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Genetic protection of wheat from rusts and development of resistant varieties in Russia and Ukraine
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Genetic protection of wheat from rusts and development of resistant varieties in Russia and Ukraine

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Abstract Leaf rust represents the major threat to wheat production in Russia and Ukraine. It has been present for many years and epidemics occur in different regions on both winter and spring wheat. In some regions there is evidence of more frequent epidemics, probably due to higher precipitation as a result of climate change. There is evidence that the virulence of the leaf rust population in Ukraine and European Russia and on winter wheat and spring wheat is similar. The pathogen population structure in Western Siberia is also similar to the European part, although there are some significant differences based on the genes employed in different regions. Ukrainian wheat breeders mostly rely on major resistance genes from wide crosses and have succeeded in developing resistant varieties. The North Caucasus winter wheat breeding programs apply the strategy of deploying varieties with different types of resistance and genes. This approach resulted in decreased leaf rust incidence in the region. Genes *Lr23* and *Lr19* deployed in spring wheat in the Volga region were rapidly overcome by the pathogen. There are continuing efforts to incorporate resistance from wild species. The first leaf rust resistant spring wheat varieties released in Western Siberia possessed gene *LrTR* which protected the crop for 10-15 years, but was eventually broken in 2007. Slow rusting is being utilized in several breeding programs in Russia and Ukraine, but has not become a major strategy.

Introduction

Wheat has been cultivated on the territory of Russia and Ukraine since ancient times (Merezhko 2001) and as the human population expanded, so did the wheat area. The diet of Slavic people is historically based on cereals. Average per capita wheat consumption in 2003-2005 was 247 kg in the Russian Federation and 226 kg in Ukraine compared to 113 kg in the USA (Dixon et al. 2009). The last 100-150 years witnessed a gradual change from primarily rye bread consumption to wheat bread. In 1900 rye covered 24 m ha of the 106 m ha of arable land in Russia and wheat was cultivated on 11 m ha (Zhuchenko 2004). During 2000-2008 the wheat area in Russia varied between 20.06 and 26.7 m ha (Table 1) whereas the rye area did not exceed 3.7 m ha and has continued to decline over the last 2-3 years (http://faostat.fao.org). In addition to high domestic consumption the importance of wheat for the national economies in both Russia and Ukraine is also substantiated by export potential. An estimated 24 m ha of arable land in Russia is not cultivated, but could be utilized for wheat production and export (Zhuchenko 2004). The yield potential of Ukraine could also be enhanced for exports.

TABLE 1 HERE

The enormous territory of Russia and Ukraine has a wide diversity of soil and climatic environments reflected in the diversity of wheat varieties cultivated in the region. In general the wheat growing area of Russia and Ukraine can be subdivided into three major overlapping regions (Fig. 1). Region 1 covers southern Ukraine and Russia. This is primarily a winter wheat growing region with relatively mild winters and variable precipitation. Spring wheat is planted on a commercial scale only in some years to replace winter wheat that did not survive the winter, as in Ukraine, for example, in 2003. This region stretches from the northern Caucasus and north shore of the Black Sea to 55-57^{o}N, or beyond

the latitude of Moscow. Moving northwards, the amount of annual rainfall increases from 300-350 mm per year to 500-600 mm. The second region stretches from northern Ukraine and central European Russia to the Ural Mountains. Low temperatures in winter limit cultivation of winter wheat in this region and spring wheat occupies a significant share of the crop area. Precipitation is variable and decreases eastwards from 600 mm in the Moscow region to 300-350 mm beyond the Volga River. The third wheat production region is Siberia, which due to its cold climate, grows almost entirely spring wheat. Western Siberia is the major production region, with a frost free period of 110-120 days and precipitation varying from 300-350 mm in the south to 450-500 mm at the northern border with the forest belt. This region comprises one uniform spring wheat production area of 15-17 m ha including northern Kazakhstan. Wheat breeding and rust research in Kazakhstan is not reviewed here, but was previously covered in separate publication (Morgounov et al. 2007). There are several smaller sub-regions of wheat cultivation like the Far East and northwestern part of European Russia, but their contributions to overall wheat production are minor. The objective of the current paper is to provide a review of wheat breeding for rust resistance and relevant rust research in Russia and Ukraine relating to the regions described above and with a focus on the current status.

FIGURE 1 HERE

Institutional framework for wheat breeding and rust research in Russia and Ukraine

The agricultural research system of Russia and Ukraine with public centralized institutions was inherited from the Soviet Union. Although some countries of the former USSR went through radical re-organizations of their research systems, the situation in Russia and Ukraine is largely unchanged (Morgounov and Zuidema 2001). Wheat breeding and research in Ukraine are primarily conducted by the research institutions of the National Academy of Agrarian Sciences. Five research centers and 52 research institutes and stations are part of the Academy with the total arable land exceeding 0.5 m ha (http://www.uaan.gov.ua/). The key wheat breeding programs are located throughout the country at Odessa in the south (Plant Breeding and Genetics Institute), Kiev region in the center (Mironovka Wheat Institute) and Kharkov in the northeast (Ukrainian Institute of Plant Industry). The Institute of Plant Physiology and Genetics (located in Kiev) belonging to the National Academy of Sciences is also involved in wheat breeding as well as several other institutes and universities. A few private wheat breeding companies are mostly involved in testing and promotion of foreign germplasm from major agricultural companies abroad. Pathology research related to rust is conducted in the same institutions as wheat breeding.

The Russian Academy of Agricultural Sciences is much larger comprising 51 research centers and 196 research institutes with a total arable area of 1.7 m ha (http://www.agroacadem.ru) . There are several All-Russian research centers and institutes with a national mandate and these primarily address more narrow topics across the whole country. In the case of wheat the All-Russian Research Institute of Phytopathology located near Moscow and the All-Russian Research Institute of Crop Protection located near St. Petersburg with respective branches and stations cover more basic aspects of pathogen research. Wheat breeding is conducted by regional research institutes which serve specific areas of the country with new technologies including wheat varieties. Applied rust pathology research is also conducted by the same institutes. There are more than 30 wheat breeding programs in the country. The role of universities is limited though some of them do have active and successful breeding programs. As in Ukraine, private companies serve the needs of foreign seed companies. Wheat breeding and rust research in both Russia and Ukraine underwent economic crises after the disintegration of the USSR in the 1990s. However, the core breeding and research programs were maintained and some even strengthened. The 2000s witnessed a revival of the wheat industry and wheat research to meet greater demands from the farming community for new varieties. Agricultural research funding is also recovering through public sources, research grants and royalties, as well as direct contracts with the producers.

Winter wheat breeding for rust resistance in Ukraine

Among the three wheat rusts, the least damaging in Ukraine are yellow rust (caused by *Puccinia striiformis*) and stem rust (*P. graminis*). Yellow rust occurs occasionally on highly susceptible varieties, but yield losses do not exceed 5-10%. Over the last 20 years yellow rust infections reaching commercially significant scales were observed in 1991, 2001, 2005 and 2007. The *P. striiformis* population is dominated by races (European nomenclature) 0E0, 6E0 and 6E16 which lack virulence to *Yr3c*, *Yr5*, *Yr9*, *Yr10*, *Yr15* and *Yr17* (Babyants et al. 2009) (Table 2). Stem rust is not common and has not damaged the crop in the last 10 years. In the past (1930-1970) it was observed on late maturing winter wheats (especially winter durums) in western and central Ukraine (Babayants et al. 2004a) with yield losses of 15-20%. Effective *Sr* genes are listed in Table 2. Leaf rust (*P. triticina*) is the most common rust across the country and reaches epidemic dimensions on susceptible varieties every two to three years in five (Babayants et al. 2004b). Over the last 10 years leaf rust epidemics occurred in 2000, 2001, 2004 and 2006 with average yield losses of 10-30%. A similar frequency of epidemics took place in the previous 50 years. More than 50 races have been identified in Ukraine with races 77 and 144 being dominant (Traskovetskaya 2009). Effective *Lr* genes are listed in Table 2.

TABLE 2 HERE

A detailed description of the history of wheat breeding in Ukraine was presented by Litvinenko et al. (2001). In breeding for rust resistance three main stages are clearly defined:

1) Breeding based on selection from local varieties and landraces and the development of the first varieties originating from crosses. Most of the varieties bred and utilized during that time were susceptible to prevailing diseases including leaf rust and other rusts. This period lasted until the 1960s.

2) The development and release of landmark varieties Bezostaya 1, Aurora, Kavkaz and Mironovskaya 808 in the early 1960s began a new era of rust resistance breeding. Aurora and Kavkaz possess the 1B.1R translocation with a combination of resistance genes. Bezostaya 1 possesses the slow rusting *Lr34* gene. Mironovskaya 808, although susceptible to leaf rust, had broad adaptation and a capacity to provide high yields in the presence of the pathogen. These varieties not only contributed to production but served as common parents for numerous other varieties, thus, transferring their resistances/responses to the next generation of germplasm. Early CIMMYT germplasm resistant to leaf rust was also utilized in crosses and contributed to new rust resistant germplasm.

3) The current period of resistance breeding is characterized by accumulation of rust resistance from different sources and incorporation of resistance from wild relatives. The Plant Breeding and Genetics Institute in Odessa introgressed resistances to leaf rust and stem rust from *Aegilops cylindrica*, *Triticum erebuni* and Amphidiploid 4 (*T. dicoccoides* x *Ae. tauschii*), and stem rust from *Aegilops variabilis* (Babayants et al. 2010). New winter wheat varieties such as Knyaginya, Olga, Lastivka Odeska and Vihovanka Odeska possess some of these genes and display high degrees of resistance to one or more rust pathogens. Rust resistance breeding at Mironovka Wheat Institute was initially based on crosses and backcrosses with triticale which resulted in variety Mironovskaya 10 (Novohatka 1976). More recent efforts are based on incorporation of resistance from foreign, primarily European, germplasm. A list of modern Ukrainian winter wheat varieties from Odessa, their reactions to rusts and nature of resistances are provided in Table 3.

Winter wheat breeding for rust resistance in the European part of Russia

The southern part of European Russia or North Caucasus region is the breadbasket of the country due to its favorable soils, climatic conditions and dynamic farming community. The average yields in the

Krasnodar and Rostov regions covering 2-3 m ha normally exceed 4-5 t/ha or double the national average. However, this favorable environment is also very suitable for wheat diseases, including the rusts, and especially leaf rust. The early 20th century witnessed devastating leaf rust epidemics leading to widespread hunger in the region. In the period 1939-1953 leaf rust epidemics occurred in 1939, 1949, 1941, 1946, 1948, 1952 and 1953. No wonder when P. Lukyanenko, breeder of Bezostaya 1, started his breeding work in Krasnodar in the early 1930s resistance to leaf rust was a primary objective. His first rust resistance breeding efforts were based on crosses of local winter wheats with resistant spring wheat varieties from USA such as Marquis and Kitchener (Lukyanenko 1973). His paper on methodology of breeding winter wheat for rust resistance, published in 1941, classified the types of resistance into seedling and adult plant, listed the sources of resistance, and described the screening methodology. Eventually, his efforts resulted in development of variety Bezostaya 1 which was a landmark both in production and as a future breeding parent.

The entire history of wheat breeding in the north Caucasus of Russia represents a continuous race between the breeders and the evolving leaf rust pathogen. The first widespread leaf rust resistant variety developed by Lukyanenko was Novoukrainka 83 which lost its resistance in 1948 due to the appearance of race 77. In the 1950s this variety was replaced by Bezostaya 4, Skorospelka 3b (*Lr3a*) and Bezostaya 1 with field resistance to leaf rust (Voronkova 1980). In the early 1970s new higher yielding varieties Avrora and Kavkaz with specific resistance against races 58 and 77 (1B.1R translocation with *Lr26*) were released to replace Bezostaya 1. However, a leaf rust epidemic in 1973 devastated 80% of the area covered by these varieties, demonstrating that major genes alone were not able to protect the varieties. The following genes protected wheat from leaf rust when the resistance of Avrora and Kavkaz was broken: *Lr9*, *Lr19*, *Lr23*, *Lr24* and *Lr25* (Alekseeva 1986). Genes *Lr10*, *Lr14a*, *Lr14b* and *Lr18* also contributed to resistance. In the early 1980s race 77 became dominant and a new generation of winter wheat varieties was grown in the region (e.g. Olimpya, Krasnodarskaya 57, Prikubanskaya, Partizanka, Obriy). Intensification of production in the late 1980s resulted in the release of semi-dwarf varieties Spartanka and Yuna (*Lr23* + *Lr26*) – the latter alone occupying almost 38% of the total wheat area (Romanenko et al. 2005). The leaf rust pathogen responded by evolving new virulent races. In the early 1990s race 25 became dominant. Variety Yuna became susceptible within five years after release. The changes in virulence in the pathogen population in the north Caucasus region are shown in Table 4.

In the 1990s the strategy of deployment of varieties with different bases for resistance (both major genes and adult plant resistance genes) was adopted. This approach denies domination of a single variety as occurred in the past and demanded that any variety could not exceed a maximum 15-20% of the wheat area. In 2001, for example, there were 14 winter wheat varieties with the area covered by any single variety varying from 1 to 14%. This strategy might be the main reason for gradual reduction of leaf rust occurrence in the region (Fig. 2). At the same time the number of pathogen races identified in the region increased (Table 4). The last outbreak of leaf rust took place in 2004, affecting varieties Krasnodarskaya 39, Polovchanka and Knyazhna which jointly did not exceed 20% of the total area. Varieties developed at Krasnodar Agricultural Research Institute are classified into 4 groups based on their reaction to leaf rust: viz. Group 1: practically immune with necrotic flecks (varieties Yara, Veda); Group 2: slow rusting genotypes with low AUDPC both under natural and artificially inoculated conditions (Rannaya 12, Krasnodarskaya 6, Umanka, Zimorodok, Afina, Starshina, Kuma, Doka, Valentin); Group 3: varieties reaching infection levels of 60% under inoculated nursery conditions, but still possessing a degree of resistance (Delta, Kroshka, Deya, Yubilejnaya 100); Group 4: varieties susceptible to rust (Bezostaya 1, Krasota, Polovchanka). Yellow rust and stem rust are not common in the region, but all materials are routinely evaluated during the breeding process.

The second largest wheat producing region in southern Russia is Rostov province which has its own research institute and a highly successful winter wheat breeding program. The environment of this region requires varieties with higher winter hardiness and drought tolerance than in the Krasnodar area. However, leaf rust resistance remains an important breeding objective. The modern germplasm from the Rostov region is based on Bezostaya 1 and its derivatives. Varieties Donskaya Bezostaya,

Donskaya Polukarliovaya, and more recently Don 85, Ermak, Stanichnaya, Zarnitsa and Dar Zernograda, are slow rusting (Kovtun et al. 2001). The winter wheat producers of the central part of European Russia are served by a breeding program located near Moscow. The main challenge there is the combination of semi-dwarf stature with winter hardiness as the temperatures decline as wheat production areas move north. Leaf rust resistance remains a high priority and yellow rust is observed in some years. Breeding is based on interrupted back- and top crosses utilizing Mironovskaya 808 type germplasm to maintain high degrees of winter hardiness with different sources of *Rht* genes and rust resistance (Sanduhadze et al. 2001). The resulting varieties (Inna, Moskovskaya 39) possess field resistance to leaf rust and are broadly adapted essentially extending the northern and eastern limits of winter wheat cultivation due to their superior winter hardiness.

TABLES 3 AND 4 HERE
FIGURE 2 HERE

Spring wheat breeding for rust resistance in European part of Russia

This important wheat production region stretches as a belt from west of Moscow to the Ural Mountains below 50-55^{o}N. It is a traditional spring wheat production area since winter wheat is frequently killed during severe winters. However, climatic changes over the last 5-10 years have made winter wheat more reliable, and in some locations it competes with spring wheat in area and profitability. The amount of precipitation is variable and a large part of the region (especially the middle and lower Volga region) is subjected to moisture stress during the season. Except in severely dry years, leaf rust is common throughout the region. A study of 42 spring wheat varieties released in the USSR during 1967-69 indicated that none was resistant (Nettevich 2008). Systematic monitoring of races started in the 1970s. Between 1971 and 2004 epidemics occurred in 1973, 1974, 1976, 1978, 1989, 1994, 2000, 2001 and 2004, or one year in three (Markelova 2009). The virulence of the rust population in the Volga region is similar to that in the north Caucasus winter wheat region suggesting that inoculum from the south moves to the north with the wind, and earlier maturing crops in the south serve as a source of spores for more northern and eastern areas of spring wheat production. Table 5 presents data for yield losses during the epidemic years as well as associated changes in the virulence pattern in the Volga region. The data clearly demonstrate the continuous co-evolution of wheat and rust. Once breeding programs started to use gene *Lr23* new races possessing corresponding virulence were detected. The same happened with varieties protected by *Lr19*. Long term research by Markelova (2009) in Saratov concludes that currently only gene *Lr9* continues to maintain a high degree of resistance in the region. At the same time evaluation of *Lr* Thatcher isogenic lines in Bezenchuk (Samara Agricultural Research Institute) demonstrated that acceptable resistance was conferred by *Lr9*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr36*, *Lr38* and *LrTR*.

The two main research institutions involved in spring wheat breeding and rust research in the region are the Agricultural Research Institute of the South East in Saratov and Samara Agricultural Research Institute in Bezenchuk located 300 km apart on the Volga River. Unlike the winter wheat breeding programs in southern Russia relying primarily on slow-rusting, the strategy of rust resistance breeding in the Volga region is based on continuous screening for possible sources of resistance and incorporation of resistance genes from wild relatives and related wheat species. The first leaf rust resistant spring wheat varieties developed only in the 1980s, utilized *Lr23* and *Lr19* which soon lost their effectiveness. Syukov et al. (2006) introduced a highly effective resistance gene, *LrAg*, from *Elytrigia intermedia*. Varieties Tulaikovskaya 5, Tulaikovskaya 10, Tulaikovskaya 100 and Lutescens 101 possess this gene which is different from *Lr24, Lr25, Lr28, Lr29, Lr36, Lr38* and *LrTR*. Genes combinations, even including some defeated genes such as *Lr19+Lr26* and *Lr19+Lr23*, are also considered part of a viable strategy at Samara Institute. The Agricultural Research Institute in Saratov has been developing drought tolerant superior grain quality varieties well adapted to the dry Volga region. Most of them lack resistance to leaf rust as it is asumed that the pathogen affects wheat less during drought. The specific effort in rust resistance research at Saratov has concentrated on screening

a huge number of germplasm accessions (50,000) over the last 20 years to select resistant germplasm. A number of foreign wheats, including CIMMYT germplasm, were selected and utilized in crosses. Wide crosses have also been utilized as well as a combination of alien translocations with known *Lr* genes (*Lr19* + *Lr26*) (Sibikeev et al. 2009). Leaf rust reactions of spring wheat varieties currently released in the Volga and Ural regions are provided in Table 6. Among 81 varieties tested, 21 (25%) were resistant mostly due to introgressed major genes.

Spring wheat breeding for rust resistance in Siberia

A recent historical analysis of the wheat rust situation in northern Kazakhstan and western Siberia and respective breeding efforts were described by Morgounov et al. (2007). Wheat production areas in the Siberian part of Russia are divided into three major areas: Western Siberia, Eastern Siberia and the Far East. The importance of wheat as a crop decreases eastwards and by far the most important region is Western Siberia. Early 20th century reports on wheat and diseases do not emphasize rust, but pay more attention to loose smut and common bunt. The massive commercial wheat production in western Siberia started in the 1960s after implementation of the program of virgin land exploration. The wheat area increased several fold and leaf rust started to damage the crop. Until the 1980s epidemics were on average one to three years in ten (Chulkina et al. 1998). There was, and still is, a common belief among breeders and wheat producers that leaf rust comes late during the maturing stages and does not damage the crop. However, numerous data suggest significant yield losses of up to 20-30% during epidemics (Koyshibaev 2002). In the 1990s leaf rust affected spring wheat in western Siberia in seven years out of ten, with 1993 and 1995 being epidemic years. In the 2000s leaf rust occurred every year, with 2001, 2005, 2007 and 2008 being epidemic years. The important issue is that it occurs during more favorable wet years when higher average yields mask losses from the disease.

There are different opinions in regard to the sources of infection for leaf rust in Western Siberia. Turapin (1991) summarizing the rust research in Kazakhstan during the Soviet Union era suggested that cultivated and wild grasses (such as *Aegilops*, *Agropyrum* and others) may play an important role in maintaining infection during winter. Mostovoy and Berezhnova (1985) compared the pathotypes of leaf rust collected in the atmosphere of northern Kazakhstan with those collected in European Russia and found many common ones suggesting there was movement of spores from the western part of Russia to Siberia and northern Kazakhstan. Koyshibayev (2002) suggested an important role of rust infected winter wheat as a 'green bridge' to preserve infections during winter. Odintsova and Shelemova (1987) compared the virulence pattern of *P. triticina* across different regions of the country and concluded that the pathogen population in Siberia is relatively closely related to the population in the European part of the country, but differs from the population in Central Asia (Kazakhstan and Uzbekistan) and the Far East. This conclusion supports the possible movement of spores from west to east with the prevailing winds. The data from the Siberian Agricultural Research Institute in Omsk for 2003-2005 obtained on Thatcher near-isogenic lines demonstrated that genes *Lr9, Lr28* and *Lr36* provided complete resistance. The genes *Lr19, Lr24, Lr25* and *Lr37* conferred resistant or moderately resistant responses depending on the year. Some genes (e.g. *Lr12, Lr29, Lr30*) seemed to have slow rusting effects with moderately susceptible reaction types, but with rust severities not exceeding 50%.

TABLES 5 AND 6 HERE

The reactions of 55 spring wheat varieties released in the USSR from 1975 to 1991 were studied in Mexico (Singh et al. 1995). Almost half of the cultivars had high or moderate degrees of adult plant resistance. Leaf tip necrosis associated with *Lr34* was observed in 20 varieties. The most common known leaf rust resistance genes were *Lr10* (14 varieties), *Lr3* (7 varieties) and *Lr13* (5 varieties). However, none of these genes is effective in Russia. Despite substantial efforts in breeding new spring wheat varieties with undoubted progress in yield potential, grain quality and other traits, little improvement was made in leaf rust resistance (Morgounov et al. 2010). One important reason

for this was utilization of a limited number of leaf rust susceptible parents (e.g. Saratovskaya 29) in the crossing programs to maintain and enhance adaptation and yield. The sources of leaf rust resistance most frequently used in the region can be classified into three groups: viz. Those used in winter wheats, local spring wheats, and foreign spring wheats with variable genetic basis of resistance. The development of more input-responsive semi-dwarf winter wheat varieties in Russia in the 1970s and 1980s resulted in increased yields and enhanced resistance to diseases. Some of the most successful winter wheat varieties were utilized in crosses to improve spring wheat. One example is variety Bezostaya 1 used as a parent in a number of spring wheat varieties grown in Northern Kazakhstan and Siberia (e.g. Tulunskaya 12, Sibakovskaya 3, Omskaya 9, Kazakhstanskaya Rannespelaya) (Morgounov 2001). Since Bezostaya 1 possesses *Lr34* and possibly some other genes it has contributed to leaf rust resistance in Russia. On the other hand, utilization of winter wheat for spring wheat breeding raised a concern that the same genes and gene combinations would be protecting the crop across a huge continuous area, increasing vulnerability to the disease.

Starting from the mid-1990s CIMMYT initiated broad germplasm exchange and cooperative breeding efforts with the region to enhance leaf rust resistance while maintaining the general adaptation and grain quality. Testing of CIMMYT germplasm showed that resistance effective in Mexico was also effective in northern Kazakhstan and Siberia. The resulting shuttle breeding of germplasm was intensively tested in the region and proved competitive for achieving rust resistance and general adaptation. Incorporation of gene *LrTR* from an Australian breeding line (designated i-286064 by the Vavilov Institute) made a tremendous impact on production when it was initially used in varieties such as Tertsia, Sonata, Duet and Sibakovskaya Yubileynaya. Gene *LrTR* located on chromosome 6B (Tsilke 1984) provided good protection for more than 10 years with varieties possessing the gene covering up to 25% of wheat area in the region in 2005. However, virulent pathotypes were detected on varieties possessing *LrTR* in 2007 . The initial source material for *LrTR* was possibly brought by I.A. Watson or R. McIntosh during their visits to to the USSR in the 1970s and given the time and the fact that the gene was located on chromosome 6B, it is very likely to be *Lr9* (McIntosh pers comm). The leaf rust reactions of spring wheat varieties released and cultivated in the region are provided in Table 7. Several varieties released after 2003 continue to have acceptable levels of resistance.

TABLE 7 HERE

Utilization of wheat genetic resources in rust resistance breeding

Vavilov (1965) in his fundamental work titled “The laws of natural immunity of plants to infectious diseases” emphasized the importance of genetic resources for rust resistance. His studies covering a wide range of environments in the USSR demonstrated high levels of rust resistance in species *T. boeoticum*, *T. monococcum* and *T. timopheevii*. The Vavilov Institute was always at the forefront of wheat pathology and wheat rust research emphasizing not only the host, but also the pathogen. Research by I. Odinstova and her colleagues in 1980s provided theoretical and practical bases for resistance breeding including consideration of different strategies for gene deployment. Evaluation and utilization of genetic resources for rust resistance involved both cultivated wheat considered globally, and wild and related species. Two recent catalogs (Mitrofanova 2004, 2007) list rust resistant germplasm of winter and spring wheats, respectively.

Conclusions

Genetic protection of wheat in Russia and Ukraine against leaf rust has employed different strategies involving utilization of major genes, introgression of major genes from wild relatives, alien translocations and slow rusting. The use of major gene protection proved as in other countries that sooner or later the pathogen evolves to overcome the resistance. Thus future efforts must focus on ways to find new resistance alternatives. Although being used by a number of breeding programs in

Russia, slow rusting has yet to be adopted as a main strategy for wheat protection, and its use in conjunction with major genes is yet to be better understood. Monitoring of the rust population has occurred across the country and there are reliable data on the structure of the pathogen population. This, coupled with close cooperation between pathologists and breeders, is an important pre-requisite for successful rust resistance breeding. Yellow rust and stem rust seem to be of only minor regional importance at present.

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Table 1 Wheat areas harvested and average yields in the Russian Federation and Ukraine, 2000-2008 (http://faostat.fao.org)

Year	Russian Federation		Ukraine	
	Area harvested, m ha	Yield, t/ha	Area harvested, m ha	Yield, t/ha
2000	21.34	1.61	5.16	1.97
2001	22.83	2.06	6.88	3.10
2002	24.48	2.07	6.74	3.04
2003	20.06	1.70	2,46	1.47
2004	22.92	1.98	5.53	3.17
2005	24.68	1.93	6.57	2.84
2006	23.05	1.95	5.51	2.53
2007	23.50	2.10	5.95	2.34
2008	26.07	2.45	7.05	3.67

Table 2 Wheat rust resistance genes effective against the pathogen populations in Ukraine

Genes effective at both the seedling and adult plant stages	Genes effective at the adult stage only
Leaf rust	
Lr9, Lr19, LrAc[1], *LrTe*[2], *LrAd4*[3]	*Lr24, Lr25, Lr37, Lr42*
Yellow rust	
Yr3c, Yr5, Yr9, Yr10, Yr15, Yr17	*Yr2, Yr3a, Yr3b, Yr4a, Yr4b*
Stem rust	
Sr14, Sr31, SrTe[2], *SrAv*[4], *SrAd4*[3], *SrAc*[1]	*Sr24, Sr25, Sr26, Sr27, Sr36*

[1]*LrAc*, *SrAc* – genes originating from *Aegilops cylindrica*

[2]*LrTe, SrTe* – genes originating from *Triticum erebuni*

[3]*LrAd4, SrAd4* – genes originating from Amphiploid 4 (*Triticum dicoccoides* x *Triticum tauschii*)

[4]*SrAv* – gene originating from *Aegilops variabilis*

Table 3 Reactions of modern winter wheat varieties from the Plant Breeding and Genetics Institute, Odessa, Ukraine, to rusts, 2007-2009

Variety	Pedigree	Leaf rust		Yellow rust		Stem rust	
		Reaction	Type of resistance/genes	Reaction	Type of resistance/genes	Reaction	Type of resistance/genes
Otaman	Zoryanka/Viktoria//Obriy/Albatros// Nikonia	R	Major genes, *LrZ*	R	Major genes, *YrZ*	MS	-
Nebokray	Strumok/Yubileynaya 75	R	Major genes, *LrTt*	MR-MS	-	R	Major genes, *Sr5, Sr36*
Vychovanka Odeska	Obriy/Tr.erebuni//Od162/2*Ukrainka	R	Major genes, *LrTe*	R	Major genes, *YrUkr*	R	Major genes, *SrT*e
Knyaginya Olga	Obriy/Tr.erebuni//Od162/2*Ukrainka	R	Major genes, *LrTe*	R	Major genes, *YrUkr*	R	Major genes, *SrTe*
Lastivka Odeska	Don.polukarl/Ae.variabilis//Ukrainka/ Nikonia	MR-MS	Non specific resistance	R	Major genes, *YrUkr*	R	Major genes, *SrA*v
Erythrospermum 139/09	Od.polukarl/Ae.cylindrica// Od.polucarl./ Kyriya	R	Major genes, *LrAc*	R	Major genes, *YrK*	R	Major genes, *SrA*c
Erythrospermum 249/09	Obriy/Tr.erebuni//Od16/2*Ukrainka// Selyanka	R	Major gene, *LrTe*	R	Major genes, *YrUkr*	R	Major genes, *SrT*e

Table 4 Changes in the virulence of the leaf rust pathogen population in North Caucasus region of Russia, 1980-2000 (Volkova et al. 2002)

Years	*P. triticina* races	Frequency of virulences against *Lr* genes		
		High	Low	Absent
1980 – 1984	5, 7, 15, 21, 25, 54, 58, 62, 77, 105, 122, 130, 144, 149, 192	*1, 2a, 2b, 2c, 3, 11, 14b, 17, 20, 30*	*10, 14a, 16*	*9, 19, 23, 24, 25*
1991 – 1994	2, 15, 16, 21, 25, 52, 62, 77, 92, 122, 123, 130, 144, 192	*3a, 11, 12, 13, 14b, 17, 30*	*18, 21, 28, 34 (+13)*	*9, 19, 24, Tr*
1998 – 1999	2, 5, 6, 15, 16, 21, 25, 28, 44, 51, 52, 57, 61, 62, 77, 92, 105, 122, 140, 141, 144, 149, 163, 169, 170, 179, 192, 222	*3a, 11, 12, 13, 14b, 17, 22a, 22в, 30, 37*	*18, 20, 21, 24, 26, 32*	*9, 19, 38, Tr*
2000	2, 5, 6, 15, 16, 17, 21, 25, 28, 33, 44, 51, 52, 58, 62, 77, 92, 93, 105, 122, 124, 129, 140, 141, 144, 149, 152, 161, 169, 170, 175, 179, 192, 214, CK1, CK2, CK3, HP8, HP9	*2c, 3a, 10, 11, 12, 13, 14a, 14b, 15, 16, 17, 22a, 22в, 27+31, 28, 29, 30, 37*	*18, 21, 24, 32, 33*	*9, 19, 38, Tr*

Table 5 Yield losses during epidemics years and associated changes in the virulence pattern of *P. triticina* in the Volga region, Bezenchuk, 1964-2005 (Vjyushkov et al. 2008)

Epidemic years	Grain yield, t/ha		Correlation coefficient between yield and Leaf rust infection rate[1]	Changes in the pathogen-host interaction
	Susceptible variety	Resistant variety		
1964	1.10 Saratovskaya 29	2.10 Eritrospermum 132-76		Race 77 spread and appearance of pathotypes virulent to *Lr3* (Mironovskaya 808, Bezostaya 1). Vareity Bezenchukskaya 98 loss of resistance to some pathotypes.
1974	2.10 Bezenchukskaya 98	3.20 Zhigulevskaya (*Lr3+LrB*)		Maximum spread of race 77/1.2 with virulence to *Lr26*. Loss of resistance by varieties Avrora and Kavkaz.
1983	2.06 Saratovskaya 46	2.93 Olimp (*Lr23*)	-0,854**	Spread of race 77/c with virulence to *Lr10* and *Lr14a*. Loss of resistance by varieties Saratovskaya 46 and Kutulukskaya.
1990	2.83 Saratovskaya 42	4.40 Samsar (*Lr19*)	-0,836**	Virulence to *Lr23* and increase in the frequency of virulence to *Lr26*. Loss of resistance by variety Zhigulevskaya.
1993	1.53 Saratovskaya 29	3.54 Olimp (*Lr23*)	-0,460*	Increase of pathotypes with virulence to *Lr23*. Varieties with *Lr23* maintain good field resistance.
1994	2.11 Saratovskaya 42	3.13 Tulaikoskaya 5 (*LrAg*)	-0,681*	Areas under varieties with *Lr19* (L 503, Samsar) expanded. Virulence to *Lr19* detected. Frequency of virulence to *Lr23* reached 80%.
2000	0.96 Saratovskaya 42	2.06 Tulaikoskaya 10 (LrAg)	-0,674*	The infection of varieties possessing gene *Lr23* increases. Frequency of virulence to *Lr19* exceeds 33%. Loss of resistance by variety Prohorovka with pyramided genes *Lr26*+.
2005	0.88 Prohorovka	1.85 Tulaikoskaya 100 (LrAg)	-0,730**	Maximum spread of virulences to *Lr23* and *Lr19*. Variety Prohorovka shows high susceptibility.

[1]Based on Yield Trial data consisting of 20-30 genotypes with variable responses to leaf rust

Table 6 Leaf rust reactions of spring wheat varieties released in Volga and Ural Mountain regions of Russia, Bezenchuk, 2008. Response = infection type (0-4 scale)/area affected

Variety	Year of release	Pedigree	Lr genes	Leaf rust response	
				At release	2008
Albidum 188	1996	Rodina/FS-3//Ershovskaya 32/3/Albidum 43/4/Saratovskaya 55		4/80	4/100
Albidum 28	1987	Kransnokutka 4/Albidum 2759		4/80	4/100
Albidum 29	1994	Saratovskaya 46// Albidum 43/Kransnokutka 3		4/80	4/100
Albidum 31	2001	Line 23/Saratovskaya 55// Albidum 28		4/80	4/100
Albidum 32	2008	With participation of Saratovskaya 46		4/40	4/60
Amir	2001	Rodina/2*Priokskaya		4/90	4/90
Bashkisrskaya 24	1994	Saratovskaya 46/Leukurum 87		4/90	4/90
Bashkisrskaya 26	2004	Zhnitsa/Kazakhstanskaya 10		4/90	4/90
Belyanka	1999	L-23/Saratovskaya 55//AS-13/Pysar 29/3/ AC38BC	*LrBel*	0	0
Boevchanka	2009	L.70-94/L.196-94-6		3-4/40	3-4/40
Varyag	1997	Saratovskaya 46/NP 876		4/80	4/100
Voevoda	2008	L-504/Kransnokutka 10//L-504/Belyanka	*LrBel*	0	0
Volgouralskaya	2001	Albidum 653*2/Lutescens 29	*Lr19*	4/30	4/70
Voronezhaskaya 12	1998	Bezostaya 1 spring/Kamyshinskaya 3//Kharkovskaya 93		4/90	4/90
Dobrynya	2002	Albidum 28/L-401//Saratovskaya 55/3/L-503	*Lr19*	4/40	4/100
Duet	2003	Erythrospermum 59//Tselinnaya 20/ANK-102	*LrTR* +	0	0
Zhigulevskaya	1984	Bezostaya 1/Bezenchukskaya 98		3/20	4/80
Zhnitsa	1983	Strela/mixture of varieties		4/60	4/90
Zemlyachka	1999	Isheevskaya//Bezostaya 1/Saratovskaya 29/3/ Red River 68		4/80	4/90
Zlata	2009	Ivolga/Prohorovka		4/70	4/70

Iren	1998	Irgina/Krasnoufimskaya 90		4/80	4/80
Isheevskaya	1992	Tr.durum/Tr.aestivum// Albidum 21/3/ Zhigulevskaya		4/60	4/90
Kazanskaya yubileynaya	2004	Omskaya 20/ Lut.204-80-1//Lut.3-86-6		4/80	4/90
Kazakhstanskaya 10	1990	Priboy/Strela		4/70	4/90
Kamyshinskaya 3	1972	Albidum K-19100/Sarrubra		4/80	4/100
Kinelskaya 59	1995	Saratovskaya 35//Lee/Mironovskaya 808	*Lr23/lr23*	4/30	4/90
Kinelskaya 60	1998	Kinelskaya 40/ Nadadores		2-4/5	4/20
Kinelskaya 61	2005	ISWRN-225 / Kutulukskaya // Zavolzhskaya		4/80	4/100
Kinelskaya niva	2007	Tulaykovskaya 1/L-503	*Lr19+ Lr23*	4/25	4/40
Kinelskaya otrada	2009	Tulaykovskaya 1/ K-56395	*Lr23+*	0	0
L 503	1993	Saratovskaya 52/Pysar 29//Saratovskaya 29*6/ Rescue/3/Saratovskaya 46	*Lr19*	0	4/90
L 505	1996	Saratovskaya 55*6/Sonora 64//L-503	*Lr19*	4/20	4/90
Lebedushka	2009	Belyanka/L-1089	*LrBel*	0	0
Lyuba	1988	Minskaya/Leningradka//Bezostaya 1/3/ Moskovskaya 35		4/60	4/80
Lyubava 5	2009	PV-18/Saratovskaya 29//World Seeds 1812/ Saratovskaya 29	*Lr23+ ?*	0	0
Margarita	2008	Krestyanka/Isheevskaya//Simbirka/L.355-83	APR	4/40	4/40
MIS	2003	Trippel/Priokskaya		4/80	4/80
Moskovskaya 35	1975	Minskaya/Bezostaya 1		4/40	4/90
Niva 2	1997	Solo/Kavkaz//Irtyshanka 10		3/10	4/80
Novosibirskaya 15	2003	Bezenchukskaya 98/Irtyshanka 10//Tulunskaya 10/3/ Novosibirskaya 92		4/80	4/80
Novosibirskaya 89	1993	Moskovskaya 21/Saratovskaya 29		4/90	4/90
Omskaya 18	1991	Omskaya 11/Gaines		4/60	4/90
Omskaya 33	2002	L 137-87-39/Omskaya 28		4/90	4/90
Omskaya 35	2004	Omskaya 29/Omskaya 30		4/80	4/80

Omskaya 36	2007			4/80	4/80
Orenburgskaya 13	1993	Pembina/ Albidum 18-73		4/70	4/100
Pamyati Azieva	2000	Saratovskaya 29/3/Irtyshanka 10//Grekum 114/ Kavkaz		4/60	4/90
Pamyati Ryuba	2006	Tertsiya/Erythrospermum 19542	*LrTR*	0	0
Piramida	2000	Kuibyshevskaya 1/Ershovskaya 32	*Lr23+ Lr13*	4/40	4/70
Prohorovka	1996	Omskaya 9/3* Ershovskaya 32 (?)	*Lr26+ ?*	2/1	4/100
Samsar	1994	Saratovskaya 52/Pysar 29//Saratovskaya 29*6/ Rescue/3/Saratovskaya 46	*Lr19*	0	4/70
Saratovskaya 29	1957	Albidum 24/Lutescens 55-11		?	4/90
Saratovskaya 42	1973	Albidum C-1616/Saratovskaya 38		4/80	4/100
Saratovskaya 55	1986	Saratovskaya 29/Saratovskaya 51		4/80	4/100
Saratovskaya 64	2000	Erythrospermum C-1976/Saratovskaya 60		4/80	4/90
Saratovskaya 66	2000	Saratovskaya 46/ Albidum C-1872		4/80	4/100
Saratovskaya 68	2003	Tselinnaya 20/Saratovskaya 60		4/80	4/80
Saratovskaya 70	2002	Albidum C-2015/Leukospermum C-1983		4/80	4/100
Saratovskaya 73	2008	L-2014/Tr.timopheevii		4/40	4/40
Simbirka	1986	Minskaya/Bezostaya 1//Saratovskaya 36		4/80	4/90
Simbirtsit	2007	Krestyanka/Isheevskaya//L-503	APR	4/25	4/25
Tertsiya	1995	ANK-1/ANK-2//ANK-3/3/ANK-7A	*LrTR*	0	0
Timer	2007	Meshinskaya/ Lyuba		4/100	4/100
Tulaykovskaya 10	2003	Albidum 653/Tulaykovskaya 5	*LrAg*	0	0
Tulaykovskaya 100	2007	Albidum 653/Tulaykovskaya 5	*LrAg*	0	0
Tulaykovskaya 5	2001	Erythrospermum 865/Agis 1	*LrAg*	0	0
Tulaykovskaya Zolotistaya	2006	Albidum 653/Tulaykovskaya 5	*LrAg*	0	0
Tulaykovskaya Stepnaya	1998	Hope/Timstein*6// Saratovskaya 29/3/ Saratovskaya 55	*Lr23*	4/40	4/90

Tuleevskaya	2002		?	0	0
Uchitel	2001	Orenburgskaya 1/Tselinogradka//Moskovskaya 35/ Leukurum 51/3/Orenburgskaya 7		4/90	4/100
Favorit	2007	L-2033/Belyanka	*LrBel*	0	0
Fora	1996	Tezanos Pintos Precoz/Carazinho// Siete Cerros 66/3/2*Kinelskaya 30		4/90	4/90
Chelyaba 2	2005	Tezpishar/2*Irtyshanka 10//Tselinnaya 20/ANK-102	*LrTR*	0	0
Ekada 6	2005	Krestyanka/Samsar	*Lr19*	4/60	4/90
Ekada 66	2009	Volzhanka/Hja 21677//Tulaykovskaya Yubileinaya	APR	3-4/15	3-4/15
Ekada 70	2007	Volzhanka/Hja 21677//Tulaykovskaya Yubileinaya	APR	4/25	4/25
Erythrospermum 59	1994	Chaika/Irtyshanka 10		4/40	4/80
Ester	2004	Eta/Line 52-4		4/40	4/40
Yugo-vostochnaya 2	1999	PPG-596/Uralochka/4/Ershovskaya 32/ Rodina//Saratovskaya 46/3/Saratovskaya 55		2/1	4/60
YUV 4	2002	PPG-596/Uralochka/4/Ershovskaya 32/ Rodina //Saratovskaya 46/3/Saratovskaya 55		4/40	4/80
Yuliya	2002	Lutescens 770/Lutescens 29	*Lr19*	4/40	4/100

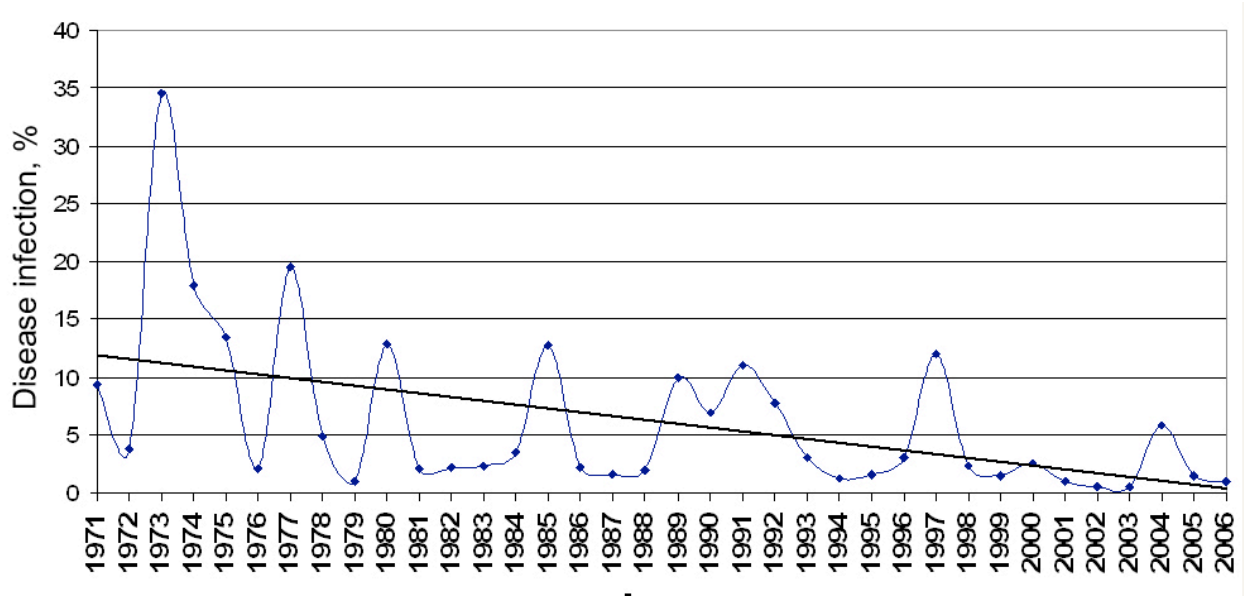
Table 7 Leaf rust reactions of spring wheats released in western Siberia, Omsk region, 2008

Variety	Year of release	Pedigree	Possible genes	Infection, %	Reaction type
		Early maturing			
Pamyati Azieva	2000	Saratovskaya 29/ Lut.99-80-1		85	S
Altayskaya 92	1995	Novosibirskaya 67/ Lut.4029		68	S
Omskaya 32	2001	Almaz/Omskaya 16// Omskaya 18/3/Chris		84	S
Chernyava 13	2001	ANK-17/2*OmGAU-6		73	S
Strada Sibiri	2002	Rang/Hybrid 21// Irtyshanka 10/3/ TR-55p-6628/ Lut.1633-617		58	S
Tuleevskaya	2003			4	R
Novosibirskaya 15	2004	Bezenchukskaya 98/Irtyshanka 10//Tulunskaya 10/3/ Novosibirskaya 92		10	R
Kazanskaya Yubileynaya	2007	Omskaya 20/ Lut.204-80-1//Lut.3-86-6		50	MS
Omskaya 36	2007			40	MR
Katyusha	2008			78	S
Boevchanka	2009	Lut. 70-94/ Lut. 196 -94-6		10	R
		Medium-maturing			
Omskaya 29	1999	Lut.204-80-1/Lut.99-80-1		90	S
Sibakovskaya 3	1980	Bezostaya 1/Saratovskaya 29		86	S
Tertsiya	1996	ANK-1/ANK-2//ANK-3/3/ANK-7A	*LrTR*	4	R
Niva 2	1998	Solo/Kavkaz// Irtyshanka 10		76	S
Rosinka 2	1999	Mutant 797 (Tselinnaya 21)		66	S
Slavyanka Sibiri	2002	Mutant 777 (Lutescens 65)		53	S
Sonata	2005	Tselinnaya 20/Tertsiya	*LrTR*	20	MR
Omskaya 33	2002	Lut.137-87-39/Omskaya 28		10	R
Duet	2004	Erythrospermum 59//Tselinnaya 20/ANK-102	*LrTR*	3	R

Svetlanka	2004	Omskaya 23/Tselinnaya 26		49	MS
		Late-maturing			
Omskaya 35	2005	Omskaya 29/Omskaya 30		60	S
Omskaya 18	1991	Omskaya 11/Gaines		88	S
Omskaya 24	1996	Sibiryachka 8/ Milturum 1578// Krasnodarskaya 39		96	S
Omskaya 28	1997	Omskaya 12/Semidwarf (Canada)+ free polination		73	S
Erythrospermum 59	1994	Chaika/Irtyshanka 10		57	S
Omskaya 37	2009	Lut. 61-89-100 / Lut.350-89-9		4	R
Sibakovskaya Yubileynaya	2010	Lut. 121*2/ANK-102	*LrTR*	8	R

Fig. 1 Wheat production areas in Russia and Ukraine: Region 1 – winter wheat area of Ukraine and European Russia; Region 2 – spring wheat area of European Russia; Region 3 – spring wheat area of Siberia

Fig. 2 Occurrence of leaf rust and the level of infection in Krasnodar region of Russia, 1971-2006



Virulence and molecular characterization of *Puccinia triticina* and *Puccinia graminis* f. sp. *tritici* populations in Russia (abstract)

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The structures of *Puccinia triticina* (*Pt*) populations were studied in the Central, Central-Chernozem, North Caucasian, Middle Volga, Low Volga, West Siberian and Northwest regions of Russia. Virulence analyses of *Puccinia graminis* f. sp. *tritici* (*Pgt*) in central Russia were also conducted. Using the North American leaf rust race notation, the prevailing *Pt* races were MBTT, MGTT, PBKT, PBTT, PGTT, TBTT, TCTT and THTT. Lines with *Lr3a, Lr3bg, Lr11, Lr17a, Lr18, Lr30, Lr3* and *LrB* were susceptible to all isolates. Virulence to lines with genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3ka, Lr10, Lr14a, Lr14b, Lr15, Lr16, Lr19, Lr20, Lr23, Lr25, Lr26, Lr27+Lr31, Lr28, Lr32, Lr36, Lr39, Lr40, Lr44,* and *Lr46* varied and depended on years, regions and host cultivars. Lines with *Lr9, Lr24, Lr29, Lr38, Lr41, Lr42, Lr45* and *Lr47* were effective against all isolates. Molecular analyses (RAPDs) revealed a high degree of genetic variability in *P. triticina* populations between West-Siberia, East-Siberia and Volga-Vyatka, but the polymorphisms did not necessarily explain variation in pathogenicity. The stem rust pathogen in the Central region was characterized by relatively low pathogenic variability with prevalent races TKNTF, TKSTF and TKNTC and high RAPD diversity based on host genotype.

International surveillance of wheat rust pathogens - progress and challenges

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Abstract Surveillance of wheat rust pathogens, including assessments of rust incidence and virulence characterization via either trap plots or race (pathotype) surveys, has provided information fundamental in formulating and adopting appropriate national and international policies, investments and strategies in plant protection, plant breeding, seed systems, and in rust pathogen research. Despite many successes from national and regional co-ordination of rust surveillance, few attempts were made to extend rust surveillance to international or even global levels. The Global Cereal Rust Monitoring System was established to address this deficiency. It is underpinned by an information platform that includes standardized protocols for methods and systems used in surveys, preliminary virulence testing, data, sample transmission and management at the field and national and global levels, and includes two web-based visualization tools. While considerable progress has been made towards a global system for monitoring variability in the wheat stem rust pathogen, and linking this to the threat posed by this pathogen to regional wheat production, some challenges remain, including ongoing commitment to support rust surveillance, and the ability to share and compare surveillance data.

Keywords pathotype, *Puccinia,* race, surveys, *Triticum aestivum*

Introduction

Following the demonstration of races (pathotypes) in the wheat stem rust pathogen in the early 1900s (Stakman and Piemeisel 1917), many countries established pathotype surveys, and/or networks of trap plots to assess the presence/absence of rusts and in some cases to provide information on the occurrence of virulence for specific resistance genes (e.g. Wellings et al. 2009). These rust surveillance efforts provide knowledge of the distribution and incidence of rust pathogens and pathotypes, and in regions where there is an understanding of deployed resistance genes, the potential impact of pathotypes on wheat production. This information is fundamental in formulating and adopting appropriate national and international policies, investments and strategies in plant protection, plant breeding, seed systems, and in rust pathogen research.

The genetic control of rusts has had considerable success in regions where pathotype surveys are closely integrated with pre-breeding and breeding efforts targeting rust resistance, and with post-release management of wheat cultivars (Park et al. 2009). This is particularly so in Australia, which along with New Zealand, is known to represent a single (Australasian) rust epidemiological zone (Luig 1983). Long-term monitoring of pathogenic variability in wheat rust pathogens including *Puccinia graminis* f. sp. *tritici* (*Pgt*) across the Australasian region has shown clearly rapid and unimpeded rust migration within this region, and provided arguably the best evidence supporting

periodic long-distance intercontinental spread of wheat rust pathogens (Watson and de Sousa 1982; Wellings 2007). The geographic isolation of Australia from other wheat growing areas and its political uniformity permitted a nationally co-ordinated effort in rust control, led by the (currently) Australian Cereal Rust Control Program.

While regional co-ordination of wheat rust surveillance has functioned well in some cases, there have been few attempts to extend this to the international or even global levels. At the First International Congress of Plant Pathology in London, 1968, a resolution was passed recommending that global surveys of several plant pathogens, including *Pgt*, be undertaken to determine regional differences in virulence. Luig (1983) published the results of an international virulence gene survey for *Pgt* that involved 18 countries over 3 years, and interestingly identified the two initial problems in this work as being "the lack of an accepted up-to-date classification system dealing with variation in *Pgt* which was applicable throughout the world", and "our inability to introduce rust cultures from other continents into our laboratory...". Both of these problems, but particularly the second, continue to impede global co-ordination of cereal rust surveillance.

The Global Cereal Rust Monitoring System (GCRMS)

The existence of evidence supporting intercontinental spread of rust pathogens reinforces the need to couple efforts on improving understanding and capacity in pathotype analysis with a system that ensures rapid and free sharing of international information. Similar challenges were faced in establishing an international system for desert locust monitoring, which was successfully achieved by developing the Desert Locust Information Service (DLIS), located within the FAO's Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES) (Hodson et al. 2009). The long-term success of DLIS and ability of FAO to enter into dialogue with UN-member countries was therefore an excellent framework within which to develop an international rust surveillance system.

A comprehensive outline of the GCRMS was provided by Hodson et al. (2009). It is underpinned by an information platform that includes standardized protocols for methods and systems used in surveys, preliminary virulence testing, data, sample transmission and management at the field, national and global levels. In order to provide access to stem rust information in a timely fashion, two web-based visualization tools were developed that are linked to a centralized database and released in the public domain: *Rustmapper* (http://www.cimmyt.org/gis/RustMapper/index.htm), a networked Google Earth application and *RustMapper Web* (http://www.cimmyt.org/gis/rustmapper/RustMapper_Web.html), a browser-based tool. Both tools incorporate updated stem rust survey data, near-real time wind trajectories, country level germplasm susceptibility estimates and distribution of major wheat growing areas. Both the tools and database are updated on a routine basis, hence delivering the most recent information relating to stem rust in a timely manner.

Success of the GCRMS is dependent on the international exchange of quality information. To assist in this, four workshops involving training in cereal rust survey (including sampling) techniques were held (Syria, Central Asia, India and Egypt), at which GPS units were distributed to ensure geo-referencing of survey data. By the end of 2009, data from over 3000 survey sites were incorporated into a centralized database, with 15 countries regularly submitting standardized field data. Stem rust pathotype summaries from 14 countries in 2008 provided an initial baseline dataset. These achievements represent significant progress towards the establishment of the foundations for an operational GCRMS.

Variability in rust pathogens and the "Ug99" lineage

The detection of virulences for the stem rust resistant wheat cultivars Eureka (*Sr6*), Gabo (*Sr11*), Spica (*Sr17*) and Festival (*Sr9b*) soon after their releases in Australia was attributed by Waterhouse (1952) and Watson (1958) to independent mutational changes in *Pgt* that resulted in the pathogen acquiring virulence for each resistance gene. Although the precise molecular basis of this process in

Pgt remains unknown, it is generally accepted that mutation to virulence occurs in nature and that most new pathotypes identified in Australia over the past 80 years, for example, were generated by this process. This is consistent with experiments under controlled greenhouse conditions that demonstrated the acquisition of virulence for resistance genes following exposure to the chemical mutagen ethyl methane sulphonate (EMS) (Luig 1978). Long term studies of pathogenic variation in *Pgt* (90+ years; Watson 1981; Park 2007), *P. triticina* (90+ years; Park et al. 1995) and of *P. striiformis* f. sp. *tritici* (30+ years; Wellings 2007) provide the best evidence of clonal lineages comprising closely related pathotypes in wheat rust pathogen populations, derived from periodic incursions of exotic pathotypes acting as "founding ancestors" that in time, underwent sequential mutations in genes conferring pathogenicity.

Similar results are now being obtained with what has become known as the "Ug99" lineage, comprising at least seven pathotypes that differ for virulence on resistance genes *Sr21*, *Sr24*, *Sr31* and *Sr36* (Table 1, Fig. 1; Jin et al. 2008, 2009; Pretorius et al. 2010). Studies using microsatellite markers showed that most of these pathotypes have identical fingerprints, consistent with them having arisen from a common ancestor via single-step mutation (Pretorius et al. 2010). Significantly, surveys in Turkey, Egypt, Pakistan and India over recent years have failed to detect any of these pathotypes, and three *Pgt* isolates collected from Pakistan in 2009 were clearly shown to differ from pathotype TTKSK in their SSR fingerprint (Karaoglu and Park unpublished) and virulence profile (Fetch unpublished). Regular and ongoing monitoring of *Pgt* pathotypes is considered to be of vital importance.

TABLE 1 HERE
FIGURE 1 HERE

Wheat has been grown in Kenya since the beginning of the 20th Century (Martens 1975), and although all three rusts caused considerable damage to susceptible cultivars, stem rust was identified as historically the main threat by Luig (1983). Pathotype identification in *Pgt* in Kenya began in 1928 (Luig 1983), with detailed published accounts available of analyses made in 1968 (Green et al. 1970) and 1969 and 1970 (Harder et al. 1972). Martens (1975) examined virulence dynamics in *Pgt* in Kenya over the 14 year period 1957-1971, and concluded that "virulence in the pathogen, relative to the host, has not changed as rapidly as the literature suggests".

"Ug99" is avirulent for *Sr28* (Singh et al. 2008), present in the stem rust differential Kota, which also carries *Sr7b* and *Sr16*. Virulence analyses of isolates of *Pgt* from many countries by Huerta-Espino (1992) reported significant levels of avirulence for *Sr28* among isolates from Ethiopia and Nepal, but in other regions, virulence for this gene was common. Using the nomenclature of Stakman et al. (1962), pathotypes TTKSK, TTKSF, TTKSP, TTKST and TTTSK all key out to standard race 218, and pathotypes PTKSK and PTKST as standard race 143. Harder et al. (1972) reported five races as avirulent on Kota, one of which was standard race 143 (aka EA17) isolated on one occasion from Tanzania in 1970. Although it is impossible to know if this or other pathotypes identified in this work are the progenitors of "Ug99", it is plausible that the "Ug99" lineage has been present in eastern Africa since at least the early 1970s and that pathotype TTKSK was derived via sequential acquisition of virulence over the intervening years. However, while previous studies suggested that barberry was non-functional in the life-cycle of *Pgt* in Kenya (Guthrie 1966, cited by Green et al. 1970), recent reports of aecial infections on this host in Kenya (Jin unpublished) could mean that sexual recombination in *Pgt* may occur there. Interestingly, reports of aecial infections of barberry have also been made from New Zealand (Waipara et al. 2005), and Azerbaijan (Nazari unpublished), Georgia (Sikharulidze unpublished), India (Prashar unpublished), Iran (Nazari, unpublished), Kazhakstan (Park unpublished), Nepal (Thapa pers comm) and Turkey (Mert pers comm. These observations suggest that sexual recombination within *P. graminis* may not be rare; however, further work is needed to determine the pathogen species, or *forma specialis*, present on

barberry in these countries are, especially in view of the recent finding that barberry is also the alternate host of *P. striiformis* (Jin et al. 2010).

Challenges in global rust pathogen surveillance

Despite the progress that has been made in establishing the GCRMS to date, many challenges remain. The incidence of rust pathogens varies between years, making it difficult to sustain funding for ongoing surveillance. A global decline in the incidence of stem rust over the past 40 years, attributable at least in part to resistance breeding and the widespread deployment of the resistance gene *Sr31*, led to many countries abandoning stem rust surveillance and an alarming reduction in the global skill base in rust race analysis and general rust pathology. The lack of capacity and resources to undertake rust surveillance and monitoring are a major challenge being faced in establishing the GCRMS, as are barriers limiting the exchange and free-flow of surveillance information and rust isolates.

Building capacity for in-country race analysis

Biosecurity concerns have and will continue to make the exchange of rust isolates between countries difficult, meaning that comprehensive pathotype analyses will in most cases require building in-country capacity. Declining support to agriculture has left many, but not all, national systems without the resources or capacity to undertake even cereal rust field surveys on a regular basis, let alone implement race analysis. A final area of concern relates to the in-country / regional capacity and the will to undertake reliable and routine pathotype analysis. Considerable progress has been made in some countries, but a sustained effort over a long period is required to achieve the overall goal. Developing in-country capacity for rust pathotype analysis at its simplest involves equipping scientists with the required skills and ensuring the provision of adequate infrastructure. Establishing a Global Reference Center for stem rust, as has already been done for stripe rust (Hovmoller et al. 2009), will overcome these problems to some extent, but in-country capacity for pathotype analysis will still be needed because it is expected that such a center would not be able to serve all countries in which rusts are an important constraints in wheat production. Developing capacity for in-country race analysis is challenging because of a lack of commitment by some countries both in providing ongoing infrastructure and personnel (particularly problematic when rust incidence is low), the time taken to train scientists in race analysis (at the very least 3 months), and the promotion or movement of trained personnel into other areas. Successful ongoing pathotype analysis is usually typified by long-term commitment of the scientists undertaking such research. For example, in Australia and North America some scientists have been doing this work for in excess of 20 years.

Sharing information

The GCRMS can only be successful with broad agreement on the timely sharing of information on the incidence of rusts and the pathotypes present. Political sensitivity, especially with respect to the "Ug99" lineage of *Pgt*, is one factor limiting the free exchange of information. The role of FAO as an international neutral broker with the ability to assist member countries over potentially sensitive rust issues is considered to be vital, and advances have already been made in this regard.

Comparing information

The appearance of *Yr9* virulence, initially in east Africa and then progressively throughout Asia in the 1990s, suggests that much of this region comprises a single rust epidemiological unit (Singh et al. 2006). However, very little information concerning the *Pst* pathotype(s) present in the region at that time is available and as convincing as the hypothesis of migration of a single *Yr9*-virulent pathotype may seem, supporting scientific evidence is lacking. Where information on pathotypes actually present is available, disparities in the differential genotypes, and to a lesser extent, the pathotype

nomenclature systems used by laboratories undertaking pathotype analyses, have made precise comparisons of pathogen variability between regions difficult. For example, the origins of 11 exotic wheat rust isolates identified in Australia since 1925 are largely unknown (Table 2), as are most entry pathways. These knowledge gaps reinforce the importance of obtaining and sharing information that can be compared if long-distance spread of rust pathotypes is to be tracked. It must be stressed, however, that two isolates of a given pathotype may not in fact be the same genotype. For example, three isolates of *Pgt* pathotype 21-0 isolated from Australia in 1954, 1994 and 2006, were shown to have different SSR fingerprints (Zhang, Park and Karaoglu unpublished).

Despite inconsistencies in the differential genotypes being used by laboratories involved in pathotype analyses, the data that emerged from recent stem rust surveillance in east Africa and beyond has already provided valuable information for stem rust control efforts in identifying countries where members of the "Ug99" lineage are present, as well as providing useful insight into migration of rust pathogens. The utility of DNA-based marker systems such as SSRs in confirming the genetic relatedness of *Pgt* isolates has also been clearly established in this process (e.g. Visser et al. these proceedings). A uniform set of differential genotypes and one system for naming pathotypes would add significant precision to international efforts to track rust migration. The difficulties in achieving this were addressed by several rust workers (e.g. McIntosh et al. 1995; Fetch et al. 2009; Pretorius and Nazari 2009). Without a standard set of differential genotypes and agreement on analytical procedures, it is not possible to have a single pathotype nomenclature system.

Differential genotypes For pathotype information to be relevant to local wheat breeding programs, the differential genotypes used must include lines carrying deployed resistance genes, which are often different between regions. Because of this, and the impracticality of including all known resistance genes in a differential set, there will always be regional differences in the genotypes used to fully characterise the pathogenicity of rust isolates. McIntosh et al. (1995) proposed the use of an internationally agreed set of differentials for use in the international exchange of information, to which researchers could add local differential testers. This issue was further addressed by Fetch et al. (2009), who proposed adopting 20 standard differential genotypes, which carry the 20 stem rust resistance genes currently used in North America to characterise pathotypes of *Pgt*. If agreement is reached on a core set of international genotypes for pathotype identification, ensuring the purity of each will be of paramount importance.

Pathotype nomenclature Providing a simple name for a new rust pathotype that conveys something of its features is important in getting a clear message across to the wider community. For example, in 1968, influenza type A strain H3N2 killed about 40,000 people worldwide. The virus was commonly referred to as the "Hong Kong flu", in the same way as we have referred to pathotype TTKSK as "Ug99". The latter term has been used widely in the press, probably because it is easily remembered and conveys the origin and year of description of rust pathotype TTKSK. Similarly in Australia, new pathotypes have at times been named after the cultivar first affected, or most affected, to assist in extension. For example, a new pathotype of stripe rust with virulence on triticale, first detected in 2008, was called the "Jackie" pathotype because of its virulence on cv. "Jackie" (Wellings unpublished). The identity of the resistance gene(s) in "Jackie" for which this pathotype is virulent is not known.

In addition to proposing 20 standard international differential testers, Fetch et al. (2009) suggested adopting the North American system of naming pathotypes of *Pgt*, which uses alpha characters and was originally proposed by Roelfs and Martens (1988). An advantage of this system is that it condenses a great deal of information into a concise "code", as does the octal system originally proposed by Gilmour (1973). The brevity of both of these systems however make it very difficult to infer evolutionary relationships between pathotypes, and impossible to convey information about features such as intermediate levels of virulence (Watson and Luig 1968). Should the North American code system be accepted in international exchange of information on rust pathotypes, virulence/avirulence formulae should also be provided to allow firstly communication of virulence for resistance genes that may only be present in local differential testers, and secondly in simplifying the ability of non-specialists to assess the threat of pathotypes to commercial wheat cultivars.

Making it work – surveillance and rust control

Where pathotype surveys have been conducted in a robust and relevant way, they have provided both information and pathogen isolates that have 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.

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, together with periodic long-distance migration events. Reaching global agreement on the composition of a core set of differential testers for stem rust should make it easier to track rust migration over large distances, improving our understanding of how these pathogens move around. It should be noted however that two isolates identified as the same pathotype may in fact be different genotypes, and because of this, DNA-based genome profiling of isolates will be an important part of global rust surveillance. Combined rigorous DNA fingerprinting coupled to virulence phenotyping has already proved useful in the detection of global dispersal events for *Pst* (Hovmoller et al. 2008).

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Table 1 Wheat stem rust pathotypes identified within the "Ug99" lineage

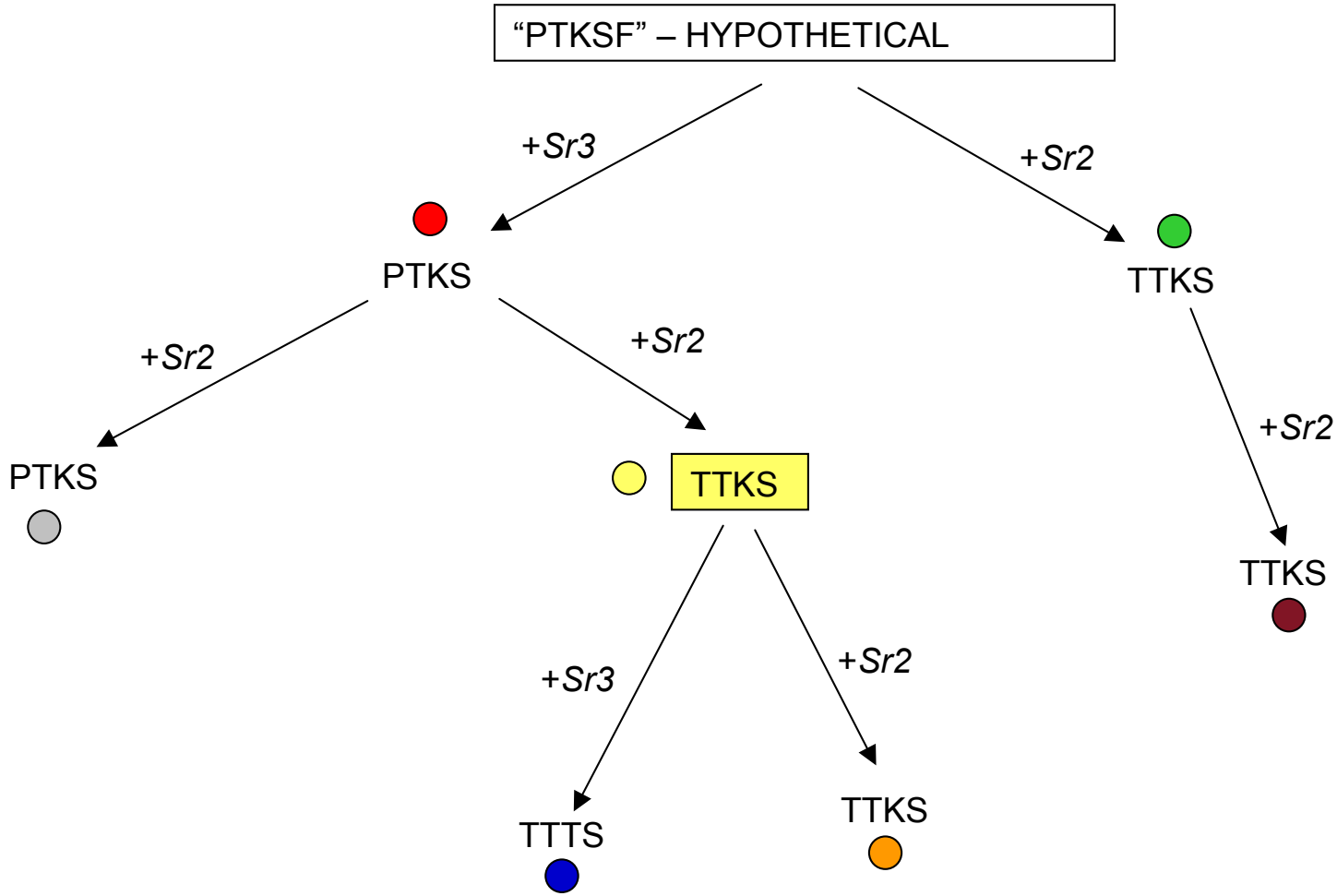
		Differs from "Ug99" (TTKSK)		
Pathotype[a]	Standard race[b]	Virulence	Avirulence	Known geographic distribution
PTKSK	143		*Sr21*	Kenya, Ethiopia
PTKST	143	*Sr24*	*Sr21*	Kenya, South Africa
TTKSK	218	-	-	Uganda, Kenya, Ethiopia, Sudan, Yemen, Iran
TTKSF	218	-	*Sr31*	South Africa, Zimbabwe
TTKSP	218	*Sr24*	*Sr31*	South Africa
TTKST	218	*Sr24*	-	Kenya
TTTSK	218	*Sr36*	-	Kenya

[a]According to the North American system for pathotype designation in *P. graminis* f. sp. *tritici* (Jin et al. 2008)
[b]According to Stakman et al. (1962)

Table 2 Documented incursions of exotic isolates of wheat rust pathogens into Australia from 1925 to 2005

Disease/Pathogen	Year detected	Possible origin	Reference
1. Wheat stem rust/*Puccinia graminis*	1925	?	Waterhouse (1952)
2. Wheat stem rust/*P. graminis*	1945	Africa?	Luig (1977)
3. Wheat stem rust/*P. graminis*	1969	Africa?	Watson and de Sousa (1982)
4. Wheat stem rust/*P. graminis*	1969	Africa?	Watson and de Sousa (1982)
5. Wheat stripe rust/*P. striiformis*	1979	France?	Wellings and McIntosh (1990)
6. Wheat leaf rust/*P. triticina*	1981	New Zealand	Luig et al. (1985)
7. Wheat leaf rust/*P. triticina*	1984	?	Park et al. (1995)
8. Wheat leaf rust/*P. triticina*	1996	New Zealand	Park (unpublished)
9. Wheat stripe rust/*P. striiformis*	2002	USA?	Wellings et al. (2003)
10. Wheat leaf rust/*P. triticina*	2004	?	Park (unpublished)
11. Wheat leaf rust/*P. triticina*	2005	?	Park (unpublished)

Fig. 1 Putative evolutionary pathways for the development of known pathotypes of *P. graminis* f. sp. *tritici* within the "Ug99" lineage



Improving wheat stripe rust resistance in Central Asia and the Caucasus (abstract)

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Wheat is the most important cereal in Central Asia (Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan) and the Caucasus (Armenia, Azerbaijan and Georgia). Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* is considered the most important disease of wheat in Central Asia and the Caucasus (CAC). Although stripe rust has been present in the region for long time, it has become a serious constraint to wheat production in the past 10 years. This is reflected by the occurrence of four epidemics of stripe rust in the CAC region since 1999, the most recent in 2009. Several wheat varieties occupying substantial areas are either susceptible to stripe rust or possess a low level of resistance. Information on the stripe rust pathogen in terms of prevalent races and epidemiology is not readily available. Furthermore, there is an insufficient understanding of effective stripe rust resistance genes in the region, and little is known about the resistance genes present in the commercial varieties and advanced breeding lines. The deployment of resistant varieties is further complicated by putative changes in virulence in the pathogen population in different parts of the CAC. Many improved wheat lines received through international nurseries or other exchange programs have shown high levels of resistance to stripe rust, leaf rust and powdery mildew to local pathogen populations. It is anticipated that this germplasm will play an important role in developing stripe rust resistant wheat varieties either through direct adoption or using them as parents in breeding programs.

Global status of stripe rust

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Abstract Stripe rust, caused by *Puccinia striiformis*, has been an important disease of wheat, barley, rye, triticale and certain graminaceous hosts for centuries. The significance of the disease on cultivated cereals has waxed and waned according to the vagaries of climate, inoculum levels and susceptible varieties. A progressive understanding of pathogen biology has revealed levels of specialisation between and within host groups, and these had varying impacts on the hosts concerned. The most economically important form is *P. striiformis* f. sp. *tritici* (*Pst*), the causal pathogen of stripe (yellow) rust of wheat, which is the major focus of this paper. The recent discovery of the perfect stage of *Pst* on *Berberis* spp. will encourage further work to uncover the potential importance of the sexual stage in pathogen biology in regions where *Berberis* spp. occur. A review of the evolution of pathotypes within *Pst* over the past 50 years reveals recurrent pandemics emanating from a combination of specific virulence in the pathogen population, wide scale cultivation of genetically similar varieties, and agronomic practices that led to high yield potential. When these factors operate in concert, regional stripe rust epidemics have proven to be dramatic, extensive and serious in terms of the magnitude of losses and the economic hardships endured. A review of these epidemics suggests that little progress has been made in containing the worst effects of epidemics.

The current status of stripe rust was gauged from a survey of 25 pathologists and breeders directly associated with the disease. It was evident that *Pst* remains a significant threat in the majority of wheat growing regions of the world with potential to inflict regular regional crop losses ranging from 0.1 to 5%, with rare events giving losses of 5 to 25%. Regions with current vulnerability include the USA (particularly Pacific North West), East Asia (China north-west and south-west), South Asia (Nepal), Oceania (Australia) and East Africa (Kenya).

The resources deployed to contain the worst effects of *Pst* will need to find a balance between training a new generation of breeders and pathologists in host-pathogen genetics, and an investment in infrastructure in IARCs and NARs.

Keywords international status, pathogen variability, *Puccinia striiformis*, yellow rust, wheat

Introduction

It is a long and widely held view that stripe rust (syn. yellow rust), caused by *Puccinia striiformis* West. (*Ps*), is a prevalent but sporadic disease of temperate cereals. Biffen (1931) noted that even an "intense attack" failed to destroy crops in any comparable manner to the "killing power" of stem rust. However history informs us that stripe rust epidemics have been significant in certain geographic regions and the losses incurred required serious financial outlays to address disease control and crop loss mitigation. Several reviews deal with the range of biological issues of importance in understanding the regional nature and distribution of *Ps*, including Zadoks (1961), Stubbs (1985), Line (2002), Chen (2005) and Wellings (2007). This paper will consider some of the regional milestones that characterize the economic significance of the disease, and then attempt to place the current status of stripe rust in a global context. Emphasis is given to wheat stripe rust (caused by *Puccinia striiformis* f. sp. *tritici, Pst*) and the remaining *formae speciales* will be given less consideration.

Historical impact of stripe rust

Rust caused great concern to cereal cropping enterprises since the earliest recorded history. References to rust epidemics are found in the Mosaic books of the Old Testament and in the writings of early scholars (Large 1940). Epidemics reported in England in 1725 and Sweden in 1794 may have been stripe rust (Chester 1946).

The principle outcome of stripe rust epidemics is reduced yield and quality of grain. Early studies undertaken by Bever (1937) in greenhouse experiments using a susceptible spring wheat cultivar demonstrated yield losses as high as 65%. The extent of the losses were measured in reduced dry matter production, root growth, plant height, size and number of flowering spikes, and the size and number of grains. These effects were more pronounced with infection beginning at the seedling stage, although infections initiated at anthesis were also associated with reduced root weight and grain yield. Crop loss studies since this early work have essentially served to confirm the magnitude of losses and the physiological mechanisms determining reductions in yield potential.

Infection of spikes by *Pst* is a symptom expression of great concern to growers, and yet has received relatively little attention. Early work by Purdy and Allan (1963) established yield losses of 20% due to spike infection among cultivars resistant to foliar infection. Surveys by Cromey (1989) in New Zealand demonstrated mean grain weight losses of 11%, and noted that the wide range of losses in commercial fields appeared to be related to the timing of infection and the duration of moisture over the flowering period.

Epidemics of national and regional significance

An historical snapshot of the episodic nature of stripe rust epidemics and associated crop losses across various regions of the world is given in Table 1. These examples are not exhaustive, but simply illustrate the significant losses that have occurred in certain regions. Stripe rust epidemics have typically been the outcome of classic interactions between vulnerable host materials, conducive environmental conditions and sufficient quantities of pathogen inoculum. Several examples illustrate these principles.

TABLE 1 HERE

The stripe rust pandemics reported in the 1970s were often associated with the failure of Siete Cerros and related cultivars over a wide area, including North Africa, the Indian Subcontinent, Middle East, east African highlands and China (Saari and Prescott 1985). It is presumed, although not definitively proven, that an important basis of these epidemics was the failure of *Yr2* that is known to be present in the varieties most affected (McIntosh 2009).

The occurrence of virulence for *Yr9* in East Africa and the subsequent migration of this pathotype north and east through the majority of wheat producing regions in the Middle East (Syria, Turkey, Iran) and the Indian Sub continent (Pakistan, India) caused considerable crop damage in the 1990s (Singh et al. 2004). The origins of this series of pandemics is widely considered to be mutation to virulence for *Yr9* arising in the Red Sea region (Ethiopia, Yemen, Eritrea) as early as 1987 (Louwers et al. 1992). However, these authors cautioned that independent single mutational changes in existing pathotypes in Syria and Turkey may also have led to the same pathotype. The situation became exacerbated by the deployment of wheats carrying *Yr9*, notably Seri 82 and derivatives, over large areas in these regions. Similar, although independent, events occurred in China with up to 80% of released cultivars carrying *Yr9* in the late 1980s (Wan et al. 2004), with the result that virulence for *Yr9* in 1985 laid the foundation for the historical epidemic in 1989-1990 (Chen et al. 2009).

Rusts have been significant limiting constraints for cereal cropping in Australia since European colonization. *Pst* was recorded in Australia for the first time in 1979 and caused significant problems throughout the eastern wheat belt in the mid-1980s (Wellings 2007). The failure of the *YrA* resistance in 1981 caused a sudden localized epidemic in certain areas where this gene was deployed in cultivars which unknowingly had minimal supporting adult plant resistance (Wellings et al. 1988). The recent severe epidemics throughout eastern Australia since 2003 were the result of a new

pathotype incursion originating in Western Australia in 2002 (Wellings et al. 2003). New incursions are by nature unpredictable, and a large proportion of contemporary cultivars were vulnerable to this new pathotype. This led to an increased role for chemical control in Australia, and from 2003-2006 there was an estimated $40-90 million annual expenditure in fungicide applications (Wellings 2007).

Current status of stripe rust

Life cycle

Stubbs (1985) surveyed early attempts, dating from Eriksson and Henning's work in 1894, which failed to establish a role for presumed candidate genera *Berberis, Valerianella* and *Mahonia* as alternate hosts. The microcyclic life cycle of *Pst* remained dogma for more than a century. The recent discovery of the sexual host of *Pst* (Jin et al. 2010) was greeted with great surprise and clearly resolved an issue that eluded generations of researchers. Although the nature and extent of sexual recombination in *Pst* in nature remains to be determined, the compelling evidence for recombination based on molecular and virulence diversity reported in certain regions such as China (Enjalbert 2009; Duan et al. 2010) may now be explained by a sexual phase in the life cycle.

International effort will now be directed to *Berberis* species during surveys in regions of interest in order to confirm the role of the host in nature. The host species reported, in particular *Berberis chinensis* (Caucasus barberry), are likely to be distributed in stripe rust prone regions. Jin (these proceedings) predicts that more *Pst* susceptible *Berberis* species are likely to be discovered.

The role of *Berberis* species will include not only the potential for virulence recombination, but also a potentially important means of pathogen survival between cropping seasons. In this respect, it is of interest to note the observation of short dormancy periods in teliospores and rapid production of basidiospores. On this basis Rapilly (1979) predicted that should an alternate host be discovered it would likely play a minor role as disease escape would be frequent in situations where basidiospores are quickly exhausted.

Pathogen nomenclature

Wheat stripe rust (*Puccinia striiformis* f. sp. *tritici* [*Pst*])
Although the predominant host of *Pst* is wheat, a noticeable increase in frequency of isolates recovered from wild *Hordeum* spp. stimulated an investigation of the evolutionary development of *Pst* on this host in Australia. Observations indicated that isolates of standard *Pst* pathotypes showed differential variation on clones of *H. glaucum* and *H. leporinum* (Wellings et al. 2000a). However, it was concluded that pathotype evolution within *Pst* on the weedy *Hordeum* species was independent from, and therefore likely to have little impact on, that occurring on wheat. It was also concluded that while *Pst* can cause stripe rust on rare genotypes of cultivated barley, it was not a threat to production (Wellings 2007).

Barley stripe rust (*P. striiformis* f. sp. *hordei* [*Psh*])
Isolates of the stripe rust pathogen that occur on cultivated barley, were described as *Psh* by European workers in the late nineteenth century. Barley stripe rust caused significant problems in winter barley production, particularly in the UK and The Netherlands in the 1960s (RW Stubbs pers com). The introduction and spread of *Psh* race 24 in Colombia in 1975, and its adaptation and dispersal throughout South America in the 1980s caused crop losses approaching 70% in some regions (Dubin and Stubbs 1986). The disease subsequently spread northwards into Mexico, Texas and western USA resulting in seasonal epidemics and significant economic losses (Marshall and Sutton 1995). *Psh* remains a pathogen of sporadic occurrence and severity, notably in certain seasons in UK, Russia and Europe (Macer 1972); North and South America, Middle East, South Asia (India) and East Africa (Stubbs 1985). A collection of stripe rust from barley stubble in northern Kazakhstan (Wellings 2009 unpublished) may represent an extremity of the geographical range of this pathogen, although the

identity of the isolate remains under investigation (Hovmoller pers comm). Reviews of barley stripe rust in a North American context can be sourced from Brown et al. (2001) and Line (2002).

Barley grass stripe rust (*P. striiformis* f. sp. *pseudo-hordei* [*Psp-h*])
A new form of *P. striiformis* was described in Australia by Wellings et al. (2000a). This form of the stripe rust pathogen first occurred on grassy species of *Hordeum* including the naturalised barley grass communities in eastern Australia. *Psp-h* causes disease on certain barley cultivars and barley breeding lines (Wellings et al. 2000b), and showed very low infection types on all wheat differential testers with the exception of Chinese 166 (*Yr1*). Contrasting isozyme loci and unique molecular phenotypes compared to Australian *Pst* pathotypes (Keiper et al. 2003) provided evidence for a unique *forma specialis* within *P. striiformis* (Wellings 2007). Recent evidence using fingerprinting markers implied that *Psp-h* shares genetic features with *Pst* (Loladze and Karaoglu unpublished). The international distribution of this form remains unclear. McIntosh (2009) predicted from personal observations that *Psp-h* is likely to be common in South America, especially Chile, and also in California, but the actual identities were not confirmed in greenhouse or laboratory studies. The US *Pst* pathotype PST-21, which seems to be very similar in virulence/avirulence phenotype to *Psp-h*, as well as being a constant outlier in molecular studies, was recorded mostly from California (Line 2002; Chen 2005).

Cocksfoot stripe rust (*P. striiformis* f. sp. *dactylidis* [*Psd*])
Stripe rust infecting cocksfoot (*Dactylis glomerata*) was described by Manners (1960) as a form with distinctive urediniospore dimensions compared to *Pst.* On this basis it was described as a variety of *Ps*, viz. *P. striiformis* var. *dactylidis*, and by implication *Pst* became *P. striiformis* var. *striiformis.* These distinctions were not widely accepted. Recent molecular fingerprinting suggests that *Psd* diverges significantly from other *Ps* variants in Australia, and may be a distinct species (Loladse, Karaoglu and Wellings unpublished). The global distribution of *Psd* remains uncertain, although it has not been recorded in Western Australia. Pasture improvement programs have focussed on the breeding and selection of *Dactylis glomerata* in Australia's eastern states, and released varieties are currently being evaluated for possible vulnerability to *Psd*.

Stripe rust on Kentucky bluegrass (*P. striiformis* f. sp. *poae* [*Psp*])
Psp was described by Tollenaar (1967) as the pathogen causing stripe rust of Kentucky bluegrass (*Poa pratensis*) in the USA. Temperature optima for urediniospore germination (12-18°C) and the close association between pathogen isolates and the host suggest that this is a distinctive *forma specialis*. The disease is noted to be widespread in western USA (Chen 2007) although the geographic distribution of *Psp* outside the USA remains unclear. Interestingly, it was the observation of stripe rust on *Poa pratensis* following random inoculation with aeciospores collected from *Berberis* spp. that gave the first suggestion of a sexual host for *P. striiformis* (Jin et al. 2010).

Host resistance

Efforts to contain stripe rust have largely focused on breeding for resistance. A review of early literature, including an accessible account of the substantial contributions of German researchers, was provided by Robbelen and Sharp (1978). Progressive genetic studies led to the description and characterisation of resistance genes (e.g. McIntosh et al. 1995; Singh et al. 2004; Boyd 2005; McIntosh et al. 2008), many of which are available in genetic stocks for use in research and breeding programs. Molecular markers associated with nominated resistance genes were summarized by Singh et al. (2004). Minor gene resistances, including QTLs and associated molecular markers, were reviewed by Singh et al. (2004) and Boyd (2005). This paper will not attempt to address the detailed status of genetics and breeding for resistance to stripe rust, and the reader is directed to the well documented literature.

Pathogen evolution

Studies of pathogenic variation in *Pst* trace back to the early work in the 1930s by Gassner and Straib (1932) in Germany. The development of differential host testers was driven historically by interests in pathogen population studies, epidemiology and breeding for resistance. An attempt was made to establish an international system of pathotype nomenclature (Johnson et al. 1972). This was adopted by Stubbs' group in The Netherlands who reported variability in European *Pst* populations and received support to conduct analyses of pathogen isolates internationally. Australian studies were also based on this set, although additions became necessary in order to address important pathogen variability related to commercial cultivars (Wellings and McIntosh 1990). A similar nomenclature and methodology is employed in India (Prashar et al. 2007), Middle East (Yahyaoui et al. 2002) and South Africa (Boshoff et al. 2002). However, current stripe rust research groups in UK, Denmark, France, USA, China, and Mexico use differential sets of varying composition and apply differing methodologies for designating pathotypes. Clearly a unified system for international pathotype designation remains a distant hope.

Despite differences in approaches to differential sets, international colleagues have monitored *Pst* populations over many years and regions. A summary of regional developments in pathogen evolution from the 1970s was provided in Wellings et al. (2009). A detailed review of this work is beyond the scope of this paper, although several common themes are worthy of comment:

1. Long term regional and continental studies consistently reveal the close relatedness of newly emerged pathotypes with pre-exisiting pathotypes. The evident clonal nature of *Pst* populations leads to the conclusion that single step mutation remains the predominant cause of variability (Justesen et al. 2002; Chen 2005; Wellings 2007; Chen et al. 2009).

2. New pathotypes with specific adaptation facilitated by acquisition of specific single gene virulences, and simple virulence combinations, were major factors underlying regional epidemics. The acquisition of virulence for *Yr2* in the 1970s, *Yr9* in the 1990s, and *Yr27* in recent years contributed significantly to regional and continental epidemics, and crop losses (Wellings et al. 2009).

3. Comparative studies using *Pst* isolates drawn from widely dispersed geographical regions showed distinct genetic contrasts including evidence for putative recombinants (Enjalbert 2009; Duan et al. 2010). Chinese workers frequently reported wide variability in pathogenic features in *Pst* populations in Gansu province (e.g. Chen et al. 2009). While the lack of evidence for a sexual host remained undiscovered, these claims were unsupported. The situation has now clearly changed, and greater emphasis will be placed on determining the distribution and importance of candidate *Berberis* spp. as alternate hosts in these regions.

Pathogen aggressiveness

The apparent aggressiveness of certain isolates of *Pst* has been a subject of speculation for many years. Johnson and Taylor (1972) were among the first to demonstrate that differences in urediniospore yield between isolates of identical pathotype were associated with the cultivar source of the isolate. However the emergence of destructive *Pst* pathogen populations in North America, Australia and elsewhere over the past decade provided the impetus to undertake detailed studies of pathogen aggressiveness in relation to temperature. While earlier European workers suggested that *Pst* may have become adapted to warmer temperatures (Macer 1972; Zadoks 1979), it was the detailed work of Milus et al. (2009) that provided a basis for the claim of temperature adaptation within newly emerging *Pst* populations. This work demonstrated that a recently introduced pathogen population in North America was capable of producing more urediniospores in shorter time periods and at higher temperature profiles. Australian work confirmed a shorter pathogen cycle in aggressive isolates of *Pst* presumed to be of similar origin to those in the USA, but with less clear evidence for adaptation to selected temperature regimes (Loladze et al. 2009).

Pathogen movement

Pathotype surveys were used in Australia to demonstrate important aspects of disease epidemiology. The early seasonal occurrence of the disease, frequently involving different pathotypes recovered from distant locations, provided evidence for the independent localized survival of *Pst* within major wheat growing regions. In Australia, there is no evidence for specific survival areas.

In Europe, various studies demonstrated clonality in *Pst* populations with clear evidence for long distance dispersal. Justesen et al. (2002) demonstrated extinction (1996) and re-colonization (1997) of *Pst* in Denmark using molecular and pathogenicity markers. Hovmoller et al. (2002) provided further evidence to indicate that *Pst* populations in northwestern Europe frequently migrate across the region. These, and other studies, argue strongly for a co-ordinated regional and continental approach to disease control.

Documentation of rapid and long distance dispersal in *Pst* is relatively recent. The first detection of *Pst* in Australia in 1979 was considered an outcome of inadvertent urediniospore transfer from Europe (Wellings et al. 1987). A similar mode of introduction presumably led to the discovery of *Pst* in South Africa in 1996 (Pretorius et al. 2007) since the initial pathotype was more closely related to North Africa and the Middle East than prevailing pathotypes in west Africa. The occurrence of a new pathotype lineage in North America in 2000 (Chen et al. 2002; Chen 2007) and detection of a near-identical pathotype in previously stripe rust free Western Australia in 2002 (Wellings et al. 2003) highlight the potential for rapid international transfer of *Pst* pathotypes. Hovmoller et al. (2008) compared a set of *Pst* isolates representing recently emerged (post 2000) near identical pathotypes collected from various continents and regions. They concluded that two subsets, or strains, of the apparent widely dispersed pathotype could be distinguished based on AFLP fingerprints: Strain 1 was common to USA and Australia, and Strain 2 was recovered from Eritrea, West Asia (Iran, Azerbaijan) and Central Asia (Kazakhstan, Uzbekistan, Kyrgystan).

The widespread international occurrence of this newly emerged *Pst* lineage is cause for concern. International travel must play a significant, although currently unmeasured, role in cross boundary plant pathogen transfer. International tourism has grown from 25 million passenger movements per annum in the 1950s to 760 million in the current decade (R. Delane pers comm). These challenges must be embraced by agriculturalists and policy makers concerned with implementing effective disease management strategies.

Current international stripe rust status

Singh (2004) presented an overview of the relative importance of the three cereal rust diseases, and concluded that stripe rust was currently the most damaging cereal rust across international locations. In order to update and further clarify this conclusion, an attempt to gauge the current status of *Pst* was undertaken for the purposes of this paper. Colleagues with experience in wheat stripe rust were contacted and asked to assess the incidence and severity of the disease over the period 2000-2010 in the regions for which they had immediate knowledge. Assessments were based on a simplified method for disease incidence, adapted from Murray and Brennan (2009), using scores to express the frequency of epidemics and the approximate wheat production area affected during an epidemic (Table 2). Assessment scores for stripe rust severity (Table 3) were based on an average expectation for crop losses encountered in nominated regions.

TABLE 2 HERE
TABLE 3 HERE

Assessments provided by 25 colleagues are summarized in Table 4. Although comparisons were not sought with other wheat diseases, the survey provides evidence for the widespread incidence and severity of stripe rust in major wheat production zones of the world. The most ‘at risk’ regions for

stripe rust could be considered those where epidemics are experienced in most seasons with expected regional losses ranging from 5 to 25%. These regions include the USA (Pacific North West), East Asia (China north-west and south-west), South Asia (Nepal), Oceania (eastern Australia) and East Africa (Kenya). A majority of regions have stripe rust incidence on a regular basis (incidence scores of 3 and 4) with estimated losses of 1 to 10%. This survey was brief and would clearly improve with greater attention to assessments for more precisely defined regions. Should this development occur, then it may be possible to arrive at regional estimates of the current costs incurred by stripe rust and other significant wheat diseases, thereby developing a basis for prioritizing R&D investments. The work of Murray and Brennan (2009) in Australia, who estimated the current national losses due to stripe rust at $AUD17.82 per hectare or $AUD127million across the national wheat growing regions, could serve as a basis in developing an international economic assessment of the impact of stripe rust on world wheat production. Such projections are likely to generate a staggering estimate of the economic magnitude of the stripe rust problem internationally.

Conclusions

This brief survey and overview of published work on stripe rust strongly supports the conclusion that wheat stripe rust continues to be a major limiting factor in world wheat production. Despite the regular and significant impact of the disease, there appears to be a limited international capacity to respond to epidemics, much less to anticipate and proactively prepare farming communities to minimise the effects. With few exceptions, there appears to be a lack of regionally co-ordinated R&D programs that link active pathogen monitoring with known deployed resistances in farmers fields and resistance breeding programs. The international rust trap nursery system implemented and co-ordinated by IARCs (CIMMYT and ICARDA) has been helpful in monitoring variety responses to local *Pst* populations, and the application of NILs has added a useful dimension to this work (Wellings et al. 2009). The recent establishment of a Global Reference Centre for Yellow Rust at the University of Aarhus, Denmark, is a significant step towards sealing the vacuum that occurred when IPO Wageningen ceased international testing and training. The latter program, which was eminently led by RW Stubbs and colleagues and continued until the early 1990s, was an invaluable assistance to those monitoring *Pst* in developing countries. Experience informs us that new and important pathotypes are generally recovered initially at low frequency, and if these can be identified and their significance can be interpreted, opportunities are presented for timely responses to minimise crop losses. The Global Reference Centre for Yellow Rust will provide valuable support, but is unlikely to have the capacity required to manage large sample numbers from regional epidemics. Investments in resources and training for regional centres of expertise in cereal rust pathology and breeding should be a priority if we are to gain maximum advantage from global IARCs.

A review of historic and contemporary experiences with stripe rust in global wheat production zones inevitably results in a cascade of apparent unresolved issues. Why is it that we seemingly remain vulnerable to successive waves of pathotype lineages across entire regions and continents? Where does the pathogen survive in vulnerable locations and what can be achieved in designing containment strategies that seek to reduce the dangers of early infection in these locations? What can be done to strengthen national programs to effectively monitor *Pst* populations in the context of locally deployed resistant genotypes? How can we synchronise informative and timely pathotype data with a whole-of-industry approach to resistance breeding and post release management of varieties? These are not new questions!

Biffen (1931), buoyed by early success in the application of Mendelian genetics in breeding for stripe rust resistance, predicted that ‘the solution of the most important problems of rust control is in sight’. Forty years later, Manners (1969) referred to Biffen’s statement as ‘perhaps still true, but the view is more distant than Biffen anticipated’. Johnson (1992) reflecting on a further thirty year period in stripe rust research found some agreement with Manners but added his own personal view: ‘although one can still optimistically look forward to the final solution, it is rather seen through the

wrong end of a telescope'. If the light of solutions to stripe rust indeed resides at the end of a tunnel, it would seem that we still have a good deal of work to do to ensure the train is heading towards us and not in the opposite direction. If this is viewed as a light hearted conclusion, then we should be galvanised to action by the thought that without continued effort and resources, international wheat productivity clearly remains at serious risk from recurring stripe rust epidemics. Currently, stripe rust is spreading rapidly in a region stretching from Turkey, Syria and northern Iraq to southern Uzbekistan; the potential for crop loss is in the billions of dollars (Abdallah et al. 2010).

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Table 1 Historical review of major stripe rust epidemics with comments on severity and losses

Region	Period	Nature of epidemic and losses	Reference
United Kingdom	1966 1969 1988-1989	Rothwell Perdix epidemic Joss Cambier epidemic Sleijpner, Hornet epidemic	Macer 1972 Johnson 1992 Bayles et al. 1989
Australia	1983-1986 2002-2010	Losses up to 80% Annual fungicide expenditure $AUD 40-90m (2003-2006)	Murray et al. 1994 Wellings 2007
New Zealand	1980-1981	60% crop loss	Beresford 1982
Iran	1993	National losses: 1.5 m tonne	Moghaddam et al. 2009
Chile	1976-1988, 2001	Regular epidemics	German et al. 2007
USA	1957-1958 1960-1964 2000 2003	Widespread in 10 states $USD15-30m losses (Wa State) Widespread in 20 states National losses 3.4% or 11.7m tonnes	Chen et al. 2002 Line 2002 Chen et al. 2002 Chen 2005
China	1954, 1964, 1990, 2002	6.00 m tonne loss 3.20 m tonne loss 2.65 m tonne loss 1.40 m tonne loss	Chen et al. 2009 Wan et al. 2004
Spain, North Africa	1978	Siete Cerros epidemic	Zadoks and Bouwman 1985
Republic of South Africa	1996-1999	Annual fungicide expenditure ZAR5-28m	Boshoff et al. 2002
India	1994-2004 2001	Recurrent epidemics PBW343 (*Yr27*) failed	Prashar et al. 2007
Pakistan	2005	$USD100m losses (NWFP)	Duveiller et al. 2007
Italy	1977-1978	Undetermined; widespread epidemic with susceptible cultivars	Vallega and Zitelli 1979
Czechoslovakia	1977	30% loss in susceptible cultivars	Slovencikova and Bares 1978

Table 2 Descriptions and scores for stripe rust incidence (adapted from Murray and Brennan 2009)

Score	Description	Incidence	
		Epidemic years (%)	Production area affected (%)
0	Not recorded.	0	0
1	Rare. Generally 1 season in 10 over scattered locations.	20	25
2	Localised in some seasons. Approximately 2 seasons in 5 over 25% of wheat growing area.	40	25
3	Present in most seasons but localized. Occurring 2 seasons in every 3 over 25% of wheat growing area.	67	25
4	Widespread in some seasons. Approximately 2 seasons in 5.	40	100
5	Widespread in most seasons. Approximately 2 seasons in 3.	67	100

Table 3 Severity and associated crop loss estimates for stripe rust (adapted from Murray and Brennan 2009)

Score	Crop loss classification	Estimated Percent Loss
0	No losses	0.0
1	Negligible	0.1
2	Light	1.0
3	Moderate	5.0
4	Severe	10.0
5	Very severe	25.0

Table 4 Incidence and severity[1] of wheat stripe rust across cereal producing continents and regions provided by collaborator assessments for 2000-2009

Continent	Region, Zone	Incidence	Severity	Collaborator
North America	Canada –western prairies (Alberta)	3	2	B McCallum, X Chen
	Canada – eastern prairies (Manitoba, Saskatchewan)	2	1	B McCallum, X Chen
	Pacific North West USA	5	3	X Chen, R Singh
	Great Plains USA	4	2	X Chen, R Singh
	South West USA, Mexico	4	2	X Chen, R Singh
South America	Bolivia, Columbia, Peru, Ecuador	4	4	R Singh
	Chile	4	4	R Madariaga
	Argentina, Brazil, Uruguay, Paraguay	1	3	S German, R Singh
Western Europe	UK – eastern England	4	2	R Bayles, M Hovmoller
	UK – other areas	2	1	R Bayles
	Germany	3	2	K Flath, M Hovmoller
	Denmark	2	2	M Hovmoller
	France - north	3	2	C Pope, M Hovmoller
	France – south, Spain	2	1	C Pope, M Hovmoller
	Italy	1	1	M Pasquini, M Hovmoller
Eastern Europe	Romania, Poland, Ukraine, Yugoslavia	2	2	X Chen
	Czech Republic	3	1	A Dreiseitl
	Russia – north Caucasus	2	2	G Volkova
	Russia – Siberia, north Kazakhstan	1	2	A Morgounov, C Wellings
West Asia	Syria, Iran, Iraq, Afghanistan	3	4	R Singh, K Nazari, A Yahyaoui, A Morgounov
	Turkey – south/south east	2	3	Z Mert
	Turkey – Central Anatolia	3	4	Z Mert, A Morgounov
	Turkey – north	2	2	Z Mert
	Iran – north, west, south, central	3	2	K Nazari
	Iran – east	1	2	K Nazari

Central Asia	Kazakhstan – south/south-east	3	4	S Rysaliev, A Kokmetova, C Wellings, A Morgounov
	Kazakhstan - north	1	3	A Kokmetova, C Wellings
	Uzbekistan	4	4	K Nazari, A Morgounov, R Singh, C Wellings
East Asia	China	4	4	R Singh
	China – north west	5	5	W Chen, X Chen
	China – south west	5	4	W Chen, X Chen
	China – south east	3	2	W Chen
South Asia	India, Pakistan	4	3	M Prashar, K Nazari
	Nepal - eastern foot hills/river basin	5	4	S Sharma, G Ortiz-Ferrara
	Nepal - eastern lower and mid elevation hills	4	3	S Sharma, G Ortiz-Ferrara
	Nepal – central valleys and hills	4	4	S Sharma, G Ortiz-Ferrara
	Nepal – western foot hills/river basin	4	3	S Sharma, G Ortiz-Ferrara
	Nepal – western mid elevation hills	5	4	S Sharma, G Ortiz-Ferrara
	Nepal – far west mid/high elevation hills	3	3	S Sharma, G Ortiz-Ferrara
Oceania	Australia – eastern states	5	3	C Wellings
	Australia - west	4	3	C Wellings
	New Zealand	4	3	C Wellings
North Africa	Morocco, Tunisia	2	4	A Yahyaoui
	Egypt	1	2	A Shahin, K Nazari
East Africa	Kenya	5	4	D Singh, K Nazari
	Ethiopia	4	4	D Singh, K Nazari, A Badebo
	Yemen	4	3	D Singh, K Nazari
	Tanzania	3	3	D Singh
	Uganda	3	3	D Singh
Southern Africa	South Africa, Zimbabwe	3	3	Z Pretorius, R Singh

[1] Scores from 0 to 5 for incidence and severity are based on scales described in Tables 2 and 3.

Global status of wheat leaf rust caused by *Puccinia triticina* (abstract)

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Leaf rust caused by the fungus *Puccinia triticina* is the most common and widely distributed of the three wheat rusts. Losses from leaf rust infections are usually less damaging than those from stem rust and stripe rust, but leaf rust causes greater annual losses due to its more frequent and widespread occurrence. Yield losses from leaf rust are mostly due to reductions in kernel weight. Many laboratories worldwide currently conduct leaf rust surveys and virulence analyses, and significant information is available. Most important races have either evolved through mutations in existing populations or migrated from other, often unknown, areas. Many leaf rust resistance genes are catalogued, and high levels of slow rusting adult plant resistance are available in high yielding CIMMYT wheats. This paper summarizes the importance of leaf rust in the main wheat production areas as reflected by estimates of yield losses, the complexity of virulence variation in pathogen populations, the role cultivars with race-specific resistance play in pathogen evolution, and the control measures currently practiced in various regions of the world.

Status of wheat rust research and control in China

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Abstract In China, wheat is grown on approximately 24 million hectares with an annual yield of 100 million tonnes. Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is a threat mainly to spring wheat in northeastern China. Leaf rust, caused by *P. triticina*, occurs on crops in the late growth stages in the Yellow-Huai-Hai River regions. Stripe rust, caused by *P. striiformis* f. sp. *tritici* (*Pst*), is destructive in all winter wheat regions and is considered the most important disease of wheat in China. During the last 20 years, widespread stripe rust epidemics occurred in 2002, 2003, and 2009, and localized epidemics occurred in many other years. In recent years, major yield losses were prevented by widespread and timely applications of fungicides based on accurate monitoring and prediction of disease epidemics. A total of 68 *Pst* races or pathotypes have been identified using a set of 19 differential wheat genotypes. At present, races CYR32 and CYR33 virulent to resistance genes *Yr9*, *Yr3b*, *Yr4b*, *YrSu* and some other resistance genes are predominant. Moreover, these races are virulent on many cultivars grown in recent years. Of 501 recent cultivars and breeding lines 71.9% were susceptible, 7.0% had effective all-stage resistance, mostly *Yr26* (= *Yr24*), and 21.2% had adult-plant resistance. Several resistance genes, including *Yr5*, *Yr10*, *Yr15*, *Yr24/Yr26*, *YrZH84* and some unnamed genes, are still effective against the current *Pst* population. All have been widely used in breeding programs. Lines with one or more of *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9* and other unnamed resistance genes are susceptible to currently predominant races. Durable adult plant resistance sources are being increasingly used as parents in breeding programs. Progress has been made in genomics and population genetics of *Pst*, molecular mapping of resistance genes, and cytological and molecular mechanisms of the host-pathogen interactions involved in stripe rust.

Keywords disease management, epidemiology, leaf rust, stem rust, stripe rust, wheat

Introduction

Wheat (*Triticum aestivum* L.), the second largest food crop in China, was grown on approximately 24 million hectares in 2009 producing more than 100 million tonnes of grain. Barley (*Hordeum vulgare* L.) is annually grown on about 1 million hectares in the Yangze River valley, Huanghe River valley and Qinghai-Tibet Plateau where leaf rust and stripe rust are now the most important diseases of barley. Stripe rust is especially destructive on barley in Tibet (Wang ZH et al. 1989; Niu et al. 1994). Oats (*Avena sativa* L.) are planted on roughly 1.2 million hectares in Inner Mongolia, Hebei, Shanxi, Gansu, Shaanxi, Yunnan, Sichuan, Ningxia, Guizhou and Qinghai. Stem rust and crown rust on oats occur in the oat-growing areas, and are the most important diseases in some regions. Oat stem rust in

particular, can cause severe epidemics in Inner Mongolia and northeastern China in some years (Yang 1984).

Leaf rust (brown rust), caused by *Puccinia triticina* Eriks., was earlier a serious disease of wheat in southwestern China and the Yangze River valley. In the last three decades, it has become more important in northern, northwestern and northeastern China. Significant yield losses were caused by leaf rust in northern China in the 1970s (Zhang and Liu 2001). Research on leaf rust of wheat is mainly conducted at Hebei Agricultural University, Baoding, Hebei.

Stem rust (black rust), caused by *P. graminis* Pers. f. sp. *tritici* Eriks. & Henn., causes severe yield reductions in some years in northeastern China and the Yellow-Huai-Hai River areas (He et al. 2008). Currently, investigations on wheat stem rust are mainly carried out at Shenyang Agricultural University, Shenyang, Liaoning. The spread of race Ug99 (TTKSK) with *Sr31* virulence from Africa to Asia is a potential threat to wheat production in China, but the current risk level is considered to be low. Of 700 Chinese major wheat cultivars and breeding lines tested in Kenya, only four were highly resistant, 10 were moderately resistant, and the remaining 686 genotypes were highly susceptible (He et al. 2008). Therefore, if race Ug99 were to appear in China severe stem rust epidemics could follow.

Stripe rust (yellow rust), caused by *P. striiformis* Westend. f. sp. *tritici* Eriks., is the most destructive disease in all winter wheat growing regions in northwestern, southwestern, and northern China, as well as in the spring wheat areas in the northwest (Li and Shang 1989; Wan et al. 2004; Chen WQ et al. 2009). Severe epidemics in 1950, 1964, 1990, and 2002 resulted in yield losses of up to 6.0, 3.2, 1.8, and 1.3 million tonnes, respectively (Wan et al. 2007; Chen WQ et al. 2009).

Stripe rust is much more important than leaf rust or stem rust based on historical data and current disease situations. Plant pathologists, breeders, farmers, and governmental organizations have given much attention to research on, and control of, stripe rust. Nationwide cooperation on various aspects of the disease has occurred since 1950, mainly focusing on epidemiology, race identification, breeding resistant cultivars, and integrated management. In this paper, we will summarize recent research on, and control of, wheat stripe rust in China, including occurrence and control, evolution of virulence, and molecular research. Also, challenges and strategies for controlling stripe rust in China will be discussed.

Epidemiology, occurrence and control of wheat stripe rust in recent years

China is considered the largest independent epidemic region in the world (Stubbs 1985). Based on historical epidemiological data for stripe rust, the wheat-growing regions can be divided into the western over-summering areas, the over-wintering areas, and the eastern epidemic areas (Li and Zeng 2002). The over-summering areas include the northwestern (Shaanxi, Gansu, Sichuan, Ningxia, and Qinghai), southwestern (Yunnan and Guizhou), and Xinjiang regions (Li and Shang 1989; Li and Zeng 2002). Stripe rust can complete its year-round cycle in Xinjiang, Yunnan, southern Gansu and northwestern Sichuan where wheat can be grown from lowland valleys at 1,000 m to highland terraces at 3,300 m which provide a “green bridge” for pathogen migration from late-maturing highland areas to early-sown wheat plants in the lowlands (Li and Zeng 2002; Zeng and Luo 2006; Chen WQ et al. 2009). The over-wintering regions are mainly in Sichuan, Hubei and Shaanxi. The eastern epidemic areas cover the largest wheat-producing areas, including Henan, Hubei, Shandong,

Shanxi, Hebei, Sichuan, Shaanxi and Anhui. In over-summering areas, the infected autumn-sown wheat and volunteer wheat in the lowlands, and late-maturing spring wheat in the highlands serve as inoculum sources for local recycling throughout the year (Li and Zeng 2000). Previous studies showed that urediniospores from the over-summering regions, particularly the northwestern and southwestern areas, spread eastward to eastern China. Fall-sown winter wheat in the over-wintering regions becomes infected during late autumn and early winter and the pathogen survives mainly as latent mycelial infections until temperatures increase in spring when inoculum then moves to the major winter wheat regions to the northeast (Li and Zeng 2002; Zeng and Luo 2006). Thus, interregional disease distribution in time and urediniospore spread are mainly from west to east in autumn and from south to north in spring (Li and Zeng 2002; Zeng and Luo 2006). The over-summering areas provide initial inoculum for the eastern plain regions of China (Li and Zeng 2002; Zeng and Luo 2006).

Over the last decade wheat stripe rust has remained at high levels posing a threat to wheat production across the entire country. This follows the development and spread of races CYR32 detected in 1994 and CYR33 found in 1997 which are now the predominant races (Chen WQ et al. 2009). The main reason is that winter wheat cultivars with *Yr9*, *Yr3b*, *Yr4b* and *YrSu*, now susceptible to both races, account for 90% of total area of winter in the entire country. Survey data from the last ten years show that the areas affected annually by stripe rust (Table 1) were on average about 4 million hectares. For example, stripe rust affected 6.6, 4.9, and 4.08 million hectares in 2002, 2003, and 2009, respectively (Table 1). It is considered that the stripe rust epidemic in 2002 was the most widespread in the past three decades (Li and Zeng 2002; Wan et al. 2004).

TABLE 1 HERE

In 2009, the early occurrence of stripe rust, 10-55 days in earlier than usual, posed the greatest threat for many years, following widespread over-wintering of urediniospores due to mild winter and favorable early spring conditions. It was estimated that the total area of wheat infected by stripe rust reached 4.08 million hectares (Table 1). Fortunately, intense disease monitoring and forecasting allowed timely application of fungicides which effectively prevented losses and further spread to the wheat production regions further east. Thus, a potentially huge yield loss nationwide was avoided through timely use of fungicides based on earlier accurate disease forecasts. Moreover, the surveys and forecasting of disease provided information to pathologists, growers, county agents, extension services, the fungicide trade, and government administrators from village level to the Ministry of Agriculture enabling them to make decisions on chemical intervention to minimize yield losses. Fungicides, spray equipment, and personnel training were well-organized in advance.

In the present situation where most currently grown cultivars are susceptible, fungicide use will continue to play a key role in control of wheat rusts until resistant varieties can be grown across large areas. Seed treatment and foliar sprays are the major means of application to decrease infection of seedlings of autumn-sown wheat in the over-summering and over-wintering areas. This can reduce the available inoculum for later windborne dispersal to more eastern regions (Chen YL et al. 1988). For other areas, generally a single foliar application is recommended in early spring when disease incidence reaches 2 to 4% or when disease severity reaches 1% at the jointing and stem elongation

stages. The areas of stripe rust occurrence and fungicide treatment during 1999-2009 are shown in Table 1. In 2008, about 80% of seed autumn-sown in southern Gansu, northwestern Sichuan, and southern Shaanxi was fungicide-treated and this delayed the occurrence stripe rust in these regions in the 2008-2009 crop seasons.

Effective control of stripe rust by the "family-unit" cultivation system is becoming increasingly difficult as many young villagers in the rural areas migrate to the cities for employment. Currently, government administrators from the townships and ministries guide growers in implementing disease control. This involves training, technical guidance, fungicide supply, seed treatment, and timing of fungicide application. Further measures are being taken to help growers understand the potential threat of stripe rust on grain yield and disease management generally, through television programs, internet websites, radio broadcasts, and cell phone messages.

Race identification and virulence evolution

Stripe rust race identification in China is conducted by the Chinese National Wheat Rust Collaborative Group (CNWRCG) comprising the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Institute of Phytopathology, Northwest A&F University, Yangling, Shaanxi, and the Academies of Agricultural Sciences in the provinces of Gansu, Sichuan and Yunnan. Rust collections in the different provinces are sent to the corresponding branches of the CNWRCG. Methods described by Li and Zeng (2002) for collecting rust samples, testing on differentials, and data recording and analysis are used.

To date, a total of 68 *Pst* races have been identified (Wan et al. 2007; Chen et al. 2009) based on avirulence/virulence patterns using a set of 19 differential wheat genotypes consisting of Trigo Eureka (*Yr6*), Fulhard, Lutescens 128, Mentana, Virgilio (*YrVir1*, *YrVir2*), Abbondanza, Early Premium, Funo (*YrA*, + (+ = additional resistance genes)), Danish 1 (*Yr3*), Jubilejina 2 (*YrJu1*, *YrJu2*, *YrJu3*, *YrJu4*), Fengchan 3 (*Yr1*), Lovrin 13 (*Yr9*, +), Kangyin 655 (*Yr1*, *YrKy1*, *YrKy2*), Suwon 11 (*YrSu*), Zhong 4, Lovrin 10 (*Yr9*), Hybrid 46 (*Yr3b*, *Yr4b*), *Triticum spelta album* (*Yr5*) and Guinong 22 (Li and Zeng, 2002; Wan et al. 2004, 2007; Chen WQ et al. 2009). Of the 68 races, 33 are designated as "races" CYR1 (Chinese Yellow Rust) to CYR33 in chronological sequence and 35 are described as "pathotypes". A "race" is named based on the avirulence/virulence pattern on major differentials and a significant frequency (>3%), whereas a "pathotype" is named based on a virulence variation on a minor differential or an additional differential wheat genotype and a low frequency (<3%) (Wang KN et al. 1986). Changes in races and frequencies occur with changes in wheat cultivars (Wan 2001). Since most of the differentials are used only in China, and since the genetic bases of their resistances are not fully established on an international basis, it is difficult to compare Chinese *Pst* races with those in other countries. Near-isogenic lines (NILs) developed by Australian and Chinese scientists, along with Chinese local supplementary cultivars, may resolve some of these difficulties in the near future.

None of the current races is virulent on *Triticum spelta album* (*Yr5*) or Guinong 22 (*Yr*+) (Chen WQ et al. 2009). Virulence to Zhong 4 (*Yr*+) occurs in a pathotype identified 2009 (Wang BT et al. 2009). All races are virulent to Fulhard and Early Premium, except pathotype CYR18, and to Abbondanza, except Su11-1. CYR32 possesses the widest virulence spectrum, comprising a

combination of virulence to the differential genotypes Lovrin 10 and Lovrin 13 (both *Yr9*), Hybrid 46 (*Yr3b,Yr4b*) and Suwon 11 (*YrSu*). In contrast, CYR18 has the narrowest virulence spectrum, being virulent only to Lutescens (*Yr*+), Abbondanza (*Yr*+), and Danshi 1 (*Yr3*). Races CYR17 to CYR27 were first detected before 1980. The 31 races identified after 1980 were divided into three groups, viz. the Lovrin group (LvG), the Suwon 11 group (SuG), and the Hybrid 46 group (HyG) (Chen WQ et al. 2009). Nine races (CYR32, CYR33, Su11-4, Su11-7, Su11-5, CYR31, Hy46-8, Su11-11 and Hy46-7) were detected with frequencies of 29.6, 17.4, 6.2, 5.5, 4.2, 3.6, 2.9, 2.3, and 2.2%, respectively, during 2003-2007 (Chen WQ et al. 2009). The remaining races had low frequencies of less than 2%. Races CYR18, CYR19, CYR27, CYR30, Lv10-3, Lv13-2, and Hy46-4 were occasionally detected in some years or in some regions, and occurred with frequencies less than 1% during 2003-2007 (Chen WQ et al. 2009). The most frequently identified race from 2003 to 2009 was CYR32 accounting for a frequency of 29.6% throughout the country. CYR33 (previously called pathotype 'Su11-14') with a frequency of 17.4% was detected in all 15 wheat-growing provinces except Shandong (Chen WQ et al. 2009). Importantly, CYR32 and CYR33 have broad virulence and high fitness which makes them widely distributed and adapted to popular cultivars, and therefore, will likely continue to be the major races to cause widespread epidemics of stripe rust in the near future.

From 2001 to 2007, the frequencies of CYR33 and Su11-7 increased from 4.21% and 1.5% in 2001 to 26.72% and 9.46% in 2007, respectively. However, frequencies of CYR30, CYR31, and Su11-4 decreased from 7.33%, 9.51%, and 9.51% in 2001 to 0.51%, 2.26%, and 4.93% in 2007, respectively. CYR32 was first found in 1994, and then became a predominant race at a frequency of 11% in 2000, and remarkably increased to 28.79% in 2001 and 34.60% in 2002 (Wan et al. 2002). Later, the frequency of CYR32 slightly declined but was still at 29.23% in 2003 and 29.97% in 2007. Pathotype CYR33 (named Su11-14 before 2008) was first detected at a frequency of less than 1% in 1997, and subsequently increased from 4.21% in 2001 to 26.72% in 2007. Pathotype Su11-7, first identified in 1995, gradually increased to 10% in 2007. Pathotypes Su11-5, Hy46-7, and Hy46-8 occurred at average frequencies of 4.20, 2.86, and 2.23%, respectively, ranging between 0.11 and 6.28% from 2003 to 2007 (Chen WQ et al. 2009).

Evaluation of stripe rust resistance in wheat cultivars

Growing resistant cultivars is considered the most effective, low-cost, and environmentally safe approach to control stripe rust (Röbbelen and Sharp 1978; Line and Chen 1995). To better understand resistance characteristics of wheat cultivars, and the distribution and utilization of resistance genes in major wheat-growing regions, we evaluated 501 wheat cultivars and advanced breeding lines from 13 provinces in the northwest, north, and Yangze River Valley from 2006 to 2009. Testing was performed on seedlings in the greenhouse and on adult-plants in fields at Yangling, Shaanxi, and Tianshui, Gansu. The recorded data showed that 35 lines (7%) had all-stage resistance, 110 (22.0%) had adult plant resistance, and 356 (71.0%) were highly susceptible. Pedigree analyses indicated that most of the resistant cultivars had *Yr26*/*Yr24*. Also, Guinong 22 and wheat-*Thinopyrum intermedium* derivatives, as well as some genotypes from CIMMYT (e.g. synthetic hexaploid wheat (*Triticum turgidum* × *Aegilops tauschii*)) had high resistance levels (Han DJ et al. unpublished data). There were distinct differences in the frequencies of resistant cultivars tested in several regions as shown in Table

2. The results revealed a low number of all-stage resistant cultivars and a low diversity of resistance genes in Chinese cultivars. Currently, *Yr26* is the most frequent effective resistance gene both in wheat breeding programs and among currently resistant cultivars. Once resistance of *Yr26* is overcome by a new race, there will be severe epidemics and consequent yield losses. Therefore, the continuing widespread use of *Yr26* is a major concern. Earlier, from a study of 98 Chinese cultivars with 26 CYR races, Li GQ et al. (2006) reported that 42.9% had *Yr9*, and 19.3% had *Yr24*/*Yr26*. Wan et al. (2004) evaluated approximately 200 cultivars or breeding lines each year at 20 different field sites located in many provinces. The successive field and greenhouse tests indicated the presence of high-temperature adult-plant (HTAP) resistance and slow-rusting in Chinese cultivars (Wan et al. 2000a, 2000b; Guo et al. 2008). Many studies in China and elsewhere (e.g. Line and Chen 1995; Singh et al. 2000; Chen XM 2005; Li ZF et al. 2006; Lin and Chen 2007, 2009) characterized durable non-race specific HTAP and slow-rusting resistances under field conditions. The advantages of utilizing HTAP and slow-rusting resistances must be considered for sustainable control of stripe rust in the major wheat-growing areas in China. Dr R. P. Singh, CIMMYT, Mexico, is working with breeders in Sichuan and Yunnan to introduce *Yr18* and other APR genes into local varieties.

TABLE 2 HERE

Developing resistance sources to stripe rust

To date, more than 70 stripe rust resistance genes have been reported (McIntosh et al. 2008 http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp); most confer race-specific all-stage resistance, and some confer adult-plant or HTAP resistance (Chen XM 2005; Lin and Chen 2007, 2009). Based on recent evaluations in China, genes *Yr5*, *Yr10*, *Yr15*, *Yr18*, *Yr24*/*Yr26, Yr36, Yr39* and *Yr41*, as well as the source lines possessing *Yr12*, *Yr13*, *Yr14* and *Yr16*, and some temporarily designated genes are still effective and could be used in breeding programs (Wan et al. 2004, 2007). Resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr20*, *Yr21*, *Yr22*, *Yr25*, *Yr27* and *Yr29* are ineffective to the currently prevalent races (Han DJ, unpublished data). Thus, identification and development of new resistance sources are needed. Chinese scientists have made contributions to this research area. Ma JX et al. (2001) documented *Yr26* conferring all-stage resistance on chromosome 1BS. *Yr41* on chromosome 2BS, and conferring resistance to most prevalent races, was identified in cultivar Chuannong 19 by Luo et al. (2005, 2008). Zeng et al. (2007) identified a resistance gene on chromosome 1B in line 101-3. *YrY201* on chromosome 7DL derived from *Aegilops tauschii* was identified in accession Y201 (Zhang YH et al. 2008). Zhou et al. (2008) identified a *Haynaldia villosa*-derived resistance gene *YrV1* on chromosome 3B, and Feng et al. (2008) found that wheat cultivars Aquileja and Xian Nong 4 had quantitative resistance. Cultivars of the Chuanmai series with good stripe rust resistance (*Yr24/Yr26*) were developed by the Sichuan Academy of Agricultural Sciences through crossing synthetic hexaploid wheat (*Triticum turgidum* × *Aegilops tauschii*) lines from CIMMYT with locally adapted cultivars (mainly wheat cultivar Fan 6 derivatives). Lin RM et al. (2007) characterized the genetics of resistance to stripe rust in Zhong 4 (partial amphiploid, 2n=56), which was resistant to all races, except the recent T4, and widely used in wheat resistance breeding programs. Several derivatives of this cultivar have been released in Gansu

province, e.g. Zhongliang and Lantian cultivars or lines.

Additionally, resistance from related species, including *Haynaldia villosa*, *Secale cereale*, *Thinopyrum intermedium* and *Th. bessarabicum* and *Aegilops tauschii* were transferred into wheat backgrounds to further develop wheat germplasm (Yang et al. 1999; Chen YF et al. 2003; Li GR et al. 2006; Chen QZ et al. 2008;).

Genetics and molecular markers for resistance genes

Molecular markers linked to resistance genes can improve selection efficiency in breeding programs. Although different kinds of molecular markers have been developed by gene mapping research, not all are applicable to breeding platforms where resistance to stripe rust is only one of many trait objectives. Thus, except in exceptional circumstances breeders will demand markers that are accurate (preferably perfect or functional markers), low cost, and built upon a single screening platform. Markers for various stripe rust resistance genes developed in China include amplified fragment length polymorphyism (AFLP) and random amplified polymorphic DNA (RAPD) markers for *Yr15* (Peng 2000), expressed sequence tag–simple sequence repeat (EST-SSR) and sequence tagged site (STS) markers for *Yr26* (Ma JX et al. 2001; Wang CM et al. 2008; Wen et al. 2008). AFLP and STS markers for *Yr10* (Shao et al. 2001; Wang LF et al. 2002), RAPD and SSR markers for *Yr5* (Zhong et al. 2002; Sun et al. 2002), SSR markers for *YrCH42* (identical to *Yr24/26*) and *YrZH84* (Li GQ et al. 2006), and SSR and AFLP markers for *YrC591* (Li et al. 2009) have been reported. The use of markers in pre-breeding and breeding programs not only circumvents the need for disease testing during the breeding process, but also allows the combining of genes including those for all-stage and HTAP or AP resistances. Many of the individual genes (or QTL) for APR do not confer sufficient protection when present alone, but often act additively permitting high levels of resistance to be achieved (Singh 1992). The use of this type of durable resistance in the US Pacific Northwest since the 1960s provides good evidence for its usefulness in sustainable control of stripe rust (Line and Chen 1995; Chen XM 2005). Molecular mapping of HTAP resistance and slow-rusting in Chinese wheat cultivars Xiaoyan 6 and Pingyuan 50 were studied byJin JX et al. (2007) and Yuan et al. (1995). We are using molecular markers to detect the presence or absence of the resistance genes (e.g. *Yr9* and *Yr18*) in current wheat cultivars in order to identify potentially new resistance genes or to help in deploying cultivars with different resistance genes in oversummering, overwintering and spring epidemic regions.

Mechanisms of *Pst* virulence variation

Growing resistant cultivars is the major component of integrated control of stripe rust. However, “breakdown” of resistance following the introduction of new genes for resistance is a major problem. According to Gassner and Straib (1932), Little and Manners (1967) and Godard (1976), virulence in *P. striiformis* can result from mutation or heterokaryosis.

Research on mechanisms of virulence variation in *Pst* has been conducted in China since the 1980s. Results from a series of studies suggested that asexual recombination acts as a possible mechanism of virulence variation for *Pst* (Kang et al. 1993a, 1994a, 1994b; Ma Q et al. 1993). Four new isolates of *Pst* were obtained from inoculating susceptible wheat genotypes with urediniospore mixtures of 120 combinations of 38 single-spore isolates. The virulence patterns of the new isolates were different

from those of the original isolates and the frequencies of urediniospores with three or more nuclei in the new isolates were much higher than those in the original isolates. Ma Q et al. (1993) observed germ tube fusions with one to four nuclei on the surface of wheat leaves. Kang et al. (1993b) observed that fusion also occurred between intercellular hyphae within the wheat leaf tissue. Fusions between germ tubes and intercellular hyphae provide the essential conditions for exchanging nuclei. Further studies by Kang et al. (1994b) showed that most urediniospores and germ tubes had two nuclei, but intercellular hyphae had various numbers of nuclei. About 20% of hyphal cells had two nuclei and 80% of the hyphal cells had three or more. They also found that haustorial mother cells and haustoria commonly had three or more nuclei. When *Pst* formed uredinia, all hyphal cells and urediniospores had just two nuclei (Kang et al. 1994b). Nuclear re-assortment could occur at the stage of differentiation from multinuclear cells to two-nuclear cells. Under natural infection conditions, the same leaves can be infected by more than one race allowing such events to occur. In contrast to Kang et al. (1994b), urediniospores with tri- and tetra-nuclei were found at rates of 0.42 and 0.55%, respectively, among field collections from Tianshui in Gansu from May 1997 to November 1998 (Wang Y et al. 2004). There is now increasing molecular evidence for somatic recombination as a likely mechanism of variation in Chinese *Pst* populations in the highland areas of Gansu (Lu et al. 2009; Duan X et al. 2010), and Mboup et al. (2009) suggested that the genetic recombination of *Pst* in the Tianshui area could be from either sexual or parasexual cycles.

Virulence in *Pst* can be produced by UV-irradiation of urediniospores and seven mutants were obtained in a screening experiment on wheat cultivars after UV treatment of urediniospores (Jin et al. 1992; Shang et al. 1994; Huang et al. 2005; Wang XL et al. 2009). The detectable mutation rate was estimated to be 10^{-4}~10^{-7}. The virulence patterns of the mutants were different from those of the wild-type isolates, suggesting that mutation may be one of the mechanisms of virulence variation for the asexual population of *Pst*.

However, it remains an open question as to whether virulence in *Pst* in China is from mutation, somatic recombination, or a sexual cycle.

Histology and cytology of wheat-*Pst* interactions

Pst development and host responses were examined by light and transmission electron microscopy in compatible and incompatible interactions in wheat cultivars Xinong 85 and Huxianhong and *Pst* race CYR25 (Kang et al. 1993b, 2002). The wheat cultivar Xinong 85 is highly resistant to CYR25, showing reaction type 1 according to the Stakman et al. (1962) scale, whereas cultivar Huxianhong is highly susceptible with reaction type 4. The infection process for *Pst* was similar to those of other cereal rust pathogens except for lack of an appressorium or development of an occasional small appressorium-like strucure over the stomata. However, *Pst* differs from the other cereal rust pathogens by the frequent occurrence of more than two nuclei in the intercellular hyphal cells, haustorial mother cells and haustoria (Kang et al. 1994b). The haustorial mother cells of *Pst* often invaginate their own intercellular hyphae in infected host tissues (Kang et al. 1993b). There is a striking difference in the fungal development and host responses between susceptible and resistant wheat cultivars following infection with *Pst*. The pronounced higher number of hyphae observed in the infected wheat leaves of susceptible cultivar Huxianhong compared to the corresponding tissues in resistant cultivar Xinong 85

indicated that the fungal development in wheat leaves was restricted in the resistant cultivar. Structural defense reactions such as formation of cell wall appositions, collars or papillae, and encasements were essentially more markedly expressed in the infected leaves of the resistant cultivar than in the susceptible genotype. Immunogold labelling of lignin showed markedly higher labelling densities in host cell walls of infected resistant leaves than in susceptible leaves (Kang et al. 2002). Immuno-labelling studies demonstrated higher accumulations of chitinase and ß-1,3-glucanase in host cell walls, cell wall appositions, intercellular hyphal cell walls and extrahaustorial matrices in the incompatible interaction than in the compatible interaction (Kang et al. 2003). The two hydrolases may contribute to defense reactions against stripe rust along with other defense responses such as depositions of lignin and callose, formations of cell wall appositions, collars or papillae and encasements.

Generation and accumulation of reactive oxygen species (ROS), superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2) were studied in leaves of wheat cultivar 'Suwon 11' infected with avirulent pathotype CYR23 and a virulent pathotype CYR31 (Wang CF et al. 2007, 2010). Generation of O_2^- and H_2O_2 was measured histochemically using nitro-blue tetrazolium (NBT) and 3,3-diamino-benzidine (DAB), respectively. At the pre-penetration stage, both avirulent and virulent races induced H_2O_2 accumulation in guard cells. In the incompatible interaction, rapid increases of O_2^- and H_2O_2 generation at infection sites were detected. The percentage of infection sites showing NBT- and DAB-staining was 36.1% and 40.0%, respectively, 12 h post inoculation (hpi). During the next 12 h H_2O_2 levels further increased, whereas O_2^- accumulation declined. The stage from 12 hpi to 24 hpi coincides with primary haustorial formation in mesophyll cells. In contrast, in the compatible interaction, O_2^- and H_2O_2 could not be detected at most infection sites. In the incompatible interaction, intensive DAB staining was also observed in mesophyll cells, especially in cell walls, surrounding the infected cells 16-24 hpi; thereafter, these cells contained fluorescing compounds and underwent the typical hypersensitive response (HR). The number of necrotic host cells surrounding the infection sites increased continuously from 20 hpi until 96 hpi. Thus, H_2O_2 accumulation during the early infection stage should be associated with the occurrence of hypersensitive cell death and the resistance response should result in stopping the growth of the avirulent race. In the compatible interaction at 96 hpi, H_2O_2 accumulation was observed in mesophyll cells surrounding infection sites.

Genomics of wheat-*Pst* interactions

To acquire further insights into wheat-*Pst* interactions, transcription profiles of genes involved in wheat-*Pst* interactions were generated. From a compatible interaction between wheat cultivar 'Suwon 11' and pathotype CYR31, 2,743 unisequences were obtained from a cDNA library consisting of 5,793 ESTs (Ma JB et al. 2009). Among the unisequences, 52.8% were highly homologous to plant genes, 16.3% to fungal genes, and 30% were non-hit. Nineteen genes had significant homologies to fungal pathogenicity/virulence factors. Thus, a new database was constructed for identifying functional genes involved in the wheat-*Pst* compatible interaction. To isolate differentially expressed genes during a compatible interaction, a suppressive subtractive hybridization (SSH)-cDNA library was constructed from the wheat variety 'Suwon 11' and race CYR31. A total of 787 unisequences were obtained from 1,707 ESTs, of which 397 unisequences (50.4%) were of unknown function. The

potential functions of these genes may have unique roles in the compatible interaction between wheat and *Pst*. Some host defense-related genes were also isolated from the compatible interaction. To determine the expression patterns of a wheat-*Pst* compatible interaction, cDNA-AFLP profiles were generated with 64 primer pairs at different time points after inoculation with a virulent race (Wang XJ et al. 2009b). In this study, 2,306 of 54,912 transcript-derived fragments (TDFs) displayed altered expression patterns after inoculation, of which 966 were up-regulated and 1,340 down-regulated. Interestingly, 48% and 35% of the differentially expressed genes showed different degrees of change during the periods 6-24 and 120-168 hpi, respectively. In contrast, only 17% of genes were differentially expressed at 48-96 hpi. The expression changes of these genes corresponded quite well to the different infection stages and were also supported by the histological study of compatible interaction between wheat and *Pst*. One hundred and eighty six TDFs were isolated, of which 9 were of pathogen origin as validated by PCR-based assays followed by sequencing. Moreover, low expressions of several host defense-related genes were also detected in the compatible wheat-*Pst* interaction in this study.

To study genes involved in an incompatible interaction, a SSH library was generated from Suwon 11 inoculated with avirulent race CYR23. A total of 652 unisequences were obtained, of which 31 were determined as genes involved in signal transduction and 77 were predicted to encode defense-related proteins (Yu et al. 2010). The expression of wheat signal transduction genes increased soon after inoculation. Various defense-related genes, including reactive oxygen species, ATP-binding cassette (ABC) transporters, pathogenesis-related proteins, and genes involved in the phenylpropanoid pathway were induced. These defense genes are known to work in a sequential and concerted manner resulting in HR. Wang XJ et al. (2010) identified transcriptionally regulated genes during an incompatible interaction between wheat and *Pst* using the cDNA-AFLP technique. A total of 52,992 (TDFs) were generated with 64 primer pairs, and 2,437 (4.6%) of them displayed altered expression patterns after inoculation, from which 1,787 were up-regulated and 650 down-regulated. A fascinating discovery in this study was the quenching of divergent expression of *Pst*-regulated genes in both incompatible and compatible interactions in the middle stages of *Pst* infection. By comparing TDFs identified for the incompatible interaction and those identified in the previous study for the compatible interaction (Wang XJ et al. 2009b), 161 TDFs were shared by both interactions and 94 were expressed specifically in the incompatible interaction. The specificities of 43 selected TDFs determined using quantitative real-time PCR (qRT-PCR) indicated that 11 expressed only in the incompatible interaction. The involvement of shared genes, but with different expression levels, indicated that plant responses in compatible and incompatible interactions are qualitatively similar, but become quantitatively different soon after *Pst* infection.

In addition, a number of candidate genes from wheat challenged by the stripe rust fungus, such as hypersensitivity induced reaction genes (*Ta-hir1*, *Ta-hir2*, *Ta-hir3*, *Ta-hir4*) (Yu et al. 2008; Zhang Y et al. 2009), a transcription factor gene *TabZIP1* (Zhang Y et al. 2009), a novel wheat NAC gene *TaNAC4* (Xia et al. 2010), a wheat HSP70 gene *TaHSC70* (Duan YH et al. 2010), a wheat ß-1,3-glucanase gene *TaGlu* (Liu et al. 2010), and a pathogenesis-related thaumatin-like protein gene *TaPR5* (Wang XJ et al. 2009a), were characterized in wheat-*Pst* interactions. The expressions of these genes in Suwon 11 wheat leaves infected with CYR23 (avirulent) and CYR31 (virulent) of *Pst* were also assessed following exogenous treatments with hormones, such as methyl jasmonate (MeJA),

salicylic acid (SA), ABA, and ethylene (ET). The expression levels of the above genes were significantly higher in the incompatible relative to compatible interaction again indicating that they may function in wheat defense response to *Pst*.

Genomics and population structures of *Pst* in China

Although *Pst* is economically important, little is known about its genome and gene functions. Zhang YH et al. (2008) constructed a cDNA library with RNA isolated from *Pst* urediniospores. A total of 4,798 ESTs were sequenced from a germinated urediniospore library and assembled into 315 contigs and 803 singletons. About 23.9% and 13.3% of the resulting 1,118 unisequences were homologous to functionally characterized proteins and hypothetical proteins, respectively, and 62.8% had no significant homolog in GenBank. Several ESTs shared significant homology with known fungal pathogenicity or virulence factors, such as HESP767 of the flax rust pathogen and PMK1, GAS1, and GAS2 of the rice blast fungus. They selected six ESTs (Ps28, Ps85, Ps87, Ps259, Ps261, and Ps159) and observed their expression patterns during urediniospore germination and infection of wheat seedlings using qRT-PCR. All showed the highest transcript levels in germinated urediniospores and much lower levels in ungerminated urediniospores and infected wheat leaves. The transcript level of Ps159 also increased at later infection stages. The data suggested that these genes that are highly expressed in germinated urediniospores, may have important roles in fungal-plant interaction during the early infection stages. In collaboration with Washington State University, USA, a cDNA library of *Pst* haustoria was generated (Yin et al. 2009); 5,126 EST sequences were also generated from *Pst* haustoria, and 287 contigs and 847 singletons were obtained from them. Approximately 10% and 26% of the 1,134 unique sequences were homologous to proteins with known functions and hypothetical proteins, respectively. The remaining 64% of unique sequences had no significant similarities in GenBank. Fifteen genes were predicted to be proteins secreted from *Pst* haustoria. Analysis of ten genes, including six secreted protein genes, using quantitative RT-PCR revealed changes in transcript levels in different developmental and infection stages. Although studies on *Pst* were focused on analyzing *Pst* transcripts, this work detected a number of candidate host genes possibly involved in fungal-plant interactions during the early infection stages.

Recently, some species of *Berberis* spp. were shown to be infected by *Pst* (Jin et al. 2010). Although barberry plants are widely distributed in different regions of China, including the provinces of Gansu, Sichuan and Shaanxi, an association of barberry plants and *Pst* has not been reported. The current view is that the fungus reproduces mostly if not always asexually by dikaryotic urediniospores. Despite asexual (clonal) reproduction new pathotypes are often found, especially when they are able to overcome resistance genes in previously resistant cultivars. Development of durable and effective control methods against stripe rust is largely based on the knowledge of the pathogen population structure and its potential for adaptation to new cultivars (Boshoff et al. 2002). To study the population genetic structures of *Pst* in China, DNA fingerprinting probes (moderately repetitive DNA sequences-PSR sequences) specific to the *Pst* genome and a group of SSR markers derived from expressed sequence tags of *Pst* were developed by Shan et al. (1997) and Chen CQ et al. (2009). Using these markers, high genetic diversity was reported in Chinese *Pst* populations from different regions (Shan et al. 1998; Zheng et al. 2000). Lu et al. (2009) showed that populations of *Pst* in Gansu

possessed a high level of genetic diversity, but there was a lower genetic differentiation using SSR markers. Duan X et al. (2010) also found that *Pst* possessed high diversity in Gansu, compared with European and Australian populations based on data reported in Hovmøller et al. (2002) and Steele et al. (2001). Although the over-summering areas in Gansu have attracted much attention concerning *Pst* virulence variation and population diversity in the past, we now have new knowledge and superior molecular tools for further study in the future.

Monitoring stripe rust plays an important role in its control. A rapid and reliable detection of the pathogen in latent infections of wheat leaves during overwintering of the fungus should contribute to the early determination of the initial inoculum potential for improving effective management of the disease. To achieve this goal, molecular markers were identified and specific PCR primers were developed to differentiate predominant races (Zhao et al. 2007; Wang XJ et al. 2008; Wang BT et al. 2010). Detection of *Pst* in the infected wheat leaves from greenhouse and field demonstrated that these primers were highly specific and sensitive for *Pst* detection and the predominant races. However, an appropriate sampling strategy will be needed for *Pst* detection in wheat leaves lacking visible symptoms under field conditions using these molecular methods.

Strategies for sustainable control of wheat stripe rust

Based on epidemiological considerations, it has been suggested that different resistance genes for controlling wheat stripe rust should be deployed in the defined over-summering, over-wintering, and eastern spring epidemic regions (Li and Zeng 2002; Wan et al. 2007). This would be best achieved by the use of different all-stage resistance genes in the oversummering and overwintering areas and of APR and HTAP resistances in the eastern areas. Historically, virtual monocultures of single resistance genes were deployed throughout the country leading to widespread 'boom and bust' cycles as exemplified by Bima 1, Lovrin varieties and derivatives (Wan et al. 2004), Fan 6 and derivatives especially in Sichuan, and predictably varieties with *Yr24/Yr26* in the near future. Furthermore, previous research indicated that southern Gansu, northwestern Sichuan, southern Shaanxi, and Yunnan are favorable for *Pst* over-summering due to their unique geographic features, climatic conditions and farming systems (Li and Zeng 2002). *Pst* can complete its asexual cycle in these areas where cropping at altitudes ranging from hundreds meters to over 2,000 m, creates a huge variation in seeding and harvesting times, as well as favorable climatic conditions (Li and Shang 1989; Li and Zeng 2002). Undoubtedly, these areas are hotspots for stripe rust and its survival in China (Li and Zeng 2002). Moreover, they are reserviors of pathogenic variability since new races are usually detected there (Li and Zeng 2002). Therefore control of stripe rust to reduce inoculum levels in these regions will have a profound effect on inoculum levels throughout the country. The following strategies have been suggested to control stripe rust in the over-summering areas:

(1) Use of resistant cultivars with multiple or different resistance genes, or effective multilines capable of reducing the build-up of inoculum. If varieties become susceptible they should be withdrawn from use.

(2) Reduction of the wheat area, particularly in highland regions. Farmers are being encouraged to grow alternative crops such as rapeseed, potato, beans, Chinese medicinal herbs and vegetables (Li and Zeng 2002). Thus, the pathogen population and disease severity are decreased, and pathogen

mutation and survival rates are also expected to decline.

(3) Use of seed treatments to reduce autumn-sown wheat infection (Chen et al. 1988).

(4) Removal of self-sown wheat in lowland areas where it acts as a host for over-summering *Pst*, by physical removal, plowing, spraying or grazing.

(5) Adjustment of seeding time to reduce or avoid early infections.

Actually, these strategies are already being put into practice and areas of wheat being grown in the over-summering regions, especially in southern Gansu and northwestern Sichuan, have significantly declined. For example, the area of wheat in southern Gansu declined by nearly 30,000 ha during 2003-2006, being replaced by potato, vegetables, fruit trees, corn, rapeseed, walnut, tea and Chinese medicinal herbs (http://www.gs.gov.cn). This accounts for 16.8% of the planned reduction in wheat grown above 1,600 m. However, the issue remains as to how much the area has to fall in order to protect the country's eastern wheat-growing areas. Farmers in the over-summering regions must keep some land for wheat in order to produce their own food.

Thus effective control of stripe rust in China remains a huge challenge as in many other parts of the world. A national project for the integrated control of stripe rust in the over-summering areas was initiated in 2009. Breeding wheat cultivars with effective and durable resistance has received much attention and is supported nationwide. The current situation of high dependence on fungicides to reduce yield losses has to be addressed by the development and greater use cultivars with adequate and durable resistance particularly in the over-summering and over-wintering regions of the country. Varieties in the more eastern high production areas should have at least some resistance for protection in years of high disease risk.

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Table 1 Areas of stripe rust occurrence, and control using fungicides, 1999-2009

Year	Area of occurrence (m ha)	Area of fungicide treatment (m ha)	Amount of fungicide used (tonnes)
1999	1.34	1.43	1502
2000	0.73	0.77	806
2001	3.13	2.77	2910
2002	6.60	5.65	5928
2003	4.95	4.59	4817
2004	3.56	4.66	4900
2005	3.19	4.53	4760
2006	3.43	4.76	5003
2007	2.89	4.31	4533
2008	1.81	2.71	2842
2009	4.08	5.94	6225

Table 2 Status of resistance to stripe rust in wheat cultivars tested in the epidemic regions in 2009

		Number and frequency		
Epidemic region	Total number	ASR[a]	of APR [b]	Susceptibie
South Gansu	80	12 (13.8%)	20 (26.2%)	48 (60.0%)
Sichuan basin	86	15 (17.5%)	45 (52.3%)	26 (29.2%)
Central Shaanxi & east Gansu and south Shanxi	106	4 (5.6%)	29 (26.8%)	73 (67.6%)
South Shaanxi-northwest Hubei-south Henan	72	3 (4.2%)	9 (12.5%)	60 (83.3%)
Yellow-Huai-River valley and Middle-lower reaches of Yangze River valley	172	0	20 (11.6%)	152 (88.4%)

[a]ASR, all-stage resistance

[b]APR, adult plant resistance

Implications of climate change for diseases, crop yields and food security

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Abstract Accelerated climate change affects components of complex biological interactions differentially, often causing changes that are difficult to predict. Crop yield and quality are affected by climate change directly, and indirectly, through diseases that themselves will change but remain important. These effects are difficult to dissect and model as their mechanistic bases are generally poorly understood. Nevertheless, a combination of integrated modelling from different disciplines and multi-factorial experimentation will advance our understanding and prioritisation of the challenges. Food security brings in additional socio-economic, geographical and political factors. Enhancing resilience to the effects of climate change is important for all these systems and functional diversity is one of the most effective targets for improved sustainability.

Key words: complex interactions, CO_2, extreme events, ozone, quality, resilience, temperature.

Introduction

Climates continually change and there is evidence for the effect of recent accelerated warming on many biological systems (IPCC 2007). Not least of these are the effects on the geographic distributions of pests and pathogens (e.g. Woods et al. 2005; Admassu et al. 2008; Elphinstone and Toth 2008), with potentially serious implications for food security. However, cropping systems will also change in response to climate, with consequent impacts on their interactions with pests and pathogens.

The focus of many assessments of climate change effects on crops has been the direct effects on potential yields driven largely by changes in temperature, CO_2 and water (Gregory et al. 2009). Pests and pathogens have major effects in determining actual yields in practice (Gregory et al. 1999) and the effects of climate change on pests and pathogens have been evaluated in some experimental and modelling studies (Garrett et al. 2006), but their consequences for yield were rarely assessed (e.g. Evans et al. 2008). Although in intensive monoculture a few major pathogens tend to cause the dominant epidemics, most crops are not only affected by a single pest or pathogen, but each will be affected in different ways.

To evaluate effects of climate change effectively we should consider the whole crop system, recognising multiple trophic levels of organisms, each of which may be affected differently by climatic variables. Not least are the effects on the community of organisms in the soil upon which the overall health of the crop substantially depends. As these comprise complex and dynamic communities of pests, pathogens, mutualists and parasites (degrees of symbiosis) that change in response to environmental triggers (Newton et al. 2010b), one might expect difficulty in being able to predict the consequences of climate change. The complexity of these interactions is illustrated in an ecological context where, in an extensive synthesis of published studies, various sub-sets of microbe-plant interactions are placed in several strength / frequency influence scenarios (Tylianakis et al. 2008). These show how the main drivers of change alter competitive interactions and generally increase pathogen infection.

To grasp the true picture of the complexity of biological interactions in crop systems and how they might interact, and to contribute effectively to the food security and climate change debates, we must also comprehend issues of scale and how to translate findings at plot-scale over a few seasons to larger spatial and temporal dimensions and subsequently their implications for food systems (Tubiello et al. 2007). The interacting driving forces of population increase, income growth, urbanization and globalization on food production, markets and consumption have changed food and agricultural systems worldwide (Von Braun 2007). Climate change and its increased variability compound the

effects of these changes and have serious consequences for food production and food security (Parry et al. 2004).

In this paper, we review the effects of climate change on some diseases and on the incidence and severity of some pathogen vectors, focussing on implications for yield. In particular we consider crop systems where organisms interact, possibly resulting in outcomes that differ from those predicted from responses of the organisms singly. It is in this latter context that outcomes must be considered in terms of implications for food security which also encompass important geographic, socio-economic and political considerations.

Local scale climate effects on crop production systems

As climate change has already affected crop production and predictions are of accelerated effects, we will look at how climate change affected one region local to the authors (Scotland) where detailed records were kept since the 1960s. These show not only changes in line with those predicted for Scotland, but also seasonal and regional shifts that can change field operations and thereby agricultural production in practice (Barnett et al. 2006). For example, winters have become over twice as wet in western regions, but in the east the increase in precipitation has been smaller and restricted to the autumn. Summers have become drier in some regions, particularly the main arable areas, but there has been no change or an increase in rainfall in others. Temperature changes also vary both regionally and seasonally, with no change in western areas in autumn, but up to a 2°C increase in the south-east during winter. Air frost and days with snow cover also show spatial and temporal variation, with changes ranging from no reduction to around 40 days less.

In practice, this means longer growing seasons but the lack of chill can affect development of some crops. For example, blackcurrants suffer uneven bud break and therefore lack of synchrony in ripening due to insufficient cumulative temperature below 7°C (Jones and Brennan 2009). This can reduce yield and quality as the whole crop is harvested by machine on one date. High summer temperatures can cause sterility in wheat ears (Porter and Gawith 1999). Wetter autumn or winters in some regions can affect access to the land for both harvest and sowing (Cooper et al. 1997), consequently it may not be possible to take advantage of the longer growing season. Summer drought can also severely limit yield in sensitive crops like potato, and cause premature senescence. The date of first or last frost also does not necessarily correlate with total frost days. The spatial variation in change may affect cropping patterns in different ways in different regions. Furthermore, year to year variability is very large so it is difficult to capitalise on changes over shorter periods. However, the requirements for breeding varieties suitable for resilience to such conditions are clear.

Macro-scale implications of climate change for agriculture and food security

Agricultural systems worldwide over the last 40-50 years have responded to the effects of the interacting driving forces of population increase, income growth, urbanization and globalization on food production, markets and consumption (Von Braun 2007). To these forces can be added the twin elements of climate variability and climate change which have direct effects on both food production and food security (Parry et al. 2004).

Although climate change may benefit crop production in northern latitudes above about 55°, where warmer temperatures may extend the growing season, in the developing world (especially sub-Saharan Africa) the projected changes are likely to have negative impact and will further complicate the achievement of food security. This is due to the observed and predicted deleterious impacts of climate change on agriculture, in particular in tropical and sub-tropical countries (Fischer et al. 2001; Parry et al. 2004; Stern 2007). Fischer et al. (2001) modelled the spatial variation in effects of climate change anticipated in 2050 on potential yields of rain-fed cereal crops worldwide and demonstrated that cereal producing regions of Canada, and northern Europe and Russia might be expected to increase production, while many other parts of the world would suffer losses, including the western edge of the USA prairies, eastern Brazil, Western Australia and many, though not all, parts of Africa.

Overall, the results of this and subsequent work demonstrated that climate change would benefit the cereal production of developed countries more than the developing countries even if cropping practices evolved to allow more than one rain-fed crop per year (Fischer et al. 2002; 2005). They concluded that production losses in some 40 poor, developing countries (mainly in sub-Saharan Africa) with a combined population of 2 billion, including 450 million under-nourished people, could drastically increase the number of those under-nourished.

There are three main factors underlying concerns about food security and climate change in the developing world. Firstly, many parts of the developing world are expected to experience significant changes in temperature and rainfall patterns. Climate assessments for southern Africa, for instance, conclude that the region will become warmer and drier (Hulme et al. 2001). A temperature increase of 2-5^{o}C is predicted over coming decades (IPCC 2007) and increasingly variable rainfall is anticipated, with the region becoming generally drier, especially in the east (Scholes and Biggs 2004). An increase in extreme events (both droughts and floods) is also anticipated (Tyson et al. 2002). The consequence of these combined changes is expected to be reduced yields of several staple food crops (Jones and Thornton 2003; Lobell et al. 2008). It is noteworthy, though, that seasonal rainfall variation in semi-arid Africa is already large, raising the question as to whether, in the medium-term, farmers will have to cope with anything that they are not already dealing with (Cooper et al. 2009). Secondly, developing economies are particularly sensitive to the direct impacts of climate change given their often heavy dependence on agriculture and natural resources, and because of their high poverty levels and geographic exposure (Stern 2007). Thirdly, many people in the developing world depend directly on agriculture as their primary source of food, and negative impacts on crop yields will affect total crop production and thereby overall food supply at the local level.

Effects of climate change on crop diseases

Disease complexes

Taking a traditional pathologist's approach, we might focus on pathogens, the 'causal agents' of disease and the effect of climate change on them. However, crop loss is attributable to direct and indirect effects of pathogens together with the effects of other biotic and abiotic agents. Direct effects of pathogens or other organisms can be the induction of resistance or susceptibility and its associated cost or benefit to the host plant (Newton et al. 2010a). Disease symptoms such as development of necrotic lesions can be due to a combination of a pathogen or pathogenic complex and in some circumstances non-pathogens of that crop such as bacterial species, exploiting an advantageous trophic environment (Newton and Toth 1999; Newton et al. 2004). Each component of the host-pathogen/microbe interaction and the pathogenic complex will have a different interaction with the environment, and thus will change under different climates (e.g. Stonard et al. 2008; Xu and Nicholson 2009). However, such associations of organisms in the disease complex are very variable due to their opportunistic nature and therefore represent another area of uncertainty in predicting the effects of climate change.

Many climate change experiments involve the manipulation of single parameters such as temperature or CO_2 and therefore do not simulate likely change scenarios where several parameters change simultaneously. These parameters will probably affect the host, disease and pathogen/pest complexes, in different ways through different mechanisms. Nevertheless, some trends can be shown that highlight where more resilience needs to be built into the system. Furthermore, the effects of many stresses are interactive, so it is frequently not possible to generalise.

For the major arable crops, particularly under intensive monoculture, a few pathogens often dominate. Epidemics can be both fast and severe where epidemiologically successful races of pathogens have adapted to the prevailing resistance mechanisms deployed. These are likely to have the most damaging economic impact, particularly rusts on cereals (Chakraborty et al. this volume) and are likely to have a large impact in terms of food security under climate change scenarios. The fact

that the above examples are from monocultures under high input situations underlines the vulnerability of such systems to change.

Temperature effects

There is evidence of the growing season being extended in many places, i.e. number of day-degrees (e.g. Barnett et al. 2006). In many countries this will enable earlier sowing and more crop species to be grown, but generally greater opportunities for increased disease pressure (Peltonen-Sainio et al. 2009). The effect of this is shown in the number of days after planting when the first outbreak of late blight occurs on potato in Finland, which decreased progressively over the 1990s (Hannukkala et al. 2007). However, it is difficult to find other examples of changes in disease incidence or severity that can be attributable directly to climate change rather than changes in cultivar, crop protection, agronomy or atmospheric pollution. For example, the abundance of the two common 'septoria' diseases of wheat, caused by *Phaeosphaeria nodorum* and *Mycosphaerella graminicola*, were studied from archive samples dating back to 1844 and, whilst seasonal variability could be attributable to weather factors, the only long-term variation was correlated with national SO_2 emissions (Shaw et al. 2008).

Many pathosystems, and it seems the cereal rusts in particular, are affected by temperature. At times about half of the wheat cultivars on the UK Recommended List (e.g. HGCA 2003) showed differential resistance expression when tested against isolates of brown rust (leaf rust) (caused by *Puccinia triticina*), either effective at 10°C and not at 25°C or *vice versa* (Jones and Clifford 1986-2002; Jones 2004-2007). The effect was not necessarily attributable simply to resistance gene expression response to temperature as isolates too showed differential temperature responses independent of resistance responses, and other authors reported similar effects (Dyck and Johnson 1983; Browder and Eversmeyer 1986). These differential responses to disease expression at different temperatures were also found against other rusts such as stripe rust (Gerechter-Amitai et al. 1984) and oat stem rust (Martens et al. 1967). In wheat stem rust the classical temperature sensitivity of the *Sr6* gene was extensively reported by Samborski and co-workers (e.g. Harder et al. 1979). However, such examples are not unique to rusts on cereals, but extend to other pathosystems involving monocot and dicot hosts and very different pathogens (Gregory et al. 2009). In rice, for example, some resistance genes, such as *Xa7* effective against some *Xanthomonas oryzae* pv. *oryzae* races, are more effective at high temperatures than low, whereas other resistance genes are less effective (Webb et al. 2010).

Greenhouse gas effects

In a majority of the examples reviewed by Chakraborty et al. (2000) disease severity increased with elevated CO_2 concentration, but in some examples it decreased. There can also be direct effects on pathogen growth; for example. the enhanced growth of *Colletotrichum gloeosporioides* infecting *Stylosanthes scabra* at high CO_2 (Chakraborty and Datta 2003). CO_2 can also affect pathogen fecundity which was shown to increase under elevated CO_2 levels leading to enhanced rates of pathogen evolution (Chakraborty and Datta 2003). *Arabidopsis thaliana* was more susceptible to *Erysiphe cichoracearum* under high CO_2 concentration, correlated with increased stomatal density and guard cell length, but there were inherent differences between ecotypes in this response (Lake and Wade 2009). In rice, enhanced susceptibility to *Magnaporthe oryzae* under elevated CO_2 was attributed to lower leaf silicon content (Kobayashi et al. 2006). Elevated CO_2 also reduced expression of induced resistance (Pangga et al. 2004) or affected expression of resistance more directly (Plazek et al. 2001; Plessl et al. 2005), but in resistant cultivars it also enhanced resistance by boosting phytoalexin production (Braga et al. 2006). In soyabean elevated CO_2 increased *Septoria* brown spot but reduced downy mildew (Eastburn et al. 2010). Pathogen-specific effects are common, and different stages of infection can be affected differentially with the cumulative effects resulting in changed disease levels (Plazek et al. 2001; Plessl et al. 2005). These cumulative effects may be due to specific differential responses to climate change at different stages of infection (Hibberd et al. 1996).

Elevated ozone can have a similar range of effects (Plazek et al. 2001; Plessl et al. 2005), such as a 3- to 5-fold increase in rust infection on poplar, but this response is reduced by elevated CO_2 (Karnosky et al. 2002). However, wheat stem rust was found to be strongly inhibited by ozone but unaffected by CO_2 (Tiedemann and Firsching 2000). High CO_2 also compensated for the negative effects of ozone on plant growth, but not the negative effects of the rust on yield.

Water, other effects and extremes

Debilitated crops under drought stress can have reduced impact or symptoms from pest or pathogen attack because they have reduced trophic value (Pennypacker et al. 1991; Huber and Gillespie 1992). However, resistance expression can also be changed and in particular reduced (Christiansen and Lewis 1982). Loss of resistance expression can be temporary due to stress or stress relief. For example, in barley a sudden relief of drought stress compromised the effectiveness of the *mlo* resistance gene against powdery mildew (caused by *Blumeria graminis* f. sp. *hordei*) (Newton and Young 1996). The significance of this is that speed of pathogen recognition and response is often crucial to effective defence and this effect is probably common to other resistance sources, but notably in *mlo* as it is particularly critical for its efficacy of expression. Stress-related and defence-related gene expression were shown to be compromised in stress-relieved barley plants compared to non-stressed plants (Barker 1998), with the differences in accumulation of defence gene transcripts correlated with levels of resistance breakdown in different barley genotypes (Stewart 2002). Enhanced levels of free radicals were found under drought-stressed conditions in the barley genotype showing the greatest loss of resistance, whereas other genotypes did not differ significantly (Goodman and Newton 2005). The same breakdown problem occurred in response to sudden relief of cold stress but not salt stress (Stewart 2002). We cannot therefore assume that resistance genes currently in use will continue to be equally effective under environments with changed abiotic stress such as water stress, as found in the temperature response of many rust resistance genes cited above.

Actions need to be taken to counter the effects of drought stress and to improve drought stress tolerance of crops generally, and the effects on resistance mechanism expression in particular. A first step will be more screening under stressed environments. However, we should also consider the role of other organisms in the crop environment or within the crop itself. For example, the grass *Elymus virginicus* was only half as much affected by drought if infected with an endophyte *Epichloe elymi* (Rudgers and Swafford 2009). Endophytes have largely been excluded by the approaches used in major crop breeding programs as well as common agronomic practices.

Pathogen adaptation

Plant pathogens are generally highly adaptable and likely to exploit any compromise in plant defence caused directly or indirectly by climate change. At a population level, the adaptive potential of pathogens may prove to be the most important predictors of the magnitude of climate change effects (Garrett et al. 2006). However, some changes, such as increased drought, may result in fewer periods with good infection conditions as the presence of free water, high humidity or open stomata is required by many pathogens for initial infection.

Instability of variation is common in many pathogens, although the mechanisms are often not known (Newton 1988). Under stressed conditions some organisms enhance their ability to generate variants by, for example, stimulation of retrotransposon activity in pathogens such as *Fusarium oxysporum, Ophiostoma ulmi* and *Ophiostoma nono-ulmi* (Anaya and Roncero 1996; Bouvet et al. 2008), and certain transposon activity regulation mechanisms in response to particular stress factors are known (Twiss et al. 2005). Elevated mutation rate mechanisms in response to stress have been found in other fungi (Hastings et al. 2000), particularly in response to increased reactive oxygen species (Blanco et al. 1995). Other mechanisms found in fungal pathogens might include alternative intron processing (Costanzo et al. 2007; Ho et al. 2007), alternate splicing of mRNA (Halterman et al. 2003) and other post-translational regulatory mechanisms, all of which may be a way of generating

enhanced levels of variation for adaptation without adding to a disadvantageous mutation load (Newton 1988). This leads to enhanced genome plasticity enabling organisms to respond rapidly to environmental changes. Such changes can later become fixed through conventional mutation and recombination.

Effects of climate change on vectors of crop pathogens

The effect of climate change on invertebrate pests and vectors is gradually becoming better characterised, based largely on evidence from both historical records and experimental manipulation. In the case of historical records, the best studied examples have examined past trends for phenological events and prevailing air temperatures (Harrington et al. 1995; Zhou et al. 1995). In particular, aphid vectors of crop diseases have been recorded over several decades using suction traps across Europe (http://www.rothamsted.ac.uk/insect-survey/) (Harrington et al. 2007). This has established clear linkages between winter temperatures and first emergence of key aphid pests, with those that overwinter in non-egg stages (e.g. as adults or larvae) being particularly tightly correlated with winter temperatures (Harrington et al. 1995; 2007). For example, date of first recorded occurrence of the peach-potato aphid (*Myzus persicae*) was negatively correlated with mean January and February temperatures across 14 sites in the U.K. (Fig. 1). Amongst this extensive dataset, important vectors of cereal diseases include the bird cherry-oat aphid (*Rhopalosiphum padi*) and the grain aphid (*Sitobion avenae*). Since winter temperatures are widely predicted to increase (IPCC 2007), it seems inevitable that some aphid vectors will arrive, or become active, earlier (Harrington et al. 2007). In terms of managing plant diseases, this will require increasingly early pest management procedures (e.g. pesticide applications). This is problematic, not only because of increasingly restrictive pesticide legislation (e.g. Directive 91/414/EEC) (Copping 2008), but also the unfavourable logistics and costs of treating crops earlier in the season (e.g. water logged fields).

FIGURE 1 HERE

Whilst not explicitly concerned with crop pests and disease vectors, examination of historical records recently showed the earlier emergence of numerous animal and plant species between 1976 and 2005 (Thackeray et al. 2010). Many of the invertebrates included in the study were likely pests of crops and vectors of plants diseases, but many were also likely natural enemies and parasitoids which may help to control populations of such pests. In particular, this study suggested that such secondary consumers were slowest to respond to advances in timing, which may therefore lead to asynchrony between the life-cycles of predators and prey (Thackeray et al. 2010). To our knowledge, the effects of climate change on such eco-system services have not been explicitly considered, but understanding how populations of antagonists of crop pests will respond to changes in prey density and attendant changes in the climate must warrant further attention. Gregory et al. (2009) point to the dramatic escalation in the numbers of ladybird predators in the U.K. following increases in aphid numbers during particularly warm summers of 1975 and 1976, so the extent to which these will be affected by extreme events should also be considered.

In terms of experimental approaches to investigating how climate change will affect plant diseases vectored by insect pests, there are still surprisingly few examples (Gregory et al. 2009). However, there is evidence that elevated CO_2 could compromise crop resistance to some insect pests (Zavala et al. 2008; 2009). Most recently, this has been reported for resistance in red raspberry (*Rubus idaei*) to the European large raspberry aphid (*Amphorophora idaei*) (Martin and Johnson 2010). As a vector of at least four plant viruses (including black raspberry necrosis virus, raspberry leaf mottle virus, raspberry leaf spot virus and Rubus yellow net virus) *A. idaei* is considered to be the most significant disease vector of this crop (McMenemy et al. 2009). Martin and Johnson (2010) reported that under elevated CO_2, the number of aphids on a cultivar containing A_1 resistance (McMenemy et al. 2009) became equivalent to an entirely susceptible cultivar (Fig. 2a), with corresponding increases in body mass (Fig. 2b). Plants with A_{10} resistance (McMenemy et al. 2009) were seemingly more

resistant at ambient and elevated carbon dioxide conditions (Fig. 2), but this still represents a potentially worrying development in terms of disease transmission in raspberry.

FIGURE 2 HERE

Direct effects of climate change on yield and quality

The major emphasis of climate change/food security research over recent years has been concerned with the impacts of climate change on crop yield. For example, Gregory et al. (1999) summarized experimental findings on wheat and rice crops that indicated decreased crop duration (and hence yield) of wheat as a consequence of warming, and reductions in yields of rice of about 5% per °C rise above 32°C. More recently, the modification of crops and management systems to cope with changed temperatures demonstrated what adaptation might be possible. For example, simulation of production for cropping systems in northern and central Italy showed that the combined effects of increased [CO_2] and climate change would depress crop yields by 10-40% if current management practices were unamended largely because the warmer air temperatures would accelerate the phenology of current cultivars (Tubiello et al. 2000). Through a combination of early planting of spring and summer crops and the use of slower-maturing winter cereal cultivars, though, the model indicated that it should be possible to maintain present yields. However, a major caveat to this conclusion was that 60-90% more irrigation water was required to maintain grain yields under conditions of climate change; this water was assumed to be available (Tubiello et al. 2000). More recently, elements of crop adaptation to extreme weather events have been explored with genotypic variation and adaptation able to compensate for several of the negative impacts on unadapted productivity (e.g. Fuhrer 2006; Challinor et al. 2007).

New cropping systems which are resilient to changed climate conditions are required. It is highly probable that the changes of climate and [CO_2] will occur sufficiently slowly that changes to sowing date, cultivar, crop and other management practices will allow at least some adaptation of the production system by farmers. Several adaptations are conceivable in the timescale available including:

- Crop selection to determine mechanisms and sources of durable disease resistance
- Crop selection to identify mechanisms and sources of resistance/resilience to abiotic stresses including drought and cold
- Genetic enhancement to cope with more variable growing conditions
- Development of new crops to take advantage of more favorable growing conditions

Extreme events may also become more frequent in the future increasing the risks to crop yields, although this is not always so and is often dependent on the precipitation scenario that is considered (Porter and Semenov 2005). The effect of extremes is illustrated by events in the summer of 2003 in parts of Europe, where temperatures were 6°C warmer than long-term means and precipitation deficits of up to 300 mm were recorded (Tubiello et al. 2007). As a consequence, parts of the European Union such as the Po valley in Italy, reported a record reduction in maize yield of 36% (Ciais et al. 2005).

Whilst model predictions of crop responses to projected climate changes are numerous, relatively few assessments have been made of the effects on crop quality. Quality is a complex issue involving the whole food chain from growth through storage and processing to cooking and consumption (Porter and Semenov 2005). One of the few quality traits investigated was that of rheological properties of wheat flour for bread-making (the Hagberg Falling Number, HFN). HFN is a measure of α-amylase activity in which a high HFN indicates a low activity – a desirable property of wheat for bread-making. Kettlewell et al. (1999) demonstrated how climate variation, expressed in terms of the North Atlantic oscillation (NAO, linked to sea surface temperatures) from year-to-year influenced the HFN of wheat in the U.K. during the period 1972 to 1996. HFN was positively

correlated with NAO index for January and February and this had major consequences for the bread-making industry. In years of low NAO and HFN (wet years) about 45% of U.. milling wheat was imported decreasing to 25% in years of high NAO and HFN.

Pests and diseases: implications for food security

The impacts of pests and diseases on yield in current conditions are well known, but the consequences of climate change on pests and diseases are complex and, as the preceding descriptions attest, are still only imperfectly understood. Scherm et al. (2000) highlighted the importance of pests and diseases both as important yield-reducing factors and as early indicators of environmental changes because of their short generation times, high reproductive rates and efficient dispersal mechanisms. It is already clear that some pests will be able to invade new areas and become increasingly problematic for the maintenance of biodiversity, the functioning of ecosystems and the profitability of crop production. Some pests which are already present, but only occur in small areas, or at low densities, may be able to exploit the changing conditions by spreading more widely and reaching damaging population densities. Aphids for instance, key pests of agriculture, horticulture and forestry throughout the world, are expected to be particularly responsive to climate change because of their low developmental threshold temperature, short generation time and dispersal abilities (Sutherst et al. 2007).

Many assessments of climate change effects on crops have focused on potential yields (e.g. Fischer et al. 2001) but factors such as pests and pathogens have major effects in determining actual yields (Gregory et al. 1999). The importance of including pathogens can be seen, for example, in the work of Butterworth et al. (2010). They combined a crop yield simulation model with a weather-based epidemiological model and showed that under 2020 and 2050 climate change scenarios fungicide-protected oilseed rape will increase in yield in Scotland by 15%, whereas in England yields could decrease by 50%.

Ingram et al. (2008) point out that more mechanistic inclusion of pests and disease effects on crops would lead to more realistic predictions of crop production on a regional scale and thereby assist in the development of more robust regional food security policies. Economic and social issues are often dominant determinants of household food security, but changed pest and disease incidence under climate change is an essential influence on future regional food security.

Another aspect of disease control is the CO_2 cost. Yield has a cost in terms of inputs that can be calculated in CO_2 equivalents per hectare. It therefore stands to reason that preventing yield loss increases efficiency that can be given a value. If it is achieved through use of crop protectants, their costs of production and application must be deducted, and similarly any additional cost of elite germplasm if it is achieved through plant resistance. It is calculated that control of disease in oilseed rape in the U.K. by fungicides reduces emissions by 100 kg CO_2 eq. t^{-1} of seed (Mahmuti et al. 2009). For wheat it is estimated that in the U.K. fungicides save 59 kg CO_2 eq. t^{-1} of grain and that a further 14 kg CO_2 eq. t^{-1} could be achieved if all diseases were eliminated by fungicides or resistance that carries no yield penalty (Berry et al. 2008). Therefore efficient pest and disease control is important for both adaptation to, and mitigation of, climate change.

Managing crop systems for resilience

Biodiversity is regarded as a valuable source of novel resistance. Whilst undoubtedly true, major resistance genes obtained from alien germplasm are rarely durable, but their value should be measured by the opportunities they give for deployment. This can be through combining several different and preferably contrasting genes into a single genotype, but also the ability to deploy many such different new cultivars. In the latter case, this lessens the selection pressure to overcome resistance in any one cultivar (Newton et al. 2009). Therefore, the scale of cultivar deployment should also be considered.

Epidemics are a feature of crop monocultures under high input situations. Contrast the opposite extreme of long-established natural ecosystems where plant, pests and pathogens are all found in much greater genotypic diversity, and we find small and localised epidemics but an absence

of large epidemics. Under climate change we would expect such ecosystems to adapt, using the wealth of diversity to confer resilience on the system. We need to deploy this functional diversity intelligently in agricultural systems to confer resilience at appropriate scales (Newton et al. 2009). In the context of pests and pathogens, this means heterogeneity of deployment of resistance at scales appropriate to their dispersal mechanisms (Marshall et al. 2009). This can easily be implemented within field or between fields, but is difficult to implement at a regional scale since cultivar breeding has become more international.

Changes in land use will have many implications for climate change (Dale 1997). Introduction of crops to regions where they were previously not grown and consequent changes to crop rotations in response to climate change may also influence the future prevalence and importance of specific pathogens. An example would be growing maize for forage or grain in more northern latitudes. In a rotation with cereals this will leave residues in which pathogens such as *Fusarium* species could build up high levels of inoculum causing severe Fusarium Head Blight (FHB) on subsequent wheat and barley crops (Maiorano et al. 2008). Effective deployment of crop diversity across all scales from individual plant interactions to regions results in slowed progress of crop epidemics, improved resource utilisation and enhanced stability of yield and quality. These, in turn, combine to enhance crop resilience to the effects of climatic stress, and stresses in general, thereby improving crop performance (Newton et al. 2009).

Phytosanitation will reduce Fusarium problems, but not eliminate them. Pesticides can and should be used in conjunction with effective resistance deployment strategies to reduce pest and pathogen populations, but not in a way that selects strongly for pesticide resistance. International phytosanitation measures, plant passports, and quarantine measures will slow the progress of pathogens entering new areas, but rarely prevent them for ever. However, all these measures – new disease resistance, deployment of resistance heterogeneously, and phytosanitation, all have roles to play in building resilience to changes likely to accelerate due to climate change. These need to be supported at local, national, regional and international levels through policy and extension service bodies where some degree of agreement and co-ordination would be desirable, including legislation (Fig. 3).

FIGURE 3 HERE

We noted above that a grass was only half as much affected by drought if colonised by an endophyte (Rudgers and Swafford 2009). Indeed endophytes may confer both abiotic and biotic stress tolerance in grasses, including cereals, and this feature could be targeted as a breeding objective (Newton et al. 2010a). Endophytes are a component of grassland breeding and development, and are widely recognised as conferring advantages for productivity. They are largely absent from major crop varieties and high soil disturbance agronomic approaches mitigate their exploitation. However, in minimum and no-tillage systems they might be exploited to enhance resilience to abiotic stresses in general. Recent evidence shows that under long-established reduced tillage situations many genotypes of barley respond differentially (Newton et al. 2010c). This is likely due not only to adaptation to changed soil physical conditions including water availability, but also the soil microbial environment and its component balance of pathogens, parasites and mutualists (symbionts) conferring enhanced resilience on some crop genotypes (Newton et al. 2010b).

Conclusions

The implications of climate change for crop pests and diseases cannot easily be summarised except to point out that they are highly adapted to exploit opportunities in general. Specific predictions will be difficult because of the complex multi-trophic relationships between many microbial organisms including pathogens, their vectors and their respective host plants. Nevertheless, such mechanistic understandings under likely multi-factorial climate change scenarios need to be obtained through linked experimentation and modelling.

The effects of disease on crops may be large but climate change will also have direct effects, complicating analyses. Diversity to give more options and build spatial and temporal heterogeneity into the cropping system will enhance resilience to both abiotic and biotic stress challenges. Other resilience sources will be more robust genetic resistance and biochemical response mechanisms.

Food security is a complex issue as it is the summation of these effects and many other socio-economic, geographic and political factors. However, our understanding of the relative importance of these factors is improving, particularly as models hitherto discipline-bound are being linked together. Nevertheless, major epidemics in staple crops grown in non-resilient agricultural systems can have large and significant impacts on food security notwithstanding these complexities.

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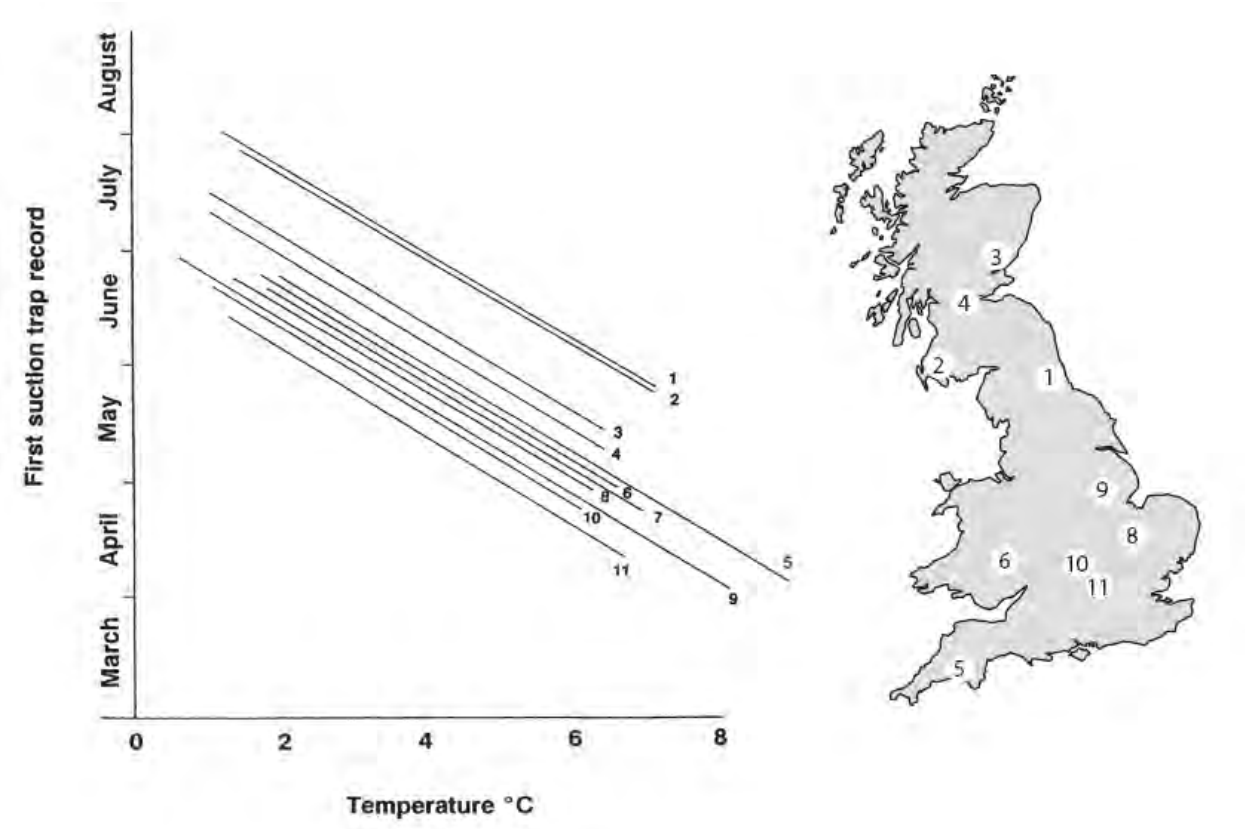

Fig. 1 Date of the first record of *Mysus persicae* (regression lines shown) in relation to mean temperature in January and February at different latitudes across the U.K., indicated on the map (1) 55.2° Newcastle, (2) 55.5° Ayr, (3) 56.5° Dundee, (4) 55.9° Edinburgh, (5) 50.6° Starcross, (6) 52.1° Hereford, (7) 51.2° Wye (8) 52.3° Broom's Barn, (9) 52.9° Kirkton, (10) 51.8° Rothamsted, and (11) 51.7° Writtle. Reproduced from Harrington et al. (1995)

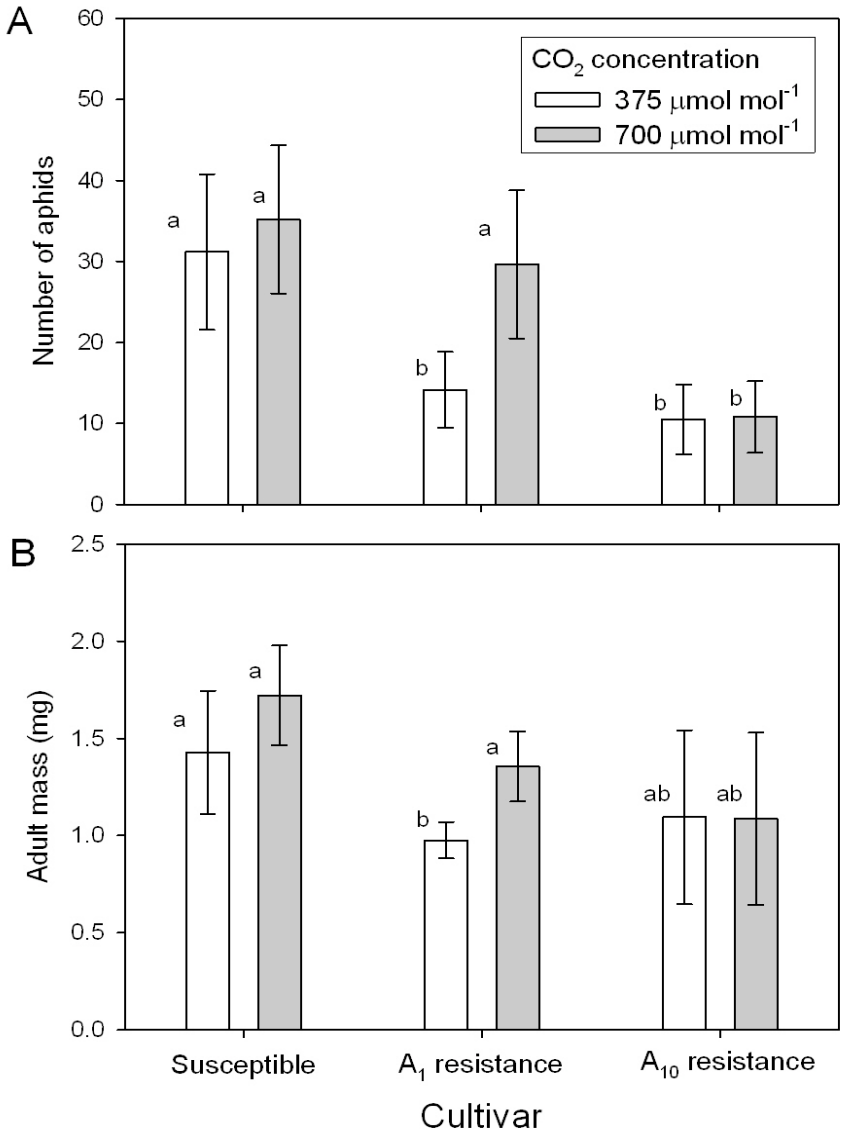

Fig. 2 Performance of European large raspberry aphid (*Amporophora idaei*) on three cultivars with different levels of resistance; susceptible (Malling Jewel), A1 gene (Glen Lyon) and A10 gene (Glen Rosa) at ambient and elevated CO_2 conditions. (A) Total population size and (B) Individual adult mass. Mean values ± S.E. shown (N = 6). Lowercase superscripts indicate statistically significant ($P < 0.05$) differences. Reproduced from Martin and Johnson (2010)

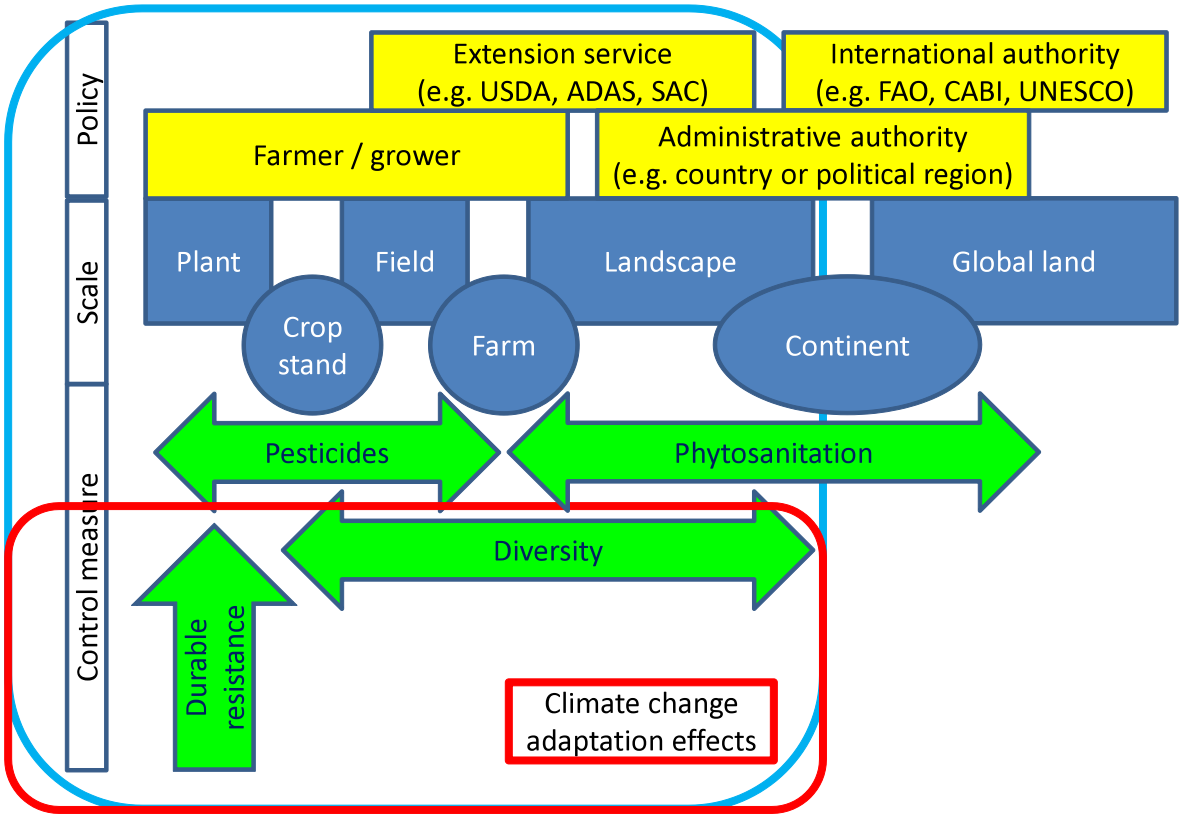

Fig. 3 Influences on pest and disease threat to food security. Yellow boxes are policy influences ranging from individuals to international organisations that can co-ordinate appropriate actions and transfer and exchange knowledge (KTE). Blue shapes are crop production scale relative to KTE bodies. Green arrows are strategies or measures that contribute to controlling disease that may be applied at different scales. The red box enclosed measures are particularly relevant to climate change effects, the blue box indicating the scale and relevant type of KTE bodies. Acronyms: USDA = United States Department of Agriculture; ADAS = Agricultural Development Advisory Service; SAC = Scottish Agricultural College; FAO = Food and Agriculture Organisation; CABI = Commonwealth Agricultural Bureau International; UNESCO = United Nations Educational, Scientific and Cultural Organization

Rust-proofing wheat for a changing climate

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Abstract This paper offers projections of potential effects of climate change on rusts of wheat and how we should factor in a changing climate when planning for the future management of these diseases. Even though the rusts of wheat have been extensively studied internationally, there is a paucity of information on the likely effects of a changing climate on the rusts and hence on wheat production. Due to the lack of published empirical research we relied on the few published studies of other plant diseases, our own unpublished work and relevant information from the vast literature on rusts of wheat to prepare this overview. Potential risks from a changing climate were divided into three major groups: increased loss from wheat rusts, new rust races evolving faster and the reduced effectiveness of rust resistances. Increased biomass of wheat crops grown in the presence of elevated CO_2 concentrations and higher temperatures will increase the leaf area available for attack by the pathogen. This combined with increased speed of the pathogen's life cycle, may increase the rate of epidemic development in many environments. Likewise, should the effects of climate change result in more conducive conditions for rust development there will also be a corresponding increase in the rate of evolution of new and presumably virulent races. The effectiveness of some rust resistance genes are influenced by temperature, crop development stage and even nitrogen status of the host. It is likely that direct and indirect changes on the host from climate change may influence the effectiveness of some of these resistance genes. Currently the likely effects of climate change on the effectiveness of disease resistance is not known and since disease resistance breeding is a long term strategy it is important to determine if any of the important genes may become less effective due to climate change. Studies must be made to acquire new information on the rust disease triangle to increase the adaptive capacity of wheat under climate change. BGRI leadership is needed to broker research on rust evolution and the durability of resistance under climate change.

Keywords climate change, elevated CO_2, epidemiology, evolution of virulence, rust resistance, wheat rust

Introduction

According to the latest assessment by the Intergovernmental Panel on Climate Change, atmospheric concentration of radiatively active gases including CO_2 has risen rapidly since 1750 (IPCC 2007). These gases trap solar radiation creating a 'greenhouse effect', which along with other factors increase the surface temperature of the earth. Changing rainfall patterns and the frequency and severity of extreme weather events, such as droughts, floods, heat waves, and hurricanes, are among other effects of climate change. Rising CO_2 concentrations and perturbations in climate are increasingly influencing agriculture (Stern 2007) by shifting crop growing regions and affecting food production.

Depending on nutrient and water supply, crop yields can increase by up to 30% from a 'fertilization effect' at elevated atmospheric CO_2 (Ainsworth and Long 2005) and increases of 20% are common for wheat (Norton et al. 2008; Högy et al. 2009). These projections do not consider the effect of other climatic factors or the impacts of pest and diseases. Changes in temperature, for example, can potentially reduce wheat productivity in regions such as the high yielding Indo-Gangetic Plains (Ortiz et al. 2008). In Australia, the likely impacts of increased CO_2 concentrations and associated temperature and rainfall changes on wheat yields will vary among regions, with significant yield reductions in Western Australia, but a moderate yield increase in north-eastern Australia (Howden and Jones 2004). Similarly, pests and diseases will interact with crops to limit yield increases with rising CO_2 but how this might affect future food security has not been addressed (Gregory et al. 2009; Mahmuti et al. 2009).

Climate influences the frequency and severity of disease epidemics and some studies link the abundance of wheat pathogens to changing atmospheric composition (Shaw et al. 2008). Wheat scab (caused by *Fusarium graminearum*) and wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*) severity in China and stem rust (*P. graminis* f. sp. *tritici*) severity in the USA have been linked to the El Niño-Southern Oscillation Index (Scherm and Yang 1995), and the prevalence/severity of wheat diseases, including leaf rust and stripe rust in Sweden (Wiik and Ewaldz 2009) to long-term climatic conditions. Among diseases of other crop plants, changes in the severity of potato late blight (Hannukkala et al. 2007) and pine needle blight (Woods et al. 2005) severities were also linked to long-term climate changes. These studies indicate that climate change modifies disease and pest risks and increases uncertainty in risk predictions associated with climate change.

Models predicting changes in geographical distribution of host plants and their diseases under a changing climate can serve as a guide to future disease prevalence (Bergot et al. 2004). However, whether current disease management strategies including the use of resistant varieties will be effective cannot be ascertained without detailed knowledge of host-pathogen biology under conditions simulating future climates. New rust pathogen races, such as *Puccinia graminis* f. sp. *tritici* race 'Ug99' (Pretorius et al. 2000), can pose significant threats to global wheat production. This race is virulent to the resistance gene *Sr31* that has been effective worldwide for more than 30 years (Wanyera et al. 2006). For example, with a 10% yield loss, damage from Ug99 could amount to US$1–2 billion in Asia alone (Duveiller et al. 2007). Screening of advanced lines in East Africa combined with a program to pyramid resistance genes is now underway through the Borlaug Global Rust Initiative (BGRI, http://www.globalrust.org). Because there has been no research on rust micro-evolution under rising temperatures and CO_2 levels, future appearance of new races under climate change cannot be predicted from current knowledge. Little is known about the potential effect of changing atmospheric composition or physical climate on the biology of cereal rusts, rust resistance of the host plant or the host-pathogen interaction and no assessments of climate change impacts on wheat rusts could be found in the literature. There were only a few published empirical studies on diseases other than rusts under realistic field conditions. The effects of elevated CO_2 in these studies varied with the host-pathogen combination (Karnosky et al. 2002; McElrone et al. 2005; Kobayashi et al., 2006; Eastburn et al. 2010; Melloy et al. 2010), but none addressed wheat rusts. Therefore, it is difficult to project 'generalized effects' of climate change on the rusts of wheat.

In a recent two-year scoping study we considered some aspects of wheat stripe rust biology in the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility. This paper gives an overview from this and other relevant studies on the potential effects of climate change, and suggests research needs to address climate change in combating the threat of wheat rusts.

Direct effects of climate change and variability

Changes in atmospheric composition and the physical climate including temperature, rainfall and humidity will no doubt affect the economic importance, geographical distribution and management of rusts of wheat ultimately effecting wheat production and food security. These effects are conveniently grouped in two classes. Firstly, changes in the effectiveness of host plant resistance and pathogen

fitness due to modified host and pathogen biology, and host-pathogen interaction. The effectiveness of resistance in wheat varieties could change under a changed climate and/or resistance may be overcome quickly by new races, which may evolve at a different rate in a changing climate. Secondly, geographical distribution of wheat rusts and consequently their relative economic importance may change. If the new environments are highly suitable for wheat, the rusts may become more damaging further increasing economic importance. If however, wheat growing regions become less suitable for wheat growth, rusts may become less important. Changes in host plants, pathogen populations, host-pathogen interactions and the geographical distribution of wheat cropping areas will each potentially affect the economic importance and management of rusts.

Effects mediated through changes in the host plant

Plants grown at elevated CO_2 have changed morphology, anatomy, chemical composition and gene expression profiles in addition to increased biomass and yield. For instance, the stimulation of photosynthesis at elevated CO_2 levels lead to increased production and accumulation of carbohydrates in plant organs, generally leading to reduced protein in grain (Kimball et al. 2000), which can influence bread-making quality of wheat flour (Rogers et al. 1998). However, changes in fiber and protein content of wheat do not change forage quality or digestibility (Akin et al. 1994). Increased starch and decreased protein concentration in the grain coincide with increased width of chloroplasts, whose shape changes from elliptical in ambient to round in elevated CO_2 (Sinha et al. 2009). The implications of these changes are not clearly understood. Stomatal density and conductance, wax and epidermal cell layers, number of mesophyll cells and fiber content are among other changes occurring at elevated CO_2 concentrations (Wittwer 1995). These changes are modulated by rising temperature and other changes in climate including the microclimate of an enlarged wheat canopy (Pinter et al. 2000), but insufficient knowledge makes any impact assessment speculative. Wheat varieties differ in their response to elevated CO_2 but this has only been considered in a very few recent studies (Ziska 2008).

The physiology, biochemistry and molecular biology of host-pathogen interactions have been extensively studied in the rusts of wheat and many reviews are available (Eversmeyer and Kramer 2000; Line 2002; Singh et al. 2002; Leonard and Szabo 2005). In stem rust, for example, a urediniospore germinates to produce a germ tube, which swells to form an appressorium over stomatal openings. Further development is dependent on a reduction of CO_2 concentration in the stomata among other factors and penetration can be inhibited by high CO_2 (Yirgou and Caldwell 1968). Infection by *P. graminis* alters the direction of phloem transport and massive accumulation of cytokinins and sugars in the lesion area resulting from highly regulated host and pathogen signaling. Detailed studies on how altered host plant physiology at elevated CO_2 concentrations influence rust infection are lacking, but reduced stomatal density, production of papillae and accumulation of silicon at the sites of appressorial penetration (Hibberd et al. 1996a) and changed leaf chemistry at high CO_2 concentrations increased resistance to powdery mildew (*Blumeria graminis*) in barley (Hibberd et al. 1996b) and other pathogens in other crops (McElrone et al. 2005; Hassan et al. 2008). In some crops, increased production of secondary metabolites at elevated CO_2 has been linked to increased disease resistance. At high CO_2 phenylpropanoids, coumarin scopoline and scopoletins are significantly increased to enhance resistance to potato virus Y in tobacco (Matros et al. 2006); and in soybean, phytoalexin glycceollin production is stimulated in a resistant, but not in a susceptible variety (Braga et al. 2006).

In wheat, the expression of many genes for resistance to leaf rust (*P. triticina*) (Kolmer 1996), stripe rust (Singh et al. 2000; Datta et al. 2009) and stem rust (Leonard and Szabo 2005) is influenced by temperature and/or plant developmental stage. Some resistances may become less effective at high temperature. For example, rust reaction of wheat varieties with the stem rust resistance gene *Sr15* can change from resistant at 15^{o}C to nearly fully-susceptible at 20^{o}C (Roelfs 1988). Anecdotal evidence of a weakening of stripe rust resistance and pathogen adaptation due to temperature increases has come from annual race surveys in the Eastern USA (Markell and Milus 2008), but these findings may

also reflect a change in the pathogen population, described in the following section. Conversely some stripe rust resistance genes, such as *Yr18*, are known to be temperature mediated and become more effective at higher temperatures (Park et al. 1992), therefore in varieties with this resistance there may be an enhancement of the effectiveness of resistance in a warming climate.

Changes in plant physiology, morphology, anatomy and gene expression will also influence the effectiveness of other rust control measures including the use of fungicides. The crucial role of plant physiology and morphology in the uptake and translocation of fungicides is well known (O'Leary and Jones 1987) as is the influence of systemic fungicides on plant physiology including photosynthesis and gas exchange (Grubler et al. 2009). Effectiveness of systemic fungicides could be reduced by changes such as smaller stomatal opening or thicker epicuticular waxes that slow down uptake rates. On the other hand, increased metabolic rates at high temperature could increase uptake, but empirical studies are lacking to allow realistic predictions. Other climatic factors will also interact, including more frequent rainfall events that will wash away residues of contact fungicides, triggering more frequent applications. Currently the potential effect of climate change on the efficacy of foliar applied fungicides for the control of rusts of wheat is not known. It is likely that should any changes in the host due to climate change reduce the efficacy of fungicides, higher rates will be warranted.

Effects mediated through changes in the pathogen / host-pathogen interaction

In a growth cabinet study of wheat, Tiedemann and Firsching (2000) observed strong inhibition of leaf rust development at elevated O_3, but not at elevated CO_2. By enhancing photosynthesis, elevated CO_2 compensated for the adverse effects of high O_3 in both leaf rust-inoculated and non-inoculated plants. Pfleeger et al. (1999) contended that whether a disease is influenced by O_3 depends on the timing of pollutant exposure and the infective periods of a particular host/pathogen combination. These authors concluded that while obligate parasitism is generally inhibited by elevated O_3, infection by facultative pathogens can increase, decrease, or remain unchanged. Ozone predisposition of plants to pests and diseases was recently summarized by Chakraborty et al. (2008) and is not considered here.

During the 2007 and 2008 wheat seasons we studied progress of stripe rust development and changes in the biology of *P.*f. sp. *tritici* on adult plant susceptible H45, the partially resistant Janz with the adult plant resistance genes *Yr18*, and others grown at ambient (380 ppm) and elevated (820 ppm) CO_2 concentrations in the AGFACE facility at Horsham, Victoria, with or without irrigation. Description of the AGFACE facility appears in Mollah et al. (2009). Stripe rust was induced by inoculating a section of each row. Latent period was assessed as the number of days from inoculation to the appearance of the first visible stripe rust lesion.

Stripe rust severity was visually assessed over time as the percentage of leaf area affected enabling determination of the area under the disease progress curve for each treatment. The partially resistant cultivar significantly reduced disease severity, and while the application of irrigation increased disease severity, this effect was not significant in either season. CO_2 did not have a significant effect on disease severity in either 2007 (Fig. 1) or in 2008. The strong varietal effect and the smaller irrigation effect on disease progress were as expected. There was no interaction between CO_2 and variety to indicate any change in the ranking of the two varieties and the effectiveness of the partial resistance within the cultivar Janz was not changed in the presence of elevated CO_2. This is important information for plant breeders developing wheat cultivars for future climates, particularity with increased effort in the breeding of wheat with multiple genes conferring partial resistance. In this experiment total leaf area, and total spore production, was not measured, so it is not known if the increased leaf area from the elevated CO_2 concentrations resulted in increased spore production from the crop canopy.

FIGURE 1 HERE

Latent period, determined from the 2008 season only, remained unchanged at 14 days at both CO_2 concentrations for both varieties. This result was unexpected, because, even in the absence of elevated CO_2, the two varieties otherwise were expected to differ in latent period and the study needs to be repeated.

For fecundity assessment infected leaves were sampled and the cumulative number of urediniospores per unit pustule area was determined by washing and counting spores using a haemocytometer. Following the first wash, leaves were incubated in the field in moist Petri dish for 24 h at the respective CO_2 concentrations and a further urediniospore suspension was recovered. The daily rate of urediniospore production was similarly determined from the second suspension. Leaf segments were blotted dry, scanned and the area covered by pustules was calculated and analysed using 'Image Tool' image analysis software from the University of Texas Health Science Center at San Antonio and downloadable from the web. Results from 2007 showed no significant differences between CO_2 concentrations or wheat varieties. Similar findings came from the 2008 data with no significant difference between CO_2 concentrations, but the susceptible H45 produced significantly more urediniospores per day than the partially resistant Janz. However, as with stripe rust severity, at a plant or paddock level *P. striiformis* f. sp. *tritici* inoculum would increase many fold even though urediniospore production per unit leaf area did not increase.

Previous growth chamber studies with rubber vine rust (caused by *Maravalia cryptostegiae*, Chakraborty et al. unpublished) and the anthracnose pathogen, *Colletotrichum gloeosporioides* (Chakraborty et al. 2000) showed significant increases in fecundity at high CO_2 for both pathogens. The AGFACE results highlight the difficulty in obtaining precise data on fecundity and latent period from the variable field environment and future studies should consider using more controlled environments. However, even with no intrinsic change in fecundity, a 20-30% increase in wheat biomass under elevated CO_2 (Kimball et al. 1995) will significantly increase inoculum size to potentially increase their dispersal and severity, but further studies are required to test this hypothesis.

It is likely that rusts of wheat will be able to adapt to increased temperature associated with climate change. Recent studies suggest that rust pathogens can adapt to different optimal temperatures. Annual race surveys in the Eastern USA showed that contemporary *P. striiformis* f. sp. *tritici* races have different temperature optima and virulence profiles than races collected before 2000 (Markell and Milus 2008). Latent period and spore germination studies indicated that the contemporary population was better adapted to high temperature (Milus et al. 2006). Both populations had similar latent period and spore germination rates at 12ºC, but at 18ºC, isolates of the new population had shorter latent periods and higher germination rates than the old isolates. Such adaptation may have been responsible for the more widespread stripe rust epidemics covering at least 20 central and southern states of the USA in 2000 (Milus et al. 2006). Further studies showed that recent severe stripe rust epidemics were most likely magnified by increased pathogen aggressiveness at higher temperature. This demonstrates adaption of *P. striiformis* f. sp. *tritici* to warmer temperatures to cause severe disease in previously unfavorable environments (Milus et al. 2009).

In parts of Canada, where rusts do not survive winter temperatures, milder winters may allow overwintering and/or earlier starts to rust epidemics, potentially making them more severe (Boland et al. 2004). In a similar way, increased out-of-season rainfall will provide increased opportunities for over-summering of rusts in parts of Australia. Changes in the ability of rust pathogens to survive from one season to the next need to be considered when determining the effects of a changing climate on rust severity.

At a larger scale and longer time-frame, changes in wheat rust severities are associated with temporal scale characteristic of the El Niño-Southern Oscillation Index (SOI), with a 2-10 year periodicity between SOI intensity and stripe rust in China (41 years data from 1950 to 1990) and a 6-8 year periodicity of stem rust in the USA (42 years data from 1921 to 1962) (Scherm and Yang 1995). Further analyses of this data showed that a mid-latitude atmospheric circulation pattern, the Western Atlantic teleconnection, better described the observed association between SOI and severe stripe rust in China (Scherm and Yang 1998). These examples serve to illustrate how changes in circulation

pattern in the upper atmosphere influence dispersal of rusts in the northern hemisphere. Increasing climate variability projected under climate change will make these associations harder to predict.

Effect of changed geographical distribution of wheat and its rusts

A coupling of crop simulation and climate change models for the year 2050 scenario predicts significant shifts in wheat mega-environments (Ortiz et al. 2008). The 12 or so mega-environments for spring wheat are demarcations of wheat growing regions of the world based on biotic and abiotic constraints, cropping systems and other characteristics (Braun et al. 1996). Mega-environment 1, comprising 32 million hectares of northwestern Mexico, the Indo-Gangetic plains and Nile valley, for example, has high yield potential. According to the simulation study, mega-environment 1 would suffer a 51% reduction in area by 2050 whereas the high latitude mega-environment 6, covering the cool temperate regions of North America and northern Eurasia and bound by the 55^{o}N latitude, would shift northwards to 65^{o}N (Ortiz et al. 2008).

At the very least, the major breeding targets would need to change in order to reflect changes in relative importance of diseases within each altered mega-environment. If these shifts in climatic suitability are not matched by soil fertility, farming systems, infrastructure and other agricultural inputs, crops could suffer from chronic water, nutrient and other stresses. Alternatively, if crops continue to be grown in areas that no longer have an ideal climate, but have the necessary soil, infrastructure and other inputs, they could also suffer chronic stress. Necrotrophic and soil-borne pathogens, among others, may become more damaging to stressed plants. Biotrophic pathogens like rust fungi associated with particular wheat varieties will follow the geographical shift in mega-environments. Migrating pathogens will interact with organisms endemic to the region. New disease complexes and/or novel biological control opportunities may arise as a consequence. Breeding targets (Ortiz et al. 2008) will change for each mega-environment with changing pathogen spectra, disease dynamics and relative economic values.

Predicted shifts in the distribution of forests and their pathogens in response to global warming are expected to be upwards along altitudinal gradients and towards the poles (reviewed by Chakraborty et al. 2008). Other modelling studies predict range shifts with rising temperature and other weather variables for important crop pathogens such as those for black sigatoka (*Mycosphaerella fijiensis*) in banana (Ghini et al. 2008), grapevine downy mildew (*Plasmopara viticola*) (Salinari et al. 2007), and phoma stem canker (*Leptosphaeria maculans*) on oilseed rape (Evans et al. 2008). We found no published modeling study on changed geographical distribution of wheat rust pathogens as a result of climate change, although with long distance inter-continental dispersal, uredinial inocula will easily keep up with changes in the distribution of cropping regions. In discussing the effect of climate changes on stripe rust development, Line (2002) summarized research on modeling the weather-dependency of stripe rust epidemics, but did not predict stripe rust severity, prevalence or distribution under future climate scenarios.

Using a process-based modeling tool, Dymex (Maywald et al. 2000), Chakraborty et al. (2002) linked a wheat growth model (O'Leary and Connor 1996) to *P. striiformis* f. sp. *tritici* life cycle through damage functions. The model outputs were validated using data collected at selected wheat-growing regions in New South Wales, Australia, and were further extended to study the potential effect of changed climate scenarios according to a CSIRO model for the period 1990 – 2100 (CSIRO 1996). The *P. striiformis* f. sp. *tritici* model included three stages in the life cycle: viz. spores, infective stages and lesions, each with parameters dealing with mortality, growth and state transfer functions. The leaf area and lesion area were linked through various feedbacks that limit lesion growth and also reduce the transpiration efficiency of the host plant. A location effect was clear with diseased leaf area decreasing under future climates in some areas, but stripe rust levels were generally higher for 2070 at many sites (Fig. 2). Yield loss resulted from an interaction between the change in climate, cultivar phenology and effect of disease.

FIGURE 2 HERE

These models did not include interacting factors such as host-pathogen adaptation and temperature sensitivity of host resistance and thus can only be used for broad guidance. Temperature sensitivity of *P. striiformis* f. sp. *tritici* may be a limiting factor, and one assessment has predicted reduced stripe rust incidence for some regions and cultivars under climate change (Chakraborty et al. 2002). Nevertheless, higher winter and lower spring temperatures in the Pacific Northwest of the USA were implicated for increased frequency and severity of stripe rust epidemics (Coakley 1979), possibly due to pathogen adaptation to high temperature (Milus et al. 2009).

Evolution of virulence

The histories of stripe rust in Australia and New Zealand (Steele et al. 2001; Wellings 2007) and the USA (Line 2002) illustrate how rust pathotypes evolve. Similar evolutionary trends were reviewed for *P. graminis* f. sp. *tritici* in Australia (Park 2007). Since the initial introduction of wheat stripe rust to Australia in 1979 at least two further introductions occurred in 1999 and 2002. A fourth stripe rust incursion in Australia in 1998 involved a putative *forma specialis* adapted to barley grass (*Hordeum murinum* complex) (Wellings 2007). Over the 28 years many new pathotypes were detected, and a subset of these became economically important and dominant components of the pathogen population (Wellings 2007).

Following exotic introduction to particular areas new pathotypes arose presumably by mutation and possibly somatic hybridization between or within the immigrant and local populations (Wright and Lennard 1980; Steele et al. 2001). Although sexual reproduction was recently reported with the discovery of *Berberis* spp. as alternate hosts (Jin et al. 2010), mutation is probably the most prevalent evolutionary force in the stripe rust pathogen (Line 2002). Similar exotic incursions followed by mutation and/or other asexual means of reproduction are the main sources of variation as appropriate alternative hosts are rarely present in cultivation areas for any of the three wheat rusts (Leonard and Szabo 2005; Bolton et al. 2008). For *P. graminis* f. sp. *tritici* the frequency of mutants were estimated at 8.3×10^{-6} per uredinial generation for a heterozygous locus dominant for avirulence to 6.9×10^{-11} for a homozygous locus (Schafer and Roelfs 1985). Mutation rate varies with the avirulence gene (reviewed by Leach et al. 2001). Mutation of avirulence to virulence in *P. graminis* f. sp. *tritici* is a case in point, where genes corresponding to *Sr5, Sr15* and *Sr21* had very high mutation rates whereas those for genes like *Sr13, Sr24* and *Sr27* rarely mutated (Luig 1979). A pathotype can also evolve at different rates at different locations; for example, two mutants of *P. striiformis* f. sp. *tritici* pathotype 134 were recorded in Australia since its detection in 2002 (Wellings 2007) and another two ('Jacky' and the 'Jacky + *Yr27*') strains were recorded since the 2007 publication. In contrast, at least 17 variants appeared in the USA within 4 years of its arrival in 2000 (Chen 2005). However, the success or failure of a new pathotype depends on several factors including the relative fitness of virulent mutants (Leonard and Czochor 1980; Leach et al. 2001).

The probability of mutation to virulence increases with increasing population size. Although there are only a few studies on pathogen biology under elevated CO_2 concentrations, the majority have demonstrated increased fecundity in both biotrophic and necrotrophic pathogens. Fecundity was significantly increased at high CO_2 levels in the rubber vine rust (*Maravalia cryptostegiae,* Chakraborty et al. unpublished), barley powdery mildew (Hibberd et al. 1996a), anthracnose (*Colletotrichum gloeosporioides*, Chakraborty et al. 2000) and crown rot (*Fusarium pseudograminearum*, Melloy et al. 2010) pathogens. However, fecundity was not increased in *P. striiformis* f. sp. *tritici* in the present AGFACE study. In the case of the anthracnose pathogen, increased fecundity was characteristic of newly isolated highly aggressive strains with a novel molecular haplotype after 25 sequential infection cycles at high CO_2 levels (Chakraborty and Datta 2004).

Rising atmospheric CO_2 and temperature will extend the wheat growing season, enlarge crop canopy to increase the amount of susceptible tissue and make the canopy micro-climate more conducive to rust development. Temperature is an important factor that controls rate of reproduction and sporulation in rusts (Clifford and Harris 1981; Dennis 1987). High CO_2 and temperature will also

influence host plant resistance. A combination of these and other factors including raised pathogen fecundity, will increase the number of uredinial generations to potentially accelerate the evolution of new pathotypes. Any assessment of future risk must anticipate changes in host-pathogen biology and evolution under climate change. With plant breeders controlling the evolution of wheat crops, pathogen evolution must be an important component of any fact-based risk assessment.

Summary of potential effects

Only four diseases of field crops have been studied under realistic field conditions in a FACE facility (Kobayashi et al. 2006; Eastburn et al. 2009; Melloy et al. 2010). The unpublished study on stripe rust summarized here, is the only empirical study dealing with climate change and a rust of wheat in a FACE. This is despite the economic importance of rusts, the massive global investment in rust management, advanced understanding of the genetic and molecular bases of host-pathogen interaction and the wealth of knowledge on the effects of atmospheric composition and climate on wheat production (Kimball et al. 2000; Ziska 2008). Many studies consider the effect of elevated CO_2 on pathogens and diseases in greenhouse and growth cabinets and one has studied the influence of elevated CO_2 and ozone levels on leaf rust (Tiedemann and Firsching 2000). However, given the variable responses of pathogens and host-pathogen interactions to altered atmospheric composition and climate for diseases studied so far, the only option is to undertake empirical studies to gather new knowledge on the rusts. Based on current understanding, there are no 'rules of thumb' for determining the specific effects of wheat rusts under a changing climate. We essentially have a 'best guess scenario' that is certainly not definitive.

Predicted effects on rusts of wheat will apply equally to endemic pathotypes as well as to exotic races, such as Ug99, yet to get a foothold in many new regions. The risks can be outlined as follows:

1. *Risk of increased loss from wheat rusts*. Increased over-summering / overwintering, enlarged crop canopy and extended growing season, among others, will increase rust inoculum to increase yield loss. Effectiveness of management options using fungicides may be reduced and/or frequency of applications may increase. Self-sown wheat and other grasses in new and existing areas opened up for wheat growth due to changing climate will serve as reservoirs of inoculum.
2. *Risk of new pathogen races evolving faster*. Large pathogen populations from increased crop biomass and increased wheat areas may undergo increased numbers of infection cycles in expanded wheat growing season to accelerate pathogen evolution on large spatial scales. If there is increased pathogen fecundity, this will further contribute to the population size.
3. *Risk of reduced effectiveness of rust resistance*. Effectiveness of some temperature-sensitive rust resistance genes may be reduced; pathogen strains adapted to higher temperature may inflict more damage and other potential physiological, structural and genetic changes may make some rust resistances ineffective.

Research needs

A lack of empirical knowledge means that the potential effects of climate change on rusts of wheat are far from clear. Studies must be made to acquire new information on the host-pathogen-environment interaction for better impact assessments. However, research must expand beyond impact assessment to develop adaptation strategies, such as new varieties and/or other rust management options that will retain their effectiveness under a changing climate thus increasing the adaptive capacity of wheat (Chakraborty et al. 2008). Whether current rust management strategies will deliver this outcome can only be determined from a rigorous scrutiny via targeted R&D and assessing the impacts of climate change will be an essential component of this.

Changes in atmospheric composition and climate will not happen quickly but will occur gradually and plant breeding and other research on rust management is expected to keep pace with gradual changes in host-pathogen interaction due to climate change. For instance, if new races evolve

at a frequency that has been driving the well-known 'boom and bust' cycles (Eversmeyer and Kramer 2000), then current breeding efforts should be adequate to cope with the new variants. If on the other hand, combinations of high temperature and CO_2, altered crop physiology, increased inoculum, and increased numbers of infection cycles lead to explosive changes in the wheat rust pathosystem, current breeding programs may not be able to keep pace with the accelerated evolution of new pathotypes. Potential changes in farming systems due to climate change will also bring new challenges.

Research on the following areas are essential to project realistic effects of climate change on rusts of wheat and to better plan and manage the threat of wheat rusts under a changing climate:

Rust evolution

As evident from the heightened global concern over Ug99, the appearance of new rust pathotypes with new virulence combinations is the biggest risk facing agriculture and food security. Even before Ug99, management of wheat rusts was one of the best examples of internationally-coordinated research efforts with perhaps the most significant investment in all cereal producing countries. Currently there is limited information available as to the likely effects of a changed climate on the evolution of rusts of wheat and therefore the likely rate of development of new pathotypes with new virulence combinations. Empirical research on rust micro-evolution under a changing climate is needed to predict future appearance of new races and this must be an essential component of any risk assessment from rust under a changing climate so that the international plant breeding community can respond accordingly.

Effectiveness of rust management strategies

Much of the current debate is on how agriculture and food security may be affected by shifting disease dynamics under climate change (Gregory et al. 2009; Mahmuti et al. 2009), but there is little on strategies that will prove effective in managing diseases. For instance, whether the current physical, chemical and biological control tactics including disease resistant varieties would offer effective protection or whether there is a need to develop and deploy new management strategies has never been addressed. Changing farming systems will influence rust epidemiology and additional measures will be necessary to reduce pathogen over-summering / overwintering opportunities. Likewise, the efficacy of fungicides in crops with altered physiology and under changing physical weather conditions, including temperature and rainfall, must be determined. Most wheat cultivars maintain resistance for five or more years (Singh et al. 2002), which is about the agronomic lifespan of a common cultivar. Some resistance genes in cultivars have failed before they were used extensively, and some genes have lasted many years (Leach et al. 2001). Testing the performance of rust resistant wheat varieties under rising CO_2 and temperature levels is essential to ascertain their longevity under future climates. Pre-emptive breeding can commence to replace resistance sources once the most vulnerable genes/gene combinations are identified (McIntosh and Brown 1997). Long distance dispersal of urediniospores on continental scales means that R&D efforts must be globally coordinated, perhaps following a similar model to the BGRI, to evaluate risks from both endemic and exotic pathotypes. Led by the late Nobel Laureate Dr. Norman E. Borlaug, the BGRI, among other activities, currently supports screening of advanced germplasm and lines in East Africa combined with a research program to combine resistance genes.

Breeding for resistance

The probability of a rust pathotype to acquire simultaneous mutations in several avirulence genes is small (Schafer and Roelfs 1985), thus the reasoning that durability can be achieved with multiple effective resistance genes. Research in Australia is aimed at developing triple adult plant resistances to rusts by combining stripe rust, leaf rust and stem rust resistance genes through marker-assisted

selection (Bariana et al. 2007). Another approach has been the use of cloned resistance genes in cassettes of linked transgenes and novel genes to develop transgenic plants with multiple rust resistance genes at a single locus (Ellis et al. 2007). Genetic improvements for rust management under changing climates will need to rely on both traditional selection/breeding and genetic transformation routes. Knowledge of host-pathogen interaction at a molecular level can lead to the identification of novel resistance genes and recent studies on secondary metabolites (Matros et al. 2006; Braga et al. 2006) and gene expression (Miyazaki et al. 2004; Zou et al. 2007; Lake and Wade 2009; Leakey et al. 2009;) at elevated CO_2 levels can serve as a guide to similar studies on rust resistance.

Rust biology and epidemiology under changing farming systems.

Changes in geographical distribution of cropping areas can alter the dynamics and epidemiology of rusts. Changing proximity and relationships of wheat crops to refuge and alternate hosts will influence the complex temporal interrelationship between hosts, pathogens and the environments (Eversmeyer and Kramer 2000). For instance, in Australia survival of all three wheat rust pathogens is critical during the non-cropping phase in the warm to hot summer period in Australia (Wellings 2007). Increased summer rainfall events associated with a changing climate will increase the growth of volunteer wheat during the non-cropping part of the season and thus increase the opportunity for rust to survive the summer and increase the likelihood of earlier and more severe rust epidemics in the winter crop. In parts of Canada overwintering and/or earlier starts to rust epidemics will be the main issue (Boland et al. 2004). Implications of altered pathogen biology and epidemiology on rust management and ways to improve management must be a central theme of future research in a farming systems context. Linking rust development models with those predicting changes in wheat productions zones and climate will assist in forecasting the likely changes in rust severity in the future. New tools in gene expression profiling can also be used to address epidemiological questions (Leakey et al. 2009).

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Fig. 1 Stripe rust severity (% leaf area affected) visually assessed on 13 occasions on susceptible H45 and resistant Janz wheat varieties grown at ambient and elevated CO_2 concentrations, with and without additional irrigation within the AGFACE facility at Horsham, Victoria, 2007

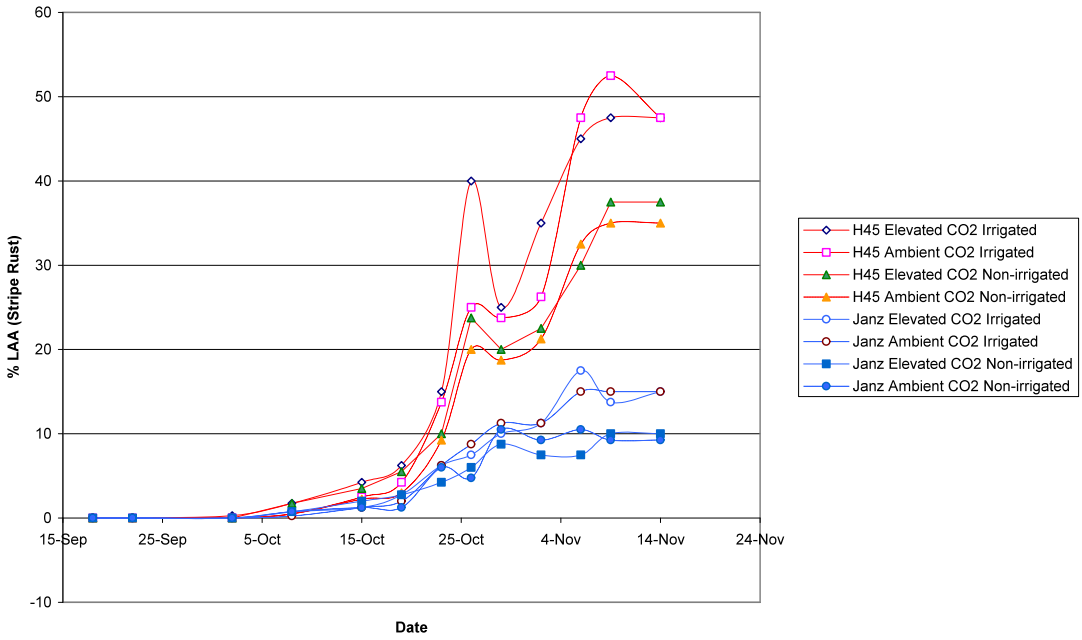
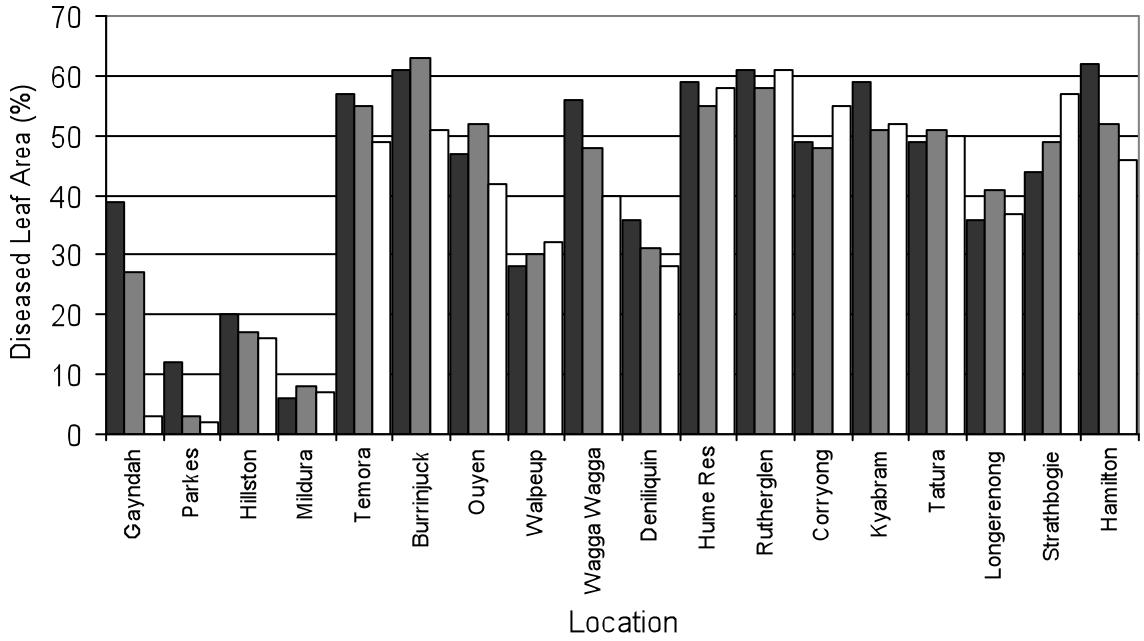


Fig. 2 Diseased leaf areas relative to present conditions (white bars) under climate change scenarios for 2030 (black) and 2070 (grey) for wheat variety 'Meering' at selected locations in NSW and Victoria, Australia. The effects of climate change on the growth of the host and the pathogen are combined



Shifting boundaries: Challenges for rust monitoring

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Abstract Significant changes have occurred in the global wheat-rust disease landscape over the last century. At the turn of the century pioneering rust research was driven by the need to combat devastating stem rust epidemics. A fundamental understanding of rust genetics and dispersal pathways emerged from this early work. Stem rust epidemics were also the driver behind the breeding programs that resulted in the green revolution of the 1960/70s. Successful incorporation of durable stem rust resistance into high yielding semi-dwarf wheat cultivars would change the wheat-rust disease situation beyond recognition. Associated intensification of the wheat cropping systems would also produce dramatic changes. Despite localized outbreaks, by the early 1990s stem rust was a disease under control. During this period of low stem rust incidence, yellow rust began to emerge as a significant threat. Breakdown of *Yr9* resistance resulted in damaging epidemics in Asia; an exotic incursion introduced yellow rust into Australia with highly significant subsequent impacts. The Australian incursion was almost certainly transmitted via an air traveller and the exponential growth in international air travel has increased the probability of other cross-continental movements. By the start of the 21st century, new rust threats had emerged. Durable stem rust resistance was broken down by the Ug99 race lineage identified in East Africa; new aggressive strains of yellow rust adapted to warmer temperatures were identified and spread across continents at a rapid rate. As in the past, significant rust developments are one driver of global initiatives to mitigate the threat. Climate change and the response of pathogens to changing conditions are possible emerging issues, although other changes may prove to be more significant. Throughout this century of change a consistent theme is the adaptability of wheat rust pathogens to cause recurring damage. Maintaining the gains of the previous decades will require sustained, collaborative, multi-disciplinary efforts. To keep pace with the evolving threats posed by wheat rusts there is a clear need for continuous vigilance and surveillance of both pathogen and host. Current international monitoring and surveillance efforts are described along with associated challenges.

Keywords air-borne pathogens, surveillance, rust surveys, pathotyping, global monitoring systems

Introduction

Throughout recorded history, rusts were a recurrent threat to cereal crop production. The three species of rust pathogens that infect wheat, namely; (*Puccinia triticina* (the leaf rust pathogen), *Puccinia striiformis* f. sp. *tritici* (*Pst*) (yellow or stripe rust) and *Puccinia graminis* f. sp. *tritici* (*Pgt*) (stem rust) are the most important economically damaging diseases affecting cereals. In all regions in which wheat is grown rusts have caused periodic severe epidemics and major losses have occurred (Roelfs et al. 1992). Historically, wheat stem rust was the most feared disease of wheat inflicting devastating damage on a periodic basis. Under highly favourable conditions, complete crop loss is possible on susceptible cultivars. Major wheat stem rust epidemics in the 20th century were the driving force in the development of significant national and international mitigation and control efforts for rusts. These mitigation efforts played a major role in the global reduction of stem rust to near insignificant levels in the last 20-30 years.

In the first half of the 20th century, wheat stem rust damaged wheat crops on a massive scale across continents. Significant epidemics included: Central India, 1946-47, estimated losses of 2 million tonnes (m t) (20% of total production); Eastern Europe / Russia, 1932, estimated losses of 5-20% across several countries; North America, 1904-54, a series of 5-6 devastating epidemics with losses ranging from 1.6-3.7 m t per epidemic; Mexico, 1947-48, estimated losses of 30% in the Bajio region; Chile, 1951, estimated 40% loss in production; Australia, 1947-48, estimated losses of

270,000 t in NSW. In the latter half of the 20th century stem rust epidemics occurred, but they have tended to be less frequent, of lower severity and more localized. Notable exceptions include; South-east Australia, 1973, estimated losses of 25-30%, north-west India, 1971-73, combined stem rust and yellow rust epidemics with estimated losses of 2.3 m t; Ethiopia, 1993-94, an estimated yield loss of 42% in highland areas (loss data extracted from Dubin and Brennan 2009). By the mid 1990s stem rust was largely considered to be under control, with low levels of inoculum worldwide (Roelfs et al. 1992).

Obviously substantial changes in the global wheat environment in the last century influenced the impact of rusts, and stem rust in particular. Despite many successes, rust diseases still pose considerable challenges and maintaining the gains of the last 50 years is a non-trivial task.

In recent years, significant changes saw the re-emergence of wheat stem rust as a threat to wheat production, and also an increasing threat posed by wheat yellow rust in many regions. The current wheat landscape is characterized not only by changing pathogen populations, but also uncertainty about changing climate and changing environments. This brief review will examine some of the historical, current and possible future factors influencing rusts and the key role of pathogen monitoring in current and future mitigation efforts. Current international efforts to monitor emerging rust threats will be highlighted. The focus will be on wheat stem rust and yellow rust, as both are currently pathogens of concern and a subject of considerable international attention.

Early 20th century: Pre-green Revolution era

The wheat farming systems and rust knowledge in the first half of the 20th century were markedly different from what we know today. Tall, traditional wheat cultivars with long growth cycles were grown under non-intensive management practices. Use of extensive irrigation, inorganic fertilizers and fungicides were not components of wheat cropping systems. Barberry, the main alternate host of stem rust, was a significant factor in new race appearance and a source of early inoculum in many parts of Europe and North America; and large-scale eradication programs were being undertaken (Leonard 2001a). In the broader context, international travel was extremely limited, especially rapid air travel; hence probability of accidental human-borne transmission of pathogens was low. During this period wheat stem rust was the major disease of concern, with epidemics causing periodic devastation across all continents.

Stem rust epidemics of the early 1900s in North America were the driving force behind the pioneering studies undertaken by E.C. Stakman and co-workers. Significant outcomes were the discovery that stem rust had distinct forms or races (Stakman and Piesmeisel 1917), and the elucidation of the *Puccinia* pathways in which rust pathogens over-winter in southern USA or Mexico and re-colonize wheat areas in the Great Plains and further north, borne by south-to-north winds as the wheat crop season progressed (Stakman 1957). By the 1950s there was recognition that race non-specific slow rusting occurred (Stakman and Harrar 1957) and the gene for gene concept between host and pathogen was elucidated by Flor (1956). These advances resulted in a fundamental understanding of rust genetics and are the basis of modern approaches. Prior to these advances, rust resistance breeding progress was largely reliant on race specific, major gene resistance resulting in “boom and bust” cycles typified by the devastating North American stem rust epidemics of 1950-54 resulting from race 15B (Leonard 2001b).

Mid to late 20th century: Green Revolution onwards

This was a period of highly significant change, most notably for stem rust on the global scale. The devastating stem rust epidemics of the early 1950s were a major factor driving the breeding program of Dr N.E. Borlaug in Mexico under the Mexico-Rockefeller wheat program. Resistance to stem rust was successfully incorporated, first into tall traditional varieties like Yacqui 50, and subsequently into semi-dwarf varieties like Sonalika and Siete Cerros. These varieties formed the backbone of the green revolution and were distributed worldwide. The resistance that Dr Borlaug put into these varieties

included the slow rusting and durable *Sr2*-complex (e.g. Singh et al. 2008). Widespread use of resistant semi-dwarf varieties is undoubtedly the major factor in significantly reduced incidence of stem rust worldwide. Early maturity of semi-dwarfs was an important complementary factor (Saari and Prescott 1985). Stem rust usually appears late in the season hence disease escape or reduction in time for inoculum build-up through early maturity was another influence.

Sporadic epidemics of stem rust did still occur. The 1973 Australian outbreak was considered the most severe in the history of the Australian wheat industry (Park 2007), although it must be noted that this occurred prior to the adoption of resistant semi-dwarf varieties in Australia. One outcome of this epidemic was a nationally coordinated approach to control rusts and the creation of what became the Australian Cereal Rust Control Program. This program would go on to have significant impacts on the control of rusts in Australia and make major contributions to rust research internationally.

The more intensive management practices associated with the green revolution of the 1960s, transformed wheat production systems in many regions. Intensive irrigation and application of inorganic fertilizers became widespread in countries like India (Fig. 1). Total wheat production in India has seen a corresponding rapid growth in response to this intensification of management practices and use of improved varieties. Total Indian wheat production leapt from approximately 11 m t in 1961 to around 79 m t in 2008 (FAOSTAT 2010). These intensive irrigated systems do raise a potential vulnerability issue for rusts, since as obligate parasites the pathogens thrive on green tissue. Large intensive areas planted to susceptible varieties, if exposed to sufficient pathogen pressure under favourable environmental conditions, are at risk of incurring damaging losses.

FIGURE 1 HERE

The massive eradication programmes for barberry, virtually eliminated the alternate stem rust host from the major wheat regions of North America and Europe. This dramatically reduced the number of new races detected in North American surveys and eliminated a source of early infection in northern areas (Kolmer et al. 2007).

Following on from the breeding gains started by Dr Borlaug, it has been argued that the largest impact from wheat breeding in the period 1960-90 was the maintenance of disease resistance. Without any doubt, rust resistance has contributed a major component of yield gains (Dixon et al. 2006). Breeding strategies of major programs like CIMMYT focused more on non-specific resistance and the introgression of alien resistance genes, such as *Sr31* from rye, proved remarkably durable. By 1995 prolonged use of resistant cultivars led to declines in levels of stem rust inoculum on a global scale. Of 13 major wheat epidemiological zones, stem rust was historically considered of major importance (i.e. severe losses without the cultivation of resistant varieties) in 11 of them (Saari and Prescott 1985). By the early 1990s stem rust was considered to be of major importance in only one of them (East Africa) (Roelfs et al. 1992). This is testament to the significant impact of global efforts, many of which were catalyzed by the devasting epidemics of previous years.

Other significant developments during this period were the emergence of damaging yellow rust outbreaks in several regions and the near exponential growth in international air travel (Fig 2). Over a ten year period (1986-1997/8) a *Yr9* virulent race of *Pst*, with presumed origin in East Africa, caused major epidemics throughout the Near East and into South Asia (Singh et al. 2004). In 1979, yellow rust was first detected in Australia – the result of exotic incursion most likely transmitted by an air traveller from Europe. The pathogen adapted to Australian conditions and became endemic, causing significant production losses (Wellings 2007).

FIGURE 2 HERE

Development and increasing use of fungicides was an important control strategy in certain regions. Irrigated or high rainfall, intensive high yielding environments have seen the greatest application for economic reasons. Western Europe is heavily dependant on chemical control (Fig. 3), although legislative efforts are underway to reduce this consumption. Under epidemic conditions

fungicide use may increase, with significant increased costs for farmers as a result. Wellings (2007) cites chemical control costs of AU$40-90 million per year during 2003-2005 to control yellow rust epidemics in Australia. In many parts of the world, fungicides are unaffordable or unavailable (note Pakistan in Fig. 3). Control strategies based on resistant cultivars are considered the most cost-effective, and sustainable long-term option.

FIGURE 3 HERE

Present day – early 21st century

In recent years, several significant changes reinforced the recurrent nature of the threat posed by wheat rusts. The notable successes of the previous decades resulted in a complacency that stem rust was a vanquished disease, with a subsequent shifting in priorities. Many countries stopped breeding or monitoring activities for stem rust. The longer the resistance genes remained effective, the more they were relied on and deployed. However durable, all specific resistances will breakdown at some point and there must be preparedness for this eventuality. An evolving threat as posed by rusts needs constant vigilance and surveillance of both the pathogen and the host. E.C. Stakman described rusts as "shifty enemies" and Dr Borlaug is famously quoted as saying "rust never sleeps". The identification of stem rust race TTKSK (Ug99) from Uganda in 1999 (Pretorius et al. 2000) proved the point. The broad virulence range of this race, accounting for most of the resistance genes of wheat origin and key alien genes like *Sr31* and *Sr38*, render a large proportion of the world's wheat cultivars susceptible to stem rust (Singh et al. 2006). Race TTKSK has spread since detection, with confirmed occurrences in Kenya, Ethiopia, Sudan, Yemen and Iran. The pattern of spread was largely as predicted from regional winds (Hodson et al. 2005; Singh et al. 2006; 2008). Since the initial identification of Ug99, the pathogen has continued to evolve. Four additional variants are now known in the Ug99 race lineage. Virulence has occurred for two additional important stem rust resistance genes, viz. *Sr24* (race TTKST) and *Sr36* (race TTTSK) (Jin et al. 2008; 2009). Loss of these genes in this virulence background increases the vulnerability of global wheat cultivars (e.g. Olson et al. 2010). These two new variants are currently only known in Kenya, but spread to other areas is a near certainty. TTKST caused epidemics on the *Sr24* carrying cultivar KS Mwamba in 2007, one year after its initial discovery. Appearance of other variants is considered a likely possibility.

The epidemiology of yellow rust is also changing, resulting in a surge of serious outbreaks across many regions. Yellow rust is now considered to be of major importance (i.e. severe losses without the cultivation of resistant varieties) in 12 of the 15 global wheat regions (RP Singh pers comm). Compared to only 4/13 regions listed by Saari and Prescott (1985). Since 2000, two closely related aggressive strains of *Pst* were identified in three continents (North America, Australia and Europe) in less than three years (Hovmøller et al. 2008). Increased aggressiveness was demonstrated (Milus et al. 2006; 2009), in terms of the ability to produce two to three times more spores per day at temperatures once considered too warm for yellow rust. This aggressiveness and adaptation to warmer temperatures led to serious yellow rust epidemics in areas previously considered unsuitable for the disease, e.g. South-Central USA. Global spread was extremely rapid; the aggressive exotic race detected in North America in 2000 is considered identical to the aggressive race detected in Australia in 2002 (Wellings 2007; Hovmøller et al. 2008). Dramatic increases in spore production by these races may increase the probability of rare long distance air-borne transmission, but human-borne transmission across continents is probably more likely. Loss of the *Yr27* resistance gene in Asia is another significant factor, as this gene is present in many of the major cultivars throughout the CWANA region (Nazari et al. 2009). Virulence for *Yr27* is spreading and being sampled in increasing frequency in regional trap nurseries distributed by ICARDA (K. Nazari pers comm). Yellow rust is without a doubt the most economically damaging wheat rust globally at the current time.

Whilst yellow rust is increasingly occurring in warmer environments, there are some indications that the Ug99 lineage of stem rust might be tolerating cooler environments. Recent observations in Kenya saw stem rust regularly appear at cool, high elevation (3,000 m) sites.

Previously, stem rust was absent from such environments in Kenya (R. Wanyera pers comm). Substantially increased inoculum levels in Kenya may be one factor, but controlled temperature experiments indicated some degree of increased competitiveness at cooler temperatures. At cooler infection temperatures (16°C and 12°C), race TTKSK (Ug99) had a significantly increased mean sporulating area compared with the dominant North American race QFCSC 13 days after inoculation (Rouse and Jin 2009).

Globalization and the associated exponential growth in air travel and international trade has a significant impact on the potential for pathogens to move into new areas. Several examples have already been given of likely accidental transmission of wheat rusts across continents. Exotic incursions of wheat rusts into Australia have been closely studied and illustrate increasing trends associated with globalization. Of the 11 known exotic incursions of wheat rusts, three were in the last 8 years, four were in the 30 year period 1970 to 2000, and the remaining four were in the 35 year period 1925-1970. At least two of the post-1970 incursions were probably human-borne rather than air-borne (Park and Guest 2008). Scherm and Coakley (2003) reported a similar situation in the USA with the rate of exotic pathogen invasion increasing from about five instances per decade from 1940 to 1970, to more than three times this during the 1990s. These increases were attributed to increased global trade in plant produce.

Future - global change

In addition to the current significant shifts occurring in the wheat-rust environment, climate systems are changing. The Fourth Assessment of the Intergovernmental Panel on Climate Change (IPCC FAR 2007) concluded that "Warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice, and rising global average sea level". The report classifies the warming trends as "very likely (i.e. >90% probability) due to the observed increase in anthropogenic greenhouse gas concentrations". These changes are occurring across continents in a relatively consistent manner. Global warming and associated changes in rainfall distribution and the increase in CO_2 will undoubtedly impact agricultural systems. Hodson and White (2009) provide an overview of some of the likely affects of climate change on wheat systems. Diseases, including wheat rusts, will undoubtedly respond to global change; however, as a result of the complex dynamics between hosts and pests/pathogens, and the large variation in pest/pathogens response to climatic conditions and CO_2 levels, trends are difficult to predict. Temperature and moisture regimes are obviously critical to rust development, but interactions with other components of the disease pyramid (environment, pathogen, host, time and human activity) are complex.

South Asia is one region predicted to experience significant effects on cropping systems from climate change (Lobell et al. 2008). For this brief review India will be used as an illustrative example, as many of the key issues relating to climate change are relevant. In India, factors that might influence the productivity of wheat are of major concern hence climate change investigation is a high priority. There is considerable evidence that short-term climatic changes are occurring. Sidhu and Singh (2009) reported that during the past 30 years in the Punjab, both maximum and minimum temperatures increased during the '*rabi*' (winter) season, whilst rainfall and solar radiation declined. Perhaps surprisingly, relative humidity also increased during the same period. Rising trends in relative humidity in north-west India were also reported by Jenamani (2007). Increased agricultural irrigation was not ruled out as a potential contributing factor to this trend. Agarwal (2007) reported an overall significant warming in surface temperature of 0.4°C over the period 1901-2000, although there was some spatial variation in trends. Pathak and Wassman (2009) analysed the last three decades of meteorological data from Ludhiana, Punjab, and found an increasing trend of 0.06°C $year^{-1}$ in minimum temperature. Sharma et al. (2007) reported a steady increase in minimum March temperature at 5 sites in eastern India over the period 2000-2005. Future climate models consistently predict substantial warming to occur over India, with most models indicating a 3-4°C increase towards the end of the 21st century (DEFRA 2005; Agarwal 2007; IPCC 2007). With warming,

Hodson and White (2007) predict that up to 50% of the current high potential irrigated wheat mega-environment of the Indo-Gangetic Plains (IGP) might be re-classified as a heat-stressed, irrigated, short-season production mega-environment by 2050. Not only would this mean a substantial loss of high yielding environments due to increasing temperature, but there are also likely to be impacts on grain quality and on the incidence of pests and diseases (Agarwal et al. 2009).

For rainfall, there was no discernable trend in the overall monsoon rainfall over the last 100 years at the national level (Agarwal 2007), but there was a significant increase in the frequency and magnitude of extreme rainfall events and a corresponding decrease in moderate rain falls over the last 50 years in Central India (Goswami et al. 2006). Future climate models are more variable in their prediction of rainfall. Most models predict an increase in total summer monsoon rainfall and an increasing frequency of extreme rainfall events. However, considerable spatial variation is expected and rainfall may decline in the north-west (DEFRA 2005).

Against this backdrop of changing climate, what might be the influence on wheat diseases? At present there is little concrete evidence. Sharma et al. (2007) reported an increasing severity of spot blotch (Helminthosporium leaf blight), caused by *Cochliobolus sativus,* in the eastern Indo-Gangetic plains, linked to increasing night-time March temperatures. Spot blotch is a characteristic disease of the warm, irrigated heat-stressed mega-environment outlined by Hodson and White (2007) and reported observations appear to correspond with the predicted expansion of this mega-environment.

FIGURES 4, 5, AND 6 HERE

Appearance of stem rust in north India in the 1960s and 1970s was related to temperature-dependant movement of spores from south to north India (Nagarajan and Joshi 1978). Agarwal et al. (2009) indicated some visual correlation between recorded appearance of stem rust in India and the 14°C isoline (Fig. 4). If such a relationship held, then it could be hypothesized that warming temperatures might lead to the possibility of earlier occurrence of stem rust in the key wheat producing regions of north-west India. As an illustrative example, examination of the 10 day minimum temperature data (ECMRWF 2008) for 1990, 2000 and 2009 for the area close to Ludhiana, Punjab, indicates some possibility that 14°C minimum temperatures might already be occurring earlier in the season (Fig. 5). Similar analysis using long-term normal mean monthly data (WorldCLIM, Hijmans et al. 2005) and downscaled future climate data from 3 major Global Circulation Models (GCMs) for 2050 under the IPCC A2a scenario (IPCC 2000) indicates a predicted similar trend (Fig. 6). Model outputs suggest that 14°C minimum temperatures may possibly occur at least one month earlier than at present in the north-west plains. Whilst temperature is an important factor for stem rust it is not the only factor and exploratory analyses of this nature are gross over simplifications of any likely disease outcome. This 14°C isoline is nothing more than at best a very approximate indicator; it will have little predictive value in any given year as specific conditions will vary and a multitude of other complex interactions will influence disease outcome. It should also be noted that in the cool highland areas of East Africa, currently severely affected by stem rust, long-term normal monthly minimum temperatures never reach 14°C (typical values are 6-9°C using the same WorldCLIM dataset). In conclusion, influences of moisture, crop phenology, altered crop management and inoculum pressure are all likely to play an equivalent or greater role in stem rust occurrence. Uncertainty around precipitation levels, especially in the north-west, may result in a warmer drier climate that is totally unsuitable for stem rust development. Available evidence for India, and other areas, suggests that increasing abiotic stresses resulting from climate change are likely to be much more significant. Although climate change will undoubtedly have some influence on pests and diseases, whether these effects will be as significant as other current changes being observed in the wheat-rust landscape i.e., pathogen change, breakdown of resistance, exotic incursions and adaptation to new environments, remains to be seen.

From this brief review of significant changes in the wheat–rust environment one consistent theme emerges. To keep pace with the evolving and recurring threats posed by wheat rusts there is a

need for continuous vigilance and surveillance of both the pathogen and host. Dubin and Brennan (2009) considered that such a system might include, among others, an international early warning system, trap nurseries and rust population monitoring. Current emerging rust threats, notably the Ug99 lineage, have catalyzed efforts to put just such an international surveillance and monitoring system in place.

Emerging international monitoring and surveillance systems

The recent global concerted efforts to combat the threat of cereal rusts, which arise from the alarm raised by Dr Borlaug over the potential threat of race Ug99 (CIMMYT 2005) supported this notion of an absolute requirement for continuous vigilance and surveillance of the pathogen and host. Implementation of a Global Cereal Rust Monitoring System (GCRMS) is being undertaken by an international coalition coordinated by the Food and Agriculture Organization of the United Nations (FAO). Key elements of this emerging monitoring system, with the Ug99 stem rust race lineage as its main focus, are already described in detail (Hodson et al. 2010).

A rapidly expanding coordinated international surveillance network is now emerging. In 2009, 15 countries were reporting standardized field survey information on disease incidence and severity. This represents a substantial increase over the two reporting countries in 2007. Several countries have now formally nominated national focal points to coordinate surveillance efforts at the national level. All these developments represent a strengthening of the international network; the resulting outcome being a clearer picture of stem rust incidence and severity over a wide geographical area. It should be noted that survey data on all three wheat rusts are held in the core database of the GCRMS, but dissemination tools and information products are currently only available for stem rust.

Summarized stem rust pathotype data was incorporated as a core component of the GCRMS. Predominant *Pgt* pathotypes in 14 countries for the year 2008 is the current baseline dataset. Pathotype data presents many unique challenges and these will be outlined later, but this probably represents the first time that a significant proportion of the global *Pgt* pathotype data has been compiled in one location.

Core tools of the GCRMS, like Rustmapper developed by CIMMYT, have been maintained and enhanced. Other tools, including a pathotype tracker and smart PDF maps have been incorporated. Regularly updated situation reports are now routinely included into the system. All available information is now being disseminated in three UN languages (English, Arabic and Russian) via a web portal – "Rust SPORE" released in April 2010 (http://www.fao.org/agriculture/crops/rust/stem/en/).

Significant on-going developments include an improvement in the core data management system and toolset. Harmonization is taking place with the existing pathogen data management system that underpins the Global Reference Centre - yellow rust at the University of Aarhus, Denmark. The overall aim is that in the future there will be a common information platform for both stem and yellow rust. The first functional common pathotype information tools have already been implemented in the Rust SPORE platform.

Serious challenges still remain. These encompass the entire spectrum from international coordination and capacity building to technical issues on data handling. When Ug99 emerged it not only exposed the vulnerability of a large proportion of the global wheat cultivars, it also highlighted the limited capacity for pathogen surveillance and monitoring in many regions. Re-building or strengthening that capacity is a priority, but will take time. Pathotyping capacity is a serious limitation. Excellent technical backstopping by advanced laboratories, notably in North America, has provided much of the current knowledge on the Ug99 lineage, but restricted sampling periods and low numbers of viable samples are limiting factors. Strong national capacity backstopped by a Global Reference Centre with year-round capacity is seen as a critical future priority. Differing nomenclature systems and analytical methods for pathotyping, raise some difficulties when trying to have an international harmonized information system. Despite encouraging progress in the development of

international surveillance networks there is still a long way to go before a fully operational system with efficient information exchange is in place.

The changes that have been highlighted in the global wheat-rust continuum represent a further set of challenges for effective monitoring. The shifting nature of the pathogen represents a continuously moving target. Pathogens populations are dynamic and unexpected events can occur at any time or place. Ug99 or new aggressive strains of the yellow rust pathogen, simply represent the latest events of concern in this continuum. Effective systems must be in place to detect any future significant events in as timely a manner as possible. The Australian rust monitoring system is one the most advanced in the world and clearly demonstrates the benefit of relevant, regular and effective pathogen monitoring. Wellings (2007) gives two examples of new virulent yellow rust pathotypes being detected by the annual pathoytype surveys several years in advance of any economic damage. Such a system has great value as an early warning mechanism that gives farmers sufficient time to adjust cultivar deployment. Replication of such a system at the international level would have highly significant benefits.

Pathogen mobility and the ever-increasing possibility of accidental incursions into completely new areas, emphasizes the need for vigilance even in regions considered to be at "low-risk". Pathogen adaptation to new environments or environment shifts due to management practices or climatic factors re-enforces the notion that careful consideration needs to be given regarding "risk" and surveillance. The persistence of the threat from rusts and irregular outbreaks are other critical factors. Stem rust race 15B in North America illustrates the point; it was 11 years after first detection that this race caused devasting epidemics. In the absence of regular major disease outbreaks it is understandable that priorities shift. This has a major bearing on the sustainability of monitoring, but when dealing with wheat rusts or any other disease/pest that affects the welfares of huge proportions of the world population it is a high risk strategy to abandon routine surveillance activities.

Conclusion

There have been some very significant changes in the global wheat-rust landscape over the last century. Very significant progress has been achieved, often driven out of necessity to combat serious rust epidemics. Despite many successes, the significant threats posed by wheat rusts remain. Maintaining the gains of the previous decades will require sustained, collaborative, multi-disciplinary efforts. Re-emergence of wheat stem rust has exposed not only the high vulnerability of a large proportion of current global cultivars, but also a limited capacity for monitoring and a degree of complacency regarding the durability of resistance. Yellow rust has shown the pathogen's ability to adapt and acquire new traits that give it a competitive advantage in new environments. Exponential growth in international travel is increasing the possibilities for human-borne transmission of rusts into new areas, often with significant consequences. Effects of climate change on rusts in the future are difficult to predict, but whether they will be more significant than either shorter term pathogen evolution or exotic incursions is a debatable point. As in the past, significant rust events are one factor driving global efforts to address emerging threats. Re-emergence of stem rust as a disease of concern has triggered significant global actions under the framework of the Borlaug Global Rust Initiative (BGRI). One component of these efforts is the establishment of international monitoring and surveillance systems. Sustained monitoring, coupled to strengthened national capacities for mitigation, are vital if early detection and response to new rust threats are to occur.

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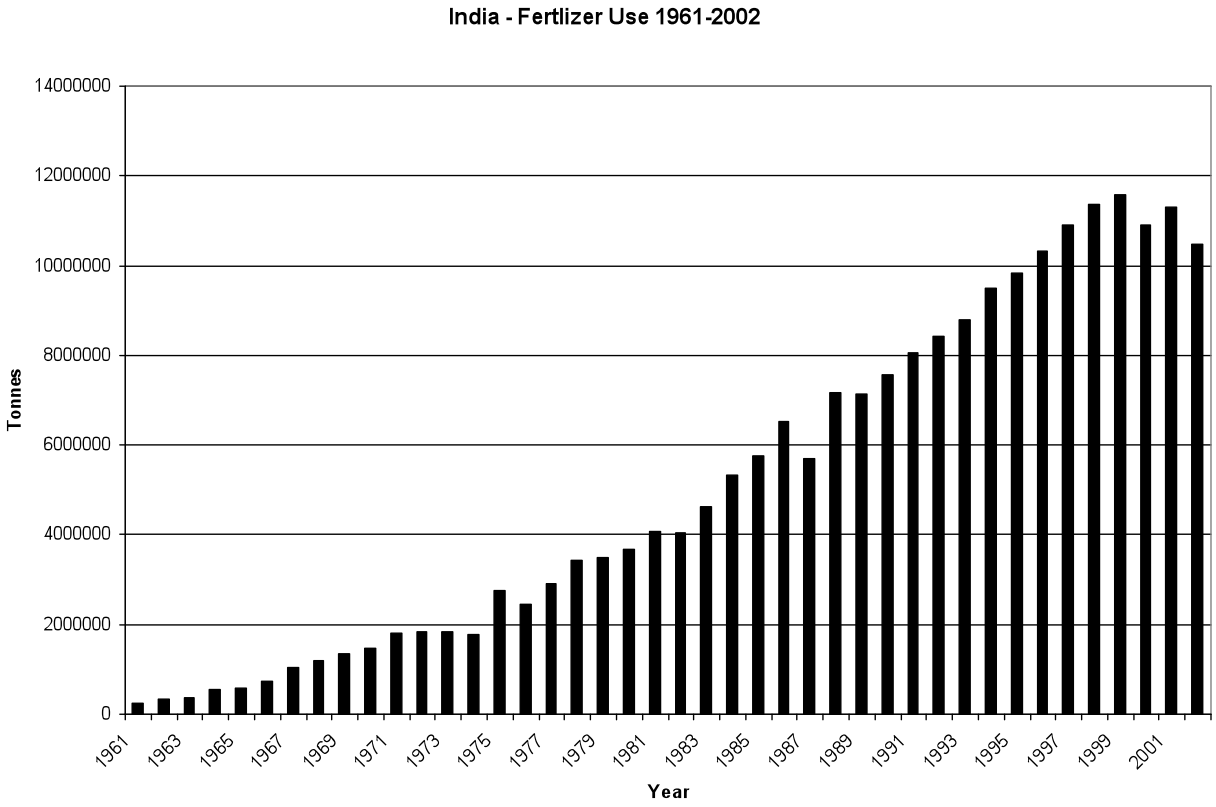


Fig. 1 Total nitrogenous fertilizer use in India (1961-2002) Source: FAOSTAT (2010)

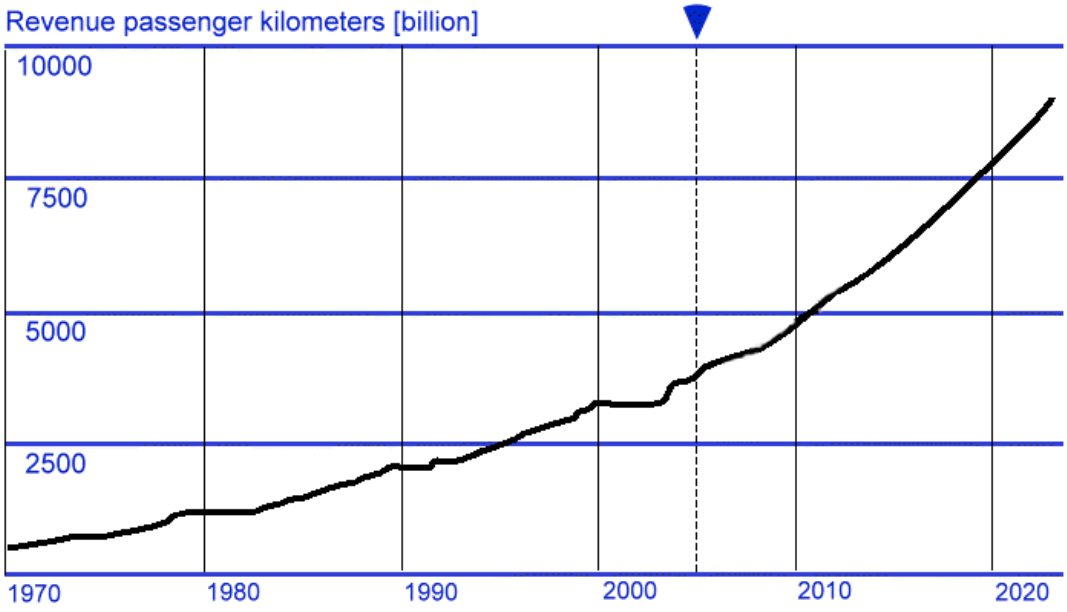


Fig. 2 Actual and predicted trends in global air travel since 1970.Source: International Civil Aviation Organization

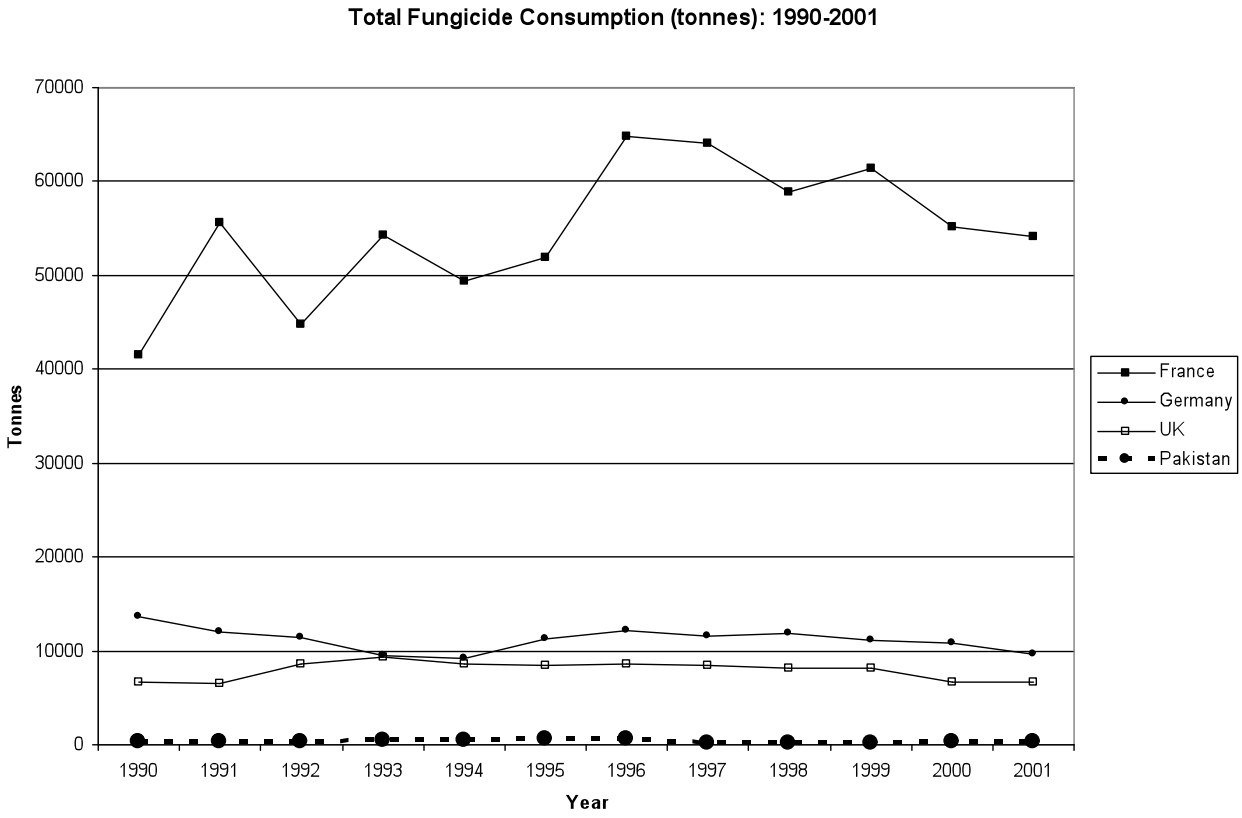
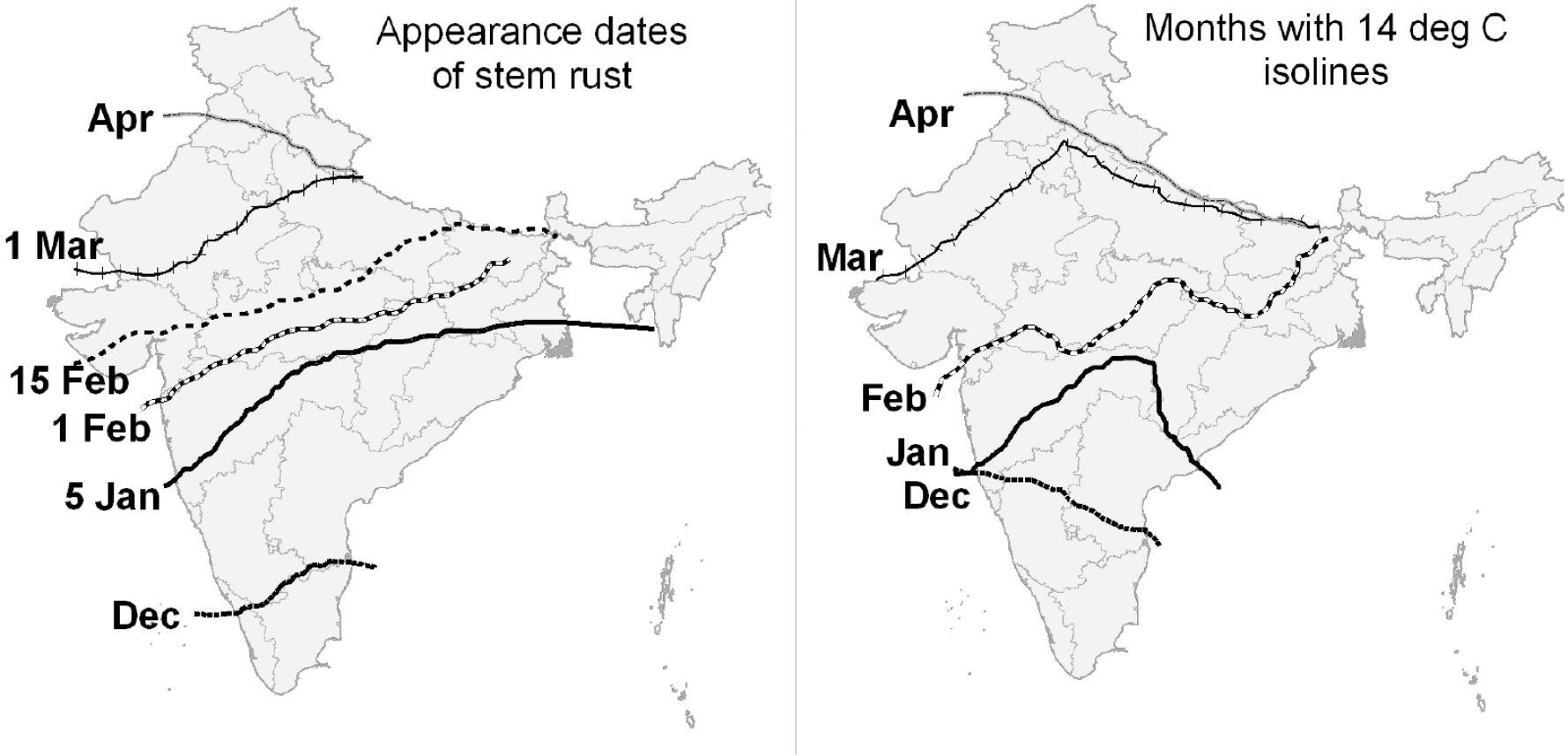


Fig. 3 Total fungicide consumption for selected countries 1990-2001 Source: FAOSTAT (2010)

Fig. 4 Appearance dates of stem rust in India and months with 14°C isolines (after Agarwal et al. 2009)



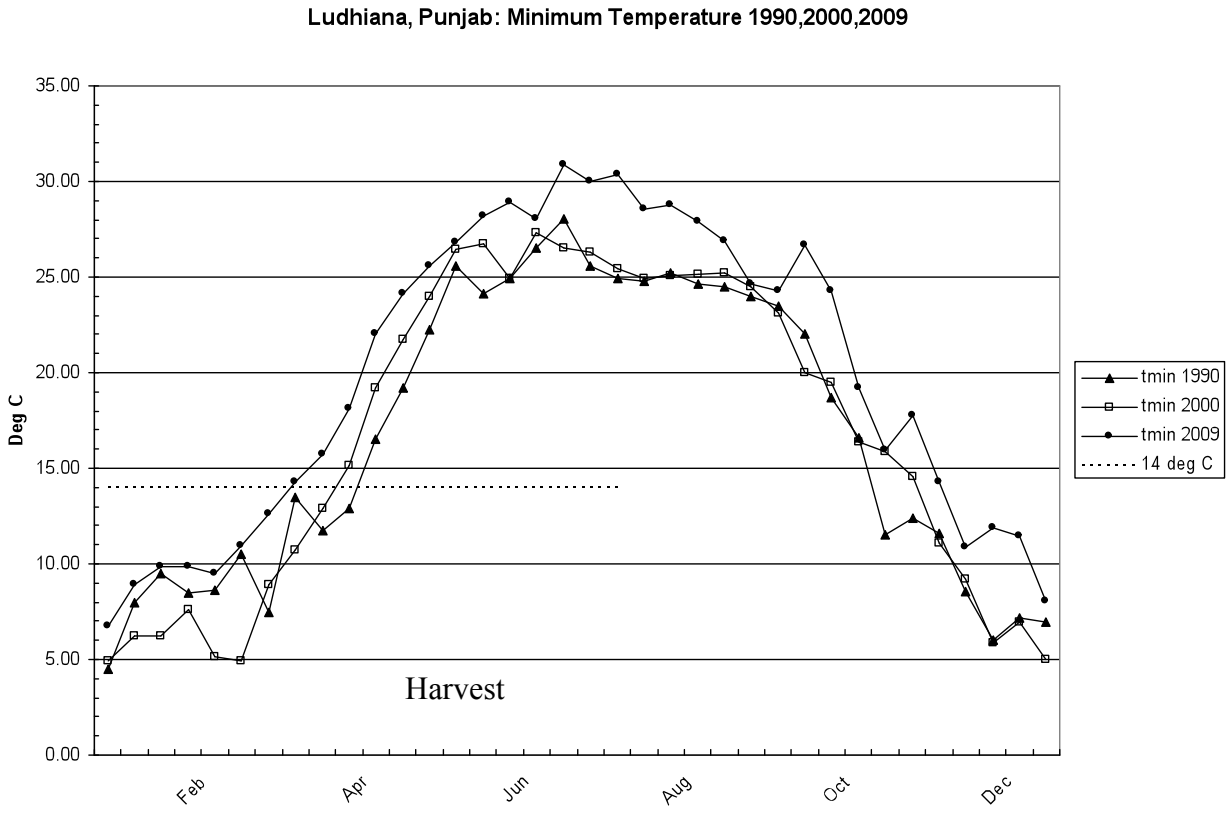


Fig. 5 Minimum 10 day temperatures for selected years (1990, 2000, 2009) in the area around Ludhiana, Punjab

Nutrient uptake in rust fungi: How sweet is parasitic life?

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Abstract A better understanding of the fundamental principles of host-pathogen interactions should enable us to develop new strategies to control disease and to eliminate or at least manage their causative agents. This is especially true for obligate biotrophic parasites like the rust fungi. One vital aspect in the field of obligate biotrophic host-pathogen interactions is the mobilization, acquisition and metabolism of nutrients by the pathogen. This includes transporters necessary for the uptake of nutrients as well as enzymes necessary for their mobilization and metabolism. In a broader sense effector molecules reprogramming the host or triggering the infected cell into metabolic shifts favorable for the pathogen also play an important role in pathogen alimentation.

Keywords nutrient acquisition, nutrient utilization

Introduction

On a global scale, some of the most serious fungal plant pathogens are *obligate biotrophic* parasites (Brown and Hovmøller 2002). The term obligate biotroph characterizes a specific lifestyle in which the host as a whole suffers only minor damage over an extended period of time. The pathogen in turn is completely dependent on the living host plant to complete its life cycle (Staples 2000). This form of parasitism stands in sharp contrast to necrotrophic parasites, which kill their hosts, or host cells, quickly and subsequently thrive on the dead plant material (Staples 2001). Hemibiotrophic fungi, like *Colletotrichum* spp., are characterized by a more or less extended biotrophic phase, before switching to necrotrophic growth and killing of their host (Perfect and Green 2001).

In order to clearly distinguish true obligate biotrophic fungi from hemibiotrophs or necrotrophs the following six criteria were suggested: 1. Obligate biotrophs are not culturable *in vitro* (at least not to a point representing the parasitic phase), 2. They form highly differentiated infection structures (variations of the normally tubular cell shape, which are necessary for pathogenesis), 3. They have limited secretory activity, 4. They establish a narrow contact zone separating fungal and plant plasma membranes, 5. They engage in a long-term suppression of host defense responses, 6. They form haustoria (specialized hyphae that penetrate host cells) (Voegele et al. 2009). According to these criteria the range of organisms designated as true obligate biotrophs comprises the downy mildews (Oomycota), the powdery mildews (Ascomycota) and the rusts (Basidiomycota). Looking at this broad phylogenetic spectrum it seems more than likely that the obligate biotrophic lifestyle has arisen more than once in the course of evolution and represents a specific adaptation of these organisms to the close interaction with their respective host plants (Hahn et al. 1997). Therefore, it does not seem appropriate to restrict the analysis of the molecular basis of this type of host-parasite interaction on members of a single phylum, assuming that these findings will also hold true for members of a different phylum. While some progress has been made especially in the analysis of powdery mildew – plant interactions (Zhang et al. 2005), the analysis of other obligate biotrophic systems is lagging behind. Over the last decade our group has been trying to elucidate the molecular basis for the interaction of rust fungi with their host plants using *Uromyces fabae – Vicia faba* as a model system.

The haustorium

The haustorium represents one of the hallmarks of obligate biotrophic parasites. These structures have generated the interest of plant pathologists ever since their first description by Zanardini about 150 years ago (von Mohl 1853). Already in naming these structures [fr. L. haurire (haurio, hausi, haustum): to drink, to draw] de Bary (1863) proposed one of the possible functions for haustoria - the uptake of nutrients from the host. However, until recently there was evidence for an involvement of haustoria in nutrient uptake only for powdery mildew fungi (Ascomycota) (reviewed in Hall and Williams 2000).

The dikaryotic rust haustorium develops from the haustorial mother cell with a slender neck and a haustorial body that forms distally to the neck (Heath and Skalamera 1997). During formation of the haustorium the cell wall of the host cell is breeched. The expanding haustorium invaginates the host plasma membrane and new membrane is probably synthesized. Therefore, the haustorium is not truly intracellular, it remains outside the physiological barrier of the host cell (Fig. 1).
FIGURE 1

With the development of the haustorial body a zone of separation between the plasma membranes of parasite and host is formed. It is composed of the fungal cell wall and the extrahaustorial matrix (Hahn et al. 1997). The extrahaustorial matrix resembles an amorphous mixture of components, mainly carbohydrates and proteins, partly of fungal but primarily of plant origin (Harder and Chong 1991). It seems likely that this zone of separation plays an important role in maintaining the biotrophic lifestyle. This hypothesis is supported by the cytological analysis of hemibiotrophic parasites. The initial biotrophic phase of hemibiotrophs, for example *Colletotrichum* spp., is also characterized by the presence of a narrow contact zone between host and parasite (Mendgen and Hahn 2002; Perfect and Green 2001). Upon the switch to necrotrophic growth the host plasma membrane surrounding the hyphae disintegrates and parasitic growth continues with narrower unsheathed hyphae. It therefore seems likely that this zone of separation plays an important role in the maintenance of the biotrophic lifestyle. Undoubtedly the extrahaustorial matrix represents a formidable trading place for the exchange of nutrients and information between the host and the fungus (Heath and Skalamera 1997). In a study on *Puccinia hemerocallidis* on daylily Mims et al. (2002) showed long tubular extensions contiguous with the extrahaustorial matrix. Similar structures were already described by Stark-Urnau and Mendgen (1995) for monokaryotic haustoria (haustoria derived from basidiospore infection) of *U. vignae*. These structures reach far into the host cytoplasm and exhibit vesicle-like bodies at their tip. However, it still remains to be shown if there is any kind of trafficking linked to these structures.

There is some evidence that the cytoplasmic membrane of the host enclosing the haustorial body, the so called extrahaustorial membrane, is modified and therefore no longer resembles a conventional plant plasma membrane. Harder and Chong (1991) summarized results obtained by freeze fracture electron microscopy with bean rust and oat crown rust. In both interactions the extrahaustorial membrane lacks intramembranous particles, and exhibits a dramatic reduction of sterols (Harder and Mendgen 1982). Cytochemical studies on powdery mildew haustoria (Gay et al. 1987; Manners 1989) and later work by Baka et al. (1995) on rust haustoria suggested that the extrahaustorial membrane lacks ATPase activity. This implies that there would be no control over solute fluxes from the host cell, at least not in the direction of the haustorium. Further support for a modification of the extrahaustorial membrane comes from recent results obtained with GFP-tagged membrane proteins in the pathosystem *Erysiphe cichoracearum / Arabidopsis thaliana* (Koh et al. 2005). In this study eight different plasma membrane markers were excluded from the extrahaustorial membrane and accumulated in a collar-like formation around the haustorial neck. This neck region is characterized by electron-dense material, apparently joining the two plasma membranes of host and parasite (Harder and Chong 1984). This "neckband" seals the extrahaustorial matrix against the bulk apoplast, not unlike the Casparian strip in the endodermis (Heath 1976). Based on the sealing by the neckband and the presence of the plant plasma membrane surrounding the whole structure it was

suggested that the extrahaustorial matrix should be considered a symplastic compartment (Heath and Skalamera 1997). However, it might also be regarded as a highly specialized portion of the apoplast, providing conditions different from those present in the bulk apoplast.

Analyses of the potential role(s) of rust haustoria were hampered by the fact that haustoria are exclusively formed *in planta* and that their isolation encountered numerous problems (Bushnell 1972). As a result, haustoria were mostly studied using cytological techniques (Harder and Chong 1991). The introduction of molecular biology into the field of phytopathology opened a new dimension to investigate the role(s) of haustoria. A picture is beginning to emerge indicating that haustoria do not only serve in nutrient uptake - the task postulated for these structures ever since their discovery. In fact, they seem to perform biosynthetic duties and are thought to be engaged in the suppression of host defense responses and in redirecting and/or reprogramming the host's metabolic flow.

The dawn of a new era

Because haustoria are only formed *in planta* and cannot be produced in axenic culture, there have been a number of attempts to establish protocols for the isolation of haustoria from infected plant tissue. A procedure developed to isolate haustoria of the powdery mildew fungus *Erysiphe pisi* from diseased pea tissue involved sucrose density gradient centrifugation (Gil and Gay 1977). However, this method proved too laborious and inefficient when applied to rust fungi (Cantrill and Deverall 1993; Tiburzy et al. 1992). A milestone in the research involving rust haustoria was the introduction of a chromatographic method to isolate haustoria by Hahn and Mendgen (1992). The method is based on a selective binding of oligosaccharides present in the haustorial wall to ConcanavalinA immobilized on a Sepharose 6MB backbone. Repeated cycling of cell extracts of infected broad bean leaves yielded considerable quantities of highly pure haustoria. Whereas research involving haustoria up to this point in time was largely based on cytological techniques, this method provided the basis for biochemical and molecular analyses of rust haustoria.

Of rusts, beans, and *PIG*s

One of the first publications arising from molecular work on rust haustoria based on this technique was the seminal paper by Hahn and Mendgen (1997) on the characterization of 31 *in planta*-induced rust genes isolated from a haustorium-specific cDNA library. These *in Planta*-Induced Genes, or *PIG*s, show exclusive or at least preferential expression in rust haustoria compared to their expression in other, earlier infection structures. This in turn was taken as evidence for a role of the corresponding gene products in structure and/or function of haustoria. An interesting finding was that homologs for only about one third of the genes could be found in publicly accessible databases. Among the sequences for which a function could tentatively be assigned based on these homology searches were highly expressed genes encoding two amino acid permeases. This result seemed to corroborate the long standing hypothesis that rust haustoria are responsible, at least in part, for nutrient acquisition. However, the two most highly expressed genes in haustoria were found to encode components of vitamin B1 biosynthesis. The vitamin B1 derivative thiamin pyrophosphate is a vital co-factor for a number of catabolic and anabolic reactions. It can therefore be concluded that besides their role in nutrient acquisition, haustoria also fulfill vital biosynthetic functions. Another interesting finding was the fact that the remaining two thirds of the *PIG*s did not exhibit homology to known genes. This was taken as evidence that at least some of these genes might be linked to virulence or pathogenicity of rust fungi in general or *U. fabae* in particular. The initial screen for *PIG*s by Hahn and Mendgen (1997) was recently followed up by Jakupovic et al. (2006). Using an EST sequencing project and microarray hybridization the authors concluded that in rust fungi a strong shift in gene expression takes place between germination and the biotrophic stage characterized by haustoria.

The method to isolate haustoria from infected plant tissue developed by Hahn and Mendgen (1992) paved the ground for a better understanding of the molecular aspects governing host-pathogen interactions in general and the role(s) of rust haustoria in particular.

Rusty power plants

As shown by Hahn and Mendgen (1997) in the rust fungus *U. fabae* the switch from early stages of host plant invasion to parasitic growth is accompanied by the activation of a number of *PIG*s. Two of them, *THI1* (former designation *PIG1*) and *THI2* (*PIG4*), were highly expressed in haustoria (Sohn et al. 2000). We showed that transcripts of both genes together make up more than 5% of the total haustorial mRNA (Sohn et al. 2000). The genes exhibit homology to genes involved in thiamin biosynthesis in yeast. Based on these homologies, THI1p is likely to be involved in the synthesis of the pyrimidine moiety, whereas THI2p seems to participate in the synthesis of the thiazol moiety of thiamin. Their functional identities were confirmed by complementation of *Schizosaccharomyces pombe* thiamin auxotrophic *THI3* and *THI2* mutants, respectively. In contrast to thiamin biosynthesis genes of other fungi that are completely suppressed by thiamin, *THI1* and *THI2* expression was not affected by the addition of thiamin to rust hyphae either *in vitro* or *in planta*. Western blot analysis revealed decreasing amounts of THI1p in extracts of spores, germlings, and *in vitro* grown infection structures. Immunofluorescence microscopy of rust-infected leaves detected high concentrations of THI1p in haustoria, whereas only low amounts were found in intercellular hyphae. In sporulating mycelium, THI1p was found in the basal hyphae of the uredinia, but not in the pedicels and only at very low levels in urediniospores. These results indicate that the haustorium is an essential structure of the biotrophic rust mycelium for the biosynthesis of metabolites such as thiamin. Therefore, haustoria can be considered power plants providing essential nutrients through *de novo* synthesis.

A function for the name

Already in naming these structures de Bary (1863) suggested the uptake of nutrients from the host as a possible function of haustoria. Earlier attempts to elucidate a potential role of haustoria in nutrient acquisition for the parasite mainly involved feeding experiments. Martin and Ellingboe (1978) employed ^{32}P-labeled substances and Manners and Gay (1982) used $^{14}CO_2$ to analyze substrate translocation in members of the *Erysiphales*, while Mendgen (1979, 1981) applied ^{3}H-labeled amino acids using *Uromyces* spp. These experiments gave indirect evidence for a role of haustoria in nutrient uptake without providing conclusive proof.

Employing the haustoria isolation protocol developed by Hahn and Mendgen (1992), Struck et al. (1996) showed a strong increase in the activity of a plasma membrane H^+-ATPase [EC 3.6.1.35] in haustoria compared to other fungal structures. A detailed characterization of the *PMA1* gene and the corresponding gene product PMA1p followed two years later (Struck et al. 1998). In contrast to the increased PMA1p activity in microsomal vesicles derived from haustoria, only reduced amounts of the corresponding transcripts were found in haustorial preparations. Analysis of wild type enzyme and a C-terminal deletion mutant in a heterologous expression system indicated a role of the C-terminus in auto-regulation of the enzyme. The observed net activity increase could be explained on the basis of a strong biochemical auto-activation paired with the decrease in mRNA level. These results suggested that the electrochemical gradient generated by the H^+-ATPase of haustoria plays an important role in their function, possibly by promoting nutrient uptake from host cells. Trans-membrane solute transport systems can be arranged in four classes: group translocating systems, traffic ATPases, facilitators, and ion-solute co-transporters (Voegele et al. 1995). As indicated by the designation, the transport process of the latter class is energized by ion co-transport. Ion co-translocation occurs either as symport or as antiport, and the co-translocated ion in most of the cases is a proton. Ion-solute co-transporters together with facilitators form one of the two transporter superfamilies, the Major Facilitator Superfamily (MFS, [TC 2.A.1.]) (Marger and Saier Jr. 1993; Saier Jr. 2000). However, the second superfamily, the traffic ATPases [TC 3.], and the group translocating systems [TC 4.] are

usually found only among prokaryotes (Saier Jr. 2000). This leaves the members of the MFS as the main solute translocation systems in eukaryotes. Therefore, an increased plasma membrane H^+-ATPase activity may be an indicator for increased transport activity in haustoria (Hahn et al. 1997).

Among the *PIG*s, putative secondary transporters for amino acids were identified (Hahn and Mendgen 1997; Hahn et al. 1997). These findings supported the potential role of rust haustoria in nutrient uptake (Hahn et al. 1997). However, while an exclusive localization of AAT2p (PIG2p) in haustoria could be shown, no transport activity could be detected (Mendgen et al. 2000). AAT1p (PIG27p) was recently characterized as a broad specificity amino acid secondary active transporter with a main specificity for L-histidine and L-lysine (Struck et al. 2002). However, a localization of the transporter is still to come. AAT3p, another amino acid secondary active transporter identified, exhibits a substrate preference for L-leucine and the sulphur containing amino acids L-methionine and L-cysteine (Struck et al. 2004). However, there have been no localization studies, so far, and RT-PCR analyses indicate that expression of *AAT3* is not restricted to haustoria. Taken together it seems that amino acid uptake in *U. fabae* is not limited to haustoria, but the transporters characterized are clearly energized by the proton-motive force, and show a preference for amino acids present in low abundance in infected leaves (Struck et al. 2004).

Sugar uptake on the other hand seems to proceed exclusively via haustoria (Voegele et al. 2001). HXT1p was localized preferentially at the tip of monokaryotic haustoria (Voegele and Mendgen 2003), and in the periphery of the body of dikaryotic haustoria (Voegele et al. 2001). No specific antibody labeling could be found in intercellular hyphae. Neither nested PCR, nor genomic Southern blot analyses under low stringency conditions provided evidence for additional hexose transporters present in *U. fabae* in any of the developmental stages tested (Voegele et al. 2001). This stands in sharp contrast to the pronounced redundancy of sugar transporters in many other organisms, for example *Saccharomyces cerevisiae* for which 20 different hexose transporters were identified (Boles and Hollenberg 1997). Based on its primary sequence, HXT1p can be placed into the sugar porter subfamily of the MFS [TC 2.A.1.1.]. HXT1p was characterized biochemically by heterologous expression. The data revealed that HXT1p is a proton-motive force driven monosaccharide transport system. Specificity was found for D-glucose and D-fructose with similar K_M-, and V_{max}-values (Voegele et al. 2001). This means that in contrast to the situation with *Am*-MST1p, the sole hexose transporter in the ecto-mycorrhizal fungus *Amanita muscaria* and closest homolog of HXT1p, HXT1p seems to transport both hexoses with similar efficiencies. Our work on *HXT1*/HXT1p provided the first conclusive proof that rust haustoria are indeed nutrient uptake devices (Voegele et al. 2001).

Overall a picture is beginning to emerge which indicates that *U. fabae* makes use of several strategies to cover its nutritional demands. Uptake of amino acids seems to occur via haustoria and also via intercellular hyphae. Uptake of carbohydrates on the other hand seems to be limited to haustoria. Substrate translocation is executed by secondary active transport systems which allow direct coupling of transport to the proton gradient established by the H^+-ATPase.

Where do the sweets come from?

Elucidating the mechanism and specificity of carbohydrate uptake in *U. fabae* provided an important advance for understanding the obligate biotrophic relationship, but at the same time put forward a series of new challenging questions (Szabo and Bushnell 2001). One of the most important questions to address was to clarify the source of the substrates of HXT1p (Voegele et al. 2001).

Earlier research had shown that the level of free hexoses is fairly low in *V. faba* leaves (Lohaus et al. 2001). However, a carbohydrate that is present in abundance in virtually every plant is the long-distance transport form of carbohydrates between source tissue and sink tissue, the disaccharide sucrose [1-ß-D-Glucopyranosyl-2-ß-D-fructofuranoside] (Weber and Roitsch 2000). Source tissue designates plant organs which act as net exporters of carbohydrates; for example, mature leaves in which carbon fixation predominantly takes place in higher plants (Williams et al. 2000). Heterotrophic tissue such as roots, or reproductive structures are net importers of sugars and hence referred to as sink tissues. The major source tissue infected by a plant pathogen is thought to be

converted into sink tissue (Ayres et al. 1996; Wright et al. 1995). With the major plant carbohydrate transport form, sucrose, being diverted directly to the plant pathogen, it seems obvious that the pathogen should try to use this nutrient source and Manners (1989) suggested that sucrose is indeed the major metabolite absorbed by powdery mildews. However, Mendgen and Nass (1988), Aked and Hall (1993), and later Sutton et al. (1999) were able to show that D-glucose is a more likely candidate carbohydrate for uptake by powdery mildew fungi. These data together with the substrate specificity determined for HXT1p in *U. fabae* clearly indicate that it is not sucrose itself which seems to be utilized by the parasite. However, considering the building blocks of sucrose, D-glucose and D-fructose, it appears likely that sucrose may well be the source of the carbohydrates consumed by the pathogen, but an enzymatic cleavage of the disaccharide has to precede the uptake.

Carbon partitioning in higher plants, the re-distribution of sucrose, seems to be largely determined by the activity of sucrose cleaving enzymes, such as invertases (Sturm 1999; Sturm and Tang 1999). Invertases (ß-D-fructofuranoside fructohydrolase [EC 3.2.1.26]) catalyze the hydrolysis of terminal non-reducing ß-D-fructofuranoside residues in ß-D-fructofuranosides, with sucrose being the major substrate (Myrbäck 1960), and are widely distributed among bacteria, fungi, and plants (Yanai et al. 2001). Plants contain different isoforms of invertases, which can be distinguished by their subcellular localization, pH optimum, and isoelectric point (Godt and Roitsch 1997). The invertase(s) responsible for sucrose partitioning, and therefore determining sink strength, are the insoluble acid invertases located in the apoplastic space (Eschrich 1989; Tymowska-Lalanne and Kreis 1998). Hence, this invertase isoform may also be responsible for phloem unloading at the site of pathogen infection. There have been a number of reports of increased invertase activity in plants upon wounding or infection (Benhamou et al. 1991; Heisterüber et al. 1994; Sturm and Chrispeels 1990; Tang et al. 1996; Wright et al. 1995), and this might be explained on the basis of an increased demand for nutrients, for example, for defense reactions. However, for most of the systems analyzed, it has not been possible to distinguish the contribution of plant or fungus to the observed invertase activity increase (Billett et al. 1977; Callow et al. 1980; Krishnan and Pueppke 1988; Tang et al. 1996; Williams et al. 1984). Using reverse-transcriptase-PCR Voegele et al. (2006) showed that whereas transcript for cell-wall bound invertase 2 of *Vicia faba* (CWINV2p) was present in roots, but absent from leaf tissue, infection with *U. fabae* induced expression of *CWINV2* in leaves and elevated expression of the gene in root tissue. Although not resolved to the single cell level, these results provided strong support for the hypothesis that infection with a pathogen establishes a new sink, which stands in competition with already existing sinks.

However, it seems highly unlikely that a pathogen would solely rely on a host enzymatic system in order to satisfy its nutritional demands. Nevertheless, so far it has only been possible to demonstrate a contribution of the fungus to the increased invertase activity during infection in the pathosystem *Botrytis cinerea / Vitis vinifera* (Ruffner et al. 1992; Ruiz and Ruffner 2002). In the course of our research involving carbohydrate metabolism in *U. fabae* we identified a gene with homology to invertases, cloned the gene, and characterized the gene product using heterologous expression. The gene is expressed during parasitic growth and the gene product catalyses the irreversible breakdown of sucrose into D-glucose and D-fructose (Voegele et al. 2006). INV1p was the first invertase described for an obligate biotrophic pathogen. Our results indicate that a rise in host invertase expression as well as a fungal sucrolytic activity contribute to the overall increase in invertase activity in this pathosystem (Voegele et al. 2006).

Another source at least for the HXT1p substrate D-glucose could be BGL1p, a ß-glucosidase (ß-D-glucoside glucohydrolase [EC 3.2.1.21]) (Haerter and Voegele 2004). ß-glucosidases are a subgroup of O-glycosyl hydrolases, and occur widely in prokaryotes and eukaryotes (Bhatia et al. 2002). In fungi and bacteria, for example, ß-glucosidases are involved in cellulose and cellobiose catabolism as part of the cellulase complex and thus play a role in the process of biomass conversion (Leah et al. 1995). BGL1p has the capacity to use cellobiose, a breakdown product of cellulose, as a substrate, and consequently could make degradation products of the plant cell wall available for fungal nutrition (Haerter and Voegele 2004). However, *BGL1* shows a different expression pattern

than *HXT1*. Therefore, there might be alternative or additional roles for this enzyme (Haerter and Voegele 2004).

Where do the sweets go to?

Another important question to address was the fate of the monosaccharides D-glucose and D-fructose once they are taken up by haustoria through the action of HXT1p.

The level of free hexoses such as D-glucose and D-fructose has to be tightly regulated since especially D-glucose is not only an excellent nutrient, but also a powerful regulator of gene expression (Leon and Sheen 2003; Sturm and Tang 1999). There was evidence from an EST sequencing project that both glycolysis and the pentose phosphate pathway (PPP) are operational in haustoria of *U. fabae* (Hahn unpublished results; Jakupovic et al. 2006). In any case, the first enzyme to act on the monosaccharides translocated by HXT1p should be a hexokinase (ATP:D-hexose 6-phosphotransferase [EC 2.7.1.1]), if both D-fructose and D-glucose are funneled into these two pathways, or a glucokinase (ATP:D-glucose 6-phosphotransferase [EC 2.7.1.2]), if only D-glucose is metabolized this way. In the course of our studies targeting nutrient mobilization in obligate biotrophic host-pathogen interactions, we cloned a *U. fabae* glucokinase (*GLK1*) using degenerate primers derived from *S. cerevisiae* hexokinases (Seibel and Voegele unpublished results). The sequence of *GLK1* was determined at both cDNA and genomic DNA level. Analysis of the amino acid sequence displayed considerable sequence homology with other fungal glucokinases. A detailed biochemical analysis of GLK1p was performed using heterologous expression of *GLK1* in *Escherichia coli*. Biochemical assays revealed substrate discrimination between aldoses and ketoses defining GLK1p as a glucokinase. Both, real-time PCR assays and immunolocalization data using two GLK1p-specific antibodies revealed expression of *GLK1* exclusively/preferentially in haustoria. The presence of a glucokinase in haustoria hints at a preferred if not exclusive usage of D-glucose as substrate in the pathways mentioned above.

But what about the second substrate of HXT1p, D-fructose? During the initial characterization of *PIG*s, one gene, *PIG8* that exhibited substantial homology to short-chain alcohol dehydrogenases, was identified (Hahn and Mendgen 1997). Subsequent analysis revealed strong homology to a mannitol dehydrogenase from *Agaricus bisporus* (Accession number O93868) (Stoop and Mooibroek 1998). Functional characterization of the gene product in the heterologous expression system *S. cerevisiae* revealed that PIG8p constitutes a $NADP^+$-dependent mannitol dehydrogenase (D-mannitol:$NADP^+$ 2-oxidoreductase [EC 1.1.1.138]). As a result the designation of *PIG8* was altered to mannitol dehydrogenase 1, *MAD1* (Voegele et al. 2005). Thermodynamic evaluation of our kinetics data suggested that although termed mannitol dehydrogenase, the equilibrium of the reaction lies far on the side of the reaction educts, which in turn means that the enzyme is more likely to act as a D-fructose reductase (reverse reaction). To illustrate this scenario, we plotted the net reaction velocity as a function of mannitol and D-fructose concentration (assumptions for these calculations were: neutral pH and equimolar concentrations of $NADP^+$ and NADPH).

FIGURE 2 HERE

As shown in Fig. 2 the reaction proceeds in the direction of mannitol formation under almost all conditions. Only under extremely high mannitol concentrations and only negligible amounts of D-fructose does the reaction proceed in the direction of D-fructose formation. MAD1p could therefore be responsible for utilization of the HXT1p substrate, D-fructose, in haustoria of *U. fabae*. Acyclic polyhydroxy alcohols or polyols are secondary metabolites typically associated with the fungal kingdom (Lewis and Smith 1967). A variety of different physiological functions were attributed to these polyols, including a role in carbohydrate translocation and storage (Jennings 1984). This role becomes especially important given that while some plants are able to synthesize mannitol from D-mannose (mannitol:NAD^+ 1-oxidoreductase [EC 1.1.1.255]) (Jennings et al. 1998), most plants are not able to utilize it. The production of mannitol would therefore be an ideal strategy for a pathogen to

store carbohydrate in a soluble form that is freely diffusible in the mycelium, but cannot be accessed by the host.

Our metabolite analyses indeed indicated a dramatic increase in mannitol in infected leaves and large amounts of mannitol in urediniospores (Voegele et al. 2005). Assuming a water content of spores of 20 %, the concentration of mannitol found in spores would be around 1 M, which is close to the solubility level of this polyol. Deposition of sugar alcohols in spores has been described for a number of fungi, including closely related rust species (Maclean and Scott 1976; Reisener 1969). Such a mechanism might suggest a role as a carbohydrate storage compound and/or in stress protection. Our results indicate a role for mannitol as a carbohydrate storage compound because of its rapid disappearance upon germination without ruling out a role in stress protection. There is no doubt that lipids and proteins constitute the major substrates during spore germination (Bago et al. 1999; Shu et al. 1954; Solomon et al. 2003). However, utilizing the pool of mannitol first would enable a quick start of glycolysis, since the conversion of mannitol to D-fructose is a single enzyme step. At the same time, oxidation of mannitol to D-fructose provides reducing power for anabolic processes. In this contest it is interesting to highlight the fact that while *MAD1* transcript was only detected in haustoria, MAD1p could be found in the lumen of haustoria and in urediniospores (Voegele et al. 2005). Our enzymatic characterization clearly identified the mannitol dehydrogenase activity associated with spores as being due to MAD1p. The high level of mannitol in spores in combination with low D-fructose concentrations (Lohaus unpublished results) provides the ideal ground for the forward reaction of MAD1p (Fig. 2). Therefore, MAD1p seems to be responsible for the formation of mannitol from D-fructose in haustoria and for the mobilization of mannitol for metabolism in germinating urediniospores (Voegele et al. 2005).

This scenario requires the presence of a hexokinase [EC 2.7.1.1] in spores of *U. fabae*. The glucokinase, GLK1p, identified by us (Seibel and Voegele unpublished results) would not be suited to catalyze this reaction. Furthermore, GLK1p could not be localized in spores. This hints at the presence of an additional yet to be identified hexose phosphorylating activity in *U. fabae*..

Another important aspect of our work on hexose metabolism in *U. fabae* was the identification of a novel enzyme (Link et al. 2005). D-arabitol dehydrogenase 1 (D-arabinitol:$NADP^+$ oxidoreductase [EC 1.1.1.287]), ARD1p, acts on D-ribulose, D-xylulose, and D-arabitol using $NADP^+$/H as a cofactor. The enzyme could be localized in the lumen of haustoria. High levels of D-arabitol were found in infected leaves and ungerminated urediniospores. Again, upon germination D-arabitol diminished rapidly, also suggesting a role of this polyol in carbohydrate storage. However, since no ARD1p was detected in spores, utilization of D-arabitol in germinating spores has to proceed via a different enzymatic pathway. Mannitol and other acyclic polyhydroxy alcohols accumulate in a variety of fungi (Lewis and Smith 1967). For example, axenic cultures of *P. graminis* produce substantial quantities of both D-sorbitol and mannitol (Manners et al. 1982, 1984). Many other fungi showed accumulation of mannitol and D-arabitol (Maclean 1982). Our own work indicates that *U. fabae* has a strong preference to accumulate both mannitol and D-arabitol. In contrast to the situation in *P. graminis*, there is no indication that D-sorbitol plays a role in *U. fabae*. Thus, there might be considerable differences in the polyol patterns even between closely related fungal species (Pfyffer et al. 1986).

Our work regarding hexose mobilization, uptake and utilization clearly identified a number of important genes / gene products in *U. fabae* (Fig. 3). Haustoria are clearly uptake devices for some of the most important nutrients, but at the same time they are also the place where major parts of the ensuing metabolic pathways are carried out. In addition, haustoria also seem to be responsible for the production of polyols used as storage compounds in spores. However, it remains to be elucidated how the polyols are translocated from haustoria, through the mycelium and deposited in the urediospores. Additional functions for these polyols are highly likely (see below).

FIGURE 3 HERE

A link from nutrients to the obligate biotrophic lifestyle

In order to establish the obligate biotrophic relationship with the host, the pathogen needs to evade or suppress host defense reactions. Rust fungi seem to have evolved a number of mechanisms to avoid recognition by host surveillance systems.

Analyses of the structural components of early rust infection structures indicated the most obvious differences between infection structures produced on the outside of the leaf, and those produced once the fungus has entered the tissue (Freytag and Mendgen 1991b; Kapooria and Mendgen 1985). One explanation for these differences would be the modification of chitin containing rust infection structures through the action of acidic cellulases and proteases (Freytag and Mendgen 1991a). Another explanation would be the conversion of chitin to chitosan by the action of a chitin deacetylase (El Gueddari et al. 2002). General recognition of patterns common to a whole group of pathogens, so called Pathogen Associated Molecular Patterns, or PAMPs, by the plant innate immune system is thought to be one of the basic defense responses of a plant (Nürnberger and Brunner 2002; Parker 2003). Therefore, masking of fungal infection structures by obscuring or modifying for example chitin, might be one possibility for the pathogen to avoid recognition by the host.

The ß-glucosidase BGL1p (see above) might also play a role in the suppression of host defenses (Haerter and Voegele 2004). The protein shows high homology to other fungal ß-glucosidases involved in the detoxification of saponins. It is therefore quite possible that BGL1p has additional or alternative functions than providing substrate for HXT1p. This hypothesis, however, awaits verification.

Since some of the carbohydrates under investigation are not only superb nutrients, but also powerful regulators of gene expression (Leon and Sheen 2003; Sturm and Tang 1999), it is also conceivable that alterations in the concentration of one or more of these compounds could result in altered gene regulation in either host or parasite, or both. There is evidence from the pathosystem *Albugo candida* / *Arabidopsis thaliana* that the level of all three carbohydrates rises as a result of infection (Chou et al. 2000; Tang et al. 1996). However, there is still no conclusive proof that alterations in gene expression are the direct result of altered carbohydrate levels. In the pathosystem *U. fabae / V. faba* there seem to be no significant changes in the level of free hexoses or sucrose (Link et al. 2005; Voegele et al. 2005). The balancing of the levels of the different carbohydrates may actually be another way for the pathogen to evade detection by host surveillance systems. For example, by keeping the level of D-glucose within certain limits, it might be possible for the pathogen to prevent the induction of host signaling cascades involved in mounting defense responses.

Infection with *U. fabae* has far reaching effects on host metabolism exceeding the boundary of the infected cell. This has been shown by expression analysis of *V. faba* genes in response to attack by the pathogen (Wirsel et al. 2001). Several of the genes we analyzed showed altered expression patterns in the infected organ as expected. However, some of the analyzed genes also showed alterations in expression in far remote organs, such as stems and roots. Our work therefore clearly shows that the influence on host metabolism by a leaf pathogen is not limited to the infected organ alone. Our results regarding the expression of host and pathogen invertases also show far reaching effects on host metabolism caused by infection with *U. fabae* (Voegele et al. 2006). Alterations in the expression level of plant invertases indicate systemic effects of infection. An attractive explanation for the observed expression of the fungal invertase INV1p in early infection structures stems from the role insoluble acid plant invertases have in the determination of the sink strength of a plant organ. Apoplastic hydrolysis of sucrose would limit export of carbohydrates from the infected tissue via the phloem and therefore would condition the tissue for a conversion from a source to a sink, which then stands in competition with naturally occurring sinks (Voegele et al. 2006).

There is also evidence that mannitol and D-arabitol are released from the fungal mycelium into the apoplast (Link et al. 2005; Voegele et al. 2005). Results from mammalian (Chaturvedi et al. 1996) and from plant (Jennings et al. 2002) pathosystems indicate that at least mannitol can effectively be used to suppress host defense responses involving reactive oxygen species. The concentrations of mannitol and D-arabitol in infected *V. faba* tissue were shown to be sufficient to effectively quench

reactive oxygen species (Link et al. 2005; Voegele et al. 2005). In essence, there might be a direct link between nutrient acquisition and at least parts of the pathogens' system to prevent activation of host defense responses. But surely this cannot be the only strategy of the parasite to establish a long lasting obligate biotrophic relationship with its respective host.

There is more to establish and maintain an obligate biotrophic lifestyle

As already mentioned, the establishment and maintenance of biotrophy requires the evasion or suppression of host defense reactions. Besides masking fungal structures, the usage of sugar alcohols to quench reactive oxygen species, the use of detoxifying ß-glucosidases, and the potential control of the level of regulatory carbohydrates, rust fungi seem to have evolved further strategies to avoid recognition through host surveillance systems.

Analysis of the morphology of extrahaustorial membranes produced by *P. graminis* or *P. coronata* on oat for example revealed several differences. This in turn suggests that formation of the fine structure of the haustorial host-parasite interface is under the control of species-specific signals from the fungus (Harder and Chong 1991). Such signals may include suppressors, which have been implicated in maintaining basic compatibility between the parasite and its host plants (Bushnell and Rowell 1981). Evidence for such suppressors comes from a phenomenon called induced susceptibility. French bean tissue already infected by *U. vignae* supported additional infections by several non-host pathogens (Fernandez and Heath 1991). Suppressors for plant defense responses have been described, but they are either poorly characterized or non-proteinaceous (Basse et al. 1992; Knogge 1997; Moerschbacher et al. 1999). Nevertheless, it is reasonable to assume that fungi, like their bacterial counterparts, have evolved mechanisms to deliver proteins as effectors to take control of the hosts' metabolism.

Papers from Australian researchers working with the pathosystem *Melampsora lini / Linum usitatissimum* indicate that there are a number of haustorium-specific secreted proteins that interact directly with corresponding host resistance gene products (Catanzariti et al. 2006; Dodds et al. 2004, 2006). This work was done using heterologous expression systems and biochemical assays and confirms the gene for gene hypothesis put forward by Flor (1955, 1956) more than 60 years ago at the molecular level. However, it has to be kept in mind that the interaction of avirulence gene products and resistance gene products results in an incompatible interaction; that is, a failure of the pathogen to establish infection fully. While this is certainly an interesting aspect with respect to the basic understanding of resistance reactions and the identification of new avirulence gene – resistance gene combinations is also advantageous for breeders, this situation does not reflect the true obligate biotrophic lifestyle, which is based on a long lasting interaction of host and parasite.

Recently, Kemen et al. (2005) showed that one of the *PIG*s identified by Hahn and Mendgen (1997) is actually not only secreted into the extrahaustorial matrix as expected from its targeting sequences, it is further transferred to the host cell cytoplasm and nucleus. It remains to be shown if Rust Transferred Protein 1 (RTP1p) acts as a suppressor or has other functions. However, RTP1p distribution seems to be limited to the infected host cell. Since RTP1p does not have any homologs in publicly accessible databases, it is not possible to deduce potential functions from sequence homologies. *In silico* analysis of RTP1p revealed the presence of potential targeting signals and domains. We also identified *RTP1* homologs in the closely related rust fungus *U. striatus* (Kemen et al. 2005). Both proteins share an overall identity of 71%. However, if only the C-terminal halves of the proteins are compared the level of identity increases to more than 91%. This C-terminal portion also contains one N-glycosylation site common to both proteins which seems to be essential for proper folding and secretion of the protein (Kemen 2006a). The C-terminal half of the proteins also contains a ß-aggregation domain often associated with prion like proteins. Indeed, our attempts to overexpress, purify, and crystallize RTP1p have been hampered by the tendency of the protein to aggregate. Using the method developed by Lee and Eisenberg (2003), we showed that amorphous aggregates of RTP1p can be converted into fibrils (Kemen 2006b). The stability of these filaments may be linked to a potential function of RTP1p: the prevention of a collapse of the host cell and the

protection of the haustorium against plant defenses. However, this hypothesis still awaits proof. At the same time it is still unclear how RTP1p is transferred from the extrahaustorial matrix into the infected host cell, and how it reaches the host cytoplasm, once it has entered the cell.

While our initial hypothesis was that *RTP1* distribution might be limited to a few species, i.e. *U. fabae* and some closely related rust fungi, it now appears that *RTP1* homologs are present in a number of rust fungi. So far, we have identified 30 *RTP1* homologs in a broad spectrum of rust fungi (Pretsch and Voegele unpublished results). It now seems that RTP1p might be a protein specific for rust fungi in general, since to date no homologs were found outside the Uredinales.

Outlook

Our analyses of the roles played by haustoria in establishing and maintaining the obligate biotrophic relationship have come a long way. We have shown that haustoria are indeed nutrient uptake devices that additionally seem to function as small power plants for the pathogen. There seems to be a tight link between standard metabolic pathways and potential suppression of host defense reactions. Our research has also shown that, like their bacterial counterparts, rust fungi are also able to deliver effector proteins into the infected host cell, although the mechanism of this transfer is still elusive. Establishing a system for the stable transformation of *U. fabae* will enable us to lift our research to a higher level. There is certainly much more to the obligate biotrophic lifestyle awaiting to be elucidated.

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Fig. 1 Schematic representation of a dikaryotic rust haustorium. Structures derived from the fungus are depicted in blue, structures contributed by the plant are shown in green. The extrahaustorial matrix is shown in light blue and the extrahaustorial membrane in yellow. Drawing from Voegele (2006), with modifications.

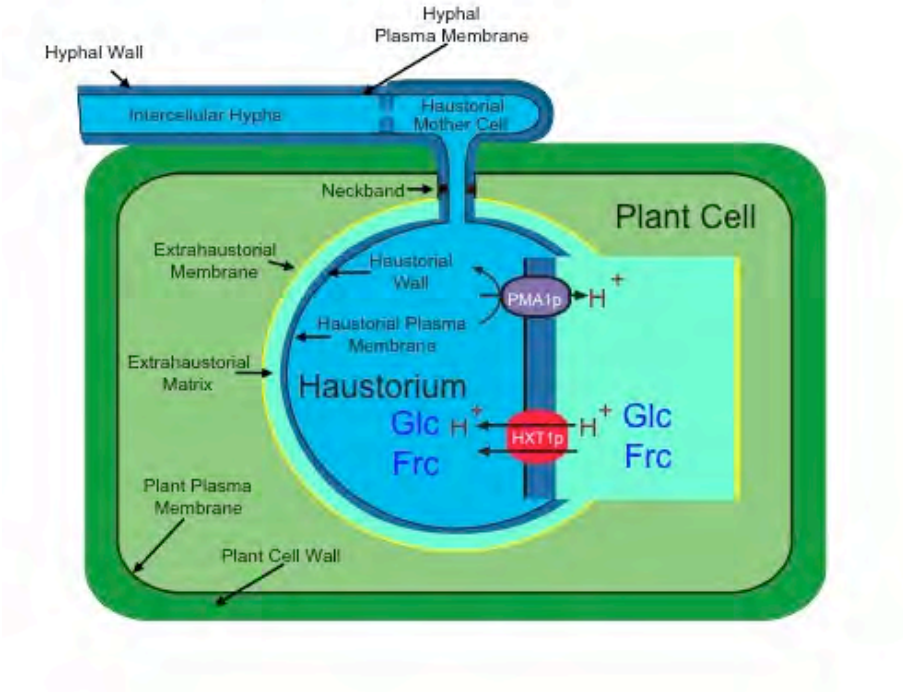


Fig. 2 Net-reaction velocities of the MAD1p-catalyzed reaction. Reaction velocities were calculated as a function of D-fructose and mannitol concentration. The following assumptions were made: Velocities were calculated for neutral pH and an assumption of equimolar concentrations of $NADP^+$ and NADPH.

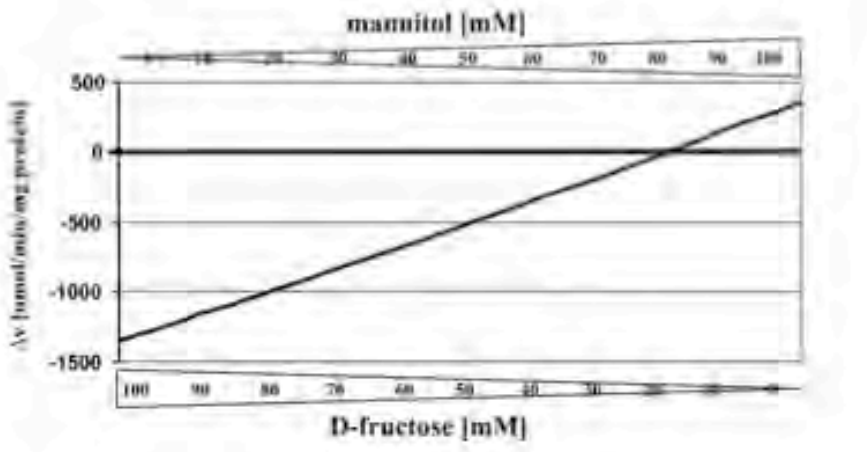
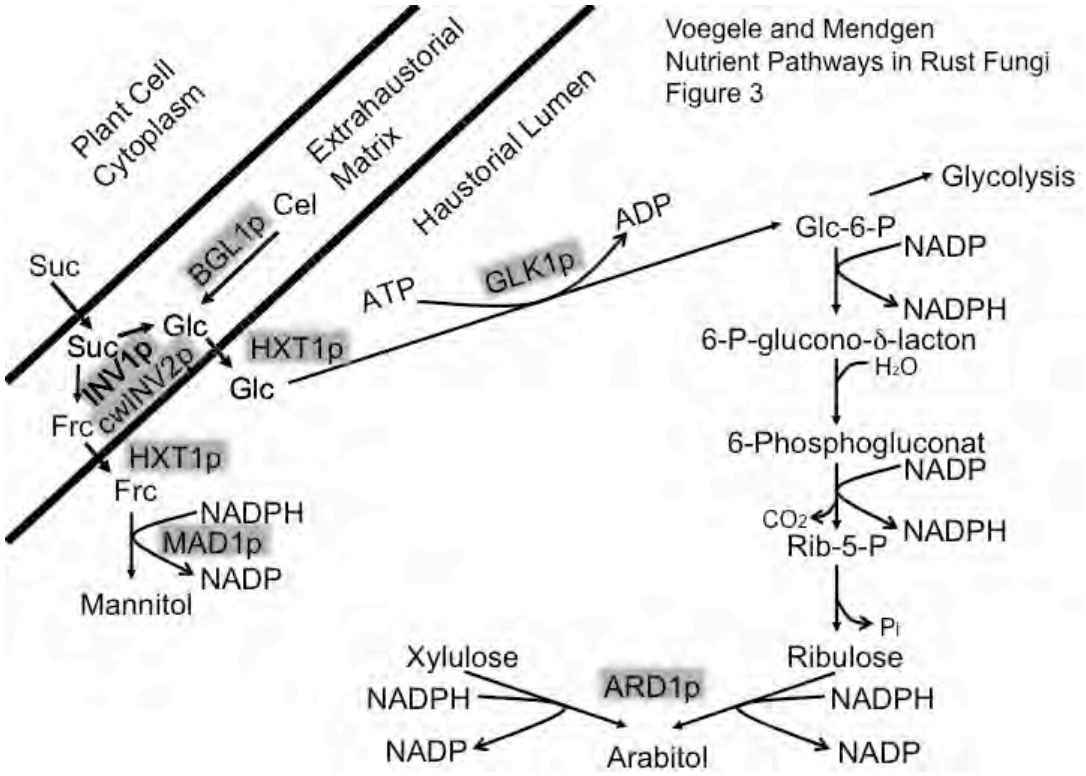


Fig 3 Mobilization, uptake, and utilization of hexoses in haustoria of *U. fabae*. Sucrose (Suc) is released from the infected plant cell either actively or passively. The disaccharide is cleaved by the fungal invertase INV1p and possibly the action of plant enzymes. The resulting monosaccharides, D-glucose (Glc) and D-fructose (Frc), are taken up via the hexose transporter HXT1p. Glc might also be provided through the breakdown of cellobiose (Cel) by the action of BGL1p. Glc is funneled into glycolysis and the pentose phosphate pathway (PPP) by phosphorylation through GLK1p. D-fructose on the other hand is converted into mannitol by the action of MAD1p. At the bottom of the PPP, the novel enzyme ARD1p is generating D-arabitol.



Comparative genomics of wheat rust fungi (abstract)

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Rust fungi (*Pucciniales*) cause some of the most devastating plant diseases on all major cereal crops. We sequenced the genomes of the three major rust pathogens of wheat: *Puccinia graminis* f. sp. *tritici* (*Pgt*, the wheat stem rust or black rust pathogen) and *Puccinia triticina* (*Pt*, wheat leaf rust or brown rust pathogen), and *Puccinia striiformis* f. sp. *tritici* (*Pst*, wheat stripe rust or yellow rust pathogen), using Sanger, a hybrid of Sanger and 454, or 454 technologies, respectively. The genomes are large compared to other fungi; the *P. graminis* assembly is 82 Mb, and a draft assembly of *P. triticina* is 127 Mb; a large fraction of each assembly consists of repetitive sequence. The genomes are also highly polymorphic; dikaryotic spore stages were sequenced and ~1 SNPs/kb was identified between the two haplotypes using Illumina data. Additional strains within each species were sequenced using Illumina to identify genome wide polymorphisms. For *Pgt*, the sequenced strains include 'race Ug99' and other African races, and we identified SNP positions which vary between these strains. We initially predicted a total of 20,567 protein coding genes in the *Pgt* assembly, and subsequently flagged 2,326 as dubious calls. Recently we predicted an initial set of 11,638 protein coding genes in the *Pt* assembly, and identified orthologs between *Pgt* and *Pt* and other fungi. To generate evidence for predicted genes and evaluate stage-specific expression patterns, we examined *Pgt* gene expression for different developmental stages as well as infection on either barley or wheat hosts by sequencing cDNA libraries or hybridizing to a *Pgt* microarray. For *Pt* and *Pst*, we are generating EST-like data for gene expression and gene structure refinement by sequencing mRNA samples. Open access to these genomes is provided through the Broad *Puccinia* database (http://www.broadinstitute.org/annotation/genome/puccinia_group) and NCBI.

Role of *Berberis* spp. as alternate hosts in generating new races of *Puccinia graminis* and *P. striiformis*

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Abstract The common barberry and several other *Berberis* spp. serve as the alternate hosts to two important rust pathogens of small grains and grasses, *Puccinia graminis* and *P. striiformis*. Barberry eradication has been practiced for centuries as a means to control stem rust. Diverse virulence variations have been observed in populations of *P. graminis* f. sp. *tritici* that were associated with susceptible barberries in North America. Barberry likely has played a role in generating new races of *P. striiformis* f. sp. *tritici* in some regions in the world. Several North American stem rust races, namely races 56, 15B and QCC, initially originated from barberry, were subsequently responsible for generating large-scale epidemics. Thus, sexual cycles on *Berberis* spp. may generate virulence combinations that could have serious consequences to cereal crop production.

Keywords barberry, life cycle, stem rust, stripe rust, wheat

Introduction

The associations of barberry with cereal rusts were known long ago as evidenced by a law in a French town in the mid-1600s requiring the destruction of barberries near grain fields (Zadoks and Bouwman 1985), even though the specific fungal species remains unknown. Many interpreted the pathogen to be *Puccinia graminis* (the causal agent of stem or black rust) after de Bary in 1864 proved heteroecism, i.e. established that stem rust on cereals and aecia on common barberry (*Berberis vulgaris*) were incited by the same fungus, *P. graminis*. This in turn provided the scientific basis for barberry eradication as a means to control stem rust that was practiced by many countries for more than two centuries. Barberry serving as the alternate host of *P. striiformis* (the causal agent of stripe or yellow rust) was discovered only recently (Jin et al. 2010). In light of this finding, it is equally probable that, in some cases stripe rust rather than stem rust, might have been the intended target for control by the destructions of barberries practiced in the 17th and 18th centuries.

Barberry as a source of genetic variation for *Puccinia graminis* f. sp. *tritici*

The intended purpose of eradicating common barberry plants in the vicinity of grain fields was to eliminate initial inoculum of *P. graminis* f. sp. *tritici* (*Pgt*) coming from barberries to grain fields (Stakman 1919) at an earlier date than might be expected from incoming wind-borne urediniospores. The role of barberry in generating new virulence combinations was not known until the concept of sex in rust fungi was established (Craigie 1927), and new races of *Pgt* were obtained in hybridization experiments (Waterhouse 1929; Newton et al. 1930; Stakman et al. 1930). A decade earlier, however, Stakman (1919) suspected that hybridization between strains might be occurring on barberry, generating new races. A careful examination of the effects of barberry eradication on stem rust in the United States by Roelfs (1982) revealed two unintended but remarkable benefits: reduction in the number of races and stabilization of the wheat stem rust population. The population of *Pgt* east of the Rocky Mountains in North America, though historically diverse (Roane et al. 1960) became asexual as a result of barberry eradication. The number of races in the population declined steadily, a trend that continues to this day. In the past decade, a single race (QFCSC) has dominated the *Pgt* population east of the Rocky Mountains, a region with approximately 60 million acres (about 24 million hectares) of wheat and barley grown annually. Most of this area is considered to be conducive for stem rust development.

In contrast to the simple race structure in the asexual population east of the Rocky Mountains, diverse races of *Pgt* were found in the Pacific Northwest region of the United States (Roelfs and Groth 1980; Burdon and Roelfs 1985). These races are presumably a part of a sexual population due to the observed diversity and the presence of aecial infections on *Berberis* spp. in the region. Recent surveys identified an active sexual population in a relatively small area bordering the states of Washington and Idaho where *B. vulgaris* and several other *Berberis* spp. are present (Y. Jin unpublished). In 2007 a barley field in northeastern Washington was severely infected by *P. graminis*. Analyses of 83 single-pustule isolates derived from a single stem rust sample collected from this field identified 23 races, many of which were further differentiated on a set of supplemental lines consisting of mostly susceptible genotypes (Rouse et al. 2009). In 2009 a total of 16 races were identified from stem rust samples collected from a single wheat field in the Palouse region bordering Washington and Idaho (Y. Jin unpublished). These races were differentiated by virulence/avirulence on six resistance genes: *Sr5* (line ISr5Ra), *Sr7b* (ISr7bRa), *Sr8a* (ISr8Ra), *Sr9a* (ISr9aRa), *Sr9d* (ISr9dRa), and *SrMcN* (McNair 701). Races of *Pgt* with broad virulence have also been isolated from barberry plants in Russia in recent years (Lekomtseva et al. 2005).

Implications of barberry for generating races of *Puccinia graminis* f. sp. *tritici* that have been consequential in North America

Race 56 of *Pgt*, a race responsible for the severe stem rust epidemics in the mid-1930s in North America, originated from barberry. The race was first found on barberries in Iowa and Nebraska in 1928 (Stakman and Rodenhiser 1958). Race 56 was virulent to Triumph (*SrTmp*+), a winter wheat cultivar developed by a farmer in Oklahoma in the 1920s. Triumph became the predominant genetic background in hard red winter wheat throughout the southern Great Plains because of its early maturity, rust resistance, and high yield. Race 56 also was virulent to Kanred (*Sr5*, a common stem rust resistance gene present in winter wheat on the central Plains during this era) and Ceres (*Sr7b,Sr28*), a spring wheat cultivar developed for stem rust resistance that dominated the area in the northern Great Plains. The combination of virulence to these genes enabled race 56 to cause epidemics because it was able to establish and multiply in the southern and central Great Plains, a necessary condition for developing epidemics in the northern Great Plains when barberry was no longer functional as a source of initial inoculum in the region. Epidemiologically, a nearly identical, but more dramatic situation happened two decades later with the epidemics of race 15B. Race 15B was first identified on barberries in Iowa in 1939 and persisted near barberry plants at a low frequency for many years before it caused epidemics in North America in the mid-1950s (Stakman and Rodenhiser 1958). Although the ability of race 15B to attack Thatcher wheat and durum cultivars might have been responsible for inciting epidemics in the northern Great Plains and Prairie Provinces of Canada, it was its ability to attack Triumph wheat that enabled inoculum to build up in the south. Stem rust isolates with virulence to *SrTmp* in North America were rare except in the race 15B lineage and race 56 that originated on barberries.

Between 1989 and 1993, race QCC caused some localized epidemics on barley crops in the northern Great Plains of United States and Prairie Provinces of Canada. This race was first discovered in 1984 in British Columbia (Martens et al. 1989), a Canadian province adjacent to the state of Washington where sexual populations of *Pgt* were known to be present. It is highly likely that QCC originated on barberry because a race with an identical virulence pattern to QCC (i.e. QCCJB) was recently identified from collections near *Berberis* spp. in the Pacific Northwest (Y. Jin unpublished). Although QCC was avirulent to the majority of wheat cultivars grown in the Great Plains, it was virulent on Karl, a popular winter wheat cultivar grown in the southern and central Great Plains. This race is one of only a few identified in the North American stem rust population that is virulent to *Rpg1*, a gene deployed in nearly every barley cultivar in the northern Great Plains (Steffenson 1992). The combination of sufficient inoculum buildup on susceptible wheat in the south and the ability to attack the barley crop in the north enabled QCC to develop into epidemics.

Frequencies of race QCC declined steadily after the removal of the susceptible wheat cultivar in the southern and central Great Plains.

Has barberry played a role in generating races of *Puccinia striiformis* f. sp. *tritici*?

A previous working assumption that *P. striiformis* f. sp. *tritici* (*Pst*) is asexual, under which interpretations were made in attempts to explain some of the observed variation for virulence, is no longer correct in light of the recent discovery of the alternate host, *Berberis* spp. We hypothesized that in areas where wheat and stripe rust-susceptible *Berberis* spp. coexist, sexual recombination has likely played an active role in contributing to the variability of *Pst* (Jin et al. 2010). Variation generated via the sexual cycle obviously can provide a more satisfactory interpretation for the observed virulence and genetic diversity in some of the stripe rust "hot spots" around the world, as in the case of western China (Chen et al. 2009; Duan et al. 2010). *Berberis* spp. are ubiquitous both in their native habitats and as introduced ornamentals in many parts of the world. Although only a few species of *Berberis* have been tested against stripe rust, the known susceptible species, *B. chinensis*, *B. holstii*, *B. vulgaris*, and *B. koreana*, represent several diverse sections in *Berberis* (Ahrendt 1961; Kim et al. 2004), a genus with nearly 500 described species. Thus, more stripe rust susceptible *Berberis* spp. are likely to be found.

Conclusions

A large number of races of *P. graminis* f. sp. *tritici* were found on or near barberry plants in North America, including those that were consequential in generating large-scale epidemics. It is safe to assume that the unique virulence combinations that equipped these races to overcome different cereal resistance genes in different regions were assembled through the sexual cycle. *Berberis* spp. likely played an active role in generating new races in *P. striiformis* f. sp. *tritici*, although evidence for this is circumstantial at this time. Old and recently emerging evidence continues to be gathered about barberries because they harbor two of the most damaging cereal rusts, *P. graminis* f. sp. *tritici* and *P. striiformis* f. sp. *tritici*. Virulence combinations generated through the sexual cycle on *Berberis* spp., however rare, can have serious and long lasting impacts on cereal crops.

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Characterization of two new wheat stem rust races within the Ug99 lineage in South Africa

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Abstract Two new races of the wheat (*Triticum aestivum* L.) stem rust pathogen, representing the fifth and sixth variants described within the Ug99 lineage, were detected in South Africa. Races TTKSP and PTKST (North American notation) were detected in 2007 and 2009, respectively. Except for *Sr24* virulence, race TTKSP is phenotypically identical to TTKSF, a commonly detected race of *Puccinia graminis* f. sp. *tritici* (*Pgt*) in South Africa. PTKST is similar to TTKSP except that it produces a lower infection type on the *Sr21* differential and has virulence for *Sr31*. Simple sequence repeat (SSR) analysis confirmed the genetic relationship amongst TTKSF, TTKSP, PTKST and TTKSK (Ug99). TTKSK, PTKST and TTKSF grouped together with 99% similarity, while sharing 88% genetic resemblance with TTKSP. These four races in turn shared only 31% similarity with other South African races. It is proposed that TTKSP arose locally as a single step mutation from race TTKSF, whereas PTKST probably represents an exotic introduction of *Pgt* to South Africa.

Key words Pathotype · *Puccinia graminis* f. sp. *tritici* · *Triticum aestivum* · Ug99

Introduction

Puccinia graminis f. sp. *tritici* (*Pgt*) is the causal agent of stem rust, a historically important (Saari and Prescott 1985) and recurring (Singh et al. 2006; 2008) disease of wheat. It is highly adapted to long distance migration through wind dispersal and rain deposition of urediniospores (Rowell and Romig 1966; Singh et al. 2006). In addition to natural dispersal mechanisms, accidental transport by means of contaminated clothing or goods may also contribute to the spread of spores (Singh et al. 2006).

Currently more than 60 numbered or temporarily designated *Sr* genes for resistance to stem rust have been listed in the Komugi Wheat Genetics Resource Database (www.shigen.nig.ac.jp, accessed 16 April 2010). Utilization of many of these genes in breeding programs has resulted in the effective control of stem rust in most countries (Singh and McIntosh 1987; McIntosh et al. 1995; Singh et al. 2008). However, the detection of Ug99 (syn. TTKSK, North American [NA] race notation, Jin et al. 2008b) in East Africa, a *Pgt* race with broad virulence (Pretorius et al. 2000; Wanyera et al. 2006; Jin et al. 2007) and adaptive capacity (Jin et al. 2008a; 2008b; 2009), indicated that continued efforts are necessary to control this disease.

The rapid adaptation of Ug99 for *Sr24* virulence (Jin et al. 2008b) was of particular concern as this gene occurs in Kenyan commercial varieties (MacKenzie 2008). *Sr24* continues to be widely used in countries such as Australia (Park and Bariana 2008) and South Africa (McIntosh et al. 1995) and was initially identified as a source of resistance to Ug99 (Jin et al. 2007).

It was recently shown that, in addition to step-wise mutations, an exotic introduction contributed to the genetic diversity of the South African *Pgt* population (Visser et al. 2009). TTKSF, the most prevalent race in South Africa since its first detection in 2000, shares an identical virulence profile with Ug99, except for avirulence towards *Sr31* (Pretorius et al. 2007). This resemblance was confirmed at molecular level using SSR and AFLP analyses (Visser et al. 2009). Ug99 and TTKSF were distinctly different from the other South African races, suggesting that TTKSF was an exotic introduction into South Africa, most probably from Ug99 ancestry in East Africa.

Two races with *Sr24* virulence, namely 2SA100 and 2SA101 (Agriculture Research Council [ARC] notation), were detected in South Africa during the mid 1980s (Le Roux and Rijkenberg 1987). These closely related races are avirulent and virulent to *Sr9g*, respectively, and were distinctly different from Ug99 (Jin et al. 2008b; Visser et al. 2009). Considering the data of Le Roux and Rijkenberg (1987) on comparable entries in the current NA differential set, 2SA100 and 2SA101 code to LSH and LTH, respectively. In 2007, TTKSP (2SA106) with *Sr24* virulence, was detected in the Western Cape, South Africa (Terefe et al. in press). Phenotypically, this race is similar to TTKST except for avirulence to *Sr31*, and similar to TTKSF except for virulence to *Sr24*. A second new race, PTKST, was detected at two locations in KwaZulu-Natal, South Africa, at the end of 2009 (Pretorius et al. 2010). PTKST is virulent to both *Sr24* and *Sr31*.

The objective of this study was to determine the relationship between TTKSF, TTKSP, PTKST, TTKSK and other *Pgt* stem rust races using SSR markers.

Materials and methods

Stem rust isolates

In this study, the South African *Pgt* races were represented by four single pustule isolates each of UVPgt50, 52, 53, 55, 56, 57, 59 and 60. UVPgt58 was represented by a single isolate. All single pustule isolates were sub-samples from the type culture of each race and do not reflect different field collections. The UVPgt notations reflect the wheat stem rust cultures held at the University of the Free State. UVPgt50, 52, 53, 55, 56, 58 and 59 are similar to races 2SA4, 2SA100, 2SA102, 2SA88, 2SA104, 2SA103 and 2SA106 named by the ARC-Small Grain Institute, Bethlehem. UVPgt57 appears to be a single-gene mutant of UVPgt56, differing only in virulence for *SrSatu*. Likewise, UVPgt58 is similar to UVPgt53 except for avirulence to *Sr9g* and UVPgt59 is similar to UVPgt55 except for *Sr24* virulence in the former. Ug99 (TTKSK, Jin et al. 2008b), from the original Ugandan collection in 1999 (Pretorius et al. 2000), was also included.

UVPgt59 (TTKSP) was received as stem rust field collection Pg-KGI-49 from the ARC-Small Grain Institute in 2007, sampled from an unknown wheat line at the Tygerhoek experimental farm, Western Cape, on 26 September, 2007. UVPgt60 (PTKST) was collected on 17 November, 2009 from a wheat cultivar suspected of carrying *Sr31* in a disease nursery near Greytown, KwaZulu-Natal. Virulence was confirmed on several wheat cultivars known to possess the *Sr31* resistance gene (Pretorius et al. in press). Both races were phenotypically characterized on a differential set (Table 1) using standard procedures for inoculation of seedlings and recording of infection types (Jin et al. 2008b). Phenotyping was repeated in at least three independent experiments. Single-pustule isolates of UVPgt59 and UVPgt60 were increased on wheat lines LCSr24Ag (*Sr24*) and Federation*4/Kavkaz (*Sr31*), respectively. Urediniospores were harvested and germinated as previously described (Visser et al. 2009).

Genomic DNA extraction for SSR analysis

Total genomic DNA was isolated from fungal tissue of the UVPgt59 and UVPgt60 isolates using CTAB according to Saghai-Maroof et al. (1984) and as described in Visser et al. (2009). For the other races, previously extracted genomic DNA (Visser et al. 2009) was used in the SSR analyses.

SSR analysis of stem rust races

SSR analysis of all isolates was done using 24 primer combinations that were developed at the Plant Breeding Institute Cobbitty, University of Sydney, Australia (H. Karaoglu and R.F. Park unpublished data). The isolates of UVPgt59 and UVPgt60 were also fingerprinted with SSR primer combinations described by Szabo (2007) and used by Visser et al. (2009). Selected isolates previously used by

Visser et al. (2009) were again fingerprinted using both SSR primer sets to facilitate correlation between the two data sets.

Each 15 µl PCR reaction contained 10 ng total genomic DNA, 10 pmol of each primer and a 1x concentration of KapaTaq ReadyMix (KapaBiosystems, Cape Town, South Africa). The amplification regime was 94°C for 1 min, followed by 31 cycles of 94°C for 30 s, 53 or 55°C for 30 s (depending on the primer pair used) and 72°C for 30 s. A final elongation step of 10 min at 72°C was included. To confirm success of the amplifications, 5 µl of each PCR reaction was analysed on a 1.5% (w/v) agarose gel (Sambrook et al. 1989). Polyacrylamide gel electrophoresis was performed as described in Visser et al. (2009).

Data analysis

A binary matrix recording specific SSR fragments as present (1) or absent (0) was generated for each isolate. Pairwise genetic distances were expressed as the complement of Jaccard's similarity coefficient (Jaccard 1908). Cluster analyses were performed using UPGMA (unweighted pairgroup method using arithmetic averages; Sokal and Michener 1958). Statistical analyses were computed using NTSYS-pc version 2.02i (Rohlf 1998; Exeter Software, NY, USA) and dendrograms were created using the SAHN programme of NTSYS-pc. The robustness of the dendrogram was tested by estimating the co-phenetic correlation values for each dendrogram and comparing them with the original genetic similarity matrix using Mantel's matrix correspondence test (Mantel 1967). Values were calculated using the COPH and MXCOMP programs. To assess the genetic variation among races and among isolates within races, analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was performed using the statistical programme Arlequin version 3.1 (Excoffier et al. 2005). AMOVA was performed to test the structure among races and groups based on UPGMA clustering results (Visser et al. 2009). The significance levels for all AMOVA tests were set at 0.05. The fixation index F_{ST} was calculated and provided a measure of genetic differentiation of groups. Values of F_{ST} greater than 0.25 indicate significant genetic differentiation (Hartl and Clark 1997).

A minimum-spanning network was constructed based on the minimum number of markers between different genotypes. The network was constructed using NETWORK 4.5.0.0 software (www.fluxus-engineering.com), employing the median-joining approach (Bandelt et al. 1999).

Results and discussion

Virulence profiles of TTKSP and PTKST

The infection type (IT) data in Table 1 show that UVPgt59 differed from UVPgt55 (TTKSF) only by its virulence to *Sr24* and it was thus coded TTKSP (Table 1) according to the NA nomenclature system (Jin et al. 2008b). In addition to *Sr31* virulence, isolate UVPgt60 differed from TTKSF and TTKSP only in regard to the IT on the *Sr21* differentials. All eight single-pustule isolates, sub-cultured from UVPgt60, produced an intermediate IT (2 to 2++) on the *Sr21* differential line CS_T mono_deriv (Table 1) and coded to PTKST. The UVPgt60 isolates also produced a clear low IT (;1=) on *T. monococcum* cv. Einkorn, the original Stakman et al. (1962) differential for *Sr21* (Pretorius et al. 2010). TTKSF and TTKSP produced IT 3 on CS_T mono_deriv and IT 2 on Einkorn. Based on stem rust phenotype, TTKSP appeared to be a single step mutant from TTKSF. Given the existence of TTKSP in South Africa, the initial conclusion was that it added virulence for *Sr31*. However, the low reaction conferred by *Sr21* suggested that PTKST may be an introduction rather than a local adaptation from TTKSP. IT data recorded during the original description of Ug99 (TTKSK) (Pretorius et al. 2000) are also included in Table 1.

TABLE 1 HERE

Marker polymorphism

Among the 24 SSR markers developed at the University of Sydney and tested on genomic DNA extracted from 33 South African and four Ug99 isolates of *Pgt*, six primer pairs failed to amplify fragments. Another two each amplified a single faint fragment. The latter amplifications were not repeatable and these eight primer pairs were therefore excluded from the analysis. The remaining 16 SSR primer combinations amplified a total of 69 alleles of which 54 (78%) were polymorphic (Table 2). The number of alleles ranged from one for primer pair A19 to 17 for primer pair A2 with a mean of 4.3 per primer pair. Fifteen monomorphic alleles were amplified by nine primer pairs. In contrast to the SSR study by Visser et al. (2009), the amplified polymorphic alleles were not only present in TTKSK and TTKSF and absent in the other races or vice versa, but polymorphisms were evident between different races that originally grouped together.

TABLE 2 HERE

Genetic diversity

Previously, SSR analysis of UVPgt50, UVPgt52, UVPgt53, UVPgt55, UVPgt56, UVPgt57, UVPgt58 and Ug99 using primer combinations developed by Szabo (2007) divided the races into two groups with one consisting of Ug99 and UVPgt55 and the second group containing the rest (Visser et al. 2009). It was not possible to distinguish between races within each group, as well as between isolates of each race, except for one isolate (56.2) of UVPgt56 which had a unique banding pattern. After combining the SSR data of UVPgt59 and UVPgt60 with that generated for the other races using the Szabo (2007) primers, a dendrogram was constructed using Jaccard's coefficient of similarity and UPGMA for clustering. Based on the available data, the 37 isolates were again divided into two groups (Fig. 1). There was a good fit between the Jaccard's coefficient matrix and symmetrical matrix produced by the UPGMA-based dendrogram with the cophenetic correlation coefficient (r) being 0.9987.

The first group (Fig. 1) consisted of UVPgt55, UVPgt59, UVPgt60 and Ug99, whereas the second group contained the remaining races. The genetic similarity between the two groups increased from 24.5% previously (without UVPgt59 and UVPgt60) to 36%. Within the first group, UVPgt55, UVPgt60 and Ug99 clustered separately from UVPgt59 with a similarity of 97%, but it was again impossible to distinguish between the other races.

FIGURE 1 HERE

To improve the resolution between the individual isolates of the different races, a dendrogram (r = 0.9958) was constructed using the 69 SSR alleles generated using SSR primer pairs developed at the University of Sydney (Fig. 2). Again, two major groups were found with the genetic similarity between the two groups decreasing to 27%. Within the first group, UVPgt55, UVPgt60 and Ug99 clustered separately from UVPgt59 with an 81% similarity between the two subgroups. Isolates Ug99.1, Ug99.2 and Ug99.3, clustering with UVPgt60, showed a genetic similarity of 97% with isolate Ug99.4 that clustered with UVPgt55.

FIGURE 2

Within the second group, two subgroups were evident (genetic similarity of 73%) with isolates from UVPgt50, UVPgt52, UVPgt53 and UVPgt57 clustering together with a similarity of 93%. Isolates of UVPgt53 fell into two smaller groups indicating genetic heterogeneity between the four isolates. The second subgroup consisted of isolates from UVPgt56 and UVPgt58 with a genetic similarity of 92%.

When the two SSR data sets were used to construct a combined dendrogram (Fig. 3), a similar pattern was observed (r = 0.9997) except that the genetic similarity of the different associations was lower than that using the Szabo (2007) primers, but higher than when the University of Sydney

primers were used. It was still impossible to distinguish between UVPgt50, UVPgt52 and UVPgt57, whereas there was some differentiation between different isolates from UVPgt53 and UVPgt56.

FIGURE 3 HERE

Analysis of molecular variance (AMOVA) was used to determine the main source of genetic variation. When isolates of all races were divided into two groups (UVPgt55, UVPgt59, UVPgt60 and Ug99 in group 1 and the other races in group 2 based on dendrogram results) 91.3% (P<0.001) of the molecular variability could be attributed to variation between the two groups, with 7.9% attributed to variation among races within groups and 0.71% among isolates within each race. The F_{ST} value of 0.993 was indicative of the high genetic differentiation among the races.

A minimum-spanning network of the combined data (Fig. 4) produced a pattern similar to Fig. 3. A total of 74 mutational events separated the two main groups. Within one group, all UVPgt59 isolates were separated by nine mutational events from three Ug99 isolates and the UVPgt60 isolates and by one further mutational step from the four isolates of UVPgt55 and one isolate of Ug99. In the other group, the cluster consisting of all four isolates of each of UVPgt50, UVPgt52 and UVPgt57 were separated by 10 events from a hypothetical intermediate isolate linked to UVPgt56 and UVPgt58. Results indicated the possibility of recombination between isolates of UVPgt56 and UVPgt58.

FIGURE 4 HERE

Conclusions

Based on infection type and SSR results, the close genetic relationship between TTKSP (UVPgt59), TTKSF (UVPgt55), PTKST (UVPgt60) and TTKSK (Ug99) was confirmed. With TTKSF being a likely exotic introduction into South Africa and TTKSP a putative mutational derivative, it can be concluded that the latter evolved locally. TTKSP is the fifth variant within the Ug99 lineage (Jin et al. 2008a). PTKST, the sixth described race in this group, most likely represents an exotic *Pgt* introduction to South Africa and highlights the vulnerability of the wheat industry to foreign pathogenic races. This emphasizes the need for regular monitoring of the stem rust pathogen, in particular isolates in the variable Ug99 lineage, as well as continued resistance breeding.

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Table 1 Infection types produced by *Pgt* races TTKSK (Ug99), TTKSP (UVPgt59) and PTKST (UVPgt60) on differentiating lines and scored according to the 0 to 4 scale (Stakman et al. 1962).

Entry	*Sr* gene	Infection type		
		TTKSK[a]	TTKSP	PTKST
ISr5-Ra	*5*	3+[b]	4	4
Cns_T_mono_deriv	***21***	**Not tested**	**3**	**22++**
Einkorn	***21***	**1**	**2**	**;1=**
Vernstein	*9e*	3+	4	4
ISr7b-Ra	*7b*	4	4	4
ISr11-Ra	*11*	3+	4	4
ISr6-Ra	*6*	4	4	4
ISr8a-Ra	*8a*	3+	4	4
Acme	*9g*	3+	4	4
W2691SrTt-1	*36*	;1=c	0;	0;
W2691Sr9b	*9d*	3+	4	4
Festiguay	*30*	3+	3++	3++
Renown	*17*	3+	3++	3++
ISr9a-Ra	*9a*	Not tested	4	4
ISr9d-Ra	*9d*	Not tested	4	4
W2691Sr10	*10*	Not tested	4	4
CnsSrTmp	*Tmp*	Not tested	2	2
LCSr24Ag	***24***	**1**	**3**	**3**
Sr31/6*LMPG	***31***	**4**	**1**	**4**
Trident	*38*	3+	3+	3++
McNair 701	*McN*	4	4	4
Barleta Benvenuto	*8b*	4	4	4
Coorong (triticale)	*27*	;	;	;
Kiewiet (triticale)	*Unknown*	;	;	;
Satu (triticale)	*Satu*	Not tested	;	;

[a]Data from the original description of Ug99 (Pretorius et al. 2000). Tester lines different from the current set were Reliance (*Sr5*), Vernal (*Sr9e*), Marquis (*Sr7b*), Yalta (*Sr11*), W2402 (*Sr9b*), Gamka (*Sr24*) and Federation4*/Kavkaz (*Sr31*)

Table 2 Number of alleles and allele sizes generated by 16 SSR primer pairs developed at the University of Sydney. Monomorphic allele sizes are underlined while the polymorphic allele sizes are in normal script.

Locus	N_a [a]	N_p [b]	Allele size (bp)
A1	2	2	405 402
A2	17	15	336 333 331 329 327 326 324 323 321 316 313 310 308 303 297 295 269
A4	3	2	241 238 230
A6	2	0	320 305
A7	10	9	311 306 299 297 294 291 285 279 276 273
A8	3	3	286 283 277
A10	3	3	594 389 358
A11	3	2	364 330 327
A12	5	2	241 235 230 222 216
A13	2	2	464 457
A15	3	2	247 243 231
A17	5	2	243 236 231 221 216
A19	1	0	244
A20	2	2	318 312
A21	5	5	233 229 225 219 134
A23	3	3	322 320 316
Total number of alleles	69	54	
% polymorphic alleles		78	
Average number of alleles per primer set	4.3	3.4	

[a] N_a, number of alleles
[b] N_p, number of polymorphic alleles

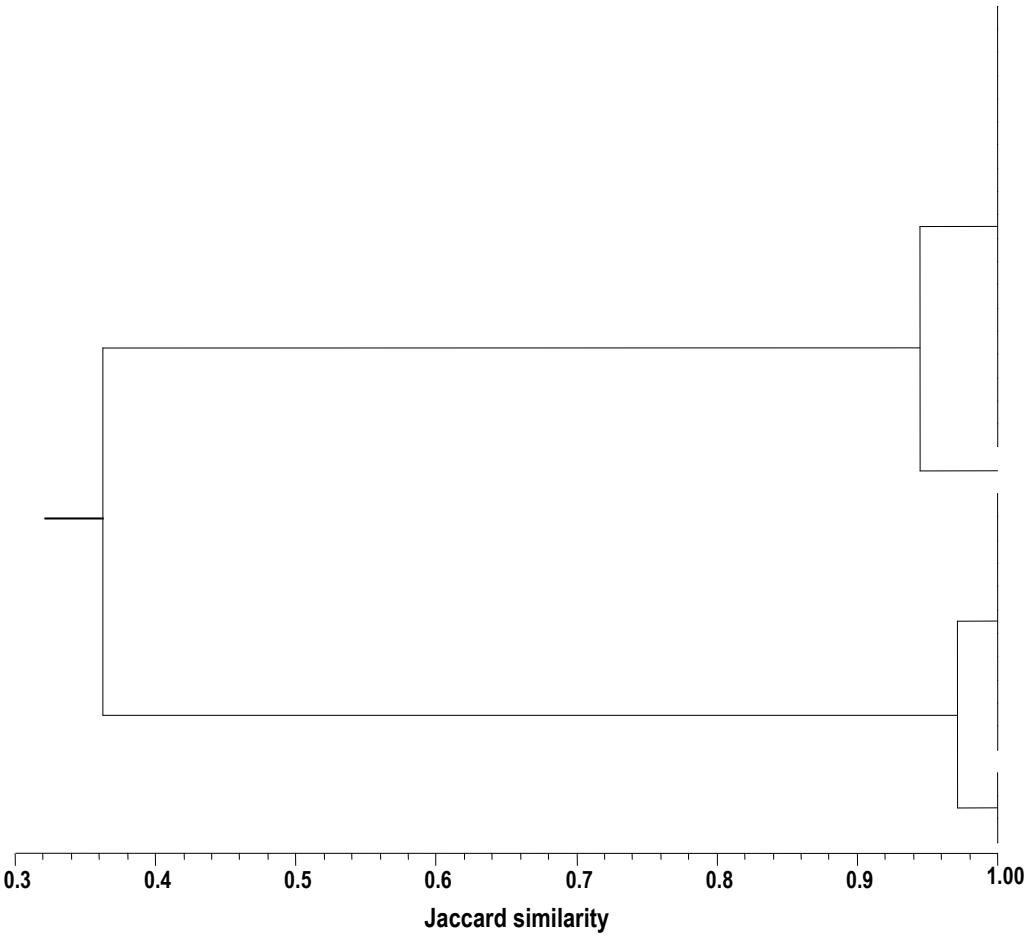

Fig. 1 Dendrogram of 37 *Pgt* isolates based on UPGMA cluster analysis and the Jaccard similarity coefficients calculated from 38 SSR alleles (Visser et al. 2009) generated using SSR primer pairs developed by Szabo (2007)

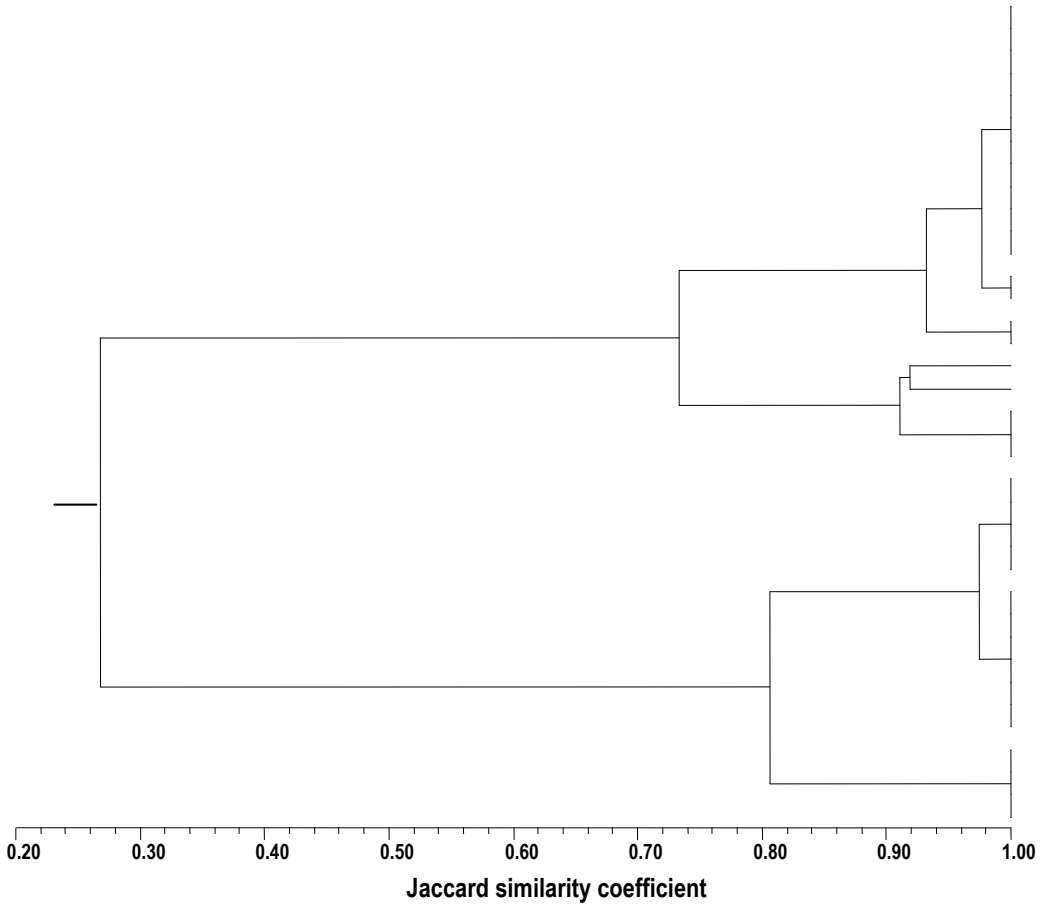

Fig. 2 Dendrogram of 37 *Pgt* isolates based on UPGMA cluster analysis and the Jaccard similarity coefficients calculated from 69 SSR alleles generated using SSR primer pairs developed at the University of Sydney

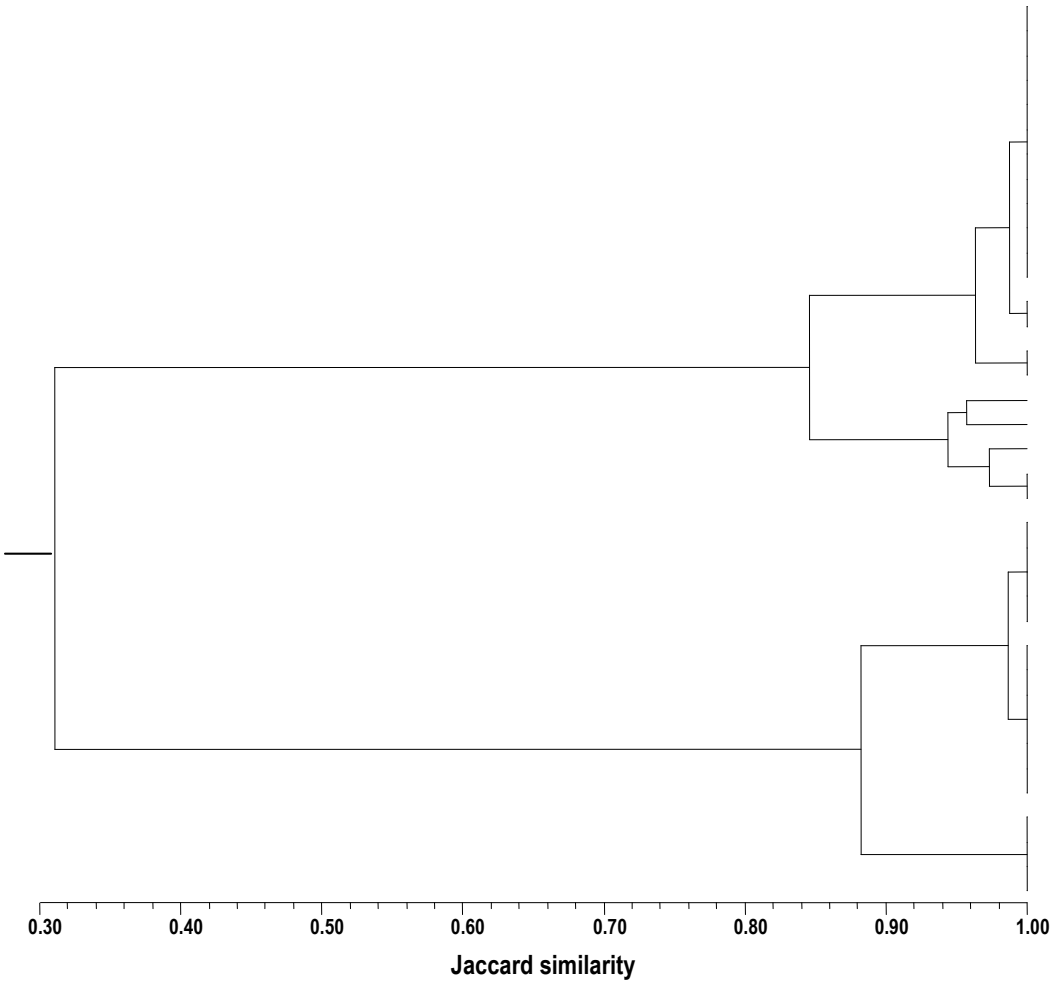

Fig. 3 Dendrogram of 37 *Pgt* isolates based on UPGMA cluster analysis and the Jaccard similarity coefficients calculated from 107 SSR alleles generated using SSR primer pairs developed by Szabo (2007) and the University of Sydney

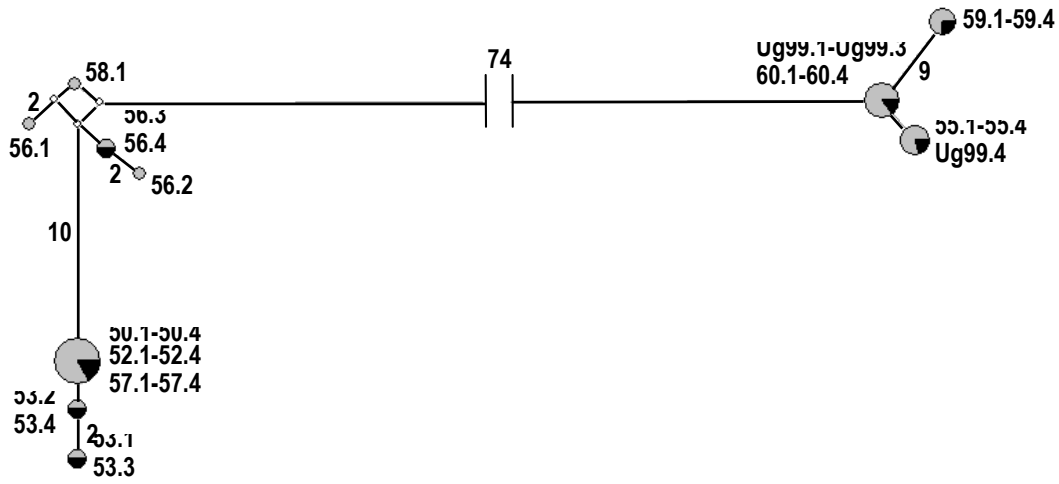

Fig. 4 Minimum-spanning network of 37 tested *Pgt* isolates based on the combined SSR data set generated by primers developed by Szabo (2007) and the University of Sydney. The diameter of each filled node is proportional to the number of rust isolates and the dark coloured sections represent a single isolate with that specific genotype. Open nodes indicate hypothetical intermediate genotypes. The number indicated next to the connecting lines represents the number of mutational events separating each genotype. No number represents a single event.

Determining the basis of nonhost resistance in rice to cereal rusts

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Abstract Cereal rusts are a constant disease threat that limits the production of almost all agricultural cereals. Rice is atypical in that it is an intensively grown agricultural cereal that is immune to rust pathogens. This immunity is manifested by nonhost resistance, the mechanisms of which are poorly understood. As part of the Borlaug Global Rust Initiative, studies are being undertaken to dissect the molecular mechanisms that provide rust immunity in rice and determine if they can be transferred to wheat via transgenesis. Microscopic analyses showed that cereal rusts are capable of entering the rice leaf via formation of an appressorium over a stomate and subsequent infection of underlying mesophyll cells. However, there is considerable variation in the extent of colonization at each infection site. Our research effort has focused on screening for increased growth of cereal rust using natural and induced variants of rice. Two collections of rice mutants, T-DNA insertional mutants and chemical/irradiation-induced mutants, and diverse germplasm accessions are being screened for compromised nonhost resistance to cereal rusts. Preliminary screening with stripe rust identified several potential mutants that allow increased fungal growth. The confirmation of these lines will serve as the foundation for the isolation of gene(s) responsible for this compromised resistance. Details of the strategies being undertaken and progress to date are provided.

Keywords disease, host- pathogen interaction, nonadapted pathogen, *Puccinia,* wheat

Introduction

Nonhost resistance (NHR) prevents plants from being infected by most potential microbial pathogens. Specialized plant pathogens infrequently cross nonhost barriers and infect new crop plant species, providing testimony to the durability of nonhost resistance mechanisms. The molecular basis of nonhost resistance is of obvious interest given the durability of this resistance and the potential to transfer this resistance mechanism(s) by transgenesis to crop plants that are hosts of a given pathogen species. 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 is the first example of transfer of NHR between species (Zhao et al. 2005).

Rust pathogens reduce the production of nearly all agricultural cereals and grasses including wheat, maize, barley, sorghum, oats, triticale, rye, sugarcane, sorghum, and millet. The emergence of a new group of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) (*Pgt*) races in the Ug99 (TTKSK) lineage that threaten global wheat production emphasises the need for durable rust resistance in most cereals (Pretorius et al. 2000; Singh et al. 2006, 2007; Stokstad 2007). In contrast, rice is the only intensively grown cereal crop for which no known rust has been identified. As part of the Borlaug

Global Rust Initiative (BGRI), research is being undertaken to elucidate the molecular basis of immunity in rice to cereal rusts.

Nonhost resistance mechanisms – what is known

Although the mechanisms of NHR are not well defined, current models describe layers of plant defense that must be circumvented by a potential pathogen for successful plant infection (Fig. 1). An obvious initial requirement is basic compatibility in that appropriate physical and chemical signals are required for microbial recognition of a potential host. Preformed plant physical and chemical (anticipins) barriers must be circumvented by an invading microbe which requires appropriate infection structures and anticipin immunity. Microbes produce a suite of highly conserved molecules (e.g. flagellin, translation elongation factors and liposaccharides) collectively known as **p**athogen **a**ssociated **m**olecular **p**atterns (PAMPs) that are recognised by plant receptor proteins (reviewed by Zipfel et al. 2008) leading to the induction of a plant defense response. A successful plant pathogen must suppress this PAMP-triggered immunity (PTI) which is achieved by introducing an array of pathogen effector proteins and products directly into plant cells that target specific plant molecules involved in the defense process (reviewed by Hogenhout al. 2009). Having circumvented PTI a successful pathogen infection is established. This microbe species is therefore an adapted pathogen of the particular host plant species. For those plant species that the microbe is incapable of infecting due to an inability to circumvent the plant nonhost resistance response it is considered a nonadapted pathogen.

FIGURE 1 HERE

The successful parasitism by a plant pathogen leads to plant host and pathogen co-evolution whereby the host plant evolves recognition mechanisms of the adapted pathogen and subsequent activation of a defense response. This recognition is most commonly, but not exclusively, mediated by NBS-LRR resistance proteins that recognise pathogen effector products either directly or more commonly by effector-mediated modification of host plant proteins. This effector triggered immunity (ETI) is the underlying basis of gene-for-gene resistance (Jones and Dangl 2006). The pathogen in turn alters or loses recognized effectors or effector functions thereby reinstating virulence.

NHR resistance is therefore multifaceted and the result of physical, chemical, preformed and active recognition processes.

A number of studies investigated the interaction of rust pathogens on nonhost plants. These include the growth of the cowpea rust pathogen (*Uromyces vignae*), wheat leaf rust pathogen (*Puccinia triticina)* and Asian soybean rust pathogen (*Phakopsora pachyrizi*) on Arabidopsis (Mellersch and Heath 2003; Shafiei et al. 2007; Loehrer et al. 2008); the growth of the barley leaf rust pathogen (*P. hordei*) on wheat (Prats et al. 2007) and *P. triticina*, *P. hordei-murini, P. hordei-secalini* and *P. persistens* growth on barley (Jafary et al. 2008). In the latter interaction barley is considered an occasional host for these pathogens with most, but not all, barley accessions being resistant to these rusts (Jafary et al. 2008). Collectively these studies demonstrated that resistance to nonadapted rust pathogens is polygenically inherited and is an active response involving salicylic acid signalling and the production of reactive oxygen species. These latter biochemical events are often also common to host resistance responses.

Infection of rice by cereal rusts

A prerequisite for examining rice NHR to cereal rust pathogens was to demonstrate that these fungi are capable of infecting rice. That is, to demonstrate that these pathogens can recognise rice as a potential plant host for colonisation and that the immunity of rice is not a consequence of a basic incompatibility. Microscopic analyses of *Pgt* infection of rice demonstrated that this pathogen is capable of producing all the infection structures necessary for successful plant colonisation including

haustoria, the specialised cells used by the fungus for nutrient acquisition (Fig. 2). At some infection sites the extent of *Pgt* growth was very large with several hundred rice mesophyll cells colonised, arguing for nutrient acquisition from the host (Fig. 2D; Ayliffe et al. 2008). Similar patterns of infection were observed with *P. striiformis* f. sp. *tritici* and *P. triticina*. Rice responded to *Pgt* challenge with an active recognition response that involved hydrogen peroxide production, callose deposition and in some instances plant cell death. To dissect the molecular basis of rice immunity to cereal rusts, several approaches are being investigated.

Analysis of genetic variation in the NHR response of rice to stem rust

Potential phenotypic variation in NHR to stem rust is being investigated both macroscopically and microscopically. Diverse rice germplasm was screened by co-inoculation with isolates of *P. graminis* f. sp. *avenae,* race TJS and *P. graminis* f. sp. *secalis,* races BBBB and 92-MN-90. To date, 9,000 rice lines have been screened, including accessions from Africa, Asia, Australia, Europe, North America and South America, in addition to accessions of wild rice and *Oryza glaberrima* (Fig. 3). In no accession did infection develop to the stage of sporulation thus demonstrating conservation of rice NHR to stem rust across this entire range of plant diversity. However, 34 rice lines showed macroscopic symptoms (lesions) to stem rust infection indicating phenotypic variation in the NHR response to these nonadapted pathogens. Microscopic analysis of the 34 lines indicated that increased fungal growth was not associated with this lesion formation.

FIGURE 3 HERE

Microscopic analysis of a number of *Pgt* infected rice lines was undertaken and average infection site areas determined by microscopic measurement. Two rice cultivars (Kyeema and Namaga) were identified that reproducibly showed the largest infection site sizes when compared with the remaining lines. An F_2 family derived from a cross between Kyeema and a highly restrictive line (IR64) typical of the remaining rice cultivars, was assessed for average *Pgt* infection site size over two replicate experiments. These datasets were shown to be correlated (P=0.008), but with only a moderate correlation coefficient (r=0.49). From these analyses we conclude that genetic factors do affect the NHR response in this cross but it is impractical to identify loci segregating in this material by QTL analyses.

Mutagenesis

The identification of genes conferring NHR by mutagenesis was possible in Arabidopsis by isolation of the *PEN* genes (reviewed by Lipka et al. 2008). Screening of EMS-mutagenized Arabidopsis plants with a barley powdery mildew fungus identified a number of plants that showed enhanced epidermal penetration by the nonadapted pathogen. Mutations that allowed enhanced penetration were identified microscopically with a mutation frequency of approximately 0.1% (Stein et al. 2006). These mutant plants were in three complementation groups that led to the isolation of three genes involved in NHR, namely *PEN1-3*, that encode a synataxin protein involved in vesicle targeting to mildew infection sites (Collins et al. 2003), a peroxisome-localised glycoside hydrolase (Lipka et al. 2005; Bednarek et al. 2009; Clay et al. 2009) and an ATP-binding cassette transporter (Stein et al. 2006; Kim et al. 2007), respectively. Both *PEN2* and *PEN3* contribute to a signalling pathway leading to callose formation following PAMP recognition (Clay et al. 2009).

Unlike nonadapted powdery mildew pathogens on Arabidopsis, microscopic analyses showed that cereal rust fungi have little difficulty in entering the rice leaf via formation of an appressorium over a stomate and subsequent infection of underlying mesophyll cells (Fig. 2D). Given the relative absence of rust penetration barriers a logical first step is therefore to screen for rice mutants that allow increased fungal growth relative to the wild type. We assembled two large collections of rice mutants for screening against rusts. The first collection consists of approximately 60,000 M4 lines of the

indica variety IR64 produced by chemical and irradiation mutagenesis at IRRI (Wu et al. 2005). The second collection consists of >20,000 mutants of the japonica variety Zhonghua 11, made by T-DNA insertional mutagenesis at Huazhong Agricultural University, Wuhan, China (Zhang et al. 2007). We are currently undertaking a systematic screen of these mutants using stem rust and stripe rust pathogens.

Screening for increased wheat stem rust growth on rice

Approximately 450 EMS mutagenized IR64 rice lines were screened microscopically for altered *Pgt* infection phenotypes. The first 300 rice lines were identified in previous genetic screens as showing increased susceptibility to compatible races of *Magnaporthe grisea* (rice blast pathogen), i.e. these plants showed reduced basal resistance to an adapted pathogen. None of these lines showed increased growth of the nonadapted *Pgt* pathogen suggesting little overlap between NHR to Pgt and basal resistance to rice blast.

Screening for increased stripe rust growth on rice

Two mutagenized rice populations are currently being screened for altered response to *P. striiformis* infection. We have so far screened 5,229 rice T-DNA lines using a local Chinese *Pst* isolate of race CYR-32. Three potential mutant plants appear to show increased fungal growth compared with wild type (Fig. 4). A second population consisting of 5,000 EMS-mutagenized M4 lines was also screened with the same isolate. A pooling strategy of 100 pools of 50 lines was employed to screen the population. Several potential mutant plants showing increased levels of stripe rust growth were identified in two pools (Fig. 5). Potential mutant plants were grown for progeny testing and, if the mutant phenotypes are confirmed, for genetic analysis. Genomic sequences that flank T-DNA insertion sites are also being isolated.

FIGURES 4 and 5 HERE

Candidate gene approach

Combining mutations in the *PEN2* and *PEN3* genes with mutations in genes (Wiermer et al. 2005) involved in basal defense, salicyclic acid signalling and effector triggered immunity to adapted pathogens *(PAD4* and *EDS1*) generated Arabidopsis plants that showed dramatic increases in the growth of nonadapted mildew pathogens. These additive mutation affects were further enhanced in a triple mutant background (*pen2pad4sag101*) whereby mutant plants became virtual hosts of nonadapted powdery mildew pathogens, allowing the formation of condiophores on this nonhost plant species (Lipka et al. 2005; 2008; Stein et al. 2006).

Unlike mildew pathogens which directly penetrate the plant epidermis, the infection structures produced by urediniospores enter the leaf through stomates. Hence *PEN*-like genes may not be of relevance in the interaction between rice plants and infection by cereal rust urediniospores (in contrast the sexual phase of cereal rust infection does involve direct epidermal penetration). We have therefore begun screening rice lines that are deficient in genes known to be involved in basal resistance and ETI, for comprised NHR to *Pgt* upon inoculation with urediniospores. Preliminary evidence suggests that rice plants deficient for the *RAR1* gene, *EDS1* gene or *CeBip* gene do not show increased growth of *Pgt*.

From these data the following conclusions can be drawn:

1) Extensive screening of diverse rice gemplasm demonstrates that rice is a true nonhost of stem rust and not an occasional host.
2) *Pgt* is capable of infecting rice and producing all the infection structures necessary for colonisation.

3) Some infection sites encompass many (hundreds) of mesophyll cells suggesting nutrient uptake from the pathogen.
4) Rice responds with an active NHR response to *Pgt* that involves the production of reactive oxygen species and callose deposition.
5) Phenotypic variation in the NHR response can be observed amongst rice cultivars both macroscopically and microscopically.
6) Loss of basal resistance to rice blast does not affect the NHR response to cereal rust.
7) Perturbation of several defense pathway molecules did not alter the NHR response to *Pgt* suggesting a great deal of redundancy in this resistance.
8) Multiple potential mutants showing increased growth of *P. striiformis* were recovered from rice mutant collections.

Future work

We will continue screening for potential diversity in NHR response to cereal rusts. Diverse rice germplasm previously screened with the oat and rye stem rust pathogens will be rescreened with wheat stem rust isolates. In addition 10,000 EMS mutagenized rice lines will be screened for macroscopic *Pgt* symptoms. Microscopic screening for altered *Pgt* infection phenotypes on mutagenized rice lines will also continue. Molecular analyses will be used to confirm the identity of T-DNA insertion mutants in rice and demonstrate that these insertions perturb the genes of interest. The genetic analyses of rice mutants that potentially allow increased growth by *P. striiformis* will continue. Demonstrating stable inheritance of increased rust growth phenotypes will be a high priority in these lines. It will also be of great interest to determine if these rice lines are also perturbed in NHR to other rust pathogens such as *Pgt*.

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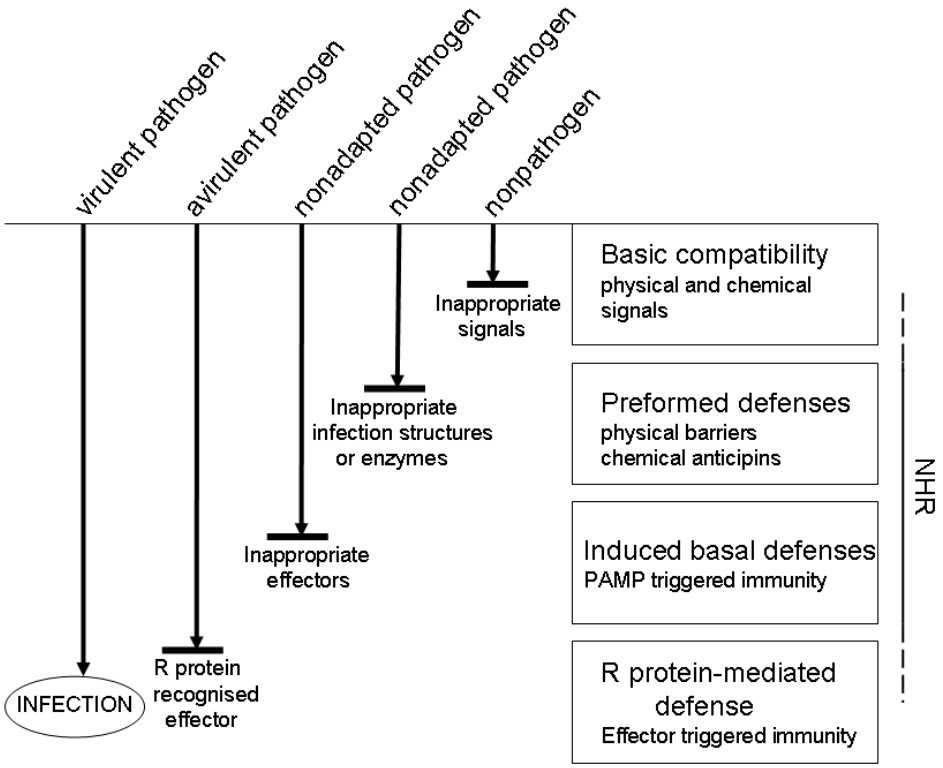

Fig. 1 Summary of plant defense mechanisms that must be circumvented by a pathogen for successful colonisation. Adapted from Ayliffe et al. (2009)

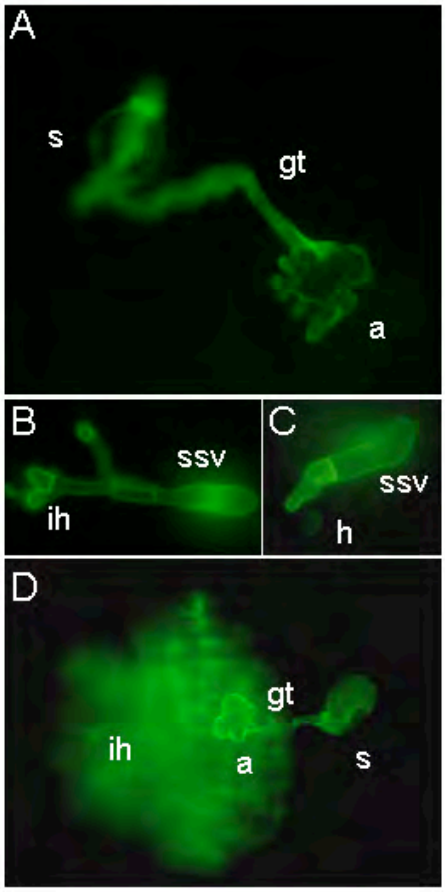

Fig. 2 Infection of rice leaf tissue by *P. graminis* f.sp *tritici* (*Pgt*). (A) Germination of a *Pgt* urediniospore (s) to produce a germ tube (gt) and appressorium (a) on the surface of a rice leaf; (B) *Pgt* substomatal vesicle (ssv) and infection hyphae (ih) within a rice leaf; (C) Production of a *Pgt* haustorium (h) within a rice leaf; (D) A large *Pgt* infection site on a rice leaf. The urediniospore (s), germ tube (gt) and appressorium (a) can be seen on the surface of the rice leaf. The large amount of fungal material underneath the appressorium is infection hyphae ramifying throughout the mesophyll. In all images fungal material was stained with wheat germ agglutinin conjugated to the fluorophore alexa 488 and visualised under blue light following the clearing of tissue. Adapted from Ayliffe et al. (2009).

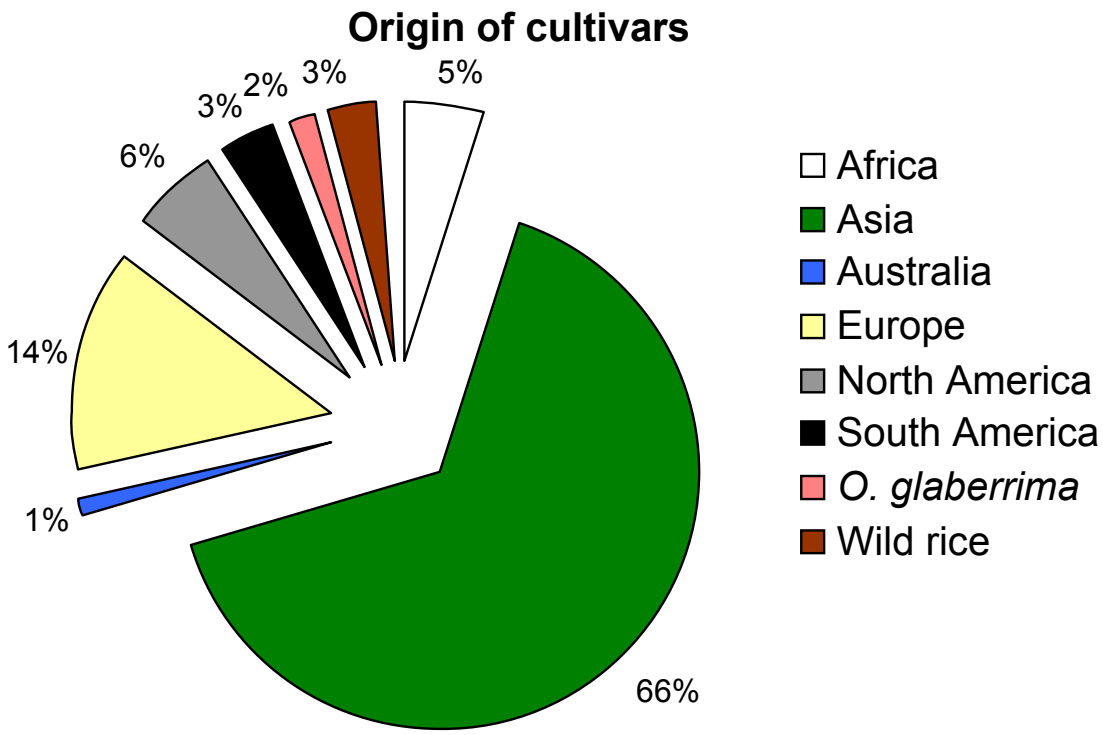

Fig. 3 Origin of 9,000 rice accession from diverse geographical locations screened with oat and rye stem rust isolates.

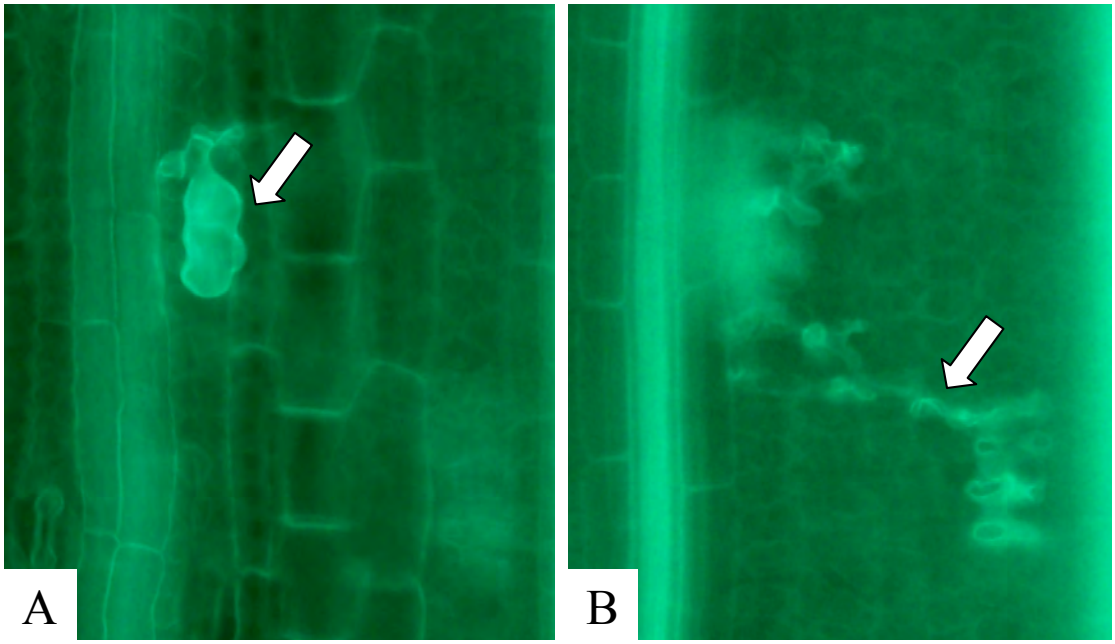

Fig. 4 A T-DNA insertion rice mutant (2611-E-2, in Zhonghua 11 background) showing increased growth of *Puccinia striiformis* f. sp. *tritici (Pst)*. (A). Substomatal vesicle (arrow) in inoculated leaf tissue 20 days after inoculation. (B) Growth of hypha in inoculated leaf tissue 20 days after inoculation (arrow). Tissues were stained by Calcofluor White M2R and observed under UV light

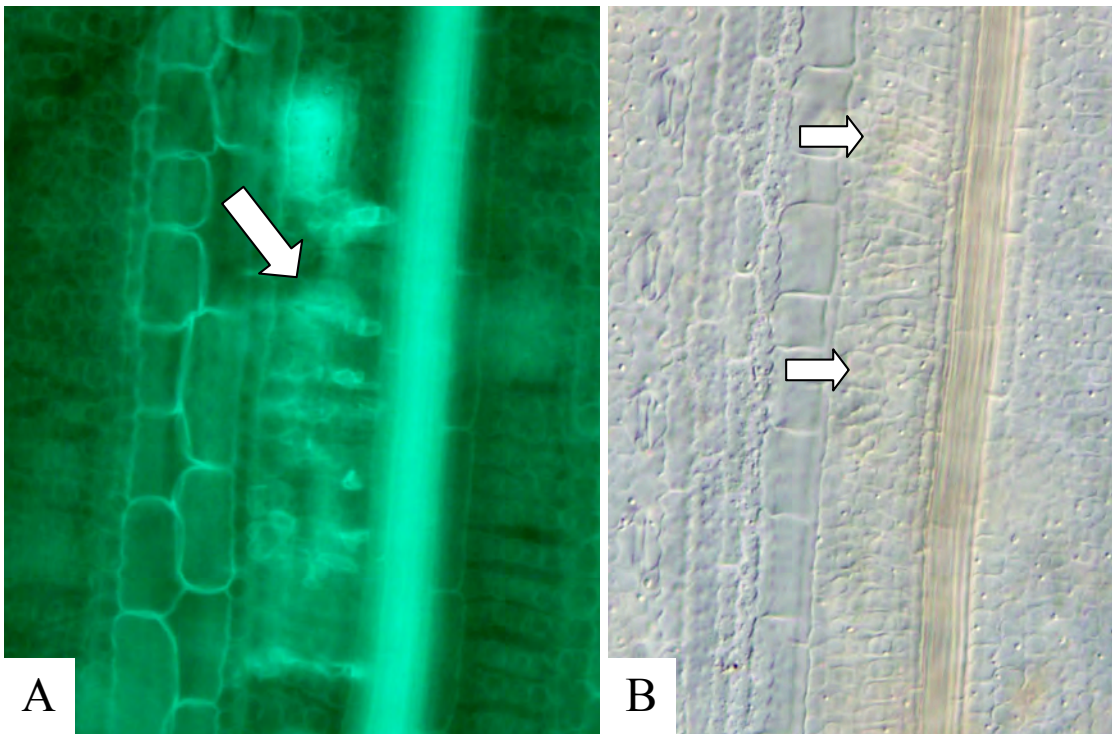

Fig. 5 IR64 mutant (B12-2) showing increased growth of *Puccinia striiformis* f. sp. *tritici* (*Pst*). A) Colonization by *Pst* hypha (arrow) 20 days after inoculation. Leaf tissue was stained with Calcofluor White M2R and observed under UV light. B) Same leaf surface as (A) shown by Nomarski interference optic. Arrows indicate altered cell structure due to hyphal colonization of mesophyll tissue

Race non-specific resistance to rusts in CIMMYT spring wheats: Breeding advances

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Abstract Rust diseases continue to cause significant losses to wheat production worldwide. Although the life of effective race-specific resistance genes can be prolonged by using gene combinations, an alternative approach is to deploy varieties that posses adult plant resistance (APR) based on combinations of minor, slow rusting genes. When present alone, the APR genes do not confer adequate resistance especially under high disease pressure; however, combinations of 4 or 5 such genes usually result in "near-immunity" or a high level of resistance. Although high diversity for APR occurs for all three rusts in improved germplasm, relatively few genes are characterized in detail. Breeding for APR to leaf rust and yellow rust in CIMMYT spring wheats was initiated in the early 1970s and the majority of the wheat germplasm distributed worldwide now possesses near-immunity or adequate levels of resistance. Some semidwarf wheats such as Kingbird, Pavon 76, Kiritati and Parula have shown high levels of APR to race Ug99 and its derivatives based on the *Sr2*-complex, or a combination of *Sr2* with other uncharacterized slow rusting genes. These parents are being utilized in our crossing program and a Mexico-Kenya shuttle breeding scheme is used for selecting resistance to Ug99. High frequencies of lines with near-immunity to moderate levels of resistance are now emerging from these activities. After further yield trials and quality assessments these lines will be distributed internationally through the CIMMYT nursery system.

Keywords durable resistance, leaf rust, *Puccinia graminis tritici*, *Puccinia striiformis, Puccinia triticina,* shuttle breeding, stem rust, *Triticum aestivum*, Ug99, yellow rust.

Introduction

The three rusts , stem (or black), leaf (or brown) and stripe (or yellow) caused by fungi *Puccinia graminis* f. sp. *tritici*, *P. triticina* and *P. striiformis* f. sp. *tritici*, respectively, continue to cause losses, often major, in various parts of the world and hence receive high attention in breeding. The rust fungi are highly specialized pathogens and significant variation exists in their population for avirulence/virulence to specific resistance genes. Evolution of new races through migration, mutation and recombination among existing genotypes, followed by selection is also frequent. Therefore, breeding for resistance has always been a dynamic process. The phenomenon of the erosion of race specific resistance genes, or their combinations, has led scientists to look for alternative approaches to resistance management. Van der Plank (1963) was the first epidemiologist to clearly define a theoretical basis of the concepts of resistance. This approach was widely recommended for breeding for leaf rust resistance by Caldwell (1968), for stem rust resistance by Borlaug (1972), and for yellow-rust resistance by Johnson (1988). The application of such concepts in breeding for leaf rust resistance, commonly known as slow rusting, has been a dominant force in CIMMYT's bread wheat improvement program for almost 40 years with major impacts (Marasas et al. 2003). Today, we understand better the genetic basis of race non-specific or durable resistance to rust diseases and this knowledge is being routinely applied in breeding. We firmly believe that development and deployment of wheat cultivars with such resistance will provide a long-term genetic solution to rust control.

Slow rusting APR to leaf rust and yellow rust

Varying levels of slow rusting resistance are commonly found in wheat germplasm; however, the level of observed APR in field trials is often inadequate. The most studied, and possibly the most effective, now cloned slow rusting leaf rust resistance gene *Lr34* located on chromosome arm 7DS, has maintained its moderate effectiveness for over 60 years of use (Dyck 1987; Krattinger et al. 2009). This gene was traced to Italian variety 'Mentana' using a gene based DNA-marker (Kolmer et al. 2008). *Lr34* is also common in Chinese landraces including 'Chinese Spring', and tall varieties 'Frontana' and 'Chris' from Brazil and USA, respectively. 'Yaqui 50', the first Mexican stem rust resistant tall variety released by N.E. Borlaug in 1950 under the Mexican-Rockefeller Program, also carries *Lr34* probably from the U.S. A. breeding line 'Frontana/Kenya 58//Newthatch' in its development. Subsequently, several first generation semidwarf wheats, such as 'Penjamo 62', 'Torim 73', and 'Kalyan/Bluebird', possessed *Lr34*. Distinct from the NBS-LRR structure underlying many race specific resistance genes, *Lr34* is a novel ABC Transporter gene belonging to the PDR (pleiotropic drug resistant) family and the mechanism of resistance seems unlike a typical gene-for-gene interaction. Moreover, the same resistance gene is also implicated in slow rusting to yellow rust and slow mildewing to powdery mildew even though specifically designated as *Yr18* and *Pm38*, respectively (McIntosh 1992; Singh 1992a; Spielmeyer et al. 2005). *Lr34* is also associated with the expression of post-flowering leaf tip necrosis (LTN) in some environments and the expression of LTN is enhanced under high leaf rust pressure (Singh 1992b).

A second designated slow rusting resistance gene, *Lr46*, located on chromosome 1BL (Singh et al. 1998; William et al. 2003), was first identified in the CIMMYT-derived Mexican variety 'Pavon 76'. This gene is widely distributed in germplasm from CIMMYT and other countries. It also confers slow rusting to yellow rust and slow mildewing to powdery mildew and is designated as *Yr29* and *Pm39*, respectively (Singh et al. 1998; William et al. 2003; Lillemo et al. 2008). *Lr46* is also associated wilh slight post-flowering LTN.

Wheat germplasm 'RL6077' developed in Canada was believed to carry *Lr34* due to the expression of APR and LTN, and because of lack of allelism, was thought to be a translocation to a different chromosome (Dyck et al. 1994). However, absence of the gene-based DNA marker for *Lr34* ruled out the presence of *Lr34* in RL6077, and subsequent studies located a new gene, *Lr67* for leaf rust resistance, and *Yr46* for yellow rust resistance, on chromosome 4DL (Herrera-Foessel et al. unpublished; Hiebert et al. unpublished). The frequency of this gene in wheat germplasm is not yet known. A fourth slow rusting resistance gene located on chromosome arm 7BL and temporarily designated as *LrP*, present in CIMMYT wheat 'Parula', is likely to be distributed widely in CIMMYT spring bread wheat germplasm.

There are other uncharacterized slow rusting genes in CIMMYT and other wheat germplasm with smaller effects than the genes described above and without the association of LTN. The diversity for such resistance genes appears to be higher for yellow rust than for leaf rust. These genes are easier to detect in mapping populations in the presence of larger effect slow rusting genes like *Lr34* and *Lr46* due to their often additive interaction effects enhancing the levels of resistance. However, phenotyping of mapping populations segregating for single slow rusting genes with minor effects remains a challenge and therefore finding tightly linked molecular markers will be very difficult using current approaches. These minor genes play an important role in enhancing the effectiveness of slow rusting genes with larger effects, such as *Lr34*, in achieving high levels of resistance comparable to immunity (Singh et al. 2000).

Genetic analyses of several CIMMYT wheats possessing high or near-immune levels of slow rusting resistance to leaf rust and yellow rust worldwide indicated additive interaction of genes such as *Lr34* or *Lr46* and three or four additional slow-rusting genes (Singh and Rajaram 1992; Navabi et al. 2003, 2004; Zhang et al. 2008). Various genetic studies conducted at CIMMYT and elsewhere led to the establishment of a simple relationship between disease progress and the number of slow rusting resistance genes present in a wheat line (Singh and Trethowan 2007). A more precise relationship is not possible because each slow rusting resistance gene has a different phenotypic effect and the

expression of individual genes is also influenced by the environment. However, the combined effect of 4 or 5 resistance genes is more stable across environments.

Slow rusting APR to stem rust

The APR gene *Sr2*, transferred to Hope and H44-24a from Yaroslav emmer wheat by E.S. McFadden in the U.S. A. and possibly to 'Khapstein' from 'Khapli' emmer wheat by W.L. Waterhouse in Australia, confers slow rusting to stem rust. 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 U.S.A., Canada and Australia, and spring wheat germplasm developed in Mexico (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 the elimination of lines in breeding programs. Under the same environmental conditions, negligible to 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. *Sr2* is either tightly linked, or pleiotropic, with *Yr30*, a minor effect APR gene that confers slow rusting to yellow rust.

Unfortunately, with the exception of *Sr2*, not much is known about the other resistance genes involved in the *Sr2* complex or their interactions. However earlier work by Knott (1982, 1988) and recent characterization in Kenya with Ug99 of various mapping populations involving crosses of APR wheats with a susceptible parent (unpublished CIMMYT studies) indicates that inheritance of complex APR is similar to that described earlier for leaf rust and yellow rust (Singh and Trethowan 2007). The accumulation of about 4 to 5 minor genes is therefore likely to delay stem rust progress to negligible disease levels at maturity under conditions of high disease pressure. Although some of the old tall varieties from Kenya, Canada and U.S. A. continue to be resistant in Ug99 nurseries in Kenya, it is important to identify and 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.

Breeding for slow rusting APR to leaf rust and yellow rust

Breeding for slow rusting resistance based on minor additive genes has been challenging and often slow, for several reasons:
1) a sufficient number of minor genes may not be present in a single source genotype,
2) a source genotype may be poorly adapted,
3) there may be confounding effects from the segregation of both major and minor genes,
4) crossing and selection schemes and population sizes commonly used by breeding programs are more suitable for selecting major genes,
5) reliable molecular markers for several minor genes are unavailable,
6) high costs associated with identifying and utilizing multiple markers.

A successful example of breeding for resistance based on minor genes is the resistance to leaf rust and yellow rust now present in many CIMMYT wheats. This achievement took about 30 years of effort. In the early 1970s, S. Rajaram, influenced by the concept of slow-rusting resistance in wheat proposed by R. Caldwell (Caldwell 1968) and of partial resistance to potato late blight championed by J. Niederhauser (Niederhauser et al. 1954), made a strategic decision to initiate selection for slow-rusting resistance to leaf rust in CIMMYT spring wheat germplasm. In the early phase of breeding he selected plants and lines in segregating populations showing 20-30% rust severities with susceptible infection types. This strategy led to the release of several wheat cultivars, such as 'Pavon 76', 'Nacozari 76', 'Rayon 89' and 'Tarachi 2000', in Mexico and other countries. These slow-rusting

lines were used heavily in the crossing program and resulted in the wide distribution of minor genes within CIMMYT spring wheat germplasm.

In the early 1990s, once the genetic bases and diversity of slow rusting resistances became clearer, high-yielding lines that combined four or five additive, minor genes for both leaf rust and yellow rust resistances showing near-immune levels of resistance were developed through 3- and 4-way crosses involving lines carrying different minor genes (Singh et al. 2000). Plants were selected from large segregating populations under artificially created rust epidemics. As far as possible, races of pathogens that had virulence for race-specific resistance genes present in the parents were used to create the epidemics. The resulting highly resistant lines formed the basis of further resistance breeding and were included in recent international trials, such as ESWYT (Elite Spring Wheat Yield Trial) and IBWSN (International Bread Wheat Screening Nursery). Fig. 1 summarizes the adult plant leaf rust severities of 360 recently developed advanced lines under high disease pressure in field trials at El Batan, Mexico, during 2009. Over 80% of lines had between 1 and 5% severities compared to the necrotic leaves of the susceptible checks. These near-immune lines were susceptible as seedlings in greenhouse tests with the same race as used in the field trial indicating that complex APR was the basis of resistance.

FIGURE 1 HERE

A similar result was observed for the yellow rust responses of 504 recent advanced lines in field trials conducted in Mexico, Ecuador and Kenya (Fig. 2). Although seedling reaction data are not available, it can be predicted from the pedigrees that at least half of the lines showing 1 to 5% disease severity carry near-immune level of APR.

FIGURE 2 HERE

Breeding for slow stem rusting APR to the race Ug99 group

Characterization of existing spring wheat breeding materials for resistance to Ug99 and its derivatives in field trials in Kenya and as seedlings in the greenhouse at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN, U.S.A. during 2005-2009 resulted in identification of several wheat lines with varying levels of APR. The best sources for APR in semidwarf wheat backgrounds were included in the 1st to 4th Stem Rust Resistance Screening Nurseries (SRRSN) annually distributed since 2006. Results were made available at www.globalrust.org and also summarized by Njau et al. (2010) for the 1st to 3rdSRRSN. Wheat lines 'Kingbird', 'Kiritati', 'Pavon 76', 'Muu', 'Parula' and a few others carry high levels of APR. The stem rust responses for the most recent CIMMYT International trials, 4thSRRSN, 31stESWYT and 43rdIBWSN, are given in Table 1. The frequencies of wheat lines with moderate, but likely adequate, levels of APR and race-specific resistance have increased significantly since screening was initiated in Kenya.

TABLE 1 HERE

Because a large proportion of high-yielding spring wheat varieties and germplasm do not carry effective race-specific resistance to Ug99, the availability of genotypes with moderate to high levels of APR provide opportunities to reconstitute high levels of APR in more recent hybrid populations. In the absence of molecular markers for APR 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 APR 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 to all three rusts. The flow of breeding materials in the "Mexico-Kenya Shuttle" is described in Table 2.

TABLE 2 HERE

In the single-backcross approach, resistance sources are crossed with adapted, high yielding wheats. A single backcross is made with the recurrent parent to obtain 350-400 BC_1 seeds. Alternatively, 3-way or top crosses are often made to a second adapted parent. The BC_1 plants are selected for desired agronomic features and resistance to leaf rust and stripe rust, and harvested as bulks in Mexico. F_2 plants derived from the BC_1, simple, and top 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 be present, 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 at Njoro for selection under high stem rust pressure during the off-season. Populations not carrying sufficient resistant plants are discarded. Selection of plants with high to adequate resistance is carried out, selected plants are bulk-harvested and plump grains are selected for establishing F_4 and F_5 populations of about 1,000 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. Selection in the main season is carried out in the same manner as off-season and about 400 plump seeds harvested from selected plants are returned to Mexico and grown at Cd. Obregon under high leaf rust pressure 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 El Batan and Toluca to select for agronomic characteristics and resistance to leaf rust and stripe rust.

Fig. 3 summarizes the stem rust responses of 761 'Mexico-Kenya Shuttle Breeding' advanced lines during the 2010 off-season at Njoro, Kenya, under high disease pressure. The parents of the lines lacked effective race specific resistance genes based on their pedigrees and field reactions. Around 25% of the lines derived from about 60 different crosses displayed near-immune levels of resistance with stem rust severities of 1-5% compared to 100% for the susceptible check Cacuke. An additional 25% of the lines displayed 10-15% stem rust severities. These lines are under yield evaluation in Mexico and stem rust resistance will be verified again during the 2010 main-season in Kenya.

FIGURE 3 HERE

Enhanced expression of moderately effective race-specific resistance genes in the presence of slow rusting APR genes

There are studies demonstrating interaction between moderately effective race specific and slow rusting APR genes. For example, German and Kolmer (1992) showed that *Lr34* enhanced the expression of several moderately effective race specific resistance genes by lowering the seedling infection types to races avirulent to the race specific genes. Singh and Huerta-Espino (1995) reported that although *Lr16* only conferred moderate levels of leaf rust resistance in the field trials in Mexico, the near-immune level of resistance in wheat varieties 'Ciano 79' and 'Papago 86' was based on the interaction of *Lr16* with two additional slow rusting genes. Similarly, immunity to yellow rust in wheat variety 'Pastor' involved moderately effective race specific resistance gene *Yr31* and slow rusting genes *Yr29*, *Yr30* and possibly one additional minor gene (Singh et al. 2003). Detection of a new race in 2008 in Mexico with virulence to *Yr31* changed the near-immunity of Pastor to a moderate level of resistance.

Several known race specific stem rust resistance genes confer moderate to inadequate levels of resistance under high disease pressure in field trials (McIntosh et al. 1995; Jin et al. 2007). Singh

and McIntosh (1986, 1987) showed that *Sr7a*, known to confer only slight resistance in seedlings, conferred high levels of seedling resistance in several wheat backgrounds including 'Chris' and 'Kenya Plume'. Interestingly both of these varieties also displayed high levels of complex APR in Australia and resistance in both varieties remain effective to Ug99 in Kenya. Several of the Ug99-effective characterized and uncharacterized race specific genes confer only intermediate levels of resistance. Adequate protection under high stem rust pressure will require enhanced expression. Enhanced expression of *Sr25* in the field, possibly due to the presence of slow rusting gene *Sr2*, in CIMMYT spring bread wheat backgrounds was reported by Njau et al. (2010). The variation in stem rust severities recorded for wheat lines that carry provisionally designated resistance genes *SrTmp* and *SrSha7* is summarized in Fig. 4. Stem rust severities for lines varied from 5 to 60% for *SrTmp* and 1 to 30% for *SrSha7*. A similar result was obtained for another moderately effective gene *SrHuw234* (data not presented). It is therefore important that slow rusting APR genes are accumulated to enhance the level of protection provided by moderately effective race specific genes under high disease pressures.

FIGURE 4 HERE

Grain yield performance of new Ug99 resistant CIMMYT wheats in target countries

Twenty-nine, high-yielding wheats identified to carry adequate levels of resistance to stem rust at Njoro in 2008 (both off- and main-seasons) were included in the 4th Elite Bread Wheat Yield Trial (4thEBWYT). Twenty-four entries had APR whereas the resistance of 4 entries was based on *Sr25* and one on *SrHuw234*. Fifty-one sets of the trial were distributed to various countries for planting during 2008-2009. Results for six countries are summarized in Table 3.

TABLE 3 HERE

Twenty-eight entries on average yielded 100-114% of the local checks used at 10 sites in India. Five entries, including 'Munal#1' (CIMMYT check) yielded 10-14% higher than the checks. Ten sites represented diverse environments in the North-Western Plain Zone (NWPZ), North-Eastern Plain Zone (NEPZ), and Central and Peninsular Zone (CPZ). Considering only the NWPZ (6 sites), all entries yielded more than the local check and 11 entries were 10-19% higher yielding than the checks (PBW343 used at most sites). 'Wheatear/Sokoll' (entry 529) with *Sr25* was the best yielder, 19% higher than the check in NWPZ. This was followed by 'Neloki#1' (entry 527) with 17% higher yield and APR to stem rust. NWPZ is the main wheat zone in India. The CIMMYT check Munal#1 has shown significant superiority over the checks in India for 3 years of testing and has potential to become a successful variety.

Trials were grown in Pakistan at 5 diverse sites from north to south. Four entries, 508, 515, 519 and 530, on average yielded 7-11% higher than the means of the local checks (different check at each site). Similarly, in Iran trials were grown at 5 diverse sites including those where facultative wheats are grown. On average eight entries had 100-108% yields compared to the checks. The best line was entry 527, Neloki#1, the entry rated 2nd in India.

Fifteen lines yielded 9-21% higher in Afghanistan based on means for three sites. One site data set was returned from Bhairahwa, Nepal, and 10 lines yielded 10-28% higher than the check. Munal#1 was the 2nd best yielder (24% higher yielding than the check) and Neloki#1, entry 527, yielded 28% higher than the check.

One site data set was returned from Kulumsa, Ethiopia. Eight entries had 9-31% higher yields than the highly popular cultivar 'Kubsa'. The top two performers, Wheatear/Sokoll (entry 529) and Neloki#1 (entry 527) were also the top two performers in India. Munal#1 (entry 2), a derivative of Kubsa, had a 12% higher yield than Kubsa and is under seed multiplication in Ethiopia.

Entries included in the 4thEBWYT were selected based on visual agronomic and disease evaluations and grain-yield performance in a single yield trial at Ciudad Obregon in Mexico, the main

breeding and testing site for the CIMMYT spring wheat program. Grain yield performance of new semidwarf wheat lines in various countries shows that significant progress in yield potential has been made over time. Varieties such as PBW343 in India, and Kubsa in Ethiopia, or Chamran in Iran were bred about 15 years ago in Mexico. We believe that changing to new higher yielding Ug99 resistant wheat varieties should enhance wheat productivity and farmers' income in addition to genetic protection from all three rusts.

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Table 1 Stem rust responses of entries included in 31stESWYT, 43rdIBWSN and 4thSRRSN when evaluated Njoro, Kenya, in 2008

	4thSRRSN		31stESWYT		43rdIBWSN	
Resistance category	No	%	No	%	No	%
Adult-plant response						
R-MR (20-30% severity)	20	27.4	15	32.6	5	2.8
MR (40% severity)	29	39.7	7	15.2	19	10.7
MR-MS (50-60% severity)			11	23.9	56	31.5
MS (70% severity)					37	20.8
MS-S (80-90% severity)			3	6.5	39	21.9
S (100% severity-necrotic)					8	4.5
Race-specific resistance gene						
Sr25	12	16.4	5	10.9	8	4.5
SrTmp	10	13.7	2	4.3	5	2.8
SrHuw234	1	1.4	2	4.3		
SrUnknown	1	1.4	1	2.2	1	0.6

Table 2 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	Location[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 bulks & 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 bulks & plump grain retained.
3	Njoro	F_3 and F_4 (800 plants) grown in stem & stripe rust nurseries. Plants with high to adequate resistance tagged and harvested as bulks. Plump grains retained.
	Njoro	F_4 and F_5 (800 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 candidates for International Nurseries 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. 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 response at Njoro.
	El Batan	International Yield Trials and Screening Nurseries prepared and distributed.
7	International	Countries with wheat seasons April-December.
8	International	Countries with wheat seasons October-June.

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

Table 3 Yield performance of entries in 4th EBWYT in six countries

Entry	Cross	Resistance Category[1]	India (10 sites)		Pakistan (5 sites)		Iran (5 sites)		Afghanistan (3 sites)		Nepal (1 site)		Ethiopia (1 site)	
			kg/ha	% Ck	Kg/ha	% Ck	kg/ha	% Ck	Kg/ha	% Ck	kg/ha	% Ck	kg/ha	% Ck
501	Local check		3460	100	3193	100	6916	100	4437	100	3644	100	3106	100
502	Munal #1	APR-MR	3828	111	3077	96	6750	98	4760	107	4519	124	3463	112
503	Kiritati/4/2*Seri.1B*2/3/Kauz*2/Bow//Kauz	APR-MR	3466	100	3272	102	6838	99	5108	115	3148	86	3001	97
504	Tarachi *2//Pfau/Weaver	APR-MR	3567	103	2670	84	7092	103	4830	109	3963	109	3270	105
505	Saar/2*Waxwing	APR-MRMS	3577	103	2912	91	6643	96	4556	103	4000	110	3490	112
506	Seri.1B*2/3/Kauz*2/Bow//Kauz*2/5/Cno79//PF70354/Mus/3/Pastor/4/Bav92	APR-MR	3504	101	3164	99	6662	96	4674	105	3852	106	2747	88
507	PBW343*2/Kukuna/3/Pastor//Chil/Prl/4/PBW343*2/Kukuna	APR-MRMS	3700	107	3334	104	6331	92	4990	112	4082	112	2992	96
508	Whear//Inqualab91*2/Tukuru	*Sr25*	3385	98	3615	113	7105	103	5385	121	4074	112	3422	110
509	PBW343*2/Kukuna//PBW343*2/Kukuna	APR-MR	3705	107	3180	100	6173	89	4617	104	2933	80	3236	104
510	PBW343*2/Kukuna//PBW343*2/Kukuna	APR-MR	3823	110	3285	103	6736	97	4705	106	3585	98	3233	104
511	PBW343*2/Kukuna//PBW343*2/Kukuna	APR-MR	3625	105	3276	103	6286	91	5041	114	3111	85	3129	101
512	Cndo/R143//Ente/Mexi_2/3/Ae.Sq./4/Weaver/5/2*Pastor/6/SKauz/Parus//Parus	APR-MRMS	3533	102	3236	101	6977	101	5072	114	3496	96	2955	95
513	Mino/898.97	APR-MR	3590	104	3045	95	6179	89	4360	98	3459	95	2962	95
514	Picaflor#2	APR-RMR	3609	104	2801	88	7129	103	5134	116	3889	107	3271	105
515	Webill1*2/Brambling	APR-MRMS	3647	105	3447	108	6777	98	5059	114	4037	111	3502	113
516	Becard	APR-MR	3857	111	3157	99	6941	100	4758	107	4185	115	2808	90
517	Becard	APR-MR	3616	105	3251	102	6959	101	5081	115	4148	114	2782	90
518	Becard	APR-MR	3581	104	3319	104	6838	99	4388	99	3259	89	3287	106
519	Prl/2*Pastor//PBW343*2/Kukuna	APR-MR	3655	106	3431	107	6454	93	4912	111	4000	110	2962	95
520	PBW343/Huites/4/Yar/Ae.Sq(783)//Milan/3/Bav92	APR-MR	3372	97	2965	93	5736	83	4602	104	3341	92	2493	80
521	Kauz//Altar84/Aos/3/Pastor/4/Milan/Cupe//SW89.3064/5/Kiritati	APR-MR	3444	100	3142	98	5968	86	4776	108	3704	102	2813	91
522	SW89.5277/Borl95//SKauz/3/Prl/2*Pastor/4/Heilo	APR-MR	3631	105	3228	101	6807	98	4676	105	3526	97	3167	102
523	Seri.1B*2/3/Kauz*2/Bow//Kauz/4/PBW343*2/Tukuru/5/C80.1/3*Batavia//2*Wbill	*Sr25*	3652	106	3286	103	6039	87	4270	96	3852	106	3303	106
524	Pfau/Seri.1B//Amad*2/3/PBW343*2/Kukuna	APR-MR	3641	105	2928	92	6502	94	4372	99	3563	98	3386	109
525	Pfau/Seri.1B//Amad*2/3/PBW343*2/Kukuna	APR-MR	3758	109	2992	94	6117	88	4578	103	3778	104	3156	102
526	Prl/2*Pastor//PBW343*2/Kukuna/3/Tacupeto F2001*2/Kukuna	APR-MR	3808	110	3060	96	6604	95	4723	106	3556	98	2766	89
527	Neloki#1	APR-MR	3937	114	3160	99	7469	108	5047	114	4667	128	3722	120
528	HUW234+Lr34/Prinia//PBW343*2/Kukuna/3/Roelfs F2007	*SrHuw234*	3623	105	3057	96	6570	95	4823	109	3556	98	3003	97
529	Wheatear/Skoll	*Sr25*	3959	114	3370	106	6881	99	5287	119	4333	119	4077	131
530	Wheatear//2*Prl/2*Pastor	*Sr25*	3541	102	3535	111	7015	101	5202	117	3348	92	3476	112
	LSD (P = 0.05)		199		299		519		754		606		363	
	CV, %		8.8		4.8		8.9		7.9		8.0		5.7	
	Heritability		0.40		0.69		0.59		0		0.74		0.79	

[1] Adult plant resistance (APR) categories RMR = 15-20%, MR = 30-40%, MRMS = 50%. Stem rust severities were recorded on adult plants in Kenya in 2008

Fig. 1 Adult-plant leaf rust severities of 360 recently developed seedling susceptible wheat lines (effective race-specific resistance genes absent) evaluated at El Batan, Mexico, in 2009 when susceptible checks were defoliated by leaf rust

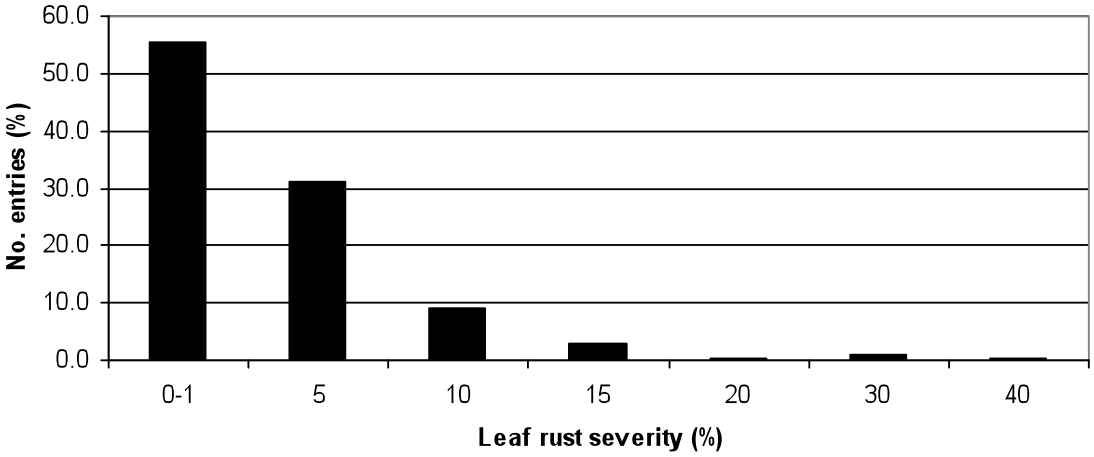


Fig. 2 Adult plant stripe rust severities of 504 recently developed advanced breeding lines at Toluca (Mexico), Santa Catalina (Ecuador) and Njoro (Kenya) in 2009. Data were recorded when the Avocet S check was defoliated in Mexico and Ecuador, and 80% severity in Kenya

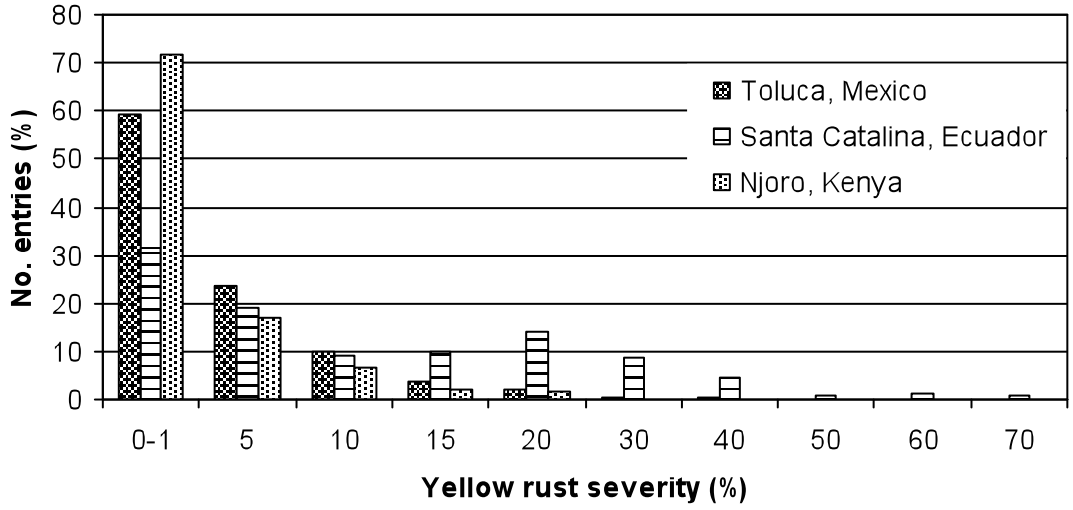


Fig. 3 Adult plant stem rust responses of 761 'Mexico-Kenya shuttle breeding-2008' wheat lines from crosses targeted for incorporating APR into high yielding wheat backgrounds and evaluated at Njoro, Kenya, during the 2010 off-season. Data were recorded 'Cacuke' displayed 100% stem rust severity

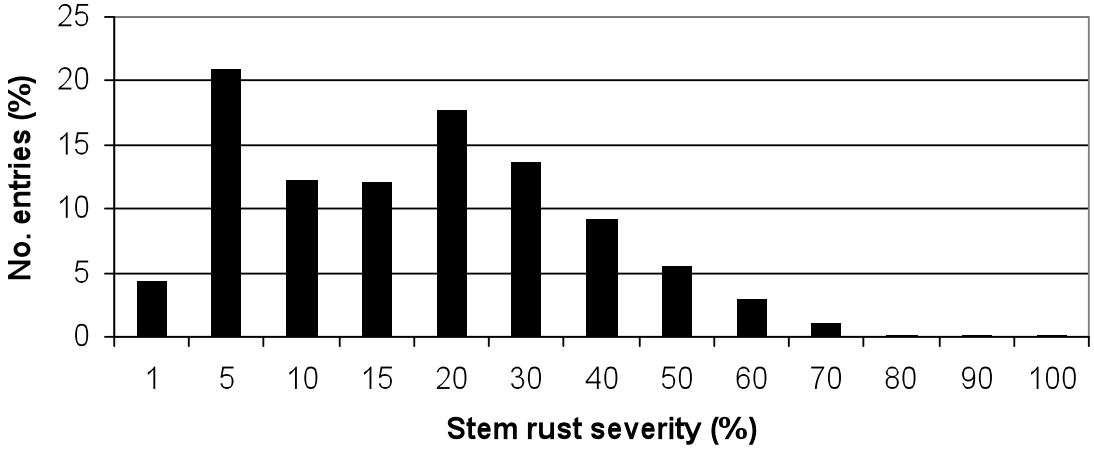
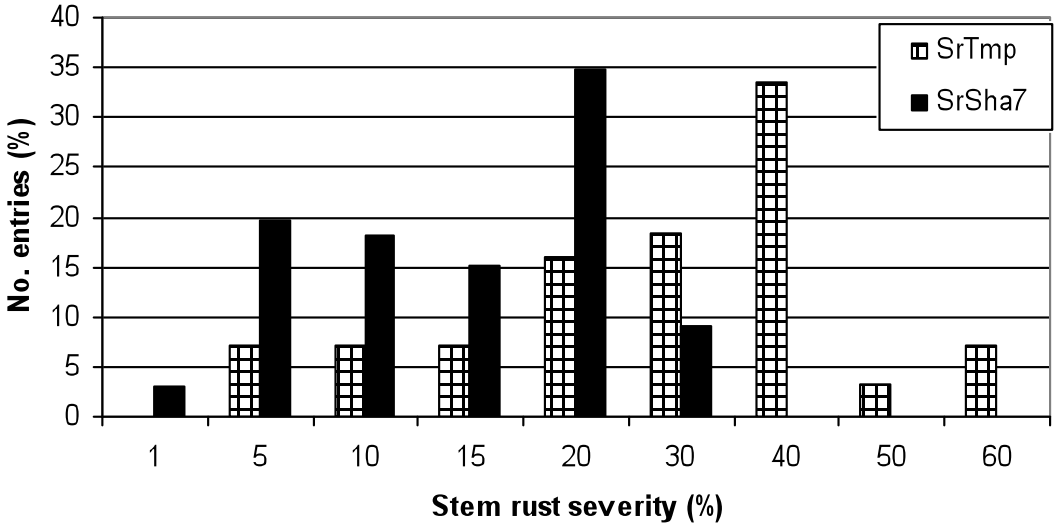


Fig. 4 Adult plant stem rust severities of 125 and 66 wheat lines carrying resistance genes *SrTmp* and *SrSha7*, respectively, at Njoro, Kenya, in 2010. Data were recorded when 'Cacuke' displayed 100% stem rust severity and reaction of lines varied from R to MR



Molecular genetics of race non-specific rust resistance in wheat

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Abstract Over 150 resistance genes that confer resistance to either leaf rust, stripe rust or stem rust have been cataloged in wheat or introgressed into wheat from related species. A few of these genes from the 'slow-rusting' adult plant resistance (APR) class confer partial resistance in a race non-specific manner to one or multiple rust diseases. The recent cloning of two of these genes, *Lr34/Yr18*, a dual APR for leaf rust and stripe rust, and *Yr36*, a stripe rust APR gene, showed that they differ from other classes of plant resistance genes. Currently, seven *Lr34/Yr18* haplotypes have been identified from sequencing the encoding ATP Binding Cassette transporter gene from diverse wheat germplasm of which one haplotype is commonly associated with the resistance phenotype. The paucity of well characterised APR genes, particularly for stem rust, calls for a focused effort in developing critical genetic stocks to delineate quantitative trait loci, construct specific BAC libraries for targeted APR genes to facilitate robust marker development for breeding applications, and the eventual cloning of the encoding genes.

Introduction

Rust resistance genes in wheat fall under two broad categories and are referred to as seedling and adult plant resistance (APR) genes. Seedling resistance genes are detected during both the seedling and adult plant stages and as such constitute an all stage resistance phenotype. APR is commonly detected at the post-seedling stage and often as field resistance, although some APR genes can be induced to express in seedlings by varying the growth temperature and light conditions. A large proportion of seedling resistance genes exhibit phenotypes of major effect and with varying infection types whereas most of the APR genes are partial in effect with varying levels of disease severity. Race specificity is more common with the seedling resistance genes; although there are some examples where virulence is yet to be documented. The seedling resistance gene *Lr21* which encodes a nucleotide binding- leucine rich repeat protein (NB-LRR) (Huang et al. 2003) is one such example where complete virulence is yet to be shown (McIntosh 2009). *Lr21* was transferred from *Aegilops tauschii* to wheat and may not have been exposed extensively in wheat cultivation for virulence to be detected. Race specificity in other NB-LRR encoded genes, *Lr1* (Cloutier et al. 2007) and *Lr10* (Feuillet et al. 2003) does exist.

Of the few APR genes that have been studied in wheat, a sizeable proportion appear to be race non-specific and a limited number are clearly race specific. Those of the race non-specific class with partial resistance are associated with a slow rusting phenotype first described by Caldwell (1968). Typically, slow rusting resistance show longer latent periods, fewer uredinia and smaller uredinia sizes within the first two weeks post inoculation when compared to susceptible plants.

Lr34/Yr18 dual APR

One of the well characterised race-non specific resistance genes is the adult plant leaf rust resistance gene *Lr34*. Earlier referred to as LrT2 (Dyck1977, 1987) it is present in older South American cultivars such as Frontana and its derivatives, some of the early crossbred wheat cultivars at the beginning of last century and landraces in particular those of Chinese origin (Borghi 2001; Kolmer et al. 2008). An important feature of *Lr34* was that virulence in the wheat leaf rust pathogen has yet to be reported and the enhanced effect of rust resistance when combined with other race specific leaf rust resistance genes has contributed to the durability of wheat cultivars with *Lr34* gene combinations (Kolmer 1996).

In a few wheat backgrounds, such as Thatcher and its derivatives, the presence of *Lr34* enhances stem rust resistance (Dyck 1987). However, cosegregation of *Lr34* with the adult plant stripe rust resistance gene *Yr18* (McIntosh1992; Singh 1992) in exhibiting dual rust resistance in numerous wheat backgrounds may have contributed to the continued widespread use of the *Lr34*/*Yr18* germplasm in wheat breeding. Subsequent observations that the *Lr34*/*Yr18* locus also contributed to partial resistance against adult plant powdery mildew (*Pm38*) highlighted the multi-pathogen nature of the *Lr34*/*Yr18*/*Pm38* locus on the short arm of wheat chromosome 7D (Spielmeyer et al. 2005; Lillemo et al. 2008)

While it is not uncommon in wheat to find regions inherited as multi-pathogen resistance loci they are typically due to absence of recombination from alien chromosomal segments such as the triple rust and mildew resistances from a rye chromosome 1RS segment or triple rust and nematode resistances from the *Aegilops ventricosa* introgressed segment on wheat chromosome 2A (McIntosh et al. 1995; Seah et al. 2000). These introgressed segments were shown to carry diverse and multiple gene clusters that encode nucleotide binding and leucine rich repeat sequences, the most common class of plant disease resistance genes (Seah et al. 2000; Mago et al. 2005) (see Fig.1). By contrast the *Lr34*/*Yr18*/*Pm38* locus of wheat has no history of alien introgression and thus suppressed recombination does not explain the multi-pathogen resistance found at this locus on wheat chromosome 7DS. From numerous wheat genetic mapping studies, component parts or all of the multi-pathogen resistance traits on 7DS have been scored as a quantitative trait locus (QTL) partly due to the partial resistance phenotype and other rust resistance loci elsewhere in the wheat genome. Delineating the locus to facilitate the molecular genetic characterisation of the multi-pathogen resistance was boosted by the development of genetic stocks in the wheat genotypes Thatcher, Lalbahadur, Avocet and Arina from which 'single gene' families were generated. The partial resistance expression of the multi-pathogen resistance QTL was shown to be inherited as a simple Mendelian trait in the single gene families.

FIGURE 1 HERE

Induced mutations to inactivate resistance genes and the use of the *ph1* mutation to permit homoeologous pairing and recombination showed that genes *Lr26*, *Yr9*, *Sr31* and *Pm8* were at different loci (Lukaszewski 2000; Mago et al. 2005). Virulence for all four genes exists, and as such they do not constitute race non-specific resistance. Chemical and physical mutagenesis were used to investigate the multi-pathogen resistance locus on wheat chromosome 7DS (Spielmeyer et al. 2008). Susceptible mutants were recovered for which there was no loss of DNA markers in the QTL interval on 7DS. These mutants were subsequently shown to be point mutations of which the chemical mutagen had created single base substitutions, and gamma irradiation resulted in single base deletions within a gene encoding ATP Binding Cassette (ABC) transporter at the multi-pathogen resistance locus (Krattinger et al. 2009). In addition to the ABC transporter six other genes cosegregated with the resistance locus. However none of the mutants (eight independent mutants) had changes in the additional genes. Thus mutagenic changes to the ABC transporter alone were adequate to confer loss of the leaf rust, stripe rust and powdery mildew resistances encoded by *Lr34*/*Yr18*/*Pm38*. Together with haplotype analysis and high resolution mapping, it was established that a single gene, an ABC transporter, conferred all three resistances (Krattinger et al. 2009).

Lr34/Yr18 haplotypes

Initial sequence analysis of the ABC transporter from the *Lr34*/*Yr18/Pm38* locus revealed two major haplotypes, exampled by Chinese Spring (CS) (resistant allele) and Renan (susceptible allele). The gene from CS and Renan were differentiated at three positions (Krattinger et al. 2009). Wheat genotypes with the resistant phenotype carried the haplotype A/TTC deletion/C corresponding to a SNP in intron 4/ an insertion-deletion (indel) in exon 11/ and another SNP in exon12, respectively. Wheat genotypes lacking *Lr34*/*Yr18* resistance possessed the haplotype T/TTC/T (Fig. 2). Sequence

changes in the haplotypes resulted in the loss of a codon for phenylalanine and the presence of a histidine residue in exons 11 and 12, respectively, in CS *Lr34*/*Yr18* resistance allele; the susceptible haplotype retains phenylalanine and tyrosine residues from the respective exons. For simplicity, the resistant and susceptible haplotypes in the reference genotypes were designated *Lr34/Yr18a* and *Lr34/Yr18b* (Fig. 2).

FIGURE 2 HERE

More recently, this gene was sequenced from a number of other genotypes, and further variation was uncovered (c to g, Fig. 2). A third haplotype, with the composition A/TTC/T identified in three winter wheats and two spelts (Lagudah et al. 2009) is designated *Lr34/Yr18c*; this haplotype was later shown to be present in 48 of 700 wheat genotypes surveyed by Dakouri et al. (2010). The cultivar Jagger retains all the polymorphic sequences found in *Lr34/Yr18a* but is susceptible to leaf rust (Kolmer et al. 2008; Lagudah et al. 2009). Complete sequencing of the ABC transporter in Jagger showed that the encoding gene carried a G/T SNP in exon 22 resulting in a premature stop codon and therefore causing a truncation in the gene product rendering it non-functional (Lagudah et al. 2009; Cao et al. 2010). The Jagger haplotype is designated *Lr34/Yr18d* (Fig. 2). Cosegregation of the defective (susceptible) *Lr34/Yr18d* haplotype was documented in a recombinant inbred line family derived from Jagger / Line '2174' from which the *Lr34/Yr18a* haplotype inherited from '2174' was associated with leaf rust resistance in field studies over three years (Cao et al. 2010). A rare haplotype (Fig. 2) was also found in introns 4 and 6 in some plants of line '2174' based on additional sequencing (Cao et al. 2010) and and this haplotype was designated *Lr34/Yr18e*.

From the studies of Dakouri et al. (2010) on 700 wheat genotypes sourced from a germplasm pool referred to as a 'world collection' and 'North American collection' two new haplotypes with sequence variants in exons were identified. A haplotype with an additional A in exon 10 (designated *Lr34/Yr18f)* which causes a frame shift mutation in the last 26 residues of the exon was found in 71 wheat genotypes. A rare haplotype which retains the TTC deletion in exon 11, characteristic of the resistant haplotype but possessing the SNP in exon 12 characteristic of the susceptible haplotype (designated *Lr34/Yr18g*) was found in two accessions originating from the Ukraine and Kazakhstan. Leaf rust severity studies conducted under Canadian field conditions suggested that the *Lr34/Yr18g* haplotype confers a susceptible phenotype. Thus the loss of the phenylalanine residue alone in the ABC transporter present in *Lr34/Yr18g* is insufficient to confer the resistance commonly found in the *Lr34/Yr18a* haplotype.

Adult plant susceptibility to leaf rust and/or stripe rust has been observed in certain wheat genotypes known to carry the *Lr34/Yr18a* resistant haplotype as reported in the Australian cultivar H45 (Lagudah et al. 2009) and Chinese landraces (L. Wu and X. Xia unpublished). From these observations it is apparent that additional factor(s) may interact with the *Lr34/Yr18a* haplotype to confer resistance. Adult plant stripe rust resistance equivalent to the *Lr34/Yr18* effect was recovered in a cross between the susceptible genotypes H45 and Avocet (Bariana H and Park R pers comm). Since H45 carries a functional *Lr34/Yr18a* allele, it may interact with a factor in Avocet to confer resistance, whereas this factor may otherwise be suppressed or not available in H45 leading to its susceptibility. It has been shown that the *Lr34/Yr18a* haplotype interacts with a stem rust suppressor locus on chromosome 7DL to inactivate the effect of the genetic suppression (Kerber and Aung 1999) and also interacts with other loci to confer stem rust resistance (Dyck 1987).

From the advances made in defining the *Lr34/Yr18* resistance gene (Krattinger et al. 2009) the encoding sequence laid the foundation for the development of perfect markers for wheat breeding (Lagudah et al. 2009; Dakouri et al. 2010). These markers have proven useful in validating and selecting for the presence of the resistance allele in wheat breeding programs. However, the challenges posed by parental germplasm sources such as H45 and haplotypes such as *Lr34/Yr18d* in Jagger means that caution needs to be exercised in using the perfect markers where these genotypes are an integral component of a breeding program. In the case of Jagger, specific markers were developed to detect and therefore select against the *Lr34/Yr18d* haplotype (Lagudah et al. 2009; Cao

et al. 2010). Derived cultivars of Jagger that also carry the *Lr34/Yr18d* haplotype have been reported in six North American wheat genotypes, viz. Fuller, Jagalene, Ok Bullet, Protection CL, Santa Fe, and Shocker (Cao et al. 2010).

Lr46/Yr29 dual APR

Strong parallels between the dual adult plant leaf and stripe rust resistance gene(s) *Lr46*/*Yr29* and *Lr34*/*Yr18* have been documented. Cosegregation of *Lr46*/*Yr29* with *Ltn2*, a second gene for leaf tip necrosis (Rosewarne et al. 2006) and adult plant powdery mildew partial resistance, *Pm39* (Lillemo et al. 2008) bear resemblance to the corresponding phenotypes of *Ltn1* and *Pm38* with the *Lr34*/*Yr18* gene. The respective phenotypes for *Lr46/Yr29* allele are comparatively weaker than those associated with *Lr34/Yr18*. They also differ in terms of chromosomal location, with *Lr46*/*Yr29* being located on chromosome 1BL (Singh et al. 1998; William et al. 2003). Despite the overall similarity of these genes, there is no evidence for gene duplication or a chromosomal segment duplication involving the respective chromosomal regions harboring *Lr46*/*Yr29* and *Lr34*/*Yr18*. Attempts at analysing the *Lr46*/*Yr29* locus for the presence of ABC transporter sequences related to the *Lr34*/*Yr18* gene have so far yielded negative results. Based on the preliminary studies, it appears the molecular basis of *Lr46*/*Yr29* differs from *Lr34*/*Yr18*.

Other attributes of partial adult plant resistance that give rise to a slow-rusting effect of leaf rust development were compared between *Lr34* and *Lr46* by Martinez et al. (2001). Both genes prolonged the latency period and caused an increase in the percentage of early aborted colonies not associated with cell necrosis, a reduction in colony size and lower disease severity relative to the susceptible background of Lalbahadur. What appears to be in doubt is the race non-specificity of the *Lr46* gene. Agarwal and Saini (2009) reported that *Lr46* is ineffective against leaf rust races in India. *Lr46/Yr29* was postulated in the Indian variety, PBW343, and cultivars such as Attila. However, it is possible that, the slow rusting nature of *Lr46* coupled with temperature-sensitive optimal gene expression may explain the ineffectiveness of *Lr46* in parts of the wheat growing zones in India. In a late season field sowing of genetic stocks possessing *Lr34/Yr18* and *Lr46/Yr29* in the cultivar background Lalbahadur in Cobbitty, N.S.W., Australia, there was no discernible difference in leaf rust severity on adult plants between the recurrent parent and the presence of *Lr46* whereas the presence of *Lr34* showed only a marginal effect. Under the normal field sowing season, using the same genetic stocks, the effects of *Lr34* and *Lr46* in reducing leaf rust severity was clearly evident. Warmer temperatures associated with the very late field sowings were considered a likely cause of the ineffectiveness of both genes, as relatively cool/mild temperatures are considered to be more favorable for the expression of leaf rust resistance in lines with *Lr34* and *Lr46*. Given the nature of the phenotypic expression of slow rusting an important factor in assessing the level of disease severity is the timing of scoring the phenotypes.

The cloning of the multi-pathogen effective *Lr34/Yr18/Pm38* and associated *Ltn1* established that all phenotypes were conferred by the same gene. In the case of *Lr46* associated phenotypes it remains to be determined if a single gene, or a cluster of genes, confers the *Lr46/Yr29/Pm39/Ltn2* phenotypes. Preliminary evidence in support for a single gene comes from mutagenesis studies, where putative point mutations in genetic stocks carrying *Lr46/Yr29/Pm39/Ltn2* lost all the associated resistance and morphological traits (Lagudah et al. 2007).

Comparative genomic approaches using micro collinear segments relative to the *Lr46/Yr29* region from the sequenced genomes of rice chromosome 5 and Brachypodium chromosome 2 enabled enrichment and development of closely linked markers to *Lr46/Yr29* (Lagudah et al. 2007; Mateos-Hernandez et al. 2007; Loughman et al. 2008). Micro syntenic rearrangements found in rice and Brachypodium, relative to the corresponding vicinity of the *Lr46/Yr29* locus, appear to indicate that there are genes in the region of the APR locus that are absent in the syntenic region of the model grass genomes (Lagudah et al. 2007)

Lr67/Yr49 a re-defined dual APR distinct from *Lr34/Yr18* and *Lr46/Yr29*

In the course of developing near isogenic lines for leaf rust resistance in the cultivar Thatcher, Dyck (1987) observed a phenotypic spectrum in line RL6077 (Thatcher*6/PI250413), that was similar to RL6058, a near-isogenic line carrying *Lr34*/*Yr18*. In subsequent studies the APR in RL6077 segregated independently of *Lr34*/*Yr18* and there was evidence for a translocation difference between the lines. As a result Dyck et al. (1994) inferred that RL6077 was a carrier of the *Lr34*/*Yr18* gene, on a different chromosome. With the development of closely linked markers, and ultimately the cloning of *Lr34/Yr18*, it became clear that RL6077 lacked the *Lr34/Yr18a* resistance haplotype present in RL6058 (Kolmer et al. 2008; Lagudah et al. 2009).

Studies on mapping populations from crosses involving RL6077 using *P. triticina* and *P. striiformis* isolates from multiple locations in Canada, Mexico and Australia, established cosegregation of the respective APRs on chromosome 4DL (Herrera-Foessel et al. 2010; Hiebert et al. 2010). These APR genes have been designated *Lr67/Yr46*.

Yr36, a temperature dependent race non-specific stripe rust resistance gene

Unlike the dual APR genes, *Yr36* is known to confer adult plant partial resistance against stripe rust at high temperatures (Uauy et al. 2005). The gene was introgressed from the tetraploid *T. diccocoides* into bread wheat in contrast to the previously described dual APR genes which are all from the primary gene pool. So far, *Yr36* has proven to be race non-specific when tested under the optimal conditions of higher temperatures (25^0C- 35^0C) in adult plants.

The gene encoding *Yr36* was identified using a map-based cloning strategy and validated via EMS mutants detected through 'TILLING' and two independent transgenic events (Fu et al. 2009). *Yr36* encodes a protein with an N terminal kinase domain and a C terminal region with a predicted steroidogenic acute regulatory protein-related lipid transfer (designated as START) domain. Analyses of cDNA clones from the *Yr36* gene revealed transcripts encoding the complete protein and five alternative variants. Gene expression studies revealed that the transcript encoding the complete protein was up-regulated at higher temperatures; in contrast the alternate transcripts were down-regulated. Given the high temperature dependence of *Yr36* resistance gene expression, the relative increase in corresponding transcript level of the complete cDNA may point to the probable mechanism of the temperature regulated resistance. The role of the START domain in binding to effectors from *P. striiformis*, or to products from the pathogen – host interaction, and how they effect defense signalling through the kinase domain of the *Yr36* protein will yield further insights into the uniqueness of this resistance mechanism.

Specific sequences were used to demonstrate the absence of *Yr36* in bread wheat and durums and its presence in some accessions of *T. dicoccoides* and the relatively few bread wheat lines into which it had been introgressed (Fu et al. 2009). As *Yr36* is introduced into more wheat genotypes and tested with more stripe rust strains across different wheat growing zones, the presumption of race non-specificity will become clearer.

Uncharacterised leaf rust and stripe rust APR genes

Undoubtedly many more leaf rust and stripe rust APR genes are yet to be fully characterised within the primary and wider gene pools of wheat. Prioritisation of which APR genes to target will in part be influenced by potential race non-specific attributes. A leaf rust APR gene, *Lr22a*, exhibiting race non-specificity was transferred from the diploid D genome progenitor species, *A. tauschii*, to wheat (Dyck and Kerber 1970) and mapped to chromosome 2DS accompanied with the development of tightly linked markers (Rowland and Kerber 1974; Hiebert et al. 2007). While *Lr22a* is associated with some components of slow rusting such as increased latent period and reduced sporulation, the presence of the gene does not confer a reduction in the number of pustules per unit area (Pretorius et al. 1990). Modifying genes independent of *Lr22a* were shown to cause variability in the slow rusting attributes. Leaf rust APR in *A. tauschii* was reported as prevalent across its natural geographic range (Gill et al.

2008) and its frequency was estimated to be around 73% among 303 accessions. What is unknown is the extent of race non-specificity, such as the *Lr22a* phenotype, what proportion constitutes dual APR types and the occurrence of multi-pathogen resistant forms such as *Lr34/Yr18/Pm38*. Field observations made on the near isogenic line of *Lr22a* in comparison with its recurrent parent, Thatcher, for stripe rust severity has as yet provided no evidence for an associated stripe rust resistance APR (unpublished data). Studies on a wide range of *A. tauschii* accessions using proxy markers for *Lr34/Yr18/Pm38* and sequencing of some of the genotypes revealed the presence of the *Lr34/Yr18b* susceptible haplotype. These observations favor the hypothesis that the resistant *Lr34/Yr18a* haplotype arose in *T. aestivum* subsequent to the natural synthesis of hexaploid wheat.

Analyses of fast neutron generated mutagenised populations of the wheat cultivar 'Hobbit Sib' led to the identification of a number of leaf rust and stripe rust APR phenotypes (Smith et al. 2004; Boyd et al. 2006). Mutant lines possessing enhanced resistance to stripe rust and leaf rust as well as stripe rust and powdery mildew were identified. However, it is yet to be demonstrated whether the same mutation events were responsible for the multiple phenotypes. While APRs are broadly described as post seedling, the precise onsets of resistances are variable and environmentally influenced. Some of the mutants recovered from 'Hobbitt Sib' expressed enhanced resistance from the third leaf stage whereas others expressed as late as the tenth and subsequent leaf growth stages under greenhouse conditions.

Tetraploid wheat genotypes were identified with slow rusting APR to leaf rust. Many showed the associated leaf tip necrosis phenotype (Hererra-Foessel et al. 2008) and the occurrence of additive genetic effects suggesting similarities to bread wheat. The novelty or equivalence of some of the tetraploid wheat APR genes to *Lr46/Yr29* on chromosome 1BL of hexaploid wheat and whether they confer dual APR or multi-pathogen resistances remain to be established.

Race non-specific stem rust resistance

Close to 50 stem rust resistance genes, derived directly from wheat or introgressed from related species, have been cataloged. Of these only *Sr2* has been characterised as an APR with a slow rusting phenotype (Hare and McIntosh 1979; Sunderwirth and Roelfs 1980). *Sr2* was originally introgressed from cultivated emmer (*T. dicoccum*) over 80 years ago by McFadden (1930) in developing the bread wheat lines Hope and H-44. Durable stem rust resistance in some older wheat genotypes was attributed to *Sr2* along with unknown minor genes with additive effects (Singh et al. 2008). As part of the ongoing evaluation of wheat cultivars at field sites in Kenya it is evident that genotypes combining *Sr2* and other seedling resistance genes exhibit enhanced levels of adult plant resistance relative to the effects attributed to the seedling resistance genes alone (Njau et al. 2010). These evaluations managed by CIMMYT in Kenya and elsewhere, enabled identification of wheat cultivars carrying APR genes additional to *Sr2* (Singh et al. 2008; Njau et al. 2010).

Sr2 shows parallels with *Lr34* and *Lr46*, in that it is associated with multi-pathogen resistance. Tight linkage between *Sr2*, the leaf rust resistance gene *Lr27*, and partial APR to stripe rust (*Yr30*) and powdery mildew were observed (Singh and McIntosh 1984; Singh et al. 2000b). Wheat plants with inactivated *Lr27* alleles from mutagenesis appear to have lost *Sr2* possibly indicating pleiotrophism (Spielmeyer et al. 2009). In addition to the plant morphological phenotypes of leaf tip necrosis associated with *Lr34* and *Lr46*, an associated *Sr2* plant morphology with dark pigmentation or necrotic region on the peduncle (Fig. 2) and glumes often referred to as pseudo black chaff has remained inseparable from *Sr2* resistance in high resolution mapping (Kota et al. 2006).

Another stem rust APR gene from a durum wheat cultivar, Glossy Huguenot, (designated *SrGH*, Fig. 3) was characterised using Australian stem rust isolates (Hare 1997). *SrGH* was considered to differ from *Sr2* due to its dominant inheritance pattern in contrast to the recessiveness of *Sr2*, the pattern of pustule distribution on the stems, and absence of the pseudo black chaff trait. Recent tests conducted in Kenya showed *SrGH* to be effective against Ug99 (Singh D pers comm) providing further incentive to continue ongoing characterization of *SrGH* in terms of gene location and transfer to bread wheat. Mutational studies on Glossy Huguenot have so far failed to recover

susceptible plants (Kota et al. unpublished). In combining the observations from the mutational analysis and ongoing mapping studies it is not certain if a single gene is responsible for all the stem rust APR in Glosssy Huguenot.
FIGURE 3 HERE

Interactions involving race non-specific resistance genes

Additive gene interactions are often attributed to the enhanced reduction of rust severity when multiple partial APR genes are combined. As an example, the accumulation of four or five slow rusting genes confers near immunity to rust infection (Singh et al. 2000a) and forms the basis of significant gains being made towards developing more durable leaf rust and stripe rust resistant wheats. What remains unclear is which specific gene combinations produce optimal additive gene effects when working with fewer genes. In crosses involving the near isogenic wheat lines RL6058 and RL6077, which carry *Lr34/Yr18* and *Lr67/Yr46*, respectively, Dyck et al. (1994) recovered a few plants with marginal reductions in leaf rust severity relative to those observed in RL6058. In this example the evidence for additive gene effects of *Lr34*/*Yr18* and *Lr67*/*Yr46* was rather weak. Lillemo et al. (2008) examined gene interactions between *Lr34/Yr18* and *Lr46/Yr29* and found that a simple additive gene model was inadequate to account for the interaction. Cultivar Parula carries several APR genes for leaf rust, stripe rust and stem rust, and more recently, a third leaf rust APR gene, designated *LrP* on chromosome 7BL, was identified in addition to *Lr34* and *Lr46* (Herrera-Foessel et al. 2009). From a recombinant inbred line derived from a cross between Parula and Avocet, there was evidence for a strong additive interaction between *LrP* and *Lr46* for leaf rust severity, whereas the combined effect of *Lr34* and *Lr46* was little different from *Lr34* alone.

A synergistic interaction involving *Yr36* and *Yr18* on stripe rust severity was inferred from observations made with the transfer of the *Yr36* into different bread wheat backgrounds (Uauy et al. 2005). Enhanced resistance of *Yr36* was most noticeable in the cultivar background of Anza, the only genotype possessing *Yr18* in the comparative study. A more definitive study is in progress using backcross-derived lines or near isogenic *Yr18* and *Yr36* transgenic lines to validate the synergistic effects.

Pre-breeding research considerations

Combining resistance genes in wheat breeding to facilitate the development of more durable resistances is a well known procedure in wheat breeding. With the advances that have been made in the wheat rust biology and resistance breeding communities, knowledge of the types of genes, whether seedling or APR, being used in germplasm improvement will increasingly be of value for germplasm assessment and eventual cultivar deployment. APR genes face more challenges as they are usually assessed in the field and, in most cases, may be limited to one growing season in a year in the absence of multi-location testing. Epistatic effects of major seedling genes over APR genes make selection of the latter impossible. Race non-specificity of the genes described in this paper, in particular those that exhibit multi-pathogen resistance are more likely to be pursued as foundational genes to which additional minor and major seedling genes are included in pyramiding strategies. Availability of robust and diagnostic markers for the race non-specific APR genes will speed up the breeding process and ensure such targeted gene combinations are present.

Given the important role that a gene such as *Sr2* has played in stem rust control, it remains the only cataloged stem rust APR gene to date. More needs to be done to identify, map and develop markers for additional stem rust APR genes. The identification of multiple stem rust APR genes in cultivars such as Kingbird and multiple APR genes to all three rusts in Parula (Singh et al. 2008) makes these genotypes useful targets for resource development, such as BAC libraries for identifying diagnostic markers and the eventual cloning of the respective genes. Several mapping families involving Kingbird and derived lines were developed as part of BGRI activities. As the APR quantitative trait loci become delineated the availability of the BAC resources will speed up

diagnostic marker development for the molecular breeding of such key traits. It is becoming apparent that some of these non-specific resistance gene targets are not present in currently available BAC libraries such as was found in the case of *Yr36* and appears to be so with *Lr46/Yr29* and *Sr2*, and therefore highlights the significance of developing specific resources based on the Kingbird and Parula genotypes.

Although the additive effects of *Lr34/Yr18* and *Lr46/Yr29* remain unclear, there is merit in combining such genes as the molecular basis of the encoding genes appear to be different and therefore may provide a useful buffer should one resistance mechanism be overcome. The observation that *Yr36* and *Yr18* have synergistic effects in enhancing stripe rust resistance, if proven, will be a useful combination to adopt during pre-breeding. Because optimal effectiveness of *Yr18* occurs under mild temperatures whereas *Yr36* is at higher temperatures, their gene combinations may provide protection under a broad temperature range. This may prove to be a vital combination in breeding wheat under climate change and the erratic nature of rust epidemics.

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Figure Legends

Fig. 1 Clusters of disease resistance genes often inherited as blocks in wheat due to suppressed recombination in alien chromosome segments, and resistance gene analogs present on alien segments (grey regions) as compared with multi-pathogen resistance traits controlled by single genes on normal wheat chromosomes (1B and 7D)

Fig. 2 Seven *Lr34/Yr18* haplotypes of the ABC transporter gene. The rectangular boxes refer to exons and horizontal lines are introns. *Lr34/Yr18a* from Chinese Spring shows SNPs A and C located on intron 4 and exon12, respectively, and the absence of the codon TTC at exon 11 is designated with the minus sign. *Lr34/Yr18b* present in Renan shows SNPs T and T at intron 4 and exon 12; the presence of the TTC at exon 11 is shown with the plus sign. Various combinations at the corresponding introns/exons are given for the remainder of the haplotypes. The asterisks in haplotype *e* show regions with additional SNP in introns 4 and 6 and the arrowed region in haplotype *f* points to the position of an additional A in exon10. A full description of the various haplotypes can be found in Krattinger et al. (2009), Lagudah et al. (2009), Cao et al. (2010) and Dakouri et al. (2010)

Fig. 3 Adult plant stem rust development on stems of durum wheat plants +/-*SrGH* and bread wheat +/-*Sr2*. The lower internodes of the +*Sr2* genotype shows regions with darkened pigmentation

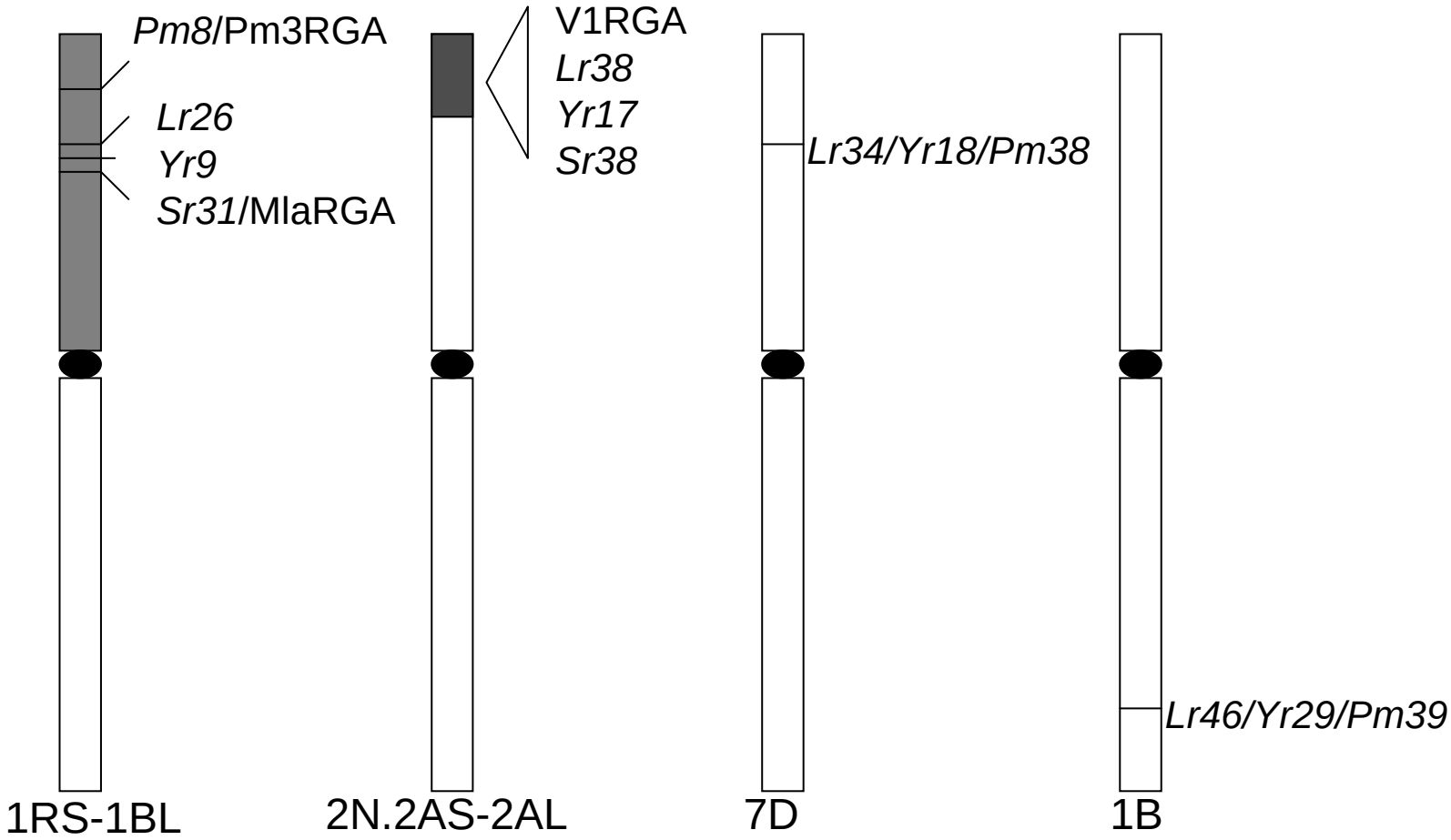
Pm8/Pm3RGA
Lr26
Yr9
Sr31/MlaRGA
V1RGA
Lr38
Yr17
Sr38
Lr34/Yr18/Pm38
Lr46/Yr29/Pm39
1RS-1BL
2N.2AS-2AL
7D
1B

Figure 1

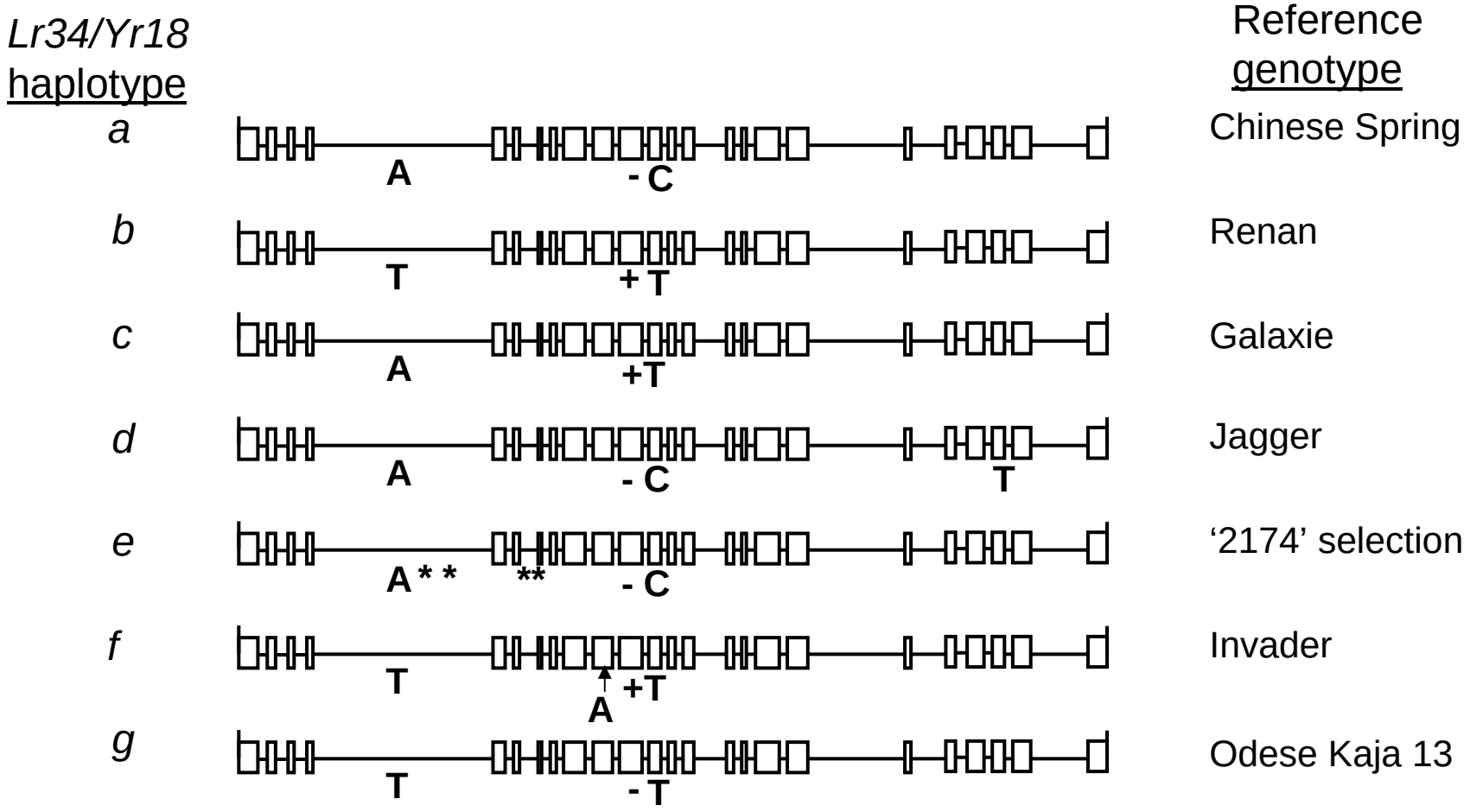
Lr34/Yr18 haplotype
Reference genotype
a
A
- C
Chinese Spring
b
T
+ T
Renan
c
A
+T
Galaxie
d
A
- C
T
Jagger
e
A * *
**
- C
'2174' selection
f
T
A
+T
Invader
g
T
- T
Odese Kaja 13

Figure 2

Figure 3

The status of genetic resources and introgressions for race-specific stem rust resistance (abstract)

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The current global emphasis and investment in stem rust pathology, germplasm enhancement, resistance genetics, and breeding have accelerated the discovery and application of genetic resistance at an unprecedented rate. Wheat scientists now have, or will soon have, the necessary tools to adequately address the stem rust threat over the next 20-plus years of wheat breeding and on-farm production. Breeding for a combination of race-specific and adult plant resistance is increasingly feasible, as genetic mapping and diagnostic marker development is of high priority for both types of resistance. The focus of this paper is to provide updated documentation for effective sources of race-specific resistance to stem rust, caused by *Puccinia graminis* f. sp. *tritici*, and to summarize relevant pathology and genetic information to enable breeding for durable stem rust resistance in wheat. Coordinated efforts to responsibly deploy available resistance sources are urgently needed. Improved germplasm sources and/or genetic markers are now available for race-specific resistance genes *Sr13, Sr22*, *Sr25, Sr26, Sr28, Sr32*, *Sr33*, *Sr35, Sr39, Sr40*, *Sr42, Sr45, Sr1A.1R, SrTmp, SrA*, *SrB*, *SrC, SrACCadillac*, and *SrR*. Additional useful resistance sources will continue to emerge from chromosome engineering projects focused on *Sr37*, *Sr43*, *Sr44*, *Sr47* and several new alien sources (resistance from *Aegilops caudata*, *Ae. geniculata*, *Ae. sharonensis, Ae. speltoides*, *Haynaldia villosa, Thinopyrum junceum*, *Th. bessarabicum*, and *Th. intermedium)* and gene discovery/introgression efforts focused on alien/wild diploid and tetraploid wheat collections. High-resolution genetic mapping of each gene will enable routine and robust marker-based selection, pyramiding, and deployment in combination with adult plant resistance.

Prospects for functional analysis of effectors from cereal rust fungi

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Abstract With the advancement of several *Puccinia* genome sequencing projects, along with gene expression data and methods for predicting secreted proteins, it is now possible to predict many effector proteins from the cereal rusts. Biological assays that can be conducted in a relatively high throughput fashion are necessary to assign specific functions, such as avirulence. Bacterial systems that had been demonstrated to introduce effectors into dicot host cells through their type three secretion system (TTSS) were tested for their suitability in wheat. *Pseudomonas syringae* DC3000 caused hypersensitive reactions (HR) when infiltrated into all tested wheat lines but only some barley lines. A variant strain with multiple effectors deleted showed a reduced HR on wheat lines. *Pseudomonas fluorescens* with an engineered TTSS system showed no HR in wheat lines but was able to deliver bacterial effectors *AvrRpm1* and *AvrRpt2* and the fungal toxin ToxA. Delivery of the bacterial effectors by *P. fluorescens* could be observed visually or by staining for hydrogen peroxide and the effects of toxin delivery could be observed by callose deposition. The bacterial systems thus showed good potential for their ability to deliver foreign proteins into wheat cells.

Keywords barley, *Pseudomonas fluorescens*, *Pseudomonas syringae*, RNAi, type-III secretion system, wheat

Introduction

A common feature of plant pathogens and parasites is the ability to produce proteins and other molecules that improve their potential for colonization, survival and reproduction on plants. These so called 'effectors' alter processes in host cells and sometimes even their structures (Hogenhout et al. 2009). A common function of pathogen effectors is to facilitate infection or interfere with host defense responses, but if their presence is recognized by host resistance (R) proteins, they cause avirulence since the recognition triggers defense responses. Since this effector triggered immunity overrides their contributions to pathogenicity on hosts carrying the specific R genes that detect them, the genes that code for these effectors have traditionally been called avirulence genes. Many bacterial, fungal and *Oomycete* effectors are proteins that are delivered directly into plant cells. Most pathogenic bacteria have specialized (type III) secretion systems (Block et al. 2008) that deliver these proteins. The R proteins that detect effectors are often present in the plant cytoplasm. Most of these cytoplasmic receptors are NB-LRR proteins but other proteins, like kinases are also involved. The delivery of effector proteins from fungal and *Oomycete* effectors is more poorly understood than bacterial effectors. It seems clear that many *Puccinia* and *Melampsora* rust effectors are recognized in the plant cytoplasm since most of the characterized rust R genes are predicted to be cytoplasmic receptors. Examples include flax rust R genes at four loci which are NB-LRR proteins (Ellis et al. 2007) as are most of the characterized cereal rust R genes, including maize *Rp1* (Collins et al. 1999) and *Rp3* (Webb et al. 2003), wheat *Lr21* (Huang et al. 2003), *Lr10* (Feuillet et al. 2003) and *Lr1* (Cloutier et al. 2007). The barley RPG5 protein also has NBS and LRR domains but is unusual in that it is fused to a kinase domain (Brueggeman et al. 2008). *Rpg1* also codes for a kinase domain and is likely cytoplasmic (Brueggeman et al. 2002).

Little progress has been made in the characterization of *Puccinia* effectors because of various technical difficulties. Genetic analysis is not trivial since the cereal rust fungi have macrocyclic life cycles, but the life cycle can be completed for several of the *Puccinia* species and has been used to genetically map avirulence genes in *P. graminis* (Zambino et al. 2000). A break through in understanding the sexual cycle of *P. striiformis* was recently made when an alternate host was identified (Jin et al. 2010). Genetic mapping of Avr genes down to small chromosomal intervals is

therefore a viable method for identifying specific Avr genes. The recent availability of *Puccinia* rust genome sequences are making this much more feasible. DNA marker sequences can be used to identify one or more regions of contiguous sequence (contigs) on which these markers, and possibly the linked Avr gene lie. The genomic sequences on these contigs can then be used to identify additional markers for fine mapping or, if the region carrying the Avr gene is delimited to a sufficiently small interval, the sequences can be searched for potential candidates. A nearly complete genome sequence for an isolate of *P. graminis* f. sp. *tritici* is now available (http://www.broadinstitute.org/annotation/genome/puccinia_group/MultiHome.html) and similar sequencing projects are underway for *P. triticina* and *P. striiformis* f. sp. *tritici*.

The first rust Avr gene was cloned from the flax rust pathogen (*Melampsora lini*) by map-based cloning (Dodds et al. 2004). This *AvrL567* gene was well-expressed in haustorial cells and encoded a 127 amino acid secreted protein delivered to the host cells across the plant membrane (Dodds et al. 2004). This knowledge was exploited to isolate three additional *M. lini* Avr genes (Catanzariti et al. 2006; Ellis et al. 2007). All four Avr genes encode small-secreted proteins (95-377 amino acids) and induced necrosis when transiently expressed in flax lines containing the corresponding R gene. Three of the Avr genes (*AvrL567*, *AvrP123* and *AvrP4*) are expressed only *in planta*, whereas *AvrM* is expressed both *in planta* and in germinated urediniospores. *AvrP123* and *AvrP4* encode cysteine-rich proteins with motifs similar to KAZAL and Cys-knot protease inhibitors, respectively. Interestingly, the AvrL567 protein interacts directly with the corresponding L5, L6 and L7 resistance proteins in vivo and in yeast two-hybrid assays (Dodds et al. 2006).

The knowledge gained from characterization of *Melampsora* Avr genes along with the DNA sequence emerging for *Puccinia* rust pathogens and the predicted cytoplasmic location of most rust R genes provides a framework for indentifying candidate *Puccinia* effector proteins. Proteins that are secreted from haustorial cells are good candidates for effectors. Methods for purification of haustorial cells that were developed for *Uromyces* (Hahn and Mendgen 1992) and used for *Melampsora* (Catanzariti et al. 2006), also work well for *Puccinia* rusts (Yin et al. 2009) and allow for the identification of haustorial transcripts. Transcripts that code for secreted proteins can generally be identified using bioinformatic approaches that find peptide motifs associated with secretion mechanisms. Some means of efficiently testing these candidate genes for avirulence activity or other phenotypes is thus important. Genetic transformation of the rust fungi would be desirable but is difficult, as with other biotrophic fungi because of the lack of good selectable markers. The use of an Avr gene was recently demonstrated for this purpose in *Melampsora*. Although Avr gene alleles typically confer avirulence on hosts carrying the corresponding R gene, Lawrence et al. (2010), designed a *Melampsora lini* transformation construct that would silence an Avr gene (AvrL567) present in the recipient strain's genome by RNAi. If the construct is expressed in an avirulent rust strain and silences this gene, the transformed strains become virulent on flax lines with the corresponding L6 resistance gene and this virulence therefore provides the selection for transformation.

Although effector proteins may have functions within the pathogen's own cells, an important aspect of their phenotype is presumably their function in host cells. Their expression in the host plant may therefore provide an indication of this function. If expression is associated with a hypersensitive response (HR), and the HR is dependent on the presence of an R gene, this provides evidence that the effector is interacting with a host R gene. HR can typically be assayed in transient expression assays without making stable transgenics. Assays for HR with *Melampsora* effectors have been performed by *Agrobacterium*-mediated transformation of flax leaf cells. Transformation was sufficiently efficient that the leaf areas infiltrated with the bacterial suspension exhibited a visible necrotic reaction. In plants like cereals, which are transformed very inefficiently by *Agrobacterium*, this is not observed. It is possible that *Agrobacterium* strains can be found or constructed that transform cereal leaf cells much more efficiently, but most strains are poorly suited. Biolistic approaches can also be used to transform individual cells. HR assays can be performed by including genes coding for visible marker proteins like GFP or GUS. A lack of marker gene expression then indicates the transformed cells have died before the proteins could accumulate. Transient assays for HR can even detect R genes that interact with effectors from pathogens that do not normally infect the host being assayed. For

example, a maize gene was detected that interacts with an effector from the rice bacterial streak pathogen, *Xanthomonas oryzae* pv *oryzicola*, and the maize gene was effective in conferring resistance after transfer to rice (Zhao et al. 2005).

Transient transformation assays, like biolistics, that express proteins in individual cells scattered among many untransformed cells have disadvantages over those that express proteins in many or most cells in a region of the host. Besides the fact that HR assays are more cumbersome, with microscopic examination of marker gene expression to monitor cell viability, they also make observations of more subtle effects, like callose deposition or changes in gene expression, less feasible. Another approach is to deliver proteins into host cells by expressing them in bacterial pathogens for delivery to host cells through their type III secretion systems. These systems have been used successfully for identification of *Avr* proteins from *Oomycete* pathogens like *Hyaloperonospora parasitica*. These proteins were expressed as fusion proteins with the N-terminal secretion-translocation signals of the well-characterized bacterial effectors *AvrRpm1* (Rentel et al. 2008) or *AvrRps4* (Sohn et al. 2007) and delivered by *Pseudomonas syringae* pv *tomato* (DC3000). Similar systems that deliver proteins to grass species could have great potential for examination of cereal rust effectors.

Materials and methods

Plant lines, bacterial strains and growth conditions

The wheat lines used included the durum cultivars Langdon (LDN) and Kulm and two ToxA-insensitive mutants derived from these lines (Faris and Friesen 2009). The Avocet S wheat near isogenic lines (NILs) with *Yr1*, *Yr5*, *Yr10*, and *Yr15* were also used. These were originally developed at the Plant Breeding Institute Cobbitty, N.S.W., Australia (Wellings and Kandel 2004). The differential wheat cultivars used in the study were Moro, Paha, Druchamp, Riebesel, Produra, Yamhill, Tyee, Tres, and Hyak (Chen and Line 1992). The twelve barley cultivars used in this study, Topper, Emir, Astrix, Varunda, Abed Binder, Trumpf, Mazurka, I5, Heils Franken, Bigo, Bancroft, and Hiproly were varieties used to differentiate barley stripe rust races (Chen et al. 1995). Wheat and barley were planted in pots containing potting mix and grown under the following greenhouse conditions: 22°C day and 18°C nights, with 23-50% RH and 16 hours of light. Seedlings were watered daily and fertilized with a dilute nutrient solution weekly. Bacterial strains used include *Escherichia coli* Top10 (Invitrogen, Carlsbad, CA) and MC4100, *Pseudomonas syringae* pv tomato (*Pst*) DC3000 and its variant CUCPB5500 (Kvitko et al. 2009). In addition, the *Pseudomonas fluorescens* strains Pf0-1, the recombinant *P. fluorescens* strains EtHAn (effector-to-host analyzer) carrying an empty vector (E.V.), EtHAn carrying *avrRpt2* (Tn7::*avrRpt2* in EtHAn) and EtHAn carrying *avrRpm1* (Tn7::*avrRpm1* in EtHAn; Chang et al. 2005) were also used. *E. coli* Top10 were grown in low-salt Luria-Bertani broth at 37^{o}C, and *Pst* DC3000 and *P. fluorescens* strains were grown at 28^{o}C in King's B (KB) liquid media, or on KB agar plates. Antibiotics were used at the final concentrations of: 30µg ml^{-1} rifampicin, 50µg ml^{-1} kanamycin for Pf0-1, 30µg ml^{-1}chloramphenicol and 25µg ml^{-1} gentamycin.

Plasmid constructs and plasmid mobilizations

The DNA fragment encoding avrRpt2$_{123-768}$ was amplified from DNA of *P. syringae* pv *tomato* (ptoJL1065) by standard PCR conditions using forward primer avrRpt2F (5'-CACCAAAAGTAGCGCTTCAAGCCCGAA -3') and reverse primer avrRpt2R (5'-TTAGCGGTAGAGCATTGCGTGT -3'). The DNA fragment encoding avrRpm1$_{129-663}$ was amplified from DNA of *P. syringae* pv *maculicola* using forward avrRpm1F (5'-CACCTCCAATGTTAACTTAATACCCAATGC -3') and reverse primer avrRpm1R (5'-TTAAAAGTCATCTTCTGAGTCAGAC -3'). DNA fragments encoding ToxA$_{48-537}$ and ToxA$_{1-537}$ were amplified from cDNA of *S. nodorum* isolate Sn4 using forward primer ToxAF1 (5'- CACC GCCCCAACGCCTGAAGCCGA -3') and ToxAF2 (5'- CACC

ATGCGTTCTATCCTCGTACTTCTT -3') and reverse primer ToxAR (5'-CTAATTTTCTAGCTGCATTCTCCAA -3'). PCR products were cloned into pENTR using the Directional TOPO Cloning kit (Invitrogen, Carlsbad, CA). DNA sequences of these constructs were verified by sequencing inserted DNA fragments using the M13F and M13R primers. The pENTR constructs were transferred to pEDV6 (Sohn et al. 2007) by performing an LR recombination reaction. Plasmids were mobilized from *E. coli* TOP10 to *E. coli* MC4100 carrying cosmid pCPP2156, *P. syringae* and *P. fluorescens* strains using electrocompetent cells.

In planta *assays*

Bacterial cells were grown overnight in KB with appropriate antibiotics at 28°C, washed with sterile ddH_2O twice and resuspended in sterile ddH_2O. *E. coli* MC4100 cells were resuspended in 0.01 M potassium phosphate buffer (pH 7.0) after harvest and then washed with sterile ddH_2O twice. For the *in planta* growth assays, *P. syringae* were suspended to an OD600 of 0.05. *E. coli* MC4100 and *P. fluorescens* were suspended to an OD600 of 1.0 for analysis of *in planta* growth and callose deposition and 1.5 for analysis of hydrogen peroxide accumulation. One ml syringes lacking needles were used to infiltrate bacterial suspensions into the second wheat leaves of 12-14 day old plants. Phenotypes were examined on wheat leaves that were harvested at 48h post inoculation. Experiments were repeated at least two times.

3,3'-diaminobenzidine (DAB) assay for hydrogen peroxide accumulation

Hydrogen peroxide accumulation was detected as described by Thordal-Christensen et al. (1997). 0.1 g of DAB powder (Sigma-Aldrich, St. Louis, MO) was dissolved in 100 mL of water by adding HCl to reach a pH of 3.8. Wheat leaves harvested 48 h post inoculation with *P. fluorescence* (four plants per treatment, experiment done twice) were immersed immediately in DAB solution, and held for 8 h under light at 25ºC in a growth chamber. The reaction was terminated by immersion of tissue in boiling 96% EtOH for 10 min and cleared with 70% EtOH. Wheat leaves were preserved in 70% EtOH for photography. Leaves inoculated with ddH_2O or *P. fluorescence* carrying an empty vector were used as controls.

Analysis of callose deposition

To visualize callose deposition, whole leaves were harvested, cleared and stained with aniline blue (Currier and Strugger 1956). Briefly, the leaves were cleared with 1:3 methanol:chloroform and dehydrated with 100% ethanol. Cleared seedlings were transferred sequentially in 50% EtOH and in 67 mM K_2HPO_4 (pH 12), and then stained for 1 h at room temperature in 0.01% aniline blue in 67 mM K_2HPO_4 (pH 12). Stained material was mounted in 70% glycerol, and fluorescence from callose was visualized with an epifluorescence microscope under UV light. For each treatment, four leaves were examined.

Results and discussion

Effector delivery into wheat by* Pst *strains DC3000 and CUCPB5500

P. syringae pv. *tomato* (*Pst*) strain DC3000, which causes bacterial speck of tomato, has been an important model organism for investigating pathogen effectors and their delivery into host cells via its type III secretion system (TTSS). Avr genes from the downy mildew pathogen *Hyaloperonospora parasitica* were delivered successfully to *Arabidopsis thaliana* using its TTSS and this triggered the hypersensitive response (HR) in lines carrying the corresponding *Rpp1-Nd/WsB* or *Rpp13-Nd* resistance genes (Rentel et al. 2008; Sohn et al. 2007). To test whether DC3000 can be used to deliver rust proteins into cereal cells, we first examined its interactions with different wheat cultivars. When *Pst* DC3000 (suspended at OD = 0.05) was infiltrated into wheat leaves it caused a similar strong HR

at 2 dpi on all tested wheat lines. In contrast, when infiltrated into barley cultivars, *Pst* DC3000 showed cultivar-dependent reactions. No noticeable necrotic or chlorotic reactions were observed in barley cultivars Emir, Astrix, Varunda, Abed Binder, Trumpf, Mazurka, Bancrof, and I5, whereas weak reactions were observed in cultivars Heils Franken and Bigo and strong HR was observed in Hiproly (data not shown). The HR in wheat and some barley lines indicated the DC3000 TTSS is delivering effectors into cereals cells and that some of these effectors are being recognized by host resistance genes which are triggering defense responses. It may therefore be possible to deliver effectors using DC3000 to some barley varieties and assay their effects, but the resistance reactions from wheat and other barley varieties would greatly complicate examining the effects of additional effectors.

Pst DC3000 is not normally a pathogen of cereals but deploys at least 28 effectors into hosts like *Arabidopsis* and tobacco (Kvitko et al. 2009). In an attempt to find a DC3000 derivative that did not induce HR in wheat, the DC3000 variant CUCPB5500 was tested. CUCPB5500 was created by deleting 18 effector genes that occur in six different clusters (Kvitko et al. 2009). When mutant CUCPB5500 and CUCPB5500 carrying the empty expression vector pEDV6 (AvrRPS4N-HA-effector) were infiltrated into wheat, very weak necrotic reactions were observed and the expression was typically delayed and reduced compared to reactions with DC3000 (Fig. 1). The fact that the CUCPB5500 variant shows reduced HR indicates one or more effector that was recognized by wheat's arsenal of resistance genes was deleted, but there are still one or more effectors recognized and causing a weak response. Responses of the barley cultivars to CUCPB5500 were, for the most part, very similar to their responses to DC3000. An exception was the cultivar Topper, which showed a weak HR to DC3000 and no visible reaction to CUCPB5500. Apparently, few of the effectors that were deleted from DC3000 to make the CUCPB5500 variant interact with resistance genes in most barley cultivars.

FIGURE 1 HERE

To test the ability of *Pst* strains to deliver specific effectors to wheat, engineered versions of the *AvrRpm1* and *AvrRpt2* genes were used. These are well-characterized T3SS effectors from *P. syringae* that trigger HR in *Arabidopsis* carrying the corresponding R genes *Rpm1* and *Rps2*, respectively. Mudgett and Staskawicz (1999) reported that amino acid residues 120-255 of AvrRpt2 were sufficient to induce *in planta* cell death in tobacco expressing RPS2.

The pEDV6 vector was developed to fuse putative effectors to the N terminus of the avrRPS4 protein to guide their delivery through the *Pseudomonas* TTSS (Sohn et al. 2007). Sequences coding for the mature AvrRPM1 or AvrRPT2 proteins were cloned into the vector pEDV6 which was then transformed into *Pst* strain CUCPB5500. When the CUCPB5500 strain carrying *avrRpt2* or *avrRpm1* was infiltrated into wheat leaves, a necrotic reaction similar to infiltration with Pst DC3000 was observed. Because the effects of the bacterial effectors were sometimes difficult to visually distinguish from the reactions with CUCPB5500, we examined callose depositions to verify a reaction to these effectors (Fig. 2). Callose depositions are associated with papillae that form as appositions inside plant cell walls and provide a useful assay for cell wall-based defenses (DebRoy et al. 2004). *Pst* DC3000 induced extensive callose deposition, whereas CUCPB5500 induced noticeably less. CUCPB5500 expressing *avrRpt2* or *avrRpm1* constructs in the pEDV6 vector induced noticeably more callose depositions than CUCPB5500 or CUCPB5500 carrying empty pEDV6 vector. This indicated that CUCPB5500 can deliver bacterial effectors to wheat via the delivery vector and the TTSS and this delivery activated plant defense reactions.

FIGURE 2 HERE

Effectors and toxin protein delivery into wheat by the modified* P. fluorescens *strain EtHAn

Recently, Thomas et al. (2009) developed a stable delivery system for individual or defined sets of type-III effectors into plant cells. They engineered the complete hrp/hrc region from *Pseudomonas syringae* pv. *syringae* 61 into the genome of *Pseudomonas fluorescens* Pf0-1. The recombinant *P. fluorescens* strain was designated EtHAn. *P. fluorescens* strains Pf0-1 and EtHAn caused no noticeable necrotic or chlorotic reaction in wheat (Fig. 3) even after infiltration at relatively high concentrations (OD600 of 1.0). The expression/delivery vector pEDV6 was used to express *avrRpt2* or *avrRpm1* in EtHAn in infiltrated leaves of wheat. Wheat leaves were also infiltrated with EtHAn carrying *avrRpt2* or *avrRpm1* as single-copy genes integrated via Tn7 into the *P. fluorescens* genome (Thomas et al. 2009). Wheat leaves infiltrated with EtHAn carrying *avrRpt2* exhibited a noticeable chlorotic phenotype through the infiltrated region that was not observed in wheat seedlings infiltrated with EtHAn or EtHAn carrying an empty pEDV6 vector. The chlorosis was observed with EtHAn carrying *avrRpt2* integrated into the genome or expressed from the pEDV6 vector. The chlorotic reaction indicates the *avrRpt2* protein is being delivered by the TTSS engineered into EtHAn and this effector is causing a chlorotic response. Wheat leaves infiltrated with EtHAn carrying *avrRpm1* integrated into the genome also showed a chlorotic phenotype. Alternatively, no obvious phenotype was observed in wheat leaves infiltrated with EtHAn carrying plasmid-borne *avrRpm1*. A similar phenomenon was reported by Guttman and Greenberg (2001). They integrated an AvrRpt2 effector into the *Pseudomonas syringae* genome as fusion protein composed of the N terminus of the heterologous AvrRpm1 effector and the C-terminal effector region of AvrRpt2. They also found that chromosomally located *avrRpt2* induced a stronger resistance response than that observed with plasmid-expressed *AvrRpt2* in plants with the cognate *R* gene, *RPS2*. It is possible that the plasmid-borne genes produce or secrete lower levels of proteins, sometimes resulting in non-physiological levels of expression.

FIGURE 3 HERE

To investigate the possibility of using modified *E. coli* strains for delivering effectors, the plasmid-borne (pEDV6) *avrRpt2* and *avrRpm1* constructs were mobilized into *E. coli* MC4100 carrying cosmid pCPP2156. This strain has been demonstrated to deliver TTSS effector proteins into *Nicotiana clevelandii* cells (Ham et al. 1998; Anderson et al. 1999). As with *Pseudomonas fluorescens* Pf0-1, *E. coli* MC4100 and *E. coli* MC4100 carrying cosmid pCPP2156 caused no noticeable reaction in wheat at relatively high concentrations (OD600 of 1.0). No noticeable necrotic or chlorotic reactions were observed in wheat leaves after infiltration with *E. coli* MC4100 carrying the plasmid-borne (pEDV6) *avrRpt2* or *avrRpm1*.

To better characterize the chlorotic reaction of wheat plants to EtHAn strains expressing *avrRpm1* or *avrRpt2*, hydrogen peroxide accumulation was examined in the wheat leaves infiltrated with EtHAn carrying *avrRpt2* or *avrRpm1* by DAB staining (Fig. 4). Wheat inoculations that exhibited chlorotic reactions also accumulated hydrogen peroxide supporting the hypothesis that the chlorotic reaction was indeed a defense response. Wheat leaves infiltrated with EtHAn or EtHAn carrying an empty expression vector accumulated little hydrogen peroxide whereas wheat leaves infiltrated with EtHAn carrying genomic or plasmid borne *avrRpt2* accumulated large amounts. The wheat leaves infiltrated with EtHAn carrying *avrRpm1* integrated into the genome showed extensive hydrogen peroxide accumulation, while wheat leaves infiltrated with EtHAn carrying plasmid-borne *avrRpm1* showed much less hydrogen peroxide accumulation. The accumulation of hydrogen peroxide was therefore very well correlated with the expression of the chlorotic phenotype. Examination of callose deposition revealed a similar trend to that of hydrogen peroxide accumulation: expression of the avrRpt2 or avrRpm1 proteins caused greater defense responses when infiltrated into wheat. While infiltration of EtHAn into wheat induced slightly more than the control infiltrated with water, infiltration with EtHAn carrying *avrRpt2* or *avrRpm1*, either integrated into the genome or plasmid-borne, resulted in noticeably more callose (data not shown). Collectively, these experiments indicate

that EtHAn can deliver effectors into wheat cells using a pEDV6 expression vector and trigger defense reactions.

FIGURE 4 HERE

While these experiments demonstrate the delivery of bacterial effectors, it is possible that many fungal proteins may be more difficult for the system to deliver. Unfortunately, no *Puccinia* avirulence gene is yet available for testing in cereal systems. ToxA is the most studied toxin in fungal-cereal pathosystems and a compatible Tsn1–ToxA interaction plays a major role in conferring susceptibility of hexaploid wheat to *Stagonospora nodorum* blotch (SNB). Toxin production by the pathogen and toxin sensitivity in the host is each conferred by a single dominant gene. The *ToxA* gene encodes a pre-pro-protein that contains a signal sequence to target the protein to the secretory system (Balance et al. 1996) and a pro-sequence that assists in proper folding and is removed prior to secretion (Tuori et al. 2000) of the mature toxin, ToxA (C-domain). Introduction of the mature toxin into the mesophyll cells of a sensitive plant by infiltration results in a necrotic response (Manning and Ciuffetti 2005). The vector pEDV6 was used to express the *ToxA* gene in the EtHAn host strain. Constructs made in pEDV6 included both the full-length *ToxA* gene and the nucleotides coding for *ToxA* without the signal peptide, fused to the amino terminus of the *avrRPS4* gene. These were then infiltrated into leaves of ToxA-sensitive wheat lines Langdon (LDN) and Kulm and ToxA-insensitive mutants LDNems138 and Kulm137. No macroscopic differences were readily observed between wheat leaves of ToxA-sensitive wheat lines and ToxA-insensitive mutants, but differences were observed in the extent callose deposition. EtHAn carrying *ToxA* with no signal peptide induced more callose deposition in leaves of toxin sensitive wheat lines compared to insensitive wheat lines which were indistinguishable from EtHAn carrying an empty vector (Fig. 5). The EtHAn strain carrying the full-length *ToxA* did not induce callose deposition. It is possible the secretion signal polypeptide either interfered with the protein's delivery or interfered with its function inside the host cell. A similar phenomenon was observed when delivering the full length downy mildew effector ATR13^{Emco5} into *Arabidopsis* via a bacterial delivery system (Rentel et al. 2008).
It was therefore concluded that the mature ToxA protein can be delivered successfully into wheat through recombinant *P. fluorescens* strain EtHAn and that the level or form that is delivered is sufficient to induce defenses but not cause a noticeable HR.

FIGURE 5 HERE

Another approach to characterization of effectors and identification of Avr genes would be feasible if these genes could be silenced by expressing RNAi constructs for these fungal genes in cells of cereals. Silencing of pest or pathogen genes in plants by RNAi expression has been demonstrated for the cyst nematode *Heterodera schachtii* (Huang et al. 2006; Yadav et al. 2006; Sindhu et al. 2009) and the parasitic weed *Triphysaria,* which forms invasive haustoria in host roots. Preliminary experiments indicated that *in planta* rust gene silencing may be feasible in cereals but results have been highly variable between different genes (C. Yin and J. Jurgensen unpublished). The ability to silence rust genes in plants could be a valuable system for functional analysis of effectors. The establishment of stable transgenics expressing RNAi constructs for numerous putative fungal effectors would likely be an arduous task for most cereal species, but more high throughput transient assays, like virus induced gene silencing (VIGS) might be more feasible. The virus infection can sometimes alter the plant-rust pathogen interaction slightly making examination of effectors with small effects on pathogenicity difficult. The BSMV VIGS system has been used successfully by several groups to scrutinize candidate host genes for involvement in rust resistance in wheat (Huang et al. 2003; Scofield et al. 2005; Cloutier et al. 2007; Zhou et al. 2007). It is therefore possible that RNAi silencing may be another method of rust effector characterization.

In conclusion, the increasing availability of genomic and transcriptome sequences is providing candidate effector genes for the cereal rust fungi. Informatics methods for refining these effector

prediction methods will eventually become available after a larger variety of rust effectors are identified. A variety of potential methods are becoming available for characterizing the function of these effectors in cereal plants. Which of these will be the most useful is still unclear. A current major deficiency is the unavailability of *Puccinia* avirulence genes that can be used to test effector analysis methods in cereals. Experiments with bacterial effectors and fungal toxin genes permit the development and preliminary examination of the utility of these systems but must be verified with several rust effectors when they become available.

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Fig. 1 Reactions of wheat leaves to infiltrations of *P. syringae* isolates. a, mock inoculated with water; b, *Pst* DC3000; c, DC3000 variant CUCPB5500; d, CUCPB5500 carrying the expression vector pEDV6. Experiments were repeated multiple times with similar results

Fig. 2 Callose deposition reactions of wheat leaves to infiltrations of *P. syringae* isolates. a, DC3000; b, CUCPB5500; c, CUCPB5500 carrying the expression vector pEDV6**;** d, pEDV6::*avrRpt2* in CUCPB5500; e, pEDV6::*avrRpm1* in CUCPB5500 f, Mock infiltration with water

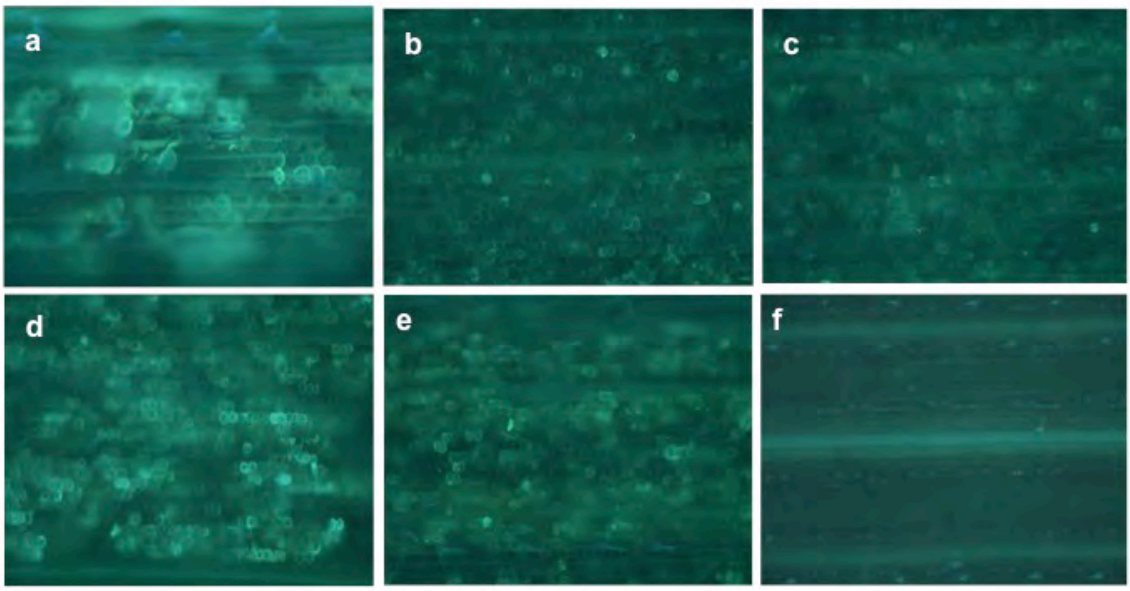


Fig. 3 Wheat leaves infiltrated with *P. fluorescens* strain EtHAn carrying different effector constructs. a, EtHAn carrying an empty Tn7 vector; b, Genomic copy of *avrRpt2*; c, Genomic copy of *avrRpm1*; d, pEDV3 empty expression vector; e, pEDV6:: *avrRpt2* f, pEDV6:: *avrRpm1*; g, Mock infiltration with water. Experiments were repeated multiple times with similar results

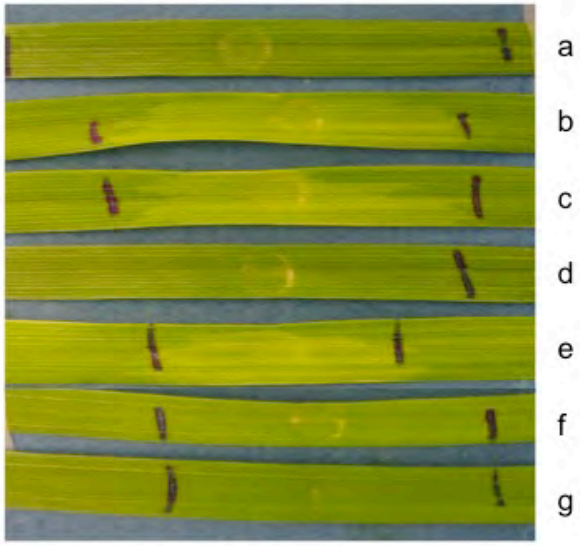


Fig. 4 DAB staining showing H_2O_2 accumulation in wheat leaves infiltrated with *P. fluorescens* strain EtHAn expressing *avrRpt2* and *avrRpm1*. a, EtHAn carrying an empty Tn7 vector; b, Genomic copy of *avrRpt2*; c, Genomic copy of *avrRpm1*; d, pEDV3 empty expression vector; e, pEDV6:: *avrRpt2*; f, pEDV6:: *avrRpm1*; g, Mock infiltration with water. Experiments were repeated multiple times with similar results

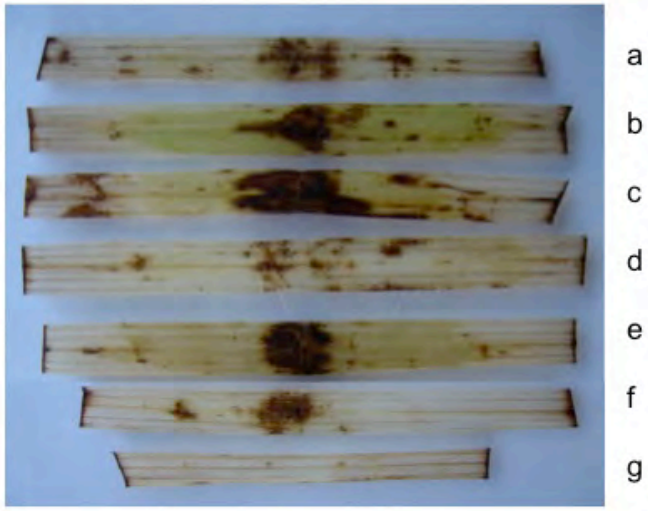
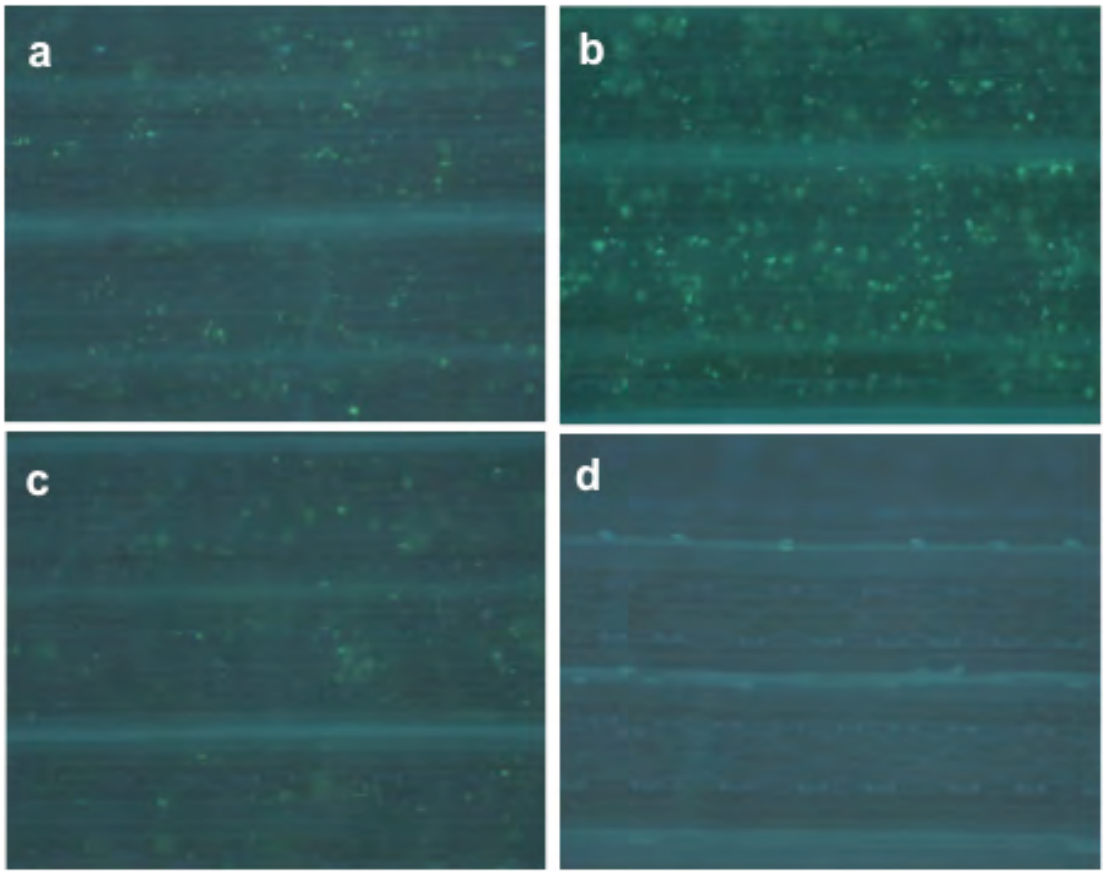


Fig. 5 Callose deposition reactions in wheat leaves infiltrated with *P. fluorescens* strain EtHAn expressing *ToxA* constructs. a, EtHAn carrying the pEDV3 vector infiltrated into durum cv Langdon; b, pEDV6::$ToxA_{48-537}$ in EtHAn infiltrated into Langdon; c, pEDV6::$ToxA_{1-537}$ in EtHAn infiltrated into wheat Langdon; d, Mock infiltration with water. The experiments were repeated multiple times with similar results.



Genomic selection for durable stem rust resistance in wheat

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Abstract Inheritance of stem rust (caused by *Puccinia graminis* f. sp. *tritici*) resistance in wheat can be either qualitative or quantitative. While quantitative disease resistance is believed to be more durable, it is more difficult to evaluate as it is expressed in mature plants, i.e. adult plant resistance (APR). Marker-assisted selection (MAS) methods for APR would be useful; however, the multigenic nature of APR impedes the use of MAS efforts that aim to pyramid only a few target genes. A promising alternative is genomic selection (GS), which utilizes genome-wide marker coverage to predict genotypic values for quantitative traits. In turn, GS can reduce the selection cycle length of a breeding program for traits like APR that could take several seasons to generate reliable phenotypes. In this paper, we describe the GS process for use in crop improvement, both specifically for APR and in general. We also propose a GS–based wheat breeding scheme for quantitative resistance to stem rust that, when compared to current breeding schemes, can reduce cycle time by up to twofold and facilitates pyramiding of major genes with APR genes. Thus, GS could be an important tool for achieving the Borlaug Global Rust Initiative's (BGRI) goal of developing durable stem rust resistance in wheat.

Keywords adult plant resistance, durable resistance, genomic election, *Puccinia graminis*, quantitative resistance, wheat

Abbreviations
AB association breeding
APR adult plant resistance
GasBLUP gene-assisted genomic selection
GEBV genomic estimated breeding value
GS genomic selection
HR hypersensitive response
LD linkage disequilibrium
MABC marker assisted backcrossing
MAS marker assisted selection
MARS marker assisted recurrent selection
RR-BLUP ridge regression best linear unbiased prediction
TP training population
TBV true breeding value
SC selection candidate

Introduction

Marker-assisted selection (MAS) enables plant breeders to more easily pyramid disease resistance genes to enhance qualitative disease resistance durability (William et al. 2007). Consequently, efforts aimed at quickly developing wheat varieties resistant to Ug99 (formally known as race TTKSK), a relatively new and highly virulent race of the stem rust pathogen (*Puccinia graminis* f. sp. *tritici*), use markers to pyramid several, effective single resistance genes to reduce the probability of resistance breakdown by pathogen evolution. It is well known that qualitative resistance to rust pathogens involves gene-for-gene relationships between the host and pathogen (Flor 1942). These genes are involved in host/pathogen recognition in which effective recognition genes (R-genes) elicit a

hypersensitive response (HR) (Stackman 1915), a programmed cell death response to prevent the spread of the pathogen. A single mutation in the pathogen can render an R-gene ineffective. In fact, new variants of Ug99 capable of infecting wheat plants carrying *Sr24* (race TTKST) and *Sr36* (race TTTSK) were already identified in 2009 (Jin et al. 2009). In germplasm with several R-genes, each must be overcome in a single generation for resistance breakdown. This is considered unlikely; however, evidence for simultaneous mutations of avirulence genes in rust fungi indicates that R-gene pyramids could be defeated in a single step (Griffiths and Carr 1961; Statler 1987). Furthermore, if R-genes used for pyramiding are present singly in some cultivars, the pathogen could become virulent to each one in a stepwise manner rendering the pyramid ineffective. A resistance mechanism other than HR should be exploited to prevent eventual stem rust resistance breakdown. Non-specific adult plant resistance (APR) to stem rust, based on multiple genes is one promising mechanism.

Adult plant resistance to stem rust describes a form of quantitative disease resistance that is detected in mature plants, is associated with non-HR and non-specific resistance (Hare and McIntosh 1979), and is quantitatively inherited (Knott 1982). Sources of quantitative resistance in crop plants have proven to be highly durable (Parlevliet 2002), making APR a promising breeding target for long-term stem rust resistance. However, the integration of APR into adapted wheat germplasm is difficult because it is multigenic (Knott 1982) thereby making backcrossing inefficient even with the assistance of molecular markers (Lee 1995). Difficulties in phenotyping APR further complicate the breeding process because APR must be screened by trained experts in specific locations and effective R-genes interfere with the evaluation and identification of APR.

Given the quantitative nature of APR and its inconvenience to phenotype, improved breeding methods and strategies for APR could be useful. A new breeding method, termed genomic selection (GS; Meuwissen et al. 2001), aims specifically at improving quantitative traits by using genome-wide marker coverage to predict breeding values of selection candidates (SCs). In turn, GS can reduce the frequency of phenotyping because selection is based on genotypic data rather than phenotypic data. GS can also reduce cycle time, thereby increasing annual gains from selection. Here, we review the potential of marker-based selection to improve quantitative traits and propose a breeding strategy that utilizes GS to select for APR. We also discuss issues that affect GS performance and the use of GS to meet the goals of the Borlaug Global Rust Initiative (BGRI).

Molecular markers in breeding

Marker assisted selection

In all breeding programs, predicting the breeding value of SCs is essential for achieving gains from selection. Molecular markers have already become important for predicting breeding value and are especially important for combining R-genes in adapted germplasm (Singh et al. 2006, William et al. 2007). The use of MAS in plant breeding is now routine in commercial breeding programs to increase gains from selection per unit time (Eathington et al. 2007). The two basic breeding strategies using molecular markers include: 1) backcrossing favorable alleles into elite germplasm, i.e. marker-assisted backcrossing (MABC) and 2) predicting an individual's breeding value for selection, i.e. marker-assisted recurrent selection or association breeding (MARS; Bernardo 2008; AB; Breseghello and Sorrells 2006). MAS has been mostly limited to improving traits with MABC of major genes (Holland 2004), as introgression of many genes required to improve traits that are quantitatively inherited is not logistically feasible for most breeding programs (Wang et al. 2007; Xu and Crouch 2008). Another drawback to MAS breeding strategies is that significant marker-trait associations must first be mapped. Typically, this step fails to detect rare or small-effect QTL, only captures a portion of the genetic variance (Goddard and Hayes 2007), can lead to overestimated marker-effects (Lande and Thompson 1990; Beavis 1998), and yields marker-trait associations that may not be relevant across breeding pools, in different environments, or after several cycles of selection (Podlich et al. 2004).

Genomic selection

Genomic selection aims to accurately predict the breeding value of SCs with genome-wide marker data through a 3 step process: 1) prediction model training and validation, 2) breeding value prediction of SCs, and 3) selection based on these predictions (Meuwissen et al. 2001). In GS model training, a training population (TP) consisting of germplasm having both phenotypic and genome-wide marker data is used to estimate marker effects. The combination of these marker effect estimates and the marker data of the SCs is used to calculate genomic estimated breeding values (GEBVs), where a GEBV is the sum of all marker effects included in the model for an individual. Selection is then imposed on the SCs using GEBVs as the selection criterion. Thus, GS attempts to capture the total additive genetic variance with genome-wide marker coverage and effect estimates. This contrasts with current MAS strategies that utilize a small number of significant markers for prediction and selection.

Accuracy of the GS model is determined by calculating the correlation of GEBVs to true breeding values (TBVs). A cross-validation approach, using a population having both genotypic and phenotypic data, is commonly used to evaluate GS accuracy. In this approach, a subset of the TP is removed from the GS model building dataset and is used as a test set for the GS model (Lee et al. 2008). Empirical GS studies for various traits in dairy cattle calculated GEBV accuracies of up to 0.85, and, in most cases, GEBVs exceeded pedigree-based breeding value accuracies (VanRaden et al. 2009; Calus 2010). These accuracies can then be used to calculate response from selection (R), where $R = ir\sigma_A$ with r = accuracy, i = selection intensity, and σ_A = the square root of the additive genetic variance (Falconer and Mackay, 1996, p. 189). R is important for determining gain per unit time and cost and for comparing breeding strategies. In dairy cattle, Schaeffer (2006) determined that the time and cost savings using GS with GEBV accuracy of 0.75 would increase genetic gain twofold and provide a cost savings of 92% when compared to the current methods. The ability to calculate highly accurate GEBVs and the potential to drastically reduce phenotypic evaluation frequency and selection cycle time facilitated a rapid adoption of GS and is revolutionizing the dairy cattle breeding industry (Hayes et al. 2009b).

GS in plant breeding programs

Greater gains from selection per unit time

To date, the only publicly available results on large-scale GS performance are from dairy cattle breeding programs. Emerging studies in crop plants indicate that GS could also be an extremely useful tool for plant breeding (reviewed by Heffner et al. 2009). The use of marker-based selection strategies to increase gain per unit time is not a new concept, especially for traits that are difficult to phenotype or crops that can utilize greenhouses or off-season nurseries (Johnson 2004; Eathington et al. 2007). Accordingly, simulation (Wong and Bernardo 2008; Zhong et al. 2009) and empirical studies (Lorenzana and Bernardo 2009) found that in plant populations GS would lead to greater gains per unit time than phenotypic selection. Furthermore, studies comparing GS with current MAS approaches support the superiority of GS for improving gain per unit time. A study comparing GS to MARS in a simulated maize breeding program found GS to have increased response from selection, especially for traits of low heritability (Bernardo and Yu 2007). Similarly, a simulation in oil palm found GS to have higher gains than MARS even at population sizes feasible for tree species (Wong and Bernardo 2008). In a recent study that accounts for cost of GS and MAS approaches, Heffner et al. (2010) report that GS with GEBV accuracies of only 0.5 could lead to a twofold higher gain per year compared to MAS in a low-investment wheat breeding program and a threefold increase in a high-investment maize breeding program.

Marker density and type

The rapid decrease in marker discovery and genotyping costs using high-throughput technologies should allow all crops to achieve genome-wide marker coverage necessary for GS. To effectively cover the entire genome, at least one marker should be in linkage disequilibrium (LD) with each QTL. The minimum number of markers to achieve genome-wide coverage therefore depends on LD decay rates which vary widely across species, populations, and genomes due to forces of mutation, recombination, population size, population mating patterns, and admixture (Flint-Garcia et al. 2003). More simply, marker density must increase with increases in *Ne**c, where *Ne* is the effective population size and c is the recombination rate between loci (Hill and Robertson 1968). In diverse populations that span many generations of recombination, *Ne* and c are large, and thus marker density must be high to achieve strong LD between markers and QTL.

In addition, marker-QTL LD should be conserved between the TP and the SCs to achieve high-accuracies (Goddard 2009). For example, if LD decay is more rapid in the SC population than in the TP, accuracies would decline because markers would segregate with the QTL they estimated in the TP. Such scenarios could result if SCs are genetically divergent from the TP or if the TP and SCs are separated by multiple recombination cycles. Therefore, TPs should have equal or greater LD decay rates than SC populations and high marker densities to reduce the accuracy loss from genetic divergence or multiple generations of selection (Muir 2007, Zhong et al. 2009). Overall, increased marker densities were shown to increase accuracy (Solberg et al. 2008) as long as the number of phenotypes also increases to allow accurate estimation of the marker effects (Bernardo and Yu 2007; Muir 2007; Hayes et al. 2009b; Lorenzana and Bernardo 2009;).

The marker platform used for GS can also affect density requirements. Most studies of GS have used SNP marker genotypes to estimate QTL effects. The data related to SNPs can be extended to other high throughput bi-allelic marker systems. In a study evaluating marker type and densities for GS, Solberg et al. (2008) found that SNP genotypes lead to higher GEBV accuracies than SNP haplotypes. However, SNPs compared to SSRs lead to similar accuracies when SNP marker density is 2 to 3 times greater than that of SSRs. Despite the increased density requirement, SNP markers are still preferred because they are easily automated and abundant.

Training population composition

The highest GS accuracies are achieved when the TP is large, consists of the parents or very recent ancestors of the population under selection (Habier et al. 2007; Goddard 2009; Hayes et al. 2009b; Zhong et al. 2009; Toosi et al. 2010), and consists of multiple generations of training (Muir 2007). The study by Zhong et al. (2009) examined the effects of different parameters on GEBV accuracy and found that doubling the size of the TP always increased accuracy. When the population under selection was one generation removed from the TP, a greater number of replications of each training individual instead of a greater number of genotypes led to higher accuracies. This is due to the improved prediction of the genetic relationship between training and selection individuals. When the population under selection was four generations removed from the TP, more genotypes rather than replicates in the TP increased GEBV accuracies because in this scenario the model relies more on estimation of marker effects due to LD with QTL. This agrees with the known fact that increased genotypes rather than replicates lead to improved estimation of QTL effects (Knapp and Bridges 1990). Thus, to apply GS models to generations far removed from the TP, markers need to be in strong LD with the QTL and the QTL effects must be correctly estimated with sufficient training records.

Accurate estimation of QTL effects in SCs is achieved by increasing the number of training records (Hayes et al. 2009b; Zhong et al. 2009) and by re-estimating QTL effects in the population where the allele frequencies (and therefore QTL effects) and LD structure change due to selection (Goddard 2009). This can be achieved by updating the GS model by including former SCs in the TP. Simulation studies have yet to account for the effect that significant QTL x genetic background interactions in the TP and SCs will have on GEBV accuracies. Ultimately QTL x genetic background effects will decrease the accuracy of estimated QTL effects. Under the assumption that QTL effects

are conserved across populations, using extreme marker densities and massive TPs for GS model training will lead to GS models able to accurately predict GEBVs of individuals distantly related to the TP (de Roos et al. 2009; Hayes et al. 2009a; Meuwissen 2009)

Biparental vs. multi-family training populations

The particular individuals to include in the TP is perhaps the most difficult variable to optimize. Currently, most studies of TPs have been conducted in cattle, where TPs are not easily constructed and generally consist of historical data. In plants, many possible TPs can be constructed, thus more studies on GS TP optimization will be required. Recent studies in plants evaluated TPs consisting of biparental crosses (Lorenzana and Bernardo 2009), doubled haploid testcrosses (Bernardo and Yu 2007) and intermated inbred lines (Zhong et al. 2009) where the validation population was directly related to the TP. The ideal situation may be to generate a new TP for each family derived from each bi-parental cross in the breeding program. This would lead to high accuracies because SCs are directly related to the TP causing LD, moderate allele frequencies, and QTL effects, and genetic background effects need to be similar between SCs and the TP. However, it also requires that a TP of individuals from each cross be phenotyped in the target set of environments prior to GS modeling. Alternatively, the use of using a TP consisting of a representative sample of the breeding program could accurately calculate GEBVs for SCs from each cross and could achieve even shorter selection cycles.

Several simulation studies in cattle assessed the potential of using a large TP of various divergent breeds to calculate GEBVs in one breed (de Roos et al. 2009; Hayes et al. 2009a; Meuwissen 2009; Toosi et al. 2010). These studies found high accuracies at very high marker densities. Such accuracies experience less decay over multiple generations of selection (Meuwissen 2009) because markers in high LD with a QTL are used to estimate the QTL effect in the model, and these linkage phases are less likely to be broken. Thus, with improved marker technology, large TPs that use a representative sample of germplasm in a given breeding program may be a good strategy for long-term accuracy over a broad range of families.

Estimating marker effects

Choice of statistical methods for estimating marker effects also can affect model accuracy. A variety of methods for genomic prediction is currently available (reviewed by Calus 2010 and Jannink et al. 2010). For brevity, we highlight three statistical methods available to train the GS model: ridge regression best linear unbiased prediction (RR-BLUP), Bayes-A, and Bayes-B (Meuwissen et al. 2001). RR-BLUP simultaneously estimates all marker effects, but assumes that markers are random effects with a common variance, and shrinks all marker effects equally toward zero. To address the common variance assumption, Meuwissen et al. (2001), proposed using Bayesian estimation methods, Bayes-A and Bayes-B, to more correctly model marker effects. In Bayes-A, the prior distribution uses an inverted chi-square distribution that is adjusted to match the mean and variance of the marker effects by adjusting the degrees of freedom and scale parameter. A modified Bayes-A method proposed by Xu (2003) and improved by ter Braak et al. (2005) also uses an inverted chi-square distribution, but places a higher probability density on marker effects close to zero. In Bayes-B, the prior distribution allows marker effects to equal zero and uses an inverted chi-square distribution for marker effects greater than zero. This adjustment yields a more realistic prior distribution because many markers are expected to have no effect on the trait of interest. The relative accuracy of these methods depends on the strength of marker effects. Where markers are in high LD with a few large-effect QTL that capture most of the genetic variance, i.e. strong marker effects, Bayesian methods are most accurate, whereas the RR-BLUP method is most accurate when many markers have small effects (Zhong et al. 2009). An additive relationship (A) matrix accounting for kinship and calculated using markers (Fernando and Grossman 1998) or pedigree can be included in the model. The A matrix accounts for QTL effects not captured in the model and thus should only significantly improve GEBV accuracy when marker density is low (Calus and Veerkamp 2007).

Trait heritability and gene number

Studies in cattle indicated that traits with lower heritability require larger TPs to maintain high accuracies (Hayes et al. 2009b). Goddard (2009) defined GEBV accuracy as a function of the number of phenotypic records and heritability; a decrease in heritability leads to lower GEBV accuracies. Although GEBV accuracies will be better when trait heritability is high, in this case the accuracy of phenotypic selection (h) will also be high; thus, GEBV accuracies (r) may not exceed h. The strength of GS is for low-heritability traits where r surpasses h when there are at least two generations included in the TP (Muir 2007). However, in a simulation study by Bernardo and Yu (2007) of GS in maize comparing scenarios where $h^2 = 0.2$, 0.5 and 0.8, GS showed a greater response to selection compared to phenotypic selection and MARS for all three h^2 values. Furthermore, GS was the most favorable in comparison to phenotypic selection when $h^2 = 0.2$ and the number of QTL = 100 Nevertheless, GEBV accuracy decreased with decreasing h^2. Clearly, traits of low heritability pose a problem for both phenotypic selection and GS because low heritability leads to decreased accuracies with both methods. However, a greater initial investment in the TP aiming to increase r will lead to more accurate selection based on GEBVs. In contrast, to select for low heritability traits based on phenotypes, many replications or laborious progeny tests need to be conducted every cycle of selection.

Facilitation of recurrent selection

Another advantage of GS is that it facilitates the use of recurrent selection especially in crop species traditionally unable to exploit this method. The success of recurrent selection has been well documented (Hallauer 1985) and is regularly exploited in outcrossing species. Although recurrent selection can be successful in wheat (Busch and Kofoid 1982; Wiersma et al. 2001) it is not a routine strategy. One of the impediments for recurrent selection in wheat is the problem of determining the breeding value of individuals because of the difficulty in producing adequate quantities of seed to make selections based on replicated trials. However, GS enables breeding value to be calculated directly based on genotype instead of phenotypes. Therefore, genomic recurrent selection in wheat requires a minimal amount of seed for intermating selections. Greater gains from selection are expected compared to bulk breeding methods because the increased opportunities for recombination events facilitate combining favorable alleles within the same line. Fig. 1 depicts a recurrent genomic selection scheme that allows up to three recombination cycles per year and presents a possible system to evaluate recombinant lines simultaneously and update the training population to ensure accuracies remain stable over cycles. New germplasm can enter the training population and recombination cycle at any time to maintain diversity.

FIG. 1 HERE

Using markers to select individuals in recurrent selection programs, MARS, is not a new idea. However, GS for recurrent selection outperforms MARS. According to simulations, the use of GS to select individuals in recurrent selection programs leads to greater responses to selection relative to MARS (Bernardo and Yu 2007). Because GS estimates breeding values of individuals in the breeding population using the sum of all marker effects, many limitations associated with detecting significant marker trait associations are bypassed and the total genetic variation for the traits of interest can be better captured by the markers.

In some self-pollinated crop species seed production from hand crosses is likely to remain a limiting factor for the use of recurrent selection. For these cases, a modified recurrent selection scheme, described as “select-recombine-self,” using GS in F2 individuals was suggested by Bernardo (2010) for crops where insufficient seed production inhibits the use of selection and recombination in the F1 generation. In this simulation study, recurrent selection where GS is applied to F2 individuals

resulted in only slightly lower response to selection than when GS was applied to F1 individuals, but the extra generation required reduces annual gain. In either case, response to selection was expected to be greater than for pure-line methods integrating GS. Thus, this modified recurrent GS scheme is a viable alternative for crops where F1 seed production limits the ability to make sufficient crosses in F1 populations.

GS for stem rust APR

The potential utility of GS for APR

The appearance of Ug99, virulent across a range of international germplasm, demonstrated the need for more durable forms of resistance such as APR. Although, several R-genes for stem rust resistance (*Sr* genes) found in unadapted germplasm confer at least moderate resistance to Ug99 (Jin et al. 2007), transferring these single genes to adapted germplasm, although easily done by conventional disease testing or by MAS, should be approached with caution because single resistance genes can be overcome via mutation or recombination (Flor 1942; Person 1959). Furthermore, although considered unlikely, pyramids of *Sr* genes can be defeated by simultaneous mutations in the pathogen (Griffiths and Carr 1961; Statler 1987), and particularly by sexual recombination and population movement. For more durable, race-nonspecific resistance, quantitative effects typical of certain types of APR should be breeding targets.

The use of genome-wide marker coverage makes GS suitable to improve traits such as APR which are based on multiple genes. The genetic characterization of APR to stripe (or yellow) rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* and leaf rust, *Puccinia triticina* Eriks., using QTL mapping studies has indicated that APR is based on several genes many having small effects (Messmer et al. 2000; Bariana et al. 2001; Mallard et al. 2005; Navabi et al. 2005; Rosewarne et al. 2008). It has also been observed that for stripe rust (Navabi et al. 2004) and leaf rust (Singh et al. 2005) different resistant parents carry different APR genes. Breeding efforts to combine four to five APR genes from different sources to improve leaf rust and stripe rust APR have been successful in creating lines with high levels of resistance (Singh et al. 2000).

Although for stem rust the genetic basis of APR is largely uncharacterized apart from *Sr2* (Sunderwirth and Roelfs 1980), inheritance studies indicate that like APR to stripe rust and leaf rust, APR to stem rust is also multigenic (Knott 1982). High levels of stem rust APR should be achievable by combining multiple APR genes as achieved for leaf rust and stripe rust. Either MAS or GS can serve as important tools for combining several APR genes from different resistant sources. GS has the advantage that APR genes do not need firstly to be mapped and QTL effects that do not exceed the significance thresholds can be selected. In contrast, MAS is limited to the use of markers deemed significant in mapping studies and is limited to the selection of a few target genes.

Strategy of breeding for APR to stem rust using GS

Cornell University is collaborating with CIMMYT to develop GS models for APR to stem rust. To initiate GS for APR, model training with known sources of APR is essential. These sources should be closely related to the parents that will be used in breeding. The TP should be screened for seedling response to ensure the APR can be evaluated without the confounding effects of effective *Sr* genes. Also, haplotyping for major genes combined with pedigree information can be used to predict the presence of major genes so that they can be taken into account in APR model development. However, screening with Ug99 minimizes the number of genes that could have confounding effects because it is virulent on many *Sr* genes present in a broad range of germplasm. Screening APR where Ug99 is not present would require the *Sr* genes effective against the stem rust races present in that location to be removed from the lines being evaluated.

The GS models can be developed using a TP consisting of CIMMYT materials with varying levels of stem rust APR. APR can be evaluated in Kenya or another location where APR expression

would not be masked by effective *Sr* genes. Screening APR in an environment such as Kenya where disease pressure is high and highly virulent races are present should provide informative phenotypic data that would allow for the training of GS models to select for APR that is effective where disease pressure is greatest. These GS models could then be used to select the SCs for APR that is effective in environments having high disease pressure without the need for evaluation of the SCs in these often remote locations.

The model development step utilizes phenotypic and genotypic data to evaluate regression models by comparing correlations between GEBVs and TBVs. Simulations of GS models that use a Bayesian method to allow unequal marker variances have been only slightly more accurate than the RR-BLUP models which assume equal variances (Hayes et al. 2009b; VanRanden et al. 2009). However, model selection depends on the distribution and size of QTL affecting the trait. In the case of stem rust APR, it is likely that many regions of the genome will have no effect on resistance and a large effect QTL for APR, such as *Sr2* (Sunderwirth and Roelfs 1980), may significantly contribute to the variance. Under these circumstances a model that allows for unequal marker variances and for some marker effects to equal zero such as Bayes-B may be the most appropriate model (Meuwissen et. al. 2001); however, this needs to be evaluated empirically. Bayes-B was also shown to perform well when marker effects are strong (Zhong et al. 2009); this is important because *Sr2* and perhaps other *Sr* genes of large effect are likely to be prevalent in the TP.

Once the GS model has been built it can be implemented in selection. Simulation results from a selected bulk scheme in wheat based on a combination of GS with MAS and phenotypic selection (Heffner et al. 2010) showed increased gains per unit time compared to MAS and phenotypic selection alone. Although pure line breeding methods such as the selected bulk scheme used in the simulation study just described are routine in wheat breeding, recurrent selection schemes for wheat using GS should be explored. Our genomic recurrent selection scheme uses parents with high agronomic performance and acceptable levels of APR. In the first recombination cycle, the parents can be recombined by performing all possible combinations of crosses. Each subsequent generation can be intermated and genotyped to identify individuals with major genes. Those individuals can either be selected or discarded depending on the breeding goals. Selected lines from each cycle using GS are then phenotypically evaluated for APR. To estimate realized gain from selection, the performance of selected inbred lines can be compared to a random sample of lines from each cycle. This project may provide proof of concept for other genomic recurrent selection programs.

New GS methods for achieving disease resistance

In addition to the selected bulk and recurrent selection methods, backcrossing using GS is another possible breeding scheme. A simulation in cattle showed that using GS in a backcrossing scheme is effective for incorporating quantitative disease resistance from an inferior population into a population with superior production traits when the model is updated after every generation of selection (Odegard et al. 2009a, 2009b). A major benefit of using GS models instead of single genetic markers during backcrossing is that quantitative traits can be introgressed from the donor parent. Also, because the GS models do not distinguish the origin of the favorable alleles, favorable alleles from both parents are selected for overall population improvement. One complication with using GS in backcrossing is that using donor and recurrent parents that are very distantly related may require very high marker densities and large training population sizes to retain accuracy (Meuwissen 2009). Although backcrossing quantitative traits using GS appears useful, empirical studies are needed to evaluate its practical efficiency. Also, selection beyond the BC_1 or BC_2 may not be productive (Bernardo 2009). In a recent simulation investigating the utility of GS for introgressing exotic germplasm in maize, Bernardo (2009) compared population improvement based on BC_2, BC_1 and F_2 populations derived from exotic and elite parents. GS always performed best when the starting population was F_2. Thus the utility of using GS in backcross breeding schemes warrants further study.

A recent simulation study of backcrossing using GS investigated the utility of using known QTL effect information in the model (Odegard et al. 2009a). The study used a gene-assisted genomic

selection (GasBLUP) model which utilizes prior QTL information that may be useful in cases when important major QTL for the trait of interest are considered known, such as *Sr2* for stem rust APR. In the simulation by Odegard et al. (2009a) using GasBLUP in a GS backcrossing scheme resulted in an end frequency of a targeted QTL that was more than twice the end frequency when standard RR-BLUP was used in backcrossing. Ensuring that a targeted QTL is not lost during backcrossing would be especially important if that QTL is at a low frequency in the initial population, has a relatively small effect, or if it is in LD with unfavorable alleles, because it would be more likely to be lost during backcrossing with standard RR-BLUP. It is uncertain whether GasBLUP would an advantage over Bayes-B, as Bayes-B already adjusts the genetic variance of each marker and simulations using Bayes-B conclude that fitting population specific marker effects provides no major advantage (Ibánẽz-Escriche 2009). Although incorporation of known QTL information in GS models is still being investigated, it is likely that in some cases where QTL information is known, incorporating this information could provide an advantage, especially if the training population lacks a major QTL or contains such a QTL only at low frequencies. In the case of stem rust resistance, incorporating *a priori* gene information may be especially useful. In order to train the model for APR based on multiple minor genes, effective R-genes conferring immunity should not be present in the TP as they mask minor gene effects on the phenotype. However, to ensure high levels of resistance, it may be desirable to combine APR with effective R-genes. Incorporating R-gene information into GS models for rust resistance would then be necessary to select for R-genes in SCs that are not present in the TP, but are present in the SCs.

GS compared to current breeding strategies for achieving APR to stem rust

A single backcross selected bulk method is one approach to breed for APR (Singh et al. 2006). The strategy is to backcross sources of APR to elite lines for one generation, bulk phenotypically desirable backcross progeny in F_1 and F_2, select Ug99 resistant lines from the F_4 generation, and continue selections for desirable traits and disease resistance until the F_6 generation. Yield trials may be conducted at the F_7 stage. This strategy, while successful, relies heavily on the success of stem rust screening nurseries and provides fewer opportunities for recombination to combine favorable alleles compared to other methods. It also may result in APR genes being lost during backcrossing because APR might not be accurately evaluated in the backcross generation.

Genomic selection for durable resistance to stem rust in a recurrent selection program could address these issues to facilitate simultaneous improvement of APR and agronomic performance in the absence of Ug99. In addition to increasing the frequency of favorable APR alleles in the population, a GS scheme could also shorten the breeding process for APR by up to two years if we consider optimal usage of greenhouse and field resources for both the proposed and current methods. Our proposed recurrent GS scheme consists of six to eight generations (depending on the number of inbreeding generations before evaluation), and all except one generation can take place in the greenhouse where three generations per year are possible. Thus, the overall duration, including one season of evaluation, is two to three years. Whereas a single backcross selected bulk method may consist of nine generations, three of which could take place during one year in the greenhouse and the remaining seven generations must be grown in the field where two generations per year are possible, leading to an overall minimum duration, including one season of field evaluations, of four years.

GS to incorporate diverse germplasm

Required marker densities and training records

Introducing genetic diversity in breeding programs is important to improve traits such as disease resistance where important alleles may not be present in elite breeding pools. Incorporating new sources of genetic diversity, e.g. landraces or exotic germplasm, will be a major challenge for breeding programs using GS. The high marker densities that are required due to high *Ne* and low

population-wide LD of diverse individuals (Hayes et al. 2009a; Meuwissen 2009) will require many phenotypes and improved marker technologies. Therefore, the required number of phenotypes and marker density will be limiting factors for determining the range of germplasm that will be included in the TP. According to Meuwissen (2009), to achieve GEBV accuracies of ~0.9 in cattle, about 10 *Ne**L markers are necessary where L is the genome size in Morgans (M) and *Ne* the effective population size. *Ne* is an indicator of genetic diversity with low *Ne* being characteristic of a bottlenecked population (Nei et al. 1975). If we suppose that the genome size in hexaploid wheat is 30 M, and the highest density marker platform surveys 5,000 loci (Akbari et al. 2006), maximum *Ne* of a GS breeding program in wheat is 16 according to Meuwissen's (2009) equations. Thus, current marker densities in wheat are not high enough for a TP composed of an extensive range of germplasm. Actual *Ne* values of a population can be estimated using genotypic data and computer software (Ovenden et al. 2007); however, *Ne* is not routinely calculated in a plant breeding context. Equations derived from empirical studies and based on routinely calculated variables such as genome-wide LD levels would be more useful.

In addition to the increased marker density required for a diverse TP, accurately estimating alleles carried by individuals newly introduced in the TP will be difficult because they will initially be at low frequency. Thus, these alleles will not have extensive multi-environment phenotypic data within the genetic background of the breeding pool. This will require increased phenotyping of new training records to immediately achieve accurate GEBVs, which may not be logistically feasible. This, along with marker density issues, could result in low GEBV accuracy and, consequently, limit the range of germplasm that will be included in the TP. In practice, an accurate method for estimating the necessary number of markers and phenotypes based on a measure of genetic distance of individuals derived from empirical data could be valuable when deciding the breadth of germplasm to include in the TP

The impact of inconsistent QTL effects

One major concern for GS performance when SCs are unrelated to the TP is that, even if sufficient markers and training records are available, marker effects will be inconsistent because of the presence of different alleles, allele frequencies, and genetic background effects, i.e. epistasis (Bernardo 2008). These factors may lead to inaccurate marker effect estimates and, consequently, poor GEBV accuracies. In an empirical study of a TP with two cattle breeds, using both breeds in the TP improved GEBV accuracies despite the possibility of inconsistent QTL effects across breeds. However, using one breed in the TP to predict the other breed led to low accuracies (Hayes et al. 2009a), thereby showing that the TP should include individuals related to the SCs for GEBVs to be accurate. More empirical studies to investigate the utility of using a TP unrelated to the SCs are needed because the consistency of LD phase of QTL effects across unrelated individuals will likely vary depending on the trait and the species.

While dissimilar allele frequencies and different alleles segregating in diverse populations are clearly issues that affect QTL effect estimates, the overall importance of the influence of QTL x genetic background interactions is not clear. Interactions of some APR genes conferring resistance to rust pathogens have been reported. Epistasis was involved in quantitative resistance to leaf rust (Messmer et al. 2000), and evidence suggests that APR to stem rust conferred by *Sr2* varies in different genetic backgrounds (McIntosh et al. 1995). For quantitative disease resistance in plants in general, QTL x genetic background interactions were detected in some cases (Young 1996). QTL mapping studies of quantitative disease resistance to rice blast and grey leaf spot in maize uncovered significant epistatic interactions of resistance loci (Wang 1994), whereas studies of soybean cyst nematode quantitative disease resistance failed to detect epistasis (Concibido et al. 1994). Overall, QTL effects across unrelated individuals may be inconsistent and could undermine GEBV accuracies if the TP consists of unrelated individuals.

Conclusions

Genomic selection could be very a useful strategy for plant breeding as it may facilitate recurrent selection and improve gain from selection per unit time and cost, especially for quantitative traits with low heritability. For crops such as wheat, where recurrent selection is currently not common because the difficulty of crossing and limited seed production inhibits selection based on replicated evaluations, GS facilitated recurrent selection schemes could provide an advantage. If quantitative disease resistance, such as APR to stem rust, is a breeding target, implementing GS in such a scheme could combine high levels of resistance based on non-HR mechanisms with good agronomic performance. Furthermore, for quantitative traits, GS can increase gain from selection over MARS and complements MAS strategies. GS can be implemented using current marker technologies and a carefully selected TP of individuals related to selection candidates. However, with the constant improvement and cost reduction of marker technologies, including a greater diversity of individuals in the TP may be possible. Emerging marker technologies, such as the Illumia GoldenGate assay based on insertion site-based polymorphisms, claim densities of one per 5.4 kb across the hexaploid wheat genome at a cost of $0.1 per assay (Paux et al. 2010). Moreover, genotyping by sequencing, the ultimate genotyping platform, should be possible for wheat in the medium term. As the cost of genotyping continues to decrease, phenotypic selection will become comparatively more expensive for a broad range of traits. Even with current genotyping costs and capabilities, GS could increase net gain from selection per unit time and cost for many crops, including wheat. Clearly, GS is a promising strategy for rapid improvements in quantitative traits. This is desperately needed as stem rust races, including Ug99 and its derivatives, continue to evolve and migrate towards major wheat producing regions that are growing susceptible varieties. Thus, GS could be an important tool for achieving the BGRI's goal of developing durable stem rust resistance in wheat.

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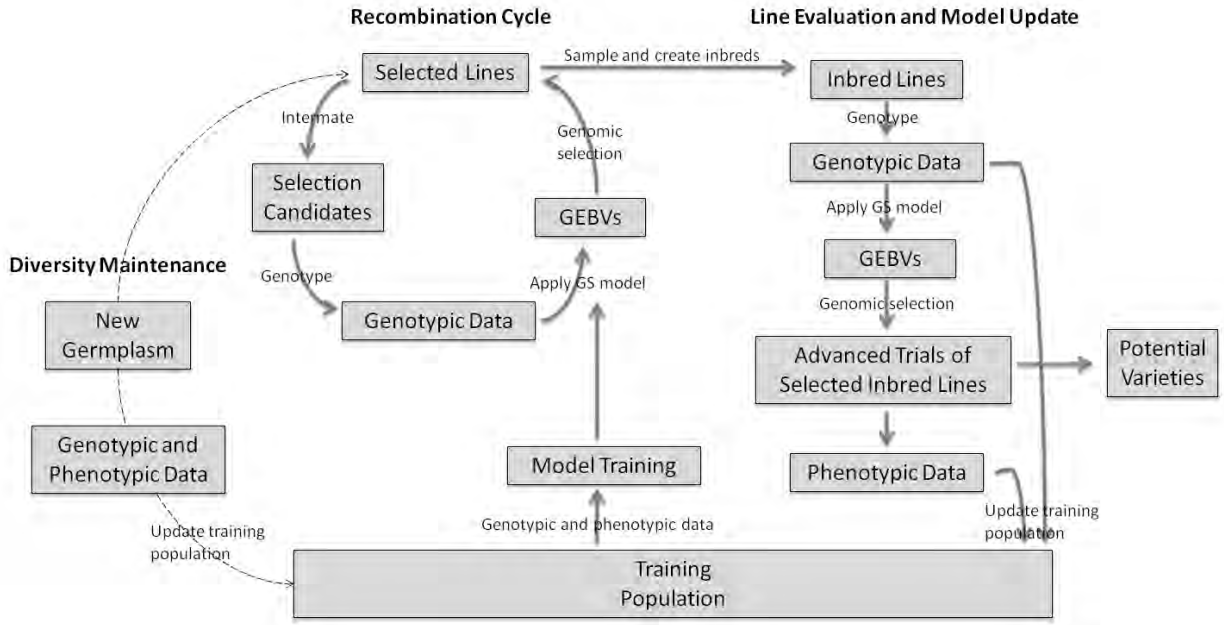
Fig. 1 A recurrent genomic selection scheme. The recombination cycle consists of rounds of intermating and selection based on GEBVs. For wheat, three recombination cycles per year are possible. Line evaluation and model updating occur simultaneously. After at least one recombination cycle, selected lines are inbred and selected again based on GEVBs or possibly phenotypes for line evaluation and training population updating. Phenotypic and genotypic data of the selected lines are used to update the model. Diversity in the breeding program can be maintained by introducing new germplasm into the recombination cycle. Genotypic and phenotypic data on the new germplasm should enter the training population to update the prediction model. GEBV: genomic estimated breeding value



Delivering rust resistant wheat to farmers: A step towards increased food security

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Abstract An important step towards reducing the vulnerability of wheat in Africa and Asia, to the Ug99 race of the stem rust pathogen, is the substitution of current susceptible varieties with superior resistant varieties. In the 2008–09 cropping season, both seed multiplication and dissemination of Ug99 resistant varieties was initiated in Nepal, Bangladesh, Afghanistan, Pakistan, Egypt, Ethiopia, Iran and India. Ug99 resistant varieties must occupy about 5% of the area sown to wheat in each country to ensure sufficient seed to displace current popular varieties. Because of the underdeveloped seed industry and the small farm sizes in most of these countries, various strategies are being applied for rapid multiplication and dissemination of resistant varieties. Approaches being used for rapid multiplication and distribution include pre-release seed multiplication whilst candidate resistant lines are being tested in National Evaluation Trials and farmers' participatory selection approaches. Resistant varieties were already released in Bangladesh, Afghanistan, Pakistan and Egypt, and more varieties are expected to be released in 2010 in these and other countries. Our results show that some new Ug99 resistant lines have yield superiority over dominant local varieties. Activities and progress in seed multiplication, using existing and new Ug99 resistant varieties, are discussed.

Keywords farmer participation, participatory varietal selection, seed distribution systems, stem rust, *Triticum aestivum*, Ug99

Introduction

Wheat is one of the most important food staples for mankind. It is cultivated on 15.4% of the arable land in the world and in almost all countries, except the humid and high-temperature areas in the tropics and high-latitude environments, where fewer than 90 frost-free days are available for crop growth (Singh and Trethowan 2007). Wheat is the primary source of calories for millions of people worldwide, accounting for around 30% of global grain production and 44% of cereals used as food, of which 18% is traded internationally (FAOSTAT 2009). Globally, approximately 220 m ha of land is sown to wheat each year, producing about 600 m t (FAOSTAT 2009), with nearly half of this area and production attributed to developing countries (Singh and Trethowan 2007). In addition, developing countries consume most of the wheat sold on the export market (Aquino et al. 2002), reflecting a huge number of consumers in these countries. In some countries, such as those in North Africa, per capita consumption of wheat is as high as 240 kg per annum (FAO 2002). Wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally.

Wheat currently faces a serious threat from stem rust, caused by *Puccinia graminis* f. sp. *tritici*, in particular race Ug99 and its derivatives (Pretorius et al. 2000; BGRI 2009). Ug99 was first identified in Uganda in 1999 and has since spread to other countries in East Africa and to Sudan, Yemen and Iran (Singh et al. 2008; IAEA 2009). Given favorable conditions, it threatens to spread into other wheat-producing regions of Africa and Asia, and potentially the entire world (BGRI 2009). The threat is particularly acute in South Asia, which produces 20% of the world's wheat for a population of 1.4 billion people (Joshi et al. 2009; BGRI 2009).

The threat of Ug99, initially in Kenya and Ethiopia, led to the launch of the Global Rust Initiative (GRI) in 2005, later renamed the Borlaug Global Rust Initiative (BGRI). The foremost objectives of the BGRI are to systematically reduce the world's vulnerability to wheat rusts, facilitate the development of a sustainable international system to contain the threat of wheat rusts, and to continue enhancements in productivity required to withstand future global threats to wheat (BGRI 2009).

Mitigating the Ug99 threat through growing resistant varieties

It was estimated that poverty in less developed countries is reduced by 0.5–1.0% with every 1% increase in wheat production (World Bank 2005). This gain may come directly from increasing yields or by reducing yield losses through the use of disease resistant cultivars. The best control strategy for most diseases of wheat grown by resource-poor farmers in the developing world, and the most environmentally friendly and profitable strategy for commercial farmers everywhere, is to grow resistant varieties (Singh et al. 2008). It has been suggested that the direct costs of rust resistance in wheat to farmers in developing countries are presently close to zero because this cost is already embedded in the seed (Dubin and Brennan 2009). Currently, the cost of protecting 1 ha of wheat from epidemics through the application of modern chemicals is USD10–80/ha. For example, Murray and Brennan (2009) estimated the cost of fungicides for foliar cereal disease control in Australia in 2008 to be approximately USD$8/ha, plus the cost of application. Therefore, an aggressive strategy to promote the use of resistant varieties in farmers' fields through large-scale quality seed production is the only viable and effective option for combating rust epidemics because chemical control is not affordable for resource-poor farmers in much of East Africa and South Asia (Joshi et al. 2008; Singh et al. 2008).

United States Agency for International Development (USAID) seed multiplication initiative in countries under threat to Ug99

Seed is the major mechanism for delivering agricultural-based technologies to farmers and therefore plays a critical role in realizing the benefits of agricultural research at the farm level. Given there is an essential link between crop improvement and seed supply, ensuring the availability and easy access to quality seed should accelerate the delivery, dissemination and adoption of new varieties by farming communities. The promotion of varieties with different resistance genes will enhance genetic diversity in farmers' fields and reduce the risk of pandemics.

Many national programs have initiated collaborations with the International Maize and Wheat Improvement Center (CIMMYT), the International Center for Agricultural Research in the Dry Areas (ICARDA) and BGRI to identify and develop suitable resistant cultivars for use in different wheat regions before Ug99 becomes established. Many varieties and genetic stocks from India, Nepal, Pakistan, Bangladesh, Iran, Egypt and other countries were previously screened against Ug99 and its derivatives in Kenya and Ethiopia and a low frequency of existing varieties and breeding materials were found to carry adequate or high levels of resistance.

To mitigate the threat of Ug99 and other wheat rusts, a seed multiplication project supported by USAID Famine Funds was implemented in Nepal, Afghanistan, Bangladesh, Egypt, Afghanistan and Ethiopia. The objective was to have sufficient seed of resistant lines to plant at least 5% of the total wheat areas by 2011. In addition, countries such as Iran, India and Kenya are also engaged in the

identification and multiplication of resistant lines. The major activities under this initiative, being implemented jointly by the national wheat programs, CIMMYT, ICARDA and BGRI are: 1. identification of suitable Ug99 resistant varieties and their pre- and post-release seed production; and 2. delivery of seed to farmers to ensure their rapid dissemination.

The infrastructures for producing seed and the strengths of the seed sectors in the six different countries are highly variable. Egypt and Pakistan have the strongest seed sectors whereas Nepal and Afghanistan are among the weakest. Consequently, the project was customized to fit the capacity and needs of each country. In countries with strong public and/or private seed sectors, such as Egypt and Pakistan, seed multiplication is through the established seed industry, whereas in Nepal, participatory varietal selection (PVS) is considered to be the best approach to achieve results. The services of emerging small scale seed companies in Nepal are also being used. In Afghanistan, seed is being produced through farmer-based seed production and marketing enterprises supported by FAO. In Ethiopia and Bangladesh, seed multiplication engages the existing public sector institutions, state farms and farmer-based seed production schemes on a regional basis.

Progress in seed production in 2008–09 under the USAID project and future projections

In 2008–09, 11 Ug99 resistant wheat genotypes from CIMMYT, Mexico, were deployed for seed production and further evaluation in the above six countries (Table 1). These lines were chosen following selection for resistance in Kenya and assessment in the CIMMYT nursery system. Several lines in this nursery out-yielded the checks by 5–15% in multi-location trials (Singh et al. 2008).

TABLE 1 HERE

The lines undergoing seed multiplication (Table 1) were also evaluated in multisite national trials. The usual process is that large-scale seed multiplication starts with the release of a cultivar. However, in this project seed multiplication starts much earlier to ensure that at the time of release large quantities of seed will be available for distribution to farmers. This approach carries the risk that some lines will be dropped from the program, even though large seed quantities were produced. Because the highest priority is to replace current Ug99 susceptible varieties as quickly as possible, parallel final stage multi-location testing and seed multiplication will permit earlier large-scale delivery of new varieties to farmers.

The area planted and seed production of Ug99 resistant lines varied across countries in 2008–09 (Table 2). The total area sown across the six countries was 52.6 ha and produced around 156 t. The maximum area (21 ha) and production (81 t) was in Egypt. In addition to these countries, a large quantity of seed of resistant varieties was produced in Iran (Table 3; 80,000 t), where Ug99 is already present. Overall, large amounts of seed, from resistant lines, were produced by the seven countries.

TABLES 2 AND 3 HERE

As in 2008–09, large-scale seed production was continued in the current 2009–10 cycle in all six countries (except Pakistan) and in Iran (Tables 4 and 5). The area and expected production in the seven countries (Nepal, Afghanistan, Bangladesh, Ethiopia, Egypt, Pakistan and Iran) is 46,899 ha and 117,364 t (Table 5). However, more than 95% of this production is expected to be contributed by Iran. Other countries are expected to produce about 1,150 t of seed (Table 5; Fig. 1). With race Ug99 already present in Iran, well organized seed production is underway. However, the expected seed production in the 2010–11 cycle portrays a much better picture. It is expected to be in the range of 0.34 to >100% (of the potential seed market) even if only 70% of the seed produced is used for planting (Table 5). It is estimated that relative to national wheat areas, the seed produced will be sufficient to cover 0.11% area in Pakistan to >100% in Iran (Table 5). Bangladesh, Egypt and Iran will be able to cover more than 5% of their respective wheat areas. Only about 30% of the seed produced in Iran will be required to cover 100% of its cropping area. Egypt and Bangladesh should

reach their projected targets by the end of the 2010–11 season allowing them to plant 5-8% of their total wheat areas (Table 5). Assuming that 100% of seed produced in 2009–10 and 2010–11 will again be used for seed multiplication, the situation will improve further. The current projected seed production figures suggest that all these countries, except Pakistan, will meet production targets by 2011–12.

TABLES 4 AND 5 AND FIG.1 HERE

Nepal

Nepal produces around 1.3 m t of wheat from 700,000 ha, representing approximately 25% of its total cereal production (FAOSTAT 2009). The main seed dissemination strategy is through PVS (Witcombe et al. 2001; Ortiz Ferrara et al. 2007). Seven Ug99 resistant lines obtained from CIMMYT and a resistant line (BL 3063) developed in Nepal were undergoing seed increase in the 2008–09 cycle (Table 1). BL 3063 (GS348/NL746//NL748) is expected to be released for farmers cultivation in 2010.

Seed production was done by the National Wheat Research Station, Bhairahwa, belonging to the National Agricultural Research Council (NARC), and Kalika Seeds, a private company. The total seed produced was 16,486 kg (Table 2), of which 11,986 kg was purchased using USAID-Famine Seed Project funds for further pre-release seed multiplication in the 2009–10 cycle. Lines undergoing seed increase in 2009–10 are shown in Table 4. About 57 ha were sown to achieve a target of 115 t of seed. The estimated seed production of resistant lines in 2010–11 will be sufficient to meet 7.3% of the effective seed market and 2.4% of the total wheat area of Nepal (Table 5).

Bangladesh

Wheat is the second most important cereal crop in the rice-based cropping systems of Bangladesh. The winter is short and mild compared to more traditional wheat-growing countries. The country produces about 0.84 m t of grain from about 400,000 ha (FAOSTAT 2009) and imports 2–3 m t of grain. Four early maturing Ug99 resistant lines (Picaflor#1, Quaiu#2, Pauraque#1 and Francolin#1) with outstanding performance in the Northeast Gangetic Plains of India (Varanasi), a similar production environment to Bangladesh, were provided. In addition, a newly developed variety, BAW 1064, recently named Bari Gom 26, is also under increase. This variety with CIMMYT lines in its pedigree (ICTAL 123/3/RAWAL 87//VEE/HD 2285), possesses adequate resistance to Ug99 and its variants and has superior agronomic performance compared to current varieties. The area under the five lines was about 7 ha and total seed produced was 12,000 kg (Table 2).

A large-scale seed multiplication of these varieties (Bari Gom 26 on 44 ha, Francolin#1 on 15 ha and Quaiu#2 on 0.8 ha) was launched on farmers' fields in 2009–10 under the supervision of the Wheat Research Centre (WRC), Dinajpur, Bangladesh and CIMMYT through 135 seed producing farmers. It is estimated that 150 t of seed will be available for planting in 2010–11. Source seed production (breeder, foundation) of these varieties is also occurring at the WRC Dinajpur Research Station and is expected to produce enough seed for farmers. The projected production of resistant lines in 2010–11 is 15.7% of the effective seed market of Bangladesh and 5.2% of the total wheat area (Table 5).

Afghanistan

Wheat is the leading staple food in Afghanistan, accounting for 80% of national cereal production. In contrast to the high per capita wheat consumption (180 kg/person/year), average wheat yields are low (2 t/ha) (FAOSTAT 2009). Wheat is produced under both rainfed and irrigated conditions. Half of all arable land is classified as rainfed (most of it in the north), but 75–80% of all wheat produced in the country comes from irrigated systems located in various regions. Most of the wheat grown in

Afghanistan is fall-sown spring wheat, although there are areas where winter/facultative wheats are grown (15–20% of the wheat area).

In 2008–09, four advanced CIMMYT lines (Chonte #1, Picaflor#1, Quaiu#1 and Munal #1) were multiplied along with two lines from the 2nd Elite Bread Wheat Yield Trial (EBWYT) (entry 14 – OASIS/SKAUZ//4*BCN/3/2*PASTOR and entry 27 – HPO/TAN//VEE/3/2*PGO/4/MILAN/5/ SSERI) on 2 ha of land (Table 2). The four major research stations used for seed production of the advanced CIMMYT lines were Baghlan, Takhar, Nangarhar and Kunduz and the two 2nd EBWYT lines were also multiplied across five sites. The total seed produced was 9,523 kg (Table 2).

Superior lines identified in 2008–09 were grown on larger seed production areas in the current 2009–10 season (Table 4). The estimated production is around 150 t which should multiply to 4,025 t in 2010–11 assuming average yields of 2.5 t/ha under irrigated management (Table 5). The current supply of seed accounts for only 5% of the total annual requirement of about 236,000 t (Kugbei 2007). Farmers in Afghanistan buy up to 31% of seed requirements from formal and informal sources, and the total effective wheat seed market is estimated to be 73,000 t (Kugbei 2007). Therefore, the estimated wheat seed production of Ug99 resistant lines in 2009–10 amounts to 0.31% of the seed market, but after 2010–11 it is projected to reach 5.5% (Table 5). However, the seed produced at the end of the 2010–11 cycle will be sufficient to cover only 1.7% of the total wheat area.

Egypt

Wheat is a main staple food in Egypt. It contributes to more than 30% of the daily caloric intake and per capita annual consumption (180–200 kg) is among the highest in the world. Owing to high population and high demand for wheat products, Egypt is particularly vulnerable to losses caused by Ug99. In 1994 severe yellow rust and stem rust epidemics caused 40% yield losses. Ug99 poses a very significant threat to Egypt because of its extremely high dependence on wheat for food and its proximity to Sudan and Yemen.

In 2008–09, in addition to the germplasm from CIMMYT, Mexico (Table 1), the Agricultural Research Center (ARC) multiplied six promising Ug99 resistant lines selected from the CIMMYT nursery system. Of these, two advanced lines were released as Misir1 (Oasis/Skauz//4*Bcn/3/2*Pastor) and Misir 2 (Skauz/Bav 92). Around 10 t of Misir1 and 7 t of Misir 2 were available for export. Of this, 1.5 t of Misir1 was exported to Afghanistan for seed production. In 2009–10, resistant varieties were planted on 67.4 ha with a target of 360 t of seed (Fig. 1, Table 5). With current progress (Table 5), Egypt is expected to have seed of rust resistant varieties for around 24.6% of its potential seed market and 8.2% of its wheat area by the end of the 2010–11 cropping cycle.

Ethiopia

Ethiopia is the second largest wheat producer in Sub-Saharan Africa (after South Africa) with an area of 1.5 m ha and annual production of about 2.8 m t. In terms of both area and production, wheat is the third (after teff and maize) most important crop. Durum and bread wheat are equally important. The country does not produce enough wheat to satisfy national demand and nearly one m t are imported annually, mainly on a concessional basis or as food aid.

Among the biotic constraints, the rusts are recognized as the most economically serious problems in Ethiopia. During the past two decades severe yellow rust and stem rust epidemics have occurred and caused significant yield losses (40–60%). Ug99 and its derivatives are a serious threat to national production and a source of inoculum to neighboring countries. Lines for multiplication were identified based on performance in 2008 multi-locational national trials and stem rust tests in Kenya. Eight Ug99 resistant lines were evaluated in 100 m^2 plots during the 2009 off-season at Kulumsa and Debre Zeit. Five lines (Picaflor#1, Quaiu#2, Munal#1, Danphe#1 and Chonte#1) were from CIMMYT whereas three (Flag 3, Flag 5 and Amir 2) were from ICARDA. In the summer of 2009, 6 ha were planted using the best five varieties (Picaflor#1, Quaiu#2, Munal#1, Danphe#1 and

Chonte#1) and 12,000 kg of seed was obtained. In the current 2009–10 cycle, seed multiplication of five lines is underway on 101 ha and 250 t of seed is expected to be produced (Table 5, Fig. 1). Based on current progress (Table 5), the country is expected to produce seed of rust resistant varieties for around 8.7% of its potential seed market but only 2.9% of its wheat area by the end of 2010–11.

Pakistan

Pakistan grows approximately 8 m ha of wheat, and produces over 19 m t of grain (Chatrath et al. 2007). For the past decade the area sown to wheat has been dominated by the variety Inqalab 91 (Joshi et al. 2007), which is susceptible to Ug99. Because of its proximity to Iran, where Ug99 is already present, the need for resistant varieties in farmers' fields is urgent. Three Ug99 resistant CIMMYT lines (Chonte#1, Quaiu#1 and Munal#1) and three previously selected lines (NR 356, NR 358 and NR 360) obtained from Mexico were grown on about 9 ha in 2008–09 (Table 2). Seed multiplication was carried out by the national program of Pakistan Agriculture Research Council (PARC) and two seed private enterprises; the Jullundar Seed Company and the Ali Akbar Seed Company. A total of 23,633 kg of seed was produced, two thirds by the Jullundar Seed Company.

Due to potential threat of a new local stem rust race, seed production of CIMMYT lines (Chonte#1, Quaiu#1 and Munal#1) was discontinued. The new race was not UG99, and appeared to be very similar to local types. It was virulent for *SrTmp*, the gene present in Quaiu#1, but its virulence on the other two genotypes is currently unexplained. Therefore seven lines including five new selections from CIMMYT (4th EBWYT No. 503, 508, 519, 529 and 530) were used for seed multiplication on about 1 ha for further increase during 2010–11. Variety V-04178 (SH88/90A-204//MH97) which is resistant to Ug99 as well as the new local race was provisionally released and is under seed multiplication. Overall, the current production estimates indicate that by the end of the 2010–11 cycle, seed of resistant varieties will be equivalent to around 0.34% of the potential seed market and 0.11% of the wheat area. With an impressive record for releasing improved wheat varieties, as demonstrated by 11 new varieties in the past two years, the seed production target may be significantly exceeded.

Seed production and dissemination in other important major countries

Iran

Iran grows around 6.5 m ha of wheat producing 14 m t of grain (FAOSTAT 2009). About 2.6 m ha or 40% is irrigated; the remainder is rainfed. The main production areas are concentrated in the northwestern region of the country. Durum wheat is grown on about 0.1 and 0.25 m ha under irrigated and rainfed conditions, respectively. Rusts are a major problem, although drought has been severe for the past two years, probably restricting the spread of Ug99.

In the 2008–09 cropping season, eight Ug99 resistant varieties were multiplied (Table 3). Most of these lines were of CIMMYT origin or developed locally with at least one parent from CIMMYT. Around 80,000 t of seed was produced, enough for planting around 0.75 m ha of land in 2009–10. This is clearly an impressive achievement. The country is expected to produce >100% of seed requirements for rust resistant varieties (Tables 4, 5). In addition to these, Iran is also currently pursuing seed production of two new resistant varieties released in 2010. The two varieties are: Behrang (Durum: Zhong Zuo/2*Green-3) and Morvarid (Milan/Sha7).

The excess seed production may have a beneficial spillover effects for adjoining countries. Iraq grows CIMMYT-derived varieties that were developed in the 1970s. Hence, any excess of new improved varieties could have a very significant impact on Iraq's wheat production. New varieties will likely have a yield potential increase of at least 10–15% over the current varieties. These lines are not only resistant to Ug99 but are also resistant to other major wheat diseases that could affect Iraq's wheat production.

India

India is the second largest wheat producer and consumer in the world, with a production of approximately 80 m t in 2008–09, which is 12% of world production. The dominant variety in South Asia, PBW343, which occupied about 8 m ha in India in 2008–09, is susceptible to Ug99 (Singh et al. 2008). In 2008–09 this variety was severely affected by *P. striiformis* f. sp. *tritici* race 78S84 which is virulent to *Yr27*. The area covered by Ug99 resistant varieties in 2007 was about 0.3% (Singh et al. 2008). The discovery that popular variety HUW234, occupying about 2 m ha in the eastern Gangetic Plains (EGP), is heterogeneous for resistance to Ug99 (Joshi et al. 2009) raises the area of resistant varieties to 8%.

Indian wheat cultivars with resistance can be accessed on the website (http://www.dwr.in) of the Directorate of Wheat Research, Indian Council of Agricultural Research. The participatory approach has also made significant impacts in some districts in the EGP by changing the varietal spectrum. The area of HUW234 declined from 4–5 m ha in the late 1990s to 2 m ha at present (Joshi et al. 2009). However, none of the varieties replacing HUW 234 are resistant to Ug99. Hence, in addition to introducing resistant material under the All India Coordinated Wheat Improvement Project (AICWIP) network, they were multiplied in farmers' fields following the PVS approach (Joshi et al. 2009). The newly developed Ug99-resistant lines under seed multiplication in 2009–10 include Picaflor#1, Pauraque#1, Becard#1, Munal#1, Quaiu#2, Francolin#1 and Danphe#1. These lines were grown on about 300 ha under participatory seed production in 2009–10 with a targeted production of at least 600 t. The official release of some of these lines in the near future is likely and it is therefore predicted that seed production in the next three years will generate enough seed to substantially reduce the threat of Ug99 in the EGP.

Conclusion

Stem rust race Ug99 and its derivatives are serious threats to global wheat production in Asia and Africa. If not addressed through effective research, seed production, and distribution of resistant varieties, Ug99 may become another cause of food shortages in many countries. The best strategy to protect wheat from the menace of Ug99 is replacement of susceptible genotypes with new high yielding and resistant varieties. The consultative group centers (CIMMYT and ICARDA) and BGRI, in collaboration with national research centers from countries under threat, have developed high yielding Ug99 resistant varieties that are now being distributed in the most threatened areas. The objective is to have sufficient seed of resistant lines to plant at least 5% of the entire wheat area by 2011. If achieved, this will be a major step towards food security.

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Table 1 Seed of Ug99 resistant wheat lines provided in November 2008, from CIMMYT Mexico, to six countries for seed multiplication during the 2008–09 crop cycle

	Countries and seed quantity (kg)						
CIMMYT name	Nepal	Bangladesh	Afghanistan	Egypt	Ethiopia	Pakistan	Total
Danphe #1	100	–	–	–	100	–	200
Picaflor #1	100	100	50	–	–	–	250
Quaiu #1	–	–	50	–	100	300	450
Quaiu #2	100	100	–	–	–	–	200
Pauraque #1	100	100	–	–	100	–	300
Becard #1	100	–	–	–	–	–	100
Munal #1	100	–	50	–	–	300	450
Francolin #1	100	100	–	–	100	–	300
Chonte #1	–	–	50	25	100	300	475
Chewink #1	–	–	–	25	–	–	25
Grackle #1	–	–	–	25	–	–	25
Total	700	400	200	75	500	900	2775

Table 2 Area planted and wheat seed of Ug99 resistant advanced lines produced during the 2008–09 crop season in six countries

Country	Area planted (ha)	Seed produced (kg)
Nepal	7.5	18,486
Bangladesh	7.0	12,000
Afghanistan	1.98	9,523
Egypt	21.14	81,166
Ethiopia (summer crop)	6.0	12,000
Pakistan	9.0	23,633
Total	52.62	156,808

Table 3 Seed production of Ug99 resistant wheat lines and varieties during the 2008–09 crop season in Iran

Variety	Pedigree	Distributed certified seed (kg)	Area (ha)	Production (kg)
Kavir	Stm/3/Kal//V543/Jit716 = Sorkhtokhm	4,192,000	22,000	45,000,000
Bam	Vee"S"/Nac//1-66-22*	2,095,000	12,000	35,000,000
Misir 1	Oasis/SKauz//4*BCN/3/2*Pastor	2,500	15	20,500
520 (2nd EBWYT)	Babax/LR42//Babax*2/3/Vivitsi	2,500	15	20,600
Munal#1	Waxwing*2/Kiritati	150	1	1,000
Parsi	Dove"S"/Buc"S"//2*Darab	1,500	10	15,000
Sivand	Kauz"S"/Azd	1,500	10	15,000
Arg	1-66-22*/Inia	2,500	15	25,000
Total		6,297,650	34,066	80,097,100

*1-66-22 is T. aest/6/Ti/4/La/3/Fr//Kal/Gb/5/*2Gb

Table 4 Ug99 resistant wheat varieties under seed increase in six countries during 2009–10

Name	*Sr* gene	Countries undertaking seed production						
		Nepal	Bangladesh	Afghanistan	Egypt	Ethiopia	Pakistan	Iran
CIMMYT name								
Danphe #1	APR (*Sr2*+)	√				√		
Picaflor #1[a]	APR (*Sr2*+)	√	√	√		√		
Quaiu #1[b]	*Sr2*+*SrTmp*			√		√		
Quaiu #2	*Sr2*+*SrTmp*	√	√	√				
Pauraque #1	APR (*Sr2*+)	√	√					
Becard #1	APR (*Sr2*+)	√						
Munal #1	APR (*Sr2*+)	√		√		√		√
Francolin #1	APR (*Sr2*+)	√	√					
Chonte #1	APR (*Sr2*+)			√		√		
NARS name								
BL 3063[c]	APR	√						
Bari Gom 26[d]	APR		√					
Misir 1[c,d]	*Sr2*+*Sr25*			√	√			√
Misir 2[e]	*Sr25*				√			
Kavir	Unknown							√
Bam	Unknown							√
Parsi[f]	APR (*Sr2*+)							√
Sivand[f]	Unknown							√
Arg[f]	Unknown							√
2nd EBWYT#20[g]	APR							√
V-04178[h]	Unknown						√	
Expected seed production (t)		115	150	230	360	250	45	116 215

√variety under seed production; [a]Released in 2009 in Afghanistan as Baghlan 09; [b]Released in 2009 in Afghanistan as Koshan09; [c]Projected for release in 2010; [d]Released as Muqawim09 in Afghanistan; [e]Projected for release in 2011; [f]Released in 2009 in Iran; [g]cross of the entry is HPO/TAN//VEE/3/2*PGO/4/MILAN/5/SSERI1; [h]Seven other resistant varieties are under small plot seed multiplication

Table 5 Area sown and expected seed production of Ug99 resistant wheat lines in the 2009–10 and 20010–11 crop cycles, and projected seed production as percentage (%) of national seed market and total national area in seven countries in Asia and Africa

Country	Area (ha) 2009–10	Expected production 2009–10 (t)	Expected production 2010–11[a] (t)	National annual seed requirement (t)[b]	National potential wheat seed market (t)	Expected seed production 2011–12 (% of national wheat seed market)	Expected seed production 2011–12 (% of national wheat area)
Nepal	57	115	1995	82000	27333	7.30	2.43
Afghanistan	115	230	4025	240,000	73000	5.51	1.68
Bangladesh	59	150	2625	50000	16667	15.75	5.25
Ethiopia	101	250	4375	150000	50000	8.75	2.92
Egypt	67	360	10080	122700	40900	24.65	8.22
Pakistan	15	45	945	846000	282000	0.34	0.11
Iran	46486	116215	1394580	573000	286500	365.07	182.54
Total	46,899.6	117,364	1,069,980	2063700	776400		

[a]Based on projected use of 70% of seed for further multiplication in six countries and 30% in Iran, with a seed multiplication ratio of 1:25 for Nepal, Bangladesh, Afghanistan and Ethiopia, 1:30 for Pakistan and Iran, 1:40 for Egypt; [b]Based on 100–120 kg/ha of seed for national wheat area

Fig. 1 Area and expected seed production of Ug99 resistant lines in six countries in Asia and Africa, 2009–10