use of existing resources and facilities additional investments may be necessary for robust response to the crisis.

Seed production is a specialized task both in field operations and post-harvest handling of seeds. Speed and time are the essence in an emergency situation. There are urgent needs for special equipment (single ear/bundle threshers, plot planters, plot harvesters, and seed cleaners/treaters) for timely operations and appropriate facilities (e.g. irrigation, storage, etc) for main or off-season seed multiplication of early generation seed. Additional field machinery such as tractors, implements, irrigation equipment, and combines, and seed processing and storage facilities may be needed for large-scale seed multiplication. A review of equipment and facilities for seed production, seed conditioning (processing) and seed storage should be undertaken to ensure that this element is not a constraint to the rapid seed multiplication and delivery of quality seed. Provisions of farm machinery and equipment should be made on a case by case basis specific to the needs of the individual countries.

4.1. Seed storage facilities Wheat seed can be effectively stored without losing its vigor and germination if well known precautions are taken. Keeping the seed as dry and cool as possible in clean stores is the best management practice because physiological processes and fungal and insect activities are low. Safe storage of wheat seed is possible as long as the moisture level is below 13%, the humidity is low, ambient storage temperature is not excessive and the infestation with storage insects is minimal. In practice, stored wheat seed should be kept at moisture levels below 12% and relative humidity below 50-60%. Van Gastel et al. (2003) cited practical guideline in choosing alternative sites for short and medium term seed storage. It is advisable to select a seed storage site, which is cool and dry (low relative humidity).

Adequate seed storage facilities should be made available particularly for the maintenance and management of carry-over stock of early generation seed and certified seed. Small quantities of seed can be stored for long periods in cold rooms; whereas properly designed seed storage facilities might be required for larger quantities.

4.2. Human resource development for seed production Specific knowledge and practical experience is needed to produce high quality seed with high standards of varietal purity, and physical and physiological health. From the outset, assistance in capacity development of NARS and national seed programs is a key to enhance their technical and managerial capacities as well as the implementation of regionally harmonized seed rules and regulation. Three levels of training could be envisaged from policy makers to farmers ranging from policy issues to practical experience: (i) workshops to create awareness and inform senior staff and policy makers; (ii) training of trainers' courses for technical managers and technicians; and (iii) practical training for farmer seed producers and growers. Private seed companies will be key partners in certified seed production and they will benefit from training programs. Training will be required in principles and techniques of variety maintenance, seed

production, seed processing, and seed quality control for technical managers, technicians and farmers whereas workshops on policy and regulatory issues would address decision makers.

Concluding remarks

The following are the key points for immediate action for policy makers to develop and establish contingency planning with wide participation, clear roles and responsibilities and necessary resources for rapid variety release, seed multiplication and distribution to counter the threat of wheat rust.

- Ensure that systems are in place for fast-tracking and rapid release of wheat rust resistant varieties
- Streamline regulatory and phytosanitary protocols to facilitate movement of varieties and seeds across international boundaries
- Create awareness about wheat rust with the public and private sectors and farmers' groups, and demonstration of resistant varieties for farmers
- Ensure that capabilities and systems are in place for initial and sustained rapid multiplication of wheat rust resistant varieties
- Determine appropriate methods to accelerate seed multiplication such as intensive management, off season production, and importation of wheat rust resistant varieties
- Provide the necessary facilities, machinery, equipment and supplies to ensure rapid seed multiplication of rust resistant varieties
- Provide training in technical aspects of seed production, processing and quality control, and policy advocacy for rapid variety release options, harmonization of seed rules and regulation.

If these points can be addressed, national seed sectors will become more efficient, better coordinated and more capable of providing quality seed of improved varieties to respond to the rust threat. A wider range of challenges include climate change, pest and disease threats, and drought. Consequently, farmers will be able to more rapidly access technologies that they will need for food security in a rapidly changing world.

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29. Ethiopia's experience with rapid seed multiplication and cultivar replacement

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Availability and access to improved seed are the constraints for improving productivity and production of crops in Ethiopia. In the case of wheat, even when available, varieties often lose their disease resistance before or soon after their adoption by farmers due to inefficient seed multiplication and delivery systems. The current production of improved seed accounts for less than 3% of all seeds needed for annual crop production. Over 97% of the seeds come from farmer sources through traditional seed exchange or seed marketing. There are two seed supply and delivery systems in Ethiopia. These include the formal and the informal sectors. Research, as a source of breeder and pre-basic seed, the public seed enterprises, and some private businesses represent the formal seed sector.

For the informal seed system, research stations serve as the initial source of seed, and farmers, farmers' co-operatives, Ministry of Agriculture and Rural Development, and non-government organizations are the main participants in seed multiplication and dissemination. Countrywide attempts at informal seed multiplication and scaling-up of crop technologies have resulted in *small success stories* in the late 1990s and early 2000s. Cereals, pulses, oilseeds and tuber crop varieties have gone through participatory seed multiplication and scaling-up with encouraging success. Recent experiences show that rendering research center-based support to both the informal and formal seed systems can improve variety adoption and the access of farmers to high quality seed. Participatory seed multiplication at the village level, supported by some training and minimum guidance, is regarded as a useful approach for rapid technology dissemination and cultivar replacement under Ethiopian conditions. This paper describes Ethiopia's experience in informal rapid seed multiplication of wheat, malting barley, faba bean, lentils, and haricot beans.

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30. Experience with rapid seed multiplication and cultivar replacement targeting race Ug99 resistant wheat varieties in the Eastern Indo-Gangetic Plains

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Abstract

The threat of stem rust epidemics caused by *Puccinia graminis* f. sp. *tritici* race Ug99 to the wheat crop on the northeastern Gangetic Plains is real. The warm and humid conditions experienced in the region are conducive to rapid disease development. Identification and breeding Ug99-resistant varieties are therefore major priorities for the region. Because of the underdeveloped seed industry and small farm sizes, various strategies are needed to disseminate resistant cultivars in a relatively short time before Ug99 reaches South Asia. Although the Indian wheat program, in collaboration with CIMMYT and KARI, has identified some existing resistant wheat varieties, the areas they occupy must increase to about 5% of the total wheat area to ensure sufficient seed for replacement of current popular varieties if necessary. In addition to national evaluation trials including advanced selections from all breeding programs, there are also farmers' participatory selection approaches in several districts in the eastern Gangetic Plains. Therby new superior lines and newly released varieties are disseminated to farmers. The objective is to enhance genetic diversity and to provide more options to farmers. The inclusion of Ug99-resistant high yielding lines distributed during the last three years (2006-2009) is enabling farmers and the region to prepare for future challenges. Some of the new lines included in this fast-track participatory approach have shown significant yield superiority over the highly popular variety HUW234, and better resistance or tolerance to other biotic and abiotic stresses that occur in the region. Moreover, the incomes of farmers, who choose to sell grain of their preferred varieties as seed, have also increased. Our results show that participatory variety selection of diverse promising lines and released varieties enables them to be disseminated to farmers in a way that enhances productivity and income simultaneously.

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Keywords

Triticum aestivum, stem rust, *Puccinnia graminis*, participatory varietal selection, participatory seed production

Abbreviations

IGP, Indo-Gangetic Plains; RWCS, Rice Wheat Cropping System; EGP, Eastern Gangetic Plains; PVS, participatory varietal selection

Introduction

Wheat is a major staple food crop in South Asia and therefore, it is of paramount importance for food security in the region (Joshi et al. 2007; Ortiz-Ferrara et al. 2007; Chatrath et al. 2007). In South Asia, wheat is grown on about 36 million ha (16% of the global wheat area) and annual production is around 100 million tonnes, or 15% of world total (FAO 2007). This region, which comprises India, Pakistan, Nepal and Bangladesh, is among the most populous in the world with a total population of around 1.5 billion people (UN 2009). The region is credited with the greatest success stories of the Green Revolution and has so far been quite successful in addressing the pressures of food security concerns. India, which accounts for approximately 12% of world wheat production (FAO 2007), is the major wheat producer in South Asia and produced a record 78 million tonnes in 2007-08 (DWR 2008). The success story of wheat production owes much to major contributions from wheat breeders based at different institutions and their partner people and organisations. A strong and ongoing disease resistance objective in breeding programs has kept diseases at bay (Singh et al. 2006). These efforts were strongly supported by the dissemination of high quality seed, a further aspect that was paramount to reaping the benefits of the research.

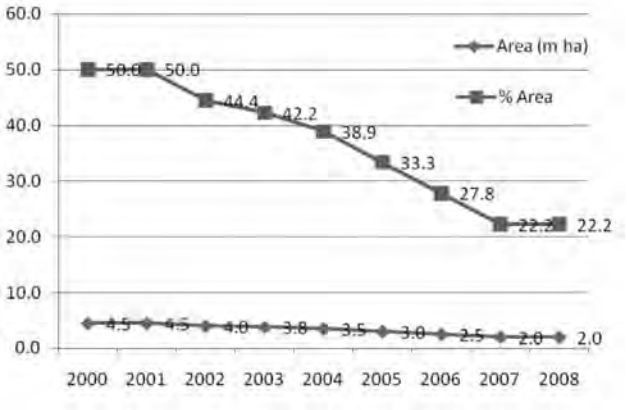
Although a number of biotic stresses had bothered wheat growers and researchers in South Asia, the unexpected emergence of stem rust race Ug99 in eastern Africa again gave stem rust the status of enemy No. 1. The reasons for this are convincing and well documented (Singh et al. 2008). The wheat varieties grown in the vast Gangetic plains covering much of Pakistan, India, Nepal and Bangladesh were susceptible (Joshi et al. 2008; Singh et al. 2008). If an epidemic of race Ug99 was to occur in South Asia, a huge population of wheat farming families would be seriously affected and there would be significant implications for rural and national economic growth (Joshi et al. 2008). The grim situation caused by Ug99 has compelled nations to monitor pathogen movement and to support wheat research, and has alerted national policy-makers to work to prevent the spread of the disease.

The two dominant cultivars of South Asia, PBW 343 and Inqualab which occupy around 8 and 7 million ha in India and Pakistan, respectively, are susceptible to Ug99 (Singh et al. 2008). Only 0.3% of a total reported area of over 44 million ha planted to known cultivars in the predicted potential epidemiologic zone of Ug99 was rated as moderately resistant (Pretorius et al. 2000; Singh et al. 2006, 2008). However, a recent finding that cv HUW 234, a previously popular cultivar on the eastern Gangetic plains, continued to occupy around 2 m ha was fortuitous. The cultivar is heterogeneous for a moderate level of resistance to race Ug99. The genetic basis of the resistance is unknown. HUW 234, was grown on nearly 5 m ha in the late 1990s. However, in the past few years its area declined to around 2 m ha (Fig. 1) due to aggressive introduction of new varieties following a number of dissemination strategies, including participatory varietal selection and participatory seed production. Therefore, of the 27 m ha wheat area of India, 24 m ha (89%) were estimated to be occupied by race Ug99-susceptible varieties. Of this, around 17 m ha are in the Gangetic plains. Hence, there is a huge challenge to increase production of quality seed of resistant varieties before Ug99 strikes this vast region.

The best strategy to check potential losses caused by Ug99 will be to identify and deploy resistant wheat genotypes adapted in the target environments in countries expected to be affected (Singh et al. 2008). It is advisable to introduce durable resistance to current cultivars and recent wheat germplasm as a long term strategy. Work done by Knott (1982) and current knowledge of durable resistance to leaf rust and stripe rust (McIntosh 1988) indicate that such resistance involves multiple minor genes with additive effects. Accumulating such complex resistance in the absence of disease pressure caused by Ug99 and lack of molecular markers will be a huge challenge.

The South Asian wheat program has initiated activities, in collaboration with CIMMYT, to identify and develop suitable resistant cultivars for rapid deployment in its different wheat zones before Ug99 arrives. Wheat varieties and breeding lines are being sent for screening with race Ug99 and its derivatives at Njoro (Nakuru), Kenya. Many of the varieties identified as resistant in the years 2005 and 2006 carried *Sr24* (Table 1). The Indian lines found to carry satisfactory resistance, but not carrying of *Sr24* (Joshi et al. 2008), are listed in Table 2. An aggressive strategy to promote resistant cultivars in farmers' fields through large scale quality seed production is the only viable option for the region as resource-poor farmers in most of South Asia cannot afford to use chemical control.

Fig. 1 ***Coverage (m ha) and per cent wheat area occupied by wheat cultivar HUW 234 in the north eastern plains of India during 2000-2008***



Evaluation of Ug99 resistant lines following participatory varietal selection (PVS) in farmers fields

Participatory varietal selection of wheat varieties was introduced to the eastern Gangetic Plains by Banaras Hindu University in collaboration with CIMMYT and Indian Council of Agricultural Research (ICAR) in 1996-97. Most Ug99 resistant varieties were included in crop season 2006-07; however, a few lines used in 2005-06 also carried moderate to low resistance. This wheat zone in India represents CIMMYT mega-environment ME5 (Singh et al. 2007). The participatory approach has made significant impacts in some districts in eastern Uttar Pradesh by changing the varietal spectrum and also in promoting zero tillage. For instance, in the Chandouli district where wheat is grown on about 100,000 ha, zero tilled wheat rose to more than half of the area within five years of its introduction (Fig. 2). Concurrently, cv. HUW 234 declined from more than 90% of the area in this district to around 30% (Fig. 2). Around 12 varieties are grown in this district at present, with the major share being HUW 510, HUW 468, PBW 343, PBW 373, PBW 154 and HD 2733. The combined effect of early sowing permitted by zero tillage and longer duration higher yielding varieties led to an increased wheat productivity from 1.6 t/ha to 2.2 t/ha in the past five years. However, none of the varieties replacing HUW 234 are resistant to Ug99. Hence, new superior Ug99-resistant lines from CIMMYT were evaluated in farmers' fields following the PVS mode.

In the last three years (2005-06, 2006-07 and 2007-08), new wheat lines developed at CIMMYT, Mexico, were grown in 8, 9 and 8 locations, respectively. Lines tested under PVS during three years along with their pedigrees and reactions to race Ug99 (in Kenya) are

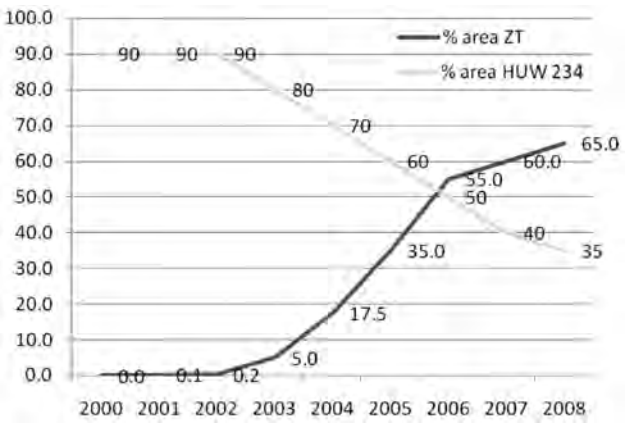
listed in Table 3. In the first year (2005-06), the lines used were the best short duration lines of the CIMMYT breeding program targeting irrigated environments, but carried only mild tolerance to Ug99 as it coincided with the beginning of the Ug99 resistance breeding program. Each year PVS followed the "mother baby" approach (Witcombe et al. 2001) with two standard check varieties, HUW 234 and HUW 468. In the current 2008-09 season, a set of ten varieties carrying resistance to Ug99 are under evaluation at ten locations.

Results for six lines that yielded 10% or above HUW234 in the year 2005-06 are given in Table 4. Three lines with 16.6 to 21.1% higher yields than HUW234 were the earliest maturing among the group with the same days to heading as new cultivar 'HUW468'. In the next two years several new lines proved superior to local checks in yield performance by over 10% (Table 5). As shown in Table 3, all these superior varieties carry higher levels of resistance to Ug99.

Inclusion of Ug99 resistant lines in the national coordinated trials

In addition to PVS trials, the superiority of many of the Ug99 resistant lines, e.g. Munal#1 (Waxwing*2/ Kiritati) and Quaiu#2 (BABAX/LR42//BABAX*2/3/ VIVITSI), has already been established through the Elite Bread Wheat Yield Trials (EBWYTs) tested across many locations of South Asia in the past three years (DWR Report, 2007; 2008). These lines were included in the All India Wheat Coordinated Trials organized by ICAR. Likewise, based on the results of PVS trials in farmers' fields, five of the Ug99 resistant varieties were included in the Indian plant protection screening nursery (IPPSN), 2008-09, and one line was promoted to the National Initial Varietal Trial (NIVT) by Banaras Hindu University. Some of these Ug99-resistant varieties have also been advanced to national coordinated trials by different research centers, i.e. Punjab Agricultural University and Directorate of Wheat Research.

Fig. 2 ***Decline in percent coverage of wheat cultivar HUW 234 and increase in zero till area for wheat in district Chandouli, Uttar Pradesh, India (wheat area = 100,000 ha)***



Seed dissemination in South Asia

Although a substantial network of organized (both public and private sectors) seed production does exist for germplasm dissemination and adoption in India, the seed replacement rate is still less than 20% (Joshi et al. 2007). The actual figure is believed to be around 10% in the eastern part of Indo-Gangetic plains (Joshi et al. 2007). It is accepted that for proper dissemination and adoption of germplasm, both the public and private

Table 1 ***Stem rust responses of wheat cultivars and advanced breeding lines from South Asia at Njoro, Kenya in 2006 and 2007***

Country of origin	No of lines			Total
	Resistant[1]	Moderately resistant[2]	Moderately sus. & susceptible[3]	
Bangladesh	4	8	112	124
India	16	7	79	102
Nepal	1	6	153	160
Pakistan	3	24	184	211
Total	24	45	528	597

[1]Disease severities up to 20% based on modified Cobb scale; small to intermediate sized uredinia with necrosis or chlorosis

[2]Disease severities between 15 and 30%; medium to large uredinia with or without chlorosis and necrosis

[3]Disease severities >40%, medium to large uredinia without chlorosis and necrosis

Table 2 *Indian wheat cultivars displaying acceptable levels of stem rust resistance at Njoro, Kenya during two years of testing (2005 and 2006)*

Species/ Cultivar	Pedigree	Max. score	Response	Year of release	Zone of release	Approx. area covered (m ha)
T. aestivum						
HW 1085	HW 2002A/CPAN 3057	10 MS	Moderately Resistant	1998	South Hill	<0.01
GW 273	CPAN 2084/VW 205	30 MSS	Moderately Resistant	1998	Central	<0.1
GW 322	PBW 173/GW 196	20 M	Moderately Resistant	2002	Central, Peninsular	<0.1
HD 2781	BOW/C 306//C 591/ HW 2004	10 RMR	Highly Resistant	2002	Peninsular	<0.01
HI 1500	HW 2002*2// STREMPELLI/PNC 5	10 RMR	Highly Resistant	2003	Central	<0.01
MP 4010	Angostura 88	5 R	Highly Resistant	2003	Central	<0.01
T. durum						
HI 8498	CR"S'-GS'S'/A-9-30-1// RAJ 911	10-15 RMR	Resistant	1999	Central	<0.01
MACS 2846	CPAN 6079/ MACS 2340	20 RMR	Resistant	1998	Peninsular	<0.1
HD 4672	BIJAGA RED/PBW 34// ALTAR 84	20 MS	Moderately Resistant	2000	Central	<0.01

sectors need to be strengthened in all developing countries of South Asia. Considerable effort is already underway. However, it is also believed that in view of the huge wheat area in the eastern Gangetic plains covering a wide range of socio-economic and environmental diversity, greater scientist-farmer interaction following a participatory mode could play a crucial supportive role to meet this objective (Ortiz-Ferrara et al. 2007; Joshi et al. 2007). The role of participatory research in varietal selection (Ferrara et al. 2002; Witcombe et al. 2001, 2003) of different crops is well documented. This assumes further importance due to the fact that in many locations farmers' access to new varieties is highly restricted, and therefore good technology takes a very long time to disseminate. For example, it is believed that a good agricultural technology takes around 10 years to spread in the eastern Indo-Gangetic plains, and the average life of a resistant variety to rust pathogens (which are still the dominant pathogens in most parts of the world, and especially, India) is believed to be around 5-6 years (Roelfs et al. 1992; Rajaram et al. 1998; Singh et al. 2000). Therefore, by the time a variety reaches the majority of farmers, it has already lost much of its potential impact due to reduced, or loss of resistance. Because the Gangetic plains cover a vast area with a complex of socio-economic issues, improvements in the availability of quality seed need to be achieved through a combination of formal and informal activities. Therefore, strengthening the capacity of farmers to undertake quality seed production following the participatory approach assumes high priority (Joshi et al. 2007). Indian research centers already work on this model and so far the participatory mode has proven quite successful (Ortiz-Ferrara 2001; Joshi et al. 2007). At many locations in the eastern Gangetic plains, farmers have started their own seed businesses. To promote further seed multiplication and dissemination, ICAR in 2003 made it mandatory for all research centers receiving support under the National Seeds Project to actively engage in participatory seed production. The recent success of the new ICAR seed project "Seed Production in Agricultural Crops and Fisheries", launched

Table 3 *New CIMMYT lines under PVS in eastern Gangetic plains of India and their reactions to Ug99*

Year/ No.	Name	Pedigree	Main season	Off season	Main season			Reponse
			SR Kenya	SR Kenya	SR-Kenya	SR-Kenya	SR-Kenya	category
2005-06			2006*	2007	2007	2007	2007	
1	Baaz	ATTILA*2/STAR/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	60S	-	15MSS	40MSS	60MSS	MS
2	Labh	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	60S	-	-	-	-	MS
3	Layak	INQALAB 91*2/KUKUNA	80S	-	-	-	-	S
4	Sundar	WBLL4/KUKUNA//WBLL1	30MSS	-	-	-	-	MS
5	Tej	WBLL1*2/KUKUNA	30M	30S	30MSS	50MSS	60MSS	MS
6	Takat	WBLL1/4/HD2281/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KAMB1	40S	-	-	-	-	S
7	Lahar	SERI.1B//KAUZ/HEVO/3/AMAD		-	-	-	-	-
8	Vishal	ATTILA*2/PBW65	70MSS	-	5MR	10MSS	30MSS	MR
2006-07								
1	Jhoola	KIRITATI/4/SERI.1B*2/3/KAUZ*2/BOW//KAUZ	-	80S	30S	50S	50S	MS-S
2	Mahak	KIRITATI//PRL/2*PASTOR	-	70S	40S	60S	60S	MS
3	Swasth	KIRITATI//ATTILA*2/PASTOR	-	80S	20S	40S	60S	MS-S
4	Agrim	KIRITATI//HUW234+LR34/PRINIA	-	10MR	10RMR	20M	30M	MR
5	Sona	KIRITATI/WBLL1	-	40MSS	30MSS	70S	70S	MS
6	Chandi	WEAVER/TSC//WEAVER/3/WEAVER/4/PRL/2*PASTOR	-	70MSS	70S	100S	100S	S
7	Panchi	PFAU/SERI.1B//AMAD/3/WAXWING	-	60MSS	40MSS	50MSS	70MSS	MS
8	Ufan	WAXWING*2/VIVITSI	-	30M	5M	20M	20M	MR
9	Uthan	WAXWING*2/TUKURU	-	60MSS	40MSS	50MSS	60MSS	MS
10	More	WBLL1*2/KIRITATI	-	30MSS	15MSS	40MSS	60MSS	MS
11	Hans	KAMB1*2/BRAMBLING	-	-	-			
12	Abhinav	KAMB1*2/KIRITATI	-	-	-			
2007-08								
1	Sarpat	KIRITATI/2*WBLL1	-	20MSS	30MS	50MSS	50MSS	MR-MS
2	Hans	HUW234+LR34/PRINIA//PFAU/WEAVER	-	5M	5M	10M	20M	R-MR
3	Koyal	ELVIRA/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/6/ VEE/PJN//KAUZ/3/PASTOR	-	40M	30MSS	40MSS	50MSS	MR-MS
4	Ravi	PFAU/WEAVER*2//KIRITATI	-	30M	-	-	-	MR
5	Guru	KIRITATI//SERI/RAYON	-	20M	5M	10M	20M	R-MR
6	Century	WAXWING*2/KIRITATI	-	20M	5MR	15M	15M	R-MR
7	Umang	WAXWING*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	-	20M	5MR	20MSS	20MSS	R-MR
Checks								
1	HUW 234**	HUW 12*/CPAN 1966 (Sparrow)						
2	HUW 468**	CPAN 1962/TONI//LIRA/PARULA						

*Data not reliable due to drought stress; **HUW 468 and HUW 234 were evaluated during 2009 off-season at Njoro. HUW 468 was susceptible whereas HUW 234 was heterogeneous for resistant and susceptible plants with 20M and 70S responses, respectively.

Table 4 *Mean grain yields, days to heading, heights and 1000 kernel weights for six CIMMYT derived advanced lines and two cultivars tested at seven sites[1] in eastern Gangetic plains of India during crop season 2005-2006*

Lines/cultivars	Mean yield (t/ha)	% over HUW 234	Heading (days)	Height (cm)	1000 kernel wt. (g)
Baz	3.88	21.1	75	87	32
Labh	3.74	16.6	75	85	32
Layak	3.82	18.9	77	87	31
Sunder	3.56	11.0	78	94	30
Lahar	3.62	12.8	75	91	30
Vishal	3.53	10.1	80	90	28
HUW 468 (Newly released cultivar)	3.28	2.2	75	83	28
HUW 234 (Most popular cultivar)	3.21		70	89	31
LSD (P = 0.05)	0.37	-	1.7	3.1	3.3

[1]The sites included six farmers' fields located in districts Varanasi, Mirzapur and Azamgarh in eastern Gangetic plains and research station of Banaras Hindu University (Source: Singh et al. 2007)

during the 10th Five Year Plan for 2005-06 and 2006-07 (http://www.teatronaturale.com/article/12.html) also includes participatory seed production, further suggesting that new varieties can be disseminated in a much faster way in South Asia.

Newly developed Ug99-resistant lines are now under seed multiplication in the eastern Gangetic plains of South Asia; these include Picaflor#1 (Kiritati//Seri/Rayon), Pauraque#1 (Waxwing*2/4/SNI/Trap#1/3/Kauz*2/ Trap//Kauz), Becard#1 (WBLL1*2/Kiritati), Munal#1 (Waxwing*2/Kiritati), Quaiu#2 (Babax/LR42//Babax*2/3/Vivitsi), Francolin#1 (Waxwing*2/Vivitsi) and Damphe#1 (Kiritati//2*PBW65/2*Seri.1B). These lines currently occupy around 15 ha under participatory seed production in the present crop season (2008-09) with a targeted production of at least 30 tonnes. The predicted production of these varieties in the next crop season (2009-10) is around 500 tonnes. This is based on the results of the seed production of some of the lines, viz. Baz, Labh and Lahar (Table 3), introduced from CIMMYT in 2005-06. These three lines with moderate susceptibility to race Ug99 each occupy about 100 ha in 2008-09, with an anticipated seed production of more than 250 tonnes. With likely official release of these lines in the near future, it is predicted that seed production in the next three years will generate seed to saturate the eastern Gangetic plains and reduce the threat of Ug99. In addition, the heterogeneous Ug99 resistant variety HUW 234 (not reselected for resistance) is also under increase in the eastern Gangetic plains to produce an estimated 25,000 tonnes of seed (including all classes, viz. breeder, foundation, certified and truthful seed). This cultivar needs to be reselected for resistance for seed multiplication till other new resistant lines get multiplied in sufficient quantity. Another new resistant variety, BL 3063 (FRTL/Chirya 7), developed in Nepal is also being multiplied in farmers fields.

Conclusion

The new stem rust race Ug99 is a serious threat to South Asia and to global wheat production. If not checked through effective research, Ug99 may become another cause of food shortage for many countries, including those in South Asia. Replacement of currently popular susceptible cultivars in these areas with high yielding resistant lines is the best strategy to protect wheat from the menace of Ug99. This will require a concerted effort involving scientists, planners, progressive farmers and extension agencies associated with governmental and non-governmental organizations. CIMMYT, in collaboration with national research centers of South Asia, has already developed several high yielding Ug99 resistant varieties. These varieties are under seed production mainly through participatory seed production in the eastern Gangetic plains of India. In Pakistan, Bangladesh and Nepal the lines are being multiplied by national research centers. A more concerted seed production and dissemination system is required to safeguard South Asia from the threat of Ug99.

Table 5 ***Mean grain yield of CIMMYT derived advanced lines that displayed 10% or higher superiority over one of two popular cultivars tested at multiplication sites[1] in eastern Gangetic Plains of India during crop season 2006-2007 and 2007-08***

No.	Pedigree	Local name[2]	Mean	% over HUW 234	% over HUW 468
	2006-07 (9 locations)				
1	Kiritati/4/Seri.1b*2/3/Kauz*2/Bow//Kauz	Jhula	3.91	11.41	12.06
2	Kiritati//Attila*2/Pastor	Swasth	3.94	12.22	12.88
3	Kiritati//Huw234+Lr34/Prinia	Agrim	3.89	10.91	11.56
4	Waxwing*2/Vivitsi	Ufan (Francolin#1)[3]	3.98	13.31	13.97
5	Waxwing*2/Tukuru	Uthan	4.08	16.09	16.77
6	Attila*2/Star/4/Sni/Trap#1/3/Kauz*2/Trap//Kauz	BAJ	3.84	9.49	10.12
Check	CPAN 1962/Toni//Lira/Parula	Malviya 468	3.49		
Check	HUW 12*/CPAN 1966 (Sparrow)	Malviya 234	3.51		
	LSD (P = 0.05)	0.29			
	2007-08 (8 locations)				
1	HUW234+LR34/PRINIA//PFAU/WEAVER	Hans	3.28	6.92	10.37
2	KIRITATI//SERI/RAYON	Guru (Picaflor#1)[3]	3.46	12.58	16.21
3	KIRITATI//HUW234+LR34/PRINIA	Ufan	3.39	10.46	14.02
4	WAXWING*2/KIRITATI	Century (Munal#1)[3]	3.14	6.71	10.15
Check	CPAN 1962/Toni//Lira/Parula	Malviya 468	3.07		
Check	HUW 12*/CPAN 1966 (Sparrow)	Malviya 234	2.97		
	LSD (P = 0.05)	0.18			

[1] Nine farmers' fields located in districts of Varanasi, Mirzapur, Azamgarh and Chandouli in the eastern Gangetic plains and research station of Banaras Hindu University

[2] Local names of varieties given by farmers in the region

[3] Name given by CIMMYT, Mexico

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31. Field efficacy of fungicides used against stem rust in Kenya

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Abstract

Field experiments were conducted at two locations during the 2006 and 2007 growing seasons to assess the effectiveness of two new foliar fungicides; viz. Nativo 300 SC (trifloxystrobin100g/L + tebuconazole 200g/L) and Prosaro 250 EC (prothioconazole 125g/L + tebuconazole 125g/L), in controlling stem rust on wheat cultivar 'Duma'. AmistarXtra 280 SC (azoxystrobin 200g/L + cyproconazole 80g/L) and Folicur 250 EC (tebuconazole) were used as checks. The treatments at each site and year included an untreated control and two spray applications of the fungicides at growth stages (GS) 55 and 65. Stem rust severities were assessed using the modified Cobb scale at 14-day intervals after application. The data were used to calculate mean rust severity (MRS). Stem rust epidemics were severe at KARI-Njoro in 2006 and the treatment effects on stem rust severities, grain yield and 1000-kernel weight were significant at both the KARI-Njoro and Mau-Narok sites. The fungicide treatments, significantly ($P \leq 0.05$) reduced stem rust severity, increased grain yield and 1000-kernel weight of the susceptible wheat cultivar 'Duma' compared to the untreated control.

Keywords

Triticum aestivum, resistance, cultivar, epidemics, crop loss

Introduction

Stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.) is one of the wheat rusts that cause severe losses throughout the world. Losses of 50-70-% have often been reported under field conditions. The actual amount of loss caused by rust can range from slight to complete destruction of the crop. Grain from infected crops is shriveled and light in weight, and therefore has reduced quality (Agrios 1988; Stubbs et al. 1986; Zadoks et al. 1974).

Currently, all three rusts threaten wheat (*Triticum aestivum* L) production in Kenya. Epidemics occur when environmental conditions during the growing season are favorable. The new virulent strain TTKS (Ug99) in the eastern African region has caused repeated stem rust epidemics since 2002, threatening wheat production. Both small- and large-scale farmers have been affected. Yield losses of up to 80% were reported (Expert Panel 2005; Wanyera et al. 2006). The current commercial wheat cultivars are highly susceptible to the new race and it is not possible to grow a profitable wheat crop without the application of a fungicide. Over the past two decades, varietal resistance to stem rust has generally provided adequate protection without the need for fungicides (Expert Panel 2005; Loughman et al. 2005). Therefore, fungicide control regimes may play a role in integrated management of the disease until new varieties become available. Limited studies have been conducted to determine the effects of foliar fungicides on stem rust severities and yields elsewhere (Dill-Macky et al. 2000), but not in Kenya. This paper reports field experiments conducted under natural infection to determine the effect of two new foliar fungicides, viz. Nativo 300 SC (trifloxystrobin100g/L + tebuconazole 200g/L) and Prosaro 250 EC (prothioconazole 125g/L + tebuconazole 125g/L) on wheat stem rust, grain yield and 1000-kernel weight.

Materials and methods

Field trials were conducted in 2006 and 2007 at Kenya Agricultural Research Institute (KARI)-Njoro and Mau-Narok (Purko Ranch), Kenya. The test cultivar was 'Duma', which is popular and recommended for low and medium elevation growing-areas. The cultivar is highly susceptible to stem rust, but is fairly resistant to stripe rust (caused by *P. striiformis* Westend. f. sp. *tritici*). Stem rust epidemics occurred naturally at both sites. A randomized complete block design with four replications was used. The cultivar 'Duma' was planted in 9 m^2 plots. Planting was on May 30 and 16 and September 19 and 29 at KARI-Njoro and Mau-Narok in 2006 and 2007, respectively. The plots were sown using an experimental seed-drill at a seeding rate of 100g/plot. A uniform application of Di-ammonium phosphate fertilizer (18% N: 46% P: 0% K) was applied at planting at the recommended rate of 150Kg/ha. The plots were sprayed with Stomp 500E (pendimethalin), a pre-emergent herbicide, at the rate of 3L/ha, to control grass weeds, and Buctril MC (bromoxynil + MCPA) at the rate of 1.25L/ha at growth stage GS 24 (Zadoks et al. 1974) to control broad leaf weeds. Metasystox 250 EC (oxy-demeton-s-methyl) insecticide was applied at the rate of 0.5L/ha to control cereal aphids. The fungicide treatments included; two new products, Nativo 300 SC (trifloxystrobin 100g/L + tebuconazole 200g/L) and Prosaro 250 EC (prothioconazole 125g/L + tebuconazole 125g/L) each applied at three rates; 0.6, 0.75 and 1.0L/ha. Two standard fungicides; AmistarXtra 280 SC (azoxystrobin 200g/L + cyproconazole 80g/L), and Folicur 250 EC (tebuconazole), each applied at the rate of 1.0L/ha, and an untreated

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control were used for comparison. The fungicides were applied twice, at growth stages (GS) 55 and 65 using a 15L capacity knapsack sprayer and recommended water volumes of 200L/ha. The applications were on the stems and the flag leaf canopy. The plots were monitored for the appearance of stem rust, and disease severities were scored on whole plots using the modified Cobb scale (Peterson et al. 1948) before fungicide application, and at two 14 day intervals following application. In 2006, the first reading prior to fungicide treatment occurred at GS 65 (flowering half way complete), on August 9 at KARI-Njoro and October 24 at Mau-Narok. The second reading was 14-16 days after the first treatment, August 23 (KARI-Njoro) and November 8 (Mau-Narok) at GS 71 (seed water ripe). The third reading was taken September 8 (KARI-Njoro) and November 22 (Mau-Narok) at GS 77 (late milk). In 2007, the first reading before fungicide treatment was at GS 59 (emergence of ear complete), July 24 (KARI-Njoro) and November 28 (Mau-Narok) at GS 45 (boots swollen). The second reading was August 7 (KARI-Njoro) at GS 61 (beginning of flowering) and December 12 (Mau-Narok) at GS 55 (one-half of ear emerged). The third reading was on August 21 (KARI-Njoro) at GS75 (medium milk) and February 19 (Mau-Narok) at GS 91 (seed hard). The reading at this site was delayed due to an unavoidable circumstance. At maturity, the plots were harvested with a Hans-Ulrich Hege 140 plot combine harvester (Saatzuchtmaschinen Hohebuch), November 6, and April 4, 2007, and October 31, 2007, April 1, 2008, for the KARI-Njoro and Mau-Narok trials, respectively. Grain yields and 1000-kernel weights were determined. Grain obtained from each plot was used to determine the 1000-kernel weight (the weight of 1000 seeds in grams). Data on MRS, grain yield, and 1000-kernel weight were analyzed using the SAS statistical package (PROC-ANOVA), and treatment means were compared by least significant difference ($P \leq 0.05$) (SAS Institute 1999; Steel and Torrie 1980).

Results

Rust severities In 2006, stem rust at both KARI-Njoro and Mau-Narok developed early in the growing season; as a result, the mean rust severities (MRS) in untreated plots were 52.5% and 40.8%, respectively (Table 1). In 2007, the MRS was moderate and the MRS in untreated control plots was 23.8 % and 16.9%, respectively (Table 2).

Effect of fungicide treatments on stem rust severity All the foliar fungicide treatments reduced stem rust on wheat cultivar 'Duma'. Fungicide applications significantly ($P \leq 0.05$) reduced MRS compared to the untreated control, with Prosaro at 1.0L/ha, Nativo at 0.75L and 1.0L/ha and the standards (Folicur 250 EC and AmistarXtra 280 SC), performing better than Prosaro 250 EC at 0.6L/ha, 0.75L/ha and Nativo 300 SC at the rate of 0.6L/ha (Tables 1, 2). The highest disease severity reductions in 2006 were generally observed in plots that were sprayed with Prosaro 250 EC at 1.0L/ha (59.4%), Nativo 300 SC and AmistarXtra 280 SC at 1.0L/ha (55.6% each) at KARI-Njoro and Prosaro 250 EC at 0.6L/ha (75.7%), Prosaro 250 EC at 1.0L/ha (75.5%), and Nativo 300 SC at 1.0L/ha (73.5%) at Mau-Narok. In 2007, the highest disease severity reduction was in plots sprayed with Folicur 250 EC at 1.0L/ha (85.3%), followed by Prosaro 250 EC at 0.75 L/ha (82.6%) and 1.0L/ha (77.8%) at KARI-Njoro and Prosaro 250 EC at 0.6L/ha (76.5%), Prosaro 250 EC at 1.0L/ha (75.5%), and Nativo 300 SC at 1.0L/ha (73.47%) at Mau-Narok.

Effect of fungicide treatments on grain yield and 1000-kernel weight The fungicide treatment effect for grain yield and 1000-kernel weight was significant ($P \leq 0.05$) at the two sites, and in both years. In 2006, the highest grain yield of 1.3 t/ha was recorded in plots treated with Nativo 300 SC at 1.0L/ha, which was 61.5% higher than the untreated control at KARI-Njoro. This was followed by Nativo 300 SC at 0.75L/ha, Prosaro 250 EC at 0.6L/ha and 1.0L/ha, and Folicur 250 EC at 1.0L/ha, which were 58.3% higher than the untreated control. Plots treated with Nativo 300 SC at 0.75L/ha and Prosaro 250 EC at 1.0L/ha had the highest 1000-kernel weights with 28.7% and 27.6% increases, respectively, over the control. In Mau-Narok in the same year, Prosaro 250 EC and Nativo 300 SC at the rate of 0.6L/ha and Folicur 250 EC at 1.0L/ha had the highest grain yields of 3.3 t/ha, 2.8 t/ha and 2.7 t/ha, increases of 75.6%, 50% and 48.1%, respectively over the untreated control (Table 1).

In 2007, treatments of Nativo 300 SC, AmistarXtra 280 SC and Folicur 250 EC, all at 1.0L/ha, had the highest grain yields of 2.3 t/ha and 2.2 t/ha. Plots treated with Prosaro 250 EC at 0.6L, Nativo 300 SC, and AmistarXtra 280 SC at 1.0L/ha had increased 1000-kernel weights of 11.8% and 11.4% over the untreated control at KARI-Njoro. In Mau-Narok, treatments of Nativo 300 SC at 1.0L/ha, Prosaro 250 EC at 0.75L/ha and Folicur 250 EC at 1.0L/ha had the highest grain yields of 4.4 t/ha and 4.3 t/ha. The thousand kernel weight was highest with Nativo 300 SC at 0.75L/ha, 1.0L/ha and Prosaro 250 EC at 1.0L/ha. The average grain yields and 1000-kernel weights across the locations varied from one treatment to another, ranging from 1.2 - 4.0t/ha and 28.5 - 45.4g, respectively. Significant ($P \leq 0.05$) grain yield increases of 57.3% and 49.7% were obtained at KARI-Njoro and Mau-Narok in 2006, while 54.1% and 44.7% increases occurred in 2007, respectively. Similar increases in 1000-kernel weight occurred at both KARI-Njoro (24.5% and 25.1%) in 2006, and 10.4% and 23.3% in 2007 (Tables 1, 2).

Table 1 *Effects of fungicide treatments on stem rust severity, grain yield and 1000-kernel weight on wheat variety 'Duma' at KARI-Njoro and Mau-Narok 2006*

Treatment[e]	KARI-Njoro							Mau-Narok					
		Stem rust severity		Grain yield		1000-kernel weight		Stem rust severity		Grain yield		1000-kernel weight	
	Rate L/ha	MRS[a]	% reduction[b]	t/ha	% increase[c]	g	% increase [c]	MRS[a]	% reduction[b]	t/ha	% increase[c]	g	% increase [c]
Untreated	-	52.5	-	0.5	-	27.9 c	-	40.8 a	-	1.4 c	-	29.4 c	-
Nativo 300 SC	0.6	31.3	40.4	1.1	54.5	33.4 b	19.7	14.7 bc	63.9	2.8 ab	50.0	39.3 ab	33.7
Nativo 300 SC	0.75	24.6	53.1	1.2	58.3	35.9 a	28.7	11.3 bc	72.3	2.6 b	46.2	41.1 a	39.8
Nativo 300 SC	1.0	23.3	55.6	1.3	61.5	33.8 b	21.1	10.8 bc	73.5	2.6 b	46.2	38.2 ab	29.9
Prosaro 250 EC	0.6	25.4	51.6	1.2	58.3	34.9 ab	25.1	9.9 c	75.7	3.3 b	75.6	41.0 a	39.5
Prosaro 250 EC	0.75	24.2	53.9	1.1	54.5	34.7 ab	24.4	14.2 bc	65.2	2.5 b	44.0	37.4 b	27.2
Prosaro 250 EC	1.0	21.3	59.4	1.2	58.3	35.6 a	27.6	10.0 bc	75.5	2.4 b	41.2	38.4 ab	30.6
AmistarXtra 280 SC	1.0	23.3	55.6	1.1	54.5	33.6 b	20.4	15.4 bc	62.3	2.6 b	46.2	37.4 b	27.2
Folicur 250 EC	1.0	25.4	51.6	1.2	58.3	35.9	28.7	16.7 b	59.1	2.7 b	48.1	38.4 ab	30.6
Mean[d]	-	24.9	52.7	1.2	57.3	28.5	24.5	12.9	68.4	2.7	49.7	38.9	25.13
Lsd (0.05)	-	7.2	59.2	0.23	-	1.74	-	6.74	-	0.58	-	3.0	-
CV	-	17.7%		15.5%	-	3.5	-	29.0%	-	15.6	-	5.5	-

[a] MRS, mean rust severity (modified Cobb scale); [b] reduction (%), (untreated MRS - fungicide-treated MRS) x 100/untreated; [c] 1000-kernel weight increase (%); (treated - untreated) x 100/treated; [d] Mean, mean of fungicide-treatments; [e] Treatment means within columns followed by the same letter are not significantly different at $P \leq 0.05$ according to least significant difference (LSD) test

Table 2 *Effects of fungicide treatments on stem rust severity, grain yield and 1000-kernel weight on wheat variety 'Duma' at KARI-Njoro and Mau-Narok 2007*

	KARI-Njoro							Mau-Narok					
Treatment[e]		Stem rust severity		Grain yield		1000-kernel weight		Stem rust severity		Grain yield		1000-kernel weight	
	Rate L/ha	MRS[a]	% reduction[b]	t/ha	% increase [c]	g	% increase [c]	MRS[a]	% reduction [b]	t/ha	% increase [c]	g	% increase [c]
Untreated	-	23.8 a	-	1.0 c	-	38.7 b	-	16.9 ab	-	2.2 d	-	34.8 a	-
Nativo 300 SC	0.6	7.8 bc	67.4	1.8 b	44.4	43.0 a	10.0	12.5 abc	26.0	4.3 ab	48.8	46.4	25.0
Nativo 300 SC	0.75	6.5 bc	72.6	1.9 b	47.4	42.6 a	9.2	6.7 c	59.2	3.2 c	31.3	44.2	21.3
Nativo 300 SC	1.0	6.9 bc	71.0	2.3 a	56.5	43.7 a	11.4	8.3 c	50.9	4.4 a	50.0	47.2	26.3
Prosaro 250 EC	0.6	8.9 b	62.6	2.1 ab	52.4	43.9 a	11.8	15.6 ab	7.7	3.6 bc	38.9	43.6	20.2
Prosaro 250 EC	0.75	4.1 bc	82.6	1.9 ab	47.4	43.2 a	10.4	18.1 a	7.1	4.3 ab	48.8	43.7	20.4
Prosaro 250 EC	1.0	5.3 bc	77.8	2.1 ab	52.4	42.9 a	9.8	11.9 bc	29.6	3.9 abc	43.6	46.9	25.8
AmistarXtra 280 SC	1.0	8.9 b	62.6	2.3 b	56.5	43.7 a	11.4	11.3 bc	33.1	4.2 ab	47.6	45.2	23.0
Folicur 250 EC	1.0	3.5 c	85.3	2.2 ab	54.5	42.7 a	9.2	9.4 c	44.4	4.3 ab	48.8	46.1	24.5
Mean[d]	-	6.5	72.7	2.1	54.1	43.2	10.4	11.8	32.3	4.01	44.7	45.4	23.3
Lsd (0.05)	-	5.2	-	0.48	-	2.5	-	5.9	-	0.77	-	5.3	-
CV	-	42.4%	-	16.9%	-	4.0%	-	33.3%	-	13.8	-	8.2	-

[a] MRS, mean rust severity (modified Cobbs scale); [b] reduction (%), (untreated MRS - fungicide-treated MRS) x 100/untreated; [c] 1000-kernel weight increase (%); (treated - untreated) x 100/untreated; [d] Mean, mean of fungicide-treatments; [e] Treatment means within columns followed by the same letter are not significantly different at $P \leq 0.05$ according to least significant difference (LSD) test

Discussion

In Kenya, wheat is grown in many agro- ecological zones that have different planting dates (Jaetzold and Schmidt 1993). These staggered plantings provide green crops for most of the year allowing urediniospores to move from one area to another. The favorable environmental conditions, and the presence of host plants year-round, favor the survival of high levels of inoculum. It is therefore difficult to prevent or reduce infection of susceptible cultivars.

There is very little published information on fungicide-use to control wheat stem rust specifically related to the rust race TTKS (Ug99) and its variants. The occurrence of stem rust infections and the onset of epidemics differed from year to year and site to site. Stem rust levels were high in 2006 at KARI-Njoro and Mau-Narok (despite early moisture stress at KARI-Njoro, rain showers at GS 65 initiated the spread of the disease). In 2007, the epidemics were moderate to low at both sites. The low disease pressure could have been due to the heavy rains at the vegetative growth stage (May-September at KARI-Njoro and October-November at Mau-Narok) and later on, drought in Mau-Narok during December, 2007, and January - February, 2008.

In environments, such as those at KARI-Njoro and Mau-Narok in 2006, where stem rust epidemics began early, followed by conditions favorable for pathogen growth and spread, stem rust can greatly reduce grain yield and 1000-kernel weight in susceptible varieties. In that year, the average grain yield and 1000-kernel weight loss at KARI-Njoro and Mau-Narok in 2006 were 50.0% and 48.0%, and 2.1% and 24.4%, respectively. In 2007, the losses were 52.4%, 45.0%, and 10.4%, 23.3%, respectively, in untreated control plots versus fungicide-treated plots. These results are consistent with losses reported in other studies (Dill-Macky et al. 1990; Dill-Macky and Roelfs 2000; Loughman et al. 2005; Paveley et al. 2003). Pretorius (1983) reported that yield losses caused by stem rust ranged from 7 to 35% depending on variety. Dill-Macky and Roelfs (2000) induced severe stem rust epidemics in barley and wheat and observed yield losses of 50 to 58%. Mayfield (1985) found a clear relationship between grain yield and disease severity by demonstrating that prevention of a 1% increase in stem rust severity saved a 2% loss in grain yield. In the present study, all fungicide applications resulted in lower disease severities and higher yields than untreated control plots.

Loughman et al. (2005) reported that Folicur (tebuconazole) provided more consistent results in terms of both disease control and yield increases than Triad (triadimefon) or Impact (flutriafol). In the current study, the new fungicide treatments Nativo 300 SC (trifloxystrobin 100g/L + tebuconazole 200g/L) and Prosaro250 EC (prothioconazole125g/L + tebuconazole125g/L) and the standards, applied at 1.0L/ha were generally effective in reducing the disease and increasing grain yield and 1000-kernel weight. The lower rates resulted in more variable disease control, grain yields and 1000-kernel weight responses.

Stem rust severity was relatively low in 2007 in the trials at KARI-Njoro and Mau- Narok compared to 2006, yet grain yields increased in response to fungicide applications. This yield increase under relatively low disease pressures may have been due, in part, to phytonic effects of the fungicides. Such stimulatory effects of fungicide treatments on growth may produce significant yield increases even in the absence of disease (Wegulo et al. 1998) and as demonstrated in this study, fungicides treatments may, if applied under high and moderate disease pressure, at critical crop growth stages, still increase yield by suppressing or eliminating the negative effects rust.

The impact of fungicide-use in the management of stem rust was well illustrated at both trial sites. The fungicide treatments had the ability to suppress disease development and protect the crop canopy, which is vital for dry matter accumulation and yield (Viljanen-Rollinson et al. 2006). The study showed that stem rust could severely reduce the grain yields of susceptible cultivars, and the adoption of foliar fungicides to combat the disease as a short term control strategy until resistant cultivars are developed, is encouraged in Kenya. The new fungicides (Nativo 300 SC and Prosaro 250 EC) were therefore recommended for commercial use in the control of stem rust.

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32. Responding to the transboundary threat of wheat stem rust (race Ug99)

Pierre J.L. Lagoda

Abstract

This paper is aimed at introducing the work of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, focusing on mutation induction assisted breeding and providing a brief overview of the roles of molecular genetics and cellular biology as efficiency enhancing bio- and molecular technologies to broaden the genetic base of germplasm available to breeders. An Interregional Project is proposed to complement ongoing international activities on wheat stem (black) rust (caused by the fungal pathogen *Puccinia graminis* f. sp. *tritici* race Ug99), providing a platform for the coordination of a network of laboratories (based on previously established laboratory infrastructures through the International Atomic Energy Agency's Technical Cooperation Projects) as a defense line against Ug99. Implementation is projected in three overlapping phases: (i) Normalization: adoption and training in the use of uniform detection and pathotyping protocols, in order to assure homogeneity of handling (capacity building); (ii) Quality control: double blind tests for identification and characterization of false positives *vs.* false negatives (periodic network performance meetings, quality management and steering); (iii) Multilocation trials of mutant germplasm in endemic hotspots/screen houses. The minimal network of Ug99 surveillance laboratories shall be comprised of: (i) Front line: Kenya, Yemen, Sudan, Egypt, Jordan, Syria, Turkey, Ethiopia, Uganda and Iran; (ii) Tunisia, Morocco and Algeria; (iii) Pakistan and South Africa. This list is not exhaustive, and *notes of interest are welcome*. Tentatively, operative co-ordination shall include: The IAEA (Vienna, Austria: scientific backstopping, Seibersdorf Laboratories, Austria: quality control/training) in close collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA, Syria), tentatively proposed as the project coordinator. The FAO (Rome) would be entrusted with the normative co-ordination. The international collaborating laboratories and institutes include: ICARDA, the International Maize and Wheat Improvement Center (CIMMYT), the United States Department of Agriculture – Agricultural Research Service (USDA-ARS). *The First Coordination and Steering Meeting is planned for the first week in May, 2009, at IAEA Headquarters in Vienna, Austria.*

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Keywords

Triticum aestivum, *Hordeum vulgare*, mutation induction, resistance

Introduction

On 1 October 1964, the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) created the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture [1] through the first arrangements concluded by Directors General of both Organizations. The goal was to combine the talents and resources of both organizations into assisting their Member States in applying nuclear techniques for providing people with more, better and safer food and other agricultural products, while sustaining the natural resources base.

Over four decades, Joint Division activities have evolved to respond to the ever-changing landscape of agriculture and nuclear technology and the expectations of national and international organizations for cooperation in nuclear research and technology transfer. Throughout this process, the Division has successfully remained at the forefront of assisting countries in fostering the uses of nuclear science and technology where these really add value. Today, the Joint Division strives to mobilize commitment and action to meeting the World Food Summit and Millennium Development Goals of reducing hunger, poverty and environmental degradation through sustainable agriculture and rural development.

The International Atomic Energy Agency (IAEA) serves as the global focal point for nuclear co-operation, mobilising peaceful applications of nuclear science, and technology for critical needs in developing countries, including fighting hunger, disease, poverty and pollution of the environment, thereby contributing to sustainable development goals of its Member States. The IAEA currently co-ordinates research networks (CRPs) [2] and supports human and institutional capacity building Technical Cooperation Projects (TCPs) [3] for integrating efficiency enhancing bio- and molecular technologies with mutation induction within the framework of national plant breeding and conservation programmes to characterize plant genetic resources and widen plant genetic diversity, and to identify and introduce agronomically and commercially useful traits.

The IAEA Program in Food and Agriculture is planned, implemented and co-financed with FAO and is known as the Joint FAO/IAEA Program. As such, its

activities – particularly in crop improvement – are conducted in close collaboration with the relevant International Agricultural Research Centers of the CGIAR with which it has a number of Memoranda of Understanding on biotechnology and other applications, and with the NARS of Member States.

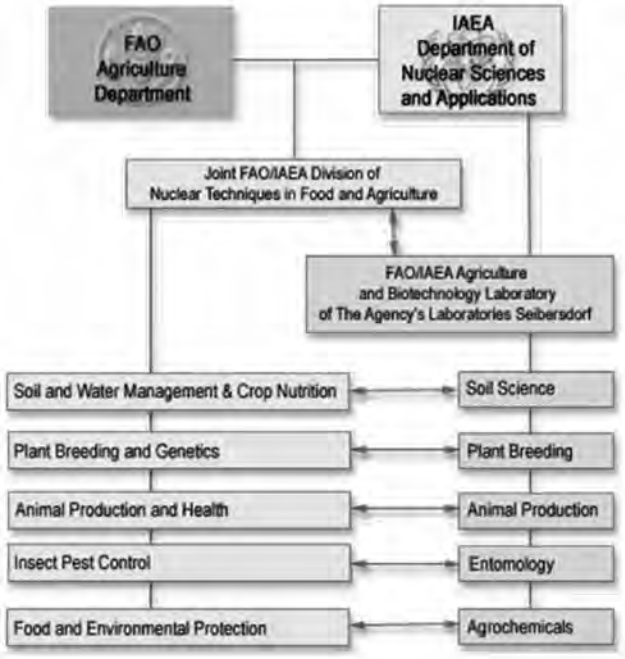
An important part of the Program is the FAO/IAEA Agriculture & Biotechnology Laboratory, set up to provide applied research, services and training to member countries. The arrangements on the Joint FAO/IAEA Program of Nuclear Techniques in Food and Agriculture were revised in 1997 and signed by the Directors General of FAO and IAEA in December 2001.

The Joint FAO/IAEA Program includes three interdependent components (Fig. 1):

- The Joint FAO/IAEA Division in Vienna, which provides normative and technology transfer support, coordinates research networks, and policy advice and public information activities to Member States
- The FAO/IAEA Agriculture and Biotechnology Laboratory (ABL) in Seibersdorf (Austria), an applied research facility, provides services and training to member countries, and plays a role as the reference center. The FAO/IAEA Agriculture & Biotechnology Laboratory – ABL - is unique within the UN system in that it provides hands-on training and gives participants the opportunity to accelerate capacity building in their respective countries. The training program is developed based on the demand for expanding expertise in developing countries
- Food and agriculture projects under the IAEA Technical Cooperation Program, which manages the implementation of operational activities in member countries

As a part of the Sustainable Intensification of Crop Production Systems (SICPS) sub-program of the Joint FAO/IAEA Program, the activities of the Plant Breeding and Genetics Section (PBG) [4] and Plant Breeding Unit (PBU) are aimed at assisting national plant breeding programs to use mutation techniques and modern biotechnologies for developing better varieties of major and under-exploited food and industrial crops. The overall aim is to increase food security and sustainable crop production by improving yields and quality for domestic use and export markets, and by enhancing crop diversification and biodiversity. In carrying out this mandate, particular emphasis is placed on the improvement of agronomically important characters for major crops with emphasis on marginal and stress prone areas. The activities further tend to focus on the improvement of local, often "neglected", crop species and the improvement and domestication of

Fig. 1 ***Organizational structure of the Joint FAO/IAEA Program***



plant species with potential value as food and export products. The use of molecular genetic and cellular biology techniques for speeding up the breeding of new crop varieties is thus receiving a lot of attention and support as these novel technologies are recognized as having huge impacts on crop improvement by increasing the efficiency of mutation induction and effectiveness of mutant selection.

The Joint Program is a successful model of cooperation within the UN System, providing necessary assistance to the needs of Member States in the peaceful application of nuclear techniques in food and agriculture.

High priority activities of the Joint FAO/IAEA Program focus on three thematic areas, viz. (i) productivity enhancement; (ii) plant, animal and consumer protection; and (iii) the conservation and sustainable use of natural resources. The Joint FAO/IAEA Program contributes to different FAO program chapters by integrating normative, policy advice, capacity building, R&D and operational technical support to their application in Member States of nuclear techniques.

In general, nuclear techniques are essential to provide unique support for these projects, and are the only solution in certain areas. The necessity for nuclear applications lies first in their capacity to bring about changes in the genetic make-up of plants, and to offer great potential to increase the biodiversity of crop plants. Second, the need for nuclear applications

lies as well in their unique sensitivity and specificity as markers. They can be used to measure - more accurately than is possible by any conventional method - basic and yet strategically essential processes which take place within and between soils, plants, and animals. Third, radiation can be effectively applied for sanitary and phytosanitary purposes in support of food safety and to facilitate international agricultural trade, as well as for specialized and successful applications such as the sterile insect technique, where leadership lies with the Joint FAO/IAEA Program.

Through IAEA-Technical Cooperation (TC) funding, the Joint FAO/IAEA Program provides technical support to more than 250 IAEA-TC projects every year, as well as capacity building and technology transfer (expert advice, training, and assisting with the procurement of experts and equipment) to Member States through these technical cooperation projects. Over the past decade, the Joint Program added each year to capacity building through over 50 training courses and workshops, 350 fellowships and scientific visits.

Through the regular budget, the Joint Program organizes symposia, conferences, consultants meetings, interregional training courses and workshops, provides normative and policy advice, disseminates information through databases, e-learning modules, and web pages. It also assists Member States through a network of coordinated research projects (CRP) and research coordination meetings (RCM) to address specific practical problems related to a range of areas: approximately 400-500 institutions and experimental stations in Member Countries cooperate in 30-40 R&D networks (CRPs) per year organized by the Joint FAO/IAEA Program.

The IAEA is the only organization within the UN family that has the mandate to promote the peaceful use of nuclear techniques. In some of the agricultural areas, nuclear techniques are an essential component and when properly integrated with other conventional and modern technologies, provide substantial added value to national and international efforts to sustainable agricultural development while at the same time creating strong synergies.

By the use of nuclear techniques, the Joint FAO/IAEA Program provides unique support not only to FAO but also to other international bodies in their efforts to enhance food quality and safety, protect consumer health and facilitate international trade in foodstuffs. All major activities of the Joint Program are within the 'public good' area both in developing and developed countries and address urgent needs and requirements from FAO and IAEA Member States. In addition, many constraints to agricultural development related to the above thematic areas, especially animal and crop pests and diseases are transboundary in nature and require an area-wide approach to be managed successfully. Regional collaboration is therefore necessary and collaboration between international organizations is best positioned to coordinate these activities.

The Joint FAO/IAEA Program is the only international body that can provide technology development and transfer, capacity building and services in this area of nuclear applications in food and agriculture to the Member States, and is in this respect it is unique.

Nuclear applications in food and agriculture

What are nuclear techniques?

Everything in the Universe, including the soil, plants and animals that we use for agriculture, and the carbohydrates, proteins and fats in the food we eat is made up of around 100 elements. These elements consist of atoms with a nucleus composed of neutrons and protons surrounded by electrons. However, not all atoms of an element have the same number of neutrons in their nucleus, i.e. they exist in different isotopic forms - some are heavier than others, some are stable, and others undergo decay and emit energy as radiation.

Applications of nuclear techniques in food and agriculture make use of isotopes to measure and track with great accuracy and precision, various events occurring in agriculturally important processes and compounds, and to manipulate those processes for greater productivity. They also make use of sealed facilities containing radiation- emitting isotopes to mimic Nature in changing the genetic make-up of plants, insects and micro-organisms in order to produce better crops, sterile insects for controlling pests and increasing the shelf-life and safety of certain foods.

Nuclear techniques, combined with the application of modern bio- and molecular technologies, are essential to providing a more efficient way, both for understanding the processes that underpin the production and transformation of biophysical resources into food and agricultural products and, directly or indirectly, for manipulating these processes to increase crop and livestock productivity while conserving and sustainably using natural resources and improving food quality and safety. The effective transfer of existing nuclear techniques to developing countries and the development of new and safe bio- and molecular technologies combined with nuclear techniques can greatly enhance the prospects for sustainably improving agricultural productivity, both currently, and in the future.

Mutation induction and breeding

The prime strategy in mutation-based breeding has been to upgrade well-adapted plant varieties by altering one or two major traits, which limit their productivity or enhance their quality value.

Scientists induce mutations in plants through the exposure of their propagules like gametes, seeds and other meristematic regions to both physical and chemical agents with mutagenic properties. Most chemical mutagens are alkylating agents or azides, the most widely used being ethyl methanesulphonate (EMS), sodium azide (NaN3) and diepoxybutane (DEB). The physical agents normally used include electromagnetic radiations such as gamma rays, X rays and UV light, and particle radiations such as fast and thermal neutrons, and beta and alpha particles. Close to 90% of the officially released mutant varieties were produced using ionizing radiations.

The global impact of mutation-derived varieties on food production and quality enhancement is difficult to monitor, even in five-year windows, given that normally the release of a new variety takes 10 to 15 years. Starting in the 1970s, the Joint FAO/IAEA Program of the United Nations sponsored extensive research on mutation induction and efficiency, enhancing both bio- and molecular technologies and the breeding of food and industrial crops. These efforts seemingly have paid off. Vast numbers of induced mutant varieties were developed with the Agency's assistance, including support on mutant germplasm exchange and dissemination around the world

Worldwide, close to 3,000 mutant varieties from 170 different plant species were officially released [5]; they were derived either as direct mutants or from mutant pre-breeding parents and are being cultivated by farmers in more than 60 countries of Africa, Asia, Australia, Europe, South America and North America. As induction of mutations with radiation has been the most frequently used method for directly developed mutant varieties, a sizable part of this success might be rightfully claimed by the Agency, either directly or indirectly through TCPs and CRPs, laboratory infrastructure creation, fellowship training, organized scientific visits and expert missions. The eight crops with the highest numbers of mutants are rice, barley, chrysanthemum, wheat, soybean, rose and common beans. China, India, The Russian Federation (formerly USSR), The Netherlands, USA, and Japan are the top six countries in terms of the numbers of officially released of mutant crop varieties. Worldwide, China with 605 mutant varieties, has released the most mutant crop varieties to farmers, followed by India with 259 varieties. The economic impacts of these mutants have continued to mirror the dramatic effects of the Norin 10 wheat mutant variety during the Green Revolution. In Pakistan for example, the textile industry relies heavily on a single cotton mutant variety, the economic benefits of which were modestly estimated at US$3 billion. A similar situation prevails in the Japanese pear industry with a high reliance on a mutant that has generated over US$250 million in additional income to growers. In 2008, a newly released mutant salt tolerant rice variety, created with the support of the Joint FAO/IAEA program, covering up to 30% of the export rice area in the Mekong Delta, benefited Vietnamese farmers by US$300 million additional income. Generally, the economic returns from the adoption and cultivation of these superior crop varieties are estimated at several billions of dollars and they occupy millions of cultivated hectares. Over 1,000 mutant varieties of major staple crops enhance rural income, improve human nutrition and contribute to environmentally sustainable food security in Asia.

Officially released mutation-derived varieties also include other important crops such as rapeseed, sorghum, sunflower, sesame, grapefruit, peppermint, banana, groundnut, pulse crops, and ornamentals. These have made major economic impacts in both developing and developed countries.

Induced mutagenesis as a crop improvement tool has been particularly useful with breeding superior varieties of vegetatively propagated and apomictic crops as evidenced by the highly efficient and reproducible protocols for generating new sources of useful variation in plants such as Bermuda grass, Japanese pear, sweet potato, peppermint, potato, sweet cherry, carnation, chrysanthemum, rose, alstroemeria, poinsettia and African violet. In these crops, where genetic variation is difficult to obtain due to limited sexual reproduction, based on sterility and polyploidy, mutation induction is the tool of choice, as it offers an alternative way of generating genetic variability. By altering the expression of only one or two traits, useful new varieties can be based on clones with proven consumer acceptance and known production requirements, and hence can be introduced to markets with minimum expense.

In several mutation-derived varieties, the changed traits have resulted in synergistic benefits on increasing yield and quality of the crop, improving agronomic inputs, crop rotation, and consumer acceptance. In contrast to either the currently protected plant varieties/ germplasm or the controversies about genetically modified organisms (GMOs), induced mutants have been freely available for plant breeding. Mutation induction is a ubiquitously applicable, flexible, workable, unregulated, non-hazardous and low-cost breeding tool.

The economic value of a new variety can be assessed in several ways. These include area planted to the variety and percentage of the area under the crop in a region, increased yield, enhanced quality, or reduced use of pesticides and fungicides (e.g. in varieties resistant to diseases and insect pests). But to make a long and complicated story short, the socio-economic impact of mutant varieties is assessed in billions of dollars and millions of hectares cultivated (Ahloowalia et al. 2004).

Many mutants have made transnational impacts on increasing yield and quality of several seed propagated crops. Induced mutations will continue to have an increasing role in creating crop varieties with traits such as modified oil content, protein and starch quality, enhanced uptake of specific metals, deeper rooting systems, and resistances to drought, diseases/pests and salinity as major components of environmentally sustainable agriculture. Future research on induced mutations will also be important in the functional genomics of many food crops.

The Agency has addressed the problems of climate variability and change, disease resistance (including resurgence, and appearance of transboundary threats due to climate change), drought and salinity stress tolerance to improve the nutrition provided by the plants and their resistance to specific environmental and geographical problems. Up to 80% of plant yields can be lost because of drought and salinity. Problems are particularly severe in developing countries in arid and semi-arid regions, with both devastating short-term effects on the livelihoods of poor people and long-term effects on food security. These are likely to increase in future as competition for water resources increases. The integration of mutation induction and efficiency enhancing bio-/molecular technologies into plant breeding and adoption of advanced selection methods can lead to the official release and wide uptake by farming communities of new varieties of basic food and industrial crops that are higher yielding, have better quality, are more nutritious and better adapted to climate change and variability.

It is noteworthy, that worldwide, more than 60% of all mutant varieties were officially released after 1985, during the era of biotechnology in plant breeding. The integration of mutation techniques and efficiency-enhancing bio-/molecular techniques that permit rapid selection of the most beneficial mutants has pushed the use of mutation induction to new and higher levels of applicability. With the integration of molecular genetic information and techniques, mutation assisted breeding is in the mainstream of progress to develop novel varieties. Mutation induction combined with bio-/molecular technologies such as plant tissue culture and molecular markers plays a very important role in crop improvement. Mutation induction is an integral part of the newest technology package in the forefront of modern and efficient methods in reverse genetics and breeding: e.g. TILLING (targeting induced local lesions in genomes), and breeding for modified starch quality traits in hexaploid wheat. Mutation induction is producing mutation grids for gene discovery and gene function analyses (e.g. Arabidopsis, rice, barley), an invaluable resource for genomics, and both reverse and forward genetics.

In recent years there has been increased interest in understanding the genome. This goes in parallel with the explosion of fundamental and strategic research to understand gene structure and function, especially in crop and model plants. The IAEA Plant Breeding and Genetics section and laboratory unit are adapting the TILLING strategy to the peculiarities of tropical orphan crops. In addition to the work on the relatively more studied crop, rice, the Joint Program has made significant progress in the development of protocols, i.e. simplifying procedures and exploring low cost options, to facilitate the use of TILLING to routinely query genomes of the scantily studied polyploid and vegetatively propagated crops that are important to the food security and livelihoods of Member States, such as cassava and bananas, thus creating an invaluable resource for reverse genetics and breeding for the global community. The widespread routine adoption of TILLING, for instance, will significantly reduce the costs and time invested in the development of superior crop varieties.

The Green Revolution was driven by spontaneous mutations affecting plant height. Two homeoalleles of the semi-dwarf gene in wheat, *Rht1* and *Rht2*, and *Sd1* in rice, produced the semi-dwarf stature that had implications for lodging resistance. The beneficial effects of spontaneous mutations continue to be reproduced in more controlled and systematic ways using mutagenic agents. In fact, the semi-dwarf characteristic was artificially induced at about the same time as "mystic" semi-dwarfing genes were being discovered, and it would be later shown that the "natural" spontaneous and "artificial" induced mutations both affected the same gene and were thus alleles.

The use of induced mutations in crop improvement, started over 80 years ago, continued to be massively used by plant breeders until the 1980's when the advent of modern techniques of molecular biology and the potential to move specific genes from one organism to another seemed to be more viable alternatives. Consequently, research on mutation induction for breeding of crops declined, especially for sexually

propagated crops. However, the trend is now reversing as scientists and policy makers recognize that induced mutagenesis remains a "clean" and inexpensive way to create novel varieties by changing single characters without affecting the overall phenotype.

It comes therefore as no surprise, that commercial companies have begun redirecting research efforts at the development of mutant crop varieties. Also, the applications of mutation techniques have expanded beyond direct use in breeding new crop varieties to the more upstream novel applications in gene discovery and reverse genetics. This partly explains the "renaissance" of mutation induction techniques in fundamental science and biotech firms.

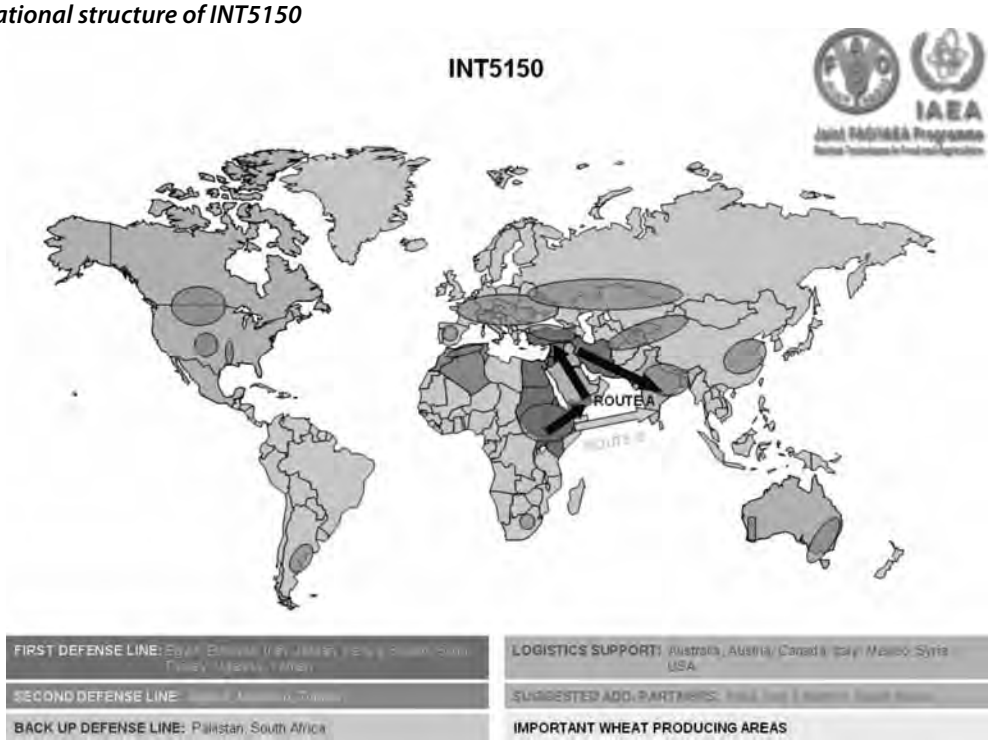
IAEA Interregional Technical Cooperation Project INT5150

An interregional project (Fig. 2) is proposed to complement ongoing international activities on wheat stem rust to provide a platform for the coordination of a network of laboratories (based on previously established laboratory infrastructures through the IAEA's TCPs) to combat the threat of race Ug99 and its derivatives. This race can attack almost 90% of all wheat varieties grown in the developing world from Africa to the Near East, and Asia, to even Europe and other continents (the Americas, Australia). Through the Global Rust Initiative (GRI) [6], ICARDA, CIMMYT and FAO started national and international awareness activities and a series of expert workshops in Rome to respond to the Ug99 threat. The 1st GRI- Workshop on Surveillance and Pathotype Analysis of Wheat Rust Pathogens was held at ICARDA in May 2007, involving a network of collaborating participants from the region.

At present, the existence of a new race is first detected through trap nurseries planted in fields in different countries. The trap nurseries include a set of wheat differential lines (agreed upon by international rust experts) that are being carefully maintained (at ICARDA) and seeds are being dispatched with clear instructions to national partners in the countries at risk. If, based on field readings in these nurseries, Ug99 or its variants is suspected, rust samples are collected and sent to specialized laboratories for molecular confirmation and characterization. Pathotyping is important for monitoring of disease movement and subsequent management as well as for breeding purposes. Based on the results of pathotyping, the list of differential lines used in the trap nurseries will be updated in order to fine-tune the monitoring capabilities.

Since transboundary movement of this race is highly risky, sending samples for testing in other countries that are still free of it should be treated very cautiously. Regarding Ug99, there are only a few laboratories, such as those in the USA (USDA-ARS, Minnesota) and in Canada with official approval to

Fig. 2 Organizational structure of INT5150



accept rust samples and to culture them under very strict conditions (timing/period and method of sending the material). This procedure is time consuming, and several of the samples sent may lose viability by the time they are tested. Thus, precious time may be lost before results are obtained and action in the field is undertaken. It is accordingly highly important that national and/or regional laboratories within the high risk regions are established or upgraded and personnel are trained in such a way that rust pathotyping takes place immediately in the country and without further risks of spreading the pathogen to other regions. Thus, testing should be done nationally, and ideally only re-confirmed in the international laboratories on a needs basis.

The Interregional Project should therefore provide a platform for the coordination of a network of laboratories as a "defense line" against the disease, implementing the following three phases:

Phase I: "Normalizing" adoption and training in the use of uniform protocols, in order to assure homogeneity of handling (capacity building)

Phase II: Quality Control: double blind tests for performance of identification and characterization false positives *vs.* false negatives (periodic network performance meetings, quality management and steering)

Phase III: Multilocation trials of mutant germplasm in endemic hotspots/screen houses

These phases are not necessarily consecutive, but overlapping.

Network of Ug99 surveillance laboratories:

1. (Front line): Kenya, Yemen, Sudan, Egypt, Jordan, Syria, Turkey, Ethiopia and Iran
2. Tunisia, Morocco, Algeria
3. Pakistan, South Africa.

This list of countries is by no means exhaustive, and notes of interest are encouraged [7]. The First Coordination and Steering Meeting is planned for the first week in May, 2009, at IAEA Headquarters in Vienna, Austria.

- Normative Coordination: FAO (Rome)
- Operative Coordination: IAEA (Vienna: Scientific Backstopping, Seibersdorf: Quality Control/ Training)
- Project Coordination: Ideally through ICARDA
- International Collaborating Laboratories/Institutes: ICARDA, CIMMYT, USDA-ARS

The training component of this project could be provided by any competent laboratory, but it should be group training to warrant homogeneity of procedures. However, besides the hands-on training, personnel who will be undertaking pathotyping in the national laboratories should have additional, complementary training on the genetic bases of changes in virulence and rust epidemiology, and in addition, should receive some field experience so that they know what they are working on and how to interpret results.

Provision of genetic resources

The genetic improvement of plants is dependent on the availability of useful and exploitable genetic variation within the genepool accessible for manipulation by the plant breeder. Such variation arises naturally through spontaneous mutations and hybridisations between wild and closely related species. Mutation induction, artificial changes to the genetic make-up of an organism generating variations in potential parental materials is thus a facilitation through artificial means of an otherwise natural phenomenon. Plant breeders engaged in the development of new superior varieties exploit such variations when they are useful. In rare cases, the mutants possess traits of agronomic or economic importance to such an extent that they require little or indeed no further manipulation before being released to farmers. Most of the time however, the mutants are just "raw materials" (pre-breeding material) that must be included in a normal varietal development mechanism. This would normally involve controlled crosses with otherwise well established varieties which lack the desirable trait identified in the mutant, followed by several cycles of field evaluation.

All the listed countries in the project have Agency supported mutation assisted breeding programs in wheat and/or barley. Through mutation assisted breeding, these member countries develop directly new high yielding cultivars with good agronomic characteristics, well-adapted and high value-added traits from any germplasm source including local landraces, which is difficult or impossible to attain through conventional plant breeding. This helps to enhance crop production for food security, increases farmer income, conserves biodiversity and enhances agro-biodiversity, thus directly contributing to the conservation and use of plant genetic resources.

Listed in the FAO/IAEA Database on Mutant Varieties and Genetic Stocks (MVGS) [5], there already are 235 officially released mutant wheat and 304 officially released mutant barley varieties. One percent of these officially released mutant varieties were created for rust resistance (2 wheat and 3 barley).

Using mutation induction techniques, an abundance of more or less advanced and characterized mutant lines have been created through different Agency sponsored cooperation projects, providing breeding material for conventional plant

breeding, but which can also be used by modern biotechnology (mutation grid). The salient fact is that this mutant germplasm is a potential source for rust resistance alleles, and for gene discovery and gene function analyses.

The interregional project proposes to test this germplasm ranging in the thousands of advanced mutant lines, from local landraces to elite accessions, as resources for potential resistance to Ug99. In a complementary program, a satellite project proposes to collect landraces, wild relatives and varieties showing resistance to Ug99 and produce mutants susceptible to Ug99 as a resource for analyzing the molecular bases for resistance to wheat stem rust (mutational analysis).

Websites

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33. The UN-FAO Wheat Rust Disease Global Program

Wafa El Khoury[1]

Abstract

The FAO Wheat Rust Disease Global Program was launched to respond to the emergence and spread of the virulent strain of wheat stem rust, Ug99, and similar virulent strains in the future. It is a part of FAO's Crisis Management Centre for the Food Chain and it reinforces and complements activities of the Borlaug Global Rust Initiative. The Program aims at contributing to global food security through prevention and management of emerging wheat rust races and the enhancement of wheat productivity. It covers the following components: 1) national preparedness and contingency planning; 2) surveillance and early warning; 3) national wheat varietal registration programs; 4) national systems for quick seed multiplication and distribution; and 5) improvement of wheat rusts field management. As a neutral information sharing forum, FAO is well positioned to lead such global efforts through linkages with national governments, regional bodies, farmers, international research and development institutions, and the donor community.

Keywords

Ug99, wheat stem rust, contingency planning, transboundary plant diseases

Introduction

Wheat is grown on more than 200 million hectares and is a source of food and livelihoods for over a billion people in developing countries. The Near East, East and North Africa and Central and South Asia alone account for some 37 percent of global wheat production (FAOSTAT 2007). In most countries in these regions, wheat is the staple food crop, providing on average some 40% of the per capita calorie supply, and is especially important in the diets of the poorest. Many people in these countries heavily rely on wheat production for their subsistence and livelihood. This livelihood has been greatly affected by the rise in grain prices which the world has witnessed over recent years. The rise in prices, largely the result of several years of severe drought, the high cost of fuel and an increased demand for grains, is not the only problem they face. With wheat crops coming under pressure from climatic stress, especially in rainfed regions, the impact of diseases is also expected to increase, resulting in severe yield losses.

A major new threat has already surfaced, and that is the widely virulent stem rust race Ug99, also known as TTKSK (Roelfs and Martens 1988; Fetch et al. these proceedings). It appeared in East Africa in 1999, and by late 2007, had reached Iran. Ug99 is highly virulent on almost all wheat varieties currently grown throughout the world, and the risk that it could cause global epidemics is very real. If this happens, wheat production will suffer devastating yield losses.

From previous experience with similar rust strains (a recent example is the breakdown of the stripe (yellow) rust resistance gene *Yr9* causing widespread epidemics between 1986 and 1998; Singh et al. 2004), and based on weather patterns, it is likely a matter of only a few years before most countries in the Near East, East and North Africa, Central and South Asia cultivating around 80 million hectares of wheat (FAOSTAT 2007) will be affected by Ug99 (Fig. 1; Wheat Rust Global Program, ftp://ftp.fao.org/docrep/fao/011/i0378e/i0378e.pdf).

Agricultural crises resulting from transboundary pests and diseases are often the result of unsound agriculturally related policies, mismanagement of resources, and poor national and regional preparedness for prevention and early response measures. For too many years, governments have reduced their investments in agriculture, especially with respect to farmer education and extension, support to agricultural research, rural development through infrastructure, and market access.

Through its Wheat Rust Disease Global Program (WRDGP), the Food and Agriculture Organization of the United Nations (FAO) is taking global action to prevent a wheat production crisis, in close collaboration with national governments, international agricultural research centers and other international institutions, to manage the Ug99 threat, and to prevent future crises caused by similar wheat rust diseases. The main objective of the Program is to contribute to global food security through the prevention and management of emerging wheat rust diseases and the enhancement of wheat productivity (Wheat Rust Disease Global Program, ftp://ftp.fao.org/docrep/fao/011/i0378e/i0378e.pdf).

FAO is well positioned to lead such international efforts because of its experience with international dimensions of other transboundary pests, such as locusts, its standing as a neutral international forum for information sharing, its experience in the emergency response and its linkages with grassroots rural communities, national governments, regional bodies, international agriculture research and development

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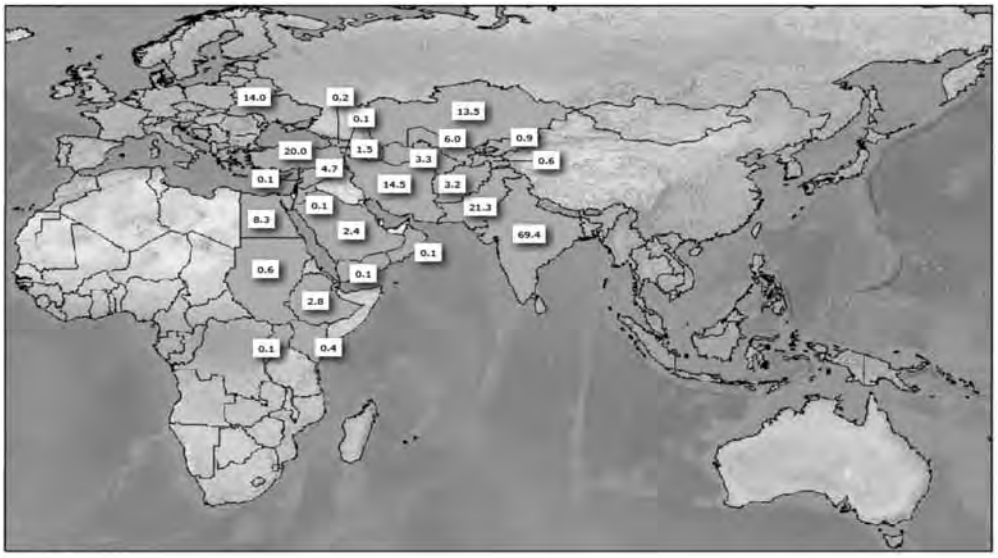
Fig. 1 *Wheat cultivation in countries affected by and at direct risk of stem rust race Ug99*



Countries threatened y Ug99 produce 37% of the world's wheat
figures in boxes are 2006 wheat production (millions of tonnes)

institutions, the private sector and the donor community.

Within FAO, the WRDGP is part of the newly established Crisis Management Centre for the Food Chain (CMC-FC) and works in close collaboration with the Initiative for the Soaring Food Prices.

Globally, the WRDGP works in full partnership with FAO's Member Countries and in full synergy and coordination with the Borlaug Global Rust Initiative (BGRI)[2]. First established in 2005 as the Global Rust Initiative (GRI), this initiative was later expanded to what is now known as the BGRI and includes in addition to the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Maize and Wheat Improvement Center (CIMMYT), Cornell University and FAO as permanent members and Dr Norman Borlaug as the Chairman of its Executive Committee. The overall objective of BGRI is to "systematically reduce the world's vulnerability to stem, yellow and leaf rusts of wheat, through advocating and facilitating the evolution of a sustainable international system to contain the threat of wheat rusts and consolidating the enhancements in productivity required to withstand future global threats to wheat" (Sounding the Alarm on Global Stem Rust, 2005; www.globalrust.org).

FAO's Crisis Management Center for the Food Chain

The Wheat Rust Disease Global Program falls within the scope of the newly established Crisis Management Centre for the Food Chain (CMC-FC). The CMC-FC was established to respond to the recent increases in the number of outbreaks of transboundary animal diseases, plant pests and food safety emergencies. Changing agro-ecological conditions, intensifying food production systems and expanding global trade increase the likelihood of animal and plant diseases and pests emerging and spreading farther and faster than ever before, and for unsafe food to reach numerous consumers in distant markets. With the advent and spread of instant mass communication, news of outbreaks can cause generalized consumer panic, market collapse and serious economic damage in regions well beyond affected areas. The CMC-FC reflects FAO's determination to address the risks to the human food chain in their assessment, management and communication dimensions in a comprehensive, systematic, inter-disciplinary, institutions-wide, collaborative approach. Recent external evaluations of FAO have highlighted the Organization's comparative advantage in this domain.

The CMC-FC acts as a broad-based international center established to bring together the entire food chain and to develop activities that allow for the forecasting, prevention and management of threats that go beyond national borders.

The organizational structure of the CMC-FC (Fig. 2) foresees three units reflecting various levels of activities; these are 1) Intelligence and Coordination unit, 2) Prevention and Early Warning unit, and 3) Rapid Response unit. The Prevention and Early Warning unit is provided by the previously established Emergency Prevention System for Transboundary Animal and Plant

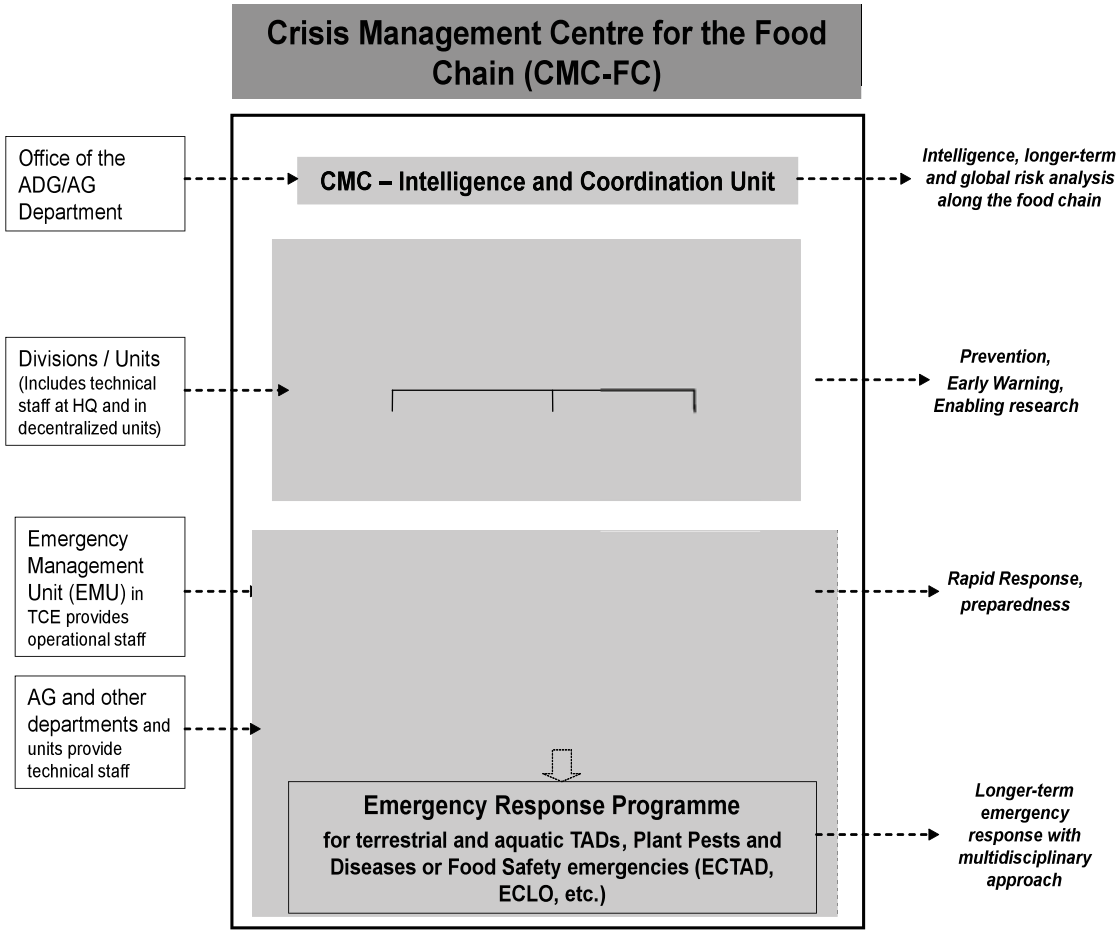
[2] Named after its chair, U.S. agronomist Dr Norman Borlaug, Nobel Peace Prize winner in 1970 and widely acclaimed as the "father of the Green Revolution"

Pests and Diseases (EMPRES) program. The EMPRES program was established in FAO in 1994 in order to minimize the risk of such developing emergencies (Emergency Prevention System (EMPRES), http://www.fao.org/EMPRES/default.htm). The establishment of EMPRES has been a phased process in which initial priority was given two components, one on animal diseases, and the other on Desert Locust control. Within the new CMC-FC structure, EMPRES has been expanded to cover a more comprehensive component for plant protection and for food safety. The EMPRES Plant Protection component covers several locust pests other than Desert Locust as well as transboundary plant pests and diseases of crops critical to food safety. Besides wheat rusts represented by the WRDGP, EMPRES Plant Protection works with critical plant diseases such as the cassava mosaic and cassava brown streak viral diseases, and banana bacterial wilt (*Xanthomonas*) disease presently threatening food security in several countries of East Africa.

The CMC-FC strategy leverages in-house expertise to build links through establishing mechanisms to facilitate inter- and intra-departmental cooperation, especially between the technical, emergency and communication units of FAO. It also aims at raising awareness of donors of the critical need for funding not only for emergency response through eradication or control of diseases and pests, but more importantly, in prevention and country preparedness for emergencies. The strategy is also to raise the awareness of FAO Member Countries at the policy level, of the importance of contingency plans and preparedness.

It is worth noting that the structural and organizational arrangements of the CMC-FC are being improved with the experience gained through the development and implementation of new programs.

Fig. 2 ***Structure of FAO's Crisis Management Center for the Food Chain***



[1] EMPRES to be activated for fisheries / aquaculture and forestry; **[2]** CMC-Rapid Response Unit function to be extended to fisheries / aquaculture and forestry

FAO's Initiative on Soaring Food Prices (ISFP):

The WRDGP has been supported by collaboration with the Initiative on Soaring Food Prices *(ISFP)* that was launched by the Director-General of FAO in December 2007 to reduce food insecurity through support to Member Countries in dealing with the food price crisis. ISFP helps countries respond to a crisis through policy and technical advice, country assessments, and through working with other donors and partners to secure funding and projects.

Recognizing the potential impact of the wheat rusts on livelihoods of small farmers in wheat-dependent countries on food security, international market prices and trade, ISFP works very closely with the WRDGP and has provided financial and technical support to its activities.

WRDGP: Strategic approach and countries covered

The WRDGP is within the FAO's CMC for the Food Chain which aims at emergency prevention, early warning and rapid response to transboundary plant diseases. Preventing the Ug99 threat from developing into a serious global crisis requires immediate action at the national level in countries at risk. However, measures can only be effective through strong regional and international collaborative action.

Most countries could benefit from policy support for contingency planning, and knowledge and information sharing. Better exchange of information on surveillance, pathogen virulence shifts, breeding results and scientific achievements is critical for decision-makers to set national priorities and contingency planning in both developing and developed countries. Some countries will also require support in capacity building, equipment and infrastructure.

The WRDGP works closely with national governments. Participatory discussions, meetings and workshops are the basis for awareness raising, needs assessments and consensus on the most adapted and effective national options and actions to take. Governmental concurrence is required for sharing national information at the global level (as with FAO's Desert Locust Information System), and for assigning focal points responsible for sharing that information internally and externally. National and regional workshops and meetings are the basis on which the Program decides on prioritization of specific activities, mode of implementation in each country and updating contingency plans.

The WRDGP has an estimated budget of around USD 74 million and is planned for an initial phase of four years. It covers 29 countries, representing most wheat-producing countries either already affected by, or at direct risk from, race Ug99 and its variants, and potential new virulent strains of wheat rusts. However, the Program does not foresee equal coverage and support to all countries. The scope of activities, the length of the implementation phase and the level of funding foreseen for the different countries depend on local needs, importance of wheat production for a particular country's food security, and the level of risk associated with epidemics of Ug99 and other wheat rusts.

Countries currently included within the WRDGP are classified within 4 categories for prioritizing the funding and implementation activities of the Program:

1) *Countries already affected by Ug99:* Kenya, Uganda, Ethiopia, Sudan, Yemen and Iran
2) *Countries at immediate risk:* Eritrea, Iraq, Oman, Afghanistan and Pakistan
3) *Countries at high risk:* Egypt, Jordan, Lebanon, Syria, Turkey, India, Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan
4) *Countries at risk:* Algeria, Libya, Morocco and Tunisia

It is worth noting that the Program foresees contingency activities for other wheat-producing countries not included in the above list, but where problems related to wheat rust epidemics may arise.

The WRDGP components

Recognizing that wheat rusts are recurrent potential threats, actions that reduce the risk of spread and allow for quick response to, and management of, these threats have to be undertaken at the national, regional and global levels. Accordingly, the Program is composed of the following five components that are implemented in full cooperation and integration with activities undertaken by other BGRI partners:

1. Advocacy and policy support to preparedness and contingency planning. Any effective national action to prevent or manage the threat of Ug99 and other virulent rust strains requires the consensus and support of national policy makers at various levels of authority. It would also require that governments devise contingency plans to improve their preparedness and rapid response in cases of rust disease outbreaks. Since such rust outbreaks can very rapidly become a global problem, coordinated regional and global efforts would also be needed.

A significant role of the WRDGP is to raise the awareness of national policy makers from the various concerned ministries and units/departments on

the risks and threats of wheat rust, and the need for national contingency planning, information sharing and international cooperation.

The WRDGP also provides the policy and technical support needed in each country for the development of contingency plans for the wheat rusts. These would include specific strategies and scenarios and corresponding actions needed so that governments can respond to potential wheat rust emergencies with minimum time, cost and loss. Support is provided to national authorities to develop their contingency plans through a participatory process that includes multidisciplinary / multi-institutional teams covering all concerned national stakeholders and sectors in the development of these national plans. For wheat rusts, these would include the plant protection units, agricultural research, breeding and seed sectors (public and private systems), extension services, and farmer organizations, as well as other public and private sector institutions involved in the production and management of wheat. Support is provided to identify organisational roles and responsibilities of the various stakeholders, the information flow systems, structures and systems that would need to be established or strengthened for emergency response, and the critical resources and functions needed for the implementation of the contingency plans.

Items that are included within the national wheat rust contingency plan discussions and preparations include advocacy and awareness raising, disease surveillance and monitoring, information exchange, enhanced breeding programs, quick and emergency varietal registration and seed multiplication systems, and actions for improved disease control and management at the field level.

This component of the WRDGP also foresees socio-economic impact assessments of wheat diseases, and specifically wheat rusts, on the livelihoods of farmers and rural communities and on farmers' coping strategies at the onset of diseases. Impact assessment studies will allow for a better rationalization and implementation of national contingency plans.

2. Surveillance, monitoring and early warning systems. National disease surveillance includes the regular assessment of disease incidence and severity as well as the detection of new emerging disease threats in the country. For wheat rusts, the identification of changes in varietal disease severities and monitoring of the spread and changes in virulence patterns is the basis for early response and reaction. Key to the process of surveillance is regularity as well as coordinated information sharing for timely decision-making and response.

The WRDGP provides countries with policy and technical support for the establishment of effective and sustainable national surveillance systems for wheat rusts. This includes the establishment of multi-institutional field survey teams, building national human and infrastructural capacity for field surveillance, rust race analysis (pathotyping) and for tracking virulence changes in the rust populations using field trap nurseries.

Many of the countries within the WRDGP still have no capacity for race analysis, which delays and complicates the process of quick detection of virulent and newly emerging races. The WRDGP foresees the provision of support to ensure that each of the concerned countries will have the capacity to undertake race analysis for all wheat rusts within the near future as part of their preparedness to wheat rust risks.

The Program also provides support to ensure that field survey results are timely shared with all national stakeholders, including plant protection units, breeding programs, varietal registration, seed multiplication and extension systems as well as farmers.

Since wheat rusts are wind-borne transboundary diseases, information sharing at the regional and global levels is also critical. The Program works closely with the national authorities to get their consensus for global sharing of national survey information. In close collaboration and integration of survey activities undertaken by other BGRI partners, the WRDGP is establishing a global wheat rust early warning system at FAO using harmonized national survey results. The early warning system will ensure that concerned countries are updated with needed information on the movement, changes and risks associated with wheat rusts.

3. Enhancement of national wheat varietal registration programs for release of resistant varieties. For a quick replacement of susceptible wheat varieties with new resistant varieties, countries will need support to enhance the effectiveness of their national systems for varietal registration and release, through which potentially resistant varieties must pass before they can be multiplied for distribution.

The WRDGP undertakes assessments of the national wheat rust disease resistance breeding capacities and needs and varietal registration systems. Based on the assessment and individual country needs, it provides policy and technical support to countries for enhancing their national strategies and varietal release procedures, including the establishment of the required multilocation adaptation trials, pest and disease resistance trials and quality testing. In addition, countries are supported in testing all their varieties and breeding materials for response to race Ug99 and its

variants in the international Ug99 nurseries established at Njoro, Kenya for bread wheat and at Debra Zeit, Ethiopia for durum wheat through support of the other BGRI partners and donors.

4. Enhancement of the seed systems for quick multiplication and distribution of resistant varieties. Before rust resistant wheat varieties are nationally registered and ready for release, a national strategy should already be in place for the multiplication and distribution of quality seed to replace rust susceptible varieties. Although many of the countries covered by the WRDGP already have a seed multiplication system, modifications may be needed to cope with the urgency of large-scale rapid multiplication and distribution of Ug99-resistant varieties, especially to serve the most vulnerable small farmers. However, production urgency should, not compromise the quality of certified seeds.

The WRDGP works with the national authorities to strengthen the systems for early generation seed multiplication of rust resistant varieties in the country, providing training and some basic equipment to maximize the yield obtained from early generation seed multiplication. The Program also works with the national authorities to develop and support a strategy for the multiplication and distribution of quality seed of rust resistant varieties to replace rust susceptible varieties through both the public and private sector. For the quick adoption of resistant varieties, support is also provided for the nation-wide establishment of demonstration plots and seed campaigns to popularize among farmers the rust-resistant varieties that will be released.

The WRDGP also provides support to countries to strengthen the National Seed Certification Agency's database through a national variety catalog of released varieties and information on quantities of certified seeds available, including information on significant pests and diseases, as well as the tolerances/responses of each variety to various races of each pest/pathogen.

5. Improving the wheat rust management at the field level. Extension services and farmer education have been given too little attention in recent years in most of the countries threatened by Ug99 and virulent strains of other wheat rusts. Participatory farmer education methods have proved to be extremely effective for empowering farmers with strong observation and decision-making abilities. Properly trained farmers will be a major support to the implementation of national contingency plans. They could help in early recognition and reporting of changes in disease severity and virulence in the field, and in understanding the risks associated with virulent strains and the importance of the various field management practices for disease development and yield improvement (planting dates, planting periods, choice of varieties, etc.).

The WRDGP works with the national authorities to jointly agree and identify the appropriate participatory methods (including, but not limited to, Farmers Field School approaches) to improve the capacities of wheat farmers in proper field management. This will include season-long participatory training of farmer groups and the development of training and reference wheat management guides for farmers and facilitators, based on local experiences.

FAO's comparative advantage

The development of the WRDGP by FAO builds on the following comparative advantages of the Organization:

1. Policy support to national authorities in emergency prevention, contingency planning, coordination and information sharing. FAO works closely and directly with governments and is therefore best situated to provide policy advice and advocacy in the areas of prevention, contingency planning and rehabilitation through the development of scenarios and action plans, including disease surveillance and monitoring, national and international information sharing, and enhancement of national varietal registration and seed systems for the quick availability of resistant replacement varieties to the most vulnerable farmers.

2. Surveillance/monitoring of disease occurrence and severity in the field coupled with analysis of changing pathogen virulence. Through the EMPRES Desert Locust Program, FAO has experience in building and training surveillance teams in the field, and in establishing monitoring and early warning systems; FAO is well positioned to enhance national coordination between the National Agriculture Research System (NARS) and the plant protection units of ministries to combine field disease survey data with scientific virulence tracking data.

3. Networking, international cooperation and knowledge sharing, including awareness raising, advocacy and early warning. By working closely with national governments, most recently through its Initiative on Soaring Food Prices, FAO is best placed to raise awareness on, and advocate with, policy- and decision-makers the importance of information sharing at the national, regional and international levels; FAO is also well positioned to lead in the development of an international early warning system for Ug99 and other potential virulent wheat rust strains.

4. National multiplication and distribution of seeds of resistant adapted replacement varieties to the most vulnerable farmers. FAO has vast experience with national seed systems in its Member Countries through policy support for national regulatory frameworks and regional harmonization, and capacity building activities in the formal and informal seed systems for multiplication and distribution of quality seeds, as well as in emergency and rehabilitation activities with national authorities and farmers.

5. Capacity building of small farmers for disease management in the field. Through Farmers' Field Schools (FFS), FAO has a large network of trained farmers and facilitators in many of the countries affected by, or at risk of, Ug99 and other wheat rusts and can therefore take a lead in supporting the disease management by farmers in the field (trained and empowered farmers provide the necessary support to governments in the implementation of contingency plans in the field).

6. Accelerated varietal registration and release procedures and regulations for quick replacement of susceptible wheat varieties. Breeding activities and identification and development of resistant varieties is beyond the scope of FAO's activities and falls within the mandate of its research partners within the BGRI. However, through its work with national governments, FAO has a critical role to play in providing policy and technical support to enhance the process of national varietal registration and release procedures. FAO's Global Initiative of Plant Breeding can also facilitate the breeding capacity building activities to be provided to national counterparts through BGRI partners.

7. FAO and emergency response. FAO has decade-long experience in emergency response, recovery and rehabilitation in a number of countries that are either affected by, or at risk of, Ug99. This is particularly true for East Africa, Pakistan and Afghanistan, where Emergency Coordination Units have contributed over the past decade to on-farm production and storage of seeds, building on the local knowledge and development of farmer-based seed enterprises. The close cooperation between the technical and emergency operational units within the newly established Crisis Management Center for the Food Chain further enhances the role of FAO in supporting the countries in the preparedness, prevention, early response and rehabilitation phases of emergencies related to transboundary pests and diseases.

Conclusions

The potential impact of wheat rust epidemics on livelihoods, food security, national economies and global markets, demands the need to address such threats in a comprehensive approach, covering all areas of preparedness, prevention and management, including surveillance, appropriate breeding and seed policies, and proper field management strategies, with a critical need for regional and global cooperation.

The placement of the WRDGP within the CMC-FC sets clearly its target stakeholders and activities in support of FAO's Member Countries and for institutional collaboration in the global governance of threats to the human food chain at all stages from production to consumption. It also allows the WRDGP to optimize the interaction between the expertises of the technical units at FAO and provide opportunities for learning lessons from other similar transboundary plant diseases, including sharing experience with animal health emergency responses. It also allows for support to be provided from the operational and fund-raising experience of the FAO emergency units and the global perspective and advocacy that the Intelligence and Coordination unit of the CMC-FC provides.

Since its launching in June 2008, the WRDGP has been able to allocate resources from FAO's core budget, its emergency trust fund budgets, and from various other donors to achieve several of its planned activities. Fund-raising efforts continue, however, since the present budget level is still below the Program needs to support country activities. The achievements of the WRDGP, cannot and should not be separated from those of its partners in the BGRI as they are usually joint or complementary activities. Many of the present WRDGP projects have been and still are jointly being prepared and implemented with CIMMYT, ICARDA, Cornell University and national programs, and each of the BGRI partners is continuously raising awareness on the wheat rust problem and raising funds for national and international activities.

The distinctive role and contribution of the WRDGP to the potential wheat rust crisis remains its outreach to the various national stakeholders beyond breeders and the national agricultural research systems, thus increasing the level of awareness and participation of policy makers from the plant protection organizations, and extension, varietal registration and seed systems. This remains very critical for the sustainability, credibility and national ownership of the strategies and actions taken in the concerned countries. The WRDGP plays also a unique and critical role in providing policy support in

the areas of contingency planning, seed systems and in improved national coordination and international information sharing and communication.

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34. A global reference center for wheat yellow rust: Pathogen variability, evolution and dispersal pathways at regional and global levels

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Following the successful plant breeding efforts to control wheat rusts during the "Green Revolution", there was a serious decline in international expertise researching the control of wheat rusts, including breeding for disease resistance. The 'Global Reference Centre: Yellow Rust' (GRC-YR) is part of the Borlaug Global Rust Initiative (BGRI), launched in April 2008 as a result of the alarm of newly emerged strains of the wheat stem rust pathogen in east Africa (http://www.globalrust.org).

Wheat yellow (stripe) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is among the most devastating crop pathogens globally, multiplying on susceptible crops and associated grass weeds and capable of both local and long distance dispersal through air currents, and human travel and commerce. Yellow rust exists in a range of genetic variants, known as "races", "strains" or "pathotypes", which are evolving continuously due to evolutionary factors, like mutation, migration, selection and occasional recombination, giving rise to new variants. Such variants may be capable of overcoming current sources of resistance in cultivars, and considered the most important factor in the development of new yellow rust epidemics around the world. The yellow rust pathogen population may possess very different characteristics on local- and regional scales, and different sources of resistance may show different degrees of 'durability' in terms of the time and areas of control. Thus, there is an urgent need to establish a global effort to follow and predict pathogen evolutionary potential and yellow rust dispersal pathways, as this is essential for future success in plant breeding and disease management. Recent research has demonstrated that new aggressive strains of *Pst* have spread to new geographical areas in central- and western Asia, Africa, Australia and North America, causing severe yield losses in these areas. The main dispersal pathways may be traced through DNA-fingerprinting techniques rather than analysis of pathogen 'races', which may be subject to rapid evolution in cereal rust pathogens. For instance, in *Pst* it was recently established that variation in virulence evolves two-three orders of magnitude faster than variation in random DNA.

GRC-YR, which is based at Aarhus University, Faculty of Agricultural Sciences, forms an umbrella for a variety of research and surveillance activities aiming at improving yellow rust management in developing countries and elsewhere. GRC-YR builds on established long-term research collaboration and training activities between Danish Research Institutes, ICARDA, CIMMYT and NARS in specific countries, and wishes to establish links to emerging research and surveillance programs within BGRI.

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35. Contribution of socio-economics to assuring impacts of global rust research investments

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Socioeconomic monitoring and assessment addresses two major issues. First, the need for vulnerability assessment and monitoring that is necessary to facilitate effective targeting of rust-resistant varieties and other damage mitigation technologies in affected and at-risk wheat production systems. This assessment involves developing farm typologies in terms of poverty status, food insecurity, nutritional deficiencies and cropping system options for risk mitigation.

The socioeconomic, policy and institutional setting particularly within the seed sector, extension services, and training of farmers are factors that influence households' overall livelihood strategies including the adoption of new crop varieties. These areas will be reviewed in the context of each country to develop sector profiles with their weaknesses. Recommendations for improving their efficiency will be formulated towards effective delivery of seed of rust resistant varieties to farmers especially in locations threatened by stem rust. Together, the information generated in the above activities will constitute the baseline against which efforts to mitigate production losses and households' vulnerability from Ug99 infestation can be measured at later dates.

Second is the ex-ante assessment to potential damage by incorporating the likelihood of rust infestation and associated risks of production losses for a given country, wheat production systems and household typology, using information gathered under Objective 1 (Tracking of Cereal Rust Pathogens). This will help extension workers, district officials, and policy makers to assist farmers in farm-level risk management while research is continuing to develop new wheat varieties.

Lack of functioning institutions and enabling economic and policy environments could lead to unavailability and inaccessibility of the new varieties where they are actually needed to prevent production losses and the negative food insecurity and livelihood impacts of the rust. In addition the absence of a comprehensive analysis of farmers' and consumers' vulnerability could lead to ineffective targeting and welfare losses. Profiling households according to vulnerability categories is crucial in making research outputs (in the form of rust-resistant varieties) accessible to target farmers.

Socio-economic Monitoring and Assessment is designed to complement breeding and pathology research for better targeting and to enhance effectiveness, impact and relevance of the undergoing research. Without establishing adequate baseline information and characterizing the vulnerability of rural livelihoods, it would be difficult (if not impossible) to assess the impact of the project (in later stages) and to target the project interventions to the benefit of small (poor) farmers.

[1] This is proposed as a joint work involving national programs in targeted countries, ICARDA, Virginia Tech University, CIMMYT and FAO.
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36. The role of the International Centers in enhancing international cooperation in wheat improvement

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The stem rust epidemics of the 1950s in North America led to the first international nursery –the International Rust Nursery (IRN). Following the success of the IRN and beginning in the 1960s with a looming global famine crisis, international exchange and co-operative testing of wheat germplasm was formalized. Led by support from the Rockefeller and Ford Foundations, teams of agricultural researchers were assembled to specifically address the needs of food production in the Developing World. This effort resulted in what is now regarded as a "global biological commons" in the exchange of genetic resources. The oldest and arguably most successful of these programs involved the exchange of wheat germplasm, facilitated by CIMMYT and ICARDA, and complemented by regional wheat nurseries organized by various National Agricultural Research Systems (NARS). It has been estimated that at least 50% of the progress made in wheat improvement in the Developing World during the past 50 years has been due to wheat germplasm exchange. The principles are (i) free distribution and redistribution of the original materials, (ii) free redistribution of materials derived from the originals, (iii) full sharing of information, including pedigrees and passport data relating to the materials, (iv) non-discrimination in participation in the networks, and (v) intellectual property rights on final materials that, if used, would not prevent their further use in research. This open source approach, which has been historically so successful for wheat improvement, is as relevant today because the challenges to increase wheat productivity to meet demands by producers and consumers remain unabated.

CIMMYT has a global mandate for wheat improvement, and within the wheat-producing countries of Central and West Asia and North Africa (CWANA) shares this responsibility with ICARDA. The Centers' contributions to wheat improvement are based on their abilities to provide to partners, germplasm adapted to a wide range of environments. Together, the Centers maintain a global resource of genetic diversity, including wheat wild relatives, land races, genetic stocks, cultivars and breeding lines that serve as valuable sources of genetic variability for breeders. The strengths of the Centers are derived from partnerships with NARS and the private sector, which ensure the effective evaluation, utilization and dissemination of new germplasm to farmers throughout the wheat growing areas of the world. With established networks that include advanced research institutes, the Centers are well placed to respond rapidly to new threats to wheat production, such as the stem rust race *Ug99* outbreak in East Africa. The open sharing of wheat germplasm has had a tremendous impact on global wheat improvement, but since the mid 1980s enactment of intellectual property rights agreements, increasingly strict quarantine restrictions and various international agreements have impacted on willingness for free exchange of germplasm, data and knowledge. Restrictions on the open sharing of germplasm and associated data need to be eased, otherwise progress in international collaborative efforts will be negatively affected.

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