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Oral Presentations

The growing threat of stripe rust worldwide

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Abstract

Stripe rust of wheat (yellow rust) is a recurring production constraint in the majority of wheat growing areas of the world. The transboundary nature of the pathogen coupled with its current virulence capabilities, favorable environmental conditions, sometimes overlapping and/or continuous cultivation of susceptible varieties in stripe rust-prone zones, and genetic uniformity of certain recent 'mega-cultivars' were major driving forces in stripe rust epidemics worldwide. Breeding for resistance must continue be the central pillar of stripe rust control, and for this to be effective there must be adequate pathogen monitoring combined with commitment to identify and incorporate diverse sources of resistance, preferably of the durable type. Deployment of resistance will only be successful if it is combined with high yield and appropriate end-use quality to meet the needs of farmers and consumers. Suitable seed systems need to be in place for timely distribution of varieties. This paper deals with the historical impacts and current status of stripe rust epidemics and highlights the need for regional and global collaboration in mitigating the global impact of this disease.

Introduction

Wheat was among the first of the domesticated food crops and for more than 10,000 years has been the basic staple food for most of the world. It is the most widely grown cereal crop in the world and one of the central pillars of global food security. About 650 million tonnes of wheat was produced worldwide on 217 million hectares in 2010 with a productivity level of about 3 t/ha^{-1} (FAO 2012). After the quantum leap of the Green Revolution, wheat yields have been rising by only 1.1% per year, a level that falls far short of the demand of a population that is growing 1.5% or more annually. According to some estimates, global wheat production must increase by at least 1.6% annually to meet a projected wheat demand of 760 million tonnes by 2020 (Dixon et al. 2007). This is however, very challenging with the current scenario of climate change, increasing drought/water shortage, soil degradation, declining supply and increasing cost of fertilizers, increasing demand for bio-fuel, and new virulent pathogen and pest strains.

Stripe rust epidemics have frequently occurred in the USA (particularly the Pacific Northwest region of North America), South America (central and southern wheat production areas), North Africa (Morocco, Algeria and Tunisia), East Africa (Ethiopia and Kenya), East Asia (northwest and southwest China), South Asia (India, Pakistan, and Nepal), Australasia (Australia and New Zealand), the Nile Valley and Red Sea (Egypt and Yemen), West Asia (Lebanon, Syria, Turkey, Iran, Iraq, and Afghanistan,), Central Asia (Kyrgyzstan, Uzbekistan, Tajikistan, and Turkmenistan), Caucasus (Georgia, Armenia and Azerbaijan), and Europe (UK, northern and southern France, the Netherlands, northern Germany, Denmark, Spain, and Sweden). Regular regional crop losses in the range 0.1–5% and sometimes up to 25% have been recorded due to stripe rust. However, individual crop losses of up to 80% were reported in the widespread epidemic in the Middle East and North Africa in 2010, when initial infection occurred on susceptible wheat varieties at early growth stages. Considering the epidemiological factors and the history of recurrent epidemics, the wheat areas in Africa (eastern and northern countries), the Middle East, the Caucasus region, and West and South Asia now appear to comprise a single epidemiological zone – hence any new pathotype that evolves in one country in the region is likely to disperse to the entire region.

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Although stripe rust is historically considered a disease of lower temperature regions, its relatively recent introduction and establishment in Australia and South Africa suggest a wider level of adaptation. The more recent spread of two new pathotypes/pathotype groups that largely replaced and expanded the range of stripe rust in Australia, central USA, and across CWANA and Europe have exacerbated the situation. These pathotypes appear not only to have the ability to adapt to higher temperatures (and therefore the potential to adapt to climate change), but have undergone rapid mutational changes in Australia, North America and northern Europe to overcome a number of specific resistance genes deployed in wheat and triticale. With current climate change predictions, winters are likely to become warmer and the likely consequence is earlier stripe rust infection and spread and hence more damaging epidemics throughout all wheat growing areas.

Regional impacts of stripe rust

Several worldwide stripe rust epidemics have occurred in recent decades with potential to inflict regular regional crop losses in the range of 0.1–5%, with rare events giving losses of 5–25% (Wellings 2011). Stripe rust can cause 100% yield loss in susceptible cultivars if infection occurs in early growth stages (Chen 2005), and this is likely to be exacerbated in regions with mild winter periods and significant levels of pathogen survival between cropping seasons.

North America

Stripe rust has been historically considered a common disease of wheat in North America since its first detection in 1915 but was not considered a destructive disease in the US from the 1930s until the late 1950s (Line 2002). However, it became increasingly important from the late 1950s and early 1960s (Chen et al. 2002). Since then, stripe rust has been considered the most significant disease of wheat in western North America, and from the 1980s became increasingly important in the south-central USA and the central Great Plains in certain seasons. Comprehensive reviews have dealt with the distribution of stripe rust, yield losses, status of resistance of commercial wheat varieties, and fungicide application in the USA (Line 2002; Chen 2005). During 2000–2007, stripe rust occurred in at least 15 US states each year with yield losses estimated at more than 6.5 million tonnes (Chen et al. 2010). However, yield loss was estimated at 2.2 million Mt (87 million bushels) in the severe 2010 epidemic, and the additional cost of fungicide application was estimated at $30 million in Washington State alone (X.M. Chen pers comm). In 2011, stripe rust was not a large problem in the Great Plains due to widespread drought, although the Pacific Northwest was even more affected by the disease than in 2010. Based on the stripe rust level in experimental fields and on crop growth stage, the potential yield loss on susceptible varieties was estimated to exceed 70%. For the 2012 crop, yield losses were predicted to reach 50% in highly susceptible wheat varieties.

Europe

Stripe rust has been considered one of the most damaging diseases of wheat in Europe for more than a century (Hovmøller and Justesen 2007). It is the most common wheat rust in a region spanning northern France, the Netherlands, northern Germany, Denmark, and the UK (Bayles et al. 2000). Northwestern Europe is considered a source of new pathotype variability due to intensive breeding for resistance that led to the use of major genes (Stubbs 1988). Epidemics have also occurred in southern Europe, but less frequently. Virulence for almost all seedling resistance genes, either present singly or in various combinations, has generally been found following their deployment in commercial cultivars (Stubbs 1985; Johnson 1988). A comprehensive survey conducted during the 1960s and 1970s estimated average annual grain yield losses of 10% in Europe (Zadoks and Rijsdijk 1984). Despite favorable environmental conditions in Europe, stripe rust has been broadly under control since the epidemics of the late 1980s and early 1990s, possibly due to successful deployment of resistance in modern European cultivars, as well as the widespread use of fungicides (Schmits 2003, cited in Hovmøller and Justesen 2007). Nevertheless, failure of resistance genes continues to be observed as consequence of mutation. Virulence for *Yr17* (widely introduced into European cultivars in the early 1990s) was first detected as a single pathotype in the UK in 1994 and this pathotype was subsequently detected in Denmark in 1997 (Justesen et al. 2002), then in France and Denmark in 1997 and 1998, respectively (Hovmøller et al. 2002). This observation indicated that northern Europe remained a single stripe rust epidemiological zone (Hovmøller and Justesen 2007). In France,

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stripe rust occurs most frequently in the north, with the most devastating epidemics occurring in the 1980s (Mboup et al. 2012; de Vallavieille-Pope et al. 2011).

In 2009, stripe rust spread rapidly and overcame resistance in triticale cultivars in Denmark. This resulted from a new pathotype, different from previously characterized *Pst* pathotypes in Denmark, and caused a 7.5 t/ha grain loss. A recent epidemic of wheat stripe rust in Spain is being investigated as a likely introduction (M. S. Hovmøller pers comm).

Australasia

Australia produces 20-25 million tonnes of wheat annually. Wellings and McIntosh (1990) stated that a single *Pst* pathotype was introduced into eastern Australia in 1979 and moved to New Zealand) in 1980. More than 20 new closely related pathotype derivatives were subsequently detected over two decades. A new exotic pathotype was reported in Western Australia for in 2002 (Wellings et al. 2003). This pathotype was virulent for *Yr6*, *Yr7*, *Yr8*, *Yr9*, and *YrA*, and avirulent for *Yr1*, *Yr2* (Heines VII), *Yr3*, *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr17*, and several uncharacterized resistances in the differential set. It was clearly exotic because it was pathogenically and molecularly distinctive from the pathogen population in eastern Australia at that time. During 2003-2006, an estimated $40-90 million was spent annually on fungicides by Australian farmers (Wellings 2007). Pathotypes virulent for *Yr17* and *Yr27* are currently considered a serious threat to wheat growing areas in Australia. Despite periodic epiphytotics and occasional exotic pathotype introductions, the national breeding program for rust resistance in Australia is considered a success in containing the worst effects of rust epidemics. Murray and Brennan (2009) estimated the value of the national breeding effort for resistance at $AUS million 438, 431 and 152, respectively, for stem rust, stripe and leaf rust.

Central and West Asia and Northern Africa (CWANA)

Reports indicate that at least three widespread stripe rust epidemics have occurred in this region since the 1970s. In each case the epidemics were considered a consequence of favorable environmental conditions, emergence and subsequent wide distribution of new virulent pathotype/s, and most notably, deployment of a narrow genetic base of resistance in recently released popular cultivars. Importantly, local susceptible cultivars in all three epidemics made very significant contributions to disease development and crop loss.

A major factor in the epidemics of the 1970s was the widespread cultivation of susceptible local cultivars together with improved varieties based on *Yr2* resistance. Siete Cerros, Kalyansona, PV 18A, Indus 66, Mexipak, Ouds and Mivhor 77 were planted across wide areas including North Africa, the Indian sub-continent, the Middle East, the East African highlands, Iran and China (Saari and Prescott 1985).

The second classical example of stepwise regional dispersal of the stripe rust pathogen was the widespread distribution of *Yr9*-virulent pathotypes during 1985–1997, following initial detection in the Horn of Africa. These pathotypes subsequently migrated northwards into CWANA, and progressively in a west-east direction that eventually included the Indian sub-continent. This caused severe crop losses in widely grown cultivars covering more than 20 million hectares. In 1993 and 1995, stripe rust epidemics occurred in most wheat-growing areas in Iran and caused in excess of 30% crop loss. Estimated grain losses were in the order of 1.5 million Mt in 1993 and one million tons in 1995 (Torabi et al. 1995). In Turkey, the wheat cv Gerek 79 grown on more than one million hectares endured losses of 26.5% due to the stripe rust epidemic of 1991 (Braun and Saari 1992).

In the southern region of West Asia, severe epidemics of stripe rust were also recorded. In Yemen losses in grain yield were in the range 10-50% during 1991-1996 (Bahamish et al. 1997). These epiphytotics occurred in crops seeded in both the main and off seasons. In Central Asia a stripe rust epidemic in Azerbaijan in 1996 caused significant yield losses. In 1997, the wheat crop in Tajikistan incurred greater than 60% loss (Yahyaoui et al. 2002). The facultative winter wheat regions of Uzbekistan and southern Kazakhstan frequently report stripe rust incidence, with recent severe epidemics occurring in 2009 and 2010.

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In Ethiopia, epiphytotics occurred in 1977, 1980-1983, 1986, 1988, and 1990. Yield losses in 1988 were severe in bread wheat, and as high as 58% on cv Dashen (Badebo and Bayu 1992). Ethiopia and Yemen form an ecological unit in regard to rust epidemiology and may have an important role in inoculum spread and virulence changes across the CWANA region.

Following the *Yr9* virulence-driven epidemics, the *Yr9*-susceptible varieties were extensively replaced with CIMMYT-derived germplasm (e.g. cvs Kauz, Atilla, Opata, Nacozari, Buckbuck, and Crow). The resistances in many of the replacement cultivars, including the mega-cultivars PBW343 (in India), Inquilab 91 and Bakhtwar (in Pakistan), Chamran and Shiroudi (in Iran), Kubsa (in Ethiopia), and Cham 8 (in Syria) were later reported to be based on *Yr27*, an all-stage resistance gene effective against the *Yr9*-predominant pathotypes of that time. The third episode of regional stripe rust epidemics developed when these resistant varieties showed increased rust levels, mainly in Pakistan, India, and southern Iran. Loss of effectiveness of *Yr27* resistance in cvs PBW343, Inquilab 91 and Chamran (in India, Pakistan, and Iran, respectively) were reported during 2002-2004. Although sporadic stripe rust outbreaks appeared in some areas, unfavorable environmental conditions possibly restricted rapid increases of the *Yr27*-virulent pathotypes until 2009 when conducive conditions resulted in severe epidemics in a number of CWANA countries (Pakistan, Morocco, Algeria, Tunisia, Uzbekistan, Turkey, Iran, Yemen, Azerbaijan, Georgia, Uzbekistan and Afghanistan). Environmental conditions favoring rust development continued into 2010, with a mild winter and adequate rainfall in several CWANA countries, resulting in early stripe rust outbreaks. The consequence was the 2010 stripe rust pandemic throughout the major wheat-growing areas in CWANA and Caucasus countries, causing very high yield losses, particularly in Syria where, for example, cultivar Cham 8 (with *Yr27*) occupied over 70% of the wheat area. Despite favorable environmental conditions in many areas in CWANA in 2011 and 2012, severe stripe rust epidemics did not eventuate, illustrating the year-to year variability of plant disease and its consequences. In 2010, the absence of resistant varieties in Ethiopia led to more than US$3.2 million expenditure on fungicides, and over 750,000 ha were sprayed against stripe rust in Iran. All major wheat cultivars grown in Uzbekistan, Morocco, Iraq, Azerbaijan, Afghanistan, and Tajikistan were susceptible. A devastating epidemic occurred across the Central Plateau in Turkey where the susceptible cv Gerek 79 predominated.

India, Pakistan and China

Following the Green Revolution in the mid-1960s, wheat production in India incrementally increased to the present level of 86 Mt in 2010-11 (Sharma and Saharan 2011). Stripe rust is an important disease in India, particularly in northwestern regions and the northern hills. During the 2010-11 season, it was severe in several areas, particularly where the majority of varieties was susceptible. However, timely fungicide intervention largely averted major crop damage. Pathotypes with virulence for *Yr9* and *Yr27* currently predominate in India (Sharma and Saharan 2011).

With 22.8 million ha of wheat and total wheat production exceeding 100 million Mt, China is the world's largest wheat producer (Wan et al. 2004). Stripe rust epidemics are major recurrent problems that can annually affect more than 20 million ha resulting in inter-regional epidemics (Li and Zeng 2000) with reported yield losses totaling 14.38 Mt in the severe epidemics in 1950, 1964, 1990, and 2002. China is considered a unique epidemiological zone and is considered to have the largest independent epidemic region. Extensive surveys in the last 60 years indicated very high pathogenic variability (Wan et al. 2004) and breeding has been the main focus of mitigation. Despite successes, stripe rust remains the most destructive wheat disease in China (W. Q. Chen pers comm).

Stripe rust is a serious threat to wheat production in northern and central-west areas of Pakistan. High production losses were reported in 1995 when cv Pak 81 (synonym Veery#5, carrying *Yr9*) predominated. This epidemic was attributed to *Yr9*-virulent *Pst* pathotypes. As elsewhere in the region, stripe rust epidemics in Pakistan fall into three periods: before 1993 when *Yr9* was effective; 1993-2002 when *Yr9*-virulence was widespread in major wheat-growing areas; and after 2002 with the occurrence of virulence for *Yr27*. The two mega-cultivars Pak 81/ Pirasabak 85, and Inquilab 91, became susceptible due to ineffectiveness of *Yr9* in 1994/95 and of *Yr27* in 2002, respectively, resulting in significant yield losses. Yield losses of 20% were estimated as a consequence of *Yr9* virulence. The high-yielding cultivar Seher 2006, which is resistant to *Yr27*-virulent

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pathotypes, was to replace Inquilab 91, but became susceptible to leaf rust –illustrating the seriousness of leaf rust in Pakistan and the need for multiple rust resistances.

Minimizing the impacts of stripe rust epidemics

A. Coordinated pathogen monitoring

The rapid spread of highly virulent and aggressive *Pst* strains, and the genetic uniformity of mega-cultivars across large areas, emphasizes the relevance of pathogen surveys covering larger areas (Hovmøller et al. 2011). In response to the need for a global rust survey, an important step towards a unified and intensive *Pst* survey was taken in 2008 when ICARDA, CIMMYT, and Aarhus University launched the Global Rust Reference Center (GRRC) at Aarhus University, Flakkebjerg, Denmark (Hovmøller et al. 2010). The Center is accessible year-round for rust samples from all countries. One purpose of the establishment of the GRRC, which has become part of BGRI, is to complement existing stripe rust and stem rust surveillance efforts by ICARDA, CIMMYT, and the NARs, particularly in developing countries. The principal objectives of the GRRC are:

1. Facilitating an early global warning system for transboundary spread of pathotypes through:

    a. Pathogen fingerprinting for rapid detection of incursions on a global scale and on understanding dispersal pathways

    b. Assessment of pathogenic variability and aggressiveness to determine wheat varieties at immediate risk

    c. Risk analysis of rust pathogen adaptation to changing climates

2. Securing unique pathogen resources to assist breeding for rust resistance

3. Providing and facilitating specialized training in epidemiology, population genetics, and pathogen evolution

4. A global source of publically available information on the cereal rusts and rust pathogen virulence surveys

The success of the GRRC will depend on global communication networks that allow rapid and free exchange of information to inform local advisory personnel in a timely and effective manner. National pathotyping capability will nevertheless be crucial in managing the large sample volumes necessary for effective regional surveillance of *Pst* populations. The GRRC will be a valuable reference for local pathology teams in gaining confidence in pathotype identity and confirming the potential of newly identified variants.

B. Resistance gene monitoring in commercial cultivars

Unless a comprehensive understanding of resistance genes in major cultivars within and between regions is established and updated, the outputs of the very best efforts to monitor *Pst* populations will remain largely irrelevant. Characterized pathotype collections of *Pst* are frequently used for postulation of resistance genes in multi-pathotype seedling tests (Perwaiz and Johnson 1986; Dubin et al. 1989; de Vallavieille-Pope et al. 1990; Nazari et al. 2008).

The development of diagnostic molecular markers has allowed some genes to be routinely screened in laboratories supporting breeding programs. The most important gene in this respect is the durable adult plant resistance gene *Yr18* which can now be conveniently monitored without the need for field disease nurseries. An international effort is needed for collaboration in marker development and utilization of linked markers, and especially in breeding for multiple gene resistance.

C. Effective resistance breeding

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Development and use of resistant cultivars is widely considered the most economically feasible and environmentally appropriate way to combat wheat rusts. The international wheat breeding programs at CIMMYT and ICARDA have been developing high yielding, widely adapted wheat germplasm with resistance/tolerance to major biotic and abiotic stresses following the classical breeding approaches and strategies whereby crossing blocks are assembled using hallmark cultivars and elite genotypes; the segregating generations are evaluated in shuttle breeding and inoculated rust nurseries, followed by key location testing of fixed lines to identify stable genotypes with appropriate combinations of desired traits. Distribution of elite material globally through the international nursery and yield trial system has resulted in the release of many high yielding, rust resistant and widely adapted wheat varieties in many countries. However, use of single resistance genes has repeatedly led 'boom and bust cycles' as the pathogens adapt and increase as evidenced above. The assembly of adult plant minor gene resistances (APR) has been the dominant breeding approach for reducing the impacts of 'boom and bust' by CIMMYT and ICARDA over the past decade. The development of molecular markers closely associated with APR genes will enable the assembly of gene pyramids to combat the evolutionary capacity of *Pst*. However, there is only one currently available marker (CsLv34 for selecting *Lr34/Yr18*) and more research and development is required in this area. Future strategies may also involve genomic selection (GS) which allows prediction of genotypic values, and thereby facilitate the selection of multiple minor QTLs associated with presumed non-race specific APR genes. Conventional breeding approaches complimented with GS and doubled haploid production systems would also enable the enhancement of breeding efficiency in developing high yielding, widely adapted genotypes with durable resistance to rusts.

D. Encouraging national action plans

An effective national strategy for combating wheat rusts has four key components: surveillance and rapid reaction plans; information sharing within and between countries; capacity strengthening – for government officials, extension services, and farmers; and participation in ongoing research programs to develop resistant wheat cultivars. A multi-faceted approach is needed by countries to combat wheat rusts. The obvious immediate response to combat rust outbreaks (whether new pathotypes or not) is fungicides wherever possible. Reducing the cropping area of susceptible cultivars across large areas is perhaps the best insurance against widespread rust damage. Countries can consider policies to plant a range of resistant wheat types in their farming systems – greatly reducing the risk of widespread epidemics. A long-term plan includes participation in international research efforts to continually monitor and develop wheat varieties that resist rust and other diseases.

One core issue for planners and policy makers is that stripe rust does not respect national borders. The rusts are 'social diseases' and can best be managed by shared agricultural practices and policies agreed across regions. The fight against rust requires good neighbors, working together. The role of policy makers and global leadership is crucial if we are to take a significant step forward in minimizing the impacts of this disease.

At the regional and international level there is a need to build a cooperative attitude for information sharing, the mutual sharing of risk analyses, and trust. The information that needs to be collected and shared across regions includes data on changing rust disease patterns, wheat variety distribution, changing agronomic practices, and climate change and weather patterns. The use of 'rust trap nurseries' across affected regions is a good example of an effective strategy for early detection and prevention of stripe rust. As rust moves across a region, researchers and planners can see the effect of new pathotypes on wheat varieties, and organize for dissemination of the most resistant varieties for the following season.

E. Accelerated seed delivery system to combat the threat of rusts

Seed is the most efficient mechanism for delivering rust-resistant wheat varieties to farmers. Availability and access to quality seed is expected to accelerate the adoption and dissemination of new durable rust-resistant varieties and associated production technologies. However, weaknesses in national seed systems threaten to impede the diffusion and adoption of replacement varieties.

For an effective seed delivery system, it is important to develop and implement the following approaches:

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a. Fast-track variety testing and release (e.g. adaptation trials) systems by pursuing flexible policy/regulatory options with partners.

b. Accelerate pre-release seed multiplication of promising lines and large-scale production of released varieties for distribution through both formal and informal channels.

c. Popularize and promote rust-resistant varieties among farmers (including targeted small-pack seed distribution) to initiate informal farmer-to-farmer seed sharing and diffusion.

d. Build capacity in technical aspects of seed production and in the provision of infrastructure (training and critical equipment).

e. Develop methods to rapidly dis-adopt cultivars that are susceptible or whose resistance is at threat from an emerging new pathotype.

Conclusions

The current challenges facing the global wheat production are complex, and addressing them requires an understanding of the drivers of past trends and prediction of future changes. Designing an effective research strategy with application of new breeding tools, such as genome-wide selection and resistance gene pyramids, needs a matching effort in establishing communication net-works and collaborations. The concept of food security involves the ability to improve and sustain production consistent with an array of economic and social measures. NARS must provide a significant contribution to this goal by improving and securing production in the long term.

Wheat cropping technologies, including varieties, are specifically important factors for controlling pest outbreaks. Developing and disseminating cultivars with progressively improved rust resistances needs to be strengthened with technological packages, such as integrated pest management (IPM). In addition to the availability of resistant varieties that are known to, and accepted by, farmers, country preparedness for stripe rust outbreaks necessitates the availability of sufficient seed in both quantity and quality. In most cases, the bottleneck for getting resistant varieties into the field is lack of local and national capacity to rapidly multiply seeds and deliver them to the market.

Improving national seed production capacity and delivery requires long-term planning and funding, and must involve government, private enterprise and farmers. There are many complex organizational, procedural and legal issues that differ between countries, but for success, coordination and timely information-sharing among all stakeholders - including pathologists, plant protection officers, breeders, seed system and extension agents, marketers and farmers - are paramount.

An international forum to discuss the way forward in stripe rust R&D was held at ICARDA headquarters in Aleppo, Syria, in April 2011. The following resolutions from that meeting continue to provide a framework for the future:

1. Long-term investment is needed to reduce the threat of stripe rust

While a significant investment has been made over the past five years in surveillance and control of stem rust, stripe rust remains the most significant endemic threat across a majority of the global wheat producing regions. In spite of its preference for cooler environments, stripe rust is rapidly spreading to new areas where it was not previously a problem. Aggressive new stripe rust pathotypes are adapting to warmer climates, causing recent outbreaks at the global level. Comparatively, investments in stripe rust R&D are small and less coordinated across countries. To reduce the current spread of stripe rust, more investment to support countries to improve surveillance and in breeding of durable varieties that resist stripe rust.

2. Strategies to address wheat stripe rust disease

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a. Surveillance and information exchange between countries.

b. Planning, awareness, and preparedness to rapidly deliver appropriate seeds and fungicides where they are needed to arrest the spread of wheat rust diseases.

c. New capacity and skills in ministries, extension services, and at the farm level to develop effective strategies for managing rust diseases.

d. Crop research for a continued, long-term effort in developing new varieties that are resistant to the emerging pathotypes of wheat rust.

3. Approaching stripe rust as a social disease

One core issue for planners and policy makers is that stripe rust does not respect national borders. The rusts are 'social diseases' and can best be managed by shared agricultural practices and policies agreed across regions. The fight against rust requires good neighbors, working together. The role of policy makers and global leadership is crucial if we are to take a significant step forward in minimizing the impacts of *Pst*.

At the regional and international level there is a need to build a cooperative attitude for information sharing, the mutual sharing of risk analyses, and trust. The datasets that need collecting and sharing across regions include information on monitoring of changing rust disease patterns, wheat variety use per region, changing agronomic practices, and observations of climate change and weather patterns. The use of 'rust trap nurseries' across affected regions is a good example of an effective strategy for early detection and prevention of stripe rust. As rust moves across a region, researchers and planners can immediately see the effect of new pathotypes of rust on wheat varieties, and organize for dissemination of the most resistant varieties for the following season.

4. Encouraging the development of national action plans

An effective national strategy for combating wheat rust has four key components: surveillance and rapid reaction plans; information sharing across countries; capacity strengthening – for government officials, extension services, and farmers; and participation in ongoing research programs to develop resistant wheat varieties. A multi-faceted approach is needed by countries to combat wheat rusts. Immediate action to combat new rust pathotypes is often the use of fungicides. Reducing the cropping of susceptible mega-cultivars across vast wheat growing areas is perhaps the best insurance policy against widespread rust damage. Countries can consider policies to plant a range of resistant wheat types in their farming systems – greatly reducing the risk of emerging virulent rust types spreading over the entire area. A long-term plan includes participation in international research efforts to continually develop wheat varieties that resist rust and other diseases.

5. Reducing the impacts of narrow range variety dependence

Diversified cropping of wheat – avoiding the sowing of mega-cultivars across large cropped areas – is another possible defense against wheat rust. In most areas of the Middle East, East Africa, and South Asia, farmers have been planting the same varieties for 20–30 years. This practice is not advisable in a situation where stripe rust pathotypes are mutating and new ones are emerging much more rapidly than in the past and overcoming resistance in current varieties.

6. Developing a clear approach to seed multiplication and farmer engagement with new, diverse varieties

Efficient and effective seed delivery systems are critical for new crop varieties to reach farmers and bring impacts in ensuring food security and improving livelihoods of farmers. However, most national seed systems operate under heterogeneous environments in terms of agro-ecology, farming systems, crops and markets. They face a broad range of constraints including policy and regulatory frameworks; inadequate institutional and organizational arrangements; deficiencies in production, processing, and quality assurance infrastructure; and

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lack of trained personnel limiting technical and managerial capacities, compounded by farmers' difficult socio-economic circumstances. It is therefore important to assist and strengthen NARS in capacity development, establish fast-track variety release systems, and participatory demonstration and accelerated seed multiplication of newly released wheat varieties to ensure fast replacement of existing vulnerable commercial varieties.

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Tracking the wheat rust pathogens

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Abstract

Rapid progress has been made towards the goal of establishing a Global Cereal Rust Monitoring System. The system has reached the point where it can now be regarded as a fully operational global disease monitoring system. Creation of a functional surveillance and monitoring network, covering 35 countries and a large proportion of the developing world wheat area is seen as a significant achievement. Through this network it has been possible to track the spread and status of important stem rust races such as those within the "Ug99 race group". New technologies are playing an increasingly important role in rust tracking. These are already having an impact in several different areas from survey data collection to pathogen diagnostics and their role is likely to increase in the future. A robust and functional data management system - the Wheat Rust Toolbox - is now in place. This includes extensive rust surveillance and race databases and a suite of dynamic visualization tools. New web resources are providing access to a wealth of information regarding rust surveillance and monitoring in ways not previously possible. Global collaboration is ensuring that key databases are shared and being integrated into different information platforms. Despite good progress, several areas are seen as priorities for further work. These include: expansion of the data management system, increased information relating to host genotypes and rust resistance genes, early warning systems and disease mitigation planning. Activities are planned in the near future in all these areas.

Introduction

The appearance of stem rust race TTKSK (Ug99) in East Africa (Pretorius et al. 2000) was the catalyst to put in place a global monitoring system for wheat rusts. Identification of a race of *Puccinia graminis* f. sp. *tritici* to which a large proportion of commercial wheat cultivars were susceptible was a clear signal that tracking rust pathogen races and monitoring disease status on a global basis was a high priority. The sheer mobility of rust pathogens coupled to their inherent ability to change through mutation, recombination or somatic hybridization makes continual monitoring across large geographical areas an absolute necessity (Brown and Hovmøller 2002; Park 2007). Significant progress in the development of such a global monitoring system has been made largely under the auspices of the Durable Rust Resistance in Wheat (DRRW) project. Progress in the conceptualization, development and implementation of a Global Cereal Rust Monitoring System (GCRMS) has been previously described in detail by Hodson et al. (2009, 2011) and Park et al. (2011). The series of reviews relating to Ug99 by Singh et al. (2006; 2008, 2011) have also provided updates and summary information regarding the GCRMS. Through the work of the GCRMS it has become clear that Ug99 cannot be regarded as a single entity, because a number of different races have been identified that are considered to belong to a clonal lineage that includes the original Ug99. For the purposes of this paper the term "Ug99 race group" is used throughout. The working definition of this term is: 'the group of *Pgt* races sharing almost identical molecular fingerprints to the original Ug99 isolate (race TTKSK). Most races in the group have virulence to *Sr31*, but *Sr31*-avirulent progenitors/relatives are also included'.

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The GCRMS is not a static entity and considerable efforts are being undertaken by an international coalition to improve and expand the existing system. Changes, progress, improvements and remaining gaps in the current GCRMS will be the focus of this paper. Technology is playing an increasingly important role in improving the efficiency of rust monitoring and these new interventions will be described. Well managed and accessible information is also critical if informed decisions are to be made to mitigate the threat of rusts. Several advances in this area will also be highlighted. It has long been recognized that any successful surveillance and monitoring system must take a holistic view, covering the pathogen, and equally important, linking this with relevant knowledge of the host as well. Progress towards this goal of a more integrated and holistic approach will be reported. Despite good progress, several areas are seen as priorities for further work to advance the current GCRMS. Future planned activities to enhance the current global monitoring efforts will be described.

Current status of the GCRMS

At the inception of the GCRMS in 2007, only two stem rust-affected East African countries (Ethiopia & Kenya) had undertaken rust surveys in a standardized way and made data available. In the subsequent years, the global network contributing to the GCRMS has expanded substantially and now 27 African and Asian wheat-producing countries have contributed surveillance data. Many of these countries are now undertaking surveys and contributing data on an annual basis. These 27 countries account for an estimated 42 million ha of wheat, approximately 20% of the world wheat area. An additional eight countries (both developed and developing) have contributed valuable data on stem rust races. In total, the GCRMS now includes consolidated stem rust race data from 21 countries. This current global network of 35 countries represents the first example of comprehensive developing country rust monitoring. The GCRMS network is now delivering routine information on the rusts on a vast geographical scale rarely seen before, enhancing knowledge not only of stem rust but other wheat rusts as well.

The global surveillance has revealed the predominance of stem rust in East and Southern Africa, with isolated pockets of the disease occurring in other areas. Stripe rust is more widespread, dominating throughout the Central, West Asia, North Africa (CWANA) region and into South Asia. There are some indications that stripe rust may be increasing in the East African highlands. In other parts of the world, e.g. Europe, China, USA and Australia, stripe rust is increasingly the major biotic constraint to wheat production. Leaf rust is the most cosmopolitan of the rusts, being recorded in all areas surveyed. An example illustrating the geographical coverage of on-going surveillance and relative importance of the three rusts in 2011 is given in Fig. 1. An increasingly rich surveillance database (over 9,000 geo-referenced records) is permitting secondary analysis and value addition to the original survey data. Geo-spatial analysis of multiple years of standardized survey data is facilitating the identification of disease hot-spots. Geo-statistical approaches such as hot-spot analysis using the Getis-Ord Gi* statistic (Mitchell 2005) permit investigation of where statistically significant clusters of high (hot-spot) or low (cold spot) disease severity occur. Spatial interpolation of disease severity data permits visualization of areas of either high or low disease severity. These approaches result in the development of risk maps that can then help guide decision making regarding disease control and mitigation measures. Examples of stem rust risk maps for Ethiopia and Kenya are given in Fig. 2. Initial risk maps have been produced for all countries with survey data, and these are being updated as soon as new data are available. Data mining of the original surveillance data to extract secondary data (e.g. climate, elevation) for possible use in disease forecasting and early warning is the next step to be undertaken.

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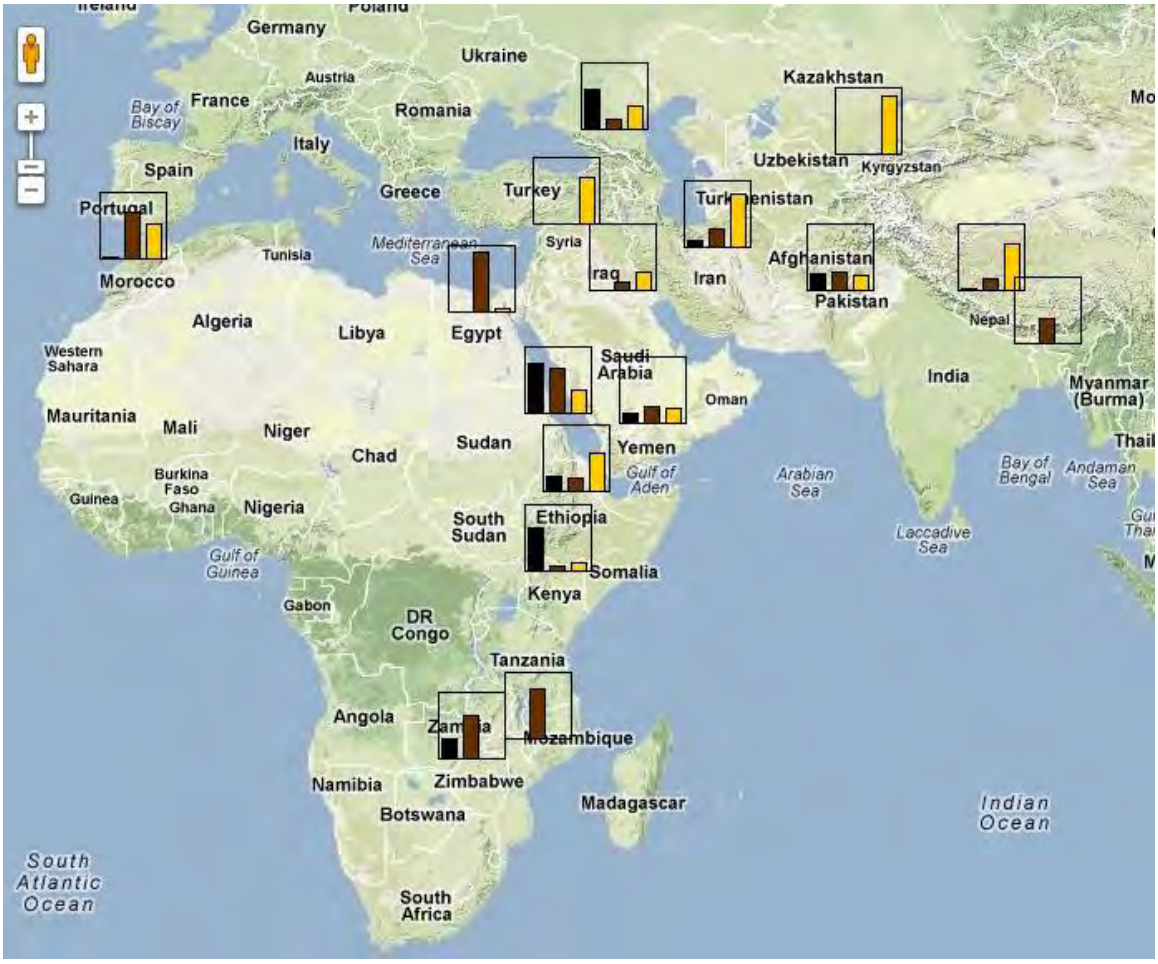

Figure 1 Relative importance of the three wheat rusts based on BGRI survey data in 2011 (Black bars = proportion of surveyed fields with stem rust, brown bars = proportion with leaf rust and yellow bars = proportion with stripe rust)
Source: Wheat Rust Toolbox

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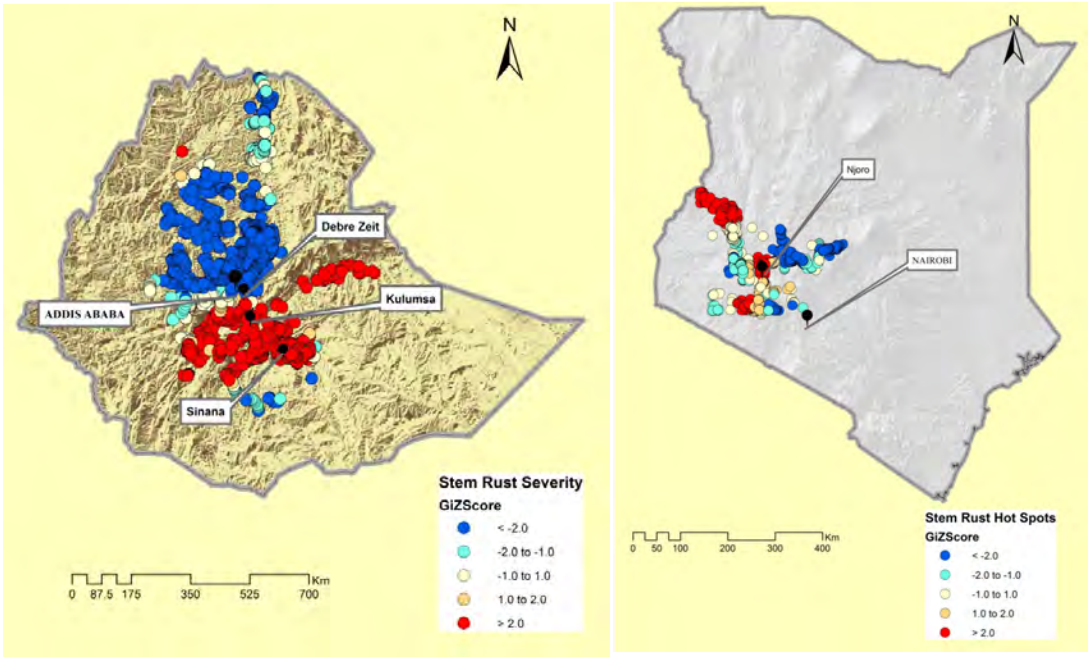
Map A



Map B

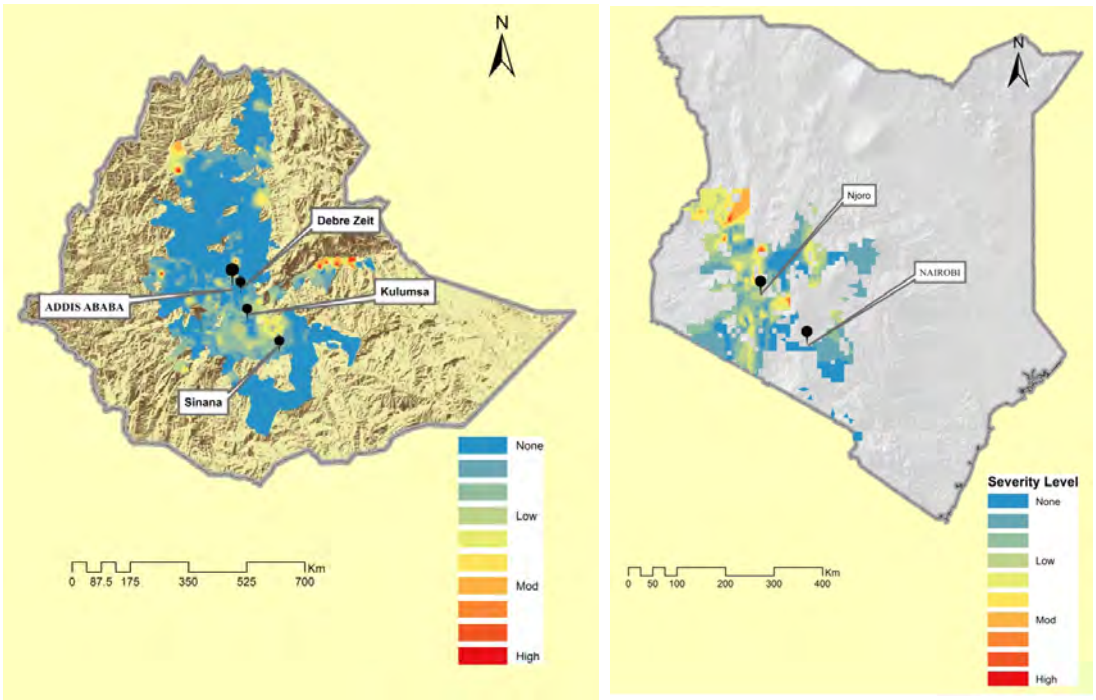


Figure 2 Examples of stem rust risk maps for Ethiopia and Kenya. **Map A** indicates geo-statistical hot-spots, with red dots representing statistically significant hot-spots of high disease severity. **Map B** shows an interpolated surface (Inverse distance Weighting) of disease severity. Surface masked to wheat growing areas

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Pathogen monitoring

Stem rust race data has been obtained from 21 countries, with multiple years of data now available for the majority of them. Data from 1,075 isolates are currently included in the GCRMS core database. The "Ug99 race group" has been the focus of much of this work. Through the global sampling and race analysis network, the "Ug99 race group" has been successfully tracked, both in terms of geographical distribution and race variation. Members of the "Ug99 race group" have been identified in 10 countries (Uganda, Kenya, Ethiopia, Sudan, Yemen, Iran, South Africa, Tanzania, Zimbabwe, Mozambique). A total of 8 races within the "Ug99 race group" have been positively identified. A summary of the known status of the "Ug99 race group" is given in Table 1. The majority of these races are present in Eastern and Southern Africa. Only the original Ug99 variant – race TTKSK – has been confirmed outside of Africa. However, future spread of additional Ug99 variants out of Africa is considered highly likely. An overview of the current geographical status of the "Ug99 race group" is summarized in Fig. 3. Within Africa, "Ug99 race group" variants with virulence to *Sr24* are increasingly widespread and are often the predominant races (Pretorius et al. 2010; Mukoyi et al. 2011; Wolday et al. 2011). These races include TTKST, PTKST and TTKSP, and one or more have been recorded in seven African countries (Kenya, Ethiopia, Eritrea, Tanzania, Mozambique, Zimbabwe, and South Africa). From current monitoring data the original Ug99 (race TTKSK) now predominates only in Ethiopia (Table 2). In all other countries, Ug99 variants other than TTKSK have been identified more frequently in analysed samples. Recent identification of race TTKSF+ in South Africa and Zimbabwe (Pretorius et al. 2012) represents the eighth race in the "Ug99 race group". No expansion of the geographical range of the "Ug99 race group" has been detected since Mukoyi et al. (2011) reported confirmation of the "Ug99 race group" in Mozambique and Zimbabwe and Wolday et al. (2011) confirmed the presence of the "Ug99 race group" in Eritrea. Recent race analysis data from Eritrea (T. Fetch, unpublished) indicates a predominance of "J" races – virulent for *Sr13* and likely originating from durum wheat – among samples collected in 2011. Only 1 isolate (out of 15) collected in 2011 was typed as a member of the "Ug99 race group" (PTKST). This contrasts to sampling in 2010 when "Ug99 race group" *Sr24* variants (PTKST, TTKST) were the only races identified in Eritrea (Wolday et al. 2011).

Table 1 Summary of the known status of the "Ug99 race group"

Race[1]	Common alias	Key virulence (+) or avirulence (-)	Country (year of 1st detection)
TTKSK	Ug99	+*Sr31*	Uganda (1998), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009)
TTKSF		-*Sr31*	South Africa (2000), Zimbabwe (2009)
TTKST	Ug99 + Sr24	+*Sr31*, +*Sr24*	Kenya (2006), Tanzania (2009), Eritrea (2010)
TTTSK	Ug99 + Sr36	+*Sr31*, +*Sr36*	Kenya (2007), Tanzania (2009)
TTKSP		-*Sr31*, +*Sr24*	South Africa (2007)
PTKSK		+*Sr31*, -*Sr21*	Ethiopia (2007), Kenya (2009)
PTKST	Ug99 + Sr24	+*Sr31*, +*Sr24*, -*Sr21*	Ethiopia (2007), Kenya (2008), South Africa (2009), Eritrea (2010), Mozambique (2010), Zimbabwe (2010)
TTKSF+		-*Sr31*	South Africa (2010), Zimbabwe (2010)

[1]Some uncertainty exists over the reaction of the *Sr21* gene (this influences the initial code letter being "T" (+*Sr21*) or "P" (-*Sr21*). Current table presents most plausible races. All names are using the North American 20 differential set nomenclature

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Table 2 Predominant *Pgt* races in African countries based on BGRI sampling studies 2008-2011 (Predominant race in year is highlighted in bold. Number in parentheses = number of isolates)

Country	2008	2009	2010	2011	Current predominant race
Eritrea			**PTKST (5)** TTKST (4)	**JRHSF (8)** JRHSC (4) JRCSC (2) PTKST (1)	**JRHSF**
Ethiopia		**TTKSK (41)** JRCQC (12) TRTTF (4) PTKST (1) PTKSK (1)	**TTKSK (56)** TTKST (3) TRTTF (1) JRCQC (2)		**TTKSK (Ug99)**
Kenya	**TTKSK (38)** TTKST (15) TTTSK (2) PTKST (2)	**TTKSK (15)** TTKST (13) PTKST (5) PTKSK (2) RRTTF (1) RTRJP (2) RTGDK (1)	**TTKST (81)** PTKST (10) TTKSK (1)	**TTKST (13)** TTKSK (3) PTKST (2)	**TTKST (Ug99 + Sr24)**
Tanzania		**TTTSK (21)** TTKST (12) TTKSK (6)			**TTTSK (Ug99 + Sr36)**
Zimbabwe		**TTKSF (3)**	**PTKST (3)** **TTKSF (3)** TTKSF+ (1)		**PTKST (Ug99 + Sr24)** or **TTKSF**
South Africa	**TTKSF (23)** TTKSP (10) 2SA105 (12) 2SA104 (5) 2SA102 (5) 2SA55 (3)	**TTKSF (49)** TTKSP (18) PTKST (18) 2SA105 (26) 2SA104 (10) 2SA103 (3) 2SA102 (16)	**TTKSF (82)** TTKSP (2) PTKST (2) 2SA105 (23) 2SA104 (3) TTKSF+ (1)	**TTKSF (35)** 2SA105 (4) 2SA102 (1)	**TTKSF (Ug99 progenitor?)**

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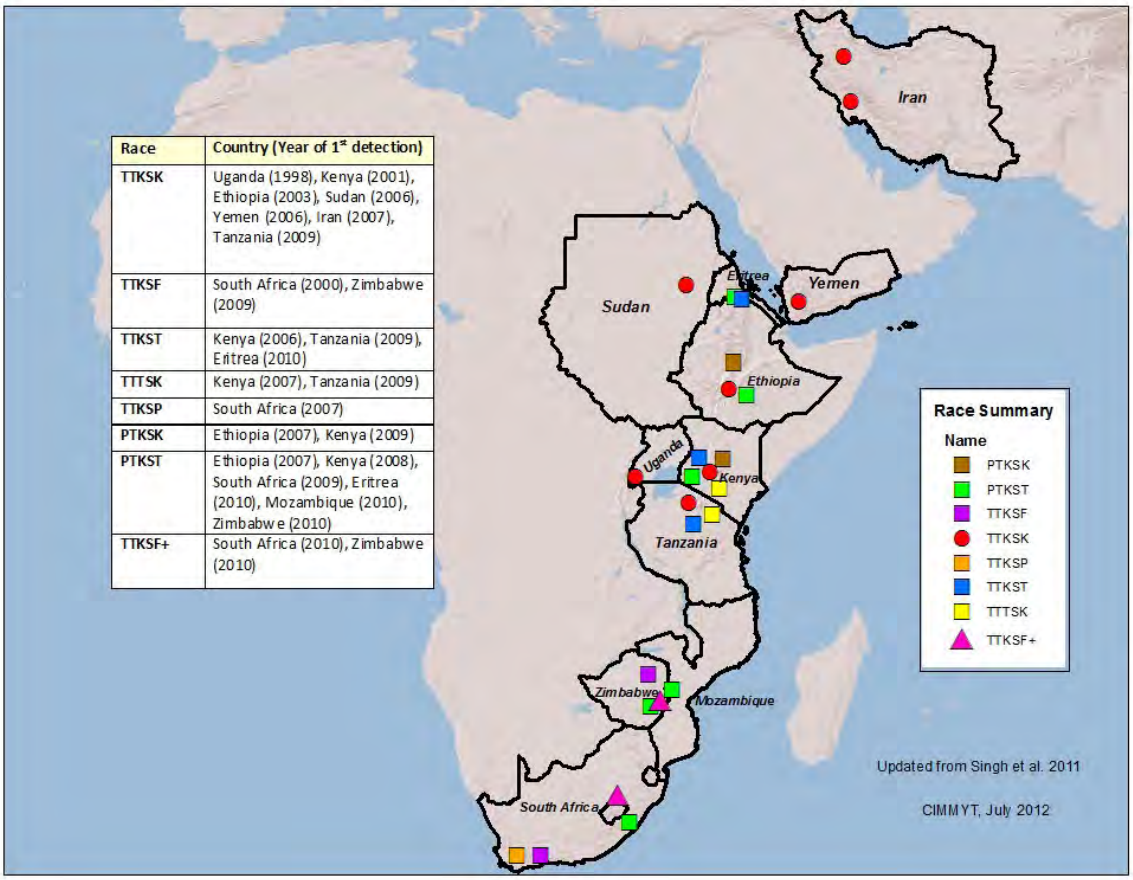

Figure 3 An overview of the current status and geographical distribution of the "Ug99 race group"

New tools for rust monitoring

Modern information and communication technologies offer increasing opportunities to enhance and improve the efficiency of disease monitoring efforts. Technological interventions are now being implemented throughout the entire rust monitoring chain from field survey data collection, through analysis, to information dissemination. Hand-held GPS provide a quick and reliable way to geo-reference survey locations and sampling sites. These have been used in parallel with standardized survey forms since global wheat rust monitoring was initiated. Whilst basic handheld GPS devices provide accurate location data, they do not permit the entry and capture of survey observation data. This limitation requires the subsequent manual integration of both data sets, which is achieved practically using traditional pen and paper format. Subsequent conversion to electronic format can be both time consuming and a source of errors. The advent of an increasing array of affordable hand-held devices offers new opportunities for field data capture and transfer.

Modern Smartphones incorporate an ever increasing array of technology options, linked to increasingly lower costs. Devices that can transfer data, run a range of custom applications, receive email, take high definition photographs or videos, and are GPS-enabled are now common-place. Tablet devices offer a similar range of functionality to Smartphones, but have the additional advantage of incorporating larger screens. Prices for both Smartphones and Tablets are now well below US$200, making them affordable options for use on field surveys.

The GCRMS has started to utilize mobile technology in field surveys. A customized version of the EpiCollect application (Aanensen et al. 2009) has been developed and deployed for Android devices and i-Phones. This customized application has four main features; (i) capture of GPS data, (ii) capture of a geo-referenced photographs, (iii) data entry using a standardized BGRI rust survey data form, and (iv) automated data transfer

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and synchronization with core, centralized databases. A similar application is currently under development for Tablet devices.

Whilst new mobile technologies offer many advantages for field surveys, it must be borne in mind that several limitations still exist. Use of any electronic devices under field conditions does require careful consideration and evaluation of issues such as screen visibility, battery life, field robustness/ ruggedness and reliability, training of users, ease of use and technology limitations, e.g. text input on small-screen devices, lack of internet access and additional costs of 3G data connections. Ultimately, it is unlikely that a single solution will fit all circumstances and user-groups, hence a range of options will be needed. The GCRMS is taking a multiple solution approach to field survey data collection, aiming to provide a range of data collection options to participating field surveyors.

In addition to field survey data collection, new technologies are also providing opportunities in other areas. Molecular diagnostics are playing an increasingly important role in supporting global pathogen monitoring activities. Crouch et al. (2010) reported the development of diagnostic SNP-based markers capable of distinguishing the "Ug99 race group" with a high degree of reliability. The advantages of such techniques include: fewer restrictions on sending dead DNA samples internationally for analysis, it avoids any problems with lack of sample viability, and permits rapid diagnosis (48 hour assay) of the possible presence of the "Ug99 race group". The major limitation is that it lacks the discriminating power for specific race identification afforded by conventional, differential-based analyses. However, this work is expanding and improved diagnostic abilities on specific, important "Ug99 race group" races is likely in the near future.

Information systems

Data management and information dissemination are critical issues for the GCRMS if it is to be successful. Using Google Earth as a platform, the CIMMYT Rust Mapper tool (http://rusttracker.cimmyt.org/?page_id=256) was the first global rust information tool and remains an integral part of the GCRMS. Several additional major advances in data management and information dissemination have been achieved recently. The Wheat Rust Toolbox data management system has been developed in collaboration with the Global Rust Reference Center (GRRC) at Aarhus University, Denmark, and this system is now fully operational. The entire Wheat Rust Toolbox has now been converted to a .NET development framework, permitting more flexibility, improved performance and enhanced functionality. Although the Wheat Rust Toolbox is entirely web-based, it is not a public-domain website. Its function is restricted to i) a data management platform regarding data entry, quality control, management and exchange of data, and ii) a test and development platform for web based data analysis and display tools. The Wheat Rust Toolbox currently has several key components and a brief description of each follows:

Core databases: Two major core databases are currently implemented and populated within the Wheat Rust Toolbox – the rust survey database and the stem rust race database. The rust survey database currently holds over 9,000 geo-referenced, standardized, field survey data records from 27 countries. Survey database records span the period 2007 to 2012 and include information on all three rusts. The stem rust race database includes data from 1,075 isolates and 21 countries. Expansion beyond the current core databases is currently in development with planned inclusion of a Barberry Database (the alternate host for *Pgt* and *Pst*), Trap Nursery Database and a Molecular Diagnostics Database. These additional databases should be available by mid 2013.

Restricted access, user data entry and visualization tools: The Wheat Rust Toolbox includes a comprehensive User Management system that permits controlled access to specific tools and functionality. Registered users have country-specific access to an on-line data entry system and a suite of country-specific data visualization options for their own data. The current system permits secure on-line survey data entry, storage in a structured database, data editing, data visualization and data export (via XML/Excel) of country-specific data. Data visualization options include: a tabular data summary, an interactive graphical display of all rust data by year, a raw data analysis tool, and an interactive map of the country survey data. An example of registered user outputs is given in Fig. 4. These options combined with the advantage of having the data analysed in a global or regional context will be a major motivation for using the system in the future. Only when country data has been checked

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and approved for publication by the data owner does it enter into the public domain, global data dissemination tools.

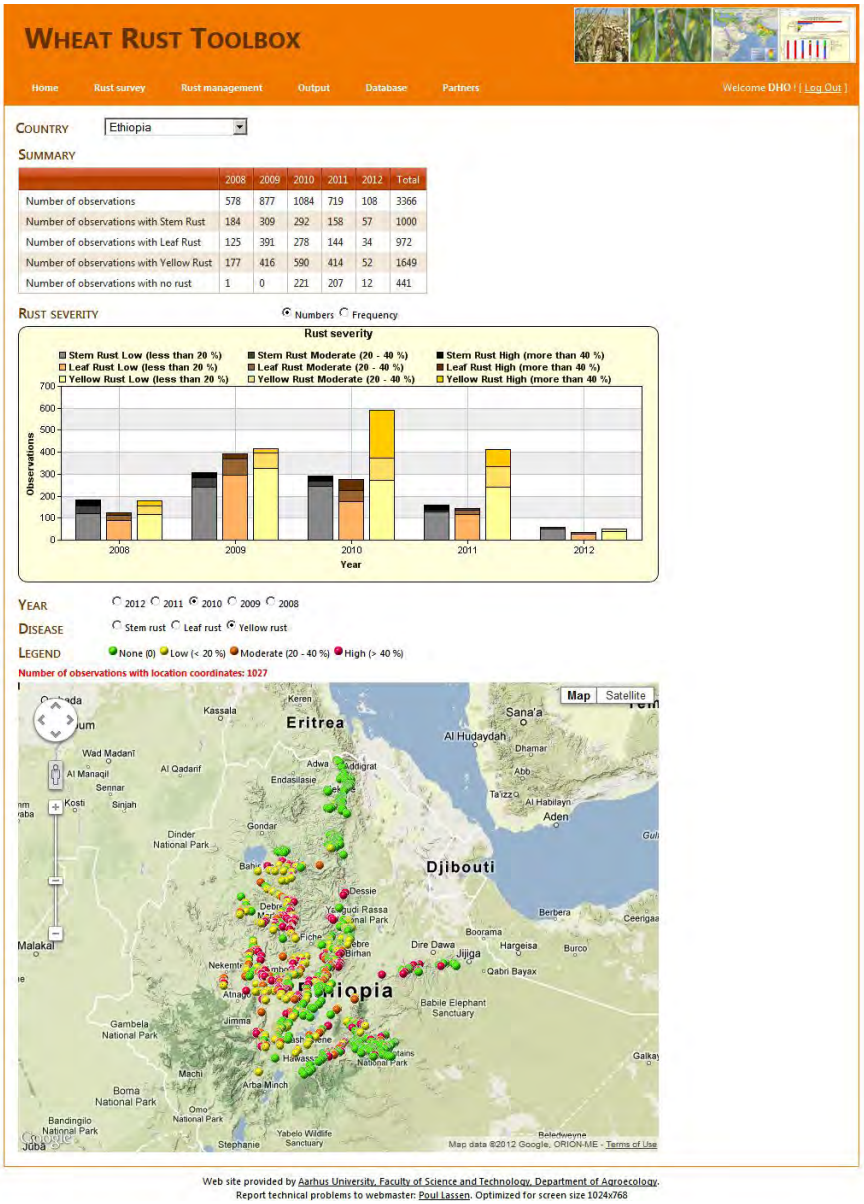

Fig. 4 An example of the Wheat Rust Toolbox registered user outputs for Ethiopia

Public domain, global data dissemination tools: A comprehensive range of visualization tools now provides access to quality-controlled survey and race data. All tools are directly linked to the under-lying databases and all provide interactive rather than static visualizations. Any updates to approved and published data are automatically displayed in the visualization tools. The current set of interactive tools includes: a survey mapping tool for all three rusts with both a country-specific and global version, an overview of the importance of all three rusts across countries (see Fig. 1), a stem rust race frequency graph and map, a stem rust race by country graph, a first detection of stem rust virulence tool, a stem rust virulence by country graph, and a stem rust virulence by year mapping tool. All of the Wheat Rust Toolbox Data Dissemination tools are accessible and implemented within public domain rust surveillance websites. A similar set of interactive race tools are likely to be made available for stripe rust in the near future.

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A set of new websites is now available that provide access to the GCRMS data sets. The principal web site for all the rust surveillance and monitoring data is RustTracker.org (http://rusttracker.cimmyt.org/). This site was developed by CIMMYT and partners as a component of the Durable Rust Resistance in Wheat (DRRW) project and is an integral part of the Borlaug Global Rust Initiative (BGRI). The aim of RustTracker.org is to provide a comprehensive set of information about global rust surveillance and monitoring. The site is directly integrated with the Wheat Rust Toolbox and includes implementations of all the data dissemination tools generated by the data management platform. Other key features include a comprehensive set of situation updates regarding current rust status and an extensive set of country-specific pages and tools covering approximately 40 countries. A limited amount of host information is also integrated directly into RustTracker.org and the plan is that this content will increase considerably in the near future. Stem rust, and the "Ug99 race group" in particular, has been an initial focus of RustTracker.org, but information and content is being expanded to include both stripe rust and leaf rust. Ultimately, RustTracker.org aims to be the most comprehensive source of information for all rust surveillance and monitoring related content.

The new GRRC website, www.wheatrust.org, is also directly linked to the outputs of the Wheat Rust Toolbox. All of the outputs from the Data Dissemination Tools are also available at this location. It is envisaged that over time, additional country-based websites hosted by national partners will provide outlets for a selected set of Wheat Rust Toolbox data outputs. The overall vision is that the data held within the GCRMS can be disseminated via a wide range of public outlets to ensure maximum accessibility and utility to the global rust community.

Whilst the Wheat Rust Toolbox and the GCRMS in general has a focus on the pathogen it is essential that linkages exist to the host, so that surveillance and monitoring information can be used to guide control and mitigation efforts. Some progress has also been made on this more holistic and integrated approach. Recent developments include collaborative work between the developers of the Genetic Resources Information System (GRIS – www.wheatpedigree.net) and the CIMMYT Wheat Atlas (wheatatlas.cimmyt.org) development team. The GRIS system already includes information on all published references to known resistance genes, and these are tagged to cultivars and pedigrees. Work is in progress to consolidate, integrate and enhance the cultivar databases currently held in the respective systems. A direct linkage between the CIMMYT Wheat Atlas cultivar data and RustTracker.org has already been established. Additional activities are on-going to update the information on areas planted to major wheat cultivars at the sub-national level. Once consolidated, this cultivar area data will be used to update responses to important races such as those within the "Ug99 race group". Direct linkages to an International Rust Screening Nursery database being developed by the DRRW project team at Cornell University is also under development. Consolidation into a single global database of all current information on rust resistance genes present in major commercial cultivars is also planned as a future activity.

Future activities

Although the GCRMS has developed into what can now be regarded as a fully operational global disease monitoring system, several areas are targeted for improvement and enhancement. The current system has a strong focus on stem rust, but it is clear that more information on the other two rusts, particularly stripe rust is needed. Timing of surveys is often geared towards the optimum for stem rust, but ways have to be found to encourage multiple surveys to obtain reliable data on stripe rust outbreaks. Improved host information and associated rust resistance genes have already been mentioned as priority areas. Expansion of the existing core databases and enhanced functionality of the Wheat Rust Toolbox are other areas on which significant progress is anticipated on a relatively short timescale. Other aspects in which it is hoped to make further progress are disease early warning and mitigation planning advice. Both these areas are under consideration to be tackled through an epidemiological modelling approach as part of a planned collaboration with Cambridge University and Rothamsted Research Institute in the UK.

Conclusion

Considerable progress has been made towards the goal of establishing a GCRMS. Within a relatively short time period, the system has now reached the point where it can be regarded as a fully operational global disease monitoring system, at least for stem rust. Creation of a functional surveillance and monitoring network, covering

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35 countries and a substantial proportion of the developing world wheat area is a significant achievement. Through the efforts of all contributing partners in this network it has been possible to track the spread and status of important *Pgt* races such as those within the "Ug99 race group". The mere fact that no major changes in the current geographical distribution of the "Ug99 race group" can be reported with a large degree of confidence indicates that the system is working. The current system has a strong focus on stem rust, but it is clear that more information on the other two rusts, particularly stripe rust, is needed. New technologies are playing an increasingly important role in the GCRMS. These are already having an impact in several different areas, from data collection to diagnostics, and their role is likely to increase in the future. Considerable attention has been given to developing a robust and functional data management system. Having the Wheat Rust Toolbox fully functional, databases populated with considerable amounts of data, and a range of operational visualization tools is a major advance. The rich, consolidated global data resource is now permitting data mining and value addition in the form of rust risk maps. New web resources are providing access to a wealth of information regarding rust surveillance and monitoring in ways that were not previously possible. Even more encouraging is that numerous groups working on rust related information systems are working collaboratively to ensure that single source databases are shared and integrated into different information platforms. This level of global collaboration around a major disease is highly commendable and likely to result in major benefits to the global wheat community. Despite good progress, several areas are seen as priorities for further work to advance the current GCRMS. These include; expansion of the data management system, increased information relating to the host and to rust resistance genes, early warning systems, and disease mitigation planning. Activities in all these areas are planned and hopefully will be initiated in the near future. A bigger challenge is to find effective mechanisms to ensure long-term, in-country sustainability of rust monitoring work versus reliance on international support. Building capacity, implementing tools and information systems to enhance efficiency will help, but sustained commitments will be needed by governments and research institutes to address the challenges posed by wheat rusts.

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Stocking the Breeder's Toolbox: An update on the status of resistance to stem rust in wheat

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Abstract

The number of designated stem rust resistance genes has increased by ~10 over the past four years. Translocations involving several broadly-effective alien resistance genes with limited or no previous agricultural deployment were enginneered to reduce the likelihood of linkage drag, and the foundations of adult plant resistance were established. This progress resulted from international collaboration, increased global coordination, and critical financial support. By buidling on these initial accomplishments and improving genetic and genomic resources over the next four years we expect to achieve: 1. more than 10 additional formally designated stem rust resistance genes conferring resistance to Ug99-complex races, 2. robust/diagnostic DNA marker haplotypes identified for most sources of resistance, 3. multiple linkage blocks of two or more resistance genes to enhance gene pyramiding efforts, and 4. knowledge of numerous additional sources of resistance complelely or partially identified. Never before have so many resources and supporting tools been available to combat the wheat rusts. It is an opportune time for the international community to strategically deploy and responsibly steward our genetic resources for durable control of wheat stem rust.

Introduction

A primary focus of pre-breeding research supported as part of the Durable Rust Resistance in Wheat (DRRW) project is to fundamentally change how host resistance to stem rust of wheat is selected and deployed. Simultaneous pursuit of effective sources of resistance from readily accessible gene pools, introgression and cytogenetic manipulation of new sources of resistance from alien gene pools, genetic mapping and development of diagnostic DNA markers for desirable sources of resistance, determination of optimal combinations of resistance genes, and germplasm development is expected to provide the materials and information to strategically confront stem rust in the coming years on a global scale in way never before possible.

Approximately 30 major genes (genes with noticeable seedling, all-stage resistance) conferring resistance to Ug99-complex races, plus at least five designated adult plant, or slow-rusting, resistance genes that contribute to stem rust resistance and ~10 additional consistent stem rust QTL have been identified to date. Several additional newly discovered resistance loci/sources are at various stages of development and validation. The following summaries provide updates for many of these genes, and most are part of ongoing DRRW research efforts. DRRW collaborators urge breeders and geneticists throughout the world to use these resources in a responsible and strategic manner, and to combine three or more major genes in future varieties relying on race-specific resistance. Development of varieties with high levels of adult plant resistance, lacking major genes, is also especially encouraged.

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Major genes (seedling, race-specific)

***Sr13*:** A number of Ug99-complex resistant durum and cultivated emmer varieties contain *Sr13* on the long arm of chromosome 6A. High-resolution genetic mapping and positional cloning of *Sr13* are underway for cloning and development of diagnostic DNA markers (Simons et al. 2011; Dubcovsky et al. unpublished).

***Sr22*:** The region surrounding this *Triticum monococcum*-derived resistance gene introgressed to chromosome 7AL was modified to minimize the *T. monococcum* chromatin, and useful DNA markers were identified (Olson et al. 2010; Periyannan et al. 2011). High resolution mapping and positional cloning are also underway (Rouse and Lagudah unpublished). *Sr22* was deployed in one Australian cultivar (McIntosh et al. 1995); deployment is not documented in other wheat production areas, but many programs are currently using *Sr22* in resistance breeding efforts.

***Sr25*:** Robust DNA markers for *Sr25* were identified (Liu et al. 2010), enabling selection of this *Thinopyrum ponticum*-derived alien resistance on chromosome 7DL. CIMMYT germplasm containing *Sr25*, and presumably *Lr19*, in combination with *Sr2* was recently released in Afghanistan (Muqawim 09), Egypt (Misr 1, Misr 2), and Pakistan (NR356) (http://www.globalrust.org/traction/project/varieties).

***Sr26*:** New translocation stocks with reduced *Thinopyrum ponticum* chromatin containing *Sr26* on chromosome 6AL were identified (Dundas unpublished). PCR-based markers that allow for routine detection (Liu et al. 2010) can be used to identify these smaller translocations. Australian varieties with the original translocation developed by D.R. Knott have been grown for over 30 years (McIntosh et al. 1995); deployment of *Sr26* is not documented in the rest of the world presumably due to real or perceived evidence of linkage drag preventing realization of other traits, but many programs are currently using the new translocation derivatives in resistance breeding efforts.

***Sr28*:** While widely ineffective against most isolates of *Puccinia graminis*, *Sr28* does confer resistance to races in the Ug99 complex. *Sr28* is of common wheat origin (Kota and Ceres) and recently reported DNA markers should aid in identification of germplasm with this gene (Rouse et al. 2012). A single nucleotide polymorphism (SNP) marker for *Sr28* has been tentatively identified, and appears diagnostic based on genotyping and phenotyping of ~1000 wheat lines from a world collection (Rouse and Pumphrey unpublished). An effort to recombine *Sr28* with other effective stem rust and stripe rust resistance genes in a single linkage block on chromosome 2B is also underway (Rouse and Xu unpublished). If linked to other effective genes, such as *SrGabo56* and *Sr47*, and used in combination, *Sr28* may be useful in wheat breeding for Ug99 resistance.

***Sr32*:** Dundas and others (unpublished) recently developed several recombinant translocation stocks with reduced *Aegilops speltoides* chromatin harboring *Sr32* on chromosome 2DS. DNA markers have not been reported for these new lines, but several laboratories have identified markers that successfully identify the original translocation. Deployment of *Sr32* has not been reported, but several programs are actively using the new translocation derivatives in resistance breeding efforts.

***Sr33*:** Documented deployment of *Sr33*, an *Aegilops tauschii*-derived gene on chromosome 1DS, is limited to Australia in the cultivar Lorikeet (Park and Bariana 2008). Single gene lines with *Sr33* show an intermediate level of resistance as adult plants in the screening nurseries in Kenya (Jin et al. 2007). The *Sr33* gene was genetically mapped (Sambasivam et al. 2008) and cloned by a map-based approach (Lagudah et al. unpublished).

***Sr35*:** DNA markers for *Sr35* were developed (Zhang et al. 2010), and candidates were identified by map-based cloning (Dubcovsky et al. unpublished). Virulence to *Sr35* is relatively common throughout the world (McIntosh et al. 1995, and references cited therein; Rouse and Jin unpublished), and while it does confer near immunity to Ug99-complex races, resistance gene combinations using *Sr35* should be chosen carefully.

***Sr37*:** The original translocation stock with *Sr37* present in a large translocated segment of chromosome 4G from *Triticum timopheevii* to wheat chromosome 4B was not deployed because of obvious linkage drag problems (McIntosh et al. 1995). Recently, secondary recombinants with smaller alien segments were produced using the

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ph1b system, but inheritance of resistance in these recombinants has been unstable to date and the usefulness of these stocks is uncertain (Xu et al. unpublished). DNA markers were developed that track these smaller alien segments.

***Sr39*:** Multiple stocks with reduced *Ae. speltoides* chromatin segments harboring *Sr39* on chromosome 2BS were recently developed (Mago et al. 2009; Niu et al. 2011); useful DNA markers for those alien segments are also available. *Sr39* has not been previously deployed and virulence has not been detected to date. Several programs around the world are currently using *Sr39* in breeding.

***Sr40*:** Dundas et al. (unpublished) developed four recombinants with reduced *T. timopheevii* chromatin surrounding this gene located on chromosome 2BS. DNA markers linked to *Sr40* in these recombinants were also identified (Wu et al. 2009), but markers useful in using the recombinant lines have not been published.

***Sr42*:** *Sr42* is derived from common wheat germplasm. Recent genetic mapping of *Sr42* resulted in identification of closely linked DNA markers on chromosome 6DS (Ghazvini et al. 2012). The similar map position of this gene and *SrCad* (Heibert et al. 2011) suggests that *Sr42* and *SrCad* may be the same gene. *SrCad* is deployed in Canadian spring wheat varieties containing the bunt resistance gene *Bt10*.

***Sr43*:** Xu et al. (unpublished) recently produced two putative translocation lines on chromosome 7D with *Sr43* from *Thinopyrum elongatum* and reduced alien chromatin. Further characterization of these lines is underway, along with identification of markers.

***Sr44*:** Liu et al. (2012) developed a compensating 7DL.7JS Robertsonian translocation with *Sr44* from *Th. intermedium*. Homoeologous recombination to reduce the size of this translocation is underway. A second stem rust resistance gene from the same source and effective against isolates in the Ug99-race complex was identified on chromosome 7JL from the original donor stock. According to information for *SrAgi* (a synonym for *Sr44*) described in McIntosh et al. (1995) this latter gene was apparently not effective in early Australian studies because races virulent for *Sr44* were virulent on addition line TAF2 which carried an entire 7JS chromosome pair.

***Sr45*:** *Sr45* is an *Ae. tauschii*-derived stem rust resistance gene on chromosome 1DS (Sambasivam et al. 2008). Deployment of *Sr45* has not been documented to date, but is possible due to the use of *Ae. tauschii*-derived germplasm in many programs; this gene is effective against TTKSK, but does not confer resistance to many other races found throughout the world. Due to their similar, but not identical, chromosome positions, efforts to recombine *Sr33* and *Sr45* into a linkage block on chromosome 1DS are underway (Lagudah unpublished). This should improve the value of deploying these genes.

***Sr46*:** Genetic mapping of *Sr46* placed this *Ae. tauschii*-derived stem rust resistance gene on chromosome 2DS (Lagudah unpublished). *Ae. tauschii* accessions with *Sr46* are resistant to TTKSK in seedling tests (Rouse et al. 2011), but other characteristics of this gene have not been extensively documented.

***Sr47*:** Faris et al. (2008) described *Sr47* in a large translocation involving wheat chromosome 2B and *Ae. speltoides* 2S. Recombinants with reduced alien chromatin were recently produced by homoeologous recombination and *Sr47* was located on a small segment on chromosome 2BL along with supporting DNA markers (Klindworth et al. 2012). *Sr47* confers seedling resistance to all races tested to date. A second gene, possibly *Sr39*, was identified in the short arm of the original translocation line with *Sr47*.

***Sr50*:** Originally temporarily designated *SrR* (Anugrahwati et al. 2008), this Imperial rye-derived resistance gene is located in an interstitial translocation on chromosome 1DS. Preliminary data suggest that recombination with *Sr33* on 1DS to form a linkage block is not feasible (Lagudah et al. unpublished), but coupling with *Sr45* or other recently identified resistance genes on 1DS may be possible. *Sr50* could be an allele of *Sr31*, but has different race specificity and is contained in a different rye translocation. A further specificity involves the *SrAmigo* gene present in a 1AL.1RS translocation present in some plants of Amigo and again, the allelic status of this gene is unknown.

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***Sr51*:** Chromosome engineering of this resistance gene in *Ae. searsii* chromosome 3SS resulted in transfer to the short arms of chromosomes 3A, 3B, and 3D as compensating Robertsonian translocations (Liu et al. 2011a). Further chromosome manipulation to isolate small translocations with *Sr51* is targeting 3D as the recipient chromosome.

***Sr52*:** This alien-derived gene from *Dasypyrum villosum* is present in a compensating 6AL.6VS Robertsonian translocation line (Qi et al. 2011). Seedling resistance conferred by *Sr52* is temperature sensitive, and the utility of this gene in wheat improvement has not been fully determined.

***Sr53*:** An interstitial translocation line was identified with the *Ae. geniculata*-derived resistance gene *Sr53* from chromosome 5M^{g}L recombined in wheat chromosome 5DL (Liu et al. 2011b). DNA markers closely linked to *Sr53* were also reported. Seedling and adult plant screening of *Sr53* stocks indicate this gene provides a moderate level of resistance.

SrWeb (Hiebert et al. 2010) and ***SrGabo56*** (Rouse et al. unpublished) are both effective against TTKSK and linked to *Sr28* with similar map positions on chromosome 2BL. Whether *SrWeb* and *SrGabo56* are allelic or identical is not clear at present. Recombinants having *Sr28* and *SrGabo56* were developed and this linkage block is being further evaluated for potential to combine with one or more additional genes on chromosome 2BL (Rouse and Xu, unpublished). Deployment of two or more race Ug99-effective genes in coupling would simplify resistance breeding efforts and enable more complex pyramiding efforts.

Other resistance genes: Additional temporarily designated major genes preliminarily mapped and likely representing new sources of resistance include: *SrZelma, Sr1662, SrND643, SrTm4, SrB, SrC, SrSha7, SrNing* (Yu et al. 2009; unpublished). In addition, preliminary evidence indicates some genes previously characterized as ineffective against the race Ug99-complex, including *Sr12*, *Sr15*, and *Sr21*, may be moderately effective in certain environments and genetic backgrounds (Rouse unpublished). Characterizations of these genes are in variable stages of development. Additional novel sources of resistance are currently being characterized and introgressed from cultivated and wild tetraploids, *Ae. tauschii* and other *Aegilops* species, *D. villosum*, *T. monococcum*, and *Thinopyrum* species.

Adult plant resistance (APR) genes

***Sr2*:** APR to Ug99-complex races conferred by *Sr2* on chromosome 3BS was validated in at least six recombinant inbred line populations characterized by CIMMYT (Bhavani et al. 2011), as well as in several other research efforts. An improved marker for *Sr2* is available (Mago et al. 2011), and continued efforts to develop "perfect" markers are underway. Significant interaction of markers linked to *Sr2* with markers linked to other resistance loci was detected in multiple association mapping panels (Yu et al. 2011, 2012). Many CIMMYT and other varieties internationally contain this gene in combination with other sources of resistance. *Sr2* is considered by many to be the foundation of APR breeding efforts.

***Lr34*/*Yr18*/*Sr57*:** The pleiotropic effect of *Lr34,* located on chromosome 7DS, on stem rust APR has been noted in multiple publications, and supported by recent screening of *Lr34* loss-of-function mutants (Singh et al. unpublished). Interaction of *Lr34* with other loci contributing to adult plant resistance to Ug99-complex races was also recently documented (Kolmer et al. 2011; Yu et al. 2012), supporting earlier work by Kerber and others using North American *Pgt* races. Marker development (Lagudah et al. 2009) following cloning (Krattinger et al. 2009) now enables routine selection and detection of *Lr34* in diverse germplasm, with a couple of exceptions still under investigation. *Lr34* is common in CIMMYT-derived germplasm, but is also present in other germplasm internationally, including Chinese landraces.

***Lr46/Yr29*:** QTL mapping of APR to Ug99-complex races in multiple populations in Kenya has detected consistent effects of a gene on chromosome 1BL, at a position consistent with the location of *Lr46* (Bhavani et al. 2011). Additional recent data suggests that *Lr46* contributes to stem rust APR, in a similar way to other "slow-rusting" genes (Singh et al. unpublished). DNA markers diagnostic for *Lr46* are not currently reported, but map-

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based cloning of this gene is well advanced (Lagudah et al. unpublished). *Lr46* is probably common in CIMMYT and other germplasm.

***Lr67/Yr46/Sr55*:** The recently identified *Lr67* locus confers a slow-rusting phenotype similar to *Lr34,* and its effect on stem rust APR was also validated (Herrera-Foessel et al. 2011; Singh et al. unpublished). Development of robust DNA markers linked to *Lr67* is underway.

***Sr56*:** APR gene *Sr56* was located on chromosome 5B of cv Arina (Bansal et al. 2008; Bansal and Bariana, unpublished). QTL conferring resistance to race Ug99 were also consistently detected on chromosome 5B in both bi-parental (Bhavani et al. 2011) and association mapping (Yu et al. 2011, 2012). Based on these reports there may be at least two genes on 5B, and the relationship of *Sr56* and other QTL is not established. Continued mapping and marker development are in progress.

Ongoing characterizations of bi-parental and association mapping populations have indicated the presence of numerous other stem rust resistance loci, many likely contributing to APR. Seven consistent QTL were identified on chromosomes 1A, 2B, 3D, 4A, 5B, 6B and 7A in mapping populations screened in Kenya (Bhavani et al. 2011). Similarly, loci on chromosomes 1A, 1B, 2B, 4A, 5B, 6B and 7B were identified by association mapping of Ug99 resistance in spring and winter wheat germplasm from CIMMYT (Yu et al. 2011, 2012). The recent availability of DNA marker platforms for detailed rapid wheat genotyping is hastening characterization of these and other sources of resistance.

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Wheat breeding efforts for achieving resistance to the rusts in Nepal

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Wheat is the major staple cereal of Nepal contributing 26% to national food requirements. It is cultivated on about 0.76 million ha with 1.85 million tonnes of production and average productivity of 2.4 tonnes per ha. Wheat is cultivated in a wide range of environments at altitudes ranging from 60 to 2,500 masl. Summer sown (late June – early July) wheat is also grown in the high hills. Summer wheat may have a role in rust survival during the off-season. Among the biotic stresses, leaf rust, stripe (yellow) rust and leaf blight are major yield-limiting factors. Stripe rust epidemics during the 1980s, mid 1990s, and in 2004-05, caused yield losses of 50 – 80%. Leaf rust epidemics are sporadic and less damaging. Early wheat breeding efforts in Nepal involved trials on introductions from CIMMYT and India, and resulted in the release of important varieties such as Sonalika, Kalyansona and UP 262 during 1970s, and Nepal 297, Triveni, Siddhartha and Nepal 251 in the mid 1980s. These varieties contributed to both expansion of the wheat area and production. With establishment of National Wheat Research Program (NWRP) in 1972, systematic wheat breeding work was initiated. The NWRP worked closely with CIMMYT and developed agronomically superior rust resistant wheat varieties (Bhrikuti, Gautam, WK 1204, Aditya and Nepal 791) that helped to mitigate rust epidemics. Leaf rust has been under control since 1985 and no yellow rust epidemics have been reported since 2005.

Following the occurrence of *Pgt* race Ug99 in Africa, we worked in close collaboration with CIMMYT and BGRI in development, and rapid dissemination, of superior Ug99 resistant varieties through participatory variety selection (PVS) with farmers. A cross (NL748/NL837) made in 1996 resulted in varieties BL 3063 and BL 3064 with adult plant resistance to race Ug99. BL 3063 was released in 2010 and named "Vijay" meaning victory. This variety now covers around 5% of the wheat area. In addition, NARC received several Ug99 resistant genotypes in 2008-09 through the USAID Famine Seed Project coordinated by CIMMYT and BGRI. The best-adapted genotypes were evaluated through PVS and on-station trials throughout the country. Pre-release seed multiplication involved both the public and private sectors. CIMMYT varieties Danphe and Francoline were identified for release, and seed is already disseminated throughout the country. Currently the NWRP is well supplied with sources of durable rust resistance from CIMMYT and is making effective use of them in the breeding program.

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Success in seed multiplication and delivery efforts at UAS, Dharwad

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Introduction

Seed is a basic, vital and central input in agriculture and in all farming systems. Timely availability of quality seeds of varieties/hybrids adapted to to different agro-climatic conditions and in sufficient quantity at affordable prices is a measure of the strength and health of an agricultural economy. Sustained increase in agricultural production requires a continuous development of improved crop varieties/hybrids, an efficient system of production, and a means of distribution to farmers.

India is one of the few countries where the seed sector has advanced in parallel with the agricultural production. However, the availability of quality seed of improved varieties and hybrids is grossly inadequate and is a major constraint to enhanced production. Studies made by several workers (Gadwal 2003, Patil et al 2004, Hanchinal et al. 2007) clearly indicate that with high-volume low-value seeds, such as wheat, groundnut, soybean and chickpea, 80% of the cropping area is sown with farm-saved seeds of old and obsolete varieties

During last few decades, a number of high yielding disease and pest resistant varieties/hybrids in different crops had 10 to 40% yield superiority over local cultivars. With the exception of high-value low-volume seeds, seed production of low-value high-volume crops is generally left to public sector agencies. The bulky nature of most self pollinated crops, and lack of adequate investment on infrastructure means low remuneration. Although there is enough breeder seed production in most of the high volume crops, further seed multiplication through the foundation and certified seed stages are major constraints to the availability of quality seed. The present rate of seed replacement (SRR) for such crops is 6 to 8%. There is a need to increase SRR to 25 to 30% in varieties and obviously 100% for hybrids.

To increase the productivity of low-value high-volume crops farmers need to have access to improved seeds of the right type, at the right time, at the right place and at a reasonable price. For supply of such seeds, both the informal seed sector (farmer managed seed systems) and the formal seed system (seed enterprises) need to be engaged. The informal seed sector is often highly effective in reaching isolated, inaccessible, small holder areas and is a sound opportunity for entrepreneurs to gradually evolve into the formal enterprises

Wheat, the most important food crop of world and backbone of global food security, belongs to the high-volume low-value seed group. Of the total area sown to both hexaploid bread wheat and tetraploid durum and emmer wheat worldwide, 44% (95 m ha) is in Asia. Of this,62 m ha are located in just three countries viz. China, India, and Pakistan (Table 1 and Figure 1). Food security and production stability are of paramount importance in most Asian countries, given that the majority of farmers are poor. The wheat rusts have historically been major biotic constraints both in Asia and the rest of the world. Stem rust has been under control since the beginning of the green revolution in South and West Asia in the 1960s. Leaf rust and stripe rust continue to be major threats to production over approximately 60 (63%) and 43 (46%) m ha, respectively, in Asia. Although, the timely application of fungicides can provide adequate control, their use adds to production costs and they are considered environmentally unsafe. Growing resistant cultivars is thus the most effective and efficient control strategy, as it has no cost to farmers and is environmentally safe. Rapid evolution of races with new virulences, or combinations of virulences, dictate a need for discovery and deployment of new resistance genes and/or resistance gene combinations.

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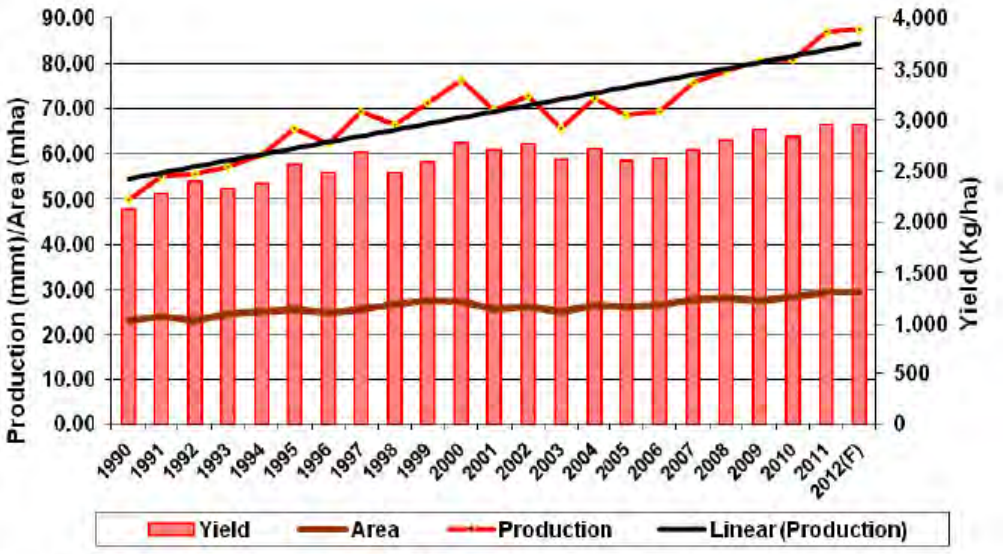
Table 1. Wheat statistics for India

Year	Area (m ha)	Production (m t)	Productivity (t/ha)
2001-02	26.3	72.8	2.8
2002-03	25.2	65.8	2.6
2003-04	26.6	72.1	2.7
2004-05	26.4	68.6	2.6
2005-06	26.7	69.4	2.6
2006-07	28.5	74.9	2.6
2007-08	28.1	78.6	2.8
2008-09	27.8	80.68	2.9
2009-10	28.5	80.70	2.8
2010-11	29.3	85.93	2.9
2011-12*	29.4	90.3	3.0

** IV advance estimate, Ministry of Agriculture, New Delhi*



Source: Ministry of Agriculture, GOI; and FAS/New Delhi estimates for MY 2012/13

Figure 1. Area, production and yield for wheat, India 1990-2012

Wheat has two properties that render rapid impact of new varieties (Tables 2 and 3): high seeding rates (100 kg/ha or higher), and a very low seed multiplication ratio. One hundred kilograms of seed planted on a hectare of land will usually produce between 3,000 and 6,000 kg of grain, that is, corresponding multiplication ratios of 30 and 60. The magnitude of the challenge of wheat seed systems is further complicated by the small size of farms and correspondingly enormous numbers of customers. Predominance of small-scale farms (1-2 ha) also renders the task of seed production more challenging, because of the number of farmers that need to be involved in producing seed.

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Table 2. Seed replacement rates in major Asian countries

Country	Crops	Seed Replacement Rate (%)
India	Wheat	25.0
	Rice	30.1
	Maize	48.5
Pakistan	Wheat	22.0
	Rice	37.0
	Maize	26.0
Bangladesh	Wheat	57.8
	Rice	47.4
	Maize	91.6
Nepal	Wheat	8.3
	Rice	6.6
	Maize	5.9

Table 3. Bridging the yield gaps

State	Area	State yield (t/ha)	Improved technology and variety			Existing varieties		
			FLD yield (t/ha)	Gap (t/ha)	Additional Production (m tons)	FLD yield (t/ha)	Gap (t/ha)	Additional Production (m tons)
Uttar Pradesh	9.67	2.88	4.27	1.39	**13.4**	3.88	1.00	**9.7**
Punjab	3.54	4.31	4.75	0.44	**1.6**	4.39	0.08	**0.3**
Haryana	2.49	4.21	4.93	0.72	**1.8**	4.76	0.55	**1.4**
Rajasthan	2.39	2.85	4.25	1.40	**3.3**	3.65	0.80	**1.9**
Madhya Pradesh	4.28	1.84	3.56	1.72	**7.4**	2.74	0.90	**3.9**
Bihar	2.23	2.08	4.04	1.96	**4.4**	3.55	1.47	**3.3**
Gujarat	0.91	2.90	3.89	0.99	**0.9**	3.53	0.63	**0.6**
Maharashtra	1.08	1.63	2.79	1.16	**1.3**	2.44	0.81	**0.9**
WB	0.3	2.31	2.79	0.48	**0.1**	2.32	0.01	**0.0**
All India	28.52				**34.2**			**21.8**

The path from plant breeding to variety uptake and utilization is a continuum of activities. New wheat varieties not only need to be rust resistant but also must have the agronomic, organoleptic and market characteristics that farmers and consumers require. Most public plant breeding programs produce just enough seed of the varieties being considered for release to supply a modest testing program. In normal circumstances, this is an

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understandable conservation of resources, but under the threat of rust and in view of ever increasing food security concerns, it is necessary to have more seed of pre-released varieties available for extensive testing and to get a head start on the seed multiplication process. This may imply the need for additional resources at an early stage. Most countries require that varieties pass independent agronomic performance tests and be examined in a DUS (distinctness, uniformity, stability) test to ensure that a candidate for release is novel.

The diversity of resistance in the Indian subcontinent can be maintained by growing cultivars that carry different resistance genes (Table 4). However, there is a general tendency for farmers to grow only one or a few favored cultivars, which, as a result, come to occupy large areas. Examples of this are varieties 'PBW343' and 'Inqualab 91' grown on 7 and 6 m ha in India and Pakistan, respectively. These cultivars are also grown in other countries under different names. Unfortunately, both cultivars carry stripe rust resistance gene *Yr27*, for which virulence has become widespread on the Indian subcontinent. Growing few cultivars that carry race-specific resistance genes leads to greater genetic uniformity and consequently greater vulnerability to disease and other threats.

Table 4. Preferred varieties in India

Wheat type	Production type			
	Normal sown	Late sown	Rainfed	Sodic soils / Others
North Western Plains Zone (NWPZ) Punjab, Haryana, Delhi, Rajasthan (except Kota and Udaipur divisions), Western UP (except Jhansi division), parts of J&K (Jammu and Kathua districts), HP (Una dist. and Paonta valley) and Uttarakhand (Tarai region)				
Bread wheat	DBW 17, PBW 550, PBW 502, PBW 343, WH 542, UP 2338, HD 2687, HD2967	WH 1021, PBW 373, UP 2425, RAJ 3077, DBW 16, RAJ 3765, PBW 590	PBW 299, PBW 175, WH 533, PBW 396	RAJ 3077, KRL-19, KRL 210, KRL 213
Durum	PBW 34, PDW 215, PDW 233, WH 896, PDW 291, PDW 314	-	-	-
North Eastern Plains Zone (NEPZ) Eastern UP, Bihar, Jharkhand, Orissa, West Bengal, Assam and plains of NE States				
Bread wheat	CBW 38, Raj 4120, K 0307, NW 1012, HUW 468, PBW 443, HD 2733, HD 2824, K 9107, HD 2967, DBW 39	HD 2643, HP 1633, HP 1744, NW 1014, HW 2045, DBW 14, NW 2036, HD 2985	HDR77,K8962,K 9465, K 8027, HD 2888, MACS 6145	RAJ 3077, KRL-19 KRL 210, KRL 213
Central Zone (CZ) MP, Chhattisgarh, Gujarat, Rajasthan(Kota and Udaipur divisions) and UP(Jhansi division)				
Bread wheat	GW 190, GW 273, DL 803-3, GW 322, GW 366, HI 1544	GW 173, DL 788-2, MP 4010, HD 2932, MP 1203, HD 2864	HW 2004, JWS 17, HI 1500, HI 1531, Sujata	RAJ 3077, KRL-19, KRL 210, KRL 213
Durum	HI 8381, HI 8498, MPO 1215	-	HD 4672, HI 8627	-
Peninsular Zone (PZ) Maharashtra, Karnataka, Andhra Pradesh, Goa, Plains of Tamil Nadu				
Bread wheat	DWR 162, MACS 2496, GW 322, Raj 4037, NIAW 917, UAS 304, MACS 6222, MACS 6273	DWR 195, HD 2501, NIAW 34, HUW 510, HD 2932, HI 977, HD 2833, PBW 533, Raj 4083, AKAW 4627	K 9644,HD 2781, PBW 596, HD 2987	_
Durum	MACS 2846, HI 8663, UAS 415, UAS 428	-	AKDW 2997-16	-
Emmer	DDK 1025, DDK 1029, DDK 1066	-	-	-

Indian assert

Although scientists that the climatic

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conditions in the major wheat belt of north India are not conducive to the spread of stem rust, including that caused by *Pgt* race Ug99, there must be a realistic consideration of the possibility that this race and its derivatives have different fitness attributes, especially given that most of the wheat varieties planted in India are highly susceptible to Ug99. ICAR and the state agricultural universities (SAUs) continuously survey and monitor the wheat crop for various rusts, including race Ug99. ICAR also screens newly released wheat varieties and advanced lines within the country and in Kenya. Because of the susceptibility of most of the local varieties, such as PBW 343, PBW 502 and HD 2687, to race Ug99, the government has been encouraging their replacement with Ug99-resistant varieties such as DBW 17, PBW 550, and HW 542.

Wheat breeding strategies for a number of years has involved the stacking or pyramiding of pathotype-specific genes. While gene stacking could be considered a short-term remedy, long-term breeding solutions through the use of durable host resistance must be strongly advocated. In order to quickly replace susceptible wheat varieties with new resistant varieties, countries will need support to enhance the effectiveness of their national systems for varietal registration and release, through which all potentially resistant varieties must pass before they can be released for distribution.

As it is well known that the seed replacement rate has a strong positive correlation with crop productivity, there is a need to enhance the seed sector through public sector seed companies, including the state seed corporations and other viable seed supply systems. It has been proved beyond doubt that the quality seed of improved varieties can alone increase the crop yields by 15-20% (Gadwal, 2003). It has been demonstrated that use of quality seed in wheat results in higher productivity (54.7 q/ha) as compared to use of farmer saved seeds (48.9 q/ha) because of higher genetic purity (99.7%) in quality seeds as against farm saved seeds (96.9%) (Gupta, 2012). It is also well recognized that the production of quality seed meeting the prescribed standards of genetic and physical purity, seed health, vigor, viability and storability is a specialized and scientifically based activity requiring detailed attention.

Production of an adequate quantity of breeder seed is a major responsibility of the National Agricultural Research System to meet the demands of the seed industry for production of foundation and certified seeds. In India, this responsibility is borne by ICAR at its research institutions along with the SAUs. To cope up with the increasing demands for breeder seed, the ICAR created Breeder Seed Production Units in almost all SAUs and crop-based ICAR institutes in 1979-80. Realizing the importance of quality seed in agricultural production the University of Agricultural Sciences, Dharwad, started a separate independent Seed Unit in 1996 with a Special Officer (Seeds) as administrative head and involving research stations The unit is responsible for seed production of a range of crops, and is also encouraged to undertake research activities related to seed production and technology, including post-harvest technologies. (Table 5)

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Table 5. Breeder seed production of wheat by UAS, Dharwad

Variety	Quantity produced				
	2006-07	2007-08	2008-09	2009-10	2010-11
DWR 162	85	200	160	100	128
DWR 1006	0	0	12	5	11
DWR 39	10	16	22	5	0
DWR 195	10	19	5	2	12
DWR 185	120	10	15	10	0
DWR 2006	0	10	5	10	0
DDK 1001	2	2	2	5	0
DDK 1009	30	8	0	5	0
DDK 1025	0	40	50	5	20
DDK 1029	0	0	20	5	28
LOK 1	600	200	369	300	80
HD 2189	450	400	333	200	192
DWR 225	0	0	60	10	0
B. Yellow	5	5	1	5	0
Kiran	5	5	1	5	0
GW 322	0	0	0	0	293
UAS 415	0	0	0	0	55
UAS 304	0	0	0	0	60
MACS 6222	0	0	0	0	29
Total	1317	915	1055	672	908

Mandates of the Seed Unit at UAS, Dharwad

As summarized by activities described below, the mandates of the UAS Seed Unit are:

- To produce adequate quantities of nucleus, breeder, foundation and certified/TL seeds of high quality as per national and state requirements
- To maintain the genetic purity of crops varieties/parental lines of hybrids and planting material
- To generate basic information on seed certification standards including seed health
- To make linkages with crop improvement projects, seed industry, seed certification agencies, NGOs, KVKs (Krishi Vigynana Kendras/Farmer Science Centers) and farmers
- To establish public-private, national and international linkages for strengthening seed production and seed research
- To augment the seed research/production program to make it relevant to the needs of the farming community
- To disseminate information through training on seed production, processing, storage and packaging, quality control and seed health and by conducting demonstrations and field day

The activities of the seed unit are summarized in Figure 2.

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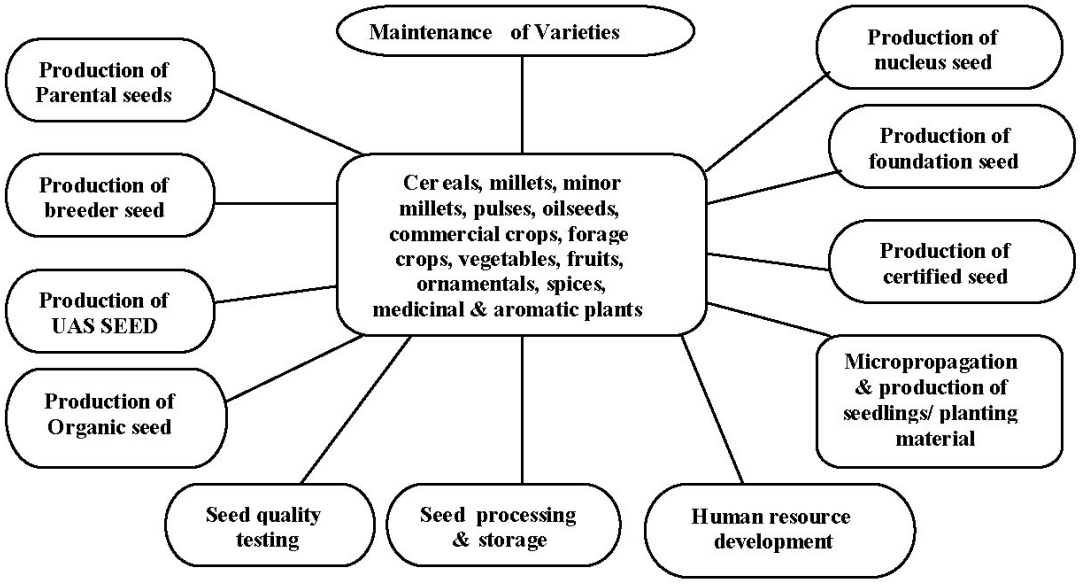

Figure 2. Crops and activities of the UAS Seed Unit

Although India is one of few countries where the seed sector has advanced in parallel with agricultural productivity, the availability of quality seed of improved varieties and hybrids is grossly inadequate and is a major constraint to enhanced production. Studies made by several workers very clearly indicate that with high volume-low value seed crops farmers use farm saved seeds for about 80% of the area sown; the area that represents old and obsolete varieties. This is more so in crops such as peanut, soybean and chickpea where seed costs alone may account for 50% of the total cost of production.

For popularizing newly developed varieties and promoting seed production of these varieties, seed mini-kits of new varieties are supplied to farmers. Seed exchange among farmers and seed producers is encouraged to popularize new/non traditional varieties. Seeds of newly developed varieties are made available to farmers with minimum time gap. In this regard, seed producing agencies are encouraged to tie up with research institutions for popularization and commercialization of these varieties. (Figure 3.)

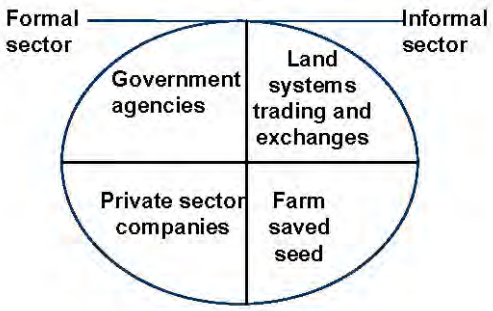

Figure 3. Seed supply systems

"Seed Village" concept

The gap between requirement of quality seeds and supply rate is large. The supply of seeds by public sector organizations and private agencies is insufficient to bridge the gap. The gap must be reduced by involving farmers in quality seed productions. A group of farmers or villages will be identified under the "Seed Village" concept to produce a particular crop/variety. Villages with a potential of producing seeds will be identified near to research stations for easier and higher quality multiplication of varieties of different crops.

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The main objective of the seed-village program is to involve farmers in seed production and thereby to make quality seed available earlier and at a reasonable price. Another objective is to demonstrate and saturate selected potential villages with improved varieties/hybrid seeds of major crops. The implementation of this program by UAS, Dharwad, has been most successful and this concept has been adopted by other institutions throughout India as the "Dharwad Model." Based on the model, UAS produces large quantities of quality seeds. University scientists monitor activities at all stages. The work also provides employment to seed growers and other villagers for seed processing, bagging and distribution.

For implementation of seed production under the Seed Village concept, villages with high potential for production are selected. Before starting the program, villagers are trained and educated about the Seed Village concept and its importance in disseminating improved production technologies and saturation of the area with quality seeds. UAS supplies genetically pure seeds of improved varieties on a credit or exchange basis. In addition, breeders of the respective crops along with Seed Unit scientists visit seed production plots in each village at 10-15 day intervals and provide technical guidance to the farmers. During the crop season, training programs are organized to educate the farmers on seed production skills. Field days and meetings are also organized by inviting all farmers of a village and of nearby villages to make them aware of improved varieties, the importance of quality seed in achieving increased yields, and providing information regarding the availability of seed. Of the seed produced 70-80% is purchased by the University for wider distribution under various government programs; the remaining 20-30%is retained by the producers who are encouraged to distribute the seed to relatives and neighbors within and outside their villages.

Under the Seed Village concept, besides the aim of popularizing improved varieties and production technologies, client oriented seed production is also undertaken. During the production programme, innovative techniques such as seed treatment with bio-agents and pest controls through IPM/bio-agent are addressed to minimize the cultivation cost.

Training on post harvest handling of produce is also encouraged and since scientists regularly visit the seed villages, problems faced by farmers in production of other crops are also addressed. This enables development of close scientist-farmer relationships resulting in transfer of new technologies, including information on varieties/hybrids, and feedback of new problems needing research solutions. In a few villages, the farmers have established Seed Growers Associations to strengthen their seed production activities.

Thus it is possible to improve the quality of farm saved seeds by improving farmers' capacity to produce, process and store quality seeds. The area, production and productivity of wheat in India have continuously grown since 1965 with the development of new high yielding varieties (HYVs), their large-scale seed production and adoption by farmers. More than 378 HYVs of bread, durum and emmer have been released since 1965 by central and state varietal release committees. This has required enormous increases in breeder and quality seed production to make new varieties available for large-scale adoption. It is essential to maintain the seed chain of breeder, foundation and certified seed production by active involvement of public and private seed agencies but with increased farmer participation. This strategy can help in increasing the seed replacement rate to replace old and obsolete varieties grown by farmers from farm-saved seeds. With new and proven seed enhancement techniques (e.g., polymer coating, accelerated aging, priming) it has been possible to boost fast-track transfer new high yielding varieties to farmers' fields and thereby to enhance overall productivity.

Before rust resistant wheat varieties are nationally registered and ready for release, a national strategy should be in place for the multiplication and distribution of quality seed of rust resistant varieties to replace the rust susceptible varieties. Production urgency should not compromise the quality of foundation/certified seeds. Many of the regions will therefore require training and some basic equipment to maximize the yield obtained from early generation seed multiplication. Support will be required for the nation-wide establishment of demonstration plots to popularize the varieties that will be released. Participatory farmer education methods have proved extremely effective in encouraging farmers with strong observations and decision-making abilities. Properly trained farmers will be a major part of implementation of national contingency plans. They can also help in early recognition and reporting of changes in disease severity and pathogen virulence in the field, and in

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understanding the risks associated with virulent strains and the importance of the various field management practices (e.g. planting dates, planting periods, choice of varieties) for disease control and yield improvement.

In order to strengthen national seed systems for rapid multiplication and distribution of resistant varieties, the following activities are emphasized:

- Work with national authorities to popularize adapted rust resistant varieties among farmers through seed campaigns, including field demonstrations
- Strengthen systems for early generation seed multiplication of rust resistant varieties in each country
- Develop and support a strategy for the multiplication and distribution of quality seed of rust resistant varieties to replace rust susceptible varieties through both the public and private sector
- Strengthen the Seed Certification Agency's database through a catalog of released varieties and information on quantities of certified seeds available and expected responses to current pathogen and pest populations
- Reach agreement with national authorities on the establishment of participatory methods such as Participatory Seed Production (PSP) to support wheat farmers in proper field management
- Support to identified or established farmer groups or PSPs through hands-on training/season-long participatory training
- Develop training and reference wheat management guides for farmers and facilitators based on local experience.

Conclusions

UAS, Dharwad has a vision to provide farmers in all the districts of Karnataka with quality seeds of improved varieties/hybrids through selected seed villages from which the seeds will spread to major cultivated areas within the shortest possible periods. This will enhance seed replacement rates and increase productivity of all major crops, including wheat. Karnataka, especially northern areas of the state has diverse climate, soil and other natural resources suitable for seed production and distribution. Potential areas with suitable natural resources can be identified and infrastructurally developed to make them "National Grids" to meet the seed requirements of neighboring states and other national regions, and even for export to other countries.

The rusts of wheat can be successfully controlled in Asia through a combination of strategies. Regional co-operation is essential for monitoring the pathogen populations and presence and movement of particular races. Information on the genetic basis of resistance is necessary will be necessary to maintain genetic diversity in farmers' fields. Traditional and molecular genetic research to further enhance understanding of slow rusting resistance based on minor, additive genes should receive priority. The targeted transfer of durable resistance into widely grown genotypes and the subsequent deployment of those derivatives is an attractive strategy for achieving long-term rust control.

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Capacity development for wheat improvement at CIMMYT

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Keywords: breeding, capacity building, Global Wheat Program, training

Abstract

Wheat breeding in national research programs worldwide deteriorated over time through lack of planning for replacement of senior breeders. Research and infrastructural support for public institutions that train plant breeders, and scientists in related disciplines, has steadily declined over the past three decades. Rapid advances in molecular genetics not only provide unprecedented opportunities to enhance breeding efficiency, but also create new challenges in training breeders with skills to integrate molecular breeding technologies with results from well-designed field experiments. The CIMMYT Global Wheat Program offers unique professional development opportunities for young scientists, particularly in the areas of breeding, genetics, pathology, physiology, end-use quality, and genetic resources. Wheat breeders must possess the research skills and knowledge needed to design and run a sustainable modern breeding program. They must be able to synthesize and utilize knowledge of diverse germplasm and new technologies for wheat improvement, while understanding the interdisciplinary nature of the work and roles of support disciplines. The Global Wheat Program has two annual training programs focused on basic and advanced wheat improvement themes to address the needs of national research programs. Junior and mid-career scientists experience a comprehensive hands-on course on breeding for durable resistance, high yield potential and stability, seed quality, and seed health issues using conventional and molecular tools. CIMMYT believes that well trained young scientists are the key to the future of national research programs. CIMMYT provides two important products: improved genetic materials and trained people.

Introduction

The CIMMYT Global Wheat Program (GWP) has a long and distinguished history of training plant breeders and continues to reinforce training at different levels to address the needs of the world wheat community. CIMMYT experienced a sharp decline in wheat training courses in the 1990s/early 2000s that was mostly due to lack of funding. Since 2005 there has been a priority to re-establish and reinforce the wheat training program. This report will highlight trends in wheat training for the period 2005-2012. This period co-incides with the resurgence of stem rust and the resulting global attention, particularly in regard to the lack of trained scientists with experience in breeding for durable rust resistance within national agricultural research programs (NARS). Over the past seven years we have established two annual training courses in Mexico. These are referred to as the basic and advanced wheat improvement training courses in order to better target and serve the needs of national programs and participating individual sientists. These courses provide hands-on training to junior scientists working with NARS as well as to students pursuing a graduate degree in topics related to wheat improvement. The courses also address the needs of mid-career scientists who desire to keep up with new scientific developments in the field of wheat improvement and related disciplines. CIMMYT is taking a pro-active approach to encourage applied research within national programs, and therefore the basic wheat improvement training course targets the needs of national programs (Kosina et al. 2007), and accommodates requests from collaborating international and national research organizations.

CIMMYT has kept up with its wheat training program despite budget limitations and has trained 168 scientists in wheat improvement and pathology since 2005 (Table 1). The vast majority of participants (57%) were from seven major wheat-producing countries, viz. Pakistan (19), India (18), Iran (16), Turkey (10), Afghanistan (11), China (11) and Ethiopia (11). A sharp decline in training occurred during the period 1988-1998. However, major efforts were made by the wheat program to sustain wheat training over the decade 1998-2007. Trends show that since 2010

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the demand for training in wheat has increased. Regular long term training courses launched in 2010 will be strengthened to reinforce the CGIAR Research Program (CRP) Wheat research agenda and to further accommodate the increasing needs of NARS partners for hands-on basic training in wheat improvement. Courses also include advanced training in selected training modules which are of interest to the wheat research community and will include degree training in association with U.S. landgrant and other universities, as well as advanced research institutions worldwide.

Table 1 Course participants (2005-2012), countries and education levels for advanced and basic wheat improvement course offered at CIMMYT HQs in Mexico

Year	No. countries	No. participants	Educational level		
			PhD	MSc	BSc
2005	4	9	1	2	6
2006	4	7	4	3	0
2007	7	10	6	2	2
2008	6	7	2	4	1
2009	10	17	7	6	4
2010	13	29	10	12	7
2011	19	50	17	26	7
2012	16	17	7	2	8
Total	34	168	54	79	35

The epidemics of *Pgt* race Ug99 in 2005 led to establishment of the Global Rust Initiative (GRI), later named the Borlaug Global Rust Initiative (BGRI). This initiative achieved a global Durable Rust Resistance in Wheat (DRRW) project. Because these events also revealed the lack of trained NARS scientists with experience in breeding for rust resistance, CIMMYT introduced intensive training courses on durable resistance organized at CIMMYT's major wheat breeding station in Obregon where 117 scientists were trained over 11 years (Fig. 1). This is seen as a resurgence of the earlier training programs that were down-sized in the 1990s and early 2000s due to lack of funding.

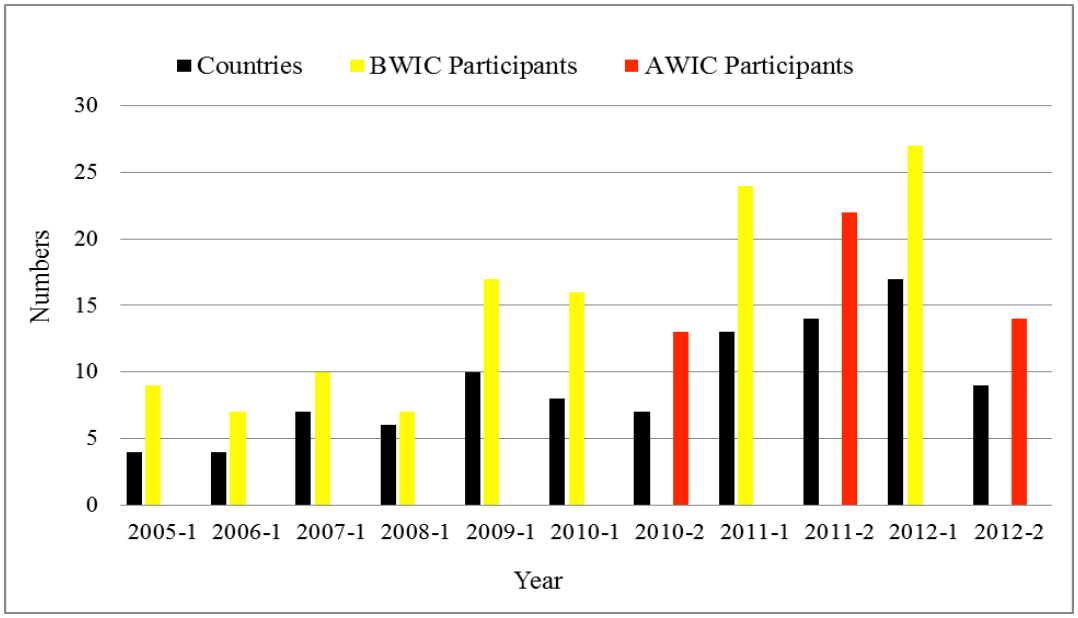

Figure 1 Number of countries and participants attending wheat improvement courses at CIMMYT HQs 2005-2012

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Wheat training has always been a critical area of importance for CIMMYT and will remain so as the demands from NARS for hands-on training courses continue to be high. To add value to the courses, we are transforming them into programs for visiting scientists, and developing advanced training courses tailored to the specific needs of mid-career scientists with different academic and scientific backgrounds. In a broader sense, capacity building also involves the building of partnerships and networks. Increasing numbers of invited lecturers are also participating in CIMMYT courses.

General objectives of the training courses

The objectives are to improve participants' knowledge and skills of the most advanced wheat breeding and pathology technologies, and to understand how these are integrated within scientific disciplines such as agronomy, statistics, physiology, biotechnology, GIS, and the social sciences. The major focus is to increase the participants' understanding of field selection for durable resistance to wheat rusts based on adult plant resistance (APR) and the increasing evidence that it involves resistance to multiple diseases, including resistance to leaf blights and Fusarium head blight (FHB). As much of the program is field-based, participants work closely with CIMMYT wheat breeders and pathologists to enable them to also improve team-work skills and to gain confidence in conducting field experiments. The general objectives of these courses are:

- To impart research skills and knowledge needed to design, manage and execute sustainable modern wheat improvement programs with specific objectives
- To familiarize trainees with new and improved wheat germplasm, CIMMYT's current research and breeding thrusts, and provide opportunities to select wheat materials that will be sent to those participants
- To encourage and develop the participants' ability to synthesize and use information and knowledge about new technologies related to wheat improvement
- To improve participants' awareness of the roles and importance of support disciplines such as pathology, wheat chemistry/quality, statistics, physiology, biotechnology, GIS, and social sciences
- To foster positive attitudinal changes among participants such as improved confidence, increased motivation, and heightened appreciation of the benefits of team work, and interdisciplinary research, and international cooperation

Basic wheat improvement course (BWIC)

The BWIC, or "A-Wheat Improvement Course", is field-oriented and focuses mainly on breeding for yield performance under conditions of biotic and abiotic stress. This course takes place at Cd. Obregon, Mexico, CIMMYT's main wheat breeding station, for a period of about three months. The major focus of the course is on breeding methodologies, with some general reviews on plant breeding, pathology, physiology, wheat quality, and biotechnology. Lectures and seminars cover these aspects.

Participants are also introduced to the agronomy program, including conservation agriculture project activities, irrigation systems, fertilizer management, research station management and seed production. During this course, participants work in groups of 5 to 8 for 3-4 weeks. During the first period, participants are exposed to different breeding projects and associated disciplines (physiology, pathology, pre-breeding, and agronomy) as well as activities of the Mexican national research program (INIFAP) through field visits and participation in national field days organized by INIFAP. Participants are then assigned to specific breeding projects for a period of 5-6 weeks where they follow all project selection activities, and are supervised by project managers.

Advanced wheat improvement and pathology course (AWIPC)

The AWIPC, previously known as the "B-Wheat Improvement Course", targets mid-career scientists at PhD and MSc levels and is conducted on a more interactive level where senior scientists present an overview of research projects and discuss various research approaches. Participants are also requested to make presentations on their research. Field research still involves about 50% of the time where participants (in small groups) conduct field selection activities with CIMMYT scientists on a rotational basis in order to have opportunities for interaction with as many CIMMYT scientists as possible.

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Weekly workshops are organized and managed by participants to discuss important topics such as food security, climate change, breeding for durable resistance, pre-breeding and physiology. Participants are also invited to attend conferences and seminars that take place at CIMMYT Headquarters. This is based at CIMMYT Headquarters (El Batan) and Toluca Experimental Station. The focus of the course is on breeding for disease resistance at Toluca which is subject to high disease pressure (especially stripe rust, Septori tritici blotch and FHB). Participants are also exposed to screening for rust resistance under greenhouse conditions and learn different methods used for race determination of rust pathogens. Participants have the opportunity to get acquainted with laboratory research on pathology, biotechnology, genetic resources and international nurseries.

Profiles of trainees

Candidates for the BWIC are junior scientists (<40 yrs of age) associated with NARS, graduate students, and self-sponsored participants (e.g. from the private sector). Candidates can be MSc or BSc degree holders or high school diploma graduates with more than five years experience. The optimum number of trainees for this course is 20-25.

Candidates for the AWIPC are mid-career scientists (<45 yrs old) including new PhD recruits in national programs, others associated with special projects, and self-sponsored candidates (Fig. 2). The optimal number is about 15, but the course can also accommodate short term visitors working with GWP scientists.

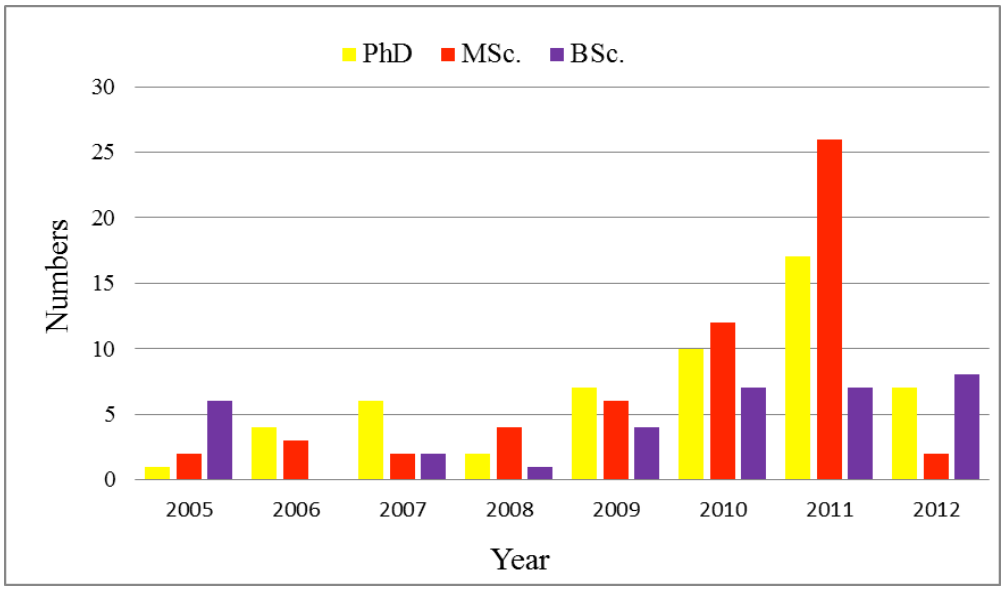

Figure 2 Number of wheat improvement training course participants and education levels

Women in Triticum (WIT) participants

The Jeannie Borlaug Women in Triticum (WIT) Award for early- to mid-career women wheat researchers established in 2010 provides professional development opportunities for women working in wheat. The Award winners are sponsored to participate in a BGRI annual meeting and to join a CIMMYT wheat training course for a period of one month. The WIT award enhances participation of women in wheat improvement, particularly those from developing countries. As shown in Fig. 3 female participation in international courses has been much less than male involvement, although the numbers of young women scientists are increasing within a number of national research programs and hence their subsequent participation in training courses (Fig. 3). Seven Awardees from 2010 and 2011 joined the basic wheat improvement and pathology course in Obregon (March 2011). They came from a range of programs in Brazil, Ethiopia, Sudan, Turkey, and the USA. They included PhD holders involved in agricultural research with universities or national research programs, or were completing PhD research (Fig. 3). They joined the BWIC that was attended by 24 participants from 13 countries (Fig. 3, 2011-1) and included 4 PhD, 15 MSc and 5 BSc graduates. Eight women, including the WIT Awardees, and 16 male participants attended the course. CIMMYT also encourages female participation in its training courses and has

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increased the participation of women in its 2011 and 2012 basic and advanced wheat improvement courses. WIT award winners will join the BWIC every second year. At least 8 participants are expected for the 2013 course.

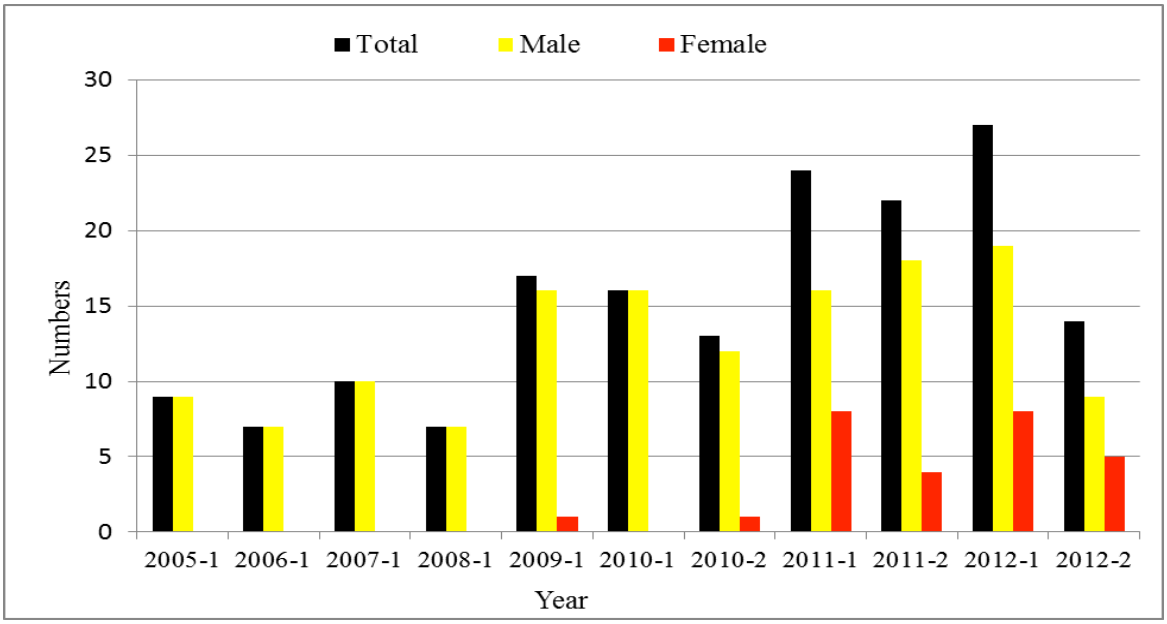

Figure 3 Number of course participants at CIMMYT's HQs and gender (2005-2012)

USDA-Borlaug Fellowship program

CIMMYT in cooperation with the USDA/Foreign Agricultural Service/Borlaug Fellows Program is committed to provide experience and facilities to support 10 Borlaug Fellows from Pakistan for the duration of (1) 3 months during the periods March - May at Ciudad Obregon and El Batan, Mexico, and (2) 2 weeks in late September/early October at Kenya Agricultural Research Institute, Njoro. The Fellows will also spend up to 6 weeks in the U.S. (Minnesota). The major (but not exclusive) objective is to facilitate efforts of Pakistani scientific institutions to minimize the adverse effects of wheat rusts (including the *Pgt* race Ug99 lineage) through surveillance and resistant varieties. This fellowship supports the objectives of the Pakistan Wheat Production Enhancement Initiative. The participants will seek to achieve the following objectives:

Objective 1: Rust pathogen surveillance. To strengthen wheat rust surveillance in Pakistan and to facilitate maximum synergies between Pakistani and international rust surveillance efforts.

Objective 2: Pre-breeding to enhance the diversity and use of rust resistant wheat breeding parents. To enhance access and contributions of Pakistani scientists to the increasingly sophisticated and diverse genetic and laboratory technologies available for pre-breeding.

Objective 3: Accelerated breeding to develop and test rust resistant, high performance candidate wheat varieties. To develop high-yielding candidate wheat varieties with durable rust resistance, as well as to strengthen capacity to develop, test, and rapidly promote such varieties.

Objective 4. Seed multiplication and distribution. To accelerate seed multiplication and improve farmer-access to new rust resistant varieties.

Objective 5: Agronomic management practices. To enhance the productivity and sustainability of rainfed and irrigated wheat systems in Pakistan.

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Future prospects

Ensuring a socially, economically, and ecologically sustainable food and agricultural system requires scientific advancements across the entire range of the discovery-integration-dissemination-application continuum, as identified by Boyer (1990). The CIMMYT wheat improvement program involves a range of disciplines that are becoming increasingly important to NARS. To respond to the needs of our collaborators, approriate shifts in training emphasis may be needed. Many national programs are now refocusing their activities to include wheat bioinformatics, molecular breeding, physiology, seed health issues, and conservation agriculture. The GWP recognizes these needs and will attempt to accommodate training courses to support national programs. The CIMMYT GWP will host highly motivated wheat scientists from developing countries working in NARS research institutions and universities. Based on individual needs, participants with clearly defined learning objectives will be assigned to relevant CIMMYT scientists/tutors in various research areas.

The AWIPC will offer four modules:

Module 1: General training This module will introduce participants to CIMMYT wheat research, generally including (1) Presentations/discussion on CIMMYT GWP research strategies, (2) Field and laboratory visits allowing participants to interact with CIMMYT scientist in various research areas, and (3) Organized workshops on relevant research topics of global importance.

Module 2: Wheat pathology This module will focus on aspects of wheat pathology, including (1) Epidemiology and study of fungal pathogens, (2) Characterization of fungal pathogens (excluding the rusts which are covered in module 3, and (3) Screening for resistance to wheat blights.

Module 3: Wheat breeding Participants are to be integrated with breeding activities carried out by CIMMYT scientists at El Batan and Toluca. This module emphasizes (1) Breeding bread wheat for increased yield potential, quality and durable disease resistance in irrigated and high and low/marginal production areas, (2) Durum wheat breeding for increased yield potential, quality and durable disease resistance, and (3) pre-breeding.

Module 4: Molecular breeding This module targets scientists who are involved in applied biotechnology research within their institutions, and who are looking for further experience in molecular breeding. The module will cover (1) Overview of molecular breeding, (2) Molecular markers, genotyping systems, genetic maps, mapping populations, (3) Marker assisted breeding – theory and practice, (4) Quantitative genetics, genetic variance, heritability, and (5) Data management, dealing with different types of data, data storage and management of data as valuable resources.

The BWIC will continue to focus on applied breeding aspects; the course will be oriented towards problem solving and will focus mainly on breeding methodology and hands-on-comprehensive training in field selection for various traits of global importance. Participants will interact with CIMMYT scientists working on specific projects. CIMMYT scientists and guest lecturers will share expertise, give lectures and discuss various topics ranging across breeding methods, germplasm exchange, yield trials, seed preparation and quality, and statistical analysis. The lectures/discussions will target basic knowledge and special discussions will be organized to respond to special needs beyond the basic information such as regular CIMMYT activities involving trainees, graduate students, staff members, interns, and visitors.

Acknowledgments

Funding support provided from various sources, including USDA, FAO, CIMMYT Special Projects (IWWIP, CSISA, Iran, Afghanistan), Cornell University DRRW project, national programs of Egypt, Paraguay, Brazil, Argentina, Italy, and China, as well as Universities sponsoring graduate students, mainly the Beachel-Borlaug scholars program, has permitted training of 168 junior and mid-career scientists from 34 countries over the past decade. CIMMYT also recognizes the contributions of its scientists and experts from various countries to both courses. They include CIMMYT scientists from both Headquarters and regional programs, and visiting scientists from other institutions, including Bob McIntosh, Perry Gustafson, Alan Roelfs and Art Klatt.

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Exploiting pathogen biology for disease resistance breeding in plants

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Eukaryotic plant pathogens, such as oomycetes and fungi, cause highly destructive diseases that negatively impact commercial and subsistence agriculture worldwide. One of the most notorious plant pathogens is *Phytophthora infestans*, the causal agent of potato and tomato late blight. The most sustainable strategy to manage late blight is to breed broad-spectrum disease resistance into potato and tomato. However, current disease resistance breeding approaches are slow and inefficient, and have taken little advantage of emerging knowledge of pathogen mechanisms. Resistance genes have been identified, bred, and deployed in agriculture without detailed knowledge of the effectors they are sensing—a "blind" approach. This presentation will illustrate how state of the art findings on pathogen biology can be exploited to drive the development of new approaches to breeding disease resistant crops with examples from the *Phytophthora infestans-Solanum* pathosystem. Furthermore, I will demonstrate how the current knowledge on effectors can be exploited for the characterization of effectors in the genome of recently sequenced rust fungi, the first step towards identifying novel resistance components for deployment in agriculture.

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How has Lr34/Yr18 conferred effective rust resistance in wheat for so long?

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Keywords: ABC transporter, durable resistance, leaf rust, leaf tip necrosis, multi-pathogen resistance, stem rust, stripe rust

Abbreviations: ABC transporter: ATP-binding cassette transporter, LR34: the protein encoded by the *Lr34* gene, LTN: Leaf tip necrosis, NBD: nucleotide-binding domain, TMD: transmembrane domain

Abstract

The *Lr34/Yr18* gene has been used in agriculture for more than 100 years. In contrast to many other resistance sources against leaf rust and stripe rust, it has remained effective and no virulence has been reported. This makes *Lr34* a unique and highly valuable resource for rust resistance breeding. The pleiotropic nature of the gene conferring partial resistance to different pathogen species, the associated leaf tip necrosis and its durability suggest a molecular mechanism that is different from major gene resistance. This is supported by the molecular nature of *Lr34* which was recently found to encode an ABC transporter. Interestingly, all tested wheat lines contain an allele of the *Lr34* gene on chromosome 7DS. In its susceptible form, the gene does not confer resistance. The difference between the encoded resistant and susceptible LR34 isoforms consists of only two amino acid changes, whereas the rest of the proteins are identical. These two changes must change the biochemical properties of the resistant LR34 transporter in such a way that the plant becomes resistant. We speculate that there is a slight conformational change in the resistant form of the protein, resulting either in modified specificity or kinetics of the transported molecule, or that the binding properties to an unknown second protein interacting with LR34 are changed, resulting in altered function. While the molecular nature of the molecule(s) transported by the LR34 protein remains unclear, it is likely that a physiological change related to *Lr34* activity is at the basis of resistance. We are currently establishing transgenic approaches in heterologous grass species to further investigate the molecular activity of *Lr34* and to better understand a physiological mechanisms resulting in disease resistance.

Introduction

The *Lr34/Yr18/Sr57/Pm38* gene is a globally important genetic resource for wheat resistance breeding against leaf rust and stripe rust (Fig. 1). It has also been shown to confer partial resistance to powdery mildew, and in some genetic backgrounds to stem rust. The locus has further been associated with tolerance to barley yellow dwarf virus. *Lr34* has been widely used in wheat cultivars worldwide and its resistance has remained effective over many years (possibly more than 100), making it one of the few known resistance genes with a generally large effect and durability despite large-scale, agricultural use. The gene was first described as *LrT2* (Dyck 1977, 1987) and later identified in several distinct groups of genetic material, e.g. CIMMYT lines, Chinese landraces and Eastern European winter wheat material (Kolmer et al. 2008; Krattinger et al. 2009). It is likely to have originated in Chinese landraces, but its use in the modern wheat breeding gene pool goes back to the Italian wheat cultivars Mentana and Ardito which were released at the beginning of the 20th century (Borghi 2001). These two lines, noted for their great adaptability and disease resistance, were also introduced to South America after the 1920s where they were widely cultivated and used in breeding (Vallega 1973). Based on lines developed there (e.g. Frontana in Brazil), the gene spread into additional lines, and was further distributed and later integrated into CIMMYT cultivars and breeding lines. Another use of the gene occurred in (Eastern) European winter wheat material, notably derivatives of the cultivar Bezostaya (Krattinger et al. 2009). The *Lr34/Yr18* gene acts quantitatively, i.e. it confers only partial resistance and generally has to be used in combination with other quantitatively acting resistance genes to provide sufficient resistance under heavy disease pressure. *Lr34* is active specifically in the adult plant stage and the flag leaf is usually evaluated in assays of plants containing the

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gene against leaf and stripe rust as well as powdery mildew (McIntosh 1992; Singh 1992; Spielmeyer et al. 2005). *Lr34/Yr18* activity is correlated with leaf tip necrosis (LTN) on the flag leaf which can be used as a phenotypic marker for *Lr34/Yr18* and which is also expressed in the absence of the pathogen (Fig. 1C; Dyck 1991; Lagudah et al. 2006). However, the expression of LTN is environmentally dependent and can vary greatly in different environmental conditions and genetic backgrounds. *Lr34/Yr18* was cloned by bi-parental mapping and positional cloning using integrated molecular marker information from several crosses as well as physical maps from the D genome of *Aegilops tauschii* and hexaploid wheat (Krattinger et al. 2009). Based on the knowledge of the *Lr34/Yr18* gene sequence, gene-specific markers were developed and have proven to be highly diagnostic for the *Lr34* gene (Lagudah et al. 2009; Dakouri et al. 2010). The analysis of eight independent mutants revealed that the same gene is in fact responsible for leaf and stripe rust resistances as well as LTN (Krattinger et al. 2009). Henceforth, we will refer only to *Lr34*, keeping in mind that it is a multi-pathogen resistance gene with a pleiotropic effect of leaf tip necrosis.

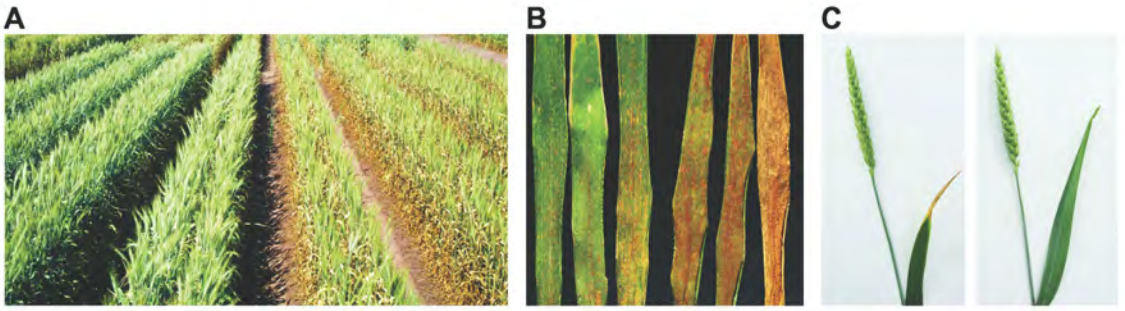

Figure 1 Phenotype of the *Lr34/Yr18* gene. (A) *Lr34* confers partial resistance. On the left there are rows of the resistant genotype Jupateco R and on the right of the susceptible near-isogenic genotype Jupateco S infected with leaf rust in Mexico. (B) Development of leaf rust on three successive leaves from the flag leaf of Jupateco R (left) and Jupateco S (right). (C) *Lr34* is associated with leaf tip necrosis shown here on a near-isogenic line 'Arina Lr34' (left) but not on the parental wheat cultivar 'Arina' which does not carry the *Lr34* gene (right). From Krattinger et al. (2009)

The molecular nature of *Lr34/Yr18*

The *Lr34* gene encodes a full-size ATP-binding cassette (ABC) transporter of the ABCG type (formerly called PDR transporter, for pleiotropic drug resistance). In contrast to half-size transporters (see Fig. 2), full-size transporters are thought to act as monomers in plant membranes. ABC transporters are integral membrane proteins that translocate molecularly diverse substrates across cell membranes. The substrates of many ABC transporters are still unknown. In addition, there is considerable mechanistic diversity of molecular transport functions within this large protein super-family (Lewinson et al. 2010). The typical eukaryotic ABC transporters are efflux transporters.

A common feature of ABC transporters is that they consist of two distinct domains, the transmembrane domain (TMD) and the nucleotide-binding domain (NBD). The TMD, also described as a membrane-spanning or integral membrane domain, consists of alpha helices embedded in the membrane bilayer. The sequences of TMDs are variable, and this variability is possibly one reason for the chemical diversity of substrates transported. The NBD or ATP-binding cassette (ABC) domain is cytoplasmic and more highly conserved. A characteristic of the NBD domain is its ATP binding. It is the hydrolysis of ATP that powers the substrate transport. In the LR34 protein the N-terminal NBD and the C-terminal transmembrane domains form a single polypeptide chain, arranged as NBD-TMD-NBD-TMD.

The three-dimensional structures of ABC transporters are predominantly derived from prokaryotic proteins so these are currently used as models for plant proteins. In prokaryotes, most exporters, such as the multidrug exporter Sav1866 (Dawson and Locher 2006) from *Staphylococcus aureus*, are made up of a homodimer (Fig. 2). There is no molecular structure yet for the plant/fungal specific ABCG/PDR type of transporters. Thus, molecular modeling is dependent on some of the bacterial ABC transporters for which the structure is known. For this reason such interpretations must be met with caution.

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The molecular analysis of the *Lr34* gene in different wheat varieties and grass species (for further evolutionary aspects also see below) has given some relevant insight into the molecular diversity. Importantly, wheat has an allohexaploid genome and we first analyzed the presence of homoeologous genes of *Lr34* on the A and B genomes (*Lr34* is located in the D genome). It was found that there is an active, expressed *Lr34*-related gene showing 97% homology at the amino acid level in the B genome, whereas the homoeolog on the A genome is inactive as it is disrupted by several transposon insertions (Krattinger et al. 2011). Thus, normal hexaploid wheat has two expressed and closely related *Lr34*-type of genes, whereas tetraploid wheat only has the B-genome copy. The most interesting finding came from the analysis of the *Lr34* gene in lines with known presence and activity of *Lr34* vs. lines lacking *Lr34*-conferred resistance. Lines without the *Lr34*-type of resistance had a closely related allelic form of *Lr34* which was called *Lr34sus* (for susceptible). In semi-quantitative comparative analysis, no expression differences were found for the two closely related alleles with largely identical promoter regions (Krattinger et al. 2009). In fact, in the complete protein with 1,401 amino acids, there are only two differences resulting in amino acid changes between the proteins encoded by *Lr34* and its susceptible allele. These are a deletion of three base pairs in exon 11 of the resistance allele resulting in the loss of a phenylalanine residue predicted to be in a transmembrane region (see Fig. 2), and a single base pair change in exon 12 converting a tyrosine to a histidine at a location that is probably localized at the cytoplasmic end of a transmembrane helix (Fig. 2).

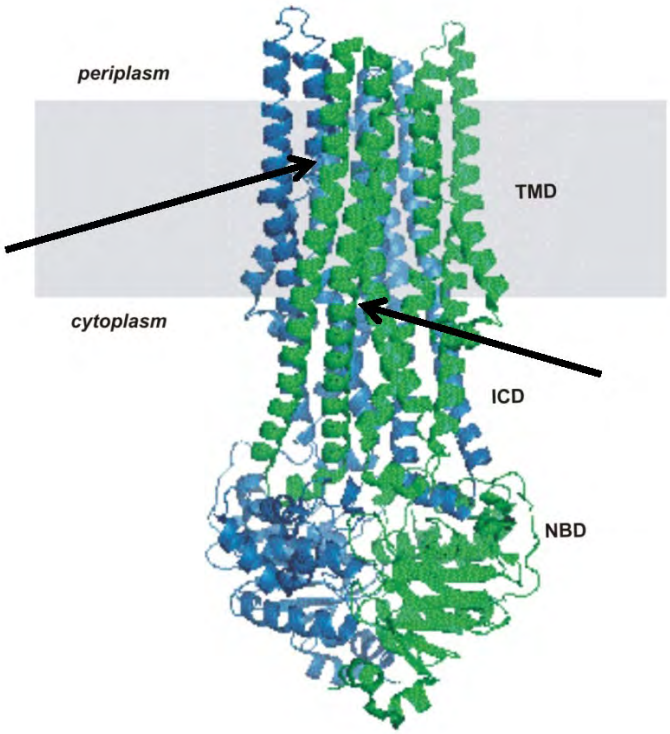

Figure 2 Structure of the ABC exporter Sav1866 from *Staphylococcus aureus* with bound nucleotide (Dawson and Locher 2006). Source: http://en.wikipedia.org/wiki/ATP-binding_cassette_transporter. Most exporters in prokaryotes, such as the multidrug exporter Sav1866, are made up of a homodimer consisting of two half-size transporters. The protein structure of the LR34 protein is unknown. However, as a full-size transporter (predicted to be active in a monomeric form), the LR34 protein certainly has a different conformation compared to the protein shown here. Nevertheless, the overall protein domain structure is expected to be similar, allowing us to derive hypotheses by protein modeling based on the protein structures of Sav1866 and related ABC transporters for which the molecular structures are available. Approximate positions of the two specific amino acid residues related to LR34

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function are indicated by arrows (deletion of phenylalanine, left arrow). TMD: transmembrane domain; ICD: intracellular domain; NBD: nucleotide-binding domain

Using transgenic approaches we are currently determining whether both polymorphisms are necessary for the resistance activity or whether one of the two changes is sufficient for the observed gain-of-function in the LR34 protein. Dakouri et al. (2010) studied the genetic diversity of the *Lr34* gene in a broad set of wheat germplasm. Among 700 wheat accessions, two lines were particularly of interest as they both had an allelic version of *Lr34* which only had the polymorphism resulting in the loss of a phenylalanine residue (characteristic for the *Lr34* gene), but not the second polymorphisms. Both these lines were described to have a susceptible phenotype after infection with leaf rust. Thus, these observations based on genetic diversity indicate that either the His to Tyr polymorphism is sufficient for *Lr34* resistance activity, or that both polymorphisms are required for resistance activity. However, the phenotyping of these two critical cultivars needs to be confirmed. In addition, it has to be established if the *Lr34* genes in these two lines are expressed and each encodes a full-length functional protein. Thus, it is important to determine if there are other polymorphisms in the *Lr34* genes of these lines, possibly resulting in an inactive gene as, for example, was found in cultivar Jagger (Lagudah et al. 2009; Cao et al. 2010) before any conclusions on protein structure – function can be made. Transgenic forms of the gene with the individual mutations will further contribute to clarification of this issue and will help to characterize the functional differences between the two allelic forms of *Lr34*. We conclude that the crucial change of function from a normal, susceptible allele of *Lr34sus* to the *Lr34* resistance gene is based on maximally two amino acid changes. These changes must result either in a change of molecular specificity of the transporter protein, a modification of the binding affinity for the substrate, or a change in the binding activity of LR34 to an unknown interacting protein, resulting in the observed effects in resistance as well as LTN (Spielmeyer et al. 2008; Krattinger et al. 2009).

Lr34/Yr18 compared to other cloned leaf rust or stripe rust resistance (*R*) genes

Three of the major leaf rust resistance genes described in the wheat gene pool have been cloned: *Lr1* (Cloutier et al. 2007), *Lr10* (Feuillet et al. 2003) and *Lr21* (Huang et al. 2003). They all encode coiled-coil, nucleotide-binding site, leucine-rich repeat (CC-NBS-LRR) proteins, a well-known class of plant resistance proteins. Their molecular modes of action resulting in leaf rust resistance are not yet known. However, based on knowledge mostly gained from molecular studies in Arabidopsis and flax resistance proteins, it is likely that they directly or indirectly recognize effectors of the fungal pathogen. As *Lr34* encodes a protein of a completely different class to the other cloned *Lr* genes, it is likely that it also has a completely different function. The leaf rust pathogen can overcome the classical *Lr* genes (such as *Lr1*, *Lr10* and *Lr21*), the different mechanism of action of *Lr34* might account for its durable resistance. No major gene against stripe rust (*Yr* genes) has yet been isolated. The cloned *Yr36* is a temperature-dependent resistance gene with no race specificity detected to date (Fu et al. 2009). *Yr36* encodes a protein with an N-terminal kinase domain a C-terminal lipid transfer (START) domain. Thus, the protein structure and possibly molecular mode of action is very different from *Lr34*.

The physiology of *Lr34* action

As described above, we assume that the LR34 protein transports a molecule which results in a defense response against multiple pathogens, as well as in leaf tip necrosis. The expression of the *Lr34* gene is regulated during development and is higher at later stages of plant growth which is in agreement with the adult plant resistance conferred by *Lr34* (Krattinger et al. 2009; Risk et al. 2012). Gene regulation under developmental control was also observed in wheat lines with a transgenic *Lr34* gene under control of its native promoter (Risk et al. 2012). The work on transgenic wheat lines was done in the genetic background of the easily transformable cultivar Bobwhite. Transformation of the genomic *Lr34* fragment (containing native promoter and terminator sequences) has confirmed that this sequence is sufficient to confer leaf rust resistance as well as LTN. Interestingly, it was observed that the transgenic *Lr34* resistance gene can result in a broader developmental range of resistance activity. In one particular genetic background, resistance was already observed at the seedling stage (Risk et al. 2012). It is not clear if this enhanced resistance is based on a slightly altered expression level and/or developmental stage of gene expression, or if it is simply an additive effect of *Lr34* with other genes present in the particular genetic background of the transformed line. In any case, this is a clear demonstration

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that the transgenic use of *Lr34* might be of practical breeding interest at least in some genetic backgrounds. It will be interesting to define in more detail the genetic components that are responsible for the additive effect of transgenic *Lr34*.

Biochemical and cytological approaches were used to study the transgenic *Lr34*-based resistance. These observations were compared with the endogenous resistance based on *Lr34*. In both the near-isogenic Thatcher line with *Lr34* as well as the transgenic lines with *Lr34*, the presence of *Lr34* did not increase pathogenesis-related (*PR*) gene induction in flag leaves to a significantly higher level (although there was a trend in that direction) after pathogen infection (Risk et al. 2012). Furthermore, callose induction which is associated with infection sites in rust-infected flag leaves was studied. However, even in the transgenic lines with increased resistance, there was no apparent alteration of callose deposition in flag leaves seven days after infection. It was concluded that all the lines had an equivalent capacity to deposit callose. Finally, no increased production of reactive oxygen species was found in *Lr34* transgenic lines. These findings are in accordance with work done by Rubiales and Niks (1995) who concluded that *Lr34* based resistance is not conferred by a hypersensitive response. All these data provide evidence that resistance in *Lr34* lines is not based on hypersensitive-response like defense mechanisms which are characteristic of the reactions of major resistance genes. Rather, a completely different type of resistance must underlie *Lr34*-based resistance.

Several intriguing observations were made concerning the molecular basis of LTN, either in lines with the endogenous *Lr34* gene and/or in lines transgenic for *Lr34*. First, it was found that the senescence-associated gene *HvS40* was induced in flag leaves of *Lr34*-containing lines when compared to lines without the gene. Further evidence for senescence-like processes involved in leaf tip necrosis came from the observation of non-fluorescent chlorophyll catabolites (NCCs) specifically in lines with *Lr34* (Krattinger et al. 2009; Risk et al. 2012). It is known that the production of NCCs is a highly controlled process in senescence, and it seems that *Lr34* can induce such processes prematurely. Importantly, this would not interfere negatively with the normal growth process relevant for agronomic parameters such as time to maturity and yield, at least in relevant agricultural environments.

Two large-scale transcriptomic studies analysed the global gene expression patterns in lines with *Lr34* compared with lines without *Lr34*, both when non-infected and after leaf rust infection (Hulbert et al. 2007; Bolton et al. 2008). Microarrays, based on 55,052 transcripts, were used for these studies. In mock-inoculated leaf tips of flag leaves with *Lr34* a total of 57 transcripts were consistently up-regulated in two different cultivars containing *Lr34*. The genes with higher expression levels in the *Lr34* genotypes are known to be ABA-inducible and related to responses to osmotic stress, cold stress and/or seed maturation. These genes are typical for plant reactions to abiotic stress. No *PR* gene induction was found in mock-inoculated leaves. After inoculation, *PR* genes were induced in both resistant and susceptible flag leaves, but expression was generally higher in resistant plants (Hulbert et al. 2007). Based on these data, the authors suggested that *Lr34* might in some way "prime" the plant for a stronger induction of defense responses. In the second study (Bolton et al. 2008), the authors identified a coordinated upregulation of key genes in several metabolic pathways. They speculated that there is an increased carbon flux particularly through the tricarboxylic cycle, resulting in a high energetic demand for the *Lr34*-based resistance in the plant.

Lr34 resistance probably does not depend on recognition of fungal effectors

One of the characteristics of *Lr34* is the improved resistance against several pathogens (leaf rust, stripe rust, powdery mildew, BYDV, and at least in certain genetic backgrounds, stem rust). *Lr34* can therefore be described as a multi-pathogen resistance gene. There are several other, cloned plant genes that have been shown to confer resistance against several diseases/pests. One of them is the *Mi* gene from tomato, which confers resistance to root-knot nematodes (Milligan et al. 1998), whitefly (Nombela et al. 2003) and aphids (Rossi et al. 1998). *Mi* encodes an NBS-LRR protein and likely guards a plant protein target that is modified by effectors of all three different organisms, similar to other proteins which confer resistance to several different pathogen species. Given the protein structure of LR34 it is unlikely to be a protein guarding a common effector target. Rather, we can assume that the molecular mechanism of resistance is completely different from other multi-pathogen resistance genes such as *Mi*.

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Evolutionary aspects and origin of *Lr34* in the grass gene pool

Evolutionary studies on the origin and divergence of genes can assist the formulation of hypotheses on gene function in different species. The *Lr34* gene seems to be specific to the grass family, as it has not been identified in a clearly orthologous form in other plant or fungal species. *Lr34* orthologs were identified in rice and sorghum (Krattinger et al. 2011). The rice ortholog has an identity of more than 80% at the amino acid level compared to LR34. Interestingly, barley is a species closely related to wheat but lacks an *Lr34* ortholog, as do maize and Brachypodium (Krattinger et al. 2011). It seems likely that all these species have independently lost the *Lr34* ortholog, whereas wheat, rice and sorghum have retained the gene. This indicates that *Lr34* can give a selective advantage under certain conditions, but that the loss of the gene is not detrimental and species can further evolve without it. A closer inspection of the rice and sorghum *Lr34* orthologs revealed that the two critical polymorphic amino acid residues for a functional resistance protein were identical to the susceptible isoform of LR34. In addition, the LR34 protein encoded by the B-genome homoeolog in wheat (see above) also carries the amino acids of the susceptible form of LR34. We conclude that the functional *Lr34* resistance gene only evolved on wheat chromosome 7D and that all the characterized orthologs in grass species (including those from the D-genome donor *Aegilops tauschii*) have the "susceptible" haplotype (no deletion of phenylalanine 546 and a tyrosine at position 634). This indicates that the critical mutations resulting in the gain-of-resistance-function in LR34 occurred after domestication and formation of hexaploid wheat (Krattinger et al. 2011).

Conclusions and next steps towards understanding *Lr34/Yr18* durable resistance

At this stage there are two essential questions on *Lr34*-based resistance which need to be answered: first, what is the molecular nature of the molecule transported by LR34 and how does the resistant form of the transporter differ in structural and biochemical properties from the susceptible form? Second, what are the molecular consequences of this transport/the transported molecule that result in durable, multipathogen resistance?

As indicated by LTN which is independent of pathogen infection, the *Lr34* gene also has an effect on plant physiology in the absence of the pathogen. Thus, it is possible that the senescence-like processes, induced by *Lr34*, simply make the tissue less conducive for a biotrophic pathogen. This might be caused by a limited availability of nutrients and/or by an accumulation of growth-inhibiting substances. Both effects would result in a quantitative limitation of fungal growth, explaining the partial and limited resistance effect in wheat. These metabolic changes would not result (at least in some relevant environments) in a yield loss or any other drastic negative consequences for growth of the wheat plant. Such a metabolic change would be deeply rooted in the physiology of the plant and could not be overcome easily by the pathogen, possibly explaining the durability of the *Lr34* resistance. Such resistance does not fit into the current models of effector and PAMP-triggered immunity and would thus represent a new type of molecular mechanism.

In an alternative model, based on transcriptomics studies, it can be envisaged that the activity of *Lr34* results in some form of priming of the plant revealed by a quantitatively higher, but not qualitatively different, induction of defense genes after infection in wheat genotypes containing the *Lr34* allele. This priming might include components from systemic acquired resistance or more likely from induced systemic resistance (ISR, Hulbert et al. 2007). It is known that ISR can be stimulated in Arabidopsis by priming, e.g. by the application of β-amino-butyric acid (Ton et al. 2005), demonstrating that a relatively simple chemical compound can result in such a primed state. However, for grasses in general or wheat specifically, no such mechanisms or phenomena have yet been described. Chemically induced resistance is known in wheat, but the genes induced there were not found to be induced in *Lr34*-containing genotypes (Hulbert et al. 2007). There are some testable consequences from the priming hypothesis, and this will represent a major research area for future experiments to understand *Lr34* gene function.

Based on the two mutations in *Lr34* resulting in an active resistance gene we propose that the evolution of the specific resistant haplotype was a unique event in evolution. This raises the question of whether such an event could be experimentally designed to enable additional durable resistance sources. However, based on the discussion above our current understanding of the underlying molecular mechanisms is still too meager to

rationally design new forms of resistance similar to *Lr34*. Thus, it is highly important and urgent to improve our knowledge on the molecular mechanisms of quantitative resistance. There are at least three areas of research which should be pursued with high priority and which will be highly relevant to reach this goal: first, there is a need to isolate additional durable resistance genes/QTL against rust diseases from wheat lines known to carry such resistances. Second, at CSIRO and UZH we are using heterologous systems transgenic for *Lr34* to study the effects of this gene in other species and to identify biological activities of *Lr34* in those genetic backgrounds. Third, it will be essential to genetically identify components of *Lr34*-based resistance to understand the molecular pathways resulting in resistance. All these approaches should finally allow us to make a big step forward in understanding quantitative, durable resistance.

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Pros and cons of utilizing major, race-specific resistance genes versus partial resistance in breeding rust resistant wheat

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Keywords: non-specific resistance, slow rusting, specific resistance, *Triticum aestivum*

Abstract

Rust control to achieve stable wheat production has been a challenge to crop scientists for over a century. The occurrence of large-scale epidemics, common in the first half of the 20th century, has decreased due to an improved understanding of disease epidemiology, the genetic basis of host-pathogen interactions, use of for diverse resistance genes, and the development of cultivars with rust resistance. The major, race-specific resistance genes (R-genes) continue to be utilized improperly to control rapidly evolving pathogens like wheat rusts, resulting in "boom-and-bust" cycles requiring cultivar replacement, often shortly after release. Although it is well known that utilization of at least two effective R-genes in combination can enhance resistance durability, the strategy is rarely implemented in the correct way when breeding or releasing varieties because rust resistance is just one of the traits required for a successful variety. Adequate to high levels of durable resistance can also be achieved through pyramiding 4-5 minor, slow rusting, or partial, resistance genes (PR-genes) that confer inadequate resistance when present alone. Breeding strategies to utilize combinations of major R-genes or pyramided multiple PR-genes with other desirable traits must be a primary goal of breeding programs aimed at achieving long-term control of wheat rusts while ensuring stable food production gains and protecting the environment. Both strategies have pros and cons and success depends on the availability of diverse resistance in adapted genetic backgrounds along with greenhouse, field and/or molecular facilities for selection. Because it is naïve to believe that all breeding programs, whether public or private, will follow the best practice of utilizing effective major R-genes in combinations, breeding for multiple PR-gene based resistance is considered a superior strategy for achieving durable resistance.

Resistance in wheat to rust pathogens

Variability in response of wheat to rust pathogens is continuous and ranges from immunity, or the lack of visual symptoms, to only slight reductions in disease severity and/or host reaction. Race-specific, or R-gene resistance tends to involve higher resistance levels and is recognized by a diverse range of hypersensitive reactions as described in Roelfs et al. (1992) and McIntosh et al (1995). The most studied and well characterized R-genes are those whose effects are easily phenotyped in greenhouse tests on seedlings or adult plants (McIntosh et al. 1995, 2003). Several small effect R-genes remain uncharacterized but can be found in breeding materials. The R-genes follow the "gene-for-gene" relationship as proposed by Flor (1956).

Niederhauser et al. (1954) used "partial resistance" terminology while working in Mexico with resistance to late blight of potato in wild species. In field trials they found that partially resistant varieties showed a degree of resistance that is exhibited equally toward all races of a pathogen and leaves of partially resistant varieties remained green longer than varieties without partial resistance. R. M. Caldwell of Purdue University, USA, elucidated the importance of breeding for slow rusting resistance in wheat (Caldwell 1968). He stated that slow rusting resistance involved mechanisms such as exclusion of the fungus, limited pustule size without hypersensitivity, or slow growth and development of the fungus. The joint action of these host characters may retard disease epidemics to a point of insignificance. Parlevliet (1975) used the term "partial resistance" to characterize slow rusting to leaf rust in barley. He maintained that partial resistance is a form of incomplete resistance characterized by a reduced rate of epidemic development despite a high or susceptible infection

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type. Slow rusting and partial resistance, abbreviated as PR in this paper, are synonymous terms and describe the same type of response.

R. Johnson of Cambridge, England, described the presence of durable resistance to yellow rust in winter wheat variety Cappelle Desprez. This moderate level of adult plant resistance had remained effective for over 20 years in the UK and such resistance was recognized as being durable. Durable resistance as defined by Johnson (1984) is resistance that has remained effective in a cultivar during its widespread cultivation for a long sequence of generations or period of time in an environment favorable to a disease or pest. This term has received wide acceptance and is popularly used in the literature to describe lasting resistance to diseases and pests.

Characterized slow rusting resistance genes are few in number, usually associated with longer latent period, reduced uredinia (pustule) size and frequency (severity), and usually not associated with hypersensitivity in the cases of leaf rust and stem rust. However, the systemic growth habit of the yellow (stripe) rust pathogen often leads to chlorosis and necrosis in stripes possibly due to overexploitation of infected cells by the pathogen, leading to their death (Singh et al. 2011b). The characterized slow rusting resistance genes confer moderate to inadequate resistance under high disease pressure and often have pleiotropic effects on multiple rust diseases. Cloning of the well characterized pleiotropic resistance gene *Lr34/Yr18/Sr57/Pm38/Sb1/Bdv1/Ltn1* showed it belonged to the ABC-transporter group and was distinct from cloned race-specific resistance genes (Krattinger et al. 2009).

Diversity for all types of resistance, large to small effect R-genes and intermediate to small effect PR-genes can be found in breeding materials and commercial varieties. Often the presence of small effect genes remains unrecognized in a variety as large effect R-genes are preferred targets for postulation studies and genetic analyses. R-genes are used widely by breeding programs worldwide due to the ease of identifying resistant plants. Although targeted utilization of PR genes is limited to only a few breeding programs, it is common to find such resistance genes dispersed in various improved wheat materials possibility because they enhance the expression of R-genes under high disease pressures in the field, and therefore the human quest to select cleaner plants favors their selection. The most successful large-scale and historical example of the utilization of PR is probably the *Sr2*-based durable stem rust resistance in North America (including CIMMYT) and Australia (McFadden 1930; McIntosh et al. 1988; Rajaram et al. 1988, Singh et al. 2008). The CIMMYT effort to breed PR-gene based leaf rust and yellow rust resistant wheat varieties over three decades and against Ug99 stem rust during recent years has been notable (Singh et al. 2008, 2011a, b).

Wheat breeding objectives

The foremost objective of most, if not all, breeding programs is continued improvement of grain yield to meet current and future demands for food. To achieve this objective, breeding programs need to have multiple but simultaneous goals. The CIMMYT international spring wheat breeding program based in Mexico is no exception and also focuses on specific traits (Table 1). Five traits are considered core traits and ideally all germplasm distributed by CIMMYT must possess them. Traits like yield potential, water-use efficiency or drought tolerance, heat tolerance and end-use quality are highly complex and need to be complemented with durable resistance to all three rusts and other diseases or pests to achieve more productive, climate-resilient, farmer-friendly wheat varieties. Progress in breeding for grain yield is slow worldwide. CIMMYT has delivered about a 1% annual yield increment in germplasm distributed through the "Elite Spring Wheat Yield Trial" during the last 15 years (Sharma el al. 2012).

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Table 1 Traits that receive high focus by the CIMMYT spring bread improvement program

Traits for improvement
I. Core traits (ideally should be present in all CIMMYT wheat germplasm)
1. High and stable yield potential
2. Durable resistance to rusts- stem (Ug99), stripe and leaf
3. Water use efficiency/drought tolerance
4. Heat tolerance
5. Appropriate end-use quality
II. Supplementary traits for specific mega-environments (MEs)
A. Durable resistance to diseases and pests
1. Septoria leaf blight (ME2/4-high rainfall/dry rainfed areas)
2. Spot blotch (ME5-optimally irrigated, warmer areas)
3. Tan spot (ME4)
4. Fusarium – scab and myco-toxins (ME2/4/5)
5. Karnal bunt (ME1-optimally irrigated areas)
6. Root rots and nematodes (ME2)
B. Enhanced Zn and Fe concentrations (ME1/5)

Pro and cons of utilizing R- and PR-genes in wheat breeding

Race-specific resistance

Breeding resistant varieties protected by single large-effect R-genes is the simplest strategy to follow as the dominant, or partially dominant, nature of these genes eases their selection and a high frequency of resistant plants with appropriate agronomic characteristics can be found in field trials. Because selection is based on near-complete resistance, even poor disease epidemics in field nurseries are sufficient to select resistant plants. Selection can also be made in greenhouse trials and resistant plants can be advanced to the next generation if methods like single-seed-descent are employed to accelerate breeding. R-genes located on alien translocations are often preferred targets for marker-assisted selection (MAS) due to the availability of linked molecular markers and the fact that any marker in an alien segment is a perfect marker for a resistance gene located within the segment.

All three rust pathogens undergo mutation and once virulence is present it is readily selected on corresponding previously resistant genotypes. The R-genes are known to have a short life, often 3-5 years (or even less) as shown for bread wheat varieties grown by farmers in northwestern Mexico during the 1970s and 1980s, and for durum varieties in the 2000s (Table 2). Races carrying new virulences can establish and survive on volunteer wheat or other accessory hosts during the off-season, and become air-borne over long distances. Continuous and relevant monitoring of pathogen populations for avirulence/virulence to R-genes deployed in commercial varieties or under utilization in breeding programs is essential in each country or epidemiological region, and even worldwide, as long distance, unpredicted and unexpected migration of new races is becoming more common (Hovmøller et al. 2008, 2010). Therefore, good communication among geneticists, pathologists and breeders is necessary to ensure that new virulences, or virulence combinations, are detected in a timely manner, their relevance to breeding assessed properly, and relevant races are used for selection by breeding programs.

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Table 2 Examples of "boom-and-bust" cycles involving deployment of wheat varieties with race-specific genes for leaf rust resistance in Northwestern Mexico

		Year		
Variety	Resistance genes	Released	Breakdown	Race
Bread Wheat:				
Yecora 70	*Lr1, 13*	1970	1973	?
Tanori 71	*Lr13, 17*	1971	1975	?
Jupateco 73	*Lr17, 27+31*	1973	1977	TBD/TM
Genaro 81	*Lr13, 26*	1981	1984	TCB/TB
Seri 82	*Lr23, 26*	1982	1985	TCB/TD
Baviacora 92	*Lr14b, 27+31*	1992	1994	MCJ/SP
Durum Wheat:				
Altar 84	*LrAlt*	1984	2001	BBG/BN
Jupare 2001	*LrAlt, 27+31*	2001	2007	BBG/BP

To avoid significant losses in farmers' fields, breeding materials must contain sufficient genetic diversity in R-genes to continue releasing new resistant varieties, and seed multiplication and distribution agencies must be well organized to rapidly multiply and distribute them. Even strong campaigns to change varieties often fail and farmers look for new varieties only after epidemics cause significant crop losses affecting their livelihoods. A positive note associated with the breakdown of R-genes is that it brings new opportunities for variety replacement and more productive varieties, if available, can be easily popularized. Spread of new, resistant varieties is much faster in post-epidemic phases especially in less developed countries where fungicide use on wheat is less common.

It is naïve to believe that unlimited R-genes are available for utilization. The reality is that only a few R-genes are used worldwide at any given time. New genes are used only after they become availabile in desirable genetic backgrounds. Several R-genes effective against the Ug99 group and other important races of *Pgt* are derived from alien species and genera and often carry undesirable traits through linkage drags (Singh et al. 2008). These genes can only be used successfully for breeding after further cytogenetic manipulation. Moreover, cytogenetic manipulations often leave genes in poorly adapted genetic backgrounds and then repeated backcrossing is required to establish them in adapted varieties. These efforts require significant, long-term scientific and financial resources to ensure that new resistance genes are identified and provided to breeding programs for utilization on a regular basis.

There are examples where R-genes have lasted for a decade or longer. Greater longevity of R-genes results in increased dependence on those genes by breeding programs and thus a higher frequency of varieties and breeding materials end up carrying them as the main source of genetic protection. This situation leads to a more serious consequence for breeding programs and farmers as the breakdown of these R-genes leads to simultaneous susceptibility of many varieties and a high proportion of breeding materials, including the most promising potential varieties. Breakdown of leaf rust and yellow rust resistance genes *Lr24* and *Yr17*, respectively, in Australia, stem rust resistance genes *Sr31* and *Sr24* in Africa (Singh et al. 2011b), yellow rust resistance gene *Yr17* in Europe, an uncharacterized leaf rust resistance gene in durum in Mexico (Singh et al. 2004), and an uncharacterized yellow rust resistance gene (Fan 6 source) in China to Chinese race CYR32 (Wan et al. 2004) are examples. Special efforts, including the use of fungicides, are required to overcome the negative impacts of such events, and recovery may take a decade or longer as seen with the breakdown of yellow rust resistance in Australian wheat germplasm leading to increased use of fungicides (Wellings 2007). Progress in increasing yield potential and other agronomic traits is hampered during the recovery phase as most resources are allocated to solution of the new rust problem.

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Utilization of effective R-genes in combinations is known to enhance resistance longevity, but such a strategy must be strictly followed by all breeding programs for long-term success. Availability of tightly linked molecular markers for some R-genes has allowed some breeding programs to aggressively undertake this strategy. For these efforts to be successful, other breeding programs must follow the same strategy otherwise the success in developing multiple R-gene based varieties is likely to be eroded due to the release of varieties that carry those genes singly. It is practically impossible to regulate the use of diverse R-genes by different breeding groups - public or private - within a country, region, or internationally. Significant changes worldwide in policies regulating resistance gene deployment strategies are not expected; indeed, in reality policies are becoming more relaxed to promote privatized breeding in many countries and regions. A strong sense of self-regulation will therefore be necessary for multiple R-gene strategies to succeed and to avoid the large-scale use of cheap, generic fungicides.

A potential downside of MAS can be a reduction in genetic diversity in breeding materials for both R- and PR-genes as only those R-genes that have tightly linked molecular markers and high phenotypic effects will be utilized by breeding programs. Most wheat breeding programs run on limited resources and therefore it is also likely that MAS changes rigorous field screening to limited field testing.

Partial resistance (PR) genes

Quantitative trait loci (QTL) studies during the last 15 years have shown high diversity for PR-genes in wheat germplasm. Over 60 and 140 QTL are reported for adult plant resistance (APR) to leaf rust and yellow rust, respectively in the literature. Consensus mapping by Rosewarne et al. (unpublished) indicated the possibility of more than 8 and 35 chromosomal regions harboring leaf rust and yellow rust resistance QTL, respectively. Recent bi-parental and association mapping studies identified at least 13 QTL associated with APR to the *Pgt* Ug99 race group in CIMMYT wheat germplasm (Bhavani et al. 2011; Yu et al. 2011). Most likely some APR QTL, especially for yellow rust resistance, correspond to small and intermediate effect R-genes that express only in adult plants. True PR-genes likely confer resistance to multiple pathogens as was shown with the four best characterized genes (Singh et al. 2011b).

Successful breeding for PR is initially more cumbersome due to one or more of the following reasons: 1) small to intermediate effects of individual PR-genes, 2) dispersed presence of PR-genes in different varieties or germplasm, 3) field selection environment lacking uniform and high disease pressure, 4) need for growing larger population sizes in various generations, 5) necessity of pyramiding 3-5 genes to achieve adequate to high resistance levels, 6) presence of R-genes in parents used in crossing programs, 7) difficulty in distinguishing small effect R-genes from PR-genes especially for resistance to yellow rust, 8) higher G x E interaction on the expression and effectiveness of PR-genes, and 9) slow progress in identifying linked molecular markers requiring a long-term commitment.

Despite various complexities described above, significant progress was made initially in breeding wheat germplasm that possesses adequate to high levels of leaf rust and yellow rust resistance and more recently with Ug99 stem rust resistance (Singh et al. 2011a, b). CIMMYT initiated deliberate selection for PR to leaf rust in the early 1970s; efforts were up-scaled in the 1990s to understand the genetic basis and pyramiding potential of multiple PR-genes in developing high or near-immune levels of APR to both leaf rust and yellow rust (Singh et al. 2000). Targeted breeding for such resistance became easier once several high yielding wheat progenitors with adequate to near-immune levels of resistance became available. Identification of some semidwarf wheat progenitors with high levels of PR-gene based Ug99 resistance, lack of effective R-genes in a large proportion of breeding materials, and access to a reliable field based selection environment have facilitated simultaneous selections for PR-gene based resistance and high yields. About 76% of the high yielding wheat germplasm derived from targeted crosses made in 2006, soon after the launch of BGRI, and distributed by CIMMYT through four international yield trials and three nurseries in 2011/2012 displayed adequate to high levels of PR-gene based APR to the Ug99 group of races in field tests at Njoro, Kenya (Table 3).

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Table 3 Levels and frequencies of *Pgt* race Ug99 resistance in 463 high-yielding bread wheat lines derived from targeted crosses made in 2006 and distributed through CIMMYT international trials and nurseries in 2011/2012

	Stem rust		Entries	
Resistance category[1]	**Severity (%)[2]**	**Host reaction[3]**	**No.**	**%**
PR-genes based APR:				
Near-Immune Resistant	1	MS-S	85	18
Resistant	1-10	MS-S	116	25
Resistant- Mod. Res.	15-20	MS-S	121	26
Moderately Resistant	30	MS-S	35	8
Inadequate APR:				
Mod. Res.- Mod. Sus.	40	MS-S	22	5
Mod. Sus.- Susceptible	50-80	MS-S	14	3
Susceptible check Cacuke	**100**	**S (Necrotic)**		
R-genes:				
Sr25	1-5	R/MR/MR-MS	10	2
Sr26	1-10	R	7	2
SrHuw234	30	MR-MS	1	0
SrSha7	5-10	R-MR	14	3
SrTmp	1-50	R/MR/MR-MS	35	8
SrUnidentified	1-10	R	3	1

[1] Resistance category is a simplified phenotypic classification based on the field performance
[2] The percent stem rust severity follow modified Cobb Scale (Peterson et al. 1948)
[3] Host reaction R = resistant, MR = moderately resistant, MS = moderately susceptible and S = susceptible (Roelfs et al. 1992)

Resistance conferred by PR-genes is considered durable, often longer lasting than the life of a variety. In contrast to the 3-5 year longevity of R-gene based wheat varieties in northwestern Mexico, varieties such as Rayon F89, Tarachi F2000, Tacupeto F2001, with adequate PR-gene based resistance and released around 1990 and thereafter remained resistant to leaf rust and were replaced only when more productive varieties were identified.

Evolution of virulence to slow rusting resistance is difficult to study due to the need for controlled greenhouses where healthy plants can be grown until the adult stage and for quantitative inoculations to be carried out. Test environments, temperatures and light intensities, sowing dates and disease pressures in field trials often affect the expression of slow rusting resistances (Lillemo et al. 2011). Multiyear and multi-environment phenotyping is therefore necessary to determine the stability of PR. The measurable effects of individual slow rusting resistance genes can be reduced if tested with races with higher aggressiveness. This is due to the shorter latent period or higher spore production ability of the pathogen allowing an additional disease cycle. However, a more aggressive race tends to reduce the effectiveness of both PR- and R-genes indiscriminately and susceptible varieties show highest disease ratings much earlier than when challenged with less aggressive races. A combination of 4-5 PR-genes tends to maintain low and stable final disease severities in field trials even when challenged with aggressive races.

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A general tendency is to associate all APR genes with durability. In reality this is not the case. Some large effect APR R-genes are also known and designated (McIntosh et al. 2003). Our inability to delineate small to moderate effect APR R-genes and small effect PR-genes, especially for resistance to yellow rust, often leads to doubts on the durability of PR-genes. However, it is possible to utilize successfully small/intermediate effect R-genes and PR-genes in breeding highly resistant varieties because both kinds of resistance genes have additive effects when combined. There are various examples with all three rusts where high levels of resistance result from combinations of a moderate effect R- gene and 2-3 additional PR-genes (German and Kolmer 1992; Singh and Huerta 1995; Rosewarne et al. 2012). In these instances the loss of resistance has been, or is expected to be, gradual as the pathogen evolves to defeat the small effect R-genes; allowing sufficient time to replace the variety.

Releasing PR-gene based varieties with moderate to adequate resistance can be a challenge in some countries where regulations require release of only clean or highly resistant varieties. Although moderate to adequate resistance is sufficient to reduce losses to non-significant levels in farmers' fields in most areas, overcoming strict regulations and a fixed mindset has been a difficult task and slow to achieve. Development of near-immune resistant varieties was a step taken by the CIMMYT breeding program to overcome this hurdle.

Deployment of wheat varieties with adequate to high levels of PR is beneficial to farmers and seed agencies as well as breeding programs. Wheat requires large seed quantities for sowing, and being a self-pollinated crop, a large proportion of farmers worldwide save their own seed. This practice is unlikely to change in the near future. Existing seed production and distribution infrastructures in the majority of countries cannot cope with high seed demands following the breakdown of R-gene based resistances. Farmers or governments need to spend significant resources to implement chemical control strategies to ensure food security during the "bust" phase until the spread of new resistant varieties. Replacement of PR-gene based varieties will be necessary only after a more productive, significantly superior variety becomes available. This reduces costs to farmers and governments as seed production and distribution can be better planned. Once PR-genes are accumulated in a large proportion of improved materials, breeding programs can allocate higher priorities to other important traits for increasing productivity while maintaining combinations of PR-genes through field selection.

Conclusions and future outlook

Both R- and PR-genes offer opportunities to achieve durable control of wheat rusts if utilized properly. "Boom-and-bust" experiences are unlikely to change in the near-future as wheat breeding and commercialization is done by many public and private institutions and requiring release and cultivation of varieties with multiple R-gene based resistances are unlikely. Privatization of wheat breeding is also unlikely to improve the present situation as experienced in some countries. Use of complex PR-gene resistance, or combinations of small effect R- and PR-genes, should be a more attractive, farmer- and environment-friendly rust control strategy. A large proportion of high-yielding spring bread wheat germplasm developed and distributed worldwide by CIMMYT has high to adequate APR to all three rusts, including the *Pgt* Ug99 group. Release and cultivation of durably resistant varieties are expected in a number of countries in the coming years. CIMMYT-distributed wheat germplasm is also the main source of new genetic diversity for many National Programs, hence the utilization and dependency on PR-gene based resistance by national breeding programs is likely to increase. An increased research focus on PR in recent years has enhanced the characterization of PR-genes and identification of tightly linked molecular markers. These advances will further aid their utilization and the exploration of new genetic diversity in landraces, related species and genera. New selection tools, such as genomic selection, can also be employed to pyramid multiple small effect PR-genes in the absence of rusts; however, field phenotyping will remain essential to verify resistance phenotypes. Finally, breeding for durable rust resistance can be simplified if multiple R- and PR-gene cassettes can be developed and inserted in high-yielding wheats through transformation; however general acceptance of genetically modified wheat for human consumption will be necessary for it to succeed.

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A pipeline for cloning resistance genes effective against African Puccinia graminis tritici races from the diploid wheat relative Aegilops sharonensis

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Abstract

The Two Blades group aims to develop durable resistance to the wheat stem rust fungus *Puccinia graminis* f. sp. *tritici*. Our approach is based on exploiting novel major dominant *R* genes from species related to wheat. These genes have received less attention in recent years because of their race specificity and propensity to break down in the field. We are developing resources in *Aegilops sharonensis*, a diploid relative of wheat, to facilitate cloning of stem rust *R* genes. We plan to work with partners to deploy the genes in multi-gene stacks that should have significantly increased durability compared to single genes, making them attractive candidate loci for breeding and deployment.

Keywords: resistance gene, Sharon goatgrass, Ug99, wheat stem rust

Origin of *Aegilops sharonensis* and potential for wheat improvement

Aegilops sharonensis Eig (Sharon goatgrass) is a wild relative of wheat belonging to the *Sitopsis* section of *Aegilops*, the genus most closely related to *Triticum* (Zhukovsky 1928). *Ae. sharonensis*, like other species in the *Sitopsis* section, contains *gametocidal* genes that restrict interspecies hybridization (Endo 1990), and limit traditional introgression of its genes into wheat, making it a largely untapped reservoir of genetic diversity for wheat improvement (Olivera and Steffenson 2009). Modern breeding techniques and transgenic technology, however, provide the potential to exploit this germplasm. Recently, *Ae. sharonensis* received attention as a source of resistance to the African *Puccinia graminis* f. sp. *tritici* race TTKSK (synonym race Ug99) and its derivatives (Olivera et al. 2007; Steffenson et al. 2007), that pose a threat to global food security (Ayliffe et al. 2008). *Ae. sharonensis* contains several attributes that make it attractive for identifying genes for wheat improvement: it is diploid with a genome related to the B genome of hexaploid wheat (reviewed in Olivera and Steffenson 2009), a diverse collection of accessions from its range along the Israeli-Lebanon coastline display high genetic diversity (Olivera et al. 2010), and pure lines can be readily maintained in the laboratory.

Resistance of *Aegilops sharonensis* to wheat stem rust: germplasm screening and population development

From an initial screen of 105 *Ae. sharonensis* accessions reported by Olivera and Steffenson in 2007, 69% were resistant to *Pgt* race TTKSK, whereas frequencies of resistance to North American races were 14% for TTTTF, 33% for TPMKC and 72% for QCCJ (Olivera et al. 2007). Only four *Ae. sharonensis* accessions (393, 1192, 1644 and G614) were resistant to all four *Pgt* races.

Screening of 14 accessions from the original collection with multiple races from Africa (TTKSK, PTKSK, TRTTF, JRCQC) and North America (TPMKC, TTTTF) resulted in three accessions of interest: 2172, 409 and 1998, all showing strong resistance to most of the races (Table 1). In addition, from another collection of 140 accessions (Olivera et al. 2010; Olivera and Steffenson unpublished), we screened 13 with races TTKSK and TTTTF and identified 10 showing strong resistance to both races (Table 1).

Table 1 Disease reactions of *Aegilops sharonensis* to six races of *Puccinia graminis* f. sp. *tritici*

Ae. sharonensis		*P. graminis* f. sp. *tritici*					
Line	Location	TTKSK[d]	PTKST[d]	TTTTF[d]	TPMKC[d]	TRTTF[d]	JRCQC[d]
396[a]	Ashdod	S[e]	Het	S[e]	S[e]	-	-
548[a]	Palmahim	S	-	S[e]	S[e]	-	-
2189[a,b]	Hefzi Bah	S	S	S	S	S	S
2232	En HaMifraz	S	Het	S	S[e]	Het	Het
542237[a]	Lebanon	S[e]	S	S[e]	S[e]	-	-
2232[a]	En HaMifraz	S	Het	S	S[e]	Het	Het
1644[a]	Ashdod	R	R	R	R	R	R
409[b]	Mikhmoret	R	R	Weak R	R	R	R
2172[b]	Qiryat Ono	R	R	Weak R	R	R	R
1998[b]	HaShikmim	R	R	-	R	R	R
575[b]	Palmahim	R	R	S	R	R	R
1193[a]	Hefzi Bah	R	R	S	S	R	R
2020[b]	Kefar Ganim	R	R	S	S	R	R
1192[b]	Hefzi Bah	R	R	S	S	R	R
4844[b]	Ashdod	Weak R	R	I	S	R	R
2000[b]	HaShikmim	Het	R	I/S	I/S	Weak R	R
2229[b]	En HaMifraz	Het	Het	Weak R	I	Weak R	R
7870[c]	Ashdod	R	-	R	-	-	-
7888[c]	Ein Hakore	R	-	R	-	-	-
7894[c]	Ein Hakore	R	-	R	-	-	-
7915[c]	Deror Junction	R	-	R	-	-	-
7919[c]	Even Yehuda	R	-	R	-	-	-
7923[c]	Even Yehuda	R	-	R	-	-	-
7924[c]	Netanya N. Reserve	R	-	R	-	-	-
7934[c]	Netanya T. Station	R	-	R	-	-	-
7935[c]	Netanya T. Station	R	-	R	-	-	-
8019[c]	Na'aman	R	-	R	-	-	-
7872[c]	Ashdod	Weak R	-	R	-	-	-
7922[c]	Even Yehuda	Weak R	-	R	-	-	-
8013[c]	Na'aman	S	-	R	-	-	-

[a]Lineage from Olivera et al., 2007.
[b]Lineage from The Harold and Adele Lieberman Germplasm Bank (http://www.tau.ac.il/lifesci/units/ICCI/genebank.html).
[c]Lineage from Olivera *et al*., 2010.
[d]Infection Type (IT) on seedling evaluation.
[e]IT from Olivera et al., 2007.

We made ~130 crosses using 38 TTKSK-resistant accessions and the susceptible accessions 2189, 2232, 548, 396 and 542237. Several F_2 populations gave interesting results (Table 2). The most promising materials for further study were the populations 548 × 1995 and 548 × 2205, which segregated 3 resistant : 1 susceptible to race TTKSK, indicative of single dominant *R* genes, and crosses 2189 × 575 and 548 × 6793, each of which segregated in a two-gene ratio, either semi-dominant (4:8:3:1) or dominant (15:1), respectively. The 2189 × 575 population also segregated 13:3 with TPMKC, indicative of one dominant and one recessive *R* genes effective against this race, but further studies will be required to establish if the dominant *R* gene is the same as one of those effective against TTKSK in the same cross.

Table 2 Segregation of resistance to TTKSK, TTTTF and TPMKC in *Ae. sharonensis* F_2 and $F_{2:3}$ populations

			Observed segregation ratio[a]			
Susceptible	**Resistant**	**Progeny**	**TTKSK**	**TTTTF**	**TPMKC**	**Model**
2189	575	94 F_2	22R:49IR:17I:6S			4:8:3:1[b] (*p*=0.97)
		94 F_2			77R:17S	13:3[c] (*p*=0.87)
2189	1644	139 $F_{2:3}$	26R:110Seg:3S			10:53:1[d] (*p*=0.46)
2232	1644	90 F_2		63R:27S		3:1 (*p*=0.71)
		114 $F_{2:3}$	29R:82Seg:3S			10:53:1[d] (*p*=0.01)
548	1995	88 F_2	65R:23S			3:1 (*p*=0.81)
548	2205	76 F_2	54R:22S			3:1 (*p*=0.43)
548	2233	74 F_2	32R:15IR:21IS:6S			7:4:4:1[e] (*p*=0.77)
548	6793	63 F_2	57R:6S			15:1 (*p*=0.28)

[a]R, Resistant; IR, Intermediate Resistant; I, Intermediate; IS, Intermediate Susceptible; S, Susceptible; Seg, segregating.
[b]Two gene model; two semi-dominant *R* genes are segregating independently and when combined give full immunity.
[c]Two gene model; one dominant and one recessive *R* gene.
[d]Three *R* gene model; three semi-dominant *R* genes are segregating independently, and any two genes give full immunity.
[e]Two *R* gene model; two semi-dominant *R* genes are segregating independently, and when any gene is at an homozygote stage it give full immunity.

Two populations having accession 1644 as the resistant parent, but differing in having either 2232 or 2189 as the susceptible parent, were tested with TTKSK in the $F_{2:3}$ and segregated for three semi-dominant *R* genes, any two of which are required for full resistance (Table 2). We tentatively mapped these *R* genes and provisionally named them *Sr1644-A*, *-B* and *-C* (see below).

Aegilops sharonensis leaf transcriptome and SNP identification

The *Ae. sharonensis* genome is estimated at 7.5 Gb (Eilam et al. 2007; Furuta et al. 1986). To reduce the complexity of the genome and identify a set of SNPs, we sequenced two *Ae. sharonensis* accessions that were chosen based on their reactions to race TTKSK. Accession 1644 is resistant to *Pgt* race TTKSK, whereas accession 2232 is susceptible (Olivera et al. 2007). Total RNA was extracted from pooled tissue of the 2nd, 3rd, and 4th leaves of the two accessions, cDNA synthesised, normalised, 454 sequencing libraries prepared, and sequenced on two plates of the 454 GS-FLX sequencer with Titanium chemistry. The initial sequencing produced 1,338,956 reads (797 Mbp) for accession 1644 and 1,355,371 reads (852 Mbp) for accession 2232. The data were processed by clipping off the sequencing adapters and removing low quality reads and curated to remove reads below 45 nucleotides, repeat sequences known in the *Triticeae*, and chloroplast, mitochondrial, and potential human contamination. Finally, we used 1,247,583 reads from accession 1644 and 1,280,912 from accession 2232 as the input data for *de novo* transcriptome assembly.

We assembled the reads using two different *de novo* assembly programs, namely CAP3, designed for assembling ESTs (Huang and Madan 1999), and Newbler 2.5.3, Roche's 454 GS assembler (Margulies et al. 2005). The two different assembly programs were employed to obtain a more extended representation of the expressed gene space in the leaf. Three different groups of assemblies were performed, two by using reads from each accession individually and one group of assemblies where all the reads from both accessions were used in combination.

The Newbler and CAP3 assemblies using the 'combined' dataset produced 35-43% and 2-13% more contigs than the single accession assemblies, respectively.

The coverage of the grass gene space for each individual assembly was calculated by counting the unique hits from each assembly contig set that are Best Reciprocal Blast Hits (BRBH) with the genes from four sequenced and annotated grass genomes, including rice (Goff et al. 2002), sorghum (Paterson et al. 2009), maize (Schnable et al. 2009) and *Brachypodium* (Initiative 2010), in addition to available barley unigenes (Close et al. 2009) and the combined set of Triticeae (wheat and barley) full-length cDNAs (Mochida et al. 2009) (Table 3). This number constitutes a measure of how many orthologous sequences each assembly has captured and is a proxy for the breadth of each assembly. Different assemblies of the same dataset generated qualitatively different coverage of the gene space: the Newbler assembler using default parameters produced the most unique BRBH against all the grass gene sets. Newbler generated a contig set that spans the grass gene space more than any other assembly (18,871 unique BRBH) and CAP3 a contig set with the highest proportion of unique BRBH (more than 50% of the CAP3 relaxed contigs have a unique BRBH; 15,703 out of 30,609 contigs) (Table 3).

Table 3 Best reciprocal BLAST hits of *Ae. sharonensis* transcripts against barley unigenes, wheat full-length cDNAs and the exomes of sequenced grasses

Assembly	Contigs	Barley35 50,938	TriFLDB 15,871	*B. distachyon* 32,255	*O. sativa* 51,258	*S. bicolor* 29,448	*Z. mays* 106,046	Unique Hits[1]
Combined Newbler Default	71,029	15,785	6,015	10,047	9,641	9,553	9,506	18,684
Combined Newbler Strict	73,109	15,800	6,020	10,044	9,610	9,548	9,518	18,351
Combined CAP3 Default	44,961	13,650	5,642	9,159	8,774	8,695	8,708	16,606
Combined CAP3 Strict	48,118	13,675	5,627	9,177	8,756	8,649	8,701	16,715
Combined CAP3 Relaxed	30,609	13,464	5,254	9,185	8,788	7,433	8,702	15,687
1644 Newbler Default	50,025	14,552	5,699	9,253	8,848	8,791	8,849	16,941
1644 Newbler Strict	51,295	14,577	5,703	9,221	8,827	8,769	8,795	16,998
1644 CAP3 Default	43,900	13,511	5,379	8,475	8,170	8,132	8,257	16,451
1644 CAP3 Strict	45,960	13,440	5,367	8,438	8,157	8,112	8,197	16,454
2232 Newbler Default	46,619	14,758	5,692	9,480	9,040	8,942	8,937	17,294
2232 Newbler Strict	47,833	14,735	5,693	9,454	9,015	8,913	8,927	17,337
2232 CAP3 Default	40,852	13,190	5,482	8,951	8,512	8,446	8,431	15,975
2232 CAP3 Strict	42,545	13,174	5,484	8,940	8,542	8,438	8,460	16,033

[1] Unique hits refer to the non-redundant sequences from the union of all best reciprocal BLAST hits from all six datasets.

We used the contigs from two of the combined *de novo* assemblies, the 'Newbler default' and the 'CAP3 relaxed', as pseudo-references to align the reads from the two accessions using the Mosaik pipeline. SNPs were called using GigaBayes, which uses a Bayesian framework to predict SNPs. The Mosaik/GigaBayes pipeline predicted 18,195 SNPs in contigs of the Newbler default assembly and 47,706 SNPs in the CAP3 relaxed assembly. The significantly higher number of SNPs in the CAP3 relaxed assembly can be explained by the fact that these contigs were constructed by the collapse of paralogs and allele/splice variants and therefore this assembly is more likely to accumulate all the polymorphisms from each sequence variant.

The high number of SNPs between these two accessions from two geographically distinct populations, 1644 from Ashdod in the Philistine Plain, southern Israel, and 2232 from En HaMifraz in northern Israel (Olivera et al. 2007), is consistent with the high degree of genetic diversity found within and between *Ae. sharonensis* populations, as revealed by a previous study using 21 SSR markers on 106 accessions (Olivera et al. 2010).

We are currently developing new marker sets for 16 additional accessions by Illumina RNAseq on un-normalized RNA and aligning those reads to our 454 transcriptome assembly. This diversity panel incorporates accessions from all major geographic regions within the habitat range of *Ae. sharonesis* and includes the parents of the most promising crosses we are pursuing.

Heterozygosity in *Ae. sharonensis* accessions 1644 and 2232

Although *Ae. sharonensis* is generally considered autogamous, outcrossing is fairly common (Dvořák et al. 1998). The rich source of SNPs provided a basis by which we could determine the degree of homo- and heterozygosity in accessions 1644 and 2232. The ratios between the most- and least-frequently observed alleles of individual SNPs with read coverage of ≥20 (460 SNP-bearing unigenes from the CAP3 relaxed assembly) produced significantly different histograms for the two accessions (Fig. 1A, 1B). Using an allelic ratio cutoff of less than or equal to 3.0 (range of 1:1 to 3:1), 10 and 165 unigenes contained a ~1:1 mixture of alleles of the SNP in accessions 1644 and 2232, respectively. Similar results were observed for the Newbler assemblies (data not shown). This suggests an overall heterogeneity of 2.1% in accession 1644 and 35.7% in accession 2232, respectively. After collection in the wild, 1644 and 2232 were maintained as inbred lines for two generations under controlled glasshouse conditions. Thus, the heterozygosity observed in accession 1644 likely reflects paralogs that have been placed into the same unigene. In contrast, accession 2232 was either highly heterogeneous when isolated in the wild or outcrossed in the glasshouse during the maintenance process.

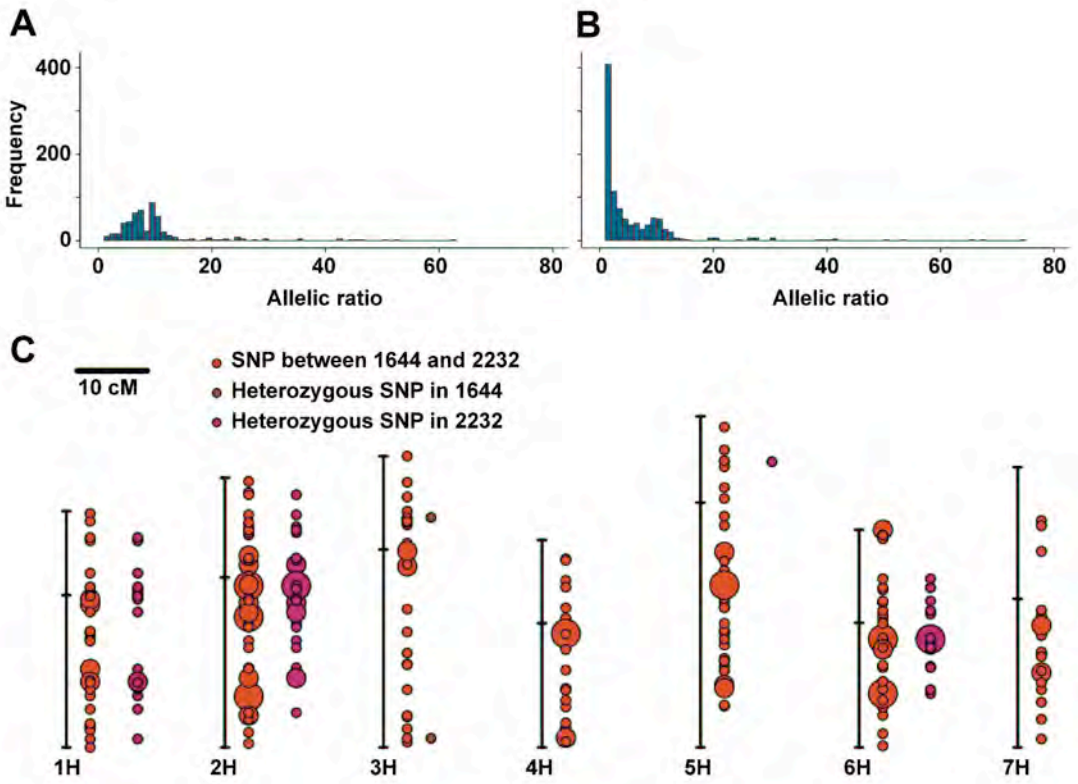

Figure 1 Analysis of heterozygosity in *Ae. sharonensis* accessions 1644 and 2232. Histograms of the ratio between the most and least frequently observed alleles of individual SNP with read coverage of ≥20 between accessions 1644 (A) and 2232 (B). Heterozygous SNP-bearing unigenes were identified using a threshold ratio cutoff of 3.0 (most versus least observed allele within a genotype) and mapped using best reciprocal BLAST hits to genes in the barley consensus genetic map

Mapping heterozygous SNPs onto a genetic map would identify the regions still heterozygous in accession 2232 and would provide support for their authenticity. In lieu of an *Ae. sharonensis* genetic map, we mapped the SNP-bearing unigenes onto the consensus barley genetic map (Muñoz-Amatriaín et al. 2011) based on BRBH (*e*-value cutoff 10^{-10}). Of the 460 SNP-bearing unigenes identified, 244 had putative orthologs that had been mapped in barley. Of these, 2 heterozygous SNPs from 1644 and 69 from 2232 were placed on the barley genetic map (Fig.

1C). Strikingly, the SNP-bearing unigenes of 2232 co-localized to chromosomes of 1H, 2H and 6H. This suggests that barley and *Ae. sharonensis* share a reasonable degree of synteny and that these SNPs within 2232 are due to a recent cross-pollination event between two distinct accessions. Given the homozygosity of accession 1644, this accession is an ideal candidate reference accession for the generation of future molecular genomics tools such as whole genome sequencing and BAC library construction.

Whole genome shotgun sequencing of *Aegilops sharonensis*

In collaboration with The Genome Analysis Centre, Norwich, UK we have commenced shotgun sequencing of the ~7.5 Gb *Ae. sharonensis* genome. An initial 31x coverage of accession 1644 with 100 bp paired-end Illumina reads (~10x each on three libraries of 514, 612, and 819 bp inserts) was assembled with the CLC Assembly Cell. This yielded 2.5 million contigs totaling 1.5 Gb (N50, 1 Kb), including 5,453 contigs larger than 10 Kb. We have also contracted with Eurofins/MWG-Operon to generate 20-30x coverage of our highly susceptible reference accession 2189, including a 180 bp overlapping short insert library, a 500 bp insert library, and 3 Kb and 8 Kb Long Jumping Distance libraries.

We expect that many of the *Ae. sharonensis* contigs can be ordered with a high degree of confidence based on synteny to other sequenced monocots. However, since accessions 2189 and 1644 are being used to generate a RIL population, we will also be able to genetically map contigs. To this end, we plan to identify a set of ~50,000 SNPs from comparison of the 1644 and 2189 genomic sequence. By performing sequence capture on marker-containing sequences (Galvão et al. 2012) on individuals from the 1644 × 2189 RIL set, we can create a high-density genetic map, to which we can anchor the assembled scaffolds. We can then use this technology to attempt association mapping among the diverse set of accessions tested for Ug99 reaction.

An integrated DArT and SNP-based genetic map of *Ae. sharonensis*

We generated an initial genetic map of *Ae. sharonensis* to provide a framework for the fine-mapping and ultimate cloning of *R* genes. The map is based on genotyping three F_2 populations (1644 × 1193, 1644 × 2189 and 1644 × 2232, with 182, 175 and 177 F_2 progeny, respectively). Markers were derived principally from a high-density custom-made *Ae. sharonensis* DArT array (Triticarte P/L, Yarralumla, Australia), and integrated with 52 gene-based SNP markers that have clear barley orthologs and are spread evenly on the barley consensus genetic map (Muñoz-Amatriaín et al. 2011) using a MassARRAY Analyzer (Sequenom, USA). Finally, 15 SSR markers were also placed in the map.

The consensus map spans 1,115 cM and comprises 1,665 markers (0.7 cM per marker) placed into seven co-linear linkage groups that correspond to chromosomes $1S^{sh}$, $2S^{sh}$, $3S^{sh}$, $4S^{sh}$, $5S^{sh}$, $6S^{sh}$, and $7S^{sh}$ (Table 4; Fig. 2). Based on the location of known markers in the wheat genome, we can detect a major translocation from the wheat chromosome group 4 to chromosome $7S^{sh}$. Future work using barley as a reference will determine the extent of rearrangement and translocation in the seven chromosomes of *Ae. sharonensis*.

Table 4 Markers in the *Aegilops sharonensis* genetic map.

	1644 x 1193	1644 x 2232	1644 x 2189	Consensus
F_2 population	182	177	175	534
Total cM	885	972	888	1115
DArT markers	495	791	750	1598
Gene SNP markers	0	49	40	52
SSR markers	15	0	0	15

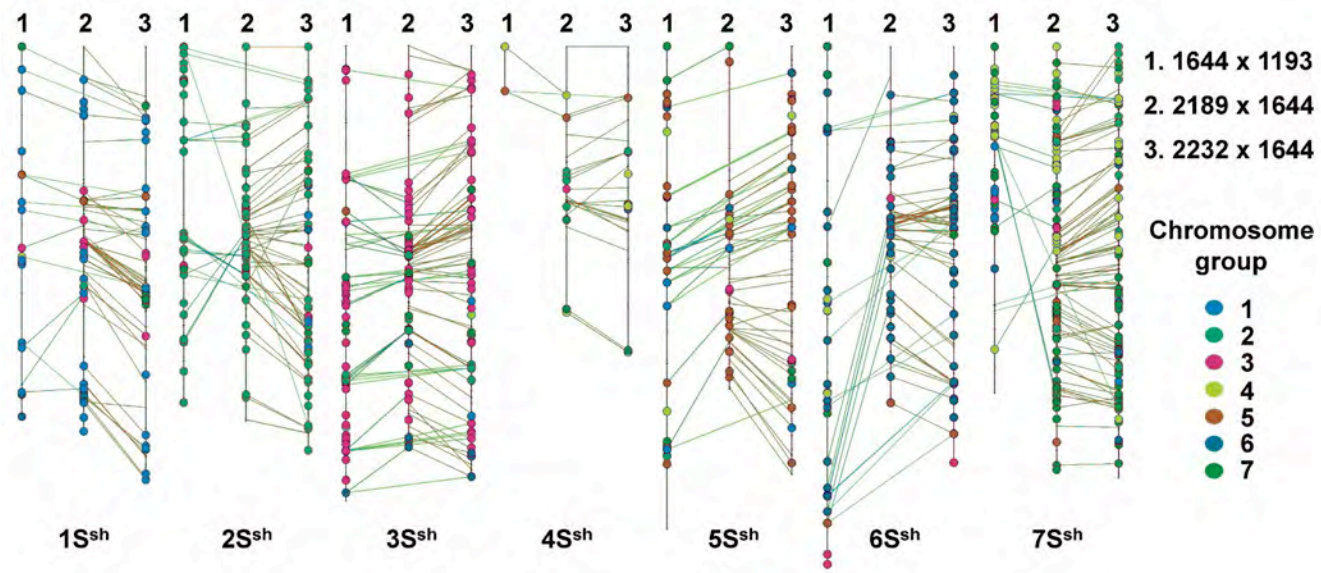

Figure 2 Integrated DArT and SNP-based genetic map of *Ae. sharonensis*. Co-linearity of the seven linkage groups from three F_2 mapping populations of *Ae. sharonensis* is indicated by lines. Chromosome assignment is based on placement of wheat DArT markers, shown as colored circles (for association, see legend on far left). Relative to wheat there is a large translocation of $4S^{sh}$ as to chromosome $7S^{sh}$.

This map provides a platform that bridges *Ae. sharonensis* with the genomic resources of wheat, barley, *Brachypodium*, rice, and other sequenced monocots.

From the RNAseq of the *Ae. sharonensis* diversity panel of 16 accessions (see above), we plan to identify single nucleotide polymorphisms (SNPs) that have a high minor allele frequency for the generation of an 1536 Illumina Golden Gate Oligo-Pooled Assay (OPA). SNPs will be prioritized when they are found to be in homologs that were mapped with the Barley OPA (Muñoz-Amatriaín et al. 2011). This genotyping platform will allow us to improve our genetic map and rapidly map genes to a genomic location. For fine mapping, we plan to use genotyping-by-sequencing (Wang et al. 2012) on resistant and susceptible bulks to saturate markers in target regions, and map the markers that we get from using Sequenome.

Map positions of *Sr1644-A*, *-B* and *-C*

DArT and SNP gene-based genotyping, used to generate the genetic map outlined above in the crosses 2189 x 1644 and 2232 x 1644, was also used to resolve the location of race TTKSK resistance in parent 1644. This initial mapping indicated the presence of a major contribution to resistance on chromosome $1S^{sh}$ and a minor contribution on chromosome $5S^{sh}$ (*Sr-1644-C*) (Fig. 3). Sequenome genotyping was used to call SNPs (previously identified by 454 sequencing) and saturate the region around the QTL on chromosome $1S^{sh}$, allowing resolution of two peaks (*Sr1644-A* and *Sr1644-B*) and for the selection of F_3 seedlings predicted to be heterozygous for one locus but homozygous susceptible at the other two loci (*i.e. Aabbcc, aaBbcc, and aabbCc*). Selected $F_{3:4}$ families were re-inoculated with race TTKSK and found to now segregate 3:1 for reaction to TTKSK. These $F_{3.4}$ families are currently being genotyped to further corroborate their genetic makeup and to fine map *Sr1644-A*, *-B* and *-C*.

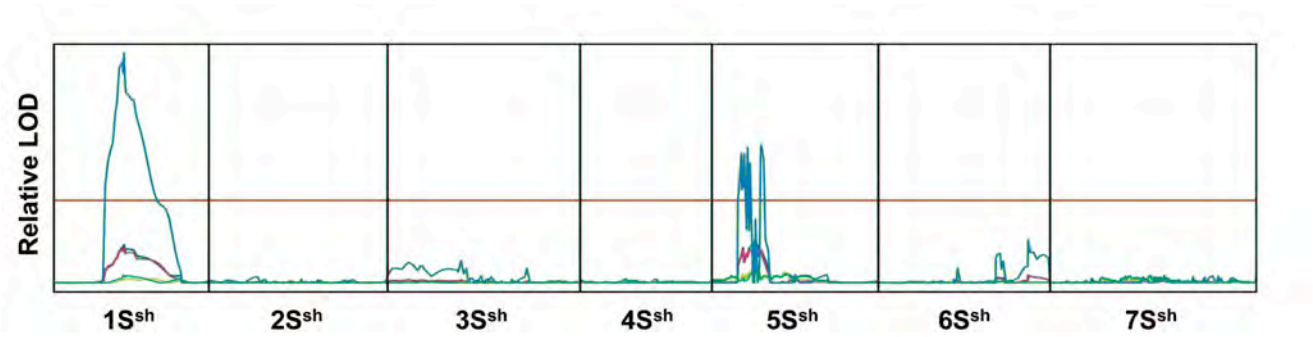

Figure 3 QTL analysis of susceptibility to TTKSK in the cross 2189 x 1644. Composite interval mapping was carried out on the frequency of infection type 3 (susceptibile) on the seven linkage groups of *Ae. sharonensis* ($1S^{sh}$ to $7S^{sh}$). Resistances conferred by significant QTL were contributed by 1644. Relative LOD values are shown with respect to the bootstrap determined threshold ($\alpha = 0.05$, 1000 bootstraps, blue line). Colors reflect tests of different hypotheses: H0:H3 (vermillion), H1:H3 (yellow), H2:H3 (bluish green), H0:H1 (orange), and H0:H2 (sky blue).

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Stripe rust resistance gene *Yr10* encodes an evolutionary-conserved and unique CC-NBS-LRR sequence

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Yr10 was isolated using a map-based cloning approach. Two linked genomic clones encoding a highly conserved and unique CC-NBS-LRR sequence in wheat were identified and characterized. Clone 4B corresponds to an expressed single copy transcript whereas clone 4E is a pseudogene. Although the two sequences show some divergence in intron and LRR sequences they appear to form a two-member gene family. Functionality of 4B (*Yr10*) was demonstrated by transgenesis and gene silencing. Transformation of stripe rust susceptible cv. Fielder with clone 4B and subsequent inoculation with *Pst* strain SRC-84 that is avirulent on Moro, resulted in an immune response similar to that on Moro. Barley stripe mosaic virus (BSMV)-VIGS transfection was employed to silence LRR and kinase domains of the 4B clone and a LRR domain in the 4E pseudogene in Moro. Silencing of all 3 domains of clone 4B (*Yr10)* in Moro proved successful as evidenced by a susceptible reaction following inoculation of the transfected treatments with strain SRC-84. This is the first report of a unique CC-NBS-LRR resistance gene with few homologs in the native wheat host, but having numerous homologs in *Aegilops tauschii, Brachypodium distachyon, Dasypyrum breviaristatum* and monocot non-hosts *Oryza sativa* and Sorghum bicolor. Related sequences were also identified in genomic databases of maize and sugarcane.

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A genome-wide SNP scan for QTL associated with adult plant rust resistances in East African bread wheat

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A recurrence of rust disease epidemics exemplified by the widely virulent *Pgt* race TTKS remains a major threat to wheat productivity in East Africa. To combat this risk, breeding wheat lines that have combinations of minor-gene adult plant resistance factors is an overriding objective. Here we report ongoing work to explore QTLs linked to APR to stem rust and leaf rust through genome-wide association mapping. A panel of 300 lines, 90% of which are past and present East African cultivars as well as a few landraces and breeders' lines, was assembled. The remaining 10% are historical Minnesota cultivars. Evaluation for disease severity was done in inoculated field trials for 2 years at 3 locations (St Paul, El Batan and Njoro). The germplasm was genotyped using a 9,000 genome-wide SNP assay leading to 5,987 high quality SNPs (minor allele frequency >0.5%). Analyses of population structure revealed four subpopulations enriched for lines associated with CIMMYT, North America, landraces and a mixed group. This distinction was further evident in a principal component analysis. Linkage disequilibrium (r^2) decay was relatively short in this germplasm set and was longest in the D-genome (18 cM). An implementation of mixed model tests integrating population structure and kinship detected ten significant experiment-wise marker trait associations for stem rust resistance (R^2 ranging from 5 to 8%) and twelve for leaf rust resistance (R^2 5 to 10%). We have increased the number of environments to test for the consistency of these QTLs.

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Identification of strategies for wheat stripe rust pathogenicity by deep transcriptome sequencing

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Stripe rust is a major constraint to wheat production worldwide. The molecular events that underlie host colonisation by *Pst* are largely unknown. The fungus creates a specialized cellular structure within host cells called the haustorium, which allows it to obtain nutrients from wheat, and to secrete pathogenicity factors called effector proteins. No effector protein has been identified to date in *Pst*. We are using genomic, transcriptomic and proteomic approaches to: 1. Understand how the pathogen establishes a compatible interaction with its host, and 2. Uncover the effector proteins that are synthesised and secreted by *Pst* during infection. We used next-generation sequencing platforms to characterise the *Pst* genome, and to generate two contrasting transcriptomes (germinating spores and haustoria). So far we have found ~900 potential secreted protein genes in haustoria and these are being tested *in vivo* to identify those with effector activity. Digital gene expression analysis comparing spores and haustoria revealed that spores mainly deploy their energy reserves for growth and development, whereas haustoria extract host nutrients for further growth and use them in biosynthetic pathways for the ultimate production of urediniospores. Finally, we developed a method to isolate highly purified haustoria, and it is being tested for proteomics analysis. This technique will be a useful aid to further effector discovery and *Pst* genome annotation. Together, these studies will substantially increase our knowledge of stripe rust effectors and provide new insights into pathogenic strategies of this important organism.

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The wheat genome sequence: a foundation for accelerating improvement of bread wheat

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Keywords: bread wheat, chromosome 3B, map-based cloning, marker-assisted selection, physical mapping, sequencing

Abstract
Genomics offers powerful tools for understanding the molecular basis of phenotypic variation as well as accelerating gene cloning, marker assisted selection, and more efficient exploitation of genetic diversity. In 2005, a group of growers, breeders, and plant scientists launched the International Wheat Genome Sequencing Consortium (IWGSC) with the goal of securing a high quality, reference sequence of the bread wheat genome. A milestone-based strategy coupled with short and long-term roadmaps provides breeders access to an increasing array of tools and resources without having to wait for the completed sequence. To reduce the complexity of the allohexaploid, highly repetitive, 17 Gb bread wheat genome, the IWGSC follows a chromosome-specific approach to develop physical maps, low coverage sequencing, and high quality sequencing of Minimum Tiling Paths before moving towards a gold standard reference sequence. Physical maps have been completed or are underway for all 21 chromosomes of the reference cultivar, Chinese Spring. To facilitate anchoring, marker development, and to gain a first insight into the gene space composition, the IWGSC launched an internationally coordinated survey sequencing initiative that provides breeders with survey sequences and a virtual gene order for all 21 chromosomes. The first physical map of a bread wheat chromosome, chromosome 3B (1 Gb) was obtained in 2008 and a high quality reference sequence was recently assembled. An overview of the IWGSC strategies and activities as well as results from the physical mapping, and sequencing of chromosome 3B and its potential application for isolating rust resistance genes will be presented.

Introduction
As the staple food for 35% of the world's population and the most widely produced crop, wheat (*Triticum aestivum*) is one of the most important crop species. Genomics is leading to a new revolution in plant breeding as it enables direct study of the relationship between genotype and phenotype for a significant number of traits and the direct study of genes underlying those traits (Tester and Langridge 2010; Feuillet et al. 2011). With a genome sequence in hand, breeders can have access to a complete, ordered gene catalog and an almost unlimited number of molecular markers that can be used for marker-assisted selection and precision breeding (Collard and Mackill 2008; Tester and Langridge 2010; Prohens 2011; Choulet et al. 2012). Despite the socio-economic importance of bread wheat and the recognition of the power that a genome sequence brings to breeding programs, bread wheat is one of the last major crops without a high quality, reference genome sequence.

The delay in developing genomic resources in wheat is largely a product of its complex genome: an allohexaploid (2n=6x=42) that is extremely large in size (17 Gb, more than 6 times the size of the maize genome) and with a very high (>90%) repetitive content thereby complicating genome assembly (Paux et al. 2008; Feuillet et al. 2011). These factors have made it more difficult and more costly to sequence the bread wheat genome than any other major crop. However, recent technological advances are enabling sequencing of the wheat genome at a reasonable cost. After a USDA-National Science Foundation funded workshop confirmed the need for sequencing the wheat genome and assessed different strategies and objectives (Gill et al. 2004), it was clear that the bread wheat genome could be sequenced as a result of the technological advances. With a mission of rectifying the paltry state of genomic resources for sequencing the wheat genome, a group of growers, breeders, and scientists launched the International Wheat Genome Sequencing Consortium (IWGSC; www.wheatgenome.org) in 2005. The underlying goal of the consortium is to accelerate wheat improvement by

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obtaining a high quality, manually annotated, reference genome sequence of bread wheat that is anchored to genetic and phenotypic maps. Using a milestone-based, adaptable strategy, the IWGSC provides breeders an increasing array of tools and resources while working towards obtaining a reference genome sequence (Feuillet and Eversole 2007).

As an organization led by growers, breeders, and scientists rather than sequencing experts, the consortium is focused on building a foundation for wheat improvement and on facilitating rapid application of the results from IWGSC-supported projects. Obtaining a "genome sequence" without any view towards the ultimate utilization of the sequence by breeders has never been a consortium goal. Thus a major consideration in designing the strategy to obtain a reference quality sequence was to understand exactly how the sequence would be used. We did not merely want to have a tool for comparing the wheat genome with other genomes. Instead, we wanted a genome sequence of sufficient quality to enable gene isolation, functional analyses, new allele discovery for pre-breeding, epigenetic modifications, polymorphism discovery for marker-assisted selection, and an increased understanding of the impact of transposable elements on gene regulation (Feuillet et al. 2011). To provide these capabilities, an integrated and ordered wheat genome sequence is essential.

Methods and results

Combined strategies are being deployed by the IWGSC to achieve a reference genome sequence of the hexaploid, bread wheat genome of cultivar Chinese Spring. These include physical mapping of Chinese Spring and *Aegilops tauschii* (the D-genome progenitor of bread wheat), as well as survey sequencing and BAC-based (i.e. the minimum tiling path of the physical map) reference sequencing of Chinese Spring. The physical map of *Aegilops tauschii* was completed in 2011 and publication of the results is expected in 2012 (J. Dvorak pers. comm.). The IWGSC follows a chromosome-specific approach for physical mapping, survey and high quality sequencing of Chinese Spring. The chromosome-based approach, made possible through technological advancements in flow-sorting of chromosomes by the group of Jaroslav Doležel (Dolezel et al. 2007), reduces the complexity of the hexaploid 17 Gb wheat genome to physical mapping and sequencing individual chromosomes or chromosome arms, the sizes of which range from 224 to 800 Mb. This chromosome-based approach facilitates international collaboration and divides the costs of obtaining a reference sequence into manageable portions. The chromosome-based approach will deliver a complete, finished reference genome sequence that, in addition to genic sequences, will provide critical information on non-coding, intergenic sequences that underlie many biological functions (Feuillet et al. 2011).

To construct the physical maps, chromosome-specific BAC libraries are created for each of the 21 chromosomes of cv. Chinese Spring. As of February 2012, almost 2.3 million BAC clones had been developed for the BAC libraries of all but 3.5 chromosomes of bread wheat (http://olomouc.ueb.cas.cz/dna-libraries/cereals). Using these BAC-libraries, physical maps are then developed. The completion of the physical map of the largest wheat chromosome (3B, ~ 1 Gb) in 2008 confirmed the feasibility of the approach (Paux et al. 2008). To date funding has been secured to construct physical maps of all 21 chromosomes and the physical maps of 9 chromosomes are already available (Fig. 1a). The next physical mapping milestone is completion of all chromosome-based physical maps; with all of the funding in place, this is expected by 2013. The information produced during the construction of the maps is useful for map-based cloning and marker development as well as increasing our understanding of the gene space organization and regulation (Choulet et al. 2010; Rustenholz et al. 2011). As an example, about a dozen genes and QTL, including a number of genes involved in disease response, are currently benefiting from access to the 3B physical map to perform chromosome landing.

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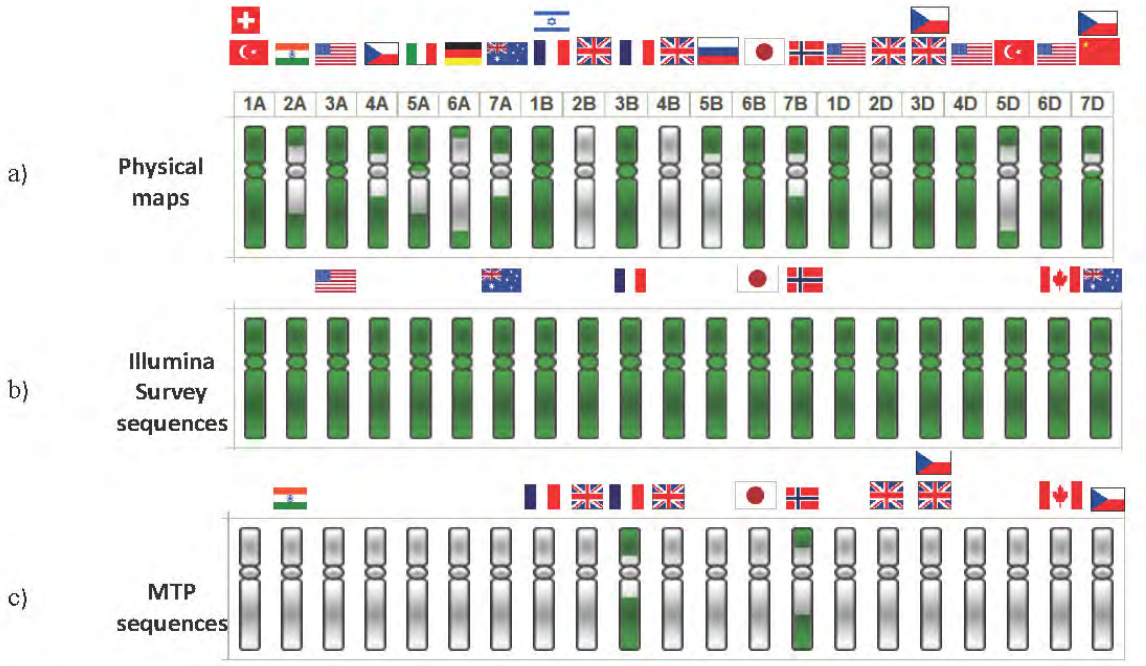

Fig. 1 Schematic representation of the current status (June 2012) of IWGSC efforts to (a) establish physical maps, (b) conduct sequence surveys by Illumina, and (c) reference sequences of each of the 21 hexaploid wheat chromosomes. Completion of each project is represented with green bars on each chromosome and chromosome arm. Four levels of completion are displayed (25, 50, 75 and 100%). Flags represent the countries of the laboratories responsible for currently funded projects. When two flags are displayed, the upper flag corresponds to the short arm and the lower flag the long arm. For those chromosomes lacking a specific flag, Illumina survey sequences were obtained by The Genome Analysis Centre (TGAC) with support from BBSRC, Biogemma, Graminor, ICARDA and INRA. For more details and regular updates, see http://www.wheatgenome.org/Projects

Physical maps serve as substrates for selecting clones containing fragments making up the chromosomes with minimal overlap. This so called Minimal Tiling Path (MTP) can then be used for sequencing and assembly of a high quality reference sequence in the form of a pseudomolecule representing each chromosome (Choulet et al. 2012). Using the sequence-ready physical map of chromosome 3B completed during the EU project TriticeaeGenome (Rustenholz et al. 2011), sequencing the MTP of chromosome 3B began in 2010 and was achieved recently (Fig. 1C). A total of 1,205 contigs covering 97% of the chromosome were assembled, and a MTP of 8,848 BAC clones was selectedand used to obtain a reference sequence of chromosome 3B using a combination of MTP/chromosome shotgun 454 Titanium /Illumina sequencing in the framework of the ANR-France Agrimer 3BSEQ funded project (http://urgi.versailles.inra.fr/Projects/3BSeq). After a first finishing step, the current assembly of chromosome 3B consists of about 5,000 scaffolds with a N50 value of 464 Kb and a total assembly size of 995 Mb. Automated annotation was performed with the Triannot pipeline (Leroy et al. 2012) using a version (V3.0) adapted to parallel computing which can annotate the 1 Gb of sequence within 5 days. After validation, a non redundant gene set of 7,975 genes was established and is under manual curation. The annotated sequences will be integrated into the physical map which is already available (http://urgi.versailles.inra.fr/gb2/gbrowse/wheat_phys_pub/) through the URGI Information System (http://urgi.versailles.inra.fr/gnpis/) that will provide links between the sequence and the genetic maps, QTL and marker information as well as phenotypic data within a single platform. The IWGSC-secured funding for the MTP sequencing of 8 additional Chinese Spring chromosomes and the next milestone is to finalize the funding for MTP sequencing of all 21 bread wheat chromosomes by 2013, which, if successful, would result in the completion of sequence production by 2015.

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While waiting for the MTP based reference sequence of each bread wheat chromosome and to facilitate marker development and map anchoring, the IWGSC engaged an effort led by the Genome Analysis Center (TGAC) in the UK to produce shotgun survey sequences of all 21 chromosomes. Sponsored by industry and government partners, the aim of the initiative was to generate sequences of at least 50x coverage and a virtual order for most wheat genes on a chromosome basis. The survey sequences provide only partial information on the order and orientation of the contiguous sequences (contigs) and do not represent a complete, reference genome. Rather, they enable *in silico* mapping, facilitate annotation of genes within contigs; allow refining of exome capture strategies, and support localized synteny studies. Sequences generated by Illumina sequencing for each chromosome arm have all been assembled (Fig. 1b) and a virtual gene order has been derived by exploiting the conserved synteny among grasses through a process called the "GenomeZipper" (Mayer et al. 2009). While whole genome analyses are underway within the consortium, the IWGSC provides immediate access to the resource for BLAST analyses, *in silico* mapping and individual gene/family analyses through its website (http://urgi.versailles.inra.fr/srs83/displayTool.do?toolName=BlastN).

Information on the various IWGSC projects as well as the availability of data can be found on the consortium website: http://www.wheatgenome.org. Information on obtaining pre-publication access to IWGSC tools and resources can be found on the website: http://www.wheatgenome.org/Tools-and-Resources.

Acknowledgements
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Identification and characterisation of effector genes from the wheat stripe rust fungus

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Keywords: Barley, effector, haustoria, metabolism, next-generation sequencing, *Puccinia striiformis*, transcriptomics

Abbreviations: *Pst* – *Puccinia striiformis* f. sp. *tritici; Pgt* – *Puccinia graminis* f. sp. *tritici;* NGS – next generation sequencing; *Avr* – avirulence; 454 – next generation sequencing on the Roche Genome Sequencer FLX; Illumina – next generation sequencing on the Illumina HiSeq or Genome Analyzer platforms

Abstract

Wheat stripe rust caused *Puccinia striiformis* f. sp. *tritici* (*Pst*) is currently one of the most significant plant pathogens on a global scale, causing widespread yield losses and overcoming the important *Yr17* and *Yr27* resistance genes. From a molecular point of view, very little is known about this organism, in particular its remarkable capacity to mutate to virulence on previously resistant wheat cultivars. In an effort to overcome this, we generated several datasets to map the genome, and describe deployment of the pathogen's genes during infection. The most important goal of these studies is to identify virulence genes called effectors, which typically are secreted by the pathogen into the host tissue to promote the infection. A subset of effectors can be recognised by the host immune (resistance) machinery, and these are designated avirulence (*Avr*) genes in the jargon. By characterising genes expressed in the fungal haustoria, we identified 531 candidate effector genes which encode secreted proteins characteristic of effectors. These are largely novel proteins which are generally not shared with those described from the related species *Puccinia graminis* f. sp. *tritici*, the causative agent of wheat stem rust. Overall, the sequences give little indication to function, and further insight will be derived from genomic characterisation of related pathogen strains with differing pathogenicity profiles.

Introduction

Cereal rusts caused by *Puccinia* spp. are significant constraints to crop production in Australia and the world. In Australia, stripe rust is the second most damaging disease of wheat, causing $127 M in lost wheat production p.a., with the potential to cause $994 M p.a. in losses in the absence of fungicide use and breeding for resistance (Murray and Brennan 2009). The cost for foliar application of fungicides to the Australian wheat crop is estimated at $359 M p.a., necessary largely because of the recent arrival of a new virulent strain that is not currently controlled by resistance. Although wheat stem rust is one of the most feared diseases on a world scale, with the recently emerged and highly virulent *Pgt* race Ug99 moving from Africa into the Fertile Crescent and potentially to South Asia (Singh et al. 2008), this pathogen is currently well controlled in Australia through genetic means (Murray and Brennan 2009). However, stripe rust is currently at epidemic levels in Australia, the Middle East, China and elsewhere, where it is causing considerable crop damage. Many experts consider that worldwide, stripe rust is currently a more significant disease than stem rust. Overall, cereal rusts are severe constraints to food security and this problem is expected to be ongoing. This is because of the variability of the pathogen – it is able to evolve quickly to overcome host resistance, due in large part to the massive numbers of spores produced from a successful infection. Thus, a low background mutation rate is sufficient to drive the evolution of virulent strains when amplified by the vast spore production. In addition, many *Puccinia* species have a complex life cycle featuring alternation of generations including a sexual stage that considerably enhances the genetic variability of the pathogen (Leonard and Szabo 2005).

Rust fungi are obligate biotrophs and as such can grow only on permissive hosts and not easily in culture. This, and the absence of hosts for generation of sexual recombinants in Australia, has been a significant drawback to

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their characterisation, especially at a genetic level. In the asexual cycle, rust fungi infect cereals from dikaryotic (two heterologous haploid nuclei per cell) urediniospores (Voegele et al. 2009). Germinated spores invade the leaf through stomata, and penetrate the host cell wall, forming a specialised cellular feeding structure called the haustorium. Haustoria are enriched for molecules associated with pathogenicity (Catanzariti et al. 2007). They invaginate the host cell, but remain extracellular as they are separated by the host plasma membrane, which differentiates to become the extrahaustorial membrane. This process reoccurs as the fungus ramifies throughout the host tissue, eventually resulting in formation of mature uredinia which release the urediniospores. This cycle can continue *ad infinitum*, but many *Puccinia* species are macrocylic; that is, are able to differentiate through a further four spore stages (Leonard and Szabo 2005). The most significant of these are the haploid (monokaryotic) pycniospores, which recombine sexually and ultimately produce dikaryotic aeciospores, which infect the grass host and restart the urediniospore infection cycle. *Puccina* species require alternate hosts for the macrocyclic, sexual stages, but these are effectively absent in Australia for all cereal rust pathogen species except the barley leaf rust pathogen *Puccinia hordei*.

Genome sequencing and assembly of *Puccinia striiformis* f. sp. *tritici* strain 104 E137 A-

The aim here is to sequence and assemble the genome of an Australian isolate of *Pst* using a combination of next-generation sequencing (NGS) technologies (Roche-454 and Illumina technologies (Metzker 2010)) and to identify fungal genes encoding virulence effectors. We received an early isolate of the original *Pst* founder strain, 104 E137 A-, that first established in Australia in 1979, as a gift of Dr. Colin Wellings, University of Sydney. The sample was purified through a single pustule, and the spores were amplified to produce enough material for sequencing. About 5 µg of high quality genomic DNA was used for the initial sequencing process using 454, and a similar quantity was used for Illumina. High-throughput sequencing technologies allow massively parallel sequencing of millions of short-read sequences. This enables fast and cheap genome sequencing, re-sequencing of large numbers of known genomes, or rapid investigation of transcriptomes under various conditions (Metzker 2010). The 454 platform produces longer reads (~400 bases) but with far less coverage than Illumina, which produces very high coverage of 75-100 base sequences. Both platforms offer the possibility of paired-end reads – sequences from the either end of larger DNA fragments, which facilitates sequence assembly because the distance between individual reads can be estimated. For this study, we generated genomic libraries composed of 75 bp Illumina paired-end (~30x coverage), 180 bp Illumina paired-end library (10 Gb data; in preparation), 3 kb 454 mate-paired (3x coverage, for genome scaffolding) and 50 bp Illumina, 2 kb paired-end (20x coverage). The paired-end libraries provide information about the physical distance between the two sequence reads, which can be used to facilitate *de novo* genome assembly. This is particularly relevant because a reference stripe rust genome is not available, and helps to resolve larger structural rearrangements (insertions, deletions, inversions) as well as repetitive regions. These reads have been assembled locally and in collaboration with Dr David Studholme (University of Exeter, UK) using a combination of software platforms. The primary aim of this analysis is to identify the sequence and organization particularly of loci encoding effector genes, as a starting point to detect sequence and copy number polymorphisms between haploid nuclei and between *Pst* strains with differential virulence on host genotypes. Ultimately, we hope to gain more insight into the identity of pathogen effector and *Avr* genes, the molecular steps underlying mutation to virulence, and the molecular strategies that explain the contributions of individual effectors to virulence.

Analysis of the stripe rust haustorial transcriptome

Haustoria are enriched for pathogenic factors and thus analysis of their expressed genes has the potential to provide considerable insights into fungal gene programming. We devoted considerable time and effort to purifying haustoria, which express surface sugars that allow affinity purification using lectins such as Concanavalin A (Hahn and Mendgen 1992). We have optimized haustorial isolation using a combination of percoll gradients and fluorescence-activated cell sorting (FACS) to recover large numbers of haustoria that are essentially free of chloroplasts (Fig. 1). We have used these preparations to generate the first *Pst* haustorial transcriptome using NGS. The transcriptome of germinated spores has also been analyzed as a baseline control to establish expression differences with respect to haustoria.

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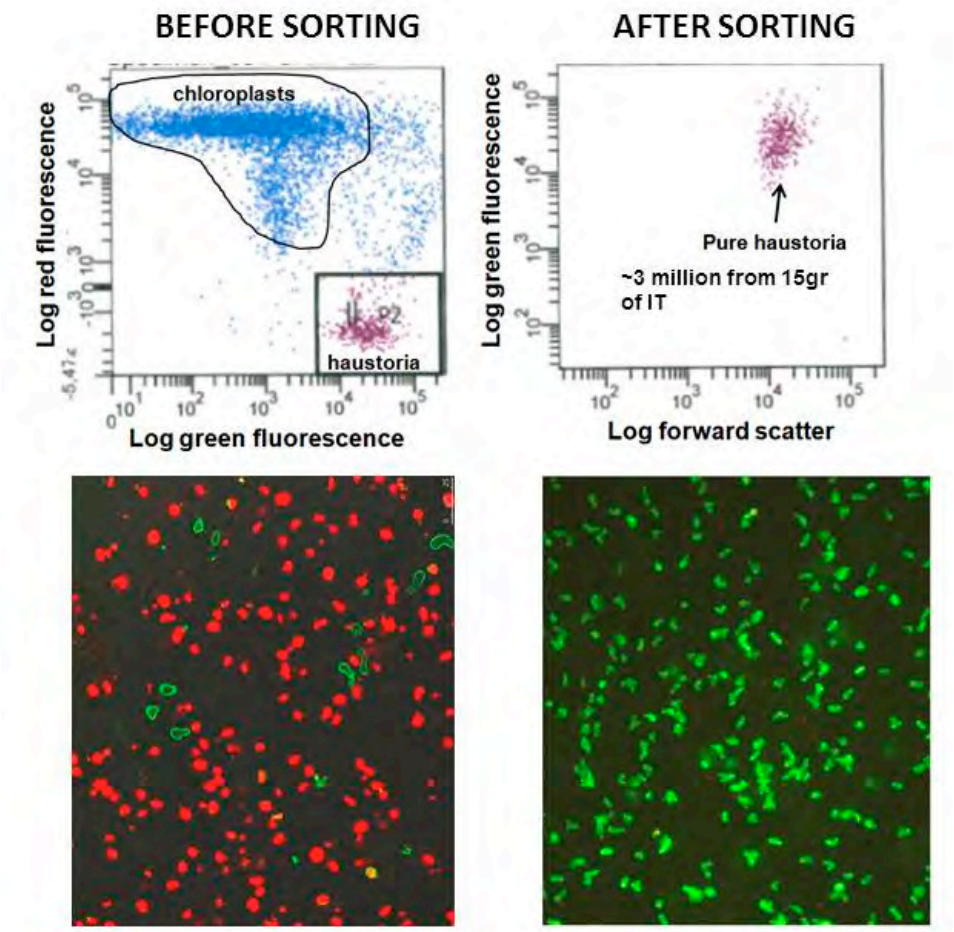

Figure 1 Purification of *Pst* haustoria by fluorescence-activated cell sorting (FACS). Top left: profile of crude rust-infected tissue after sorting showing separation of chloroplasts and haustoria. Top right: profile of purified haustoria after sorting. Bottom left: haustoria preparation showing abundant chloroplasts (red) and haustoria (green). Bottom right: pure haustoria (green purified by FACS.

The use of NGS technology to study gene transcription from mRNA preparations is known as RNAseq (Metzker 2010). We extracted mRNA from purified haustoria and germinated urediniospores for 454 sequencing, and subsequently for profiling by the higher density Illumina technology which gives statistical robustness to the analysis. For each tissue, a total of 729,036 and 457,071 single 454 reads with average sizes of 413 and 420 bp, respectively, were generated. Using CLC genomics (CLC Bio v3.9) the haustorial reads were assembled *de novo* into 16,831 contigs. The dataset was cleaned of contaminating plant sequences before mapping against the *Pst* draft genome described above, resulting in a sequence set of 14,662 fungal contigs. This set was analysed for effector genes to identify ORFs with a predicted signal peptide (SP) for protein secretion, but lacking internal membrane-spanning domains. In this way we defined 531 genes that are candidate effector genes, the majority of which were under 300 amino acid residues in length, consistent with the consensus criteria for small secreted peptides (SSPs) of which effectors are believed to be a subset (Table 1). In contrast from what has been described previously for effector proteins of rust fungi and other obligate biotrophs, we found no evidence for sequence similarity between predicted stripe rust effector proteins that could define gene families or tribes, or conserved sequence motifs (Saunders et al. 2012). Notably only a few *Pst* genes had similarity with predicted *Pgt* effectors; most were completely novel in sequence. Godfrey et al. (2010) defined the Y/F/WxC motif which was supposed to be overrepresented in the first 30 aa of candidate effector proteins. Although we could find examples of this motif within our effector set, it was present only in some predicted proteins, and at an incidence not above that predicted by chance. Thus, the importance of the Y/F/WxC motif is questioned by our findings.

Finally, many secreted fungal proteins are enriched for cysteine residues, which stabilize folded proteins by formation of disulfide bridges between cysteines. While we could find some evidence for increased numbers of cysteine residues in candidate *Pst* effectors, these were not present at particularly high levels, although some proteins that contained similar numbers of cysteines showed conserved spacing between them, suggestive of a common ancestor. Thus, not much can be learnt from candidate *Pst* effector genes by analysis of their coding sequences.

Table 1 Characteristics of 514 candidate effector proteins predicted from deep transcriptome sequencing of the wheat stripe rust fungus.

Motif Y/F/WxC	1-30 aa after SP	47
	Other location	93
	none	375
Protein length	≤ 300 aa	418
	> 300 aa	97
Cysteine content	< 4 Cysteines	256
	4 ≥ Cysteines	259
Level of expression	Spores < Haustoria	290
	Spores > Haustoria	101
	Haustoria = Spores	124
BLASTn (NCBI nt)	not available	340
	PGT Hypothetical protein	128
	Other hits	47
BLASTx (NCBI nr)	not available	122
	PGT Hypothetical protein	306
	Other hits	87

The final aspect of this work was to examine differential gene expression profiles between germinated urediniospores and haustorial datasets. To do this, a new unigene assembly of 12,464 contigs was created by *de novo* assembly of the pooled haustorial and spore reads described above. Raw reads from each tissue were independently mapped against the reference set and expression values expressed as numbers of reads per kilobase. Expression values were statistically assessed to determine differential expression between each tissue type. Digital expression analysis revealed that 2,440 genes were up-regulated in haustoria, and 2,754 were upregulated in spores (Fig. 2). This provides a considerable advance on what is known about rust pathogen gene expression during development and shows a clear difference between the genetic programming of each stage. Moreover, the function of many genes can be uncovered by comparison to public databases, which gives a very detailed description of the priorities of each tissue in completion of the fungal lifecycle. Thus, germinating urediniospores apparently use lipid reserves to generate energy used in the growth processes of DNA replication and cell division, and actively modify chitin to avoid host recognition. On the other hand, haustoria are enriched in products that transport nutrients such as sugars and amino acids from the host; use these to generate

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precursors of metabolites and energy; biosynthesise compounds necessary for the ultimate production of spores, and importantly, secrete effector proteins.

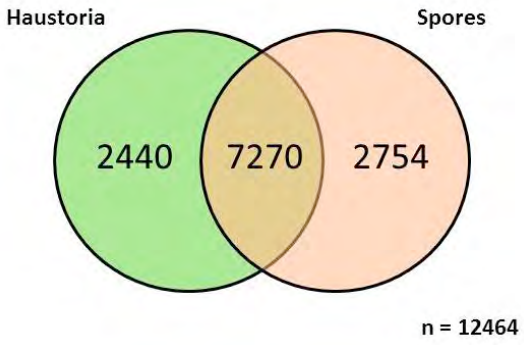

Figure 2 Differential regulation of gene expression in pure haustoria and germinated spores of the wheat stripe rust fungus.

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Antibody-mediated protection of plants against Fusarium pathogens

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Abstract: Fusarium head blight (FHB) is an economically devastating disease of wheat in China and many other countries. Innate resistance for FHB is inadequate and investigation of antibodies with resistance roles could provide an alternative strategy for breeding FHB-resistant cultivars. Analyses of FHB pathogens in wheat throughout China revealed two species, *F. asiaticum* and *F. graminearum sensu stricto*, as the predominant causal agents responsible for FHB of wheat. Cell wall-bound proteins (CWPs) from a representative strain of *F. asiaticum* from Wuhan, a region with frequent FHB epidemics, were prepared and used to generate antibodies. A chicken-derived single chain antibody, CWP2, specific to antigens displayed on the *Fusarium* cell surface was isolated from a pooled immunocompetent phage display library. This recombinant antibody inhibited fungal growth *in vitro* when fused to antifungal peptides (AFPs). Expression of the fusion proteins in transgenic *Arabidopsis thaliana* and wheat plants conferred a high level of protection against *Fusarium* pathogens, with a significant reduction in initial infection, fungal spread and mycotoxin production. Plants expressing CWP2 alone also exhibited a moderate level of resistance. Envisaged as a promising resistance germplasm for use in developing transgenic plants resistant to FHB, the CWP2 antibody was further improved *in vitro* for its affinity for FHB pathogens. One of the improved antibodies, CWPa, had 15-fold higher affinity compared to CWP2 and conferred even higher resistance to FHB. The antibody-mediated resistance based on FHB pathogens was *Fusarium*-specific and had no harmful effects on non-*Fusarium* micro-organisms. The term "pathogen-based resistance" is proposed to describe this promising strategy to control important pathogens in an environmentally friendly way. Advances in combined tools of molecular immunology, plant pathogenomics and biotechnology will further facilitate the generation of *Fusarium*-specific antibodies. In addition, these antibodies can be utilized as sources of resistance to develop transgenic plants resistant to FHB.

Keywords: antibody affinity, *Fusarium asiaticum, Fusarium graminearum*, Fusarium head blight, phage display, resistance, single chain antibody, surface antigen

Introduction

Fusarium head blight (FHB) or scab, caused by *Fusarium* spp., is an economically devastating disease of wheat, barley, corn, and other small grain cereal crops worldwide, and is particularly favored by conditions of high humidity and warm temperatures (Parry et al. 1995; Windels 2000; Bai and Shaner 2004). At least 17 species of *Fusarium* infect all members of the Gramineae and most genera of other cultivated plants. Infection takes place both in the field and during storage. FHB epidemics occur frequently in the middle and lower regions of the Yangtze River and in northeastern China (Qu et al. 2008; Zhang et al. 2012). During recent years, global climate change has aggravated the spread and severity of FHB to an even wider region, extending northward in China (Liang et al. 2007) and causing increased frequencies and intensities of epidemics in Canada, Europe and the USA, results in huge losses in yields and quality. Various trichothecene mycotoxins that accumulate in cereal grains during infection by FHB pathogens may occur in food/feed items such as flour, beer and animal feed. Recent incidences of mycotoxicosis due to the consumption of FHB-infected wheat flour were reported in China (Chen et al. 2003) and contaminated grain continues to pose a serious threat to human health.

The most effective strategy for control of fungal diseases is the prevention of infection in the field and during storage by endogenous expression of resistance genes. However, innate FHB resistant germplasm is inadequate in nature, and it is a challenge to develop resistant varieties with adequate resistance (Liu 2001). Current

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protective measures rely heavily on fungicides, generating undesirable environmental consequences and fungicide-resistant *Fusarium* strains (Chen and Zhou 2009). Therefore, introducing alien resistance genes into the wheat genome by transgenic approaches has been proposed as a strategy to protect plants against *Fusarium* pathogens and reduce mycotoxin production. Anti-fungal peptides/proteins (AFPs) expressed in wheat plants confer resistance to FHB in wheat. In most cases, the expression of individual AFPs merely delays the appearance of symptoms and does not provide effective control of the disease. Thus, development of alternative approaches to improve resistance is essential for effective control of FHB and to reduce mycotoxin loads.

Antibodies, also known as immunoglobulins, produced in very large numbers by all vertebrates recognize and bind pathogen-specific antigens; thus helping to eliminate pathogens from the body. Although plants do not innately produce antibodies as do their animal counterparts, they, as eukaryotic organisms, carry all essential components necessary for functional expression and targeting of antibodies. Full-size antibodies and single-chain antibodies (scFv) against plant pathogens are expressed, and have been characterized, in plants (Voss et al. 1995; Boonrod et al. 2004; Nölke et al. 2009). Plant-derived antibodies have been developed as defence molecules for the protection of plants against various pathogens. For example, apoplast accumulation *in planta* of a *Fusarium*-specific single-chain Fv antibody generated against *F. graminearum* from scabby wheat spikes in China was shown to protect plants against *Fusarium* pathogens (Peschen et al. 2004). Pathogen-specific scFv antibodies conferred a significant level of resistance towards *Sclerotinia sclerotiorum* in rapeseed (Yajima et al. 2010) and *F. virguliforme* in soybean plants (Brar and Bhattacharyya 2012). Thus, antibodies with defined specificity and affinity have potential to improve resistance in plants, especially in pathosystems that lack natural resistance.

The first scFv antibody, CWP2, demonstrated to confer resistance to fungal pathogens *in planta* was isolated by phage display from chickens that were immunized with cell wall proteins (CWPs) from a representative *F. asiaticum* strain 5035 isolated from a scabby wheat spike in Wuhan (Qu 2002; Peschen et al. 2004; Zhang et al. 2007). This *Fusarium* strain is highly pathogenic and produces DON and 15-AcDON. The CWP2 antibody generated reduced disease severity and mycotoxin production in plants when challenged with toxigenic *Fusarium* pathogens. More importantly, resistance to FHB was significantly enhanced in transgenic plants that expressed the antibody fused with AFPs. Furthermore, combined with molecular evolution *in vitro*, affinity-improved antibodies derived from CWP2 were isolated and used to generate a better resistance *in planta* against *Fusarium* pathogens (Liu et al. 2011). This paper summarizes current progress in antibody-mediated resistance to *Fusarium* pathogens, molecular interactions between an antibody and its antigens from *Fusarium* fungi, and reviews a few examples applied to control of other fungal diseases following similar strategies. We propose the term "pathogen-based resistance" (PBR) to describe antibody-mediated pathogen-specific resistance and other similar strategies in comparison with pathogen-derived resistance (PDR).

Fusarium head blight and associated pathogens in wheat

The first report of FHB in wheat in China was from Anhui province in 1936. Since then, FHB epidemics have become more severe and frequent in the middle and lower regions of the Yangtze River valley in central China, including the province of Hubei where there are often abundant rains coinciding with high temperatures during the wheat-flowering period. In 1985, a FHB epidemic in wheat affected up to 373 thousand hectares in Henan province alone, causing a yield loss of 900,000 tonnes. A more recent survey of wheat fields in Hubei in 2009 revealed FHB levels (percentages of the diseased wheat spikes) ranging from 36.0 to 98.7% (Fig. 1), with 12 of 14 regions being higher than 50%, the threshold of a severe FHB epidemic (Lu et al. 2001). Mycotoxin contamination ranged from 0.59 - 15.28 mg kg^{-1} in grains collected from these regions (Zhang et al. 2012). Thus, FHB is still a major threat to wheat production in the region. In addition, FHB on wheat and other cereal crops has also been reported in different continents, e.g., Africa (Boutigny et al. 2011), America (Windels 2000), other countries in Asia (Lee et al. 2004), Europe (Tóth et al. 2005), and Oceania (Akinsanmi et al. 2006).

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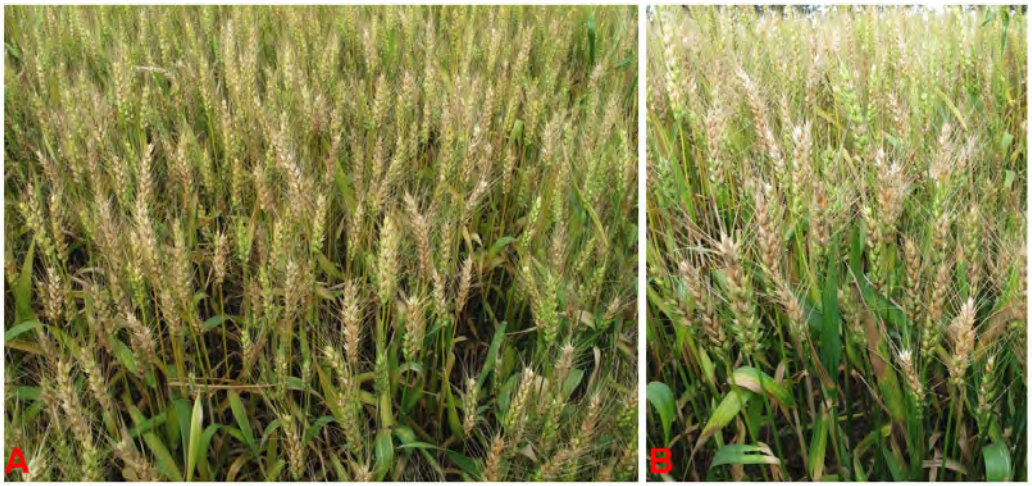

Figure 1 Symptoms of Fusarium head blight in wheat fields in Qianjiang (A) and Xiantao (B), in Hubei, China. The photos were taken on the 8th May, 2009 (For detailed data, see Zhang et al. 2012)

The causal agents associated with FHB symptoms on wheat and barley are *F. graminearum* species complex (Fg complex), which is considered a single cosmopolitan species. Using conventional classification methods, morphological species recognition fails to set clear species limits for this group. Genome sequencing of *F. graminearum* and advances in molecular biology led to the development of genealogical concordant phylogenetic species recognition (GCPSR) coupled with high-throughput multilocus genotyping (MLGT) assays of house-keeping genes and molecular marker technologies. This led to identification of 14 phylogenetically distinct, cryptic species within the Fg complex (O'Donnell et al. 2000, 2004; Wang et al. 2011). The species designation *F. graminearum* has therefore been *sensu stricto*, and accordingly, other Fg complex species designations are used (for details, see a review by Wang et al. 2011).

Earlier studies of *Fusarium* isolates from scabby wheat in China in the 1980s showed that the trichothecene mycotoxins deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON) and 15-acetyldeoxynivalenol (15-AcDON), but not nivalenol (NIV), were produced by Chinese *F. graminearum* isolates (Wang and Milier 1994). Characterization of strains collected from wheat in China in 1999 and subsequently showed that the predominant fungal species derived from FHB-infected wheat were *F. asiaticum* and *F. graminearum sensu stricto* (Gale et al. 2002; Qu et al. 2008; Zhang et al. 2007, 2012). These two species co-occurred in the middle and lower reaches of the Yangtze River, but the vast majority was *F. asiaticum* (Fig. 2). Recent genetic chemotyping and chemical analyses revealed that Fg complex strains associated with FHB on wheat in China produced various types of trichothecene mycotoxins such as DON, 3-AcDON, 15-AcDON, NIV and 4- acetylnivalenol (4-AcNIV) (Li et al. 2005; Wang et al. 2008; Zhang et al. 2012). Therefore, the predominant *F. asiaticum* strains that produce DON mycotoxins are the representative species in China, and thus have been used for subsequent analyses including preparation of antigens for generation of antibodies in our laboratory.

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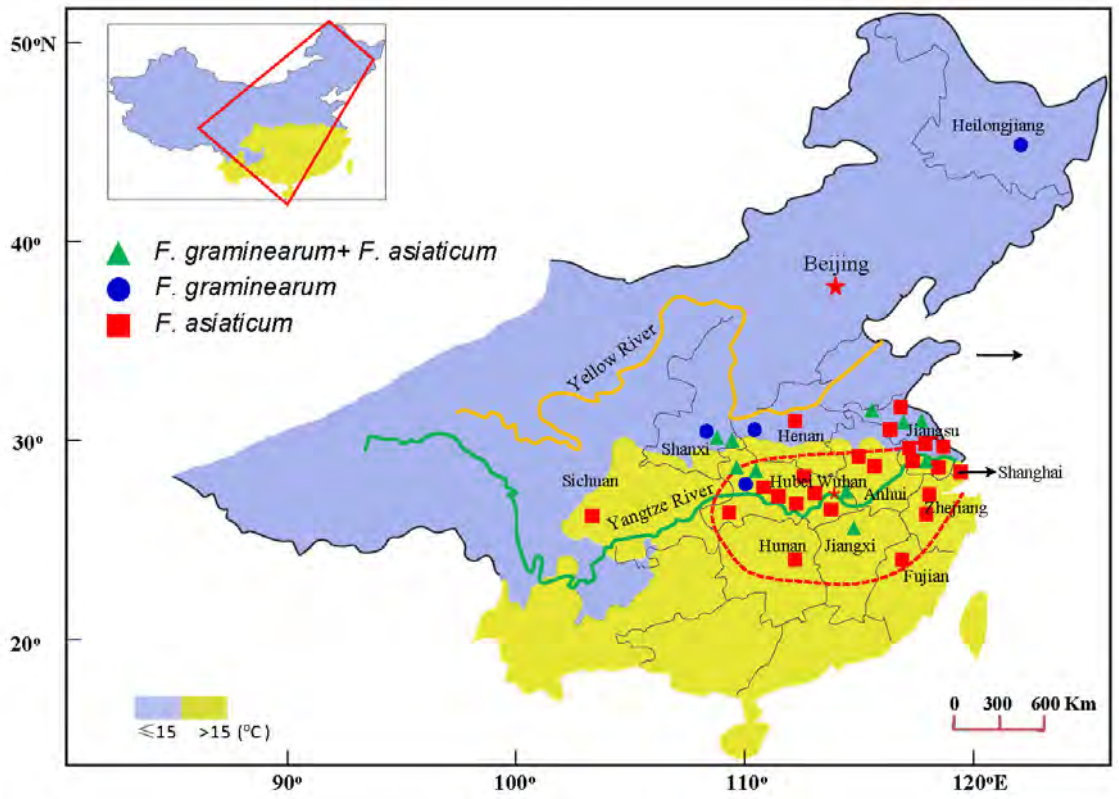

Figure 2 Distribution of *Fusarium asiaticum* and *F. graminearum sensu stricto* in China in relation to regional temperature. Temperature zones are arbitrarily divided at a mean temperature of 15° based on the annual average temperature data from 1970 to 1999 (adapted from Qu et al. 2008)

Resistance to Fusarium head blight in wheat

Genetic analyses carried out in 1980s in China showed a quantitative nature of FHB resistance for a few local Chinese wheat varieties such as Sumai 3 and Wangshuibai, with both additive and dominant effects. In addition, different resistance modes for the wheat cultivars Sumai 3 and Wangshuibai were observed. During the last decade, quantitative trait loci (QTL) analyses revealed that all chromosomes except 7D in wheat were involved in FHB resistance (Buerstmayr et al. 2009). Moreover, a few well known Chinese wheat cultivars with moderate FHB resistance, such as Sumai 3, Wangshuibai and Fanshanxiaomai, displayed a susceptible response to Fusarium seedling blight (FSB) when challenged with the same isolates causing FHB (Wu et al. 2005; Li et al. 2010). In recent studies different QTLs were attributed to FSB and FHB resistances (Tamburic-Ilincic et al. 2009). Although various efforts have been made in China during the last four decades to breed FHB-resistant wheat cultivars through conventional approaches, progress has been slow and the resistance obtained is not adequate due to the complex nature of resistance and limited availability of FHB-resistant germplasm (Liu 2001; Lu et al. 2001).

Expression of alien genes has thus been proposed to improve FHB resistance in wheat, and AFPs and various transcription factors were introduced into wheat (Chen et al. 1999; Mackintosh et al. 2007; Di et al. 2010). Constitutive expression of the alien genes usually reduced FHB disease severity to a limited degree, and for a limited period of time after inoculation. Moreover, transgenic wheat expressing an AFP may display resistance to *Fusarium* pathogens and other non-*Fusarium* pathogens. Transcription factor *NPR1* gene from *Arabidopsis thaliana* conferred resistance to Fusarium head blight in transgenic wheat, but increased the severity of Fusarium seedling blight, with concurrent activation of defense genes in response to *Fusarium* pathogens (Makandar et al. 2006; Gao et al. 2012). Therefore, it is necessary to develop alternative ways to protect plants against *Fusarium* pathogens and to reduce mycotoxin levels, preferably in a *Fusarium*-specific manner. Antibody-mediated approaches may have potential to generate such specific resistance.

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Antibodies expressed in plants (Plantibody)

Since the first demonstration of functional expression of mammalian antibodies in plants more than twenty years ago (Hiatt et al. 1989), various studies have shown that plant cells are capable of carrying out many of the post-translational modifications required for optimal biological activity of antibodies because they produce fully functional antibodies with virtually identical specificity and affinity as monoclonal antibodies produced by hybridoma cell lines (Fischer et al. 1997; 1999; Liao et al. 2006). So far, different forms of full-size monoclonal IgG and secretory IgA antibodies, Fab fragments, scFv antibodies, biscFv antibodies, diabodies and scFv antibody fusions to AFPs have been expressed in leaves and seeds of plants without loss of binding specificity or affinity (Fig. 3; Safarnejad et al. 2011). Exploiting the innate protein sorting and targeting mechanisms of plant cells can enhance expression levels of recombinant antibodies in plants, and thus make the plants carry new characters that are directly associated with the antibodies.

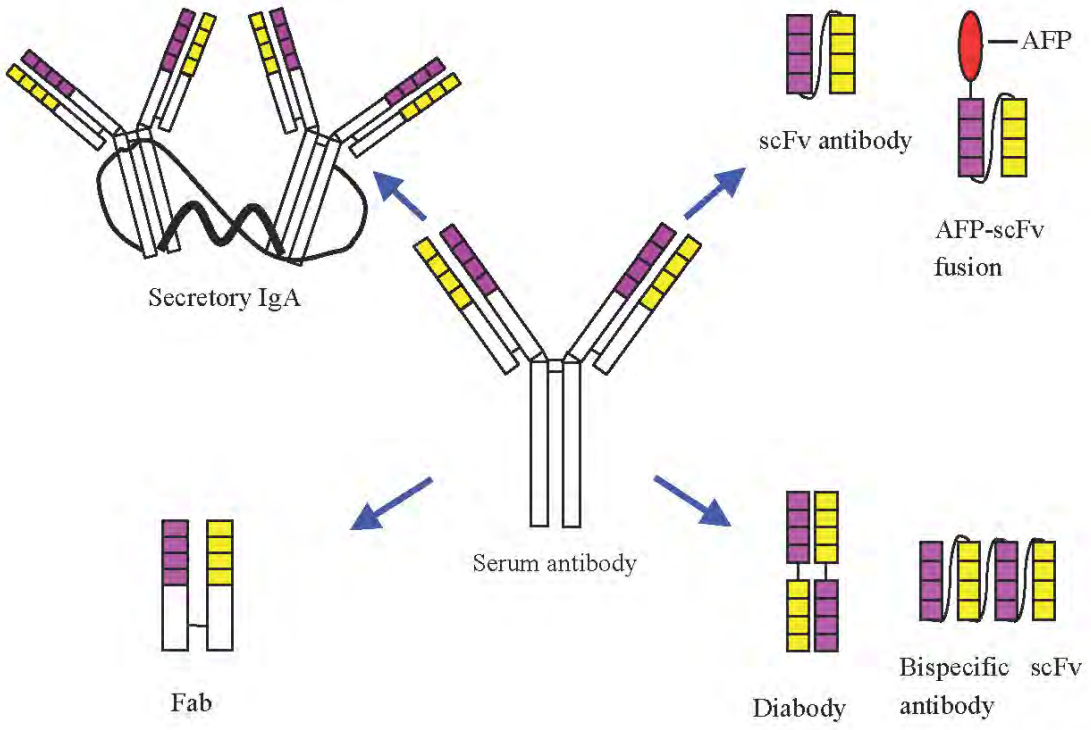

Figure 3 Different antibody formats expressed in plants

Antibodies expressed in plants are called plantibodies. Significant increases in recombinant antibody yield were observed when antibodies were targeted to the secretory pathway instead of the cytosol. Secretion of antibodies into the intercellular space leads to significant levels of expression. Plant-produced recombinant antibodies have been functionally targeted to different compartments of plant cells: viz. the intercellular spaces, cytosol, chloroplasts and endoplasmic reticulum (Spiegel et al. 1999; Boonrod et al. 2004). In most cases of cytosolic expression of scFvs, levels were found to be very low or just at the detection limit. However, cytosolic expression of a coat protein-specific scFv conferred significant resistance to tobacco mosaic virus (TMV) (Zimmermann et al. 1998), because the cytosol is believed to be the place for TMV propagation after infection. Thus, cellular compartments for functional targeting of plantibodies for resistance improvement will depend on mode and progression of infection and development of the pathogens in host plants.

Generation of recombinant antibody against surface antigens of *Fusarium* pathogens

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To prove the principle that monoclonal antibodies with defined specificity and affinity can be generated against surface antigens from phytopathogenic fungi, cell wall-bound proteins (CWPs) from mycelium of FHB strain 5035 was prepared and subsequently used for immunization of animals and screening of specific antibodies that bind to the surface of *Fusarium* pathogens. Strain 5035 is a representative strain in China belonging to the predominant phylogenetic species *F. asiaticum* from FHB epidemic regions and produces DON and 15-AcDON, the main contaminating mycotoxins in wheat (Qu 2002; Zhang et al. 2007). The CWPs were used for immunization of chickens from which spleenic RNA was isolated and reverse-transcribed into cDNA for amplification of avian variable heavy (V_H) and light (V_L) chains. The amplified V_H and V_L domains were connected by a flexible linker and cloned into a phagemid. The resulting recombinant phagemids were introduced into *E. coli* and phage display was deployed to select antibodies against the CWPs. After several rounds of panning, selected phage-displayed antibodies were subjected to phage enzyme-linked immunosorbent assay (ELISA) and highly reactive monoclonal scFv antibodies were expressed in bacteria. The soluble scFv antibodies expressed from bacteria were further analyzed by ELISA (Fig. 4 next page).

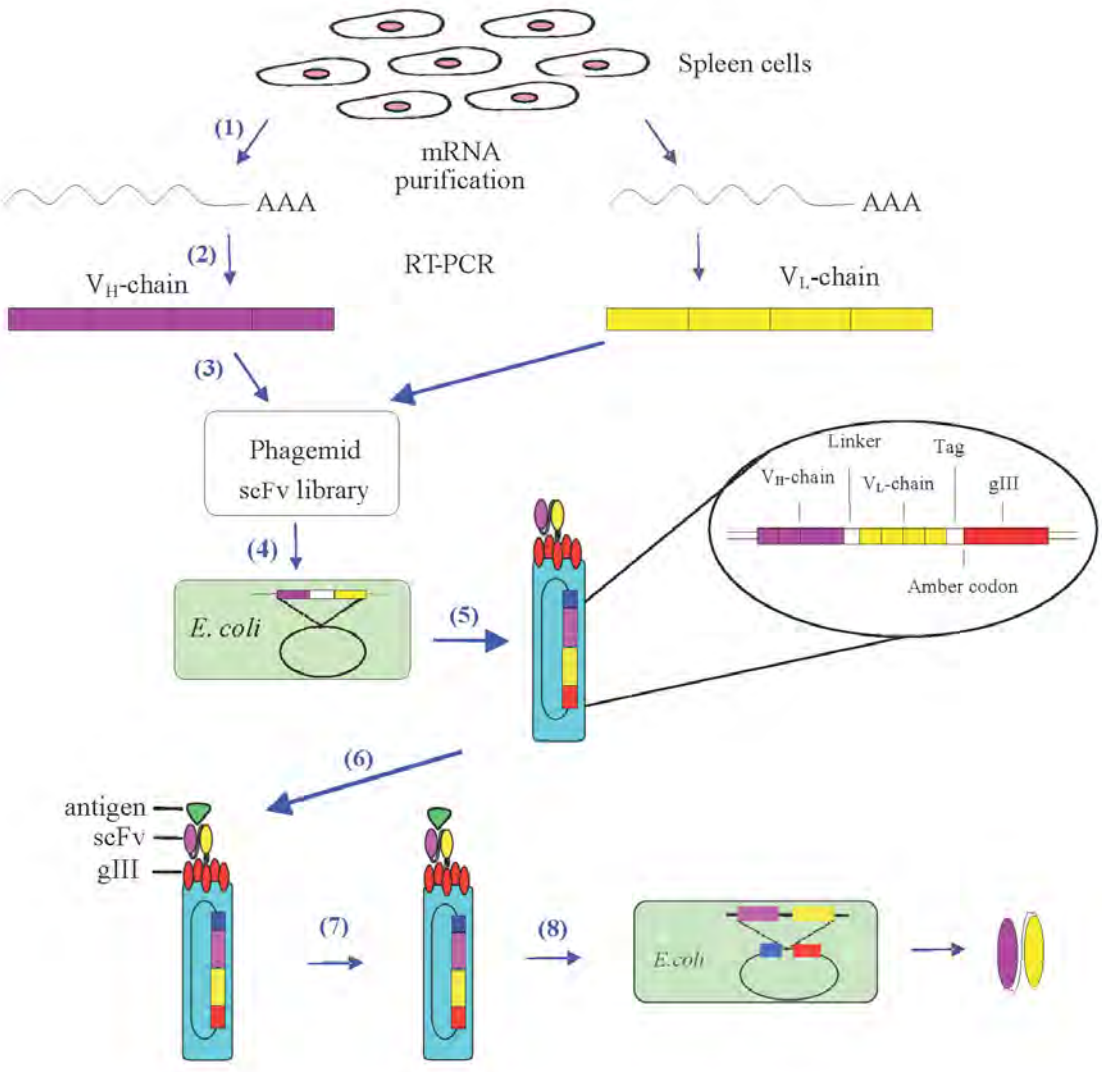

Figure 4 Generation of recombinant scFv antibodies by phage display. (1) Spleen cells from immunized animals are used for mRNA purification. (2) The mRNA is reverse-transcribed into cDNA and variable heavy chain sequences (V_H) and variable light chain sequences (V_L) are PCR-amplified. (3) V_H and V_L sequences are cloned into phagemid

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vector, such as pHEN4, to construct the scFv library. (4) The scFv library is used to transform *E. coli*. (5) scFv antibody is displayed on the surface of a phage particle. An amber codon is located at the end of tagged sequence and the scFv molecule is expressed as a fusion to the minor coat protein encoded by gIII in a suppressor strain. The phage particle contains the phagemid encoding the scFv gene, thereby linking genotype and phenotype. (6) Antigen-driven affinity selection. Phage library is panned with target antigens. (7) Affinity maturation. Phages with high binding affinity are enriched by several rounds of panning. (8) Soluble antibody production. Phagemid from the selected phages is transformed into a non-suppressor strain to express soluble antibodies for further characterization of specificity and affinity

Western blot analysis showed that the isolated scFv antibodies bound predominantly to one protein with a molecular mass of approximately 50 kDa from cell wall preparations of *F. asiaticum* and other *Fusarium* species. These results suggested that the scFv antibodies recognize the same epitopes of cell wall fractions of *Fusarium* fungi. ELISAs with a number of different *Fusarium* spp. and fungi from different genera revealed that these antibodies recognized all the *Fusarium* spp. with similar reactivity, but did not cross-react with non-*Fusarium* spp., such as *Aspergillus flavus, Rhizoctonia cerealis, S. sclerotiorum* and *Verticillium dahliae*, indicating that they were *Fusarium* genus-specific. To further identify the site of antibody binding, the antibodies were immunofluorescence-labeled and analyzed by confocal or fluorescence microscopy. The binding of one isolated antibody, CWP2 as an example, localized with bright, continuous fluorescence intensity to the cell walls of hyphae produced by germinating spores, confirming the specificity of this antibody for a cell surface target. Thus, recombinant scFv antibodies isolated against the CWP fractions of a *Fusarium* pathogen specifically recognized the surface antigens of the fungal pathogens. With similar approaches, several scFv antibodies against the CWPs from *Fusarium verticillioides*, the main fungal species responsible for *Gibberella* ear rot (GER) in maize, were isolated in our laboratory and demonstrated to recognize both surface targets in conidiospores and mycelia as visualized by immunofluorescence labeling (Hu et al. 2012). These results also suggested that surfaces of *Fusarium* spp. contain unique genus-specific components, although they are non-specific pathogens infectious to many genera of plants. These surface components appear to be highly immunogenic and may be involved in recognition between pathogens and their hosts. Dissection of these surface components may provide more information on molecular regulation and pathogenesis.

Antibody-mediated resistance in plants to *Fusarium* pathogens and mycotoxins

After *in vitro* assays, the CWP2 scFv antibody was chosen for expression in transgenic *Arabidopsis thaliana* to ascertain whether it was able to confer an enhanced resistance to *Fusarium* pathogens *in planta*. This antibody was fused to different AFPs and the AFP-CWP2 fusions (AFP-scFv fusion in Fig. 3) were assayed for inhibitory activity towards *Fusarium* pathogens *in vitro* and also introduced into *Arabidopsis* plants (Peschen et al. 2004; Hu et al. 2008). Transgenic plants with single integrations were selected and their integration sites were identified by TAIL-PCR. GFP-transgenic and non-transgenic *Arabidopsis* plants served as controls. All the transgenic and control plants were assayed for response to *F. oxysporum* and *F. asiaticum*. The results indicated that expression of a scFv antibody CWP2 alone in transgenic plants resulted in significant reductions in disease indices of up to 42% 14 days post inoculation (dpi), compared with GFP-transgenic controls. More importantly, plants expressing the AFP-CWP2 fusions displayed a particularly high level of resistance compared with those expressing GFP or AFP-PIPP (the PIPP is a scFv antibody which recognizes the human antigen hCG). After 21 dpi, the disease indices of plants expressing AFP-CWP2 fusions were 53-78% lower than those of corresponding plants expressing AFP-PIPP fusions. Furthermore, inoculation with *F. asiaticum* revealed that plants expressing CWP2 alone had a 55% reduction in disease index compared with GFP-transgenic plants. A reduction of 68%-76% was observed for the plants expressing AFP-CWP2 fusions compared with AFP-PIPP plants. As for mycotoxin accumulation, CWP2-transgenic plants displayed a reduction of 53% and AFP-CWP2 plants showed a reduction of up to 81% compared with their corresponding controls. On the other hand, transgenic *Arabidopsis* plants expressing the antibody or antibody fusions displayed a comparable susceptibility to the non-*Fusarium* fungal pathogen, *S. sclerotiorum*, indicating a *Fusarium*-specific resistance mediated by the *Fusarium* genus-specific antibody.

Enhanced resistance to FHB in wheat has also been demonstrated by constitutive expression of the antibody fusions regulated by the ubiquitin promoter. Antibody fusions that showed good resistance were selected for transformation of different wheat varieties (Li et al. 2008). Single floret injection with FHB pathogens revealed

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that transgenic wheat expressing the AFP-CWP2 fusions displayed significant reductions in disease levels of up to 80% at 21 dpi compared with non-transgenic wheat controls. Such a reduction is comparable to that observed for the FHB-resistant cultivar Sumai 3. Spray inoculation indicated that disease severities in the transgenic wheat lines were significantly lower than the non-transgenic controls, with disease reductions ranging from 20 to 41%. In comparison with FHB-resistant cultivar Sumai 3, some transgenic lines showed a significant reduction in initial infection. Furthermore, deoxynivalenol (DON) and acetylated DON levels in the transgenic wheat expressing the antibody fusion were significantly lower than those in non-transgenic controls, with reductions of up to 61%, again similar to that observed in the FHB-resistant cultivar Sumai 3. Thus, the expression of the antibody fusions enhanced the resistance of wheat plants to initial infection (type I resistance) and spread (type II resistance) as well as reducing mycotoxin levels (type III resistance).

Molecular evolution to improve affinity of an antibody with a resistance role

The CWP2 antibody conferred resistance to *Fusarium* pathogens. Envisaged as a promising resistance source for use in developing transgenic plants resistant to FHB, this antibody may be further improved *in vitro* for its affinity to *Fusarium* pathogens. To reach this objective, we used the CWP2 antibody for error-prone PCR and DNA shuffling to generate phage display libraries (Liu et al. 2011). Panning of the mutated library against CWPs from *F. asiaticum* strain 5035 by phage display enriched phage clones that were used for a further round of DNA shuffling to construct a combinatorial library. Screening of this library by phage display for variants reactive against the CWPs led to the identification of a number of clones. Comparative ELISAs revealed eight clones exhibiting a higher reactivity than the parent CWP2, and containing four different single-chain antibody sequences. Surface plasmon resonance (SPR) measurements confirmed that three mutated scFvs, CWPa, CWPb, and CWPd, displayed 15-fold, 11-fold, and 7-fold higher affinities, respectively, compared with the CWP2. Three-dimensional modeling of the antibodies illustrates a distinct conformational change of the CWPa antibody that has the highest affinity; it brings all six complementary domain regions (CDRs) on the antibody surface in one direction. This overall conformational change involving different variable regions of both heavy and light chains may contribute to the improved binding strength between the antibody and its antigen, as proven by SPR analysis.

The affinity-improved antibody CWPa was fused with different AFPs and then introduced into *Arabidopsis thaliana* for assay of their responses to FHB pathogens. Inoculation with *F. asiaticum* 5035 revealed that plants expressing AFP-CWPa showed better resistance than the AFP-CWP2, with disease reductions of up to 26%. These results suggested that the CWPa antibody with the improved affinity created by *in vitro* directed molecular evolution retained its specificity towards FHB pathogens *in planta*. Thus, specific recognition and binding of the antibody to antigens present in the invading fungus during infection appeared to be essential for enhanced resistance. Further functional expression in wheat is under way.

Antibody-antigen interaction and gene-for-gene recognition

Specificity and affinity of an antibody is defined by its corresponding antigen, whose unique structure interacts with the corresponding antibody. AFP-scFv fusions appear to have dual functions: a role of an antibody, i.e., specificity and affinity towards antigens, and the formation of an antibody-antigen complex that may in turn affect the innate functions of the antigen; and antifungal activity contributed by the AFP that directly interferes with the fungal infection process. CWP2 antibody-mediated resistance displayed a *Fusarium*-specific activity, with no harmful effect on non-target micro-organisms (Peschen et al. 2004). This specific resistance is clearly based on the uniquely intimate interaction of the antibody and the antigen present on the surface of the *Fusarium* hyphae. During infection, hyphal surfaces within plant tissues are at the fungus : plant interface thus having the antigen exposed at the surface offers the maximum opportunity for affinity and specificity of the antibody towards the antigen. Hence the fungal surface antigen should be a key component of fungal infection and development in pathogenesis. Indeed, a preliminary study through an immunoproteomics approach revealed that a gene coding for the surface antigen from FHB pathogens appeared to be involved in a specific antibody-antigen interaction (Xing et al. unpublished). FHB pathogens have a non-specific interaction with their hosts. However, the interaction of the CWP2 antibody with its antigen appears to bring this non-specific pathogen-plant interaction into a specific one, reminiscent of a gene-for-gene relationship. Based on these

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results and other analyses, a resistance mechanism deployed by pathogen-specific antibody fusions was proposed (Fig. 5; Bohlmann 2004). This resistance mode led us to assume that development of an antibody specific for a non-specific pathogen may eventually generate a specific interaction between a host and its non-specific pathogen, and subsequently, a novel strategy for specifically controlling pathogens that innately interact non-specifically with their hosts. Moreover, this model implies a proper combination of antibody fusions with different activities on plasma membranes and chitin polymers of fungi may generate a synergistic effect that results in a more durable resistance in plants.

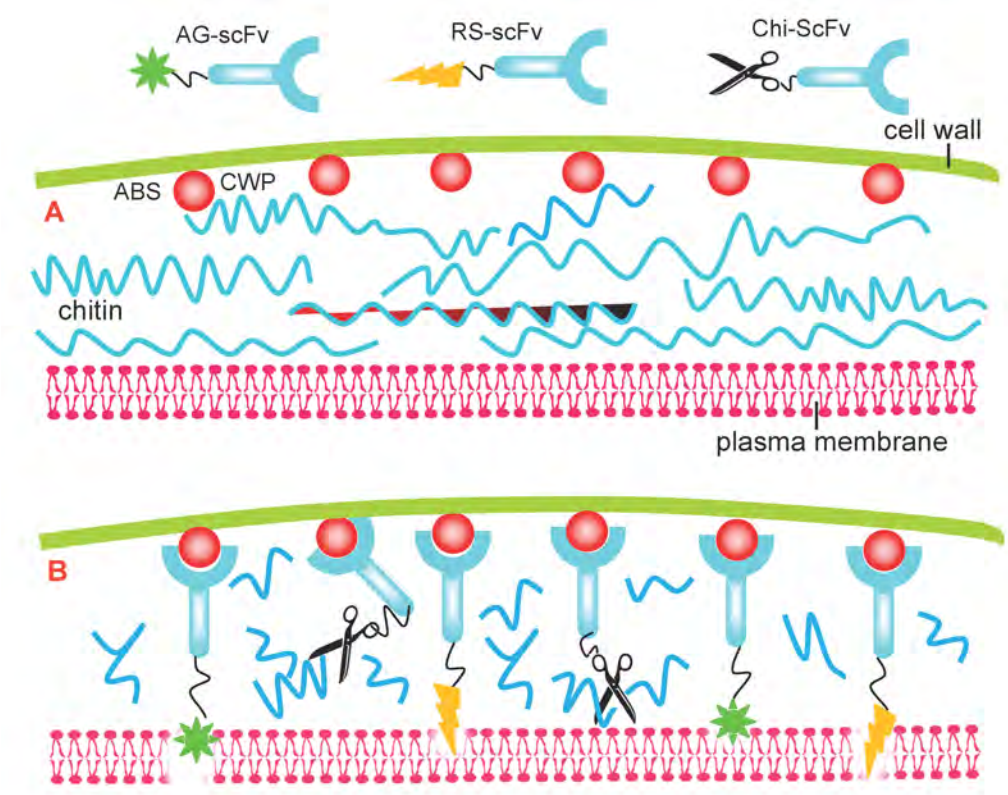

Figure 5 Resistance mechanisms deployed by antibody fusions with dual functions. (a) Fungal cell wall showing chitin and cell wall-bound proteins (CWP) as antibody-binding sites (ABS). Three constructs shown above represent different AFP-scFv fusions that gave a strong antifungal activity *in vitro* and *in planta*. (b) Antibody fusions bind to cell wall-bound proteins (ABS) via their antibody moiety. The antifungal half of the fusion protein then exerts its activity, either on plasma membrane (AG-scFv or RS-scFv) or by cleaving the chitin polymers (Chi-scFv) (adapted from Bohlmann 2004)

In antibody-mediated resistance, an antibody, especially when modified by other methods such as molecular evolution coupled with phage display (Liu et al. 2011), may serve as an ideal model for *in vitro* evolution to create mutant antibodies that are reactive to changes in pathogens, thus providing variants of resistance germplasm for molecular plant breeding. This may be particularly important for current disease and pest management strategies in agriculture. It is known that plant pathogenic fungi often undergo mutations that lead to virulence. The changed virulent fungal components (assuming no complete deletion of their coding sequences) can be used as new targets, to which corresponding specific antibodies can be selected *in vitro*. The selected antibodies can recognize and bind to the fungal targets. This specific interaction between the fungal target and antibody may be further used in resistance breeding. Our results indicated that *in vitro* molecular evolution of a resistance molecule such as an antibody may provide candidate variants that potentially interact with corresponding mutants in fungal pathogens. In our studies, the CWP2 antibody was generated with *Fusarium* strain 5035 whereas CWPa antibody was selected in 2009 with CWPs prepared from this same strain that was repeatedly

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used in field inoculations of wheat. The affinity improvement of an scFv antibody CWPa suggests that the selected scFv antibodies not only can be further used as sources of FHB resistance, but can also confer an even stronger resistance than its parent antibody CWP2 in transgenic plants. These results suggested that if any changes have taken place in the *Fusarium* strain 5035 during the inoculations in the field, the newly isolated antibody CWPa was able to specifically recognize those changes because it can still bind to the fungal targets to confer resistance.

Pathogen-based resistance in plants

The term "pathogen-based resistance (PBR)" is proposed to describe antibody-mediated disease resistance and similar phenomena in plants. The concept is based on the fact that pathogen-specific antibodies are generated by using pathogens as targets for selection and only antibodies that specifically bind to the pathogens can be isolated. This means that the characteristics of antigens derived from the respective pathogens used for the selection define the properties of antibodies generated. Thus, plant-expressed antibodies targeted into apoplasts or the cytosol, usually confer resistance to a specific pathogen or evolutionarily associated pathogen species that carries structural analogs of the corresponding antigens. A similar scenario is RNAi-based resistance in plants, in which antisense nucleotide sequences from pathogens are constructed and subsequently used for designing double-strand RNA or antisense molecules. Such RNAi-mediated resistance is also achieved through a pathogen-specific activity.

PBR may have several advantages over pathogen-derived resistance (PDR), another disease control strategy that has been successfully used to control virus-associated diseases in plant species (Beachy et al. 1990). No sequences from the pathogens are used in antibody-mediated resistance in transgenic plants, whereas in PDR, portions of gene sequences encoding a coat protein or other key components of viruses are expressed in transgenic plants. The truncated viral proteins expressed in plants interfere with virus propagation, resulting in resistance. The pathogen-derived sequences may undergo recombination to generate different virulent viruses, and are seen as potential dangers with environmental consequences (Greene and Allison 1994). Antibodies can be altered *in vitro* for their affinity and specificity as the corresponding counterpart pathogens undergo mutations affecting the host-pathogen response (Liu et al. 2011) before expression in plants. In PDR, if resistance is defeated by mutations that take place within pathogens, no *in vitro* system is available to overcome the potential problem. One epitope of an antigen recognized by a defined antibody usually consists of only a few amino acids or small structure of a chemical group, and a peptide or protein can have many different potential epitopes that interact with different antibodies and can be used for generation of diverse antibody variants. Mammalian immune systems are able to produce limitless variants of antibodies that interact with those specific epitopes present in one antigen. Diverse antibodies with different affinities but with the same specificity can be generated and used for PBR via an antibody approach. Therefore, PBR via an antibody approach, and PDR using a sequence of the pathogen, employ different mechanisms to confer resistance; the former appears to be more durable and environmentally safe.

Perspectives

The *Fusarium*-specific antibody CWP2 was the first antibody shown to confer resistance to fungal pathogens in plants (Peschen et al. 2004). Since then, this strategy has been employed to isolate fungus-specific antibodies used to improve resistance to different economically important fungal pathogens in crops, such as *S. sclerotiorum* in rapeseed (Yajima et al. 2010) and *F. virguliforme* in soybean plants (Brar and Bhattacharyya 2012). Like FHB in wheat, there is no germplasm that provides effective innate resistance to the diseases caused by these two fungi. The mammalian immunoglobulins can provide diverse antibodies that can be developed against key components of plant pathogens by different methods such as phage display as described above. Through advances in functional genomics and molecular biology of fungal pathogens (Xu et al. 2010; Chen et al. 2011; Glinka and Liao 2011), more key factors and components essential for pathogenesis have been identified and can be used as targets for antibody selection. Thus, it is anticipated that antibodies with defined specificity and affinity will play more important roles in improving disease resistance in plants, especially in situations where there is a lack of natural resistance.

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Antibody-mediated resistance displays specificity towards pathogens through molecular recognition and binding to corresponding antigens. This intimate interaction of antibody-antigen resembles a gene-for-gene relationship in terms of its high degree of specificity. Antibodies can be further improved *in vitro* for higher affinity to facilitate better binding to antigens. This advantage makes antibodies, as resistance molecules, changeable as their corresponding antigens in pathogens change, generating durable resistance. Furthermore, use of fungal surface antigens recognized by *Fusarium*-specific antibodies will assure that antibodies can easily bind to the targets as soon as the invading fungus is present in infected plant tissues. This allows AFP-antibody fusions to retain both functions from antibody and AFP, i.e. specifically binding to, and destruction of, pathogens. Thus, antibody-mediated resistance is pathogen-specific and does not affect non-specific micro-organisms. It therefore has no environmental consequences. In this sense, the resistance mechanism exploited by pathogen-specific antibodies is similar to that conferred by innate host resistance.

Antibody fusions have dual functions and may be further used as *Fusarium*-specific fungicides for biological control of *Fusarium* pathogens. Antibody-based drugs have been widely used for medicines. Antibody fusions retain high specificity and affinity toward their targets while destroying fungal infection structures. It is known that various antifungal modes have been observed for different AFPs. Some APFs directly destroy fungal cell walls while others act on cell membranes. Combinations of two AFPs with different antifungal modes were shown to confer better resistance to FHB and Fusariun seedling blight in transgenic wheat (Liu et al. 2012). Different antibodies with diverse variation can be generated against different epitopes of the same antigen. Combinations of different AFP-antibody fusion molecules with synergistic activities may generate highly reactive fungicidal compounds to control *Fusarium* pathogens.

Conclusions

FHB in wheat continues to pose a serious threat to both sustainable yields and food safety. Two phylogenetic species, *F. asiaticum* and *F. graminearum sensu stricto*, are the predominant pathogens of FHB on wheat in China. A *Fusarium* genus-specific antibody, CWP2 generated by phage display against CWPs from a local *F. asiaticum* strain, specifically binds to hyphal surfaces of FHB pathogens. This antibody alone conferred significant resistance to *Fusarium* pathogens in transgenic plants. The antibody fused with AFPs was able to confer very significant resistance to initial infection, fungal spread and mycotoxin accumulation in transgenic wheat as assayed by single floret injection, spray inoculation and chemical analyses. *In vitro* molecular evolution of the CWP2 antibody generated a mutant antibody, CWPa, that displayed a 15-fold higher affinity towards *Fusarium* pathogens, and this affinity-improved CWPa antibody conferred an even higher resistance to FHB pathogens in transgenic plants when fused with AFPs. These results suggest that *in vitro* molecular manipulation of a resistance molecule may provide candidate variants that potentially interact with new virulent mutants in fungal pathogens. The antibody-mediated resistance was *Fusarium*-specific and had no harmful effects on non-target micro-organisms. This antibody-antigen interaction appears reminiscent of a gene-for-gene relationship. This study presents an efficient approach for generation and selection of highly reactive *Fusarium*-specific antibodies as well as utilization of the antibodies as resistance molecules for development of transgenic plants resistant to FHB pathogens. Further investigation will be undertaken to evaluate resistance roles *in planta* for the antibodies which either alone or as fusion partners, serve as promising unique resistance molecules for developing transgenic plants or for use in other strategies to control FHB diseases and to reduce mycotoxin contamination in food/feed chains.

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