Oral Presentations

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Table of contents

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Putting Ug99 on the map: An update on current and future monitoring
D. P. Hodson, K. Nazari, R.F. Park, J. Hansen, P. Lassen, J. Arista, T. Fetch, M. Hovmoller, Y Jin, Z. A. Pretorius and K. Sonder *3*

Challenges in controlling leaf rust in the Southern Cone region of South America
S. German, P. Campos, M. Chaves, R. Madariaga and M. Kohli *14*

Yellow rust in CWANA in 2010 & 2011: The situation and measures taken to control it
K. Nazari, D. Hodson and M. Hovmoller *24*

Risk assessment of aerial transport of rust pathogens to the Western Hemisphere and within North America
S. A. Isard and J. M. Russo *25*

Puccinia graminis: Variation is the rule
L. J. Szabo, C. A. Cuomo, Y. Jin, M. Rouse and P. Olivera *35*

Identification of the Pgt effectors that are recognized in rice
F. Katagiri, Y. Tsuda, T. Stoddard, K. Tsuda, C. A. Cuomo, J. Glazebrook and L. J. Szabo *36*

Diversity of yellow rust resistance: Inferences from association mapping analysis of elite wheat germplasm
F. C. Ogbonnaya, K. Nazari, A. Jighly, F. Makdis, O. Youssef, E. Essa, I.S.A. Tahir, A. Yaljarouka, A. Yahyaoui, A. O. Abdalla and M. Baum *42*

Mapping of durable adult plant stem rust resistance in six CIMMYT wheats to Ug99 group of races
S. Bhavani, R. P. Singh, O. Argillier, J. Huerta-Espino, S. Singh and P. Njau *43*

Cracking the codes: genetic basis of nonhost resistance of barley to heterologous rust fungi
R.E. Niks, H. Jafary and T.C. Marcel *54*

Rpg1-mediated durable stem rust resistance: mechanisms of action
A. Kleinhofs, J. Nirmala, R. Brueggeman and B. Steffenson *64*

Towards an understanding of the molecular mechanisms of durable and non-durable resistance to stripe rust in wheat
X. M. Chen, T. Coram, X. L. Huang, M. N. Wang and A. Dolezal *70*

Unraveling the entry mechanism of oomycete and fungal effector proteins into host cells
S. D. Kale, A. C. Rumore, B. Gu, W. Shan, C. B. Lawrence, D. Capelluto and B. M. Tyler *82*

Investigating rust resistance with the model grass Brachypodium
D. F. Garvin *89*

New tools for wheat genetics and breeding: Genome-wide analysis of SNP variation
E. Akhunov, S. Chao, V. Catana, D. See, G. Brown-Guedira, A. Akhunova, J. Dubcovsky, C. Cavanagh and M. Hayden *92*

High yielding CIMMYT spring wheats with resistance to Ug99 and other rusts developed through targeted breeding
R. P. Singh, J. Huerta-Espino, S. Bhavani, S. A. Herrera-Foessel, Y. Jin, P. Njau, P. K. Singh, G. Velu, S. Singh, R. J. Peña and J. Crossa *98*

Breeding for Ug99 Resistance at ICARDA: Results from Regional Trials
Osman Abdalla, F. Ogbonnaya, I. Tahir, W. Tadesse, A. Yaljarouka, A. Yahyaoui, K. Nazari, A. Badebo, F. Eticha, S. Gelalcha, B. Hundie, Ruth Wanyera and Peter Njau ***105***

Breeding for stripe rust resistance in the U.K. and Europe
L. A. Boyd, C. Lewis, J. P. E. Melichar, L. J. Jagger, N. Powell, R. MacCormack, C. Newell and S. T. Berry ***106***

Multiple rust resistance and gene additivity in wheat: Lessons from multi-location case studies in cultivars Parula and Saar
M. Lillemo, R. P. Singh, M. William, S. Herrera-Foessel, J. Huerta-Espino, S. German, P. Campos, M. Chaves, R. Madriaga, X. C. Xia, S. S. Liang, D. Liu, Z. F. Li and E. S. Lagudah ***111***

Canadian initiatives in breeding for stem rust resistance to race Ug99 and variants
S. L. Fox, R. M. DePauw, D. G. Humphreys, P. Huc, A. K. Singh, C. J. Pozniak, P. D. Brown, R. J. Graf, H. S. Randhawa, H. D. Voldeng, C. A. McCartney, C. W. Hiebert, R. E. Knox, J. B. Thomas, T. F. Townley-Smith and T. G. Fetch ***121***

Highly expressed RPG1 protein in a five-copy Rpg1-transgenic barley line results in susceptibility to stem rust
Y. Chai, B. Steffenson, J. Nirmala and A. Kleinhofs ***127***

Fungicide strategies for managing the Ug99 stem rust threat - Australian and international perspectives
R. W. Rainbow ***132***

Release of durable rust resistant wheat varieties in certain wheat districts of Ethiopia
Y. Chai, B. Steffenson, J. Nirmala and A. Kleinhofs ***138***

Delivering quality seeds
U. B. Zehr ***139***

Looking for a needle in a haystack: Screening of the International Stem Rust Nursery in Kenya for new sources of resistance in spring wheat expo landraces
M. Acevedo, M. Newcomb, M. Rouse, H. E. Bockelman, B. J. Goates, E. W. Jackson, Y. Jin, G. Brown-Guedira, A. Kilian, P. Njau, D. Singh, R. Wanyera and J. M. Bonman ***140***

Deployment of rust resistant wheat varieties: Successes and challenges in Afghanistan
S. Kugbei and A. Habibi ***144***

Putting Ug99 on the map: An update on current and future monitoring*

D.P. Hodson[1], K. Nazari[2], R.F. Park[3], J. Hansen[4], P. Lassen[4], J. Arista[5], T. Fetch[6], M. Hovmøller[4], Y Jin[7], Z.A. Pretorius[8] and K. Sonder[5]

Abstract

Detection of stem rust race TTKSK (Ug99) from Uganda in 1998/99 highlighted not only the extremely high vulnerability of the global wheat crop to stem rust but also a lack of adequate global systems to monitor such a threat. Progress in the development and expansion of the Global Cereal Rust Monitoring System (GCRMS) is described. The current situation regarding the Ug99 lineage of races is outlined and the potential for expansion into important wheat areas is considered. The GCRMS has successfully tracked the spread and changes that are occurring within the Ug99 lineage and is now well positioned to detect and monitor future changes. The distribution of Ug99 variants possessing combined virulence to *Sr31* and *Sr24* is expanding rapidly and future spread outside of Africa is highly likely. Efficient and effective data management is now being achieved via the Wheat Rust Toolbox platform, with an expanding range of dynamic information products being delivered to end-users. Application of new technologies may increase the efficiency of the GCRMS, with mobile devices, molecular diagnostics and remote sensing all seen to have potential application in the medium to long-term. Expansion of the global capacity for race analysis is seen to be critical and integration of the Global Rust Reference Centre into the stem rust monitoring network is seen as a positive development. The current acute situation with severe epidemics of stripe rust in many countries indicates a clear need for more effective global monitoring systems and early warning for this pathogen. The existing GCRMS for stem rust is seen as a good foundation for this to occur.

Keywords

GIS, Information systems, monitoring, pathotypes, *Puccinia graminis tritici, stem rust,* Ug99

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Introduction

Detection of stem rust race TTKSK (Ug99) from Uganda in 1998/99 (Pretorius et al. 2000) highlighted not only the extremely high vulnerability of the global wheat crop to stem rust but also a lack of adequate global systems to monitor such a threat. The expert panel convened by Dr Borlaug in 2005 in response to Ug99 (CIMMYT 2005) recognized this deficiency and recommended that monitoring should be addressed as a priority. The global wheat community responded to this recommendation with a Global Cereal Rust Monitoring System (GCRMS) being developed by an international consortium within the frame work of the Borlaug Global Rust Initiative (BGRI) (Hodson et al. 2009).

Concern over races within the Ug99 lineage is justified by the well documented historical capacity for stem rust to cause severe devastation in all continents in which wheat is grown (see Dubin and Brennan 2009; Hodson 2011). The virulence profile exhibited by races in the Ug99 lineage is unique, both in terms of the range of resistance genes defeated and in the area sown globally to cultivars protected previously by these defeated genes. Combined, these factors make the Ug99 lineage unique and a clear threat to a large proportion of existing commercial wheat cultivars (Singh et al. 2008).

Rust pathogens exhibit two major characteristics that make continual monitoring an absolute requirement. Firstly, they are highly mobile trans-boundary diseases capable of rapid, long distance movements, either by wind-assisted or accidental human-mediated transmission. This mobility makes quarantine a near impossibility and implies the clear need for effective, regular monitoring at national, regional and global scales. Globalization and associated growth in air transportation has increased both the probability of human-borne transmission and the need to monitor areas geographically distant from known infected areas. Secondly, rust pathogens have an inordinate ability to change and evolve through mutation or sexual recombination (Knott 1989; Park 2007; Watson 1981). Ug99 is no exception in this regard, and is mutating and migrating rapidly. Seven variants are now recognized within the Ug99 lineage and confirmed occurrence is known in 10 countries (Singh et al. 2011; Mukoyi et al. forthcoming). Within east and southern Africa, data indicates that members of the Ug99 lineage are now the predominant stem rust pathotypes throughout the entire region.

Monitoring systems alone are obviously not the solution to prevent damaging losses from virulent rust pathotypes like Ug99. However, they are seen as an essential component in a broader, integrated mitigation and control strategy. Effective monitoring plays a vital

role in the early detection of new pathotypes and in providing reliable information on disease spread. Regular surveillance in areas of continuous wheat production e.g., the highlands of East Africa and Yemen is seen to be critically important given the opportunities for pathogen persistence and change in such areas. Strong, functional linkages to breeding programs, seed systems and control mechanisms are essential if improved decisions on both preventative and reactive control are to be taken.

This paper describes the considerable progress in the development and expansion of the GCRMS. The current, actual situation regarding the Ug99 lineage is outlined with possible indicators of change highlighted. The potential for future range expansion into important wheat areas is considered. New opportunities to advance the effectiveness of global rust monitoring, resulting from advances in information technology and molecular diagnostics, are described. Current challenges to effective global monitoring of cereal rusts are discussed along with potential solutions.

Current status – An overview

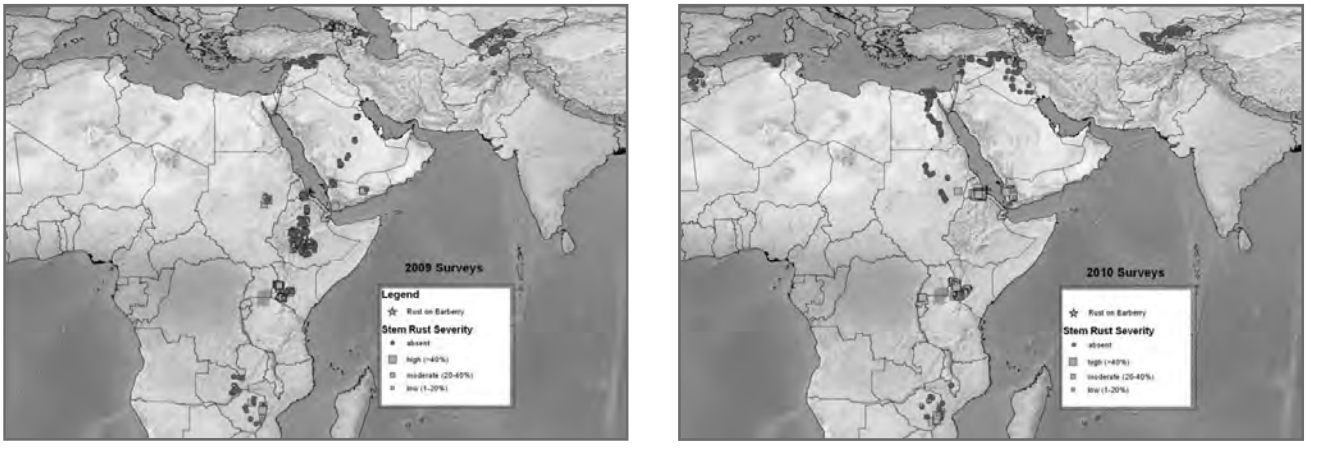
The initial concepts and early development of the GCRMS were described by Hodson et al. (2009). Regular reviews by Singh and co-authors (2006; 2008; 2011) have also provided summarized updates on the global status of the Ug99 lineage. However, since its inception, the GCRMS has undergone a series of major changes and improvements. Spatial aspects of monitoring the Ug99 lineage have been an important component, with GIS technology applied extensively. Mapping the status of Ug99, its distribution and movements – both actual and potential, has been an important communication tool of the GCRMS and this paper will describe some of the background on how Ug99 was put on the map.

The mobility of the stem rust pathogen requires monitoring to be undertaken over a vast geographical area. The trans-boundary nature of the disease requires communication and information sharing at the regional or global scale. The only means of undertaking this in effective manner is through a coordinated network of national surveillance teams collecting standardized data and contributing to a consolidated, centralized global information system. Under the auspices of the Borlaug Global Rust Initiative (BGRI), this network is now a functional reality, with a rapidly expanding number of countries now undertaking regular rust surveys and contributing with standardized data. The current geographical coverage of about 20 wheat growing countries in Africa and Asia is a substantial achievement, but further expansion is required. Ultimately, a network of 30–35 priority countries is the target for the next 2-3 years. Regular surveillance and pathogen monitoring over such an extensive geographical area will provide a solid information base to mitigate and control rust for a considerable proportion of the developing world wheat areas.

Standardized field surveys now cover most of the main wheat growing regions in the reporting countries. All data are geo-referenced using GPS, permitting survey data to be mapped accurately as soon as they have entered the centralized database and quality control has been undertaken. Consolidated datasets are starting to provide an indication of the distribution of stem rust incidence and severity in any given year on a scale that was unattainable in the past. Data showing recorded stem rust severity from 2009 and 2010 field surveys are given in Fig. 1. Obviously, field survey data provides no indication of specific races, hence these general distribution maps include all races and not only those belonging to the Ug99 lineage. The most comprehensive

Fig. 1. ***Stem rust severity from BGRI field survey data in 2009 and 2010***

a) 2009 **b) 2010**



monitoring of stem rust is occurring in eastern and southern Africa. Isolated occurrences of stem rust are regularly recorded in other regions e.g., Middle East, Caucasus and Central Asia. Increased surveillance efforts are obviously a contributing factor to the increased detection of stem rust, although other factors may play a role. An increasing number of countries are now recording potentially comparable multi-year survey data and with time these data will become more valuable as a monitoring tool. Current data are too limited to detect any long-term trends, but some possible indicators of change are apparent. Short-term changes may solely be artifacts of the survey approach or may reflect either changing pathogen populations and/or changing environmental conditions. More detailed examples of possible indicators of change are given in the following section.

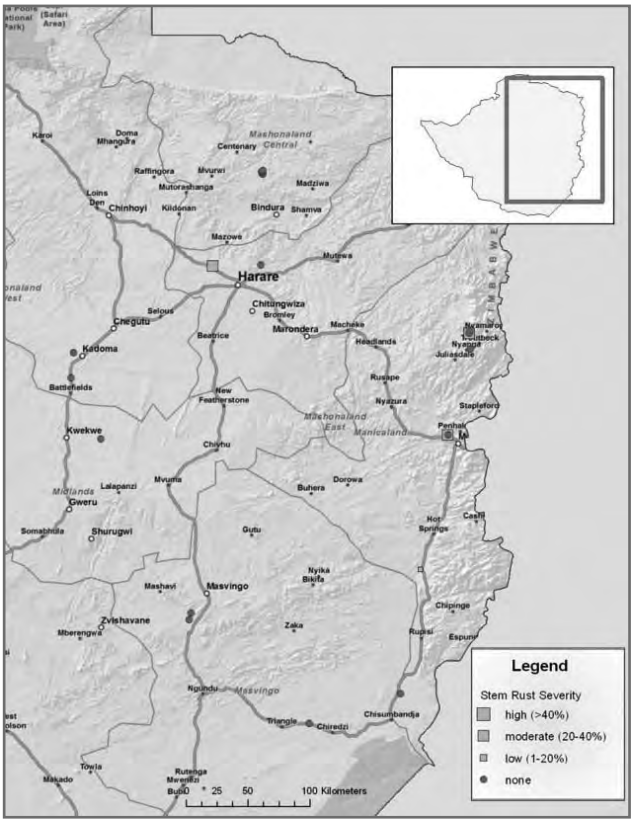
Indicators of change and emerging concerns

Use of standardized survey techniques and survey routes in consecutive years are now permitting the detection of at least indicators of potential change in terms of stem rust distribution and occurrence. Two examples of possible change are highlighted from African countries using 2009 and 2010 surveillance data. These examples also illustrate some of the difficulties in interpretation of the changes observed at the field level.

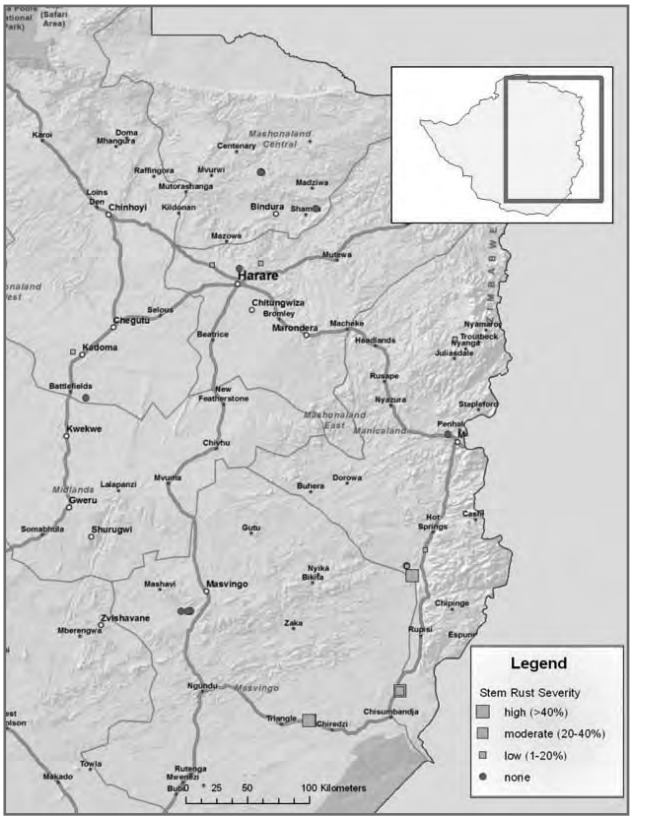
In Zimbabwe, surveys were undertaken throughout the wheat growing areas in early September 2009 (Mutari et al. 2009). A total of 21 sites were surveyed using standard BGRI methodology. Stem rust was recorded at only four sites; Gwebi Variety Testing Centre, Birchenough, Sisal Farm Mutare, and Nyanga (Fig. 2a). Very low incidence of stem rust was recorded at all sites apart from Nyanga, but high severity of infection was recorded at all sites apart from Birchenough. The latter was the only lowveld (elevation <800m) site in Zimbabwe at which stem rust was recorded in 2009. In early September 2010, repeat surveys were undertaken in Zimbabwe using an essentially identical survey route and methodology. Stem rust was recorded at 12 of the 27 sites surveyed. In contrast to 2009, stem rust was widespread in the lowveld areas with eight out of nine lowveld sites recording the disease. Very high severity of infection was observed at five of the lowveld sites in 2010 and disease incidence was also very high at three of the sites (Fig. 2b).

Fig. 2. Stem rust severity in Zimbabwe from BGRI field survey data in 2009 and 2010

a) 2009



b) 2010



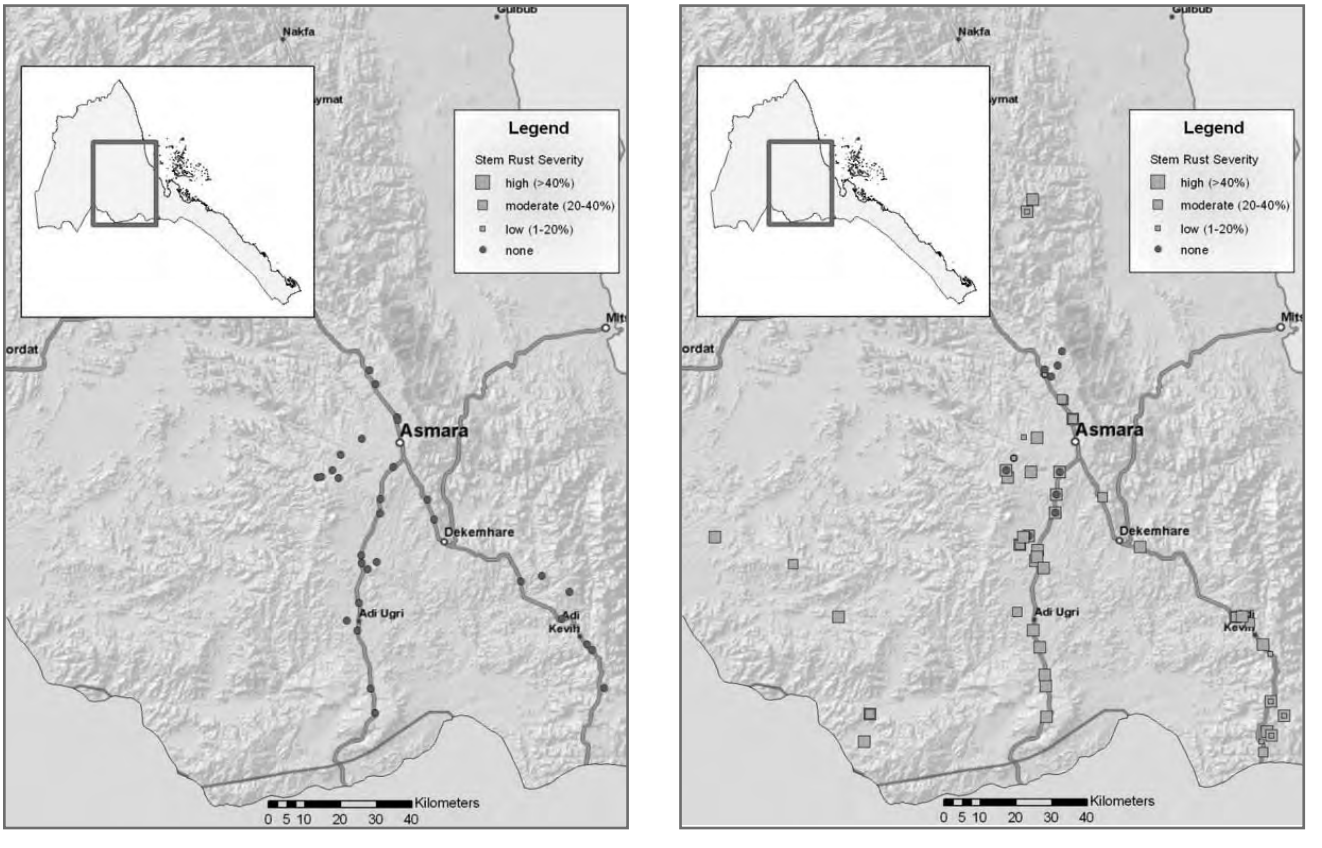
These data implied a quite dramatic change in the stem rust situation in Zimbabwe in 2010 compared to 2009. It was unlikely that this could be attributed to cultivar change, but pathogen change and/or a more conducive environment may be responsible. There is little evidence of a more conducive environment prevailing in 2010, although influences of micro-climatic factors or irrigation schedules/methods cannot be excluded. Rainfall during the preceding wet season (i.e., Nov. 2009 to March 2010) was lower than average for 1998 to 2009 (WFP 2010). It is therefore more likely that pathogen population changes have played a role in the difference in stem rust occurrence between the two years. Race analysis conducted on the 2010 samples detected the presence of the Ug99 lineage races PTKST and TTKSF (Mukoyi et al forthcoming). The presence of race PTKST, with virulence on both *Sr31* and *Sr24*, was only confirmed in 2010.

In Eritrea, an apparently analogous situation of substantial change was observed in surveys undertaken in late September 2009 and late October 2010. In 2009, no stem rust was observed in any of the 32 fields surveyed. In 2010, stem rust was recorded in 76 of the 92 fields surveyed (Fig. 3). For Eritrea, several factors may contribute to this situation. Firstly, the timing of surveys was quite different, with the 2009 surveys possibly being undertaken too early to detect stem rust reliably. Secondly, the environmental conditions were very different between years: in 2009, rainfall was below average, whereas in 2010, rainfall was above average and prolonged, resulting in excellent growing season conditions. Climatic conditions in 2010 undoubtedly favoured rust development and similar conditions in neighbouring Ethiopia were one factor behind the serious stripe rust epidemics. It is unknown at the current time if races within the Ug99 lineage were present in Eritrea in 2010, although given the close proximity of confirmed occurrence in neighbouring countries this is considered likely. Samples collected from Eritrea in 2010 are now undergoing race analysis.

The evidence available indicates that stem rust is widespread in eastern and southern Africa, and from sampling data, races within the Ug99 lineage are becoming increasingly predominant throughout the region. Obviously, several factors other than pathogen population changes are behind many of the perceived

Fig. 3. ***Stem rust severity in Eritrea from BGRI field survey data in 2009 and 2010***

a) 2009 **b) 2010**

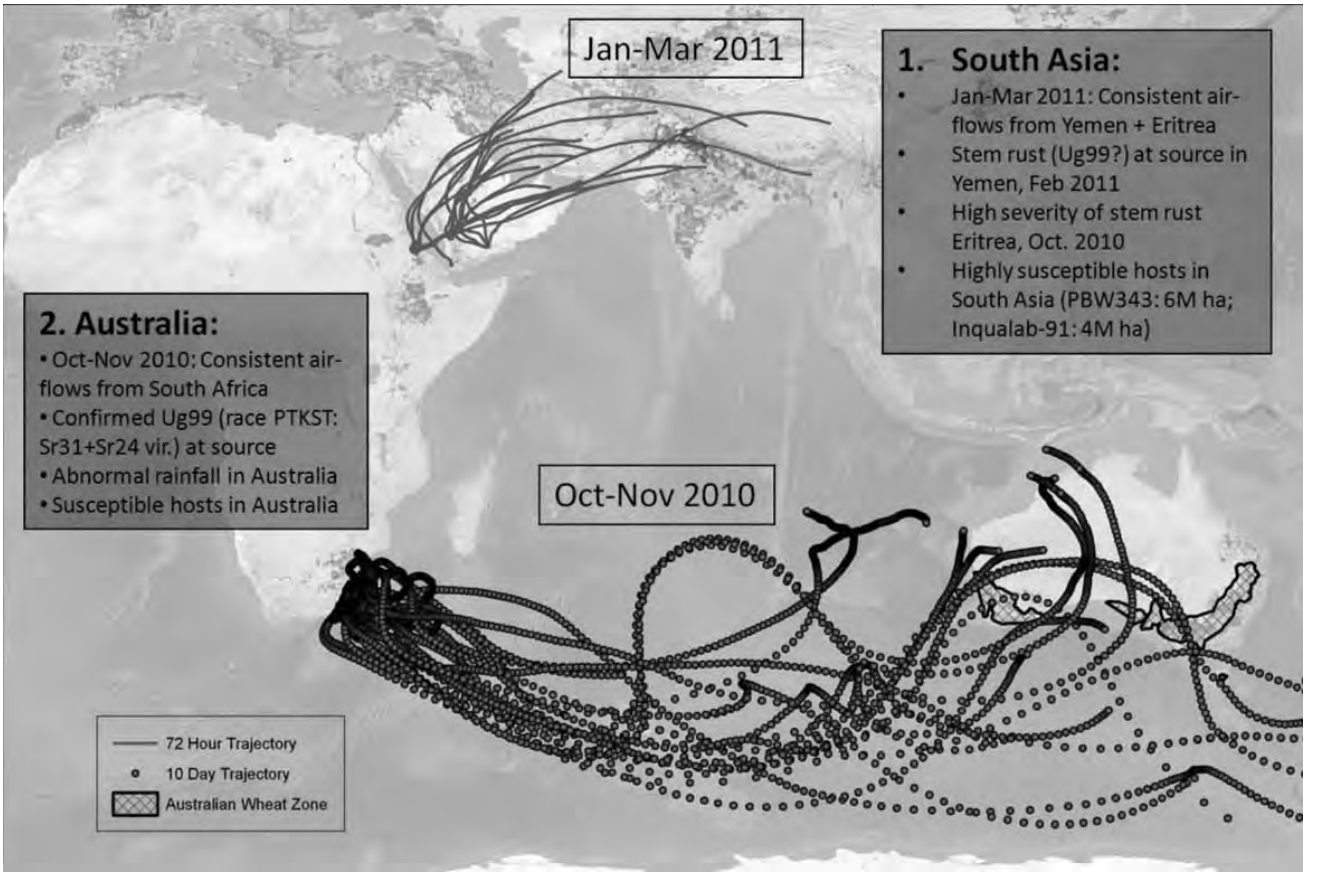


changes, with environmental factors playing a key role in all of the countries. From the very limited time-series data available, it is impossible to determine if stem rust is occurring at increased incidence compared to previous years.

Assessment of potential for movements of the Ug99 lineage beyond the known current range has also been undertaken. Prior use of the HYSPLIT wind model (Draxler and Rolph 2003) yielded valuable information regarding the likely migration of Ug99 out of Africa (Singh et al. 2006; 2008) in terms of general movement routes rather than specific time-bound events. Continued use of the HYSPLIT model is providing some insights into potential future movements. With the current known distribution of the Ug99 lineage, two potential onward movements are of concern. Potential for occurrence of these scenarios has been raised before (Singh et al. 2008; Watson and de Sousa 1982), but recent HYSPLIT model outputs and other factors give additional credence to possible occurrence at some point in the future.

Firstly, onward movement of the UG99 lineage into South Asia has been a concern since 2005 (Hodson et al. 2005). The importance of the wheat crop in this region and the known very high susceptibility of mega-cultivars like PBW343 and Inqualab-91, covering millions of hectares, are key factors. Prevailing wind patterns and historical evidence implying movements of rust pathogens from the Middle East into South Asia e.g., *Yr9*, *Yr2* (Nagarajan and Singh 1990; Singh et al. 2002; 2004) indicate the distinct possibility that rust pathogens like Ug99 may move into South Asia at some point in the future. Wind trajectories observed during the period January to March 2011 added further weight to this hypothesis. On several occasions during this period air movements were observed from known stem rust sites in both Yemen and Eritrea and reached South Asia within 72 hours (Fig. 4). Stem rust infections were confirmed in the southern highlands of Yemen during this time and although pathotypes still require confirmation there is a high probability that races within the Ug99 lineage were present. As described previously, a high incidence and severity of stem rust was observed in Eritrea in October 2010, however given the absence of any cereal crop during January–March it is unlikely that any significant amount of inoculum would have been present in Eritrea at this time. Timing of these observed wind movements coincides with the main wheat season

Fig. 4. ***Emerging concerns in 2010/11 related to the Ug99 lineage and modeled air-flow patterns***



in South Asia; hence large areas of extremely susceptible cultivars were present at this time. To date no unusual stem rust occurrence has been reported from South Asia and long distance dispersal events are always a very low probability. Several interacting factors beyond simple air movements are required for successful long-distance transport of rust spores to occur, but the existence of such wind movements coupled to known sources of inoculum at source illustrate the very real possibility for movement into this key wheat growing region.

A second cause for concern is the apparent build up of Ug99 lineage races, especially those with combined virulence for *Sr31* and *Sr24* in Southern Africa. There is good historical evidence of rare foreign incursions into Australia of rust pathogens originating in this region (Luig 1977; Watson and de Sousa 1982). There is also considerable evidence of airflows moving out of southern Africa and into Australia (Sturman et al. 1997). During October to November 2010, Ug99 race PTKST (virulent to both *Sr31* and *Sr24*) was present at two sites in KwaZulu-Natal, and one site in the eastern Free State South Africa (Z. Pretorius pers. comm.). Modeled airflows from the KwaZulu-Natal source locations indicated several air movements that crossed the Australian wheat growing areas within 6–8 days of leaving South Africa (Fig. 4). Numerous rain bearing fronts were crossing southern Australia at this time and stem rust susceptible wheat cultivars were being grown in this region of Australia. While stem rust incidence increased in south eastern Australia during 2010, no unusual instances of the disease were reported and only one race was identified in that region (34-1,2,7 +Sr38, R.F. Park unpublished). However, the series of air movement events observed during Oct–Nov 2010 illustrate the potential for foreign incursions into Australia from South Africa. Such an event would be of very low probability due to the low chances of spore release at the right time, low spore survival, and limited or no successful deposition, but the historical evidence gives warning that such low probability events are possible. It is hypothesized that a similar series of events to those observed in Oct–Nov 2010 may have been responsible for the documented foreign rust incursions into Australia from southern and central Africa.

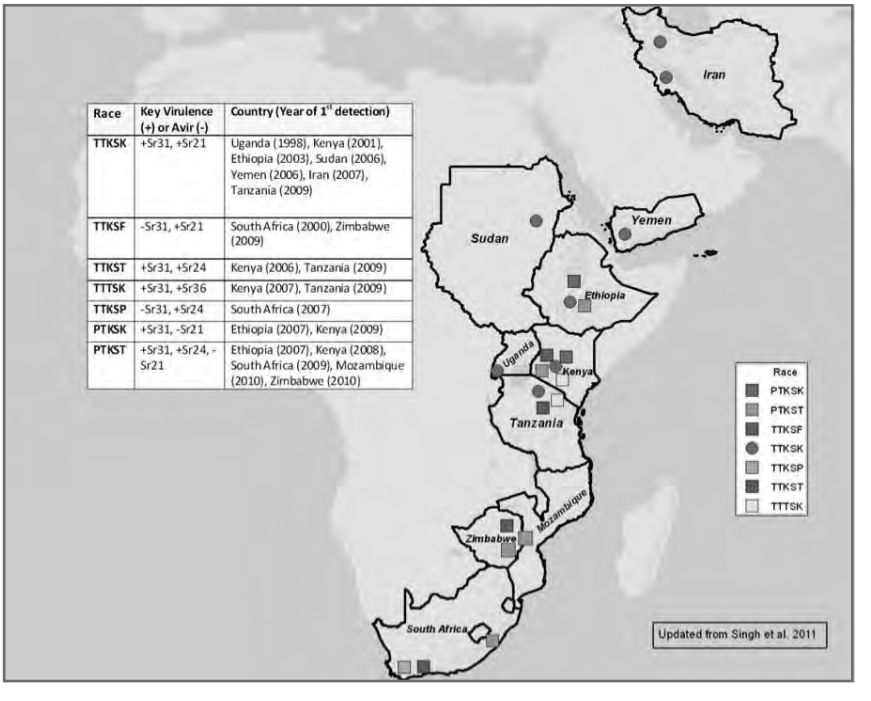
Monitoring the Ug99 lineage

Current knowledge on the Ug99 lineage has been obtained primarily from race analysis undertaken at specialist international rust laboratories. Rust laboratories in Canada, South Africa and USA have provided most of the information to date, but the expectation is that in-country laboratories will increasingly undertake routine monitoring activities. Establishment of the Global Rust Reference Centre (GRRC) in Denmark, initially for stripe rust (Hovmøller et al. 2009), and with subsequent expansion in 2011 to include stem rust analysis on foreign isolates, is expected to provide a valuable additional new resource in the international network of benchmark rust analysis laboratories.

Since regular international monitoring of Ug99 started in 2005, over 400 samples have been analyzed and race identity confirmed. Several important variations in the virulence / avirulence profile of the Ug99 lineage have been detected since monitoring started. Currently, seven variants are recognized in the Ug99 lineage (Singh et al. 2011). Notable is the acquisition of virulence to additional important resistant genes i.e., *Sr24* and *Sr36* (Jin et al. 2008; 2009). Park et al. (2011) outline a putative evolutionary pathway for the known races in the Ug99 lineage, with single step mutations considered to be the primary source of variation.

Fig. 5. ***Overview of the current known status for the Ug99 lineage of races (Nomenclature using the North American system based on 5 differential sets (Jin et al. 2008)***

Race	Key Virulence (+) or Avir (-)	Country (Year of 1st detection)
TTKSK	+Sr31, +Sr21	Uganda (1998), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009)
TTKSF	-Sr31, +Sr21	South Africa (2000), Zimbabwe (2009)
TTKST	+Sr31, +Sr24	Kenya (2006), Tanzania (2009)
TTTSK	+Sr31, +Sr36	Kenya (2007), Tanzania (2009)
TTKSP	-Sr31, +Sr24	South Africa (2007)
PTKSK	+Sr31, -Sr21	Ethiopia (2007), Kenya (2009)
PTKST	+Sr31, +Sr24, -Sr21	Ethiopia (2007), Kenya (2008), South Africa (2009), Mozambique (2010), Zimbabwe (2010)



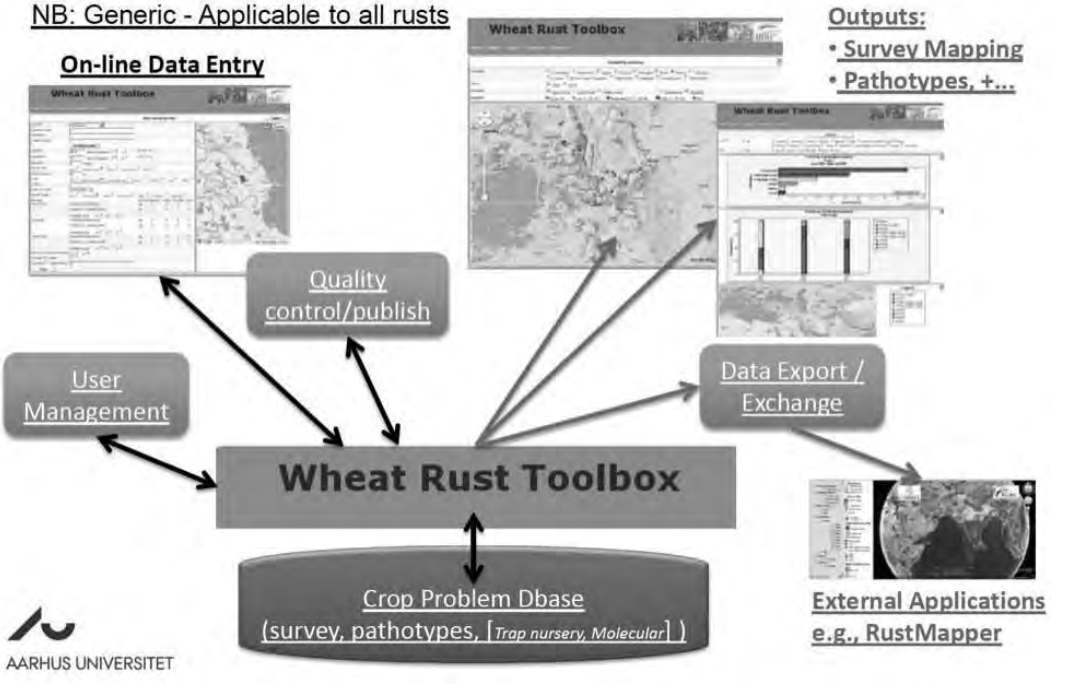
Race variants in the Ug99 lineage have been successfully detected and tracked (Park et al. 2011), and the number of countries in which these races have now been confirmed is increasing. New information from southern Africa (Mukoyi et al. forthcoming) takes the number of confirmed countries with races within the Ug99 lineage to 10 (Uganda, Kenya, Ethiopia, Sudan, Yemen, Iran, South Africa, Tanzania, Zimbabwe, Mozambique). To date, 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 status of the Ug99 lineage races is summarized in Fig. 5

Monitoring data indicates that members of the Ug99 lineage with combined virulence to *Sr31* and *Sr24* (i.e., races TTKST or PTKST) are spreading rapidly. These races now predominate in Kenya and are increasing in frequency in other countries. Throughout east and southern Africa, the results obtained indicate that races within the Ug99 lineage now predominate in the region.

Data management and dissemination

With the ever increasing amounts of data now being received by the GCRMS, effective data management is a critical issue Collaboration with Aarhus University, Denmark has permitted the development of an innovative data management platform – The Wheat Rust Toolbox –based on existing tools and experiences from information systems on potato late blight (Hansen et al. 2007) and IPM in wheat (Jørgensen et al. 2010). The Wheat Rust Toolbox is a web-based data management system that controls both inputs and outputs from a centralized database, which is currently populated with survey and race data. In the near future, expansion of this system is planned to include trap nursery data and molecular diagnostic probe data. On-line data entry permits quality controlled and standardized data to be entered into the database by any authorized national rust surveillance team. Once validated and approved, data is published and automatically disseminated via a series of graphical and mapping tools. An overview of the Wheat Rust Toolbox is provided in Fig. 6. This new data management system greatly improves the efficiency of managing and distributing cereal rust information. The Wheat Rust Toolbox also permits standardized data exchange with other applications; for example, the RustMapper tool managed and developed at CIMMYT. A dedicated web portal - Rust SPORE (http://www.fao.org/agriculture/crops/rust/stem/rust-report/en/) provides the primary dissemination mechanism for all of the information available, with several Wheat Rust Toolbox or external visualization and query tools embedded within the web portal. This distributed mode of operation provides a very rich and diverse set of information in a seamless way to end users.

Fig. 6. ***An overview of the Wheat Rust Toolbox data management system***



Current challenges and future activities

Considerable progress has been made in the implementation of an operational monitoring and surveillance system for stem rust in response to identification of Ug99. Knowledge regarding race distribution and diversity has increased, along with a much clearer picture on the occurrence of stem rust on an annual basis over a wide geographical region. However, several areas exist in which substantial improvements could be made. Emerging technology options and increasing technical capacity offer some interesting opportunities to overcome existing challenges and bottle-necks.

Advances in mobile phone technology present several opportunities to increase the efficiency of field data collection and transmission. Smart phones now permit field data entry via standard forms, plus GPS location data and photos can be collected and transmitted to a central database. Aanensen et al. (2009) describe such a system, Epicollect, based on the open-source android operating system. The Epicollect system is generic and could easily be adapted to the collection of wheat rust data. Current cost of android smart phones makes large-scale deployment of such a system quite costly, but with phone costs decreasing equipping core surveillance teams in several countries could be feasible. Quality controlled data collection at source, coupled to automatic transmission into centralized databases, would offer many advantages with considerable efficiency gains in terms of the timeliness and ease of data transmission. Disadvantages might include; limited robustness in the field, poor screen visibility or battery life, limited ease of use and user acceptance. Extensive field testing would be a critical requirement before any large-scale deployment. However, in the future field data collection through smart phones, or equivalent mobile devices, could provide a useful alternative to the existing systems of field data collection.

Another area in which mobile phone technology may play a role in monitoring and surveillance systems is via crowd sourcing techniques i.e., capturing disease information from a large number of respondents in a rapid way. Mobile phone SMS networks targeted towards farmers, extension workers and the general public could provide one mechanism for wide coverage disease reporting. For any system to be successful it would need carefully planned and widespread publicity targeted at interested user groups and also clear incentives for people to report presence of the disease. Systems that have demonstrated success usually rely on valuable information being returned to any participating users, usually in local languages. In the case of rust diseases, expert information on control measures, sources of resistant seed or rapid targeted control measures by government agencies might be options to be considered as part of any incentive system. Follow up by formal surveillance teams is likely to be necessary to confirm reported disease outbreaks.

Race analysis is another area in which improvements are required. Currently, considerable time delays can exist between a field sample being collected and final race identification. Global capacity to undertake high quality race analysis is also limited. Established rust laboratories in Australia, Canada, South Africa and the USA have done an outstanding job and provided most of the current information relating to races in the Ug99 lineage. All these laboratories face strict restrictions on receiving and working with foreign rust isolates, with good reason as accidental transfer of dangerous isolates must be avoided at all costs. For the North American laboratories, foreign isolates can only be received and analyzed during the northern hemisphere winter months, when cold and absence of hosts provide further containment. This restricted time period for analysis does however limit the possibility to analyze samples collected in countries with wheat crops that mature in the period March–September. In-country analysis of stem rust samples is an obvious and desirable solution to avoid movement of isolates, but capacity (human resources and infrastructure) remains limited. Some progress, resulting from investments in both human capacity and physical infrastructure from international and national donor sources, is now beginning. Advances in race analysis capacity are now being seen in some priority countries within Africa and Asia. Establishment of the GRRC in Denmark started as a collaborative effort between Aarhus University and two CGIAR centers (CIMMYT and ICARDA) is another very positive development. Initially focused on stripe rust, the GRRC has now been expanded with improved bio-safety facilities to incorporate analysis of foreign stem rust isolates. This facility in collaboration with the other advanced rust laboratories will permit year-round analysis of priority stem rust samples. Expansion of the capacity of the international network of rust laboratories in this manner is seen as a very beneficial development.

Rust trap nurseries are a valuable tool for the early detection of changes in rust populations and in the status of key resistance genes. The concept of trap nurseries is extremely simple, but appropriate application is often lacking. In the worst case, poorly managed trap nurseries can be extremely misleading and a source of inaccurate data. Purity of seed stocks is vital, but has long been

problematic. However, extensive seed purification undertaken by the Cereals Research Centre, AAFC, Canada and subsequent field multiplication measures undertaken by ICARDA have overcome concerns in this area. Timely reporting of trap nursery data is another critical issue, with current data reporting rates extremely low (K. Nazari pers. comm.). Planned expansion of the Wheat Rust Toolbox data management system to include trap nursery data may facilitate easier data entry and faster, more effective dissemination of trap nursery information.

Advances in molecular biology, notably molecular diagnostics, look set to have a major impact on stem rust analysis and monitoring. Considerable progress has been made in the development of diagnostic DNA probes based on single nucleotide polymorphism (SNP) markers (Crouch et al. 2010). Identification of specific stem rust races is not possible with current methods, however a panel set of probes shows considerable promise in ability to identify the general Ug99 lineage race group using a simple and rapid real-time PCR assay. Further testing of this rapid (48 hour) molecular diagnostic assay is on-going, but if proved to be reliable, it offers the possibility for fast, in-season diagnosis of Ug99 lineage group members. The other major advantage of this approach is that it may reduce the need to transport live stem rust isolates around the world. However, it must be noted that molecular diagnostic assays cannot replace traditional bioassays on live isolates, the latter being the only option to identify specific races and virulence profiles. Another unforeseen minor problem with the DNA sampling approach is the restrictions on sending flammable solvents e.g., ethanol via international couriers. However, there is little doubt that molecular diagnostics look set to play an increasingly important role in the future for the rapid detection of important pathotype groups like Ug99. Work is being initiated in order to expand the capacity of the Wheat Rust Tool box to manage molecular diagnostic probe data.

The integration of diverse datasets into the centralized Wheat Rust Toolbox platform will permit a more comprehensive assessment of emerging disease situations using harmonized and quality-controlled data. Increasing use of web-based platforms will improve timeliness of data upload, data consolidation and information dissemination. Ultimately, improved decisions regarding disease control and mitigation should be possible based on accurate and timely, integrated information.

Remote sensing technologies may have a future role in the assessment of disease spread or damage evaluation under a major epidemic. As far back as 1980's, Najaragan et al. (1984) reported the use of Landsat imagery to detect wheat crops damaged by leaf rust epidemics in Pakistan. With the serious stripe rust epidemic in Syria and neighbouring countries in 2010, disease damage was clearly visible in high resolution Quickbird imagery and a putative damage signal from premature senescence was reported (USDA, 2010). However, at present there seems to be no good example of an operational remote sensing rust monitoring or damage assessment system operating routinely over large geographical scales. Limitations might include reliable differentiation of a stress signal attributable to a specific disease, spatial resolution of detection and feasibility of routine operation over large geographical areas. However, with the rapid advances occurring in remote sensing technology future routine operational assessments may be realistic.

The acute and widespread epidemics of stripe rust that are occurring throughout the CWANA region highlight the need to have more coordinated global monitoring systems for stripe rust as well as stem rust. Existing monitoring systems now in place for stem rust provide an excellent foundation for expansion to include stripe rust. The surveillance networks, data collection, data management and information systems used for stem rust are readily applicable to stripe rust. However, several challenges will need to be addressed for an effective global stripe rust monitoring system to be implemented. Early infections mean that adjustments will be needed in survey planning, with multiple survey visits optimal. The widespread nature of the disease and multiple races causing losses add complexity to monitoring efforts. High inoculum levels increase the likelihood of accidental human-borne movements, which are beyond the scope of any model-based prediction system. Climatic and environmental factors appear to play a very major role in driving current outbreaks, so increased attention may be required on climate-based early warning systems. Systems that permit much more rapid detection linked to mechanisms that allow subsequent targeted control are likely to be required. Despite the challenges, implementation of a global monitoring system for stripe rust is seen as a high priority.

Conclusions

Through an extensive global surveillance network, routine monitoring of stem rust across a vast geographical area is now becoming a reality. The identification of Ug99 provided the impetus to put such a system in place and remains the clear focus. However, the true value of robust monitoring systems is their ability to detect any new significant change as it occurs. The current global cereal rust monitoring system has successfully tracked the spread and variation that are occurring within the Ug99 lineage and is well positioned to detect and monitor any future significant pathotype changes that might arise. Future geographical expansion of the Ug99 lineage races is a near certainty. Variants possessing combined virulence to *Sr31* and *Sr24* are exhibiting rapid range expansion and future spread outside of Africa is highly likely. Efficient and effective data management is now being achieved via the innovative Wheat Rust Toolbox platform, with an expanding range of dynamic information products being delivered to end-users. Application of new technologies may increase the efficiency of the GCRMS, with mobile devices, molecular diagnostics and remote sensing all seen to have potential application in the medium to long-term. Expansion of the global capacity for high quality race analysis is seen to be critical and expansion of the international stem rust pathogen monitoring network via the GRRC is seen as a very positive development. The current acute situation with stripe rust in many countries indicates the clear need to more effective global monitoring systems for this pathogen. The existing systems in place for stem rust can provide a good foundation for this to occur.

References

Aanensen DM, Huntley DM, Feil EJ, al-Own F, Spratt BG (2009) EpiCollect: Linking Smartphones to Web Applications for Epidemiology, Ecology and Community Data Collection. PLoS ONE 4(9): e6968. doi:10.1371/journal.pone.0006968

CIMMYT (2005) Sounding the alarm on global stem rust. CIMMYT, Mexico, DF. http://www.globalrust.org/uploads/documents/SoundingAlarmGlobalRust.pdf

Crouch J, Sakthikumar S, Cuomo C, Pretorius ZA, Szabo LJ (2010) A Panel of SNP-Based Real-Time PCR Probes for the Rapid and Accurate Detection of Ug99. International Wheat Conference Proceedings. p. 477.

Draxler, RR Rolph GD (2003) *HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory)*. NOAA Air Resources Laboratory, Silver Spring, MD. http://www.arl.noaa.gov/ready/hysplit4.html

Dubin HJ, Brennan JP (2009) Combating stem and leaf rust of wheat. Historical perspective, impacts and lessons learned. In: Spielman D, Pandya-Lorch R (eds) Millions fed: proven successes in agricultural development, IFPRI, Washington D.C., USA. http://www.ifpri.org/sites/default/files/publications/oc64ch02.pdf.

Hansen JG, Colon LT, Cooke DEL, Nielsen BJ, Cooke LR, Andrivon D, Lees AK (2007) Eucablight – collating and analysing pathogenicity and resistance data on a European scale. Bulletin OEPP/EPPO Bulletin 37:383-390

Hodson DP, Singh RP, Dixon JM (2005) An initial assessment of the potential impact of stem rust (race Ug99) on wheat producing regions of Africa and Asia using GIS. In: Abstract 7th international wheat conference, Mar del Plata, Argentina, p 142

Hodson DP, Cressman K, Nazari K, Park RF, Yahyaoui A (2009) The global cereal rust monitoring system. In: McIntosh R (ed) Proc Technical Meeting, BGRI. Ciudad Obregon, Mexico, pp 35–46

Hodson DP (2011) Shifting boundaries: challenges for rust monitoring. Euphytica 179:93-104

Hovmøller MS, Yahyaoui AH, Singh RP (2009) A global reference centre for wheat yellow rust: pathogen variability, evolution and dispersal pathways at regional and global levels. In: McIntosh R (ed) Proceedings of Oral Papers and Posters, 2009 Technical Workshop, BGRI, Cd. Obregon, Sonora Mexico, p 221

Jin Y, Pretorius ZA, Singh RP, Fetch T Jr (2008) Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 92:923–926

Jin Y, Szabo LJ, Rouse MN, Fetch T Jr, Pretorius ZA, Wanyera R, Njau P (2009) Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici.* Plant Dis 93:367–370

Jørgensen LN, Hovmøller MS, Hansen JG**,** Lassen P, Clark B, Bayles R, Rodemann B, Jahn M, Flath K, Goral T, Czembor J, du Cheyron P, Maumene C, de Pope C, Nielsen GC (2010) EuroWheat.org - A support to integrated disease management in wheat, Outlooks on Pest Management August 2010:173-176

Knott DR (1989) The Wheat Rusts-Breeding for Resistance. Springer Verlag, London, Paris, Tokyo.

Luig NH (1977) The establishment and success of exotic strains of *Puccinia graminis tritici* in Australia. Proc Ecol Soc Aust 10:89–96

Mukoyi F, Soko T, Mulima E, Mutari B, Hodson D, Herselman L, Visser B, Pretorius ZA (submitted to Plant Disease) Detection of variants of wheat stem rust race Ug99 in Zimbabwe and Mozambique

Mutari B, Musoni M, Hodson D (2009) Zimbabwe Wheat Rust Survey September 2009 – Summary Report. http://www.fao.org/fileadmin/user_upload/rust/docs/Zimbabwe%20Rust%20Survey%20September%202009.pdf

Najaragan S, Seibold G, Kranz J, Saari EE, Joshi LM (1984) Monitoring wheat rust epidemics with the Landsat-2 satellite. Phytopathology 74:585-587

Nagarajan S, Singh DV (1990) Long-distance dispersion of rust pathogens. Annu Rev Phytopathol 28:139-154

Park RF (2007) Stem rust of wheat in Australia. Aust J Agric Res 58: 558-566

Park R, Fetch T, Hodson D, Jin Y, Nazari K, Prashar M, Pretorius Z (2011) International surveillance of wheat rust pathogens: progress and challenges. Euphytica 179:109-117

Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Dis 84:203

Pretorius ZA, Bender CM, Visser B, Terefe T (2010) First report of a Puccinia graminis f. sp. tritici race virulent to the Sr24 and Sr31 wheat stem rust resistance genes in South Africa. Plant Dis 94:784

Singh RP, Huerta-Espino J, Roelfs AP (2002) The wheat rusts. In: Curtis BC, Rajaram S, Gomez Macpherson H (eds) Bread Wheat: improvement and production. Plant production and protection series no. 30. FAO, Rome, pp 317–330

Singh RP, William HM, Huerta-Espino J, Rosewarne G (2004) Wheat rust in Asia: meeting the challenges with old and new technologies. In: New directions for a diverse planet: Proc 4th Int Crop Sci Cong, Brisbane, Australia. http://www.cropscience.org.au./icsc2004/symposia/3/7/141_singhrp.htm

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua M, Wanyera R, Njau P, Ward RW (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Rev 1:54

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward RW (2008) Will stem rust destroy the world's wheat crop? Adv Agron 98:271–309

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel S, Singh PK, Singh S, Singh VG (2011) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu Rev Phytopathol DOI 10.1146/annurev-phyto-072910-095423

Sturman AP, Tyson PD, D'Abreton PC (1997) A preliminary study of the transport of air from Africa and Australia to New Zealand. J R Soc N Z 27:485-98

Watson IA (1981) Wheat and its rust (*Puccinia*) parasites in Australia. In: Evans LT, Peacock WJ (eds) Wheat science : today and tomorrow. Cambridge University Press, Cambridge, England, pp129-147

Watson IA, de Sousa CNA (1982) Long distance transport of spores of *Puccinia graminis tritici* in the southern hemisphere. Proc Linn Soc NSW 106:311–321

USDA (2010) Middle East: Yellow rust epidemic affects regional wheat crops. Foreign Agricultural Service Commodity Intelligence Report June 10, 2010. http://www.pecad.fas.usda.gov/highlights/2010/06/Middle%20East/

WFP (2010) Zimbabwe, Mozambique, South Africa. Rainfall distribution and deviations from the average rainfall (1998-2009). http://reliefweb.int/sites/reliefweb.int/files/resources/707F154C5804C19F85257743006343E4-map.pdf

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Challenges in controlling leaf rust in the Southern Cone region of South America

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Abstract

Leaf rust (caused by *Puccinia triticina*) continues to be the most important and widespread foliar disease of wheat in the Southern Cone. The *P. triticina* population of the region is extremely dynamic, leading to short-lived resistance in commercial cultivars. Some high yielding materials susceptible to leaf rust have been released and their increasing cultivation relies on fungicide applications to control leaf rust. The most important challenge of breeding programs in the Southern Cone is to incorporate durable leaf rust resistance in high yielding cultivars. These cultivars must also combine resistance to other relevant diseases and meet industrial quality standards demanded by the market. Leaf rust resistance in wheat varieties and lines lies mostly in combinations of seedling resistance genes or combinations of these with adult plant resistance (APR), including *Lr34*. Few recently released cultivars carry APR to leaf rust that might be expected to be durable. Since efforts to introduce slow rusting into high yielding adapted germplasm are increasing in most countries, more cultivars carrying this type of resistance will likely be released. If major genes are used, the introduction of effective genes not present in the regional germplasm will increase the diversity of resistance. Molecular markers are used in breeding in Argentina and are starting to be implemented in Brazil and Uruguay. Increased use of molecular tools could improve genetic progress in breeding programs, allow identification of APR genes present in current regional germplasm, and facilitate identification of new resistance genes.

Key words

Breeding for resistance, durable resistance, *Puccinia triticina*

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Introduction

Leaf rust (caused by *Puccinia triticina*) continues to be the most widespread and most important foliar disease of wheat in the Southern Cone. The general situation of the disease was reviewed previously (Germán et al. 2007, 2009) and is updated here. During 2008 and 2009 leaf rust caused moderate epidemics in all countries, but in 2010 epidemics were more intense in Uruguay and in the main wheat areas of Argentina. In the most favorable areas for leaf rust development (Brazil, Paraguay, Uruguay) leaf rust can cause grain yield losses higher than 50% in severe epidemics if fungicides are not applied (Germán et al. 2007).

The widespread occurrence of wheat leaf rust in the eastern epidemiological zone is explained by the use of susceptible cultivars in a significant proportion of the wheat areas of Argentina, Brazil, Paraguay and Uruguay. In Argentina and Uruguay, over 50% of the wheat area in 2010 was planted with moderately susceptible and susceptible cultivars. In Brazil, over 60% of the wheat varieties were susceptible to the leaf rust races present in 2008 and 2009 and the situation was similar in 2010. In Paraguay, the susceptible Brazilian cultivar CD 104, the most widely grown cultivar in Brazil and Paraguay for many years, is losing area in favor of newer less susceptible varieties. In Chile, spring cultivars have adequate levels of resistance, but winter varieties are predominantly susceptible. The release, mostly by private companies, of high yielding susceptible cultivars is becoming more common. These varieties are being introduced from France, or are derived from French breeding materials, and are being adopted by farmers on substantial wheat areas in Argentina and Uruguay.

This paper will provide updated information about the leaf rust situation in the Southern Cone region, economic importance of the disease, changes in the pathogen population, basis of breeding for resistance, resources for research, and the main challenges in breeding for resistance.

Use of fungicides to control leaf rust: an indication of its economic importance

Farmers continue to grow high yielding susceptible cultivars using fungicides. Although farmers in Argentina and Uruguay are discontinuing to plant highly susceptible cultivars that are difficult to manage even with fungicides (requiring more than one application in Argentina and one to two applications in Uruguay), those in Brazil and Paraguay where the disease situation is more complex, continue to grow susceptible cultivars if chemical use allows achievement of high yields, even when requiring three fungicide applications. In Chile

farmers demand almost immunity to leaf rust in new cultivars, the same result as with chemical control, although some old susceptible cultivars are still being used with chemical control.

Fungicides are widely used to control the disease complex present in crops, with leaf rust as the main target due to the high area occupied by susceptible cultivars. There are no official estimates of the use of fungicides in wheat. Estimates from several sources indicate that during 2008 and 2009, fungicides were applied on approximately 25% of the Argentine wheat area. During 2010, triazols and mixtures of triazols and strobilurins were used on over 40% of the 4.3 million ha sown with wheat in Argentina, with an average of 1.2 applications. Double applications were required on highly susceptible cultivars in the central area where the leaf rust epidemic was more severe.

An estimated average of 1.0 to 1.5 applications per ha of mixtures of triazols and strobilurins or triazols alone was used on 0.5 million ha of wheat in Uruguay, with the highest estimate under the moderate to severe leaf rust epidemic of 2010. Under these conditions highly susceptible cultivars required two to three applications for control.

In Brazil (2.3 million ha of wheat during 2008-2010) and Paraguay (0.5 millon ha) most of the wheat area uses chemical control (mixtures of triazols and strobilurins). Depending on the year, farmers in these countries apply fungicides once or twice to control leaf rust and other foliar diseases, but the number of applications can increase to three or four applications in the case of highly susceptible varieties such as CD 104.

In Chile, higher yields (average of 5.7 tonnes/ha during the crop season 2009-2010) make fungicides more widely used since their cost represents a lower proportion of production (100 to 150 kg of wheat per ha). Most winter cultivars require at least one fungicide application to control not only leaf rust but also other diseases, allowing attainment of over 10 tonnes/ha. Leaf rust damage is relatively low since leaf rust infections are normally observed in the flag leaf stage and the development is slow during the cool months of October and November.

Puccinia trtiticina population

The *P. triticina* population in the Southern Cone is extremely dynamic, leading to short-lived resistance in commercial cultivars (Germán et al. 2007). The prevalent races change dramatically over time according to the resistance genes present in the commercial cultivars.

Annual surveys and race analysis of the *P. triticina* population are performed in Argentina, Brazil and Uruguay. Information from Chile and Paraguay is more sporadic. Race nomenclature described by Long and Kolmer (1989) is used, with additional virulences to *Lr10* and/or *Lr20* indicated after the Prt code. The pathogen population has remained relatively stable during 2008-2009, with the same predominant races in Argentina and Uruguay. In order of prevalence during 2009, races with more than 5% frequency in Argentina were MDP, MDT-10,20, MCP-10, MFP, MFP-20, MDP-10,20, MFT-10,20, and in Uruguay, MFP, MFP-10,20, MDP, MFP-20, MDP-10,20 and MFT-10,20. Races MDT-10,20 and MFT-10,20 (Brazilian designation B55) have predominated in Brazil since first identification in the latter in 2005. New races MCT and MJP were identified in Argentina during 2009, TPR-20 in Uruguay during 2008 and MFT in 2009. Cultivars BIOINTA 3004, Don Mario Themix and Don Mario Atlax had increased leaf rust infection in Argentina during 2010. In Uruguay the situation was also stable until 2010 when increased infection was observed on cv. Atlax, due to the presence in high frequency of new races TDT-10,20 and TFT-10,20, which were previously detected in Brazil. The widely grown cultivar INIA Carpintero was resistant until 2009 and also had high leaf rust infection during 2010; races contributing to this change are under study.

Changes in the pathogen population also occurred in Paraguay as evidenced by increased leaf rusting on some commercial cultivars. Initially resistant, cv. IAN 10 has shown leaf rust infections up to 80S since 2009. High infections were also observed on cv. Itapúa 45, Itapúa 55 and Itapúa 65. No new races or associated changes in the rust resistances of cultivars were detected in Brazil since 2008, and leaf rust levels on commercial cultivars in Chile have also been stable in recent years.

The same races occur in the eastern epidemiological zone (Argentina, Brazil, Paraguay and Uruguay). Within this region, generally the pathogen populations present in Argentina and Uruguay are very similar (Ordoñez et al. 2010), probably because of the similarity of cultivars planted in both countries. The same situation of similar cultivars and likely presence of similar *P. triticina* races also occurs in Brazil and Paraguay. However, if races affect a wide range of cultivars, they spread very fast within the entire epidemiological zone. MDT-10,20 and MFT-10,20 were first identified in Brazil in 2005, and then in 2007 in Argentina and Uruguay, indicating that these races probably originated in Brazil and later migrated to the other countries. Similarly, races TDT-10,20 and TFT-10,20 (B57) were detected in Brazil in 2005 and in Uruguay during 2010. Race MDP-10,20 (B58) was first detected in Uruguay and Argentina in 2004 and 2005, respectively, and in Brazil in 2007. This pattern of appearance indicated this race probably originated in Uruguay or Argentina and later migrated to Brazil.

P. tritici isolates from South America (Argentina, Brazil, Chile, Peru and Uruguay), fell into five groups according to their virulence phenotypes and molecular genotypes (Ordoñez et al. 2010). Four of these groups were very similar in SSR genotype to previously characterized North American populations suggesting a common origin of *P. triticina* on both continents. In the fifth group there were three isolates found only in Chile. The use of durum and distinctive bread wheat cultivars in Chile, and its relative isolation from the rest of the region by mountains, results in a different *P. triticina* population, although there are some races in common with the eastern zone.

Race MCD-10,20, prevalent in both South America and North America for several years, was first detected in Mexico and later in USA (1996) and in Uruguay (1999). Different isolates of MCD-10,20 from both continents had similar SSR genotypes (Ordoñez et al. 2010), indicating likely intercontinental migration.

Genetic basis of leaf rust resistance

The presence of *Lr3a, 3ka, 9, 10, 16, 17, 24, 26, 34, 37, 41* (or *Lr39*) and *Lr47* in recent popular Argentinian cultivars was postulated using molecular markers and *P. triticina* races with different virulence phenotypes. In Uruguay, cultivars carrying genes *Lr16, 24, 34, 37* and *39* are being planted, besides others with unknown resistances. Most resistance genes were present in old cultivars and materials grown in the early 2000s. *Lr37* and *Lr39* are present in high yielding French and French-derived cultivars (e.g. Baguette cultivars and Nogal, respectively) which are planted in both countries. *Lr47* was introduced in Argentinian cv. BIOINTA 2004. There is no recent information on the genetic basis of resistance in cultivars from Brazil, Chile and Paraguay.

Resistance in wheat varieties and lines lies mostly in combinations of seedling resistance genes or combinations of these with adult plant resistance (APR). There are a few examples of recently released cultivars which only have APR likely to be of the slow rusting type (e.g. Klein Capricornio released in Argentina, and cv. Canindé 12 and Canindé 13 recently released in Paraguay).

Lr34 was postulated in Argentine cultivars and its presence has been demonstrated in several Uruguayan cultivars. Genetic studies indicate that APR conferred by genes different from *Lr34* is present in some Uruguayan cultivars. The presence of APR becomes evident after the development of races with virulence to the seedling genes present in cultivars. This leads to increased field infections on some cultivars, but with a residual partial resistance expressed as slower rusting relative to susceptible checks. Mapping data from Tc*3/Americano 25e showed that *Lr46* is present in this old line (J. Kolmer pers. comm.) which is in the pedigree of many old cultivars from Uruguay and Argentina. *Lr46* is common in CIMMYT germplasm (Singh et al. 2011) widely used directly or in breeding programs in the region. Kolmer et al. (2007) found two possibly undesignated APR genes in 'Americano 44d', another old Uruguayan land race selection released in 1918. Other uncatalogued APR genes were found in Brazilian cv. 'Frontana' (Singh and Rajaram 1992), 'Toropí' (Barcellos et al. 2000), 'BR35' (Brammer et al. 2004), and Argentinian cv. 'Buck Manantial' (Altieri et al. 2008). APR genes conferring durable resistance different from *Lr34* and *Lr46* are widely distributed in CIMMYT germplasm (Singh et al. 2011), including a slow rusting gene (*LrP*) present in Parula.

Breeding for leaf rust resistance

Breeding for leaf rust resistance is among the most important objectives for public and many private breeding programs in the Southern Cone countries, but has become less important in some programs due to the efficient control of the disease through use of fungicides. The general approach in the public programs is to avoid the release susceptible cultivars. However, some high yielding susceptible lines have been released, mainly by private companies, since there are no protocols preventing such releases. Public and some private breeding programs are trying to incorporate resistance into their germplasm, mainly APR conferred by minor additive genes, although major genes are also being used. Phenotypic selection is possible every year in most places, even without artificial inoculation. Marker assisted selection is being used in some programs to introduce specific resistance genes.

In Argentina, the control of leaf rust using resistant cultivars could be improved. Advanced lines and commercial cultivars from all breeding programs are tested in the seedling stage each year. They are also field tested in inoculated nurseries at several locations. This allows determination of the effectiveness and type of the resistance present in elite germplasm. Introduction of APR is the main objective in the INTA (National Institute of Agricultural Technology, Argentina) breeding program, although combination of APR with seedling resistance may be the best option for areas with high disease pressure. A special project in INTA focuses on introducing slow rusting to leaf rust in adapted germplasm. Selection of sources of resistance is based

on phenotype (seedling susceptibility and APR) and other available information. The materials are mainly from CIMMYT and to a lesser extent local germplasm carrying *Lr34* and *Lr46*. Local materials resistant to Ug99 and derived *Pgt* races are also being used in crosses. Besides phenotypic screening, marker assisted selection is being used to pyramid seedling-effective genes *Lr47* and *Lr19* (low in yellow pigment) with other genes present in resistant backgrounds. A molecular marker for *Lr34* is being used to confirm its presence in parental materials and in advanced lines derived from parents that carry this gene. Other breeding programs in Argentina (Klein, Buck, ACA) have released resistant cultivars selected phenotypically. Cultivars from Brazil and Uruguay planted in Argentina are generally resistant to leaf rust.

The number of genotypes with resistance to all races is very limited in Brazil and Paraguay. Most efforts in breeding for resistance are focused on the incorporation of APR in adapted germplasm. Characterized sources of resistance such as Parula (*Lr34,46,P*) and Chapio are being used, along with other sources of APR (regional and introduced germplasm). APR is identified phenotypically, based on field and seedling tests. A diagnostic marker for *Lr34* and markers already developed for the APR of Toropí are under validation and will be used in breeding. BRS 296 (PF 93232//Cook*4/VPM 1), released in 2009, has expressed high levels of APR for over seven years in experimental plots, with a final leaf rust score of 5SMS.

The resistance present in new cultivars released in Paraguay is probably complex since it has been stable for a number of years. Sources of slow rusting resistance from CIMMYT and the Southern Cone are being used heavily by the local breeding program. Field selection is facilitated by the occurrence of high levels of leaf rust infection every year, favored by the use of spreader rows consisting of mixed susceptible materials. The recent release of two varieties of CIMMYT origin carrying APR will further improve leaf rust stabilization in this country.

Chilean spring germplasm, primarily based on CIMMYT sources, generally has good levels of leaf rust resistance. Useful sources of resistance have been identified in winter germplasm; the genetic basis of this resistance is unknown but it has been effective for a long period of time. Segregating progenies of 700-1,000 crosses annually are selected under natural infection enhanced by the use of spreader rows. Several locations are used for selection. Generally the upper threshold of response for release is 20MS. Biotechnological tools were used in Chile for the development of imidazolinone (herbicide) resistant cv. Pantera and Bicentenario, but are not currently used for selection of leaf rust resistance.

In Uruguay elite resistant germplasm and recently released cultivars generally carry seedling resistance to current races. *Lr34* is present in cultivars used in crosses, and a diagnostic molecular marker will be used in the short term to postulate its presence in advanced lines and new parental materials. Germplasm with leaf rust APR derived from selected CIMMYT and regional materials was developed from crosses with adapted susceptible cultivars. The first BC_1F_5 lines with APR were evaluated for yield potential, and some have been included in the crossing block. Lines with good agronomic type and acceptable industrial quality combining resistance to leaf rust and other diseases have been identified.

Projects to identify the basis of durable resistance.

In Argentina, mapping populations derived from Buck Manatial and Sinvalocho gamma are being studied (Altieri et al. 2008; Ingala et al. 2008). A doubled haploid population derived from a Toropi cross is being developed in Brazil (Scagliusi et al. 2010; Wiethölter et al. 2010) to detect genomic regions associated with the durable APR in this cultivar. The histological mechanisms involved in the resistance expressed by Toropí are also being examined.

The basis of expressed APR in a set of Brazilian lines from the EMBRAPA (Brazilian Enterprise for Agricultural Research) wheat breeding program has been under study for several years (Almeida et al. 2010). A core collection of diverse wheat lines will be assessed for leaf rust and other relevant traits for breeding purposes in Brazil. The genomic regions associated with resistance to leaf rust and other characteristics will be identified using association mapping. Association mapping will also be used to study APR to leaf rust as part of a recently approved project in Uruguay.

Trained human resources infrastructure, funding and research coordination

Resources to work in rust pathology and breeding for leaf rust resistance differ among countries in the region.

Work on breeding for leaf rust resistance in Argentina is supported by a rust pathologist and recently a molecular geneticist is working specifically on durable resistance and marker assisted selection. The available infrastructure and specific equipment for rust research is adequate for current projects; however, an increase in the number of researchers and additional infrastructure and funding would accelerate outcomes. Collaboration between the rust pathologist and molecular geneticists in INTA-Argentina is adequate and it is improving with breeders.

Brazil has adequate personnel, infrastructure and equipment as well as teamwork among researchers of different disciplines. Breeding for resistance to rusts has funding, technical support, and infrastructure of national institutions (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Ministerio da Agricultura Pescuaria e Abastecimento, Empresa Brasileira de Pesquisa Agropecuária, Fundação de Amparo à Pesquisa do Rio Grande do Sul, Universidade Federal do Rio Grande do Sul, Universidade Estadual do Centro-Oeste do Paraná, Universidade Federal de Pelotas).

In Chile, a progressive reduction in human resources has occurred as several highly experienced breeders and pathologists have retired and were not replaced. Plant pathologists and breeders currently work in highly integrated manner. The public breeding program has adequate funding as private industry also provides financial and collaborative support. Good laboratory facilities and new greenhouses are available, but specific equipment for rust research is very old (from the early 1980s) and needs to be renewed.

Paraguay has serious deficiencies in researchers, training and infrastructure and needs to more efficiently address breeding for resistance to leaf rust or other diseases.

INIA-Uruguay wheat breeding and rust pathology programs have adequate personnel, infrastructure and equipment. A molecular geneticist recently joined INIA-Uruguay to study genetics and breeding for durable resistance to leaf rust through molecular work as one priority, and this has also improved the collaboration between disciplines.

In most countries breeding for leaf rust resistance is only one aspect of multidisciplinary breeding programs. Specific research for rust resistance provides a good contribution to breeding in general and allows a better integration between disciplines. However, better collaboration in defining sources of resistance, hybridization programs and field selection would be beneficial. In general, breeders require more training to identify and select APR to leaf rust in segregating populations.

A regional testing network and exchange of information is fundamental since most Southern Cone countries are in a single epidemiological zone, with Chile being partially contiguous; some races from the east are also found in that country (Germán et al. 2007). Past experience with regional research projects has been good, and the exchange of information among countries needs to continue. This can be done through regular contacts and regional meetings.

Challenges in controlling leaf rust in South America

Introducing durable resistance into high yielding adapted germplasm with acceptable market quality

The most important challenge for breeding programs in the Southern Cone is to incorporate adequate levels of durable leaf rust resistance into high yielding cultivars. These cultivars must also combine some resistance to other relevant diseases, including foliar and head blights, especially Septoria tritici blotch (caused by *Mycosphaerella graminicola*), tan spot (caused by *Pyrenophora triciti repentis*) and Fusarium head blight (FHB, caused by *Fusarium* spp.) in Argentina, Brazil, Paraguay and Uruguay, stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) and septoria leaf blotch in Chile, and resistance or tolerance to head blast (caused by *Magnaporthe grisea,* anamorph *Pyricularia grisea*) in Brazil and Paraguay. Besides disease resistance, varieties must meet industrial quality standards demanded by the market.

Table 1 ***Average grain yields with and without fungicides, yield reductions and disease reactions of old widely grown and two new wheat cultivars. Paraguay, 2008, 2009***

	Yield (kg/ha)						
Cultivar	**With fung.**	**Without fung.**	**% yield reduction**	**Leaf rust**	**Fusarium head blight**	**Tan spot**	**Blast**
Old							
CD 104	4672	3505	25.0	S[1]	S	MS	S
Itapúa 40	4628	4307	6.9	MS	MS	MS	MS
New							
Caninde 1	4415	4228	4.2	R	S	MR	R
Itapúa 70	4964	4986	-0.4	MR	MS	MS	MS

Source: Wheat Program, CRIA, Cap. Miranda, Paraguay (unpublished)

[1]R = Resistant, MR = Moderately resistant, MS = Moderately susceptible ,S = Susceptible

Examples of the most important challenges that local breeding programs are facing to control leaf rust, and some achievements are shown in Tables 1-6. In Paraguay, Itapúa 40 and the widely grown Brazilian cultivar CD 104 have similar grain yields compared to new cultivars when leaf rust is chemically controlled (Table 1). However, without fungicides they suffer high yield reductions. Breeding has been successful in combining resistance to leaf rust and other important diseases such as tan spot and head blast, but resistance to FHB is still not adequate. Some recently released cultivars (Canindé 12 and Canindé 13), with APR to leaf rust are susceptible to tan spot.

In Argentina some high yielding leaf rust susceptible cultivars, mostly of French origin, are planted over a large area and require one or more fungicide applications to prevent losses. In southeastern Buenos Aires province, these cultivars have higher grain yields than local resistant cultivars such as ACA 303 (*Lr10,16+*), Klein Capricornio *(Lr34+*, APR) and BIOINTA 2004 (*Lr47,34+*) when fungicides are applied, but this difference is lower when fungicides are not used (Table 2).

In Uruguay, the situation is similar to Argentina, although environmental conditions are more favorable for leaf rust infection and more fungicide applications are usually required to control leaf rust in highly susceptible

Table 2 ***Grain yield with and without fungicides, yield reductions and leaf rust infection. Balcarce, Argentina, 2010***

	Yield (kg/ha)			
Cultivars	**With fung.**	**Without fung.**	**% yield reduction**	**LR response**
French				
Baguette 19	6580	5723	13.0	90S[1]
Baguette 30	6257	5393	13.8	90S
Local				
ACA 303	5307	5350	0.0	5MS
Klein Capricornio	5233	4878	6.7	5MS
BIOINTA 2004	4773	4960	-3.9	0

RET Trigo 2010 Subregión IV INASE MAGPyA

[1] Severity: modified Cobb scale (Peterson et al. 1948), R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible (Stakman et al. 1962)

Table 3 ***Yield with and without fungicides, yield reduction, leaf rust and foliar diseases infection. La Estanzuela, Uruguay, 2010***

	Yield (kg/ha)						
Cultivar	**With fung.**		**Without fung.**		**% yield reduction**	**LR respo nse**	**Foliar spots**
French							
NT 805	8358	a[1]	6745	a[1]	19 **[2]	99S[3]	
Local							
LE 2331 (I. DON ALBERTO)	8170	a	7367	a	10 NS	2MRR	40[4]
LE 2354 (GENESIS 2354)	8131	ab	7101	a	13 NS	3MR	3
LE 2369	7592	b	7194	a	5 NS	5RMR	25

Source: Modified from Castro et al. 2011

[1] Numbers followed by the same letter in the same column are not significantly different

[2] **, highly significant, NS, non significant

[3] Severity: modified Cobb scale (Peterson et al. 1948), R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible (Stakman et al. 1962)

[4] Percentage leaf area affected by Septoria leaf blotch and tan spot

cultivars. Earlier infections result in high and significant yield losses in susceptible cultivars (Table 3). French derived germplasm, such as NT 805, has higher yield potential in the absence of leaf rust, but usually yields less when fungicides are not applied. Therefore, high yielding leaf rust resistant cultivars are required to reduce the area of susceptible wheats in Argentina and Uruguay.

Most resistant advanced breeding lines in the INIA-Uruguay breeding program have effective seedling resistance (Table 4), which may be overcome by the pathogen in a short period of time. Slow rusting is more difficult to select when these seedling resistant elite lines are used in crosses.

Specific crosses with locally adapted susceptible cultivars and lines have been made to introduce APR to leaf rust in INIA-Uruguay wheat germplasm. BC_1F_5 lines selected from these crosses were yield tested at La Estanzuela during 2010 without chemical control (grey background in Table 5). Some lines had grain yields statistically similar to the best checks (LE 2358 and LE 2346), but had higher yields than other check cultivars in the presence of leaf rust.

Some of the advanced lines developed in Uruguay combine APR to leaf rust and resistance to prevalent diseases (Table 6). Considering the susceptibility to FHB of most CIMMYT sources of APR used in crosses, the moderate resistance to FHB of derived lines such us PARULA/ORL 99192*2 is an important achievement.

Lines listed in Table 6, and others with similar characteristics, as well as new CIMMYT lines which combine APR to leaf rust and resistance to *Pgt* race Ug99 and its derivatives have also been used in recent crossing programs.

Diversity of resistance between countries in the Southern Cone and other countries in South America

A better knowledge of the bases of resistance being used in different breeding programs would be very useful for combining different sources. Few major genes appear to be present in the regional germplasm and resistance appears to depend on different combinations of these genes. For this approach to continue in the future, it will be necessary to know which genes are being combined and to introduce different effective genes in order to maintain diversity within and among the programs to reduce the risk of widespread epidemics. This is seen as an important step forward since races rapidly migrate from one country to another, and between continents in the longer term (Ordoñez et al. 2010).

Table 4 ***Seedling infection types produced on Uruguayan breeding lines to 17 P. triticina races***

Line	CHT	KDG-10,20	MCD-10,20	MCR-10	MCT-10	MDP	MDP-10,20	MDR-10,20	MDT-10,20	MFP	MFP-10,20	MFP-20	MFR-10,20	MFT-10,20	MMD-10,20	SPG-10	TDD-10,20
Facultative																	
LE 2359	0[1]	0	0	0;	0	0	0	0	0	0;	0	0	0	0;	2	0	0
LE 2366	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LE 2377	0	0;	0;	0;1=	0;	0;	0;	0	0;	0;	1-;	0	0	0	0;	0	0;
LE 2379	0	;1=	1;	2-;	;1=	1-;	2	;1=	12-	0;	;1-	0;	;1=	2=;	;	0	1;
Spring																	
LE 2354	0;	0;	;1=	;1=	;1=	;1=	;1=	0	0	;1-	0;	;	;1-	;	0;	0;	;1=
LE 2382	0	0;	0;	0;	0;	0;	2-;	,1=	2=;	0;	0	0;	0;	2=;	2=;	0;	;1=
LE 2388	0	0;	,1=	12-	2=;	1-;	1-;	;1=	0;	;1=	0;	;1-	;1=	;1=	;	0	;1=
LE 2390	0	0;	0	0;	0;	2=;	2	0;	2=;	0;	;1=	0;	0;	2-;	0;	0;	0;

[1]Infection type, Stakman et al. (1962), IT 0 to 4 scale, 0 to 2+, low, 3- to 4, high. + and – indicate larger and smaller IT, respectively

Table 5 *Yield and leaf rust infection of INIA breeding lines. La Estanzuela, Uruguay, 2010*

Line	Cross	Yield (kg/ha)		LR response
LE 2358	PI/FUNO*2/5/VLD/4/CO723595/3/TAM200*2//TAM107/ TA2460/6/LE2220	6862	a[1]	0[2]
LE 2346	PEETHREE NR2/2*OS//NWT/3/OS.VONA PYN COMP/4/ PIOPIO/5/LE2221	6766	a	0
F5 09-13158	I.TORCAZA*2//CEP8749/EMBRAPA27	6657	ab	0
F6 09-696	ITIJ/4/TRAP1/YACO/3/KAUZ*2/TRAP//KAUZ	6487	abc	15MSS
LE 2359	ITIJ/LE2266	6459	abc	0
F6 09-10081	IGAR/LE2321	6096	abcd	10MS
F5 09-13139	I.TORCAZA*2//CEP8749/EMBRAPA27	6007	abcd	0
F6 09-599	IGAV/5/CEP85155/3/CEP7780*2//H499.71A/4*JUP/4/BR23	5999	abcd	15MSS
F5 09-13049	I.TIJERETA*2/SUZ6/OPATA	5986	abcd	0
F6 09-10027	IGAR/ALSEN	5935	abcd	0
F5 09-13177	I.GORRION*2/CHAPIO	5895	abcd	30MSS
I. GARZA	ICAB/ITIJ	5856	abcd	10MS
BAGUET.19		5530	bcd	70S
BIOINTA3000		5467	cd	50S
I.TIJERETA	LE2132/ECAL	5196	de	15MSS
BIOINTA3004		4122	e	70S

Source: M. Quincke, INIA wheat breeding program (unpublished)

[1]Numbers followed by the same letter in the same column are not significantly different

[2]Severity: modified Cobb scale (Peterson et al. 1948); reaction: R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible (Stakman et al. 1962)

Table 6 *BC_1F_5 lines from INIA-Uruguay combining adult plant resistance to leaf rust and resistance to other prevalent diseases*

Line	Cross	Fusarium head blight	Tan spot	Septoria tritici blotch	Stem rust (Kenya)
R09-19126	I.TIJERETA*2/TOROPI	MR[1]			
R09-19008	PARULA/ORL 99192*2	MR			MRMS
R09-19149	PARULA/ORL 99192*2	MR			
R09-19228	LE 2304*2 / PARULA	MRMS			R
R09-19024	LE 2304*2 / PARULA				R
R09-19107	GENARO*3/PARULA//LE 2252	MRMS			R
R08-10690	LE2252*2//GENARO*3/PARULA		MR		R
R09-19200	I.GORRION*2 / CHAPIO	MRMS			
R09-19197	I.GORRION*2 / CHAPIO			R	
R09-19229	LE 2304*2 / TOROPI			R	R
R09-19249	LE 2304*2 // CEP8749/EMBRAPA27	MR			
R09-19173	I.TORCAZA*2 // CEP8749/EMBRAPA27		MR	R	
R09-19189	I.TORCAZA*2 // CEP8749/EMBRAPA27	MR		R	
R08-4898	I.TIJERETA*2 / AMADINA		MR		
R09-19318	I.TIJERETA*2 / AMADINA			R	

R = Resistant, MR = Moderately resistant, MS = Moderately susceptible

Concluding remarks

Many cultivars used in the Southern Cone are leaf rust susceptible or have short-term effective seedling resistances. However, some recently released cultivars carry APR, which is expected to be durable. Since efforts to introduce slow rusting into high yielding adapted germplasm with adequate quality are increasing in most countries, more cultivars carrying this type of resistance are likely to be released. In some of the countries it will be difficult to meet the challenge of releasing cultivars with these characteristics without increasing scientific staff, training, updating and increasing facilities, equipment, and funding. In others, improvements in these aspects will result in faster progress. Increased use of molecular tools could improve genetic progress in breeding programs, allow identification of APR genes present in current regional germplasm, and facilitate identification of new resistance genes.

Fungicides are very valuable for controlling leaf rust and protecting cultivars with specific resistance when virulent races emerge. It may also be relevant to fine-tune the usage of chemicals on genotypes with intermediate levels of disease.

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References

Almeida NP, Chaves MS, Martinelli JÁ, Graichen FAZ, Brammer SP, Bonato ALV, Mognon AP, Copetti MR (2009) Validação da resistência de planta adulta à ferrugem da folha em genótipos de trigo. In: Mostra de iniciação científica da Embrapa Trigo, 5., 2009, Passo Fundo. Resumos. Passo Fundo: Embrapa Trigo, 2009. p. 23. (Embrapa Trigo. Documentos Online, 115). Disponível: http://www.cnpt.embrapa.br/biblio/do/p_do115.htm

Altieri E, McCallum B, Somers DJ, Sacco F (2008) Inheritance and genetic mapping of leaf rust resistance genes in the wheat cultivar Buck Manantial. Available online: http://ses.library.usyd.edu.au/bitstream/2123/3277/1/P155.pdf

Barcellos AL, Roelfs AP, de Moraes-Fernandes MIB (2000) Inheritance of adult plant leaf rust resistance in the Brazilian wheat cultivar Toropi. Plant Disease 84:90-93

Brammer SP, de Moraes-Fernandes MIB, Barcellos AL, Milach SCK (2004) Genetic analysis of adult-plant resistance to leaf rust in a double haploid wheat (*Triticum aestivum* L. em Thell) population. Genetics and Mol Biol 27:32-436

Castro M, Vera M, Díaz M, González N, Vázquez D (2011) IV. Trigo con control de enfermedades en La Estanzuela. In Resultados Experimentales de la Evaluación Nacional de Cultivares de Trigo de Ciclo Intermedio. Período 2010. INASE/INIA. La Estanzuela, Uruguay, pp43-55

Germán S, Barcellos A, Chaves M, Kohli M, Campos P, Viedma L (2007) The situation of common wheat rusts in the Southern Cone of America and perspectives for control. Austr J Agric Res 58:620-630

Germán S, Chaves M, Campos P, Viedma L, Madariaga R (2009). Are rust pathogens under control in the Southern Cone of South America? In: McIntosh RA (ed) Proc Borlaug Global Rust Initiative Technical Workshop, Cd. Obregon, CIMMYT, Mexico: BGRI. pp65-73

Ingala L, Diéguez MJ, Pergolesi F, López M, Paux E, Feuilliet C, Sacco F (2008) Genetic map of wheat chromosome 3BS including SV2 an adult plant leaf rust resistance gene. In: Appels R, Eastwood R, Lagudah E, Langridge P, Mackay M, McIntyre L, Sharp P (eds) Proc 11th Int Wheat Genet Symp, Sydney University Press, NSW, Australia. Vol 3:827-829

Kolmer JA, Oelke LM and Liu JQ (2007) Genetics of leaf rust resistance in three Americano landrace derived cultivars from Uruguay. Plant Breeding 126:152-157
Long DL, Kolmer JA (1989) A North American system of nomenclature for Puccinia recondita f.sp. tritici. Phytopathology 79:525-529
Ordoñez M, Germán S, Kolmer JA (2010) Genetic differentiation within the *Puccinia triticina* population in South America and comparison with the North American population suggests common ancestry and intercontinental migration. Phytopathology 100:376-383
Peterson RF, Campbell AB, Hannah AE (1948) A diagramatic scale for estimating rust intensity on leaves and stems of cereals. Can J Res C 26:496-500
Scagliusi SMM, Torres GAM, Chaves MS, Simioni A (2010) Use of high molecular weight glutenin analysis as a tool to avoid self pollinated seeds when developing double haploid populations. In: Proc 8th Int Wheat Conf, Vavilov Research Institute of Plant Industry, Saint Petersburg, Russia, p470
Singh RP, Rajaram S (1992) Genetics of adult-plant resistance of leaf rust in Frontana and three CIMMYT wheats. Genome 35**:**24-31
Singh RP, Huerta-Espino J, Bhavani S, Herrera-Foessel SA, Singh D, Singh PK, Velu G, Mason RE, Jin Y, Njau P, Crossa J (2011) Race non-specific resistance to rust diseases in CIMMYT spring wheats. Euphytica 179:175-186
Singh RP, Huerta-Espino J, Rajaram S (2000) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting genes. Acta Phytopathologica Hungarica 35:133-139
Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiologic races of *Puccinia graminis* var *tritici*. U.S. Department of Agriculture ARS - E 6/7, 53 pp
Wiethölter P, Brammer SP, Da Silva PR, Chaves MS (2010) Uso da hidridização subtrativa como ferramenta para a identificação de genes envolvidos na resistência à ferrugem da folha do trigo. In: Pires JL; Pasinato A; Caierão E; Tibola C (2009) Trigo: resultados de pesquisa - safra. Passo Fundo: Embrapa Trigo (Documentos/Embrapa Trigo Nº 96) pp43-55

Yellow rust in CWANA in 2010 & 2011: The situation and measures taken to control it*

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Abstract

Wheat is the most important cereal crop in Central, West Asia and North Africa (CWANA). The total acreage in CWANA is approximately 53 million hectares. Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) continuously poses a serious threat to wheat production in CWANA. Several factors have contributed to the current severe epidemics of stripe rust, including; the rapid shift of virulence in the pathogen population, genetic uniformitity of mega-cultivars, favorability of environmental conditions, and an overlapping/ continuous crop calendar.

During 1985-1997 the widespread appearance of *Yr9* virulent pathotypes in CWANA, and eventually in the Indian sub-continent, resulted in several epidemics that caused a series of severe crop losses in popular cultivars known to be protected by the *Yr9* resistance gene. Following the *Yr9* virulence epidemics, susceptible cultivars were extensively replaced with CIMMYT-derived germplasm such as Kauz, Atilla, Opata, Nacozari, Bucbuc and Crow. The resistance of many of the replacement cultivars, including the mega-cultivars in India (PBW343), Pakistan (Inquilab-91, Bakhtwar), Iran (Chamran, Shiroudi), Ethiopia (Kubsa), and Syria (Cham 8) was based on *Yr27*. Breakdown of *Yr27* resistance in PBW343, Inquilab 91 and Chamran, in India, Pakistan, and Iran, respectively, was reported between 2002-2004. Although occasional stripe rust outbreaks appeared in some areas, unfavorable environmental conditions presumably restricted the increase of the *Yr27 Pst* population until 2009, when conducive environmental conditions resulted in severe epidemics in several CWANA countries e.g., Morocco, Algeria, Uzbekistan, Turkey, Iran, Azerbaijan, Georgia, and Afghanistan. Environmental conditions favouring rust development continued into 2010, with mild winters and adequate rainfall in several CWANA countries resulting in early outbreaks of stripe rust. The 2010 stripe rust outbreaks occurred throughout the major wheat growing areas in the CWANA and Caucasus countries, causing severe yield losses particularly in Syria where Cham 8 (with *Yr27*) occupied more than 70% of the wheat areas. Inspite of favorable environmental conditions in many areas in CWANA in 2011, similar severe stripe rust epidemics have not been reported to date.

Climate change now appears to be playing a major role in *Pst* population dynamics in CWANA. Direct, multiple affects of climatic changes on epidemiology of rust pathogens are expected, including the survival of primary inoculum, the rate of disease development, duration of rust epidemics, and development and distribution of rust populations. Emergence of stripe rust in non-traditional areas, changes in the frequency of new race evolution, early infection of stripe rust, shifts in predicted pathways of rust migrations, and finally wide spread epidemics of stripe rust in warmer areas as a potential indicator of adaptation to high temperatures are considered as possible consequences of climatic changes. Regional pathogen surveys indicated the widespread distribution of aggressive *Pst* pathoype (s) with adaptation to higher temperature. In the absence of resistant varieties, fungicide application remains the only practical measure to control stripe rust. Effective disease surveillance and monitoring systems, coupled to timely application of fungicides has effectively controlled stripe rust epidemics in Iran, Turkey, and Syria during 2010-11.

Regional monitoring of pathogen variability and disease development must be undertaken as a matter of high priority, and timely chemical control measures will continue to play a major role for control of stripe rust in CWANA in the short-term. In the medium to long-term, existing resistant varieties and advanced breeding lines need to be promoted and susceptible varieties have to be urgently replaced.

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Risk assessment of aerial transport of rust pathogens to the Western Hemisphere and within North America

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Abstract

The risk of aerial long-distance transport of rust pathogens from potential source locations in the Eastern Hemisphere to the Western Hemisphere and from subtropical to continental interior regions within North America is investigated. Simulations of long-distance transport of rust spores using the Integrated Aerobiology Modeling System indicate that the frequency of transport and deposition in the Western Hemisphere of viable rust spores originating from potential sources in tropical Africa, at high latitudes in Europe, and throughout eastern Asia is low. However, the frequency of trans-oceanic transport and deposition of viable rust spores in the Western Hemisphere is high for potential African source locations poleward of the tropics. The relatively short distance between Western Africa and northeastern South America coupled with the presence of persistent Northeasterly Trade Winds create an active pathway for spore transport. Western Hemisphere regions that are influenced by the Intertropical Convergence Zone have the highest likelihood of receiving viable rust spores from the Eastern Hemisphere. The risk of aerial transport of viable rust spores to U.S. regions from potential Eastern Hemisphere source regions is low. Analysis of wind streamline maps for North America indicate that strong low-level advection of air northward from the subtropics is prevalent east of the Rocky Mountains from early April to mid-May providing opportunities for long-distance transport of rust pathogens into the continental interior. After mid-June, the number of days with strong low-level advection of air from south to north across these regions and thus opportunities for long-distance spore transport decrease dramatically.

Key words

Aerobiology, Integrated Aerobiology Modeling System, long-distance transport, wind streamlines

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Introduction

Rusts are important diseases of many crops that comprise the world food supply. Where highly susceptible cultivars are grown over large areas, rust pathogens have frequently demonstrated a capability to cause regional epidemics and substantial crop losses. A single infected plant can produce enormous numbers of urediniospores and wind can disseminate these propagules onto the same or new hosts. Most liberated spores are deposited near their source (Roelfs 1972; Roelfs and Martell 1984; Aylor 1999); however, long-distance dispersal is well documented for many rust pathogens. There are two general patterns of aerial dispersal in time and space: (1) single aerial transport events that result in colonization of new regions far from a source, and (2) step-wise range expansion from a source into new regions (Stakeman and Harrar 1957). The latter is accomplished through a series of individual transport events over short to moderate distances. This study focuses on the former of these patterns of aerial dispersal - single long-distance aerial transport events. The Integrated Aerobiology Modeling System (IAMS) and wind streamline maps are used to provide a quantitative assessment of pathways for long-distance transport of rust pathogens from potential source locations in the Eastern Hemisphere to the Western Hemisphere and from subtropical to continental interior regions within North America. The assessment is conducted by enumerating: (1) the number of days per month with opportunities for rust pathogens to be transported from potential Eastern Hemisphere source locations to destinations in the Western Hemisphere, and (2) the number of days per month with opportunities for rust pathogens to be transported from potential source locations in subtropical regions of North America to the interior of the continent.

Indirect evidence of intercontinental aerial spread of passively transported organisms is abundant (Pedgely 1982; Nagarajan and Singh 1990; Isard and Gage 2001). Noteworthy examples of rust pathogens that have dispersed through the atmosphere to the Western Hemisphere and have had important agricultural impacts include: (1) *Hemileia vastatrix,* the causal agent of coffee leaf rust first observed in Brazil during January 1970 (Bowden et al. 1971); (2) *Puccinia melanocephala,* that causes brown sugarcane rust noted first in the Dominican Republic during July 1978 (Purdy et al. 1985); (3) *Phakopsora pachyrhizi,* the causal agent of soybean rust first reported in Paraguay during February 2001 (Morel and Yorinori 2002); and (4) *Puccinia kuehnii, that causes* orange rust of sugarcane first detected in Florida and Costa Rica during July 2007 (Comstock et al. 2008) and two months later in Nicaragua and Guatemala (Ovalle et al. 2008; Chavarria et al. 2009).

In North America, many rusts move long distances through the atmosphere between subtropical and middle latitudes. Seasonal changes in temperature and precipitation influence the availability of appropriate hosts at different latitudes and times of year. *Puccinia graminis f. sp. tritici*, *P. triticina,* and *P. striiformis f. sp. tritici* (wheat stem rust, leaf rust, and stripe rust pathogens), *P. sorghi* and *P. polysora* (common and southern corn rust pathogens) and *Phakopsora pachyrhizi* (soybean rust pathogen) are a few examples of rusts of agricultural importance that have spread rapidly through the air from southern regions into the interior of the North American continent during recent growing seasons (USDA 2011).

Rust pathogens have a number of characteristics that make them well-suited for long-distance aerial dispersal in addition to their capability to produce copious numbers of urediniospores. The physical characteristics (shape, size, density, and surface features) of rust urediniospores are such that the energy in a turbulent atmosphere is often able to lift large numbers of the spores from a plant canopy against the attraction of gravity and keep them airborne for long periods of time. The energy of the air is also used for spore liberation (mechanical vibration of plant parts) with the result that most urediniospores are released when the atmosphere is turbulent and thus conducive to spore transport. Rust urediniospores are able to remain viable for many days when exposed to the range of temperature and humidity conditions typically found in the lower atmosphere during the growing season. Their relatively dark pigmentation provides extended protection from the lethal effects of ultraviolet (UV) radiation. Finally, the primary mechanism that causes deposition of rust spores after long-distance aerial transport is rainfall. Precipitation can efficiently wash spores from a tall column of air onto a host and concomitantly provide the pathogen with the environmental conditions (leaf wetness and cool to moderate temperatures) required to rapidly infect a plant host.

Methods

Assessment of aerial pathways for long-distance rust pathogen transport to the Western Hemisphere

The Integrated Aerobiology Modeling System (IAMS) was built following the aerobiology pathway model (Benninghoff and Edmonds 1972; Isard and Gage 2001). It includes modules for spore production, spore release and escape from the plant canopy, atmospheric dispersion, mortality due to exposure to UV radiation, wet and dry deposition of spores, host development at destinations, and disease progress on these hosts. Together the modules can predict the progression and intensity of an epidemic in an impacted region. Details of the parameterization for IAMS simulations of long-distance aerial spread of soybean rust are provided in Isard et al. (2007).

Table 1. ***Eastern Hemisphere Source Regions and Western Hemisphere Destination Regions.***

Source region	Destination region	Source Region	Destination Region
United Kingdom	Northeastern Canada, U.S. east coast, Florida, Greater Antilles, Lesser Antilles	South Africa	Florida, Greater Antilles, Lesser Antilles, northeastern South America (10° S–12° N), southeastern South America (>10° S)
Spain/ Morocco	U.S. southeast coast, Florida, Greater Antilles, Lesser Antilles, northeastern South America (10° S–12° N)	Eastern Australia	Central America, northwestern South America, northern Chile (<30° S), central Chile (30-40° S), southern Chile (>40° S)
Sierra Leone	Florida, Greater Antilles, Lesser Antilles, northeastern South America (10° S–12° N), southeastern South America (>10° S)	Philippine Islands	Alaska, British Columbia, U.S. west coast, Hawaii, Mexico/Central America
Nigeria	Florida, Greater Antilles, Lesser Antilles, northeastern South America (10° S–12° N), southeastern South America (>10° S)	Southeastern China	Aleutian islands, Alaska, British Columbia, Washington/Oregon, California, Hawaii
Cameroon	Florida, Greater Antilles, Lesser Antilles, northeastern South America (10° S–12° N), southeastern South America (>10° S)	Northeastern China/Korea	Aleutian islands, Alaska, British Columbia, Washington/Oregon, California, Hawaii
Angola	Florida, Greater Antilles, Lesser Antilles, northeastern South America (10° S–12° N), southeastern South America (>10° S)		

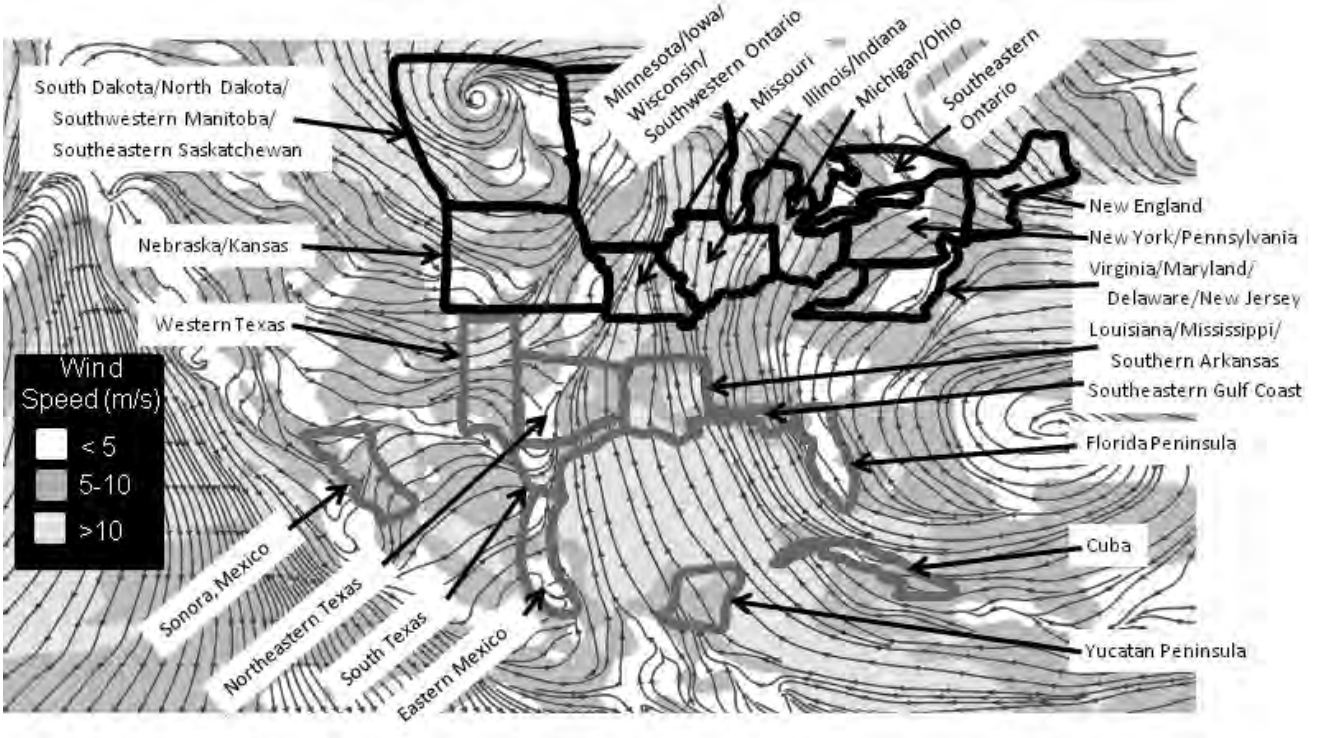
In this analysis, spore production was held constant across time and source area (see below) and only the IAMS transport modules (spore release and escape, atmospheric dispersion, mortality due to UV exposure, and wet and dry deposition) were employed. The IAMS simulations used a grid spatial resolution of 0.083 degrees (~ 14 km), a vertical resolution defined by the standard pressure levels (1000, 950, 900, 850, 800, 700, 600, 500 hPa), and a 1 hr time step. The National Center for Environmental Prediction – Department of Energy Reanalysis 2 data set (Kalnay et al. 1996; Kanamitsu et al. 2002) for the 1998-2007 period containing historical surface and upper-air meteorological observations integrated with numerical model output was used as input for IAMS simulations. Each simulation was initiated for 1 January 1998 with the daily spore production held constant for the duration of the 10-yr run. Nine grid cells (equivalent to about 125,000 ha at the equator), distributed throughout a source area, were assumed to have a healthy crop and rust infection severity of 50%. Because the size of the IAMS grid cells decrease with increasing latitude, the infection severity was increased with increasing latitude to ensure that the same number of spores per day were released from each source region. Parameters for the spore release and escape, dispersion, mortality, and deposition modules were those used in a previous soybean rust study (Isard et al. 2007).

Aerial transport of rust spores from eleven potential Eastern Hemisphere source regions to destination regions in the Western Hemisphere was investigated. The Western Hemisphere destination regions varied with source region location (Table 1). For each source area simulation, the days for which the IAMS predicted deposition of viable rust spores at each destination were enumerated. The pathway analysis for each source-destination region pair involved production and inspection of 3,652 maps and the resulting tabulations were aggregated by month.

Assessment of opportunities for long-distance rust pathogen transport from subtropical to continental interior regions of North America

Wind streamline maps for 18 UTC (noon CST) have been produced by ZedX Inc for the ipmPIPE (Isard et al. 2006) from Global Forecast System model output (Kanamitsu 1989; Kanamitsu et al. 1991) since mid-2006. Three wind speed classes (<5, 5-10, and >10 m/s) are displayed as color shadings on the maps. The wind speed and directions are averaged for the air layer between the ground and the height at which air pressure decreases 30 hPa (typically 200 m near mid-day). The potential source and destination regions are overlain on an example map in Fig. 1.

Fig. 1 ***Potential source and destination regions used in the analysis of opportunities for long-distance rust spore transport within North America overlain on an example wind streamline and speed map***



Days were enumerated as opportunities for long-distance aerial transport of rust pathogens between source and destination region pairs when two conditions were met: (1) wind streamlines connected the two regions, and (2) wind speeds equaled or exceed 5 m/s along the entire route defined by the streamlines (or a subset of streamlines) connecting the two regions. Biological factors related to the pathogens and their hosts that also govern when rusts spread through the air were not considered. The opportunity analysis for each source-destination region pair involved inspection of daily maps for the mid-2006 through 2010 period and the resulting tabulations were aggregated by two-week periods between 1 April and 30 September.

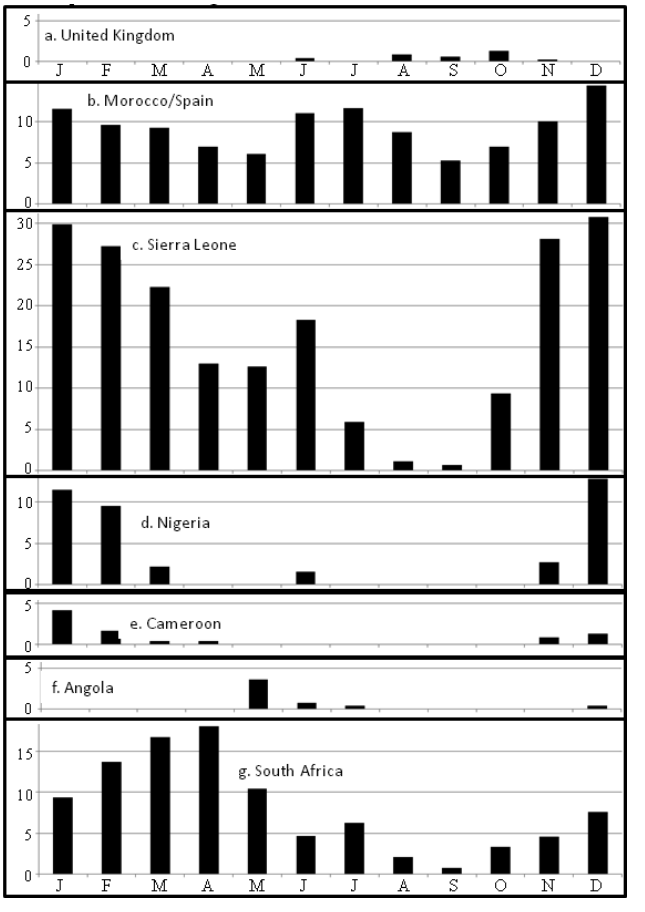
Results and discussion

Assessment of aerial pathways for long-distance rust pathogen transport to the Western Hemisphere

The assessment of long-distance transport is presented as histograms showing the average number of days per month with deposition of viable rust spores in the Western Hemisphere. These daily averages per month of spore deposition were derived from 10-years (1998-2007) of IAMS simulations for potential source regions on the west coasts of Europe and Africa (Fig. 2).

Westerly air flows dominate at high latitudes and thus the number of days on which viable rust spores originating from the United Kingdom are deposited in the Western Hemisphere is small (Fig. 2a). When trans-oceanic transport did occur, the majority of the spores were deposited in Newfoundland and Labrador. Westward trans-Atlantic Ocean transport of spores from locations near the equator is also infrequent. In these potential source regions (e.g. Figs. 2d, 2e and 2f), frequent precipitation limits spore release, rainfall wet-deposits most airborne spores close to their source, and winds are generally weak. In contrast, the strong and persistent Northeasterly and Southeasterly Trade Winds that blow from subtropical latitudes into the Intertropical Convergence Zone (ITCZ) provide frequent opportunities for trans-Atlantic Ocean movement of spores from Morocco/Spain (Figs. 2b), Sierra Leone (Fig. 2c) and South Africa (Fig. 2g) to the Western Hemisphere. The persistence of the Northeasterly Trade Winds, the frequent presence of cloud cover in the ITCZ (protects spores from exposure to lethal UV radiation), and the relatively short distance from western Africa (e.g. Sierra Leone) to northeastern South America (3,000 km), results in opportunities for trans-Atlantic Ocean spore movement on the vast majority of days when the location of the ITCZ is south of the Equator (Northern Hemisphere winter).

Fig. 2 ***Average number of days per month with deposition of viable rust spores in the Western Hemisphere as simulated by IAMS for 1998-2007 for European and African potential source regions***

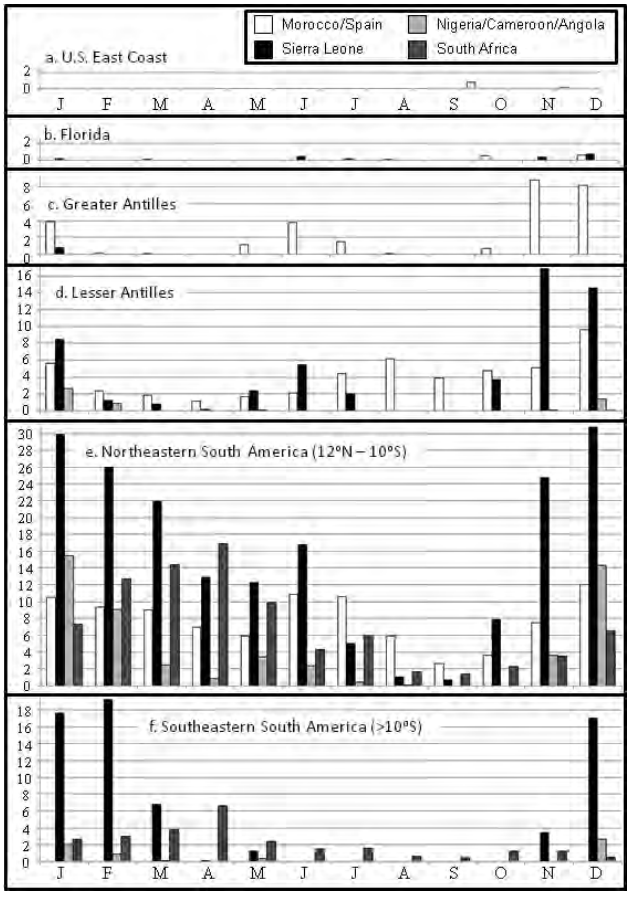


The importance of these factors in combination can be clearly seen when the average number of trans-Atlantic Ocean viable spore deposition days are plotted for destination regions (Fig. 3).

The potential for direct transport of viable rust spores from the Eastern Hemisphere to the U.S. east coast (Fig. 3a), Florida (Fig. 3b), and Greater Antilles (Fig. 3c) regions as simulated by IAMS is very low. Regions influenced more by the ITCZ (e.g. Lesser Antilles (Fig. 3d), Northeastern South America (Fig. 3e), and Southeastern South America (Fig. 3f)) have a greater likelihood of receiving viable wind-blown rust spores from the Eastern Hemisphere.

The average number of days with deposition of viable rust spores in the Western Hemisphere as simulated by the IAMS for 1998-2007 aggregated by month are plotted in Fig. 4 for potential source regions on the east coasts of Australia and Asia. The large size of the Pacific Ocean and the persistence of high pressure cells in subtropical latitudes over the Pacific

Fig. 3 ***Average number of days per month with deposition of viable rust spores originating from European and African potential source regions for destination regions in the Western Hemisphere as simulated by IAMS for 1998-2007***



Ocean in both hemispheres limit the number of trans-ocean, spore transport events (Fig. 4). Rust spores from potential Northern Hemisphere locations in eastern Asia are generally blown northeastward into the Gulf of Alaska. The vast majority of these spores are deposited in the Aleutian Islands and Alaska regions. Average number of days per month with deposition of viable rust spores originating from Asian and Australian potential source regions for the destination regions of British Columbia, Washington/Oregon, California, and Hawaii destination regions are few (Fig. 5).

The large distance (11,000 km) and the presence of a persistent high pressure cell over the South Pacific Ocean reduces the number of days on which viable spores originating in eastern Australia have the potential to be deposited in the Western Hemisphere (Fig. 4d). When trans-oceanic transport did occur, the majority of the spores were deposited in southern Chile (>40° S).

Fig. 4 ***Average number of days with deposition of viable rust spores in the Western Hemisphere as simulated by IAMS for 1998-2007 aggregated by month for Asian and Australian potential source regions***

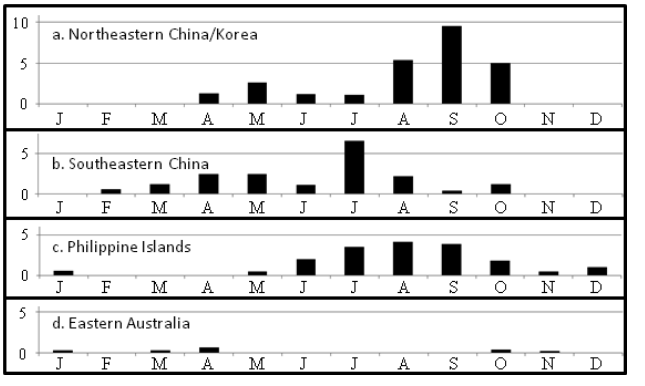
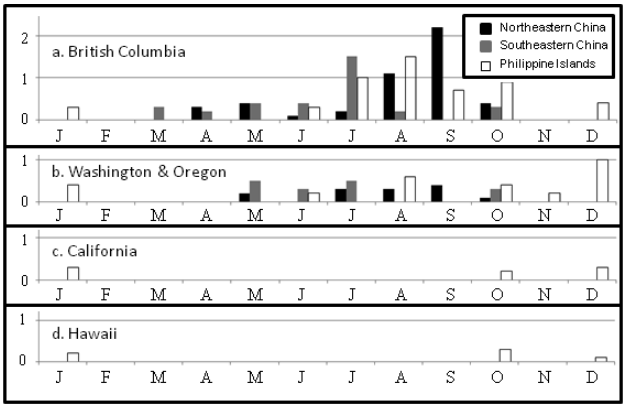


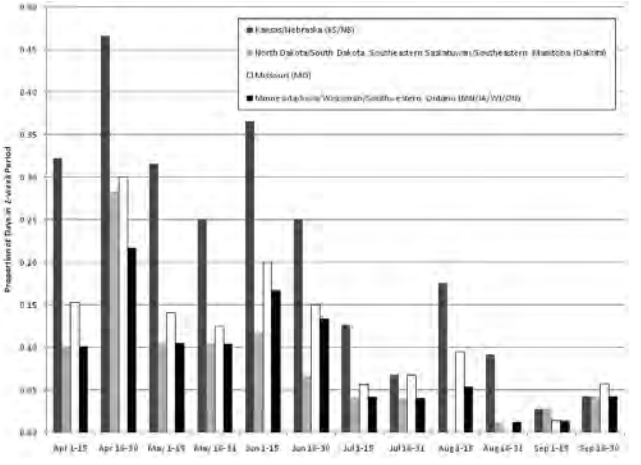
Fig. 5 ***Average number of days per month with deposition of viable rust spores originating from Asian and Australian potential source regions for destination regions in the Western Hemisphere as simulated by IAMS for 1998-2007***



Assessment of opportunities for long-distance rust pathogen transport from subtropical to continental interior regions of North America

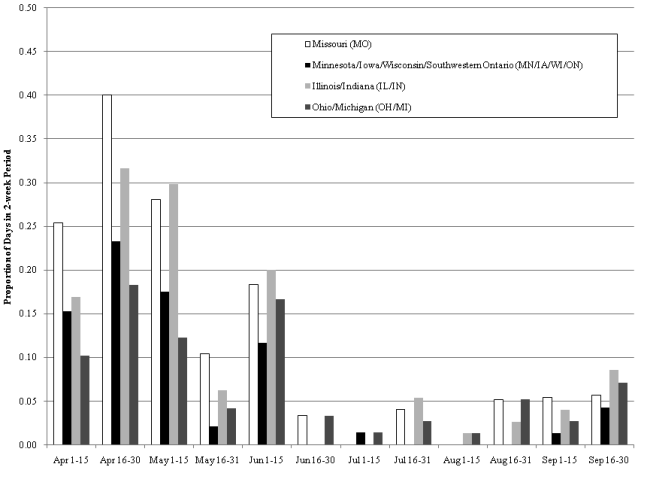
Opportunities for aerial transport of rust pathogens due to strong low-level airflows from the South Texas and Delta (Mississippi River delta covering Louisiana, Mississippi, and southern Arkansas) regions to selected continental interior destination regions are shown in Figs. 6 and 7, respectively. Advection of air northward from the subtropics is prevalent for both regions during the spring. For example, strong low-level airflows from south Texas to the Kansas/Nebraska (KS/NB) region occurred on 32-47% of days during the mid-April to mid-May period (Fig. 6).

Fig. 6 ***Frequency of strong low-level airflows from the South Texas region to designated regions in the North American continental interior for two-week periods between April and September***



The Dakota (South Dakota, North Dakota, southwestern Manitoba, and southeastern Saskatchewan), Missouri (MO), and Minnesota/Iowa/ Wisconsin /southwestern Ontario (MN/IA/WI/ON) destination regions experienced strong low-level advection of air from the South Texas region 10-28, 14-30, and 10-22% of the days, respectively, during the same period. Advection of air through the Mississippi River valley from the subtropics into the continental interior is almost as prevalent as on the High Plains during spring. For example, strong low-level airflows from the Delta region to the MO, MN/IA/WI/ON, Illinois/Indiana (IL/IN) and Ohio/Michigan (OH/MI) regions occurred on 28-40, 17-23, 30-32, and 12-18% of the days in the mid-April to mid-May periods (Fig. 7).

Fig. 7 ***Frequency of strong low-level airflows from the Delta region to designated regions in the North American continental interior for two-week periods between April and September***



After mid-June, the number of days with strong low-level advection of air from south to north across these regions decreases dramatically. On average, the Delta region experiences this flow pattern on only 2 days in the three month period from mid-June to mid-September. The corresponding decrease for the High Plains is not as dramatic as in the Mississippi River valley. For the mid-June to mid-September period, strong low-level northward advection of air from the South Texas region to the continental interior occurs on 1-2 days per month.

The pronounced seasonal pattern in strong low-level airflows and thus opportunities for aerial transport of rust pathogens from the subtropics into the continental interior coupled with an understanding of crop production practices can provide insight into differences in risk of spread of wheat, soybean, and orange sugercane rusts in North America. Maps showing the distribution of the winter wheat, soybean and sugercane production areas (USDA NASS 2011) are presented in Figs. 8-10. Note that the subtropical South Texas and Delta regions are at the southern extremes of the U.S. wheat and soybean production zones, respectively.

Stripe rust, stem rust and especially leaf rust, can be common in the wheat production regions east of the Rocky Mountains. Over the past 50 years, the first line of defense for controlling these diseases has been resistance in wheat cultivars. Durable rust resistance has been difficult to achieve due to genetic changes in the pathogens. As new resistant genotypes are introduced over time there is a mosaic of cultivars with different responses throughout the High Plains production region (Roelfs and Long 1987).

Fig. 8 ***Acres of winter wheat harvested by county for 2010. Map provided by the U.S. Department of Agriculture, National Agricultural Statistic Service (2.4 acres = 1 ha)***

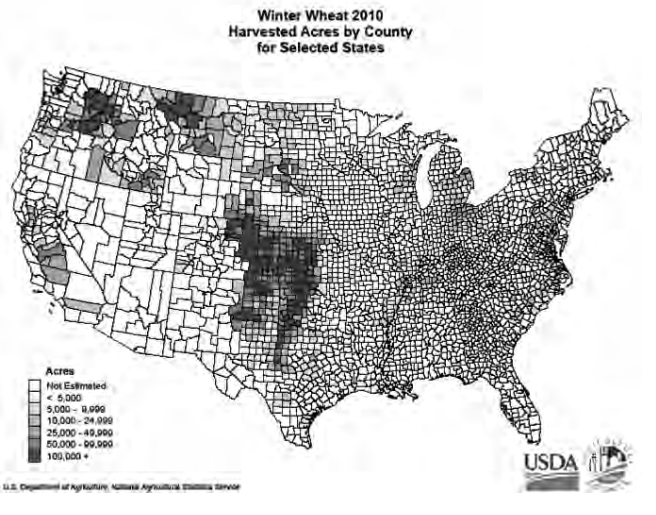


Fig. 9 ***Acres of soybean harvested by county for 2010. Map provided by the U.S. Department of Agriculture, National Agricultural Statistic Service (2.4 acres = 1 ha)***

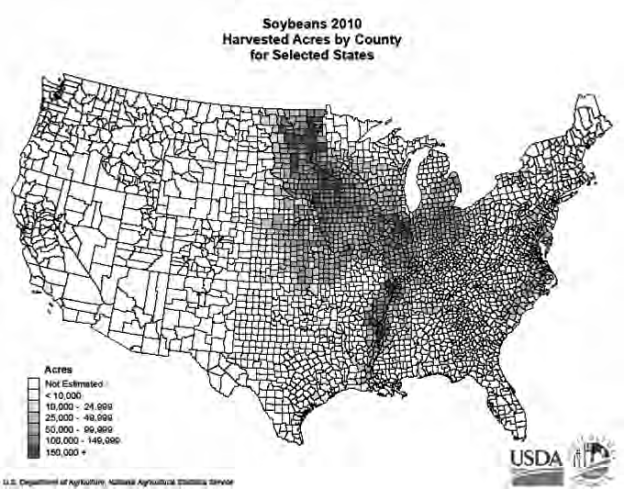
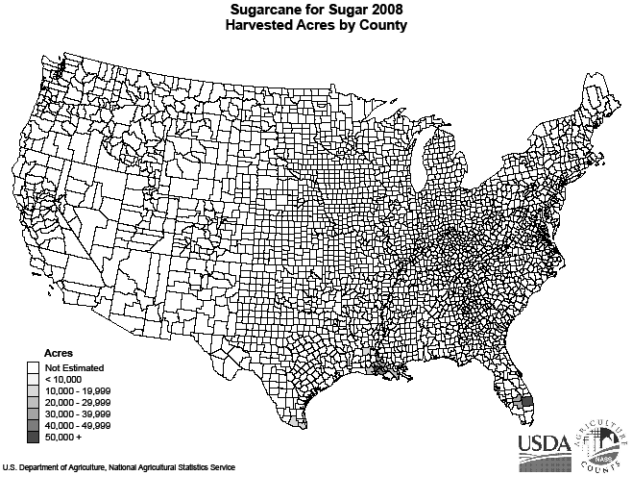


Fig. 10 ***Acres of sugarcane harvested by county for 2010. Map provided by the U.S. Department of Agriculture, National Agricultural Statistic Service (2.4 acres = 1 ha)***



In each growing season, wheat rust foci from overwintering uredinia are generally first identified in late February or early March in the South Texas region, and can become increasingly widespread as the season progresses especially when there is adequate precipitation (Roelfs 1989). By mid-April, winter wheat cultivars in the KS/NB region that are developed enough to serve as hosts, and have favorable environmental conditions, can become infected. Because large-scale southerly advections of air present opportunities for long-distance aerial transport are frequent at this time (Fig. 6), the risk of yield losses to wheat rust can be high. Opportunities for transport of wheat rusts directly from the South Texas region to the Dakota region occur less frequently than to the KS/NB region. However, since winter wheat in the Dakota region develops later than the KS/NB crop, susceptible winter wheat and spring-planted wheat cultivars can be at risk from rust spores transported from the nearby KS/NB region later in the growing season (e.g. step-wise range expansion).

Soybeans are planted in April and early May in the lower Mississippi River valley and during late April and May in the central U.S. Currently all soybean cultivars grown in the U.S. are susceptible to soybean rust (Dorrance et al. 2007). From 2005-2010, the first spring observations of rust on soybean in the Delta region occurred on June 28, 29, & 20, July 25, and June 5, & 30, respectively (USDA 2011). Opportunities for aerial transport of pathogens in strong low-level airflows from this area into the MO, MN/IA/WI/ON, IL/IN, and OH/MI soybean production regions are infrequent in late June, July, August, and early September (Fig. 7). Even with abundant precipitation and rapid inoculum build up in the Delta region's commercial crop, the risk of soybean yield losses in the continental interior production region due to rust is relatively low because of the dearth of potential long-distance spore transport events. Consequently in the absence of changes in atmospheric circulation or in pathogen/production systems, the risk of crop losses in major production regions of the North American continental interior from soybean rust is much less than for wheat rusts, especially leaf rust.

The frequencies of strong low-level advection of air and thus opportunities for aerial transport to the MN/IA/WI/ON and IL/IN regions from the Delta and Northeastern Texas source regions are shown in Figs. 11 and 12.

Fig. 11 ***Frequency of strong low-level airflows to the Minnesota/Iowa/Wisconsin/Southwestern Ontario region from the Delta and Northeastern Texas regions for two-week periods between April and September***

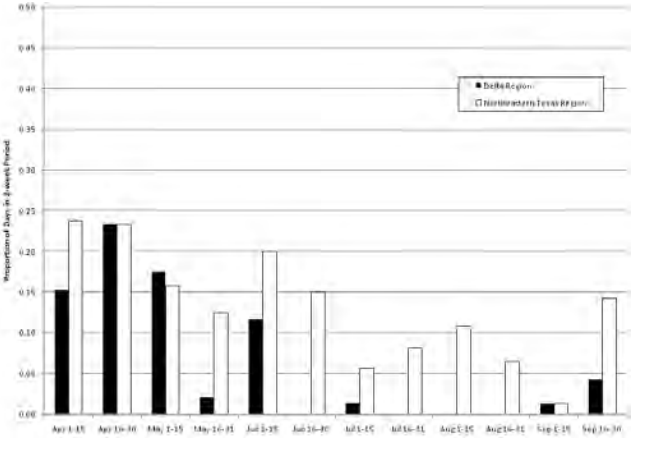
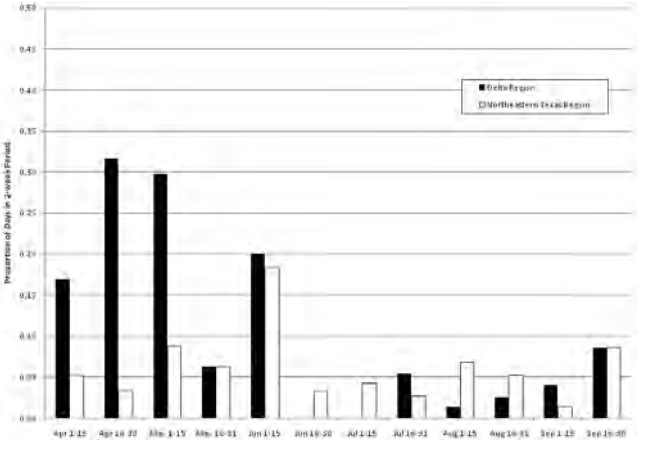
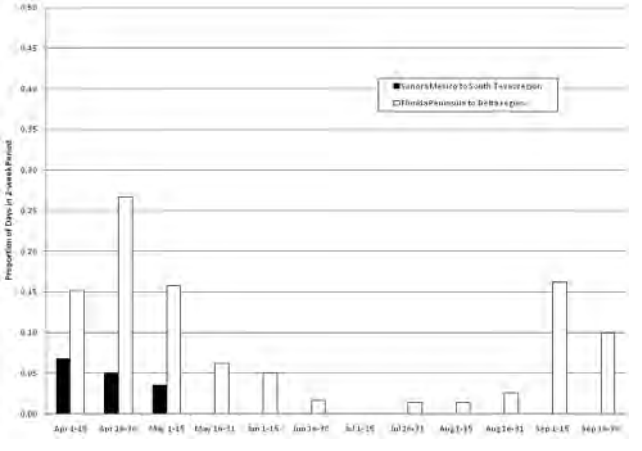


Fig. 12 ***Frequency of strong low-level airflows to the Illinois/Indiana region from the Delta and Northeastern Texas regions for two-week periods between April and September***



Although soybean production is much less in Northeastern Texas than in the Delta region, opportunities for spore transport northward from mid-June through mid-September are more frequent. This suggests that the risk of yield losses in the central U.S. soybean production region from soybean rust could be greater when the disease spreads into the Northeastern Texas region in the summer than when the pathogen is confined to the Delta region.

Fig. 13 ***Frequency of strong low-level airflows from the Sonora, Mexico, to South Texas region and from the Florida Peninsula to Delta region for two-week periods between April and September***



The frequencies of days with strong low-level airflows and thus opportunities for aerial transport of rust spores from the wheat production region in Sonora, Mexico, to the South Texas region and from the Florida Peninsula to the Delta region are shown in Fig. 13 for April-September.

Clearly opportunities for aerial transport of rust spores from Sonora to South Texas are infrequent. However, the number of potential aerial transport days peaks in April at the time when irrigated wheat in the Sonora region, if infected, could be an abundant source of wheat rust spores. At this time, susceptible wheat cultivars in the South Texas region are typically advanced enough in their development to be hosts where environmental conditions are conducive for infection and subsequent disease spread.

Orange sugarcane rust has been present in the Florida Peninsula region since 2007 (Comstock et al. 2008), but has yet to be identified in the Delta sugarcane production region. In 2010, an extensive survey of growers' fields in south Florida indicated that orange sugarcane rust spore production primarily occurs between July and September (N. Glynn pers comm). The pronounced spike in the frequency of days with strong low-level airflows and thus opportunities for aerial transport of spores from the Florida Peninsula to the Delta regions in September suggests the spread of this exotic pathogen into the Louisiana sugarcane crop is most likely to be a late season phenomenon (Fig. 13).

Summary

This study focused on assessing the risk of aerial long-distance transport of rust pathogens from potential source locations in the Eastern Hemisphere to the Western Hemisphere, and from subtropical to continental interior regions within North America. It asked two basic questions: (1) how frequently are there opportunities for rust pathogens to be transported from potential source locations to the destinations in the Western Hemisphere; and (2) how frequently are there opportunities for rust pathogens to be transported from potential source locations in subtropical regions to the continental interior of North America. The major findings of the study can be summarized as follows:

1. The potential for transport and deposition in the Western Hemisphere of viable rust spores that originate from source regions in the tropics of Africa, high latitudes in Europe, and throughout eastern Asia, is low.

2. The frequency of trans-oceanic transport and deposition of viable rust spores in the Western Hemisphere is relatively high for potential source locations poleward of the tropics in Africa. The relatively short distance from Western Africa (north of the Equator) to northeastern South America coupled with the presence of persistent Northeasterly Trade Winds create a pathway for frequent spore transport. Regions in the Western Hemisphere that are influenced by the ITCZ have the highest likelihood of receiving viable rust spores from the Eastern Hemisphere. The risk of aerial transport of viable rust spores to U.S. regions from source regions in the Eastern Hemisphere is low.
3. Strong low-level advection of air northward from the subtropics is prevalent in North America east of the Rocky Mountains from early April to mid May providing opportunities for long-distance transport of rust pathogens into the continental interior. After mid-June, the number of days with strong low-level advection of air from south to north across these regions that present opportunities for spore transport decreases dramatically.

Two methods are used in this study to provide a quantitative assessment of pathways for long-distance transport of rust pathogens, analyses of IAMS simulations and wind streamline maps. Modules for spore release and escape from the plant canopy, atmospheric dispersion, mortality due to exposure to UV radiation, and wet and dry deposition of spores are included in the IAMS simulations. Although the production of spores was assumed constant in time and equal for all source locations evaluated, the effects of the aerobiological processes captured in the other modules enabled assessment of trans-oceanic pathways for rust spore transport. Biological factors related to the pathogens and their hosts that influence the aerial spread of rusts are not included in the wind streamline analysis. Notwithstanding, the wind streamline analysis provides a useful tool for assessing spatial and temporal variations in opportunities for aerial movements of rust pathogens within the North American continent. Both types of analyses offer insights for deploying monitoring networks for rust pathogens.

References

Aylor DE (1999) Biophysical scaling and the passive dispersal of fungus spores: relationship to integrated pest management strategies. Agric and For Meteorol 97:275-292

Benninghoff WS, Edmonds RL (1972) Ecological systems approaches to aerobiology I. Identification of component elements and their function relationships. In: Benninghoff WS, Edmonds RL (eds) US/IBP Aerobiology Program Handbook Number 2. University of Michigan, Ann Arbor, MI, USA

Bowden J, Gregory PH, Johnson CG (1971) Possible wind transport of coffee leaf rust across the Atlantic Ocean. Nature 229:500-501

Chavarria E, Subiros F, Vega J, Galda G, Glynn NC, Comstock JC (2009) First report of orange sugarcane rust caused by *Puccinia kuehnii* in Cost Rica and Nicaragua. Plant Dis 93:425

Comstock JC, Sood SG, Glynn NC (2008) First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in the United States and Western hemisphere. Plant Dis 92:175

Dorrance AE, Hershman DE, Draper MA (2007) Economic importance of SBR. In: Dorrance AE, Draper MA, Hershman DE (eds) Using foliar fungicides to manage SBR. The Ohio State University, Wooster, OH, USA, pp11-19

Isard SA, Gage SH (2001) Flow of life in the atmosphere. Michigan State Press, East Lansing, MI, USA

Isard SA, Russo JM, Ariatti A (2007) Aerial transport of SBR spores into the Ohio River valley during September 2006. Aerobiologia 23:271-282

Isard SA, Russo JM, DeWolf ED (2006) The establishment of a national pest information platform for extension and education. Online, Plant Health Progress, doi:10.1094/PHP-2006-0915-01-RV. Retrieved 25 April 2011 from http://www.plantmanagementnetwork.org/pub/php/review/2006/platform/

Kalnay E, Kanamitsu M, Kistler R, Collins W, Deaven D, Gandin L, Iredell M, Saha S, White G, Woollen J, Zhu Y, Leetmaa A, Reynolds R, Chelliah M, Ebisuzaki W, HigginsW, Janowiak J, Mo KD, Ropelewski C, Wang J, Jenne R, Joseph D (1996) The NCEP/NCAR 40-year reanalysis project. Bull American Meteorological Soc 82:247-279

Kanamitsu M (1989) Description of the NMC global data assimilation and forecast system. Weather Forecasting 4:334-342

Kanamitsu M, Alpert JC, Campana KA, Caplan PM, Deaven DG, Iredell M, Katz B. Pan H-L, Sela J, White GH (1991) Recent changes implemented into the global forecast system at NMC. Weather Forecasting 6:425-435
Kanamitsu M, Ebisuzaki W, Wollen J, Yang S-K, Hnilo JJ, Fiorino M, Potter GL (2002) NCEP-DOE AMIP-II Reanalysis (R-2). Bull American Meteorological Soc 83:1631-1643
Morel W, Yorinori J (2002) Bol. Divulg. No. 44. Ministerio de Agricultura y Ganaderia, Centro Regional de Investigacion Agricola, Capitan Miranda, Paraguay
Nagarajan S, Singh DV (1990) Long-distance dispersion of rust pathogens. Annu Rev Phytopathology 28:139-153
Ovalle W, Comstock JC, Glynn NC (2008) First report of Puccinia kuehnii, causal agent of orange rust of sugarcane, in Guatemala. Plant Dis 92:973
Pedgley DE (1982) Windborne pests and diseases. Ellis Horwood Chichester, U.K.
Purdy LH, Krupa SV, Dean JL (1985) Introduction of sugarcane rust into the Americas and its spread to Florida. Plant Dis 69:689-693
Roelfs AP (1972) Gradients in horizontal dispersal of cereal rust uredospores. Phytopathology 62:70-76
Roefls AP (1989) Epidemiology of the cereal rusts in North America. Can J Plant Pathol 11:86-90
Roelfs AP, Long DL (1987) *Puccinia graminis* development in North America during 1986. Plant Dis 71:1089-1093
Roelfs AP, Martell LB (1984) Uredospore dispersal from a point source within a wheat canopy. Phytopathology 74:1262-1267
Stakeman EC, Harrar JG (1957) Principles of plant pathology. Ronald Press, New York, USA
USDA 2011. Integrated Pest Management – Pest Information Platform for Extension and Education. Retrieved 25 April 2011 from http://sbr.ipmpipe.org/cgi-bin/sbr/public.cgi
USDA National Agricultural Statistics Service. Retrieved 25 April 2011 from http://www.nass.usda.gov/

Puccinia graminis: Variation is the rule*

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For nearly a century, characterization of *Puccinia graminis* f. sp. *tritici* (*Pgt*) populations has been based on phenotypic race pathotyping. These studies have shown a wide range of diversity worldwide. In regions where the alternate host is lacking the population often have limited race diversity and are stable over long periods of time, with gradual step-like changes. Occasionally, dramatic race shifts occur, often a result of an introduction of a new race. In contrast, when the alternate host is present, local population are extremely diverse and major race shifts occur frequently. Limited genetic studies and recent genotyping using molecular markers have indicated that *Pgt* genome contains a high level of allelic variation between the two haploid genomes in this dikaryotic fungus. The recent sequencing of *Pgt* has shed new light on the complexity and diversity of the genome. The genome is much larger (88.6 Mb) than other sequenced fungal genomes and is a result of a massive expansion of transposable elements, which comprise approximately 45% of the genome. A similar genome expansion (101 Mb) driven by transposable elements was also observed in the poplar leaf rust pathogen, *Melampsora larici-populina* (*Mlp*). Although these two rust fungi are phylogenetically diverse, it was surprising that very little synteny exists between the two genomes indicating that massive genome rearrangement has occurred and may play an important role in the evolution of rust fungi. Re-sequencing using Next Generation technology demonstrated a very high rate of single nucleotide polymorphisms (SNPs) between the two nuclei within a cell (130,000 SNPs) and between isolates (240,000-479,000 SNPs). The frequency of SNPs in the *Pgt* genome is approximately twice that found in the *Mlp* genome. Using the genome sequence data, molecular markers bases on SNPs have been developed. SNP analysis of isolates of Ug99 and related races (Ug99 race group) has shown genetic diversity not only between isolates representing different races, but also isolates of the same race. In addition, SNP based markers have been used for the development of molecular diagnostic tools for rapid identification of the Ug99 race group. Genome analysis of *Pgt* and other rust fungi is providing new tools for analysis and insight into evolution of these important plant pathogens.

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Identification of the *Pgt* effectors that are recognized in rice*

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Abstract

Emergence of new strains of the wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* (*Pgt*), Ug99 and its relatives, threatens world wheat production; more than 80% of the wheat varieties currently being used world-wide are susceptible to these strains. Small wheat farmers in the developing world do not have the resources to use fungicides and rely entirely on host resistance. Therefore, rapid discovery and deployment of durable resistance (*R*) genes against *Pgt* Ug99 are urgent needs to protect such small farmers and wheat production in the developing world. It has been noted that rice is completely free of rust disease, including stem rust disease. This observation suggests that rice carries durable and broad-spectrum *R* gene(s) against *Pgt*. As rice and wheat are in the same taxonomic family, the chance that a rice *R* gene functions in wheat is expected to be high. The long-term goals of our research are: (i) to isolate multiple broad-spectrum *R* genes against *Pgt* from the non-host rice; and (ii) to elucidate the genetic architectures of non-host resistance in both rice and *Pgt*. The latter goal will enable a rational strategy of rice *R* gene deployment for durable *Pgt* resistance in wheat and for conservation of non-host resistance against *Pgt* in rice. In the short term, identification of the *Pgt* effector genes that are recognized in rice is required to achieve these goals. Here we discuss a strategy for rapid identification of such *Pgt* effecor genes.

Keywords

effectors; genetic architecture; hypersensitive response; non-host resistance; rice; *Puccinia graminis* f. sp. *tritici*; *R* gene-mediated resistance**;** transient expression; Ug99.

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Wheat stem rust

Puccinia graminis f. sp. *tritici* (*Pgt*) is the causal agent of stem rust of wheat and barley (Leonard and Szabo, 2005). Stem rust has plagued wheat production worldwide and has been the most feared pathogen of wheat due to its ability to devastate a healthy wheat field in less than a month (Roelfs, 1985). This fungal pathogen is easily spread by aerial transport of the urediniospores (asexual spores) over long distances (Christensen, 1942). Regular epidemics of stem rust in the late 1800's through the 1950's were major factors limiting wheat production. In response, Norman Borlaug and collaborators developed stem rust-resistant wheat varieties which, along with short stature, were responsible for the "Green Revolution". A key component of the rust resistance in these varieties of wheat was the 1BL.1RS translocation, which contains the stem rust resistance gene *Sr31*(McIntosh, 1998). The first *Pgt* strain (Ug99) able to defeat *Sr31* was an isolate from Uganda characterized in 1999 (Pretorius et al., 2000). In addition to *Sr31*, Ug99 can defeat over 25 additional *Sr* genes making it one of the most virulent *Pgt* strains known. Strains of *Pgt* are characterized as races based on a standard set of wheat differential lines; Ug99 is defined as race TTKSK (Jin et al., 2008). More than 80% of the currently cultivated wheat varieties worldwide are susceptible to Ug99. Ug99 has rapidly spread through Northeast Africa, the Arabian Peninsula and Iran, and currently threatens the major wheat production areas in India and Pakistan. Furthermore, strains very closely related to Ug99 that can break *Sr24* and *Sr36* (races TTKST and TTTSK, respectively) in addition to all the *Sr* genes broken by Ug99 were isolated in Kenya in 2006 and 2007 (Jin et al., 2008; Jin et al., 2009). This suggests that Ug99 and its relatives may rapidly evolve to break even more *Sr* gene-mediated resistances. Without strong control measures, an eventual pandemic of Ug99 and its relatives appears to be inevitable.

Farmers with resources have the option of using fungicides against Ug99 as a last resort. However, this is not an option for smallholder farmers in the developing world since it is too expensive. Thus, smallholder farmers rely entirely on genetic resistance to control wheat diseases. In addition, smallholder wheat farmers are heavily dependent on wheat for both food and the primary source of income. Therefore, spread of Ug99 will have severe impacts on lives of smallholder farmers. To protect smallholder wheat farmers in the developing world, durable and strong genetic resistance against Ug99 and its relatives is urgently needed. For resistance traits to be most useful, they should be easy to breed into many wheat varieties as smallholder farmers tend to grow local varieties. Thus, a single-locus trait in an elite cultivar would be ideal.

Non-host resistance and disease resistance (R) genes

For a microbe to be pathogenic on a particular species of plant, it must suppress basal inducible immunity of the plant, which is called pattern-triggered immunity as it is triggered based on recognition of molecular patterns derived from microbes (Jones and Dangl, 2006; Tsuda and Katagiri, 2010). For this purpose, pathogens adapted to the host plant deliver effectors, which are typically proteinaceous, into the plant cell. Effectors interfere with signaling for pattern-triggered immunity at various points (Block et al., 2008; Cunnac et al., 2009; Gohre and Robatzek, 2008). Plant R proteins directly or indirectly recognize such pathogen effectors, and the activation of an R protein rapidly induces strong immunity, called *R* gene-mediated resistance (Jones and Dangl, 2006). As it is triggered by recognition of effectors, *R* gene-mediated resistance is also called effector-triggered immunity (Jones and Dangl, 2006). The majority of isolated *R* genes encode proteins of the nucleotide-binding leucine-rich repeat (NB-LRR) class (Collier and Moffett, 2009).

When a pathogen cannot grow on any genotype of a particular plant species, the plant is said to be a non-host for the pathogen and to have non-host resistance. For example, rice is non-host to all the known *Pgt* strains and other cereal rust disease fungi (Ayliffe et al., 2008). The term non-host resistance is thus a phenomenologically-defined term and does not specify the molecular mechanisms underlying resistance. Different mechanisms appear to be primary mechanisms in non-host resistance for different combinations of plants and pathogens, and multiple layers of different mechanisms may be in place for some combinations. Some non-host resistance cases are associated with a hypersensitive response (HR)-like response, and others are not (type II and type I non-host resistance, respectively, defined in(Mysore and Ryu, 2004)). The HR is a hallmark of *R* gene-mediated resistance (Jones and Dangl, 2006). *R* gene-mediated resistance is probably the primary mechanism in type II, but not in type I, non-host resistance. Studies of interactions between non-host *Arabidopsis thaliana* and barley powdery mildew *Blumeria graminis* f. sp. *hordei* showed that resistance to fungal penetration of the plant cell is the primary mechanism in this non-host resistance (Collins et al., 2003). Thus, this is an example of type I. However, in mutant plants in which the penetrance resistance is impaired, the plant cell penetrated by the fungus undergoes an HR-like response, and resistance to *B. graminis* remains largely intact (Collins et al., 2003). This observation suggests that *R* gene-mediated resistance can be a backup mechanism for type I resistance.

Non-host resistance of rice to rust fungi

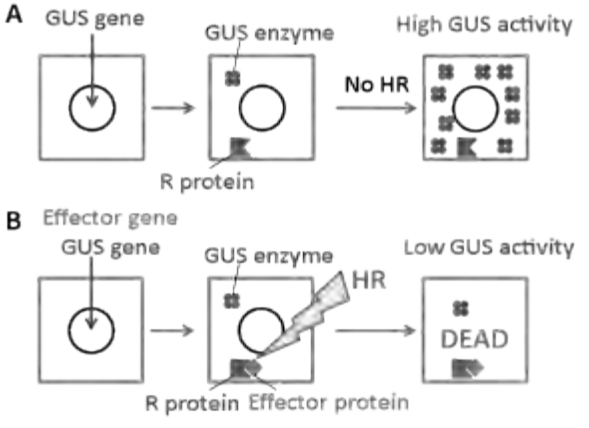
Since rice is a non-host to all the cereal rust diseases, rice must have durable and broad-spectrum genetic resistance against rust fungi. The nature of this resistance is not known. One potential explanation of the genetic resistance is that rice has durable and broad-spectrum *R* gene(s) against *Pgt*. If this is the case, it is important to know the genetic architectures of rice and rust fungi that enable such durable and broad-spectrum resistance. Does rice have one or a few *R* genes with broad spectra and/or many *R* genes with narrow spectra to collectively cover a broad spectrum? How many of the effector genes in a single strain of *Pgt* are recognized by a single rice variety? Does the list of the *Pgt* effector genes recognized vary substantially among rice varieties? What are the distributions of rust effector genes that are recognized by rice *R* genes among strains of *Pgt* and among species in the rust fungus class?

On a practical level, isolated rice *R* genes effective against *Pgt* would be valuable genetic resources for engineering wheat with *Pgt* resistance. As rice and wheat are in the same taxonomic family, the chance that a rice *R* gene can function in wheat when transgenically introduced is expected to be high. Multiple rice *R* genes could be stacked and transgenically transferred into wheat. The transgenic trait could then be bred into many wheat varieties. Gaining insights into the genetic architectures of *R* genes and effector genes for rice non-host resistance to *Pgt* will be important for deciding which *R* genes should be stacked for durable and broad-spectrum resistance in wheat and for evaluating the risk that *Pgt* would become virulent on rice if the rice *R* genes introduced into wheat should be defeated by *Pgt*.

Some techniques for detection of *R* gene functions

We invented a functional assay for *R* gene-mediated resistance using biolistic transient transformation in Arabidopsis (Leister et al., 1996; Mindrinos et al., 1994). When a pathogen effector gene is transiently expressed in a plant cell expressing the corresponding *R* gene, the plant cell undergoes HR cell death. Co-transformation with a reporter gene, such as the β–glucuronidase (GUS) gene driven by a constitutive promoter, can report cell death. If an HR occurs, the cell dies rapidly and only a low level of the reporter product can be accumulated; if an HR does not occur, the long living cell accumulates a high level of the reporter product (Fig. 1).

Fig. 1 ***Principle of the transient assay for R gene-effector gene interactions. (A) When only the GUS reporter gene is transiently expressed, high GUS activity is observed. (B) When an effector gene is expressed together with the GUS gene and the effector is recognized by an R protein, the HR occurs. Consequently, the cell does not accumulate much GUS enzyme.***



We later made the assay quantitative by including normalization biolistics with a second reporter to normalize variation in the bombardment-to-bombardment transformation efficiency (Leister et al., 1996). We also showed that an assay based on the same principle can be performed using protoplast transient transformation in Arabidopsis (Leister and Katagiri, 2000). Since Jia et al. (Jia et al., 2000) and Yoshida et al. (Yoshida et al., 2009) demonstrated that these assays can be applied to rice *R* genes and the cognate rice blast fungal effector genes, using biolistic and protoplast transient expression, respectively, the transient expression assays can be used to look for pathogen effectors that are recognized by *R* genes in rice.

To identify *R* genes by loss-of-function mutations, it is important to isolate a single *R* gene-effector gene interaction in the plant-pathogen system because *R* genes are typically co-dominant. For example, if two combinations of *R* and effector genes, *R1*-effector 1 and *R2*-effector 2, are functional in a system, knocking out *R1* will not lead to loss of the resistance response since the *R2*-effector 2 combination is still functional. If we can make only the *R1*-effector 1 combination functional, an *R1* knock-out mutation will result in loss of the resistance response. This can be achieved by using only effector 1 in the screen instead of an intact pathogen that delivers both effectors 1 and 2. Therefore, isolating effector genes from the pathogen of interest provides an opportunity to conduct *R* gene mutant screens even when the plant host has multiple *R* genes corresponding to multiple effector genes of the pathogen.

The long-term strategy of research

Our long-term goals are to isolate rice *R* genes that are effective against *Pgt* and elucidate the genetic architecture of rice-*Pgt* non-host resistance interactions. For efficient isolation of rice *R* genes, it is important to identify each of the *Pgt* effector genes that are recognized by the rice *R*-gene system in isolation from other *Pgt* effector genes. First, *Pgt* effector gene candidates will be bioinformatically identified from the genome sequences of *Pgt* strains. These candidates will be systematically screened for their ability to induce an HR-like response in rice when they are expressed or delivered in the rice cell. The effector candidate genes can be transiently expressed in rice using biolistic or protoplast transformation as described in the previous section. Such transient expression might also be achieved by use of a viral vector (Rentel et al., 2008) although a rice viral vector appropriate for transient expression has not been established. Alternatively, the effector candidate proteins may be delivered from a bacterial strain utilizing the type III secretion system of phytopathogenic bacteria (Rentel et al., 2008; Sohn et al., 2007; Thomas et al., 2009). To gain a fairly global view of the genetic architecture, different combinations of rice varieties and *Pgt* strains should be used. Therefore, it is important to use assay methods that can provide a reasonable throughput.

Once recognized *Pgt* effector genes are identified, they will be used to search for the corresponding *R* genes in rice. Loss-of-function and gain-of-function screens will be considered. Mutagenized rice populations will be screened for loss of effector recognition using transient expression or type III-delivery of each of the identified *Pgt* effectors. Once such loss-of-function rice mutants are identified, the range of possible candidates for the *R* genes that carry the mutations will be rapidly narrowed by deep-sequencing of backcrossed populations. The advantage of the loss-of-function approach is that we do not need to assume any particular gene types for the *R* genes. A gain-of-function screen can be performed using transient co-expression of a rice *R* gene candidate and one of the identified *Pgt* effector genes in a susceptible wheat variety. We used such an assay with transient co-expression of an *R* gene and the cognate effector gene in analysis of interactions between Arabidopsis

R genes and *P. syrinage* effector genes (Leister et al., 1996; Leister and Katagiri, 2000; Tao et al., 2000). We consider that biolistic transient expression is relatively adaptable to different plant species/varieties. To make the screen realistic, it will be necessary to limit the number of *R* gene candidates to screen to a small number. Since the majority of known *R* genes belong to the NB-LRR class (Collier and Moffett, 2009) and since the number of NB-LRR genes in rice is about 500 (Monosi et al., 2004), limiting *R* gene candidates to the NB-LRR genes appears to be a reasonable strategy. While the types of rice genes to be screened will be limited, the advantage of a gain-of-function approach is that *R* genes can be detected even if the rice variety used has multiple functionally redundant *R* genes. In addition, detection in this screen directly indicates that the rice *R* gene is functional as a single isolated gene in wheat for recognition of the effector and induction of the HR. Therefore, the chance that the rice *R* gene will be fully functional in wheat when transgenically transferred is high.

Provided that we capture the majority of rice R protein-*Pgt* effector combinations (assuming that there are many), surveying the distributions of the *R* genes in rice varieties and of the effector genes in *Pgt* strains will elucidate the genetic architecture underlying the non-host resistance. In this way, we will learn the genetic architecture that enables durable, strong, and broad-spectrum disease resistance. With this type of knowledge, it will be feasible to form a rational strategy for deployment of rice *R* genes in wheat to avoid breaking down the rice non-host resistance if the deployed rice *R* genes should be defeated by *Pgt* in wheat.

Identification of *Pgt* effector gene candidates

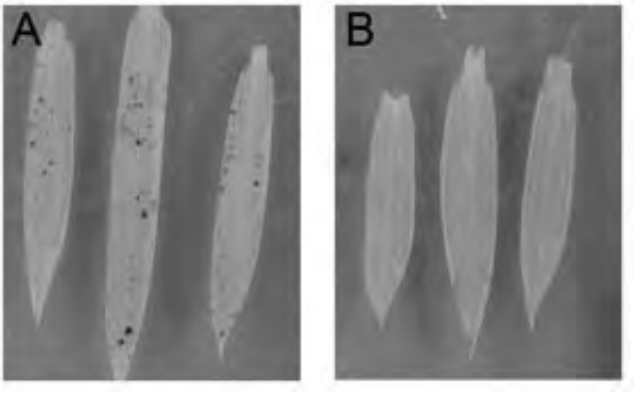
The genome assembly and predicted gene models for the *Pgt* reference strain CRL 75-36-700-3 (hereafter strain 7a) has been made public through the Broad Institute website (http://www.broadinstitute.org/annotation/genome/puccinia_group/MultiHome.html), and the sequence reads are available from the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra). The current genome assembly is composed of 392 scaffolds totaling 88.6 Mb. The annotated genome contains 18,241 predicted protein-coding genes with 44% of these proteins showing significant similarity to documented proteins (Duplessis et al.).

Studies of potential effector genes in fungi and oomycetes indicated that most effectors are small secreted proteins (Dodds et al., 2009; Kamoun, 2007). The predicted encoded proteins of *Pgt* 7a were filtered for small secreted proteins as follows: a) SignalP (Emanuelsson et al., 2007) with an HMM signal probability of at least 0.9; b) TargetP (Emanuelsson et al., 2007) to filter out potential mitochondrial-targeted proteins; c) TMHMM (Emanuelsson et al., 2007) to filter out potential transmembrane proteins; d) big-PI (Eisenhaber et al., 1999) to filter out proteins with GPI-anchor sites; and e) proteins 300 amino acids or smaller. This filter yielded 1,386 predicted secreted small protein genes. Using the Nimblegen microarray designed for mRNA profiling of *Pgt* 7a, 120 genes were selected among the 1,386 predicted secreted small protein genes that are highly expressed during infection of both wheat and barley but are not detectable in either ungerminated or germinated urediniospores. A cysteine-knot protein, which belongs to a class of known fungal effectors (Catanzariti et al., 2006), was manually added to the selected genes.

We attempted PCR-cloning of the 121 effector gene candidates from a cDNA library made from *Pgt* 7a-infected wheat leaves. In the process, we found two common types of error in the *Pgt* 7a gene models: exon-intron predictions are incorrect; and highly homologous genes are often combined together into a single gene model by automated genome sequence assembly. With the high heterozygosity of *Pgt*, alleles of a single gene, as well as gene family members, could contribute to this assembly issue. We have successfully cloned 53 effector gene candidates, of which 18 had exon-intron structures different from the model. Each of the effector gene candidates were cloned in a Gateway entry vector such that its 5′-end has an artificially introduced start codon immediately preceding the coding sequence of the mature protein predicted by SignalP. The cloned effector gene candidates were moved into a Gateway destination vector for transient expression, pUNos, which drives expression of the inserted gene with the maize ubiquitin promoter (Christensen and Quail, 1996).

The 53 effector gene candidates were screened using the biolistic transient expression assay in rice: each of the effector genes was co-bombarded with pUNos-GUS into rice seedlings, and the seedlings were histochemically stained for GUS activity with 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-Gluc). We identified five potentially positive *Pgt* effector genes by this method (Fig. 2).

Fig. 2 ***Representative results of the biolistic assay using GUS as the reporter. (A) A negative control, GFP. (B) One of the five positives identified. All the five positives identified gave results similar to this one. The other 48 effector genes screened gave results similar to GFP.***



Thus, the transient expression assay can be used to rapidly discover the *Pgt* effector genes potentially recognized in rice by *R* gene-mediated recognition mechanisms.

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References

Ayliffe, M., Singh, R., and Lagudah, E. (2008). Durable resistance to wheat stem rust needed. Current opinion in plant biology *11*, 187-192.

Block, A., Li, G., Fu, Z. Q., and Alfano, J. R. (2008). Phytopathogen type III effector weaponry and their plant targets. Curr Opin Plant Biol *11*, 396-403.

Catanzariti, A., Dodds, P., Lawrence, G., Ayliffe, M., and Ellis, J. (2006). Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. The Plant Cell Online *18*, 243.

Christensen, A., and Quail, P. (1996). Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants. Transgenic research *5*, 213-218.

Christensen, J. J. (1942). Long distance dissemination of plant pathogens. In Aerobiology, F. R. Moullon, ed. (Washington D. C., Am Assoc Adv Sci), pp. 78-87.

Collier, S. M., and Moffett, P. (2009). NB-LRRs work a "bait and switch" on pathogens. Trends Plant Sci *14*, 521-529.

Collins, N. C., Thordal-Christensen, H., Lipka, V., Bau, S., Kombrink, E., Qiu, J. L., Huckelhoven, R., Stein, M., Freialdenhoven, A., Somerville, S. C., and Schulze-Lefert, P. (2003). SNARE-protein-mediated disease resistance at the plant cell wall. Nature *425*, 973-977.

Cunnac, S., Lindeberg, M., and Collmer, A. (2009). Pseudomonas syringae type III secretion system effectors: repertoires in search of functions. Curr Opin Microbiol *12*, 53-60.

Dodds, P., Rafiqi, M., Gan, P., Hardham, A., Jones, D., and Ellis, J. (2009). Effectors of biotrophic fungi and oomycetes: pathogenicity factors and triggers of host resistance. New Phytologist *183*, 993-1000.

Duplessis, S., Cuomo, C. A., Lin, Y.-C., Aerts, A., Tisserant, E., Veneault-Fourrey, C., Joly, D. L., Hacquard, S., Amselem, J., Cantarel, B.*, et al.* (2011). Obligate Biotrophy Features Unraveled by the Genomic Analysis of the Rust Fungi, *Melampsora larici-populina* and *Puccinia graminis* f. sp. *tritici*. Proc Natl Acad Sci U S A, in press.

Eisenhaber, B., Bork, P., and Eisenhaber, F. (1999). Prediction of potential GPI-modification sites in proprotein sequences. Journal of molecular biology *292*, 741-758.

Emanuelsson, O., Brunak, S., von Heijne, G., and Nielsen, H. (2007). Locating proteins in the cell using TargetP, SignalP and related tools. Nature protocols *2*, 953-971.

Gohre, V., and Robatzek, S. (2008). Breaking the barriers: microbial effector molecules subvert plant immunity. Annu Rev Phytopathol *46*, 189-215.

Jia, Y., McAdams, S. A., Bryan, G. T., Hershey, H. P., and Valent, B. (2000). Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. Embo J *19*, 4004-4014.

Jin, Y., Szabo, L. J., Pretorius, Z. A., Singh, R. P., Ward, R., and Fetch, T., Jr. (2008). Detection of virulence to resistance gene Sr24 within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis *92*, 923-926.

Jin, Y., Szabo, L. J., Rouse, M. N., Fetch, T., Jr., Pretorius, Z. A., Wanyera, R., and Njau, P. (2009). Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. Plant Dis *93*, 367-370.

Jones, J. D., and Dangl, J. L. (2006). The plant immune system. Nature *444*, 323-329.

Kamoun, S. (2007). Groovy times: filamentous pathogen effectors revealed. Current opinion in plant biology *10*, 358-365.

Leister, R. T., Ausubel, F. M., and Katagiri, F. (1996). Molecular recognition of pathogen attack occurs inside of plant cells in plant disease resistance specified by

the Arabidopsis genes RPS2 and RPM1. Proc Natl Acad Sci U S A *93*, 15497-15502.
Leister, R. T., and Katagiri, F. (2000). A resistance gene product of the nucleotide binding site -- leucine rich repeats class can form a complex with bacterial avirulence proteins in vivo. Plant J *22*, 345-354.
Leonard, K. J., and Szabo, L. J. (2005). Stem rust of small grains and grasses caused by Puccinia graminis. Mol Plant Pathol *6*, 99-111.
McIntosh, R. (1998). Catalogue of gene symbols for wheat, Citeseer).
Mindrinos, M., Katagiri, F., Yu, G. L., and Ausubel, F. M. (1994). The A. thaliana disease resistance gene RPS2 encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell *78*, 1089-1099.
Monosi, B., Wisser, R., Pennill, L., and Hulbert, S. (2004). Full-genome analysis of resistance gene homologues in rice. TAG Theoretical and Applied Genetics *109*, 1434-1447.
Mysore, K. S., and Ryu, C. M. (2004). Nonhost resistance: how much do we know? Trends Plant Sci *9*, 97-104.
Pretorius, Z. A., Singh, R. P., Wagoire, W. W., and Payne, T. S. (2000). Detection of Virulence to Wheat Stem Rust Resistance Gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Disease *84*, 203.
Rentel, M. C., Leonelli, L., Dahlbeck, D., Zhao, B., and Staskawicz, B. J. (2008). Recognition of the Hyaloperonospora parasitica effector ATR13 triggers resistance against oomycete, bacterial, and viral pathogens. Proceedings of the National Academy of Sciences *105*, 1091.
Roelfs, A. P. (1985). Race specificity and methods of study. In The Cereal Rusts, W. R. Bushnell, and A. P. Roelfs, eds. (Orlando, FL, Academic), pp. 1565-1592.
Sohn, K. H., Lei, R., Nemri, A., and Jones, J. D. (2007). The downy mildew effector proteins ATR1 and ATR13 promote disease susceptibility in Arabidopsis thaliana. Plant Cell *19*, 4077-4090.
Tao, Y., Yuan, F., Leister, R. T., Ausubel, F. M., and Katagiri, F. (2000). Mutational analysis of the Arabidopsis nucleotide binding site-leucine-rich repeat resistance gene RPS2. Plant Cell *12*, 2541-2554.
Thomas, W. J., Thireault, C. A., Kimbrel, J. A., and Chang, J. H. (2009). Recombineering and stable integration of the Pseudomonas syringae pv. syringae 61 hrp/hrc cluster into the genome of the soil bacterium Pseudomonas fluorescens Pf0-1. Plant J *60*, 919-928.
Tsuda, K., and Katagiri, F. (2010). Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. Curr Opin Plant Biol *13*, 459-465.
Yoshida, K., Saitoh, H., Fujisawa, S., Kanzaki, H., Matsumura, H., Tosa, Y., Chuma, I., Takano, Y., Win, J., Kamoun, S., and Terauchi, R. (2009). Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen Magnaporthe oryzae. Plant Cell *21*, 1573-1591.

Diversity of yellow rust resistance: Inferences from association mapping analysis of elite wheat germplasm

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Abstract

Yellow rust (caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*)) in wheat continues to be responsible for substantial economic losses globally and to represent a major threat to food security in many developing countries. This was exacerbated by the recent loss of *Yr27* resistance in certain mega-varieties. With recent advances in gene technology, and increasing impetus to exploit natural diversity, we employed a genome-wide association study (GWAS) to identify the genes and genomic regions associated with quantitative trait loci for resistance to yellow rust in a collection of 197 elite wheat germplasms. The lines were phenotyped for both seedling and adult plant response and genotyped using the Diversity Array Technology (DArT®) marker system in addition to SSR and STS markers previously reported to be linked to yellow rust resistance in wheat. The GWAS study was carried out using a general linear model that accounted for population structure, and a mixed linear model that accounted for both population structure and relatedness among lines. Twenty one QTLs were identified for yellow rust resistance and were located on chromosomes 1AS, 1BS, 1DL, 2AL, 2BS, 2DS, 3AL, 3BS, 3D, 4AL, 4DL, 5AL, 5B, 6AS, 6BS, 6DL, 7AS, 7BL, and 7DS, each explaining from 3 to 15% of the phenotypic variation at P≤0.05. Some of the identified loci were located close to previously identified genes; other DArT markers were associated with previously uncharacterized yellow rust resistance genes on chromosomes 1DL, 4AL, 5B, 6B, 7AS, 7BL. The DArT haplotypes associated with yellow rust resistance in the germplasm suggest the prevalence of many yellow rust resistance genes in ICARDA's germplasm, including *Yr9* associated with the 1BL.1RS translocation*, Yr10, Yr15, Yr24* (1BS), the dual APR genes *Lr34*/*Yr18*/*Pm38* (7DS) and *Lr46*/*Yr29* (1BL)*, Yr30* (3BS), the defeated *Yr27* gene*, Yr31* (2BS), *Yr5* and *Yr7* (2BL). Some of the germplasms with a combination of loci consisting of major and minor effects appeared to have higher levels of yellow rust resistance. These constitute valuable gene sources for the transfer of durable yellow rust resistance into locally adapted wheat cultivars. This information will be useful in choosing parents for crossing and pyramiding yellow rust resistance genes in wheat.

Keywords

linkage disequilibrium, marker assisted selection, population structure*, Puccinia striiformis, Triticum aestivum,* yellow rust resistance.

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Mapping of durable adult plant stem rust resistance in six CIMMYT wheats to Ug99 group of races*

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Abstract

Durable resistance to wheat stem rust fungus can be achieved by developing and deploying varieties that have race-nonspecific, adult plant resistance (APR) conferred by multiple minor, slow rusting genes. Wheat lines 'Kingbird, 'Kiritati', 'Huirivis#1', 'Juchi', 'Muu' and 'Pavon 76' showed high levels of APR to Ug99 races of stem rust fungus when tested in Kenya. The F5 and F6 generation recombinant inbred line (RIL) populations developed from the crosses of moderately susceptible 'PBW343' with five resistant parents were used in mapping. The non-*Sr26* fraction of the 'Avocet' x Pavon 76 RIL population, developed earlier for leaf rust and stripe rust resistance studies, was also included. Field phenotyping of the parents and RILs were conducted at Njoro, Kenya for at least two years with Ug99+*Sr24* (TTKST) race under high stem rust pressures. The continuous variation of APR in each RIL population and genetic analyses indicated quantitative nature of resistance that was likely governed by 3 or 4 minor genes. Single and joint year analyses by Inclusive Composite Interval Mapping (ICIM) using informative DArT and/or SSR markers identified consistent APR QTLs on chromosomes 1AL, 3BS, 5BL, 7A and 7DS in Kingbird; 2D, 3BS, 5BL and 7DS in Kiritati; 2B, 3BS, 4A, 5BL and 6B in Juchi; 2B, 3BS, 7B in Huirivis#1; 2B, 3BS and 5BL in Muu; and 1BL, 3BS, 5A and 6B in Pavon 76. QTLs on each genomic regions explained 10- 46% of the phenotypic variation for APR. Pseudo-black chaff phenotype associated with APR gene *Sr2* on chromosome 3BS in all six resistant parents and identification of an APR QTL in the same region in all mapping populations confirmed the role of *Sr2* in reducing stem rust severity. The 1BL QTL in Pavon 76 was in the same region where pleiotropic APR gene *Lr46/Yr29/Pm39* is located. Similarly a 7DS QTL in Kingbird and Huirivis#1 was in the chromosomal region where pleiotropic APR gene *Lr34/Yr18/Pm38* is located. These results indicate that the above two pleiotropic resistance genes confer APR to stem rust in addition to leaf rust, yellow rust and powdery mildew. Further studies are underway to saturate the genomic regions harboring new APR QTLs with additional molecular markers.

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*This is an unedited original draft. A revised version will appear in the workshop proceedings published later this year.

Key words:

Black rust, DArT, durable resistance, molecular mapping, *Puccnia graminis*, *Triticum aestivum*

Introduction

Stem rust, caused by fungus *Puccinia graminis* f. sp. *tritici* (Pgt), is an important disease of wheat and was historically a major problem in Africa, the Middle East, Asia (except Central Asia), Australia, New Zealand, Europe, and both the Americas (Saari and Prescott 1985). Although the last major stem rust epidemics occurred in Ethiopia during 1993 and 1994 (Shank 1994) when a popular wheat variety 'Enkoy' suffered major losses, the rest of the world practically remained unaffected from stem rust for over three decades (Singh et al 2008). The widespread deployment of the rye-derived stem rust resistance gene *Sr31*, located on 1BL.1RS translocation, in the 19th century also contributed to stem rust control globally for several years. However, *Sr31* virulent race Ug99 (TTKSK) was first identified in 1998 in Uganda (Pretorius et al. 2000) and is now spread to various other countries. Of the 50 stem rust resistance (*Sr*) genes characterized in wheat, only a few are effective against Ug99 (Singh et al. 2006, 2008). Some of these effective resistance genes are associated with undesirable effects on agronomic traits (McIntosh et al. 1995). Recent detections of new variants of Ug99 with virulence to gene *Sr24* and *Sr36* reflect that the pathogen is continuously evolving.

Resistance to rust in wheat can be classified into two broad categories and are referred to as seedling and adult plant resistance (APR). Seedling resistance genes are usually provide resistance at all stages of plant growth. In contrast APR is commonly detected in post-seedling stages. Race specificity is more common with the seedling resistance genes and pathogen usually evolves to overcome them in few years leading to "boom and bust cycles" (Parlevliet 2002).

The APR genes are usually race non-specific, confer partial resistance and are associated with a slow rusting phenotype (Caldwell 1968). Slow rusting resistance is associated with longer latent periods, fewer and smaller uredinia, and reduced spore production when compared to susceptible checks. Accumulation of 4 to 5 minor genes is often expected to retard disease progress to rates that result in negligible disease levels at maturity under high disease pressure, described as "near-immunity" by Singh et al. (2000).

Combinations of effective race specific resistance genes, accumulating multiple minor APR genes, or a combination of both major and APR genes can lead to long-term effectiveness of host resistance. *Sr2* is the only catalogued APR gene and has provided durable APR for more than 30 years (McIntosh et al. 1995). *Sr2* confers slow rusting (Sunderwirth and Roelfs 1980) and is associated with the pseudo black chaff (PBC) trait (Hare and McIntosh, 1979). Combination of *Sr2* with other unknown slow rusting resistance genes possibly originating from Thatcher and Chris, commonly known as the "*Sr2*-Complex," provided the foundation for durable resistance worldwide (McIntosh 1988, Rajaram et al. 1988). Unfortunately, not much is known about the other genes in the "*Sr2*-complex" and their interactions.

Combining several minor genes by traditional breeding is cumbersome, needs large populations and is time consuming. Molecular marker techniques provide powerful tools to characterize quantitative traits such as slow rusting. Different marker platforms are used for genotyping of which DArT (Diversity Array technology, http://www.diversityarray.com) has gained significant importance as it is high-throughput and produces data at low costs compared to other available techniques (Kilian et al. 2005, Akbari et al. 2006). A number of statistical methods have been developed for QTL detection and estimation of effects. Inclusive Composite Interval Mapping (ICIM) considers marker variables in a linear model for additive mapping, and both marker variables and marker-pair multiplications are simultaneously considered for epistasis mapping (Huihui et al. 2007). ICIM increases detection power, reduces false detection rate and biased estimates of QTL effects compared to CIM in additive mapping (Li et al. 2007).

The CIMMYT-derived wheat lines 'Kingbird', 'Kiritati', 'Juchi', 'Huirivis#1', 'Muu' and 'Pavon 76' were found to be susceptible as seedlings to Ug99 and had good levels of APR when tested in Kenya and Ethiopia with Ug99. They also displayed pseudo-black chaff phenotype indicating the presence of *Sr2*. We summarize the results of various studies conducted to identify genomic regions that contribute to APR to stem rust resistance through QTLmapping.

Materials and Methods

Plant materials and APR phenotyping

Seedlings of the APR parents Kingbird, Kiritati, Huirivis#1, Juchi, Muu and Pavon 76 were susceptible to Ug99 and derivative races however adult plants showed low disease severity to Ug99 in field trials in Kenya since 2006 (Njau et al. 2010). Mapping populations were developed from crosses of the resistant parents with the moderately susceptible parent 'PBW343', except for Pavon 76 where the second parent was 'Avocet'. The F5 or F6 generations recombinant inbred lines (RILs) were field tested at the Kenyan Agricultural Research Institute (KARI), Njoro during 2009 and 2010 in two replicates at different planting dates. RILs that lacked resistance gene *Sr26* in Avocet x Pavon population were used in our study and were tested in 2007, 2009 and 2010. The stem rust responses of parents and RILs were assessed in field plots comprising two 70 cm long rows spaced 20 cm apart. To facilitate uniform disease build-up within the nursery, continuous stem rust spreader rows (mixture of *Sr31* and *Sr24* susceptible genotypes) were planted perpendicular to all entries on one side of plots in the middle of alleys and around the field. A suspension of freshly collected urediniospores of race Ug99+*Sr24* (TTKST) suspended in distilled water was injected twice in spreader plants (1-3 plants/m) just prior to booting (growth stage Z35–Z37; Zadoks et al. 1974) using a hypodermic syringe. Final disease severity responses were assessed using the modified Cobb Scale (Peterson et al. 1948) when the susceptible controls displayed 80- 100% stem rust severity about the soft-dough to mid-dough stages of plant growth.

Genetic analysis

χ^2 analyses were performed to check the goodness-of-fit of observed segregations with the expected genetic ratios of 2, 3 and 4 genes, respectively in each mapping populations. The RIL populations were classified into a resistant and a susceptible group based on the disease severity. All the non segregating families that scored between 5 and 55% severity were grouped in resistant category whereas families scoring 55% and higher were classified as susceptible.

Characterization of pseudo black chaff phenotype

Pseudo Black chaff (PBC) is a dark pigmentation developing on the glumes and internodes due to the accumulation of melanin and is linked to stem rust resistance gene *Sr2*. Kingbird and Hurivis#1 populations were scored on a 0-4 scale for PBC expression (0= no pigmentation, 4= high pigmentation) during the main season of 2010. Our study was conducted to identify the regions controlling PBC expression in Kingbird and Hurivis#1.

Genotyping

Diversity Arrays Technology (DArT)

For DArT assays, 500-1000 ng of restriction grade DNA, suspended in TE with a final concentration of 50-100 ng/µL were sent to Triticarte Pty. Ltd., Canberra, Australia (www.triticarte.com.au**)** for whole genome profiling (Wenzl et al. 2006, Neumann et al. 2010). Loci were scored as present (1) or absent (0). DArT marker names have the prefix '*XwPt*' and the number corresponding to the particular clone in the genomic representation, where w stands for wheat, P for *Pst*I (primary restriction enzyme) and T for *Taq*I (secondary restriction enzyme). The overall call rate for both populations was ~95% and the Q (estimate of marker quality) value for most markers was above 80%. The markers were named starting with 'w' if the clone was from wheat,'t' if it was from triticale, and 'r' if it was from rye libraries, respectively.

SSR and PCR based markers

SSR genotyping of Kiritati and Huirivis#1 population was conducted at Syngenta seeds, France as part of CIMMYT-Syngenta foundation collaborative research. Two hundred and seventy markers were used for mapping studies which included SSR (simple sequence repeats) and markers licensed to or owned by Syngenta.

Linkage mapping and QTL analysis

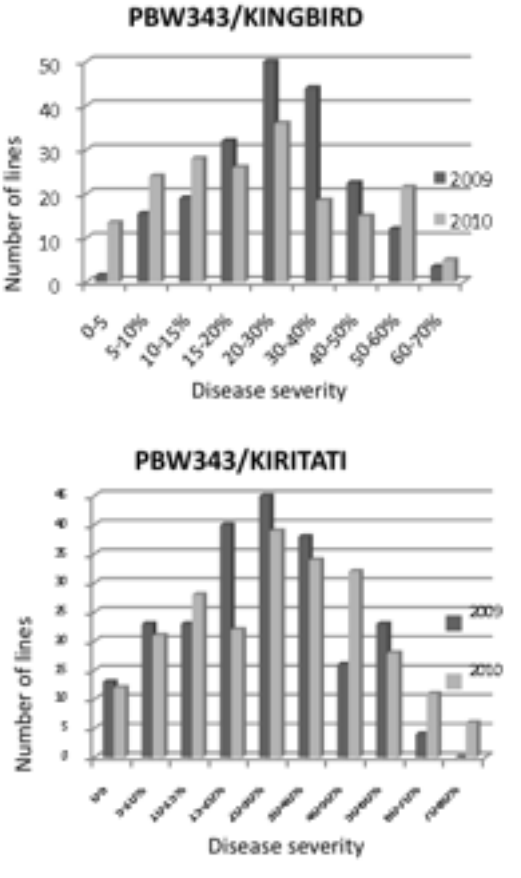
Mapping software ICIM (Li et al. 2007) was used for constructing linkage maps and QTL mapping of Kingbird, Juchi, Muu and Pavon populations, whereas Kiritati and Huirivis#1 populations were analyzed using QTL Cartographer and ICIM. ICIM is an efficient method for additive and epistasis mapping and QTL epistatic networks can be identified no matter whether the two QTLs have any additive effects (Li et al. 2008). In ICIM, marker selection is conducted through stepwise regression by considering all marker information simultaneously, and the phenotypic values are then adjusted by all markers retained in the regression equation except the two markers flanking the current mapping interval. The adjusted phenotypic values are finally used in interval mapping until the testing position moves into a new interval. A LOD score of 2.5 was set as a threshold for declaring the presence of QTL and probability in stepwise regression at 0.001.

Results

Evaluation at adult plant stage and inheritance studies

The mean disease severity for the resistant parents was 7.5 - 20% while that of PBW343 was 65-70%. The disease severity of the RILs over the years was continuously distributed (Figure 1) and differed significantly ($p<0.001$). The identification of RILs with stem rust responses lower and higher than the two parents in each cross indicated transgressive segregation and presence of different APR genes in the parents (Figure 1). The heritability (h^2) for rust severity ranged between 0.67 and 0.8. The correlation coefficient (r) for disease severity in Kingbird population ranged from 0.45 to 0.84 and for disease severity and PBC ranged from -0.28 to -0.60 (Table 1). Results obtained from the correlation analysis indicated that there was significant relationship and reliability between severities responses of RILs observed over environments. However, the lower correlation with 2009-off-season data was likely due to the low disease pressure and poor plant establishment caused by very dry conditions during the season.

Fig. 1 ***Frequency distribution of RILs in six mapping populations for adult plant stem rust severity observed during two or three years***



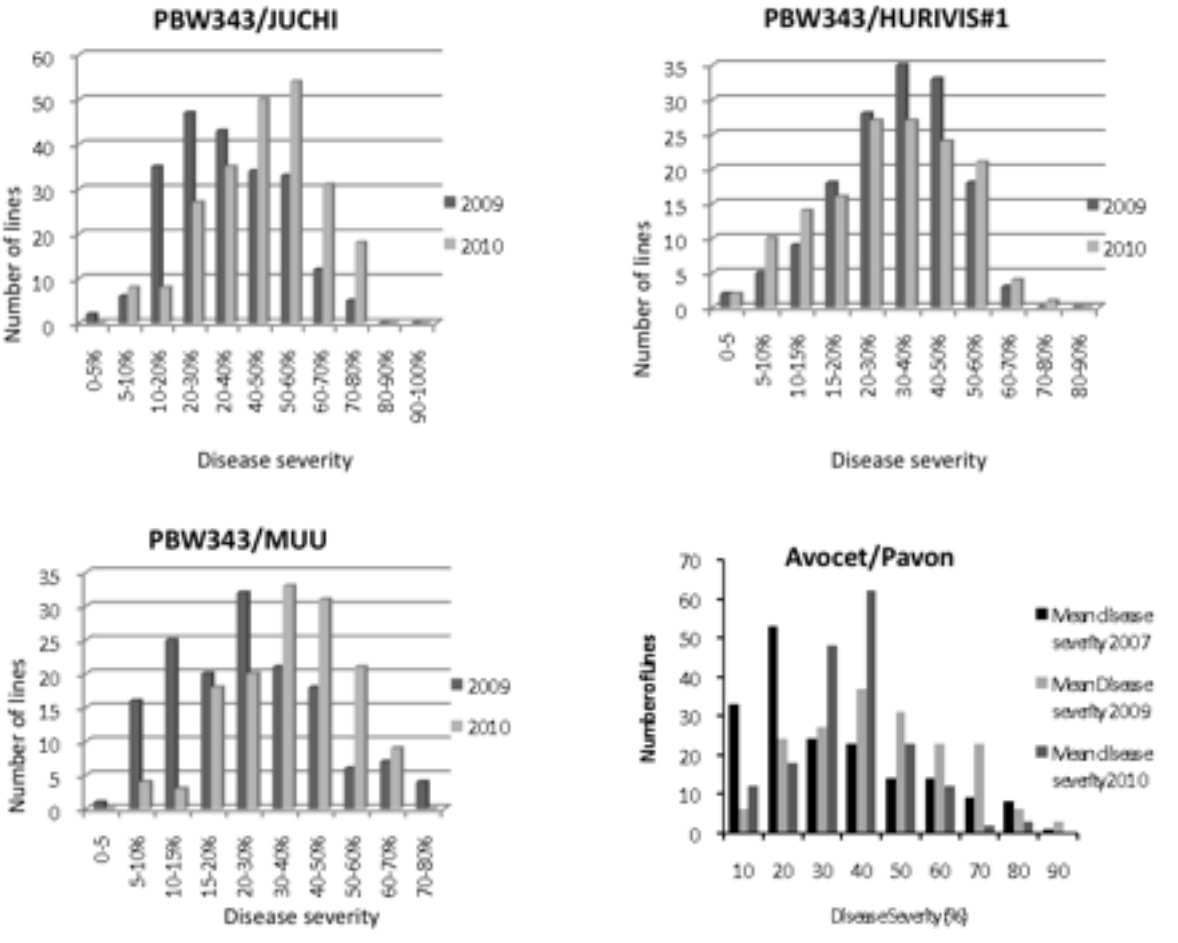


Table 1. ***Correlations (p<0.01) between the phenotypic scores for stem rust observed across years, environments, replicates and dates and PBC for the Kingbird x PBW343 RIL population during 2009 and 2010. (MS- Main season, OS-Off season-1,-2 and-3 indicate the number of notes for each replicates)***

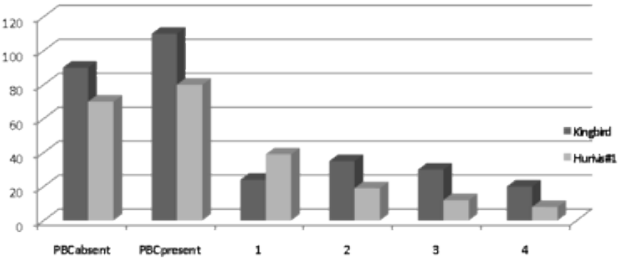
	OS09	MS09-1-1	MS09-1-2	MS09-2-1	MS09-2-2	MS10-1-1	MS10-1-2	OS10-1-1	OS10-1-2	OS10-1-2	OS10-2-2	OS10-2-3	MS10-PBC
OS09													
MS09-1-1	0.59												
MS09-1-2	0.63	0.74											
MS09-2-1	0.55	0.78	0.72										
MS09-2-2	0.48	0.60	0.58	0.77									
MS10-1-1	0.49	0.67	0.64	0.63	0.45								
MS10-1-2	0.49	0.67	0.61	0.65	0.44	0.93							
OS10-1-1	0.31	0.38	0.34	0.31	0.18	0.48	0.46						
OS10-1-2	0.58	0.71	0.59	0.60	0.45	0.73	0.72	0.60					
OS10-2-1	0.44	0.61	0.37	0.45	0.25	0.61	0.63	0.44	0.73				
OS10-2-2	0.52	0.68	0.53	0.57	0.43	0.70	0.71	0.38	0.78	0.84			
OS10-2-3	0.56	0.66	0.50	0.56	0.40	0.72	0.74	0.42	0.79	0.84	0.95		
MS10-PBC	-0.41	-0.59	-0.55	-0.55	-0.48	-0.60	-0.66	-0.28	-0.55	-0.45	-0.60	-0.60	

The RILs in each cross were grouped in two classes viz; non-segregating homozygous resistant and non-segregating homozygous susceptible based on their stem rust severities (Table 2). The number of susceptible families was fairly consistent over the two years. Chi-squared analyses of the observed distribution during each year indicated involvement of three to four APR genes in each resistant parent (Table 2).

Effect of *Sr2* linked PBC on stem rust severity

RILs showing varying degrees of PBC expressions, linked to *Sr2*, had stem rust severities ranging between 7.5-50% compared to 33-72% severities of RILs that lacked PBC (Figure 2). This indicated that RILS without *Sr2* usually had higher disease severities. The intermediate disease severities of 33-72% for some *Sr2*-lacking RILS can be attributed to other APR QTLs. All lines that were positive for PBC expression showed the 3BS alleles for the two markers *XwPt-11419* and *XwPt-3761* suggesting complete linkage. The DArT markers *XwPt-730303* and *XwPt-9067* linked to 4B QTL in Kingbird were found to enhance the expression of PBC. Lines that carried the 3BS and 4B marker alleles were given a PBC score of 2 in contrast to 0 score for lines that did not carry the allele.

Fig.2 ***Phenotypic variation for PBC expression in PBW343 x Kingbird and PBW343 x Huiviris#1 RIL mapping populations***



QTL analysis of APR

ICIM analysis based on phenotypic responses and marker data detected QTLs on different chromosomes in the RIL populations with significant LOD scores across all data sets (Table 3). The *Sr2* and PBC region on 3BS contributed to APR in all populations as shown for Kingbird and Huirivis#1 in Figure 3. Various QTLs identified in PBW343 x Kingbird population are shown in Figure 4. The 3BS QTL, or *Sr2*, explained 40-45% of phenotypic variation in Kingbird RIL population (Figure 4b). DArT markers *XwPt-3921* and *XwPt-2757* were associated with *Sr2* in Kingbird (Table 3). ICIM analysis identified chromosomes 3BS (47% PVE) and 4B (10% PVE) to be linked to PBC expression.

Table 2 ***Genetic analysis of adult plant resistance to stem rust resistance in six RIL mapping populations***

		Stem rust response		χ2 ratios[1]			
Resistant parent	Year	Resistant	Susceptible	3:1	15:1	61:1	No. of genes
Kingbird	2009	184	14	33.9**	5.3	0.2	3-4 genes
	2010	180	18	26.7**	2.1	2.7	3-4 genes
Muu	2009	125	23	7.1*	1.3	21.8**	3 genes
	2010	119	29	2.3	6.8*	44.9**	2-3 genes
Juchi	2009	196	27	19.8**	0.0	13.1**	3 genes
	2010	193	30	15.9**	0.2	19.7**	3 genes
Hurivis#1	2009	129	19	11.7**	0.0	10.9**	3 genes
	2010	127	21	13.1**	0.2	7.5**	3 genes
Kiritati	2009	196	27	19.8**	0.0	13.1**	3 genes
	2010	202	21	28.9**	1.9	3.8	3-4 genes
Pavon 76	2007	169	9	9.0**	0.4	2.2	4-5 genes
	2009	169	9	9.0**	0.4	2.2	4-5 genes
	2010	173	5	15.3**	3.6	0.1	4-5 genes

[1] The ratios 3:1, 15:1 and 61:1 are expected for segregation of 2, 3 and 4 independent resistance genes respectively.

* Significant at P=0.05

** Significant at P=0.01.

Table 3 *Genomic locations of additive effects QTLs for adult plant resistance to stem rust identified by stepwise regression mapping ICIM in six wheat mapping populations*

Year	Chromosome	Marker Position	[1]Left Marker	[1]Right Marker	[3]LOD	[4]PVE(%)	[2]Est.ADD	R^2
Kingbird-2009,2010 combined (ICIM)	1AL	251	*XwPt-0128*	*XwPt-734078*	4.5	41.5	-11.2	
	3BS	21	*XwPt-3921*	*XwPt-2757*	10.9	41.5	11.0	
	5BL	191	*XwPt-2607*	*XwPt-1733*	3.2	13.7	5.6	51.2
	7A	1201	*XwPt-8670*	*XwPt-744574*	3.2	10.1	-5.4	
	7DS	0	*XwPt-1859*	*XwPt-731810*	3.4	9.3	-31.8	
Kiritati-2009,2010 combined (Multiple regression Mapping)	2D	20	*Xbarc*095	N/A	3.6	N/A	3.7	6.0
	3BS	30	[5]SW58	N/A	17.3	N/A	-7.6	25.0
	5BL	76	*Xbarc109*	N/A	5	N/A	-3	8.0
	7DS	36	Lr34-linked	N/A	7	N/A	-5.3	12.0
Juchi-2009,2010 combined (ICIM)	2B	152	*XwPt-7829*	*XwPt-2266*	4.6	16.6	7.5	
	3BS	28	*XwPt-8056*	*XwPt-800213*	3.1	8.3	5.1	
	4A	123	*XwPt-5124*	*XwPt-6390*	2.7	12.8	6.9	42.4
	5BL	472	*XwPt-0750*	*XwPt-5896*	4.2	16.5	-7.5	
	6B	21	*XwPt-5480*	*XwPt-9532*	2.9	23.5	8.5	
Hurivis#1-2009,2010 combined (Multiple regression Mapping)	2B	0	N/A	*Xwmc257*	2.4	N/A	-4.7	6.8
	3BS	0	N/A	SW3648	6.0	N/A	-9.0	16.0
	5BL	N/A	*Xwms371*	[6]NW2012ND	3.9	49.2	1.1	23.0
	7B	N/A	N/A	NW3109ND	2.5	N/A	-5.3	6.9
MUU-2009,2010 combined (ICIM)	2B	340	*XwPt-744022*	*XwPt-1964*	2.9	5.4	-4.2	
	3BS	41	*XwPt-666139*	*XwPt-3921*	15.8	36.5	10.7	46.0
	5BL	353	*XwPt-6014*	*XwPt-3661*	3.0	7.1	-4.7	
Pavon-2007, 2009 and 2010 combined (ICIM)	1BL	278	*XwPt-1560*	*XwPt-7486*	6	23.8	N/A	
	3BS	52	*XwPt-8093*	*XwPt-7212*	13.7	18.9	N/A	68.9
	5A	8	*XwPt-6048*	*XwPt-4249*	2.9	6.3	N/A	
	6B	8	*XwPt-1541*	*XwPt-0171*	2.7	13.4	3.2	
PBC 2010 (KINGBIRD)	3BS	26	*XwPt-800213*	*XwPt-3761*	15.9	52.3	-1.0	58.3
	4B	4	*XtPt-0602*	*XwPt-1708*	3.1	7.9	0.4	
PBC 2010 (HURIVIS#1)	3BS	N/A	*Xwmc54*	*Xbarc131*	2.8	8.6	-0.4	
	4B	24	*Xwms113*	N/A	3	N/A	-0.3	8.6

[1] Closest flanking markers

[2] Estimated additive effects; the negative sign means the favorable alleles contributed by the susceptible parent that enhances phenotypic trait

[3] Logarithm of Odds as per the ICIM calculations; only QTL with LOD score above 2.5 and stepwise regression at 0.001

[4] Phenotypic variance explained by the QTL as per ICIM calculation

[5, 6] Markers developed or licensed to Syngenta

Fig. 3 *Inclusive interval analysis of QTL for PBC on chromosome 3BS in PBW343 x Kingbird RIL mapping population*

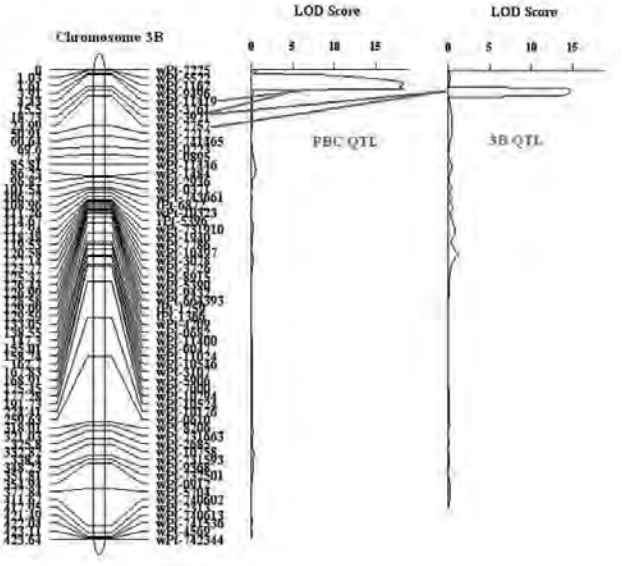
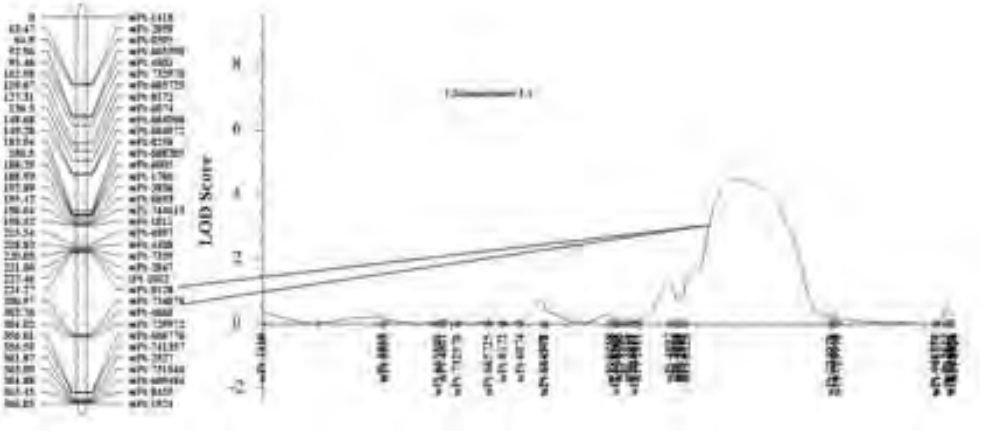
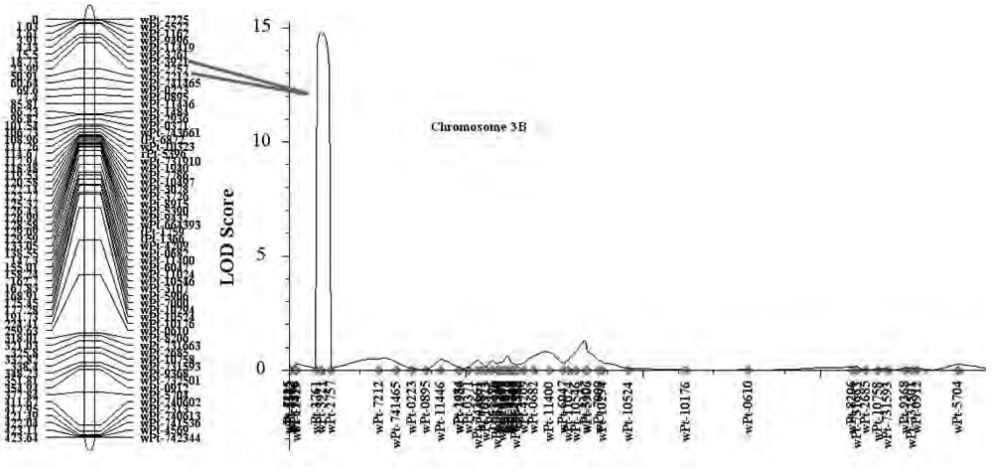


Fig.4 *Genomic locations of QTLs with additive effects for stem rust resistance identified by stepwise regression mapping ICIM in PBW343 x Kingbird RIL mapping population. (a) QTL on chromosome 1AL, (b) QTL on chromosome 3BS, (c) QTL on chromosome 5BL, (d) QTL on chromosome 7A, and (e) QTL on chromosome 7DS*

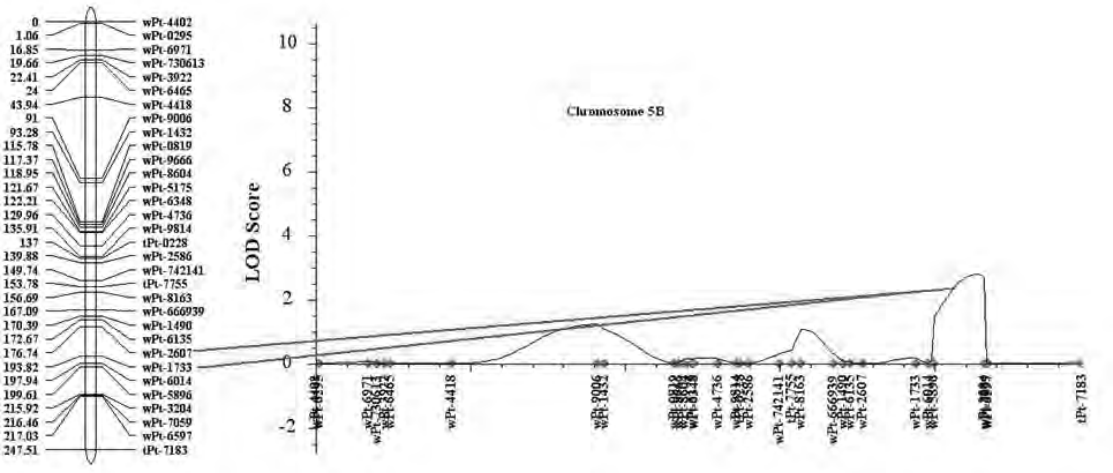
a



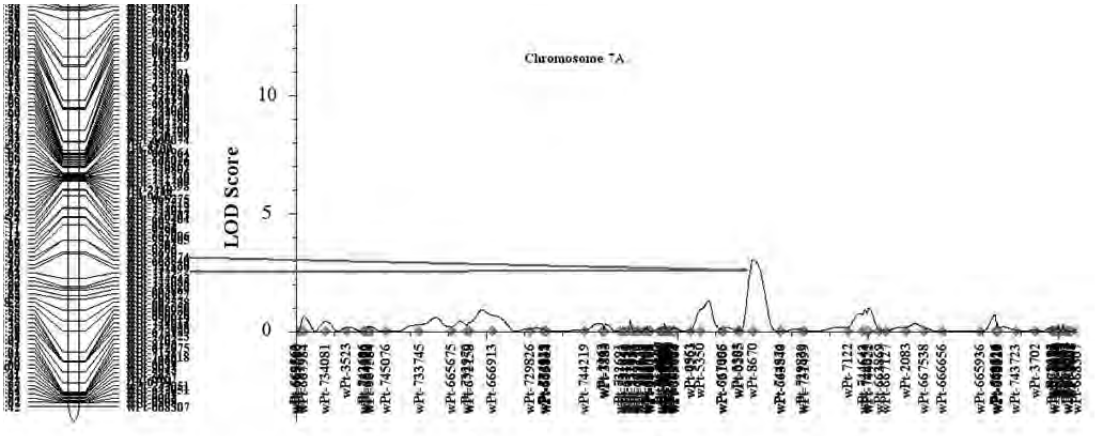
b



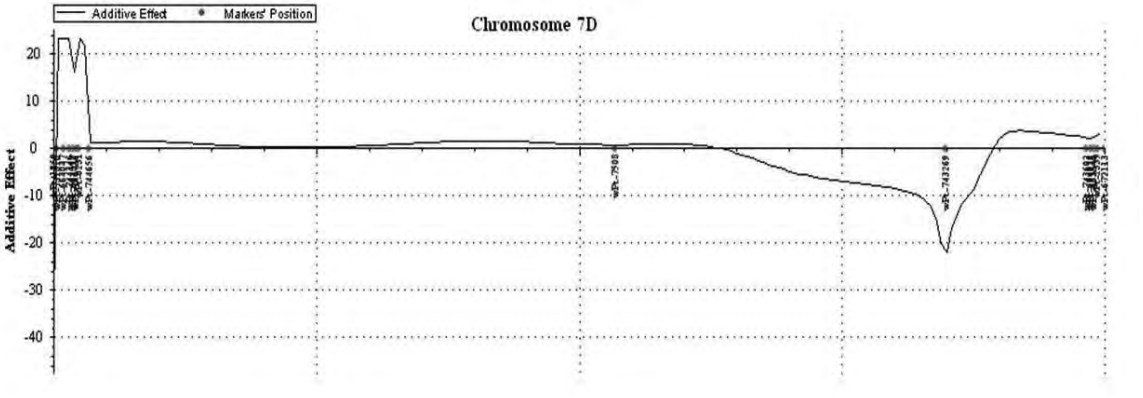
c


Chromosome 5B
LOD Score
10
8
6
4
2
0
-2
0 wPt-4402
1.06 wPt-0295
16.85 wPt-6971
19.66 wPt-730613
22.41 wPt-3922
24 wPt-6465
43.94 wPt-4418
91 wPt-9006
93.28 wPt-1432
115.78 wPt-0819
117.37 wPt-9666
118.95 wPt-8604
121.67 wPt-5175
122.21 wPt-6348
129.96 wPt-4736
135.91 wPt-9814
137 tPt-0228
139.88 wPt-2586
149.74 wPt-742141
153.78 tPt-7755
156.69 wPt-8163
167.09 wPt-666939
170.39 wPt-1490
172.67 wPt-6135
176.74 wPt-2607
193.82 wPt-1733
197.94 wPt-6014
199.61 wPt-5896
215.92 wPt-3204
216.46 wPt-7059
217.03 wPt-6597
247.51 tPt-7183

d


Chromosome 7A
LOD Score
10
5
0

e


Additive Effect
Markers' Position
Chromosome 7D
Additive Effect
20
10
0
-10
-20
-30
-40

A QTL on chromosome 5BL was detected in all populations (Table 3). A QTL for stem rust resistance on the same chromosomal region was earlier reported in European cultivar 'Arina' (Bansal et al. 2008). Two DArT markers *XwPt-2067* and *XwPt-1733* were found to be associated with the 5BL QTL in Kingbird (Figure 4c). RILs, positive for 3BS and 5BL alleles, had lower disease severities indicating the additive effects of the two QTLs in reducing disease severity. ICIM has an advantage in detecting interactive QTLs where the alleles that enhance the phenotypic trait are contributed by the alternate parent in a bi-parental cross. The individual effects of such alleles contributing to minor effects usually are underestimated however combinations of such alleles results in increased phenotypic effects, i.e. reduced stem rust severity in this case.

The QTL identified in chromosome 1BL of Pavon 76 suggested the presence of *Lr46/Yr29* in reducing stem rust severity in Avocet x Pavon RIL population. SSR marker *Xbarc80* was earlier shown to be linked to the slow rusting, APR gene *Lr46* located on chromosome 1BL (Suenaga et al. 2003, William et al. 2003). Another QTL on chromosome 7DS was associated with the stem rust severity reduction in PBW343 x Kingbird RIL population. Kingbird is known to carry *Lr34/Yr18* effective against leaf rust and yellow rust and shows leaf tip necrosis. The 7DS QTL (LOD=3.4) explained 10% of the phenotypic variation and the DArT markers *XwPt-1859* and *XwPt-731810* linked to this QTL mapped to the short arm in the region where *Lr34/Yr18* are located (Figure 4e).

ICIM also identified additional genomic regions on chromosomes 1AL, 2B, 2D, 4A, 5A, 6B and 7A to be associated with stem rust resistance in our mapping populations explaining various degrees of phenotypic variation (Table 3). These genomic regions were not reported in earlier studies on stem rust resistance.

Discussion

The distribution of disease severity for RILs in various mapping populations varied from highly resistant (<10% severity) to susceptible (>80% severity) confirming the quantitative nature of resistance to stem rust (Figure 1). Molecular mapping studies on six populations indicated presence of ten APR stem rust genes of chromosomes 1AL, 2B, 2D, 3BS, 4A, 5A, 5BL, 6B, 7A and 7DS. The only catalogued adult plant stem rust resistance gene in wheat is *Sr2* (McIntosh et al. 2003). *Sr2* is located on chromosome arm 3BS (Hare and McIntosh 1979) and was shown to be closely linked with SSR marker *Xgwm533* (Spielmeyer et al. 2003). The best known APR is conferred by the "*Sr2*- complex" (Singh et al. 2008). *Sr2* shows similarities with *Lr34/Yr18* and *Lr46/Yr29* and is associated with multi-pathogen resistance. Tight linkage between *Sr2*, the leaf rust resistance gene *Lr27*, and partial APR to stripe rust (*Yr30*) were is known (Singh and McIntosh 1984). Wheat plants with inactivated *Lr27* alleles from mutagenesis appear to have lost *Sr2* possibly indicating pleiotrophism (Spielmeyer et al. 2009). Germplasm showing adequate APR levels usually has several minor genes, each with small to intermediate effect in reducing disease severity (Singh et al. 2008). The close genetic linkage of 3BS QTL with *Sr2* and PBC as determined by the DArT markers in our studies further supports earlier results (Hare and McIntosh 1979, Kota et al. 2006). RILs that carried PBC displayed varying levels of stem rust severities ranging between 5-60% indicating that *Sr2* alone is not enough to provide adequate resistance. Lines with negligible PBC expression and low disease severity are usually selected in breeding program at CIMMYT because high expression of melanin is considered undesirable.

The QTL on chromosome 5BL explained 10-13% of phenotypic variance and was consistent during both years of testing. This QTL was earlier reported in two other studies (Kaur et al. 2007, Bansal et al. 2008). Lines carrying the 3BS and 5BL QTL usually had lower stem rust severities of 15-30% compared to lines that lacked them. The 5BL QTL is likely to be an important component of the "*Sr2* complex". The detection of 1BL QTL in Pavon and its linkage to *Lr46/Yr29/Pm39*-linked marker *barc80* suggests that the pleiotropic APR gene *Lr46/Yr29/Pm39* (William et al. 2003, Lillemo et al. 2008) also confers partial resistance to stem rust.

A QTL for stem rust resistance on 7DS mapped to same region where pleiotropic APR gene *Lr34/Yr18/Pm38* is located. Leaf rust resistance gene *Lr34* was first described by Dyck (1987) as non-suppressor of stem rust resistance in 'Thatcher'. Another feature of *Lr34* is that it has remained genetically inseparable from yellow rust resistance gene *Yr18* (Singh 1992, McIntosh 1992). Co-segregation *Lr34*/*Yr18* with other traits leaf tip necrosis gene *Ltn1*, powdery mildew resistance gene *Pm38*, and barley yellow dwarf virus tolerance gene *Bdv1* is reported in various studies (Singh 1993, McIntosh 1992, Spielmeyer et al. 2005, Liang et al. 2006). These multi-pathogen resistance traits have made the *Lr34*/*Yr18* locus one of the most valuable gene regions for disease resistance breeding in wheat. These pleiotropic resistance genes enable wider protection against wider range of pathogen and are therefore valuable in breeding.

QTL analysis of APR to stem rust also revealed various other loci in addition to *Sr2/Yr30, Lr34/Yr18* and *Lr46/Yr29*. QTLs on chromosomes 1AL, 2B, 2D, 4A, 4B, 5A, 5B, 6B and 7A suggest new genomic regions for stem rust resistance and require additional research. We plan to saturate these genomic regions with SSR markers to identify flanking markers that can be used for marker-assisted selection and fine mapping. RILs showing higher levels of APR to stem rust than the parents can be used as new sources for transferring resistance in high yielding backgrounds. Information on the effects of each APR-QTL in various genetic backgrounds should help wheat breeders in prioritizing the target loci for their breeding programs. New APR genes and associated molecular markers should be useful in the development of near-immune levels of diverse APR to stem rust that can be deployed strategically to reduce genetic vulnerability.

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References

Akbari M, Wenzl P, Vanessa C, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Rathmell B, Vaughan P, Huttner E, Kilian A (2006) Diversity Arrays Technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor. Appl. Genet. 113: 1409-1420

Bansal UK, Bossolini E, Miah H, Keller B, Park RF, Bariana HS (2008) Genetic mapping of seedling and adult plant stem rust resistance in two European winter wheat cultivars. Euphytica 164:821–828

Caldwell RM. 1968. Breeding for general and/or specific plant disease resistance. In *Proc. International Wheat Genetics Symp., 3rd,* eds. KW Finlay, KW Shepherd, pp. 263–72, Publ. Aust. Acad. of Sci., Canberra, Australia

Hare RA, McIntosh RA (1979) Genetic and cytogenetic studies of durable adult-plant resistances in Hope and related cultivars to wheat rusts. Zeitschrift Fur Pflanzenzuchtung-Journal of Plant Breeding 83:350-367

Huihui L, Guoyou Y, Jiankang W (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361-374

Kilian A, Huttner E, Wenzl P, Jaccoud D, Carling J, Caig V, Evers M, Heller-Uszynska K, Cayla C, Patarapuwadol S, Xia L, Yang S, Thomson B (2005) The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. In Tuberosa R, Phillips RL, Gale M (eds). Proceedings of the International Congress In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution, May 27-31, 2003, Bologna, Italy. Avenue Media, Pp 443-461

Kota R, Spielmeyer W, McIntosh RA, Lagudah ES (2006) Fine genetic mapping fails to dissociate durable stem rust resistance gene *Sr2* from pseudo black chaff in common wheat (*Triticum aestivum* L) Theor Appl Genet 112:492-499

Li H, and Wang J (2008) Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. Theor Appl Genet 116:243-260

Li H, Ye GY, Wang J (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175: 1-14

Liang SS, Suenaga K, He ZH, Wang ZL, Liu HY, Wang DS, Singh RP, Sourdille P, Xia XC (2006) Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. Phytopathology 96:784-789

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjørnstad Å (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor Appl Genet 116:1155-1166

McIntosh RA (1988). The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. In: Simmonds NW, Rajaram S, editors. Breeding Strategies for Resistance to the Rust of Wheat. CIMMYT, Mexico, DF. 1–9

McIntosh RA (1992) Close genetic linkage of genes conferring adult-plant resistance to leaf rust and stripe rust in wheat. Plant Path 41:523-527

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts, an atlas of resistance genes CSIRO, Melbourne, Australia

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R (2003) Catalogue of gene symbols for wheat. In: Pogna NE, Romano M, Pogna A, Galterio G (eds) Proceedings of the 10th international wheat genetics symposium. Paestum, Italy

Neumann K, Kobiljski B, Denčić S, Varshney RK, and Börner A (2010) Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.). Mol. Breeding, DOI 10.1007/s11032-010-9411-7

Njau PN, Jin Y, Huerta-Espino J, Keller B, Singh RP (2010) Identification and evaluation of sources of resistance to stem rust race Ug99 in wheat. Plant Dis 94:413-419

P. Roelfs and W. R. Bushnell, eds.), pp. 259–298. Academic Press, Orlando.
Parlevliet JE (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124:147-156
Peterson RF, Campbell AB, Hannah AE (1948) Adiagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can J Res Sect C26:496-500
Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in Puccinia graminis f sp tritici in Uganda. Plant Dis 84:203
Rajaram S, Singh RP, and Torres E (1988) Current CIMMYT approaches in breeding wheat for rust resistance. In: N. W, Simmonds and S. Rajaram (eds.). Breeding Strategies for Resistance to the Rust of Wheat. CIMMYT, Mexico, DF. Pp 101-118
Saari, E. E., and Prescott, J. M. (1985). World distribution in relation to economic losses. In "The Cereal Rusts, Vol. II: Diseases, Distribution, Epidemiology, and Control" eds. AP Roelfs, WR Bushnell, pp. 259–98. Acadmic Press, Orlando, USA
Shank, R. (1994). Wheat stem rust and drought effects on Bale agricultural production and future prospects. Report on February 17–28 assessment. In "United Nations Emergencies Unit for Ethiopia." http://www.africa.upenn.edu/eue_web/Bale_mar.txt. Addis Ababa, Ethiopia
Singh RP (1992) Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835-838
Singh RP (1993) Genetic association of gene *Bdv1* for tolerance to barley yellow dwarf virus with genes Lr34 and Yr18 for adult plant resistance to rust in bread wheat. Plant Dis 77:1103-1106
Singh RP, Hodson DP, Huerta-Espino J, JinY, Njau P, Wanyera R, Herrera-Foessel SA, and Ward RW (2008) Will Stem Rust Destroy the World's Wheat Crop? Advances In Agronomy 98: 271-309
Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P, Ward RW (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 1:1-13
Singh RP, Mcintosh RA (1984) Complementary genes for reaction to *Puccinia recondita tritici* in *Triticum aestivum*. 1. Genetic and linkage studies. Can J Genet Cytol 26:723-735
Singh, RP, Huerta-Espino, J, Rajaram, S (2000) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. Acta Phytopathol Entomol Hung 35:133-139
Spielmeyer W, Mago R, Simkova H, Dolezel J, Krattinger S, Keller B, Paux E, Feuillet C, Breen J, Appels R, McIntosh R, Kota R, Wellings C, Lagudah E (2009) Durable rust resistance in wheat is effective against multiple pathogens. Plant and Animal Genomes XVII Conference, San Diego http://www.intl-pag.org/17/abstracts/W61_PAGXVII_425.html
Spielmeyer WP, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.). Crop Sci 43:333–336
Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES (2005) Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7DS of wheat. Theor Appl Genet 111:731-735
Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881-890
Sunderwirth SD, Roelfs AP. (1980) Greenhouse characterization of the adult plant resistance of Sr2 to wheat stem rust. Phytopathology. 70:634–7
William M, Singh RP, Huerta-Espino J, Ortiz Islas S, Hoisington D (2003) Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93:153-159
Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesna J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and phenotypic traits. BMC Genomics **7**:206
Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Cracking the codes: genetic basis of nonhost resistance of barley to heterologous rust fungi

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Abstract

Full nonhost resistance can be defined as immunity, displayed by an entire plant species against all genotypes of a plant pathogen. The genetic basis of (non)host-status of plants is hard to study, since identification of the responsible genes would require interspecific crosses that suffer from sterility and abnormal segregation. There are some plant/potential pathogen combinations where only 10% or less of the accessions are at most moderately susceptible. These may be regarded as marginal host or near-nonhost, and can provide insights into the genes that determine whether a plant species is a host or a nonhost to a would-be pathogen. Barley (*Hordeum vulgare* L.) is a near-nonhost to several rust pathogens (*Puccinia*) of cereals and grasses. By crossing and selection we developed an experimental line, SusPtrit, with high susceptibility to at least nine different heterologous rust taxa such as the wheat and *Agropyron* leaf rusts (caused by *P. triticina* and *P. persistens*, respectively).

On the basis of SusPtrit and several regular, fully resistant barley accessions, we developed mapping populations. We established that the near-nonhost resistance to heterologous rusts inherits polygenically (QTLs). The QTLs have different and overlapping specificities. In addition, an occasional R-gene is involved. In each population, different sets of loci were implicated in resistance. Very few resistance genes were common between the populations, suggesting a high redundancy in barley for resistance factors. Selected QTLs have been introduced into near-isogenic lines to be fine-mapped. Our results show that the barley-Puccinia system is ideal to investigate the genetics of host-status to specialized plant pathogens.

Keywords

Barley, genetics, leaf rust, QTL

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Introduction

Plants are exposed to a huge number of potential pathogens that represent a very diverse array of microorganisms. By far most plant species are nonhost to by far most would-be pathogens. The term nonhost was defined as the resistant status of an entire plant species to all genotypes of a parasite or pathogen species (Heath 2000). This definition is widely agreed upon, but implies that a nonhost-status can never be proven. All genotypes of a plant species, even those in the past, present and future, cannot be tested to all past, present and future genotypes of a microbe species.

An important motive and justification of research into nonhost resistance, is the obviously high durability of this form of resistance. This is in contrast with the typically ephemeral effectiveness of *R* genes that determine hypersensitive resistance. Exciting new insights are accumulating, and have been summarized and discussed in recent review papers (e.g. Thordal-Christensen 2003; Mysore and Ryu 2004; Nürnberger and Lipka 2005; Ingle et al. 2006; Jones and Dangl 2006; O'Connell and Panstruga 2006; Schweizer 2007; Niks and Marcel 2009; Schulze-Lefert and Panstruga 2011). Most advances are in the understanding of perception of microbial intruders, and in the genes that play a role in signal transduction and defense. Many authors acknowledge that, despite these advances, still very little is known about the genes that determine natural variation between plant species, making one a host, and the other a nonhost, to a would-be (potential) pathogen.

In this contribution we will present the Barley-*Puccinia* rust system as a model to help elucidating the specificity aspects of (non)host-status of a plant to a would-be pathogen.

Mechanisms of nonhost resistance

The resistance of most plants to most would-be pathogens is based on a multilayered defense (Heath 2001, 2003; da Cunha et al. 2006), that comprises physical and constitutive chemical features (preformed barriers) and induced defenses. Would-be pathogens should be able to deal effectively with the defense that plant species perform against maladapted microbial intruders (Heath 1981). They may need to locate stomata on the plant leaf surface, and should tolerate or break down secondary metabolites. Since related plant species frequently have similar preformed barriers, it is reasonable to assume that, generally, preformed barriers are more likely to contribute to nonhost resistance to pathogens of other plant families (like *Arabidopsis* to the wheat leaf rust pathogen), than to pathogens of plant species that are related to the nonhost (like barley

to the wheat leaf rust pathogen). Induced defenses are effective to pathogens of plants of whatever close relationship with the nonhost.

On nonhost plant species, resistance against specialized maladapted fungal pathogens like those of the rusts and powdery mildews commonly appears as defective haustorium formation, termed pre-haustorial or penetration resistance (Heath 1974; Elmhirst and Heath 1987; Niks 1987). This defense typically leads to the formation of cell wall reinforcements, also called cell wall appositions or papillae (O'Connell and Panstruga 2006; Underwood and Somerville 2008).

Frequently, pre-haustorial nonhost resistance is backed-up by a hypersensitive post-penetration resistance for those infection units that still succeed in cell wall penetration (Heath 2002; Lipka et al. 2005). The process of this cell death in nonhost plants is not necessarily the same as in hypersensitive resistance of the host species (Christopher-Kozjan and Heath 2003).

Nonhost resistance induced by MAMPs

It seems that plants are able to discriminate between self and non-self, and this ability is the basis for the activation of induced defenses upon microbial infection. The perception of the non-self intruder in the plant tissue is probably mostly mediated by receptor-like kinases (RLKs). These recognize directly certain characteristics of microbe-associated molecular patterns (MAMPs). So, MAMP detection serves as an early warning system for the presence of non-self molecules. Also indirect perception of microbe-induced molecular patterns (MIMPs) occurs, where the product of the intrinsic activity of a microbe-derived compound will be recognized through alteration of the functional state of a host molecule (Mackey and McFall 2006). These recognition factors are also known under the acronym PAMP (pathogen-associated molecular pattern) (Ingle et al. 2006).

As a rule, MAMPs seem too conserved to explain the difference between nonhost and host pathogens. Indeed, host pathogens as well as related nonhost pathogens contain very similar or identical MAMPs. As a consequence, adapted pathogens also trigger the basal defense reaction in their host, but are able to suppress this reaction within hours (Li et al. 2005; Caldo et al. 2006; Truman et al. 2006). Indeed, the failed or successful suppression of basal defense is presumed to be the key phenomenon determining a nonhost or a host-status of the interaction (Heath 1991; Panstruga, 2003; Nomura et al. 2005; Caldo et al. 2006). The organization of plant defense to maladapted pathogens bears similarity to burglar alarm devices in buildings. The alarm is triggered by non-specific factors (motion and MAMPs, respectively) and should be suppressed in a very specific way (bypass code for authorized persons and a specific set of microbial factors, respectively). Unauthorized intruders should "crack the code" in order to enter without activating the alarm and defense system.

Effectors mediate suppression of defense

Suppression of basal resistance is assumed to be mediated by so called effectors that are delivered into the apoplast or into plant cells (Kamoun 2006). These have been particularly well studied in bacterial diseases (caused by *Pseudomonas* and *Xanthomonas* spp.) (Li et al. 2005; Ingle et al. 2006; Gurlebeck et al. 2006; Truman et al. 2006). In other pathogen classes, like rust fungi (Catanzariti et al. 2006), oomycetes (Kamoun 2006) and other plant pathogenic fungi, effectors have also been implicated. Up to now, very little is known about the identity and biochemical function of effectors delivered by fungal and oomycete pathogens. Some of these effectors may act as transcription factors (Lahaye and Bonas 2001); others may cleave specific cytoplasmic host proteins (Shao et al. 2003; Coaker et al. 2005).

What determines the specificity of pathogens?

Since related pathogens with different host ranges contain identical or very similar MAMPs, they all will activate the same basal defense in any plant. However, for example the rye leaf rust fungus (*Puccinia recondita*) is able to suppress the defense in rye but not in barley, and the barley leaf rust pathogen (*P. hordei*) in barley, but not in rye. So, the effectors of these pathogens should differ, as should the targets of their effectors, making them only effective in the plant species to which their effectors have been adapted. This suggests that the effectors and their targets are important determinants of the (non)host-status of a certain plant-microbe combination (Niks and Marcel 2009). The effectors may be the means by which the pathogen "cracks the codes" of a possible host plant.

The next question is how to find the plant targets that determine whether effectors of a would-be pathogen can or cannot suppress the basal defense. An obvious approach would be to study the inheritance of (non)host-status. This would, by definition, need an interspecific cross, viz between a host and a nonhost plant species. The overriding difficulty of this approach is that in interspecific crosses classical genetics are rarely feasible, due to hybrid sterility, abnormal segregation, lack of chromosome pairing and artifacts caused by odd plant morphology. This degree of incompatibility hampers the identification of individual genetic factors.

Exploitation of "near-nonhost" status

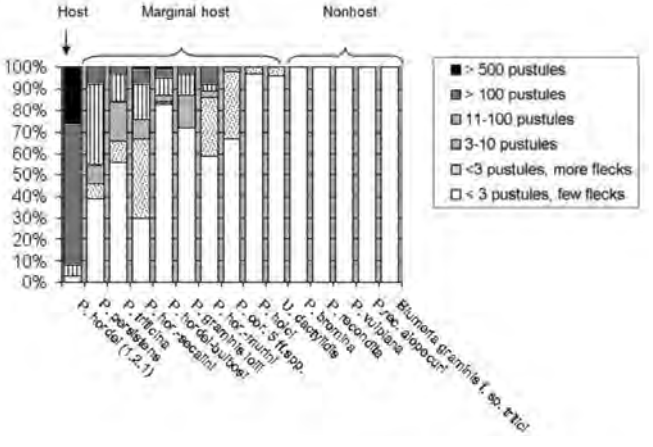
There are several plant species that are only marginal hosts to a potential pathogen. For example, plant species in which less than 10% of the accessions are at most moderately susceptible, and only at the seedling stage (Mains 1933; Niks 1987). This phenomenon is obvious in barley. Occasional susceptible barley genotypes have been found to the wheat stripe rust pathogen (*P. striiformis* f.sp. *tritici*) (Pahalawatta and Chen 2005; Sui et al. 2010) and to at least nine other rust fungal species of cereals and grasses (Atienza et al. 2004). This "near-nonhost" status offers the possibility to analyze phenotypic segregations if rare susceptible barley accessions are crossed with common immune accessions. This circumvents the need for inter-specific host x nonhost crosses. Still, it seems reasonable to presume that genes that are responsible for the resistance/susceptibility to marginal pathogens will, by extrapolation, also teach us about principles that contribute to, or even determine, the full nonhost-status to related pathogens.

We quantified the level of susceptibility of barley to several heterologous rust fungal species, all pathogenic on (other) cereals and grasses (Atienza et al. 2004, and unpublished data). Some examples are presented in Fig. 1. The high level of infection by *P. hordei* isolate 1.2.1. is as expected, since barley is the regular host species.

Other heterologous rust fungi were applied with 3-fold as much inoculum as *P. hordei*, but nevertheless several did not produce any pustule (for example, the rye leaf rust pathogen *P. recondita*); others, like the wheat leaf rust pathogen (*P. triticina*) produced more than 100 pustules per leaf on fewer than 10% of the accessions. To the latter group barley can be considered a "near-nonhost". So far, adult plants were resistant to all rusts, except to *P. hordei* (Atienza et al. 2004). The wheat powdery mildew fungus (*Blumeria graminis* f. sp. *tritici*) did not produce macroscopically visible colonies on any barley accession.

Among barley accessions with susceptibility to heterologous rusts, relatively many accessions were with naked seeds, black seeds and African and Asian land races, and relatively few were modern cultivars (Atienza et al. 2004).

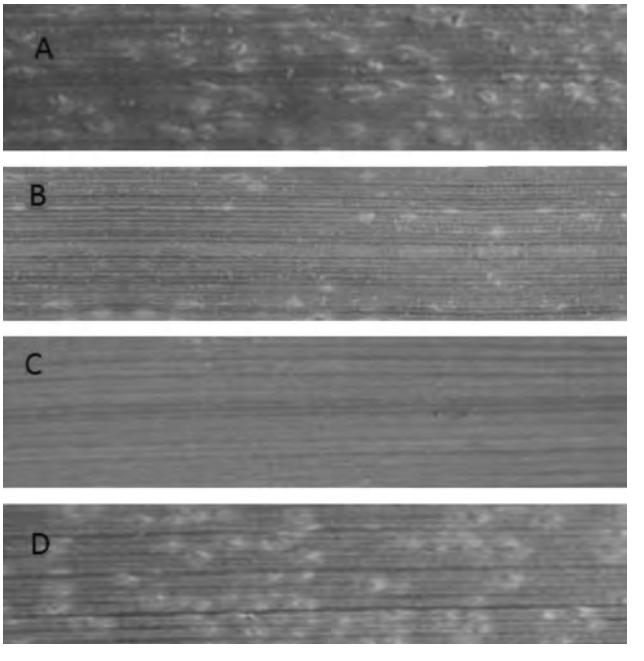
Fig. 1 ***Percentages of barley accessions (n=110) per susceptibility class for 13 heterologous rust fungi, and wheat powdery mildew and the barley leaf rust pathogens, determined at the seedling stage. After Atienza et al. (2004)***



Accumulation of susceptibility to heterologous rusts

None of the barley accessions was as highly susceptible as the regular host to the heterologous rusts. Therefore, we accumulated susceptibility alleles by convergent crossing of accessions identified as fairly susceptible, selecting in their progeny for higher susceptibility to the wheat leaf rust pathogen, *P. triticina*. This resulted in an experimental line, called SusPtrit, with high seedling stage susceptibility to *P. triticina* as a typical susceptible wheat accession (Fig. 2; Atienza et al. 2004). We followed the same procedure, starting from different parental material, to develop a line with very high susceptibility to the heterologous rust *P. hordei-murini*, called SusPmur (Atienza et al. 2004).

Fig. 2 ***Responses of the wheat leaf rust pathogen (Puccinia triticina) on (A) a seedling leaf of wheat; (B) barley line L94 (a relatively rare, slightly susceptible, barley accessions); (C) barley cv. Vada (a representative and typical "immune" barley accession); and (D) experimental barley line SusPtrit, in which genes for susceptibility to P. triticina have been accumulated***



Mapping populations

We developed two mapping populations by crossing SusPtrit, the susceptible parent, with the European cultivar Vada and the South American cultivar Cebada Capa, parents that are regularly immune to heterologous rust fungi. These mapping populations consisted of 152 recombinant inbred lines for Vada x SusPtrit (V x S), and 113 recombinant inbred lines for Cebada Capa x SusPtrit (C x S). We also found that the Oregon Wolfe Barley population (OWB; 94 DH lines, Costa et al. 2001) segregated for susceptibility to heterologous rusts for which barley was a near-nonhost. Since the parents of the OWB population were generated by convergent crosses of exotic barley accessions (Costa et al. 2001), it was not entirely unexpected that this population would segregate for susceptibility to heterologous rust fungal species.

Research questions to be addressed

The availability of the barley research lines with extreme susceptibility to heterologous rusts and of the mapping populations enabled us to address the following research questions;

- is host-status based on quantitative genes or on the stacking of several *R* genes?
- do nonhosts have specific genes for nonhost-status, each effective to a different pathogen taxon, or do the genes have a broad spectrum effectiveness?
- is the resistance of members of a nonhost species to a heterologous pathogen due to shared resistance genes, or are different members of the nonhost species each resistant due to a different set of genes?
- is nonhost resistance due to variation in genes that have been identified as playing a key role in perception, signal transduction and defense?
- are the genes that determine the (non)host-status to a heterologous pathogen also implicated in basal defense to the related adapted pathogen?

Quantitative genes or stacking of several R-genes?

The barley-rust interaction indicates clearly quantitative genes for (non)host-status. The mapping populations segregated quantitatively and continuously. Fig. 3 provides two examples of such segregation. To each heterologous rust species QTL mapping led to the discovery of two to five QTLs that explained the resistance of the resistant parent, Vada or Cebada Capa, to a particular rust pathogen species (Jafary et al. 2008; Fig. 4).

The quantitative, polygenic inheritance is also consistent with the fact that the accumulation of susceptibility by convergent crossing to produce SusPtrit and SusPmur (Atienza et al. 2004) led to a gradual increase in the level of susceptibility.

It has been proposed that nonhost resistance of plants may be due to *R* genes of the NBS-LRR type that recognize pathogen-derived Avr factors (Heath 1981; Niks 1988; Jones and Dangl 2006; Schweizer 2007; Schulze-Lefert and Panstruga 2011). If several of such *R* genes occur in combination and at high allele frequency in the plant species, and if the cognate Avr factors occur also at high allele frequency in the microbe species, this would lead

Fig. 3 ***Frequency distributions of phenotypes for resistance to two heterologous rust fungi in the barley mapping population Vada x SusPtrit. Values of the two parental lines are shown by arrows. A. Puccinia triticina isolate Flamingo; B. P. graminis f. sp. lolii***

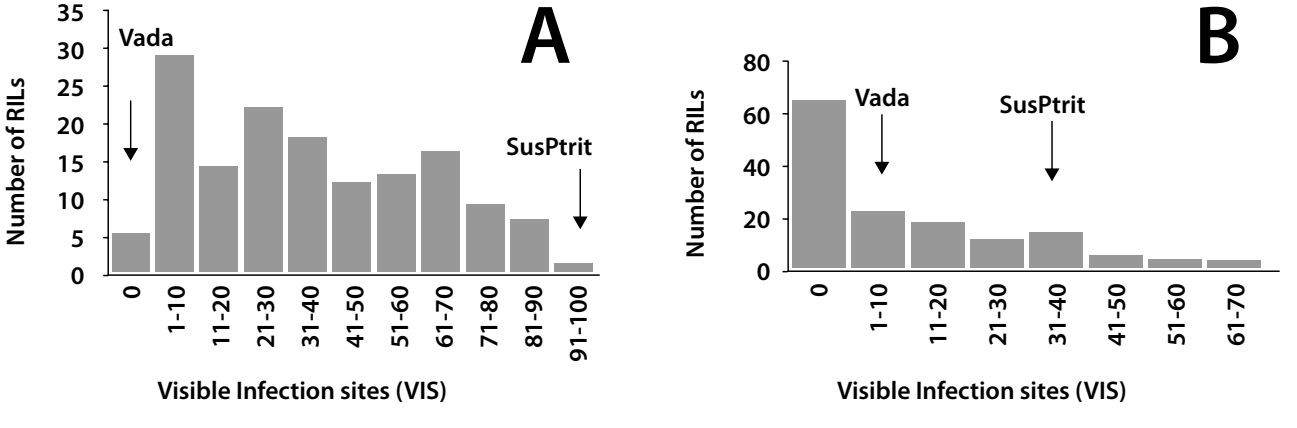
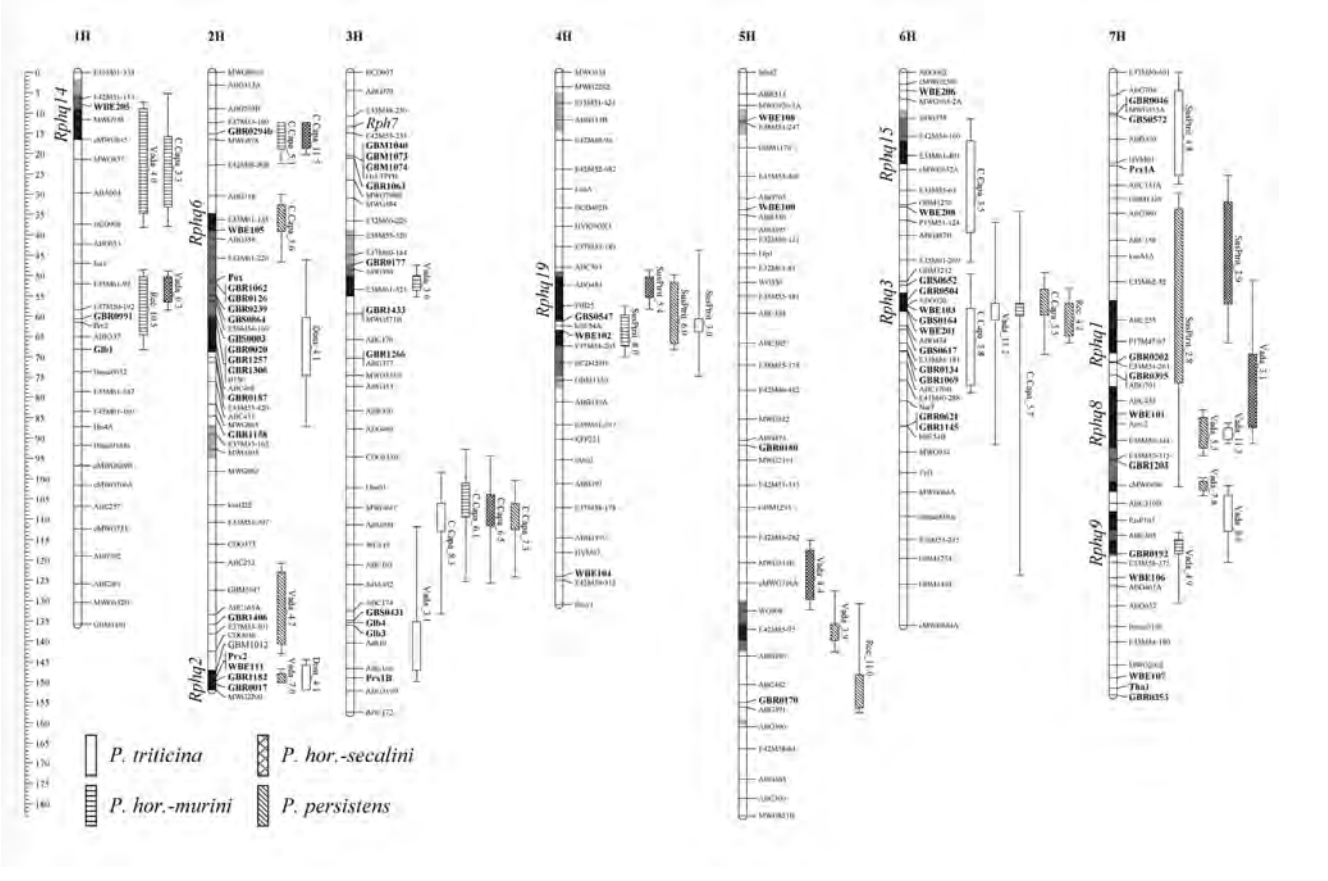


Fig. 4 *Locations of QTLs for nonhost immunity to four heterologous rust pathogen species on a BIN map extracted from a high-density consensus map of barley (Marcel et al. 2007a). The QTLs were originally mapped on three individual barley linkage maps. Length of QTL boxes (with pattern) correspond to the LOD-1 support interval (from peak marker) and QTL lines are extended to the LOD-2 support interval, based on results of rMQM. The parental line contributing the allele for resistance and the LOD value obtained by rMQM are indicated on the right side of the QTLs. Within chromosome bars, LOD-2 support intervals of QTLs for partial resistance to barley leaf rust (Marcel et al. 2007a; Jafary et al. 2008) are indicated in black if overlapping with the LOD-1 support interval, in dark grey if overlapping with the LOD-2 support interval and in clear grey if not overlapping with QTL(s) for nonhost resistance presented in this study. The name of the QTL for partial resistance (Rphq-) is indicated on the left side of the chromosome bars when its peak marker(s) was within the LOD-1 support interval of QTL(s) for nonhost resistance. The 63 loci in bold are defense gene homologue (DGH) based markers. The ruler on the left end side of the figure indicates the distance in centiMorgans (Kosambi) from the top of each chromosome*



to redundancy, and the resistance would be complete and durable. The resistance of barley to wheat stripe rust (*P. striiformis* f. sp. *tritici*) (Pahalawatta and Chen 2005; Sui et al. 2010) and of wheat to barley stripe rust (*P. striiformis* f. sp. *hordei*) (Johnson and Lovell 1994; Rodrigues et al. 2004) seem indeed to be largely due to major gene(s) for hypersensitivity resistance. However, in the crosses with SusPtrit and in the OWB we found only one locus (on chromosome 1H) carrying a major *R* gene for hypersensitive resistance to heterologous rusts; one contributed by Vada was effective to *P. hordei-secalini* (Jafary et al. 2006), and the other, contributed by Rec (the recessive parent of OWB), was effective to *P. hordei-secalini* and *P. hordei-murini* (Jafary et al. 2008). However, the *R* gene was backed-up by quantitative resistance. We conclude that *R* genes contribute occasionally to the resistance to heterologous rusts, but genes for quantitative resistance play a much more prominent role.

Pathogen specificity or broad spectrum effectiveness?

The QTLs found to underlay the near-nonhost status of barley to heterologous rust pathogens had overlapping rust specificity, i.e. they were typically effective to only one or two rusts, and only few were effective to at least four heterologous rust species (Fig. 4; Jafary et al. 2006, 2008).

This combination of specificity and broader spectrum effectiveness was also found in the analysis of barley germplasm and the development of the experimental line SusPtrit (Atienza et al. 2004). Accessions that were (moderately) susceptible to one heterologous rust had a high chance to be also somewhat susceptible to other heterologous rusts. The accessions Trigo Biasa (from Indonesia) and L94 (from Ethiopia) were rather susceptible to most heterologous rusts to which barley is a near-nonhost. The line SusPtrit, selected for susceptibility to *P. triticina*, was also very susceptible to the other heterologous rusts to which barley is a near-nonhost, again suggesting a broad spectrum effectiveness of the resistance alleles that were selected against in SusPtrit.

On the other hand, some lines were susceptible to one heterologous rust pathogen and completely resistant or even immune to others. In several cases, differential interaction occurred between barley accessions and heterologous rusts. This suggests that there are also genes with high rust species specificity.

High allele frequencies of the same resistance genes?

Is the resistance of members of a nonhost species to a heterologous pathogen due to shared resistance genes, or are different members of the nonhost species each resistant due to a different set of genes? One might expect that almost all immune barleys would carry the common, i.e. resistance, alleles for almost all loci on which SusPtrit carries the (probably rare) susceptibility allele. In that case, in crosses between SusPtrit with any immune barley almost the same loci should be found to govern the response to a certain heterologous rust pathogen. This was not what we found (Jafary et al. 2008).

In each mapping population, different sets of quantitative genes explained resistance to particular heterologous rusts (Fig. 4). For example, Vada and Cebada Capa share only one QTL from nine for resistance to *Phm* and *Phs* (Fig. 4). Therefore, immunity to a heterologous rust may be due to many different sets of QTLs, indicating a high redundancy of genes for resistance in the barley species.

These observations also suggest that even immune barleys contain some susceptibility alleles on certain QTLs, and therefore will not segregate for the same QTLs when crossed with SusPtrit. This possibility is consistent with the observation that crossing exotic barley lines with slight susceptibility to heterologous rusts, resulted in transgression towards increased susceptibility, leading to the research line SusPtrit with extreme susceptibility (Atienza et al. 2004).

Do the resistance QTLs represent defense-related genes?

At first thoughts, genes involved in plant defense are not likely candidates to determine the (non)host status of plants. These genes, like for example peroxidases, MAPKinases, super-oxide dismutase, and BAX inhibitor 1,are generally quite conserved and should be effective to a broad spectrum of the same class of pathogens. The barley lines SusPtrit and SusPmur (Atienza et al. 2004) are very susceptible to some heterologous rusts, but they are fully resistant to several rusts to which barley is probably a full nonhost. Therefore, it is unlikely that the susceptibility of these lines to some heterologous rusts is due to structurally defective key genes for basal defense. However, an option is that such basal resistance genes contain minor sequence differences in promoter and/or coding sequences that would be the point of action of effectors to specifically reprogram such genes in order to suppress defense (Niks and Marcel 2009).

The QTLs for basal resistance to *Puccinia hordei* in three barley mapping populations tended to co-locate significantly with Defense Gene Homologs (DGHs) (Marcel et al. 2007a). The DGHs that associated with the QTLs for nonhost resistance included peroxidases, MAPKinases, superoxide dismutase and BAX inhibitor 1. We extended this work by focusing on peroxidase (*prx*) genes. We followed the Motif-directed Profiling approach that targets conserved motifs in functional domains of gene family members (Gonzalez et al. 2010). On the basis of the conserved FHDCFV and VSCADI motifs of *prx* genes we mapped 200 *prx* profiling markers, inserted them into a consensus linkage map of barley (Aghnoum et al. 2010) and compared their positions with those of 63 QTLs for nonhost resistance to heterologous rust fungi, 19 QTLs for basal resistance to *P. hordei*, and 23 QTLs for basal resistance to barley powdery mildew (caused by *Blumeria graminis* f. sp. *hordei*). The 5 cM BIN system of the barley consensus map was used to test by chi-squared test for a possible association between the distribution of the QTLs and the distribution of of the *prx* profiling markers. The QTLs for resistance to heterologous rust fungi, QTLs for basal resistance to *P. hordei*, and for basal resistance to barley powdery mildew were all significantly associated with *prx* based markers (Table 1; Gonzalez et al. 2010). Our data imply that 61% of the QTLs for partial resistance to *P. hordei*, 61% of the QTLs for resistance to *B. graminis* and 47% of the QTLs for non-host resistance to other *Puccinia* species co-localize with *prx* based markers. This suggests that that *Prx* genes may represent a substantial part of the targets of effectors that aim to suppress the basal defense of plants.

Table 1 ***Chi-squared values (in bold) for independent distribution of Prx-based markers and barley QTLs for partial resistance to*** *Puccinia hordei* ***(QTLph), nonhost resistance to heterologous cereal and grass rusts (QTLnh), and to*** *Blumeria graminis* ***(QTLbg)***

	Prx	QTLph	QTLnh	QTLbg
Marker no.	200	19	63	23
BIN no[1]	63	18	47	23
O (E)[2]		11 (5.2)	22 (13.4)	14 (6.7)
χ^2		9.9*	9.9*	12.6**

[1] The number of barley BINs (5 cM) occupied by the (peak) markers for the respective class of QTLs or markers

[2] Number of BINs observed to be co-occupied by a QTL peak marker and a Prx-targeted marker (the expected number of co-occupied BINs in case of independent distribution is in brackets)

** P<0.001, * P<0.05

Are genes that determine nonhost resistance also implicated in basal defense to the related adapted pathogen?

In almost all plant-pathosystems there is variation within the susceptible class of plant accessions, ranging from moderately to extremely susceptible. Such variation is known as quantitative basal (or partial) resistance. There is evidence that this type of resistance is part of the same system as nonhost resistance;

- at least in powdery mildew and rust fungi, the quantitative basal resistance is typically predominantly pre-haustorial and associated with formation of cell wall reinforcements, also called cell wall appositions or papillae (O'Connell and Panstruga 2006), as in nonhost resistance to these pathogen classes.
- genetic segregation for resistance to rusts to which barley has a near-nonhost status tends to be associated with segregation for levels of quantitative basal resistance to *P. hordei* in barley, indicating that some of the same genes are involved (Zhang et al. 1994).

It would also make sense that nonhost resistance to heterologous pathogens and quantitative basal resistance are associated. Assuming that genetic factors determine differences between plant species in (non)host status to a would-be pathogen, it may also be expected that such genetic factors differ within a host plant species to that micro-organism, making one host plant genotype a more suitable host individual than another genotype of the same species.

Indeed, mapping of the QTLs for resistance to heterologous rust fungi (Fig. 4) confirms that many of those QTLs map to positions where QTLs for basal resistance to *P. hordei* in the same or different mapping populations are located. This association was significant ($p < 0.01$).

Perspectives

The near-nonhost status of barley to several rust fungi that are adapted to grasses and other cereals (Atienza et al. 2004) offers great perspectives in understanding the genetics and specificity of resistance to unadapted specialized pathogens. Tools that have been developed are:

- hypersusceptible lines in which alleles for susceptibility to heterologous rusts have been accumulated (Atienza et al. 2004)
- a large collection of rusts of different grasses and cereals
- a large number of mapping populations segregating for their level of (non)host resistance to rust fungi of cereals and grasses
- BAC libraries of Vada, SusPtrit (Marcel et al. unpublished), Cebada Capa (Isidore et al. 2005) and Morex (Yu et al. 2000)
- advances in the development of barley physical maps (Künzel et al. 2000; Stephens et al. 2004) and very dense marker linkage maps (e.g. Marcel et al. 2007a; Stein et al. 2007; Aghnoum et al. 2010)
- NIL-QTL lines for basal resistance have been developed in susceptible barley and barley with high levels of basal resistance (Marcel et al, 2007b, 2008) demonstrating that the QTLs are robust, and that most of them not obviously do not depend on genetic background.

In this contribution, we presented data on the genetics of (near-)nonhost resistance of barley to heterologous rusts. These rusts enter through stomata to infect mainly the barley mesophyll cells. That infection style does not allow testing of candidate genes by reverse genetics through transient transformation by biolistics (Schweizer et al. 1999), since that method only transforms epidermal cells. Such methods are relevant for powdery mildew fungi. Recently, we started to accumulate genes in barley for unusual but natural susceptibility to the wheat powdery mildew pathogen (*Blumeria graminis* f. sp. *tritici*) (Aghnoum and Niks 2010), and lines with very high and very low levels of quantitative basal resistance to *B. graminis* f. sp. *hordei* (Aghnoum and Niks 2011). That material will allow similar studies and comparisons as done here for rust fungi, but with the additional advantage of the amenability to reverse genetics approaches.

Acknowledgements

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References

Aghnoum R, Marcel TC, Johrde A, Pecchioni N, Schweizer P, Niks RE (2010) Basal resistance of barley to barley powdery mildew: connecting QTLs and candidate genes. Mol Plant-Microbe Interact 23:91-102

Aghnoum R, Niks RE (2010) Specificity and levels of non-host resistance of barley to non-adapted *Blumeria graminis* forms. New Phytologist 185:275-284

Aghnoum R, Niks RE (2011) Transgressive segregation for very low and high levels of basal resistance to powdery mildew in barley. J Plant Physiol 168:45-50

Atienza SG, Jafary H, Niks RE (2004) Accumulation of genes for susceptibility to rust fungi for which barley is nearly a nonhost results in two barley lines with extreme multiple susceptibility. Planta 220:71-79

Caldo RA, Nettleton D, Peng J, Wise RP (2006) Stage-specific suppression of basal defense discriminates barley plants containing fast- and delayed-acting Mla powdery mildew resistance alleles. Molec Plant-Microbe Interact 19:939-947

Catanzariti A-M, Dodds PN, Lawrence GJ, Ayliffe MA (2006) Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. Plant Cell 18:243-256

Christopher-Kozjan R, Heath MC (2003) Cytological and pharmacological evidence that biotrophic fungi trigger different cell death execution processes in host and nonhost cells during the hypersensitive response. Physiol Mol Plant Pathol 62:265-275

Coaker G, Falick A, Staskawicz B (2005) Activation of a phytopathogenic bacterial effector protein by a eukaryotic cyclophilin. Science 308:548-550

Costa JM, Corey A, Hayes PM, Jobet C, Kleinhofs A, Kopisch-Obusch A, Kramer SF, Kudrna D, Li M, Riera-Lizarazu O, Sato K, Szucs P, Toojinda T, Vales MI, Wolfe RI (2001) Molecular mapping of the Oregon Wolfe barleys: a phenotypically polymorphic doubled-haploid population. Theor Appl Genet 103:415-424

Da Cunha L, McFall AJ, Mackey D (2006) Innate immunity in plants: a continuum of layered defenses. Microbes Infect 8:1372-1381

Elmhirst JF, Heath MC (1987) Interactions of the bean rust and cowpea rust fungi with species of the Phaseolus-Vigna plant complex. I. Fungal growth and development. Can J Bot 65:1096-1107

González AM, Marcel TC, Kohutova Z, Stam P, van der Linden CG, Niks RE (2010) Peroxidase profiling reveals genetic linkage between peroxidase gene clusters and basal host and non-host resistance to rusts and mildew in barley. PLoS-ONE 5(8):e10495 doi:10.1371/journal.pone.0010495

Gurlebeck D, Thieme F, Bonas U (2006) Type III effector proteins from the plant pathogen Xanthomonas and their role in the interaction with the host plant. J Plant Physiol 163:33-255

Heath MC (1974) Light and electron microscope studies of the interactions of host and nonhost plants with cowpea rust Uromyces phaseoli var. vignae. Physiol Plant Pathol 4:403-414

Heath MC (1981) Resistance of plants to rust infection. Phytopathology 71:971-974

Heath MC (1991) Tansley Review No. 33. Evolution of resistance to fungal parasitism in natural ecosystems. New Phytologist 119:331-343

Heath MC (2000) Nonhost resistance and nonspecific plant defenses. Curr Opinion Plant Biol 3:315-319

Heath MC (2001) Non-host resistance to plant pathogens: nonspecific defence or the result of specific recognition events? Physiol Mol Plant Pathol 58:53-54

Heath MC (2002) Cellular interactions between biotrophic fungal pathogens and host or nonhost plants. Can J Plant Pathol 24:259-264

Heath MC (2003) Nonhost resistance in plants to microbial pathogens. In: Ezekowitz RAB, Hoffmann JA (eds) Innate immunity. Humana Press Inc. Totowa, NJ, USA, pp.47-57

Ingle RA, Carstens M, Denby KJ (2006) PAMP recognition and the plant-pathogen arms race. BioEssays 28:880-889

Isidore E, Scherrer B, Bellec A, Budin K, Faivre-Rampant P, Waugh R, Keller B, Caboche M, Feuillet C, Chalhoub B (2005) Direct targeting and rapid isolation of BAC clones spanning a defined chromosome region. Funct Integr Genomics 5:97-103

Jafary H, Szabo LJ, Niks RE (2006) Innate nonhost immunity in barley to different heterologous rust fungi is controlled by sets of resistance genes with different and overlapping specificities. Mol Plant-Microbe Interact 19:1270-1279

Jafary H, Albertazzi G, Marcel TC, Niks RE (2008) High diversity of genes for nonhost resistance of barley to heterologous rust fungi. Genetics 178:2327-2339

Johnson R, Lovell NK (1994) Genetics of resistance of wheat to barley attacking races of *Puccinia striiformis*. Cereal Rusts and Powdery Mildews Bull 22:32-40

Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323-329

Kamoun S (2006) A catalogue of the effector secretome of plant pathogenic oomycetes. Annual Rev Phytopathol 44:41-60

Künzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barely genome based on translocation breakpoints. Genetics 154:397-412

Lahaye T, Bonas U (2001) Molecular secrets of bacterial type III effector proteins. Trends in Plant Sci 6:479-485

Li X, Lin H, Zhang W, Zou Y, Zhang J, Tang X, Zhou J-M (2005) Flagellin induces innate immunity in nonhost interactions that is suppressed by *Pseudomonas syringae* effectors. Proc Natl Acad Sci USA 102:12990-12995

Lipka V, Dittgen J, Bednarek P, Bhat R, Wiermer M, Stein M, Landtag J, Brandt W, Rosahl S, Scheel D, Llorenta F, Molina A, Parker J, Somerville S, Schulze-Lefert P (2005) Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. Science 310:1180-1183

Mackey D, McFall AJ (2006) MAMPs and MIMPs: proposed classifications for inducers of innate immunity. Mol Microbiol 61:1365-1371

Mains, E.B., 1933. Host specialization in the leaf rust of grasses, *Puccinia rubigo-vera*. Papers of the Michigan Acad. Science, Arts and Letters 17:289-394

Marcel TC, Varshney RK, Barbieri M, Jafary H, de Kock MJD, Graner A, Niks RE (2007a). A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. Theor Appl Genet 114:487-500

Marcel TC, Aghnoum R, Durand J, Varshney RK, Niks RE (2007b). Dissection of the barley 2L1.0 region carrying the 'Laevigatum' quantitative resistance gene to leaf rust using near isogenic lines (NIL) and sub-NIL. Mol Plant-Microbe Interact 20:1604-1615

Marcel TC, Gorguet B, Truong Ta M, Kohutova Z, Vels A, Niks RE (2008) Isolate-specificity of quantitative trait loci for partial resistance of barley to Puccinia hordei confirmed in mapping populations and near-isogenic lines. New Phytologist 177:743-755

Mysore KS, Ryu C-M (2004) Nonhost resistance: how much do we know? Trends in Plant Science 9:97-104

Niks RE (1987) Nonhost plant species as donors for resistance to pathogens with narrow host range. I. Determination of nonhost status. Euphytica 36:841-852

Niks RE (1988) Nonhost plant species as donors for resistance to pathogens with narrow host range. II. Concepts and evidence on the genetic basis of nonhost resistance. Euphytica 37:89-99

Niks RE Marcel TC (2009) Nonhost resistance and basal resistance: how to explain specificity? New Phytologist 182:817-828

Nomura K, Melotto M, He S-Y (2005) Suppression of host defence in compatible plant-Pseudomonas syringae interactions. Curr Opinion Plant Biol 8:361-368

Nürnberger T, Lipka V (2005) Non-host resistance in plants: new insights into an old phenomenon. Molec Plant Pathol 6:335-345

O'Connell RJ, Panstruga R. (2006) Tête à tête inside the plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. New Phytologist 171:699-718

Pahalawatta V, Chen XM (2005) Inheritance and molecular mapping of barley genes conferring resistance to wheat stripe rust. Phytopathology 95:884-889

Panstruga R (2003) Establishing compatibility between plants and obligate pathogens. Curr Opinion Plant Biol 6:320-326

Rodrigues P, Garrood JM, Shen QH, Smith PH Boyd LA (2004) The genetics of non-host disease resistance in wheat to barley yellow rust. Theor Appl Genet 109:425-432

Schulze-Lefert P, Panstruga R (2011) A molecular evolutionary concept connecting nonhost resistance, pathogen host resistance and pathogen speciation. Trends in Plant Science 16:117-125

Schweizer P, Pokorny J, Abderhalden O, Dudler R (1999) A transient assay system for the functional assessment of defense-related genes in wheat. Mol Plant-Microbe Interact 12:647-654

Schweizer P (2007) Nonhost resistance of plants to powdery mildew - New opportunities to unravel the mystery. Physiol Mol Plant Pathol 70:3-7

Shao F, Golstein C, Ade J, Stoutemyer M, Dixon JE, Innes RW (2003) Cleavage of Arabidopsis PBS1 by a bacterial type III effector. Science 301:1230-1233

Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, Kota R, Varshney RK, Perovic D, Grosse I, Graner A (2007) A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor Appl Genet 114:823-839

Stephens JL, Brown SE, Lapitan NLV, Knudson DL (2004) Physical mapping of barley genes using an ultrasensitive fluorescence in situ hybridization technique. Genome 47:179-189

Sui X, He Z, Lu Y, Wang Z, Xia X (2010) Molecular mapping of a non-host resistance gene *YrpstY1* in barley (*Hordeum vulgare* L.) for resistance to wheat stripe rust. Hereditas 147:176-182

Thordal-Christensen H (2003) Fresh insights into processes of nonhost resistance. Curr Opinion Plant Biol 6:351-357

Truman W, Torres de Zabala M, Grant M (2006) Type III effectors orchestrate a complex interplay between transcriptional networks to modify basal defence responses during pathogenesis and resistance. Plant J 46:14-33

Underwood W, Somerville SC (2008) Focal accumulation of defences at sites of fungal pathogen attack. J Exp Bot 59:3501-3508

Yu Y, Tomkins JP, Waugh R, Frisch DA, Kudrna D, Kleinhofs A, Brueggeman RS, Muehlbauer GJ, Wise RP, Wing RA (2000) A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. Theor Appl Genet 101:1093-1099

Zhang H-S, de la Rosa R, Rubiales D, Lubbers HH, Molenveld JW, Niks RE (1994) Role of partial resistance to *Puccinia hordei* in barley in the defence of barley to inappropriate rust fungi. Physiol Mol Plant Pathol 45:219-228

Rpg1-mediated durable stem rust resistance: mechanisms of action

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Abstract

Stem rust, caused by *Puccinia graminis* f. sp. *tritici,* is a devastating disease on wheat and barley. A single barley gene, *Rpg1,* has provided durable resistance since its commercial introduction in the 1940s. The cloned *Rpg1* gene encodes a protein with two tandem protein kinase domains, one an active kinase (pK2) and one a pseudokinase (pK1). Function of both domains is required for resistance. The gene is constitutively expressed in all tissues with elevated levels in the epidermis. It is mostly cytoplasmic with small, but significant amount associated with the cell membrane. We have been studying this gene and protein to try to understand how it works and why it has been so durable. Here we report our most recent results showing that RPG1 is phosphorylated within 5 min after urediniospores from avirulent, but not virulent, races land on the leaf surface. Two effector proteins were isolated from the ungerminated spores and shown to work cooperatively to induce RPG1 phosphorylation and eventual degradation. The proteins were identified as a hypothetical protein (PGTG_10537.2) with a fibronectin type III and BRCA1 C-terminal domains and vacuolar protein sorting-associated protein 9 (PGTG_16791). The rapidity of the effector function and the nature of the two protein effectors indicate that a unique mechanism for effector entry and signaling in the host cell is involved. This hypothetical mechanism may be similar to what is observed in animal cells where fibronectin proteins with an RGD-binding domain act to mediate communications between the extracellular matrix and plasma membrane.

Key Words

Disease resistance, kinase, phosphorylation, RGD

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Abbreviations

aa – amino acid; bp – base pairs; cv – cultivar; eQTL – expression QTL; HR – hypersensitive response; kDa – kiloDaltons; MALDI-TOF MS – matrix associated laser desorption ionization-time of flight mass spectra; *Pgt – Puccinia graminis* f. sp. *tritici*; *Pgs - Puccinia graminis* f. sp. *secalis*; pK1 – protein kinase domain 1; pK2- protein kinase domain 2; QTL – quantitative trait loci; RGD – arginine-glutamic acid-aspartic acid

Introduction

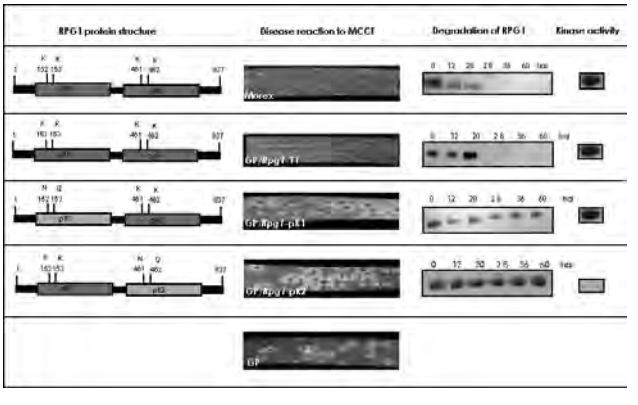
Rusts are biotrophic fungal pathogens that cause disease on almost all plants. Among them *Puccinia* is the largest genus and contains the most economically important species that attack crops such as wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), rye (*Secale cereale* L.) and many forage grasses. The stem rust pathogen *Puccinia graminis* attacks wheat and barley and is composed of several *formae speciales*; for example, *Puccinia gramini* f. sp. *tritici* (*Pgt*) attacks primarily wheat and barley whereas *P. g.* f. sp. *secalis* (*Pgs*) attacks primarily rye and barley. Development of resistant varieties is the most economical and environmentally friendly means of combating losses due to rust (Steffenson 1992; Kolmer 2001;). Stem rust resistance in wheat has been achieved by pyramiding multiple genes in cultivars whereas in barley a single durable gene, *Rpg1,* has been responsible for controlling stem rust in barley cultivars since its introduction in the 1940s. Thus, cloning and characterization of the *Rpg1* gene function has been our main emphasis. Understanding how this single gene has been effective in controlling stem rust in barley should facilitate understanding and using other stem rust resistance genes against new and virulent races such as TTKSK (Ug99) in spite of their extreme variability and virulence.

Origin of the *Rpg1* gene in barley can be traced to a bulk landrace imported by the USDA from Canton Lucerne in Switzerland in 1914. This land race gave rise to sister cvs. Chevron and Peatland. A third potential source, cv. Kindred, arose from a single rust-free barley "mutant" identified in 1935 by farmer Sam Lykken in a heavily rusted field of Wisconsin 37 barley. This presumably different stem rust resistance source, however, most likely came from a seed admixture as proposed by Steffenson (1992) and confirmed by molecular analyses that showed all three sources contain an identical allele (Brueggeman et al. 2002). We have not been able to trace the *Rpg1* gene origin beyond Switzerland since all wild barley *Hordeum vulgare* ssp. *spontaneum* lines tested only had defective *Rpg1* genes (Mirlohi et al. 2008).

Cloning and characterization of the *Rpg1* gene and protein

The *Rpg1* gene was cloned in our laboratory by a map-based approach from cv. Morex, which traces it from Kindred and Peatland in its pedigree. The correct identification was confirmed by allele sequencing and by stable transformation of cv. Golden Promise known to harbor a deletion of the *Rpg1* gene region (Brueggeman et al. 2002; Horvath et al. 2003). The 4,466 bp genomic gene sequence encodes an in silico translated protein of 837 aa and 94.5 kDa (Brueggeman et al. 2002). The protein consists of an N terminal leader sequence, two tandem protein kinase-like domains designated pK1 and pK2 and a C terminal region. The pK1 domain is a pseudokinase while the pK2 domain is an active kinase; both domains are required for stem rust resistance (Nirmala et al. 2006). The function of the pK2 domain appears to be phosphorylation while the function of the pK1 domain remains unclear. Fig. 1 summarizes the characterization of the RPG1 protein and functions.

Fig. 1 ***Summary of RPG1 characterization. Columns from left to right show cartoon RPG1 structure; phenotypes in response to race MCCF infection; RPG1 protein response to infection timed in hours after inoculation (hai); and kinase autocatalytic activity measured in vitro. Row 1 - from top indicates wild-type RPG1 with two kinase domains domains and the adjoining KK aa; cv. Morex shows resistant reaction to stem rust, the RPG1 protein is degraded 20 - 28 hai, and it is catalytically active in vitro; Row 2 – shows the same protein in the Golden Promise (GP) transgenic line, it is highly resistant to stem rust, the protein is degraded and it has an active kinase just like in the wild-type above; Row 3 – the pK1 mutant KK152,153NQ is susceptible, the protein fails to degrade, but it retains autocatalytic activity presumably due to the pK2 domain; Row 4 – the pK2 mutant KK461/462NQ is highly susceptible, the protein is not degraded and it has lost the kinase activity. Row 5 – shows the cv. GP susceptibility to stem rust for comparison with the transgenic lines. GP does not have the Rpg1 gene and it does not produce an RPG1 protein***



The *Rpg1* mRNA is expressed constitutively in all tissues tested and in similar amounts except the epidermis where the levels are elevated (Rostoks et al. 2004). The significance of this observation was not clear until our most recent experiments (to be described later) suggesting that *Pgt* effectors are detected while still on the surface of the leaf. The RPG1 protein was also studied for its distribution within the cell and found to be mostly cytoplasmic except for a small fraction associated with membranes (Nirmala et al. 2006). Surprisingly, inoculation with avirulent, but not virulent *Pst* urediniospores led to a rapid (within 20-24 h) degradation of the RPG1 protein via the ubiquitin-proteasome pathway and this degradation was essential for stem rust resistance (Fig. 1) (Nirmala et al. 2007).

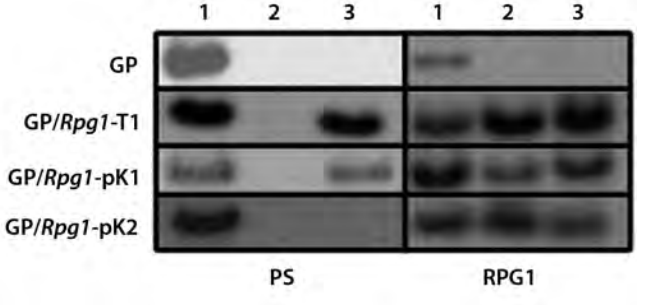
Experiments to identify other genes or proteins required for *Rpg1*-mediated stem rust resistance have resulted in several putative candidates. Mutant analysis identified a gene designated *Rpr1* which is required for *Rpg1* stem rust resistance. Map-based cloning identified the *Rpr1* gene to encode a protein kinase, but the role of this protein in stem rust resistance remains unclear (Zhang et al. 2006). Yeast two hybrid analyses identified several RPG1 interactors, among them *HvPti1* and *HvRin4* (Nirmala unpublished). Further studies with these genes, however, failed to identify any strong function in stem rust resistance. In order to identify minor QTL that might contribute to *Rpg1*-mediated stem rust resistance, we conducted eQTL mapping of *Pgt* interaction phenotypes as quantitative traits (Druka et al. 2008). This resulted in the identification of six putative loci. The strongest QTL was at the *Rpg1* locus itself, as expected. The second strongest QTL was at chromosome 2H, unfortunately in a region including several hundred genes, among them a transduction histidine kinase, which was also previously identified as highly downregulated in the *Rpg1* suppressor mutant *rpr1* (Zhang et al. 2006). Additional QTL were identified on chromosomes 3H, two on 5H(barley chromosome 7), one on 7H(1) and a weak one on 5H(7). Some of these QTL coincided with a heat shock protein 17 (*Hsp17*) gene. This is potentially significant since HSP17 was also identified as an RPG1 interactor in a yeast two-hybrid assay (Nirmala et al. unpublished).

RPG1 phosphorylation and *Pgt* effectors

Upon examination of RPG1 in vivo phosphorylation status, we discovered that the protein is rapidly (within 5 min.) phosphorylated upon inoculation of the seedling leaves with spores from avirulent, but not from virulent races (Nirmala et al. 2010). Transgenic mutants with an inactive pK2 domain, i.e. unable to autophosphorylate in vitro, fail to phosphorylate RPG1 in vivo and are

susceptible to stem rust (Fig. 2). This observation is very significant because the spores have not yet germinated on the leaf surface in 5 min. The typical route of infection is believed to be when the spores germinate, penetrate the cells through the stomata and establish a haustorium. Furthermore the haustorium is believed to be the site of avirulence protein synthesis and transport to the cytoplasm initiating the resistance response. The rapidity of the phosphorylation suggested that the effectors responsible for the phosphorylation must reside on the spore surface since it seems unlikely that there would be sufficient time for effectors inside the spores to be activated and transported to where they could interact with the barley cells.

Fig. 2 ***RPG1 phosphorylation in vivo is dependent on a functional protein kinase 2 domain. Plants were inoculated with Puccinia graminis f. sp. tritici spores, and sampled 5 min later. Proteins were precipitated with RPG1-specific antibody and phosphorylated bands detected in Western blot with phosphoserine (PS)-specific antibody (left panel; lane 3). The right panel was visualized with an RPG1 specific antibody to demonstrate protein loading in all lanes. Lane 1 – in vitro phosphorylated RPG1 control; Lane 2 – uninoculated control. For description of GP and the transgenes see Fig. 1 legend***



Armed with this knowledge, we proceeded to try to isolate the effector molecules. The breakthrough came with the knowledge that RGD peptides were shown to block adhesion of *Uromyces appendiculatus* (bean rust pathogen*)* spores and prevent appresoria formation and successful infection (Correa et al. 1996). Experimental treatment of the *Pst* spores with RGD peptides prior to inoculation on susceptible barley cvs. did indeed block infection and disease development (Nirmala et al. submitted). RGD affinity chromatography was also previously used to isolate a protein with RGD-binding capacity (Schindler et al. 1989). Therefore we proceeded with RGD affinity chromotagraphy of avirulent *Pst* spore extracts. The crude RGD chromotagraphy product when applied to stem rust resistant barley leaves induced RPG1 phosphorylation and degradation and HR (Fig. 3).

Fig. 3 A:***The crude RGD column eluate induced RPG1 phosphorylation and degradation. The eluate was applied to seedling leaves and samples taken at indicated intervals. The samples were precipitated with either phosphoserine (PS) or phosphothreonine (PT) antibodies, separated on gels and visualized with an RPG1-specific antibody. hai, hours after infection***

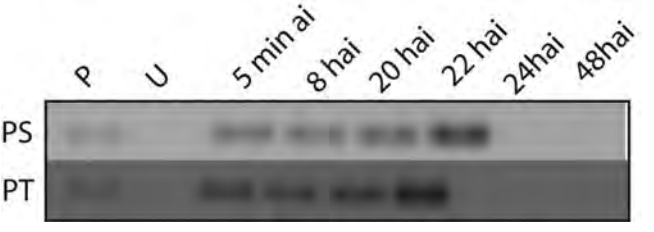
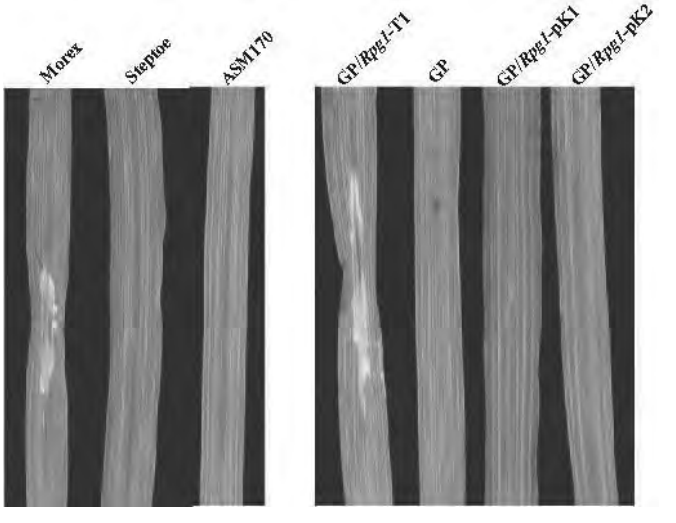


Fig. 3B:***The crude RGD column eluate induced hypersensitive responses in barley lines with afunctional Rpg1 gene. ASM170 – barley line with a recombination in the Rpg1 gene resulting in apart cv. Morex and part cv. Steptoe sequence and lack of Rpg1 function. For GP and the transgene descriptions see Fig. 1 legend***



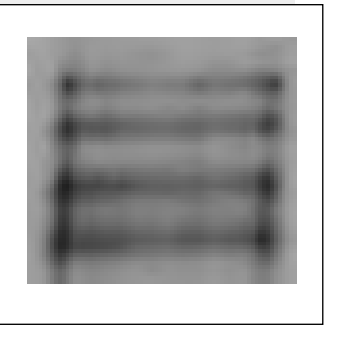
Fractionation of the crude product on gradient gel electrophoresis produced four distinct protein bands (Fig. 4). The protein bands were excised and subjected to MALDI-TOF MS/MS analysis and proteins identified by comparison with data from the *Puccinia* database (http://www.broadinstitute.org/annotation/genome/puccinia_group/GeneFinding.html).

The highest molecular weight band was identified as a hypothetical protein (PGTG_10537.2) with fibronectin type III and BRCA1 C-terminal domains. Fibronectin proteins are known to have an RGD-binding motif; therefore this protein is referred to as RGD-binding protein. The BRCA1 C-terminal domain containing proteins are widespread throughout all

Fig. 4 *RGD column chromatography followed by density gradient gel electrophoresis revealed four protein bands. The bands were identified by MALDI-TOF and MS/MS and comparison with Puccinia graminis f. sp. tritici sequence*

Band	Peptides identified	Protein	Domains
Band 1	MTGSFGGK (800.36); ***GAEEEAEPR (987.43)*;*** FSAALSDILK (1064.59); HVRTESDSEK (1187.56); ANSAPTITTISDSGR (1490.79); QHERANSAPTITTISDSGR (2041); ***SNLHSSTPISPSNTAEANSSSPGPSGASSR (2884.3)***	PGTG_10537	Fibronectin type III; BRCA1 ALQLYPVVQPSWLQACLHER (2388)
Band 2	SPVIENIK (899.5197); AIDSLLEMS (978.48); SHLSSTDDR (1017.45); TLISNLQYIQRFR (1651.29); MREIPDGPWDPR (1468.7002); SWLFTTVPQLAEKAVSKPLNAIAR (2640.4872);	PGTG_16791 HLDLSLPSDEADGFMEFAK (2121.97);	VPS9; CUE
Band 3 &	***GAEEEAEPR (987.43);***	***truncated PGTG_10537***	
Band 4	***SNLHSSTPISPSNTAEANSSSPGPSGASSR (2884.3);***		

* - Bold italic type indicates identical peptides



kingdoms and are involved with cell cycle regulation, DNA metabolism, phospho-peptide binding and protein-protein interactions. The next highest molecular weight protein was identified as a vacuolar protein sorting associated protein 9 (VPS9) with a CUE domain (PGTG_16791). The VPS9 domain catalyzes nucleotide exchange on Rab5 linking regulation of cell signaling cascades with intracellular receptor trafficking through the endocytic pathway (Carney et al. 2006). The Rab5 protein was shown to have a number of functions dealing with regulation of the endocytic pathway including signaling to the nucleus (Zerial and McBride 2001). The CUE (coupling of ubiquitin conjugation to ER degradation) domain of VPS9p binds directly to monoubiquitin (Prag et al. 2003). VPS9 proteins are not known to have an RGD-binding motif, therefore it was probably isolated due to interaction with the RGD-binding protein. The other two bands are truncated fragments of the RGD-binding protein.

The crude protein sample induced RPG1 phosphorylation and degradation and HR. In order to determine which one of the proteins were responsible for these activities, they were cloned and produced in yeast in purified form. The RGD-binding protein induced RPG1 phosphorylation, but not degradation as would be expected with spore inoculation. The VPS9 protein by itself did not induce either one of the phenotypes. Both proteins were required together to induce RPG1 phosphorylation and degradation at about 20-24 h as is the case with spore inoculation and treatment with the crude protein preparation. The same was true for the HR, which only showed up when both proteins were applied together on barley cvs. with a functional *Rpg1* gene. The HR effect mapped to the *Rpg1* gene.

Transcription analysis showed that the RGD-binding and VPS9 mRNA are present in ungerminated spores and constitutively expressed in germinating spores, infected leaves and haustoria of all three avirulent races tested (MCCF, HJHK, SCCLc7a). This suggests that haustoria are not the primary source of these effectors, but does not exclude the possibility that additional effectors synthesized there may reinforce their activity. Expression of the RGD-binding or VPS9 mRNA in the virulent race QCCJ was not detected. In the virulent *Pgs* isolate 92-MN-90 the RGD-binding protein mRNA was expressed with only a few aa changes in the predicted protein, but the VPS9 mRNA had a G insertion that resulted in a frame shift mutation, an early stop codon and a predicted truncated protein.

The co-elution of the RGD-binding and VPS9 proteins suggested that they interact in vivo. Yeast two-hybrid analysis confirmed this and also showed that each of the proteins interacts with wild-type RPG1, but not with non-functional mutated protein. These results help to explain the observations that both proteins are required to induce timely RPG1 phosphorylation and degradation and HR.

Models and speculation

Much remains to be learned about *Rpg1* gene action and mechanisms, but some intriguing details are starting to emerge. It is well established that RPG1 phosphorylation is essential, but not sufficient for disease resistance. The same is true for RPG1 degradation.

The two effectors we have isolated have interesting properties, suggesting possible modes of action based on similar protein functions in animal system. The fibronectin domain in the RGD-binding protein probably signals across the extracellular matrix (cell wall in plants), the plasma membrane, and the cytoskeleton as in animals (Giancotti and Ruoslahti 1999). RPG1 molecules associated with the plasma membrane may be the initial recipients of this signaling. This is suggested by the existence of part of the RPG1 protein in membrane bound form and the elevated levels of RPG1 in the epidermis. However, RGD-binding proteins interact with RGD motifs and RPG1 does not have an RGD motif. RPG1 does interact with the RGD-binding protein in yeast, possibly through some other domains. In plants integrin-like proteins were identified and shown to function in cell wall plasma membrane adhesion, facilitated by RGD motif proteins (Lu et al. 2007). The *Pst* RGD-binding protein may have a virulence function of disrupting these RGD motif-facilitated functions. The *Rpg1* resistance gene may have evolved to recognize this virulence function and confer resistance.

BRCA1 C-terminal (BRCT) domains are common and appear to be mostly involved with protein-protein interactions, but a small subset has DNA-binding activity (Kobayashi et al. 2010). The BRCT domain might play a role in protein-protein interactions, but how it affects disease resistance or virulence function is not clear at this time.

The VPS9 protein catalyzes GDP-GTP nucleotide exchange in Rab5 protein. Rab5 proteins are involved in regulating endocytic pathways including signaling to the nucleus. The CUE domain is involved in binding to monoubiquitin and therefore in protein degradation. RPG1 is degraded upon infection with the stem rust fungus by the ubiquitin pathway. What role the RPG1 degradation plays in stem rust resistance is not clear, but it is known to be important. Possible hypotheses include release of disease resistance complex proteins from negative regulation by RPG1 and limiting the extent of cell death.

To bring all of these elements together in one coherent model of the stem rust effector function is not possible at this time. We favor an idea that the RGD-binding protein together with VPS9 bind with the barley cell surface and interact with unknown protein(s) via the RGD motif. The complex is also recognized by RPG1, which autophosphorylates and initiates a disease resistance-signaling pathway. The RPG1 protein is known to be associated with the plasma membrane although the majority is cytoplasmic. The components of the disease resistance signaling pathway are not known at this time, but they do include another protein kinase that we have designated RPR1 (Zhang et al. 2006). Future research efforts will focus on identifying the host proteins with which the RGD-binding protein interact and the proteins involved in the disease resistance-signaling pathway.

Acknowledgments

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References

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328-9333

Carney DS, Davies BA, Horazdovsky BF (2006) Vps9 domain-containing proteins: activators of Rab5 GTPases from yeast to neurons. Trends in Cell Biol 16:27-35

Correa AJ, Staples RC, Hoch HC (1996) Inhibition of thigmostimulated cell differentiation with RDG-peptides in *Uromyces* germlings. Protoplasma 194:91-102

Druka A, Potokina E, Luo Z, Bonar N, Druka I, Zhang L, Marshall DF, Steffenson BJ, Close TJ, Wise RP, Kleinhofs A, Williams RW, Kearsey MJ, Waugh R (2008) Exploiting regulatory variation to identify genes underlying quantitative resistance to the wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* in barley. Theor Appl Genet 117:261-272. DOI: DOI 10.1007/s00122-008-0771-x

Giancotti FG, Ruoslahti E (1999) Integrin signaling. Science 285:1028-1032

Horvath H, Rostoks N, Brueggeman R, Steffenson B, von Wettstein D, Kleinhofs A (2003) Genetically engineered stem rust resistance in barley using the *Rpg1* gene. Proc Natl Acad Sci USA 100:364-369

Kobayashi M, AB E, Bonvin AMJJ, Siegal G (2010) Structure of the DNA-bound BRCA1 C-terminal region from human replication factor Cp140 and model of the protein-DNA complex. J Biol Chem 285:10087-10097

Kolmer JA (2001) Early research on the genetics of *Puccinia graminis* and stem rust resistance in wheat in Canada and the United States. In: Peterson PD (ed) Stem Rust of Wheat: From ancient enemy to modern foe. American Phytopathological Society, St. Paul, MN, pp . 51-82

Lu B, Chen F, Gong Z-H, Xie H, Liang J-S (2007) Integrin-like protein is involved in the osmotic stress-induced abscisic acid biosynthesis in *Arabidopsis thaliana*. J Int Plant Biol 49:540-549

Mirlohi A, Brueggeman R, Drader T, Nirmala J, Steffenson BJ, Kleinhofs A (2008) Allele sequencing of the barley stem rust resistance gene *Rpg1* identifies regions relevant to disease resistance. Phytopathology 98:910-918

Nirmala J, Brueggeman R, Maier C, Clay C, Rostoks N, Kannangara CG, von Wettstein D, Steffenson B, Kleinhofs A (2006) Subcellular localization and functions of the barley stem rust resistance receptor-like serine/threonine-specific protein kinase Rpg1. Proc Natl Acad Sci USA 103:7518-7523

Nirmala J, Dahl S, Steffenson BJ, Kannangara CG, von Wettstein D, Chen X, Kleinhofs A (2007) Proteolysis of the barley receptor-like protein kinase RPG1 by a proteasome pathway is correlated with *Rpg1*-mediated stem rust resistance. Proc Natl Acad Sci USA 104:10276-10281. DOI:10.1073/pnas.0703758104

Nirmala J, Drader T, Chen X, Steffenson B, Kleinhofs A (2010) Stem rust spores elicit rapid RPG1 phosphorylation. Mol Plant-Microbe Interact 23:1635-1642

Prag G, Misra S, Jones EA, Ghirlando R, Davies BA, Horazdovsky BF, Hurley JH (2003) Mechanism of ubiquitin recognition by the CUE domain of Vps9p. Cell 113:609-620

Rostoks N, Steffenson BJ, Kleinhofs A (2004) Structure and expression of the barley stem rust resistance gene *Rpg1*. Physiol Mol Plant Pathol 64:91-101. DOI:10.1016/j.pmpp.2004.05.006.

Schindler M, Meiners S, Cheresh DA (1989) RGD-dependent linkage between plant cell wall and plasma membrane: consequences for growth. J Cell Biol 108:1955-1965

Steffenson BJ (1992) Analysis of durable resistance to stem rust in barley. Euphytica 63:153-167

Zerial M, McBride H (2001) RAB proteins as membrane organizers. Nature Rev Mol Cell Biol 2:107-117

Zhang L, Fetch T, Nirmala J, Schmierer D, Brueggeman R, Steffenson B, Kleinhofs A (2006) *Rpr1*, a gene required for *Rpg1*-dependent resistance to stem rust in barley. Theor Appl Genet 113:847-855. DOI:10.1007/s00122-006-0342-y

Towards an understanding of the molecular mechanisms of durable and non-durable resistance to stripe rust in wheat

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Abstract

Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici*, continues to cause severe damage worldwide. Durable resistance is a key for sustainable control of the disease. High-temperature adult-plant (HTAP) resistance, which expresses when the weather becomes warm and plants grow old, has been demonstrated to be durable. We have conducted numerous of studies for understanding molecular mechanisms of different types of stripe rust resistance using a transcriptomics approach. Through comparing gene expression patterns with race-specific, all-stage resistance controlled by various genes, we found that a greater diversity of genes is involved in HTAP resistance. The genes involved in HTAP resistance are induced more slowly and their expression induction is less dramatic than genes involved in all-stage resistance. The high diversity of genes and less dramatic expression induction may explain the durability and incomplete level of HTAP resistance. Identification of transcripts may be helpful in identifying resistance controlled by different genes and in selecting better combinations of genes for pyramiding to achieve adequate and more durable resistance.

Keywords

Durable resistance, molecular mechanism, *Puccinia striiformis*, yellow rust.

Introduction

Stripe rust (yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (*Pst*), is one of the most important diseases of wheat worldwide (Stubbs 1985; Chen 2005). The disease continues to cause severe damage in many wheat-producing regions. Control is preferably through resistant cultivars. However, cultivars that are developed for resistance to stripe rust often become susceptible as virulent races continue to evolve in the pathogen populations and spread from one region to another. Race-specific resistance, which usually provides complete protection throughout the entire growth cycle and is therefore referred as all-stage resistance, is usually not durable when conferred by a single gene. In contrast, high-temperature adult-plant (HTAP) resistance, which expresses when the weather becomes warm and/or plants grow old, has been demonstrated to be non race-specific and durable (Qayoum and Line 1985; Line 2002; Chen 2005). Plants with only HTAP resistance are susceptible to all races in the seedling stage, but become resistant or less susceptible in late growth stages when temperatures become high. Typical HTAP resistance is identified through a four-way (seedling-low temperature, seedling-high temperature, adult plant-low temperature and adult plant-high temperature) test (Chen 2005, 2011). We routinely screen wheat germplasm and breeding lines for HTAP resistance by testing seedlings with various *Pst* races at a low temperature profile (diurnal temperature cycle changing from 4°C at 2:00 a.m. to 20°C at 2:00 p.m.) and then test adult-plants with selected races virulent in the seedling tests at a high temperature profile (diurnal temperature cycle changing from 10°C at 2:00 a.m. to 30°C at 2:00 p.m.). HTAP resistance has been successfully used in the U.S. Pacific Northwest to reduce major damage from stripe rust since Dr. Orville Vogel released wheat cultivars Gaines and Nugaines in the early 1960s. However, HTAP resistance is not perfect as it is mostly incomplete and the resistance level is influenced by growth stage, temperature, and inoculum pressure (Chen 2005). Therefore, the best approach is to combine genes for effective all-stage resistance with those for HTAP resistance. Numerous genes conferring both types of resistance have been identified. The gene structures and predicted proteins of cloned stripe rust resistance genes *Yr18/Lr34* and *Yr36* reported by Krattinger et al. (2009) and Fu et al. (2009), respectively, and unpublished *Yr10* (Laroche, personal communication) and candidate *Yr5* (Chen and associates, unpublished data) lead to a hypothesis that resistance controlled by NBS-LRR genes may not be durable, and resistance controlled by non-NBS-LRR genes may be durable. These cloned genes and others are not the only genes that contribute to resistance, but it is likely that each *Yr* gene regulates various defense and other functional genes to confer actual resistance. Here, we compare our data in a series of transcriptomics studies previously published (Coram et al. 2008a, 2008b, 2008c, 2008d, 2009, 2010) and unpublished to understand molecular mechanisms of race-specific all-stage and nonrace-specific HTAP resistance with an ultimate goal of finding a molecular basis for durable resistance.

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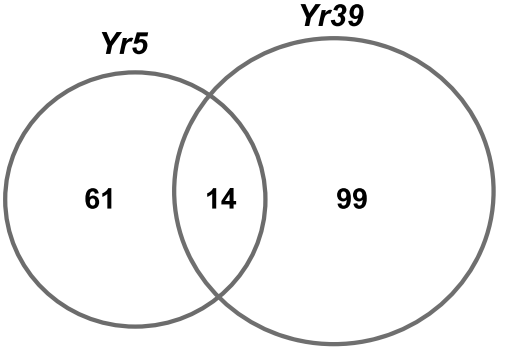
Comparison of transcripts induced during race-specific all-stage resistance mediated by *Yr5* and race non-specific HTAP resistance mediated by *Yr39*

Yr5 confers typical all-stage resistance to stripe rust, exhibiting infection type (IT) 0 (no visible symptoms) on leaves of the original donor, *Triticum spelta album* (*Ts*a) (Chen and Line 1992) and IT 1 (small necrotic flecks without uredinia) on the near-isogenic line (AvSYr5NIL) in the Avocet Susceptible (AvS) background (Yan et al. 2003). In a study to determine genes involved in *Yr5*-controlled resistance (Coram et al. 2008b, 2008d), the Wheat GeneChip (Affymetrix, Santa Clara, CA), including 61,127 probe sets representing 55,052 transcripts (www.affymetrix.com), was used to profile changes occurring in wheat near-isogenic lines (a BC_7:F_4 line of AvS x *Ts*a and AvS) at 6, 12, 24 and 48 h post-inoculation (hpi) with race PST-78 that is avirulent on the *Yr5* isoline and virulent on AvS. The microarray study identified 61 transcripts specific to *Yr5*-mediated resistance (Fig. 1). These transcripts are typical genes involved in signaling pathways and defense-related events known to occur during R-gene-mediated responses, including protein kinase signaling and production of reactive oxygen species, leading to hypersensitive responses. The gene expression pattern showed a peak at 24 hpi, which is correlated to haustorial formation. In this study, 19 transcripts were identified to be specifically induced for basal defense during the compatible interaction. However, due to lack of *R*-gene signaling, the response was weak. In contrast, *Yr5*-signalling resulted in a rapid and strong resistance response.

Fig. 1 Comparison of the numbers of transcripts associated with Yr5-mediated race-specific all-stage resistance (61) and Yr39-mediated race non-specific high-temperature adult-plant resistance (99). Fourteen of the genes were common in both types of resistance



In the study of *Yr39*-mediated HTAP resistance (Coram et al. 2008c), the same Wheat GeneChip was used to identify genes induced in two selected F_7 recombinant inbred lines (RILs) from a cross between AvS and Alpowa inoculated with PST-78 urediniospores and mock-inoculated without urediniospores at the flag-leaf stage and grown at the high temperature profile (10-30°C) after inoculation. The resistant RIL and Alpowa showed a typical HTAP resistance, IT 2-3 (necrotic stripes of 0.5-2.0 cm with occasional uredinia on the edges of necrotic stripes). Under the same temperature conditions, the susceptible RIL and AvS had IT 9 (uredinial stripes without chlorosis or necrosis). In this study, 99 induced transcripts were identified as HTAP resistance-specific (Fig. 1). This number is higher than that specifically involved in *Yr5* resistance as discussed above. Transcript accumulation peaked at 48 hpi, which is later than the peak time (24 hpi) for *Yr5*-mediated all-stage resistance, but corresponded to the time point when rust hyphae were observed microscopically and were undergoing rapid increases in fungal biomass as detected by quantitative PCR assays in the compatible interaction. More than half (50.5%) of the annotated HTAP resistance transcripts were involved in defense and/or signal transduction, including *R*-gene homologs and transcripts associated with pathogenesis-related protein production, phenylpropanoid biosynthesis and protein kinase signaling. The identification of nine *R*-gene homologs leads to a hypothesis that these genes regulated by a master gene (*Yr39*) serve as secondary master genes regulating defense and other related genes contributing to the HTAP resistance.

When we compared the *Yr39*-mediated HTAP resistance with the *Yr5*-mediated all-stage resistance, we found 14 genes involved in both types of resistance (Fig. 1). These genes include WIR1A protein (involved in cell wall structure), beta-1,3-glucanase (a PR protein), phenylalanine ammonium lyase (a phenylpropanoid phytoalexin), peroxidase (involved in oxidative stress), protein kinase and calmodulin protein (involved in signal transduction), carbohydrate (related to transport), and blue copper-binding protein (related to electron transport). The common genes may be related to host cell death involved in both types of resistance. The putative functions of genes identified in *Yr39*-mediated resistance, but not in the *Yr5*-mediated resistance, included *R* proteins, UDP-glucosyl transferase and hydroxyanthranilate hydroxyl cinnamoyl transferase involved in phenylpropanoids, pleiotropic drug resistance/ABC transporter, putative disease resistance protein, latex protein allergen,

receptor protein kinase involved in signal transduction, WRKY5 homolog involved in transcription, and amino acid/protein and ammonium/phosphate/potassium involved in transport. Some of the specific genes may explain the durability of the *Yr39*-mediated resistance, especially the R proteins. Three of the R protein genes are protein kinases with homology to RPG1 protein conferring durable resistance to stem rust in barley (Brueggeman et al. 2006). One *R* gene is a homolog of Cf2/Cf5 LRR disease resistance protein for *Cladosporium fulvum* resistance in tomato (**Dixon et al. 1998**). One putative *R* gene has homology with the putative stripe rust resistance protein *Yr10* (http://pir.uniprot.org/uniprot/Q9FR63). The remaining three *R* genes encode putative leucine-rich repeat family protein, leucine-rich repeat transmembrane protein kinase, and NB-ARC domain containing protein. In addition, a homolog of Hm1 NADPH-dependent HC-toxin reductase protein was involved in the *Yr39*-mediated resistance. *Hm1* conferring resistance to *Cochliobolus carbonum* in maize was the first cloned plant disease resistance gene (Johal and Briggs 1992). The collective contribution of these *R* genes to the *Yr39*-mediated resistance may require different *Pst* genes for recognition. The diverse *R* genes may regulate various defense genes involved in different abiotic stress responding pathways. All of the *R* and defense genes make the *Yr39*-mediated HTAP resistance diversely based, perhaps making it difficult for the pathogen to overcome.

Comparison of transcripts identified for different genes conferring race-specific all-stage resistance

In a study aimed at identifying common transcripts associated with race-specific all-stage resistance (Coram et al. 2010), genes *Yr1*, *Yr5*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15* and *Yr17* were selected because they are available in near-isogenic lines in AvS background. Due to budget limitations, we used a custom microarray instead of the Wheat Affymetrix GeneChip with a primary goal of identifying common transcripts. A total of 343 probes were selected based on their significant expression in the *Yr5* and *Yr39* studies (Coram et al. 2008c, 2008d). An avirulent race was used to inoculate two-leaf seedlings of each of the *Yr* gene lines and PST-78 was used to inoculate the susceptible background line AvS, with the same race being used for each gene when possible. A mock-inoculation was also used for each of the lines. The inoculated seedlings were grown at the low-temperature profile described in the *Yr5* study. Leaf samples were taken 24 and 48 hpi for RNA extraction and gene expression analysis. This study identified 28 genes significantly involved in resistance phenotypes across all eight *Yr* genes. Among these transcripts, those for putative blue copper-binding protein, heat-stress transcription factor, pathogen-induced WIR1A protein, and ent-kaurene synthase transcripts were the most significant. Some transcripts were uniquely significant in each *Yr* gene line, indicating transcriptional events specific to particular *Yr* gene-mediated race-specific resistances. The results confirm the activity of known *R*-gene-mediated pathway race-specific resistance, including an oxidative burst that likely contributes to a hypersensitive response, as well as pathogenesis-related protein expression and activation of the phenylpropanoid pathway.

Comparison of transcripts identified for different genes conferring race-specific all-stage resistance and race non-specific HTAP resistance in adult plants under high temperatures

Similar to the meta-analysis of transcripts for race-specific resistance mediated by various genes, we also conducted a study to identify common transcripts associated with race non-specific HTAP resistance mediated by different genes in comparison with all-stage resistance. Isogenic lines having *Yr18*, *Yr29*, *Yr36* and *Yr39* were selected as they were identified as single genes involved in HTAP resistance. The isolines with the genes (*Yr1*, *Yr5*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15* and *Yr17*) studied at the seedling stage (Coram et al. 2010) were also included. The custom microarray, experimental design, procedure, data collection and analyses were all as described for the race-specific resistance study (Coram et al. 2010), except that inoculated plants were grown in the high-temperature profile as described for the *Yr39* HTAP resistance study (Coram et al. 2008c). Adult plants at booting of NILs *Yr1*, *Yr5*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15* and *Yr17* were inoculated separately with appropriate avirulent races (PST-21 for *Yr8*, *Yr9*, *Yr10* and *Yr17*; PST-45 for *Yr1* and *Yr7*; and PST-78 for *Yr5* and *Yr15*) and virulent races (PST-17 for *Yr1*; PST-43 for *Yr10*; PST-45 for *Yr17*; PST-78 for *Yr7*, *Yr8* and *Yr9*; an Australian isolate for *Yr5*; and no isolate virulent for *Yr15*); and those of the single gene lines for *Yr18*, *Yr29*, *Yr36* and *Yr39*, together with AvS, were inoculated with PST-78 that is virulent on seedlings, but not adult-plants, with these genes.

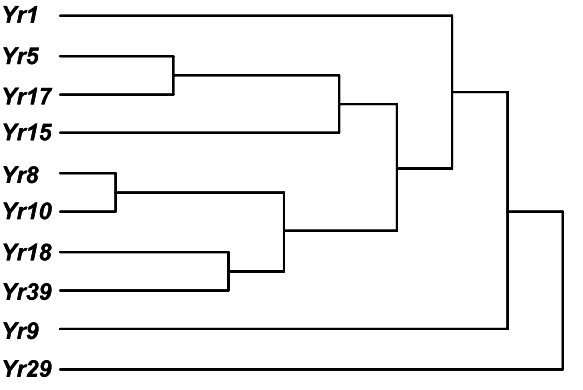
Stripe rust infection type data observed 20 days after inoculation were as expected for each compatible or incompatible *Yr* gene-*Pst* race combination, except the *Yr8*, *Yr10* and *Yr17* single gene lines in the presumed compatible interactions. Adult plants of these three lines exhibited resistance in the test with virulent races. The phenotypes of *Yr8* and *Yr17* confirmed the presence of HTAP resistance (Chen and associates, unpublished data), while that of the *Yr10* line was surprising. Because these lines have both all-stage and HTAP resistance, they were not included in the analyses of comparing transcripts of HTAP resistance with all-stage resistance.

For the race-specific adult-plant resistance gene lines, three probes were down-regulated when compared to their mock-inoculated checks and no probes were down regulated across the all-stage resistance gene lines compared to their compatible race inoculations (data not shown). Four probes were up-regulated when compared to their mock-inoculated checks and five up-regulated when compared to their compatible interactions. For the HTAP resistance gene lines, two probes were down-regulated and 4 were up-regulated when compared to AvS. When the HTAP resistance gene lines were compared with the all-stage resistance gene lines, nine probes were down regulated and two up-regulated (data not shown).

Transcript values with significant changes (2-fold or higher, P <0.10) in the adult-plant tests under the high-temperature profile (10-30°C) are shown in bold in Table 1. Seven transcripts were significant for *Yr1*, 10 for *Yr5*, 4 for *Yr7*, 31 for *Yr8*, 6 for *Yr9*, 13 for *Yr10*, 6 for *Yr15*, 5 for *Yr17*, 40 for *Yr18*, 4 for *Yr29*, 99 for *Yr39* and none for *Yr36*. Up-regulated transcripts shared by two or more *Yr* genes also can be found from this Table. For comparison, significant transcript values detected in previously published studies of seedling tests at the low-temperature (4-20°C) profile for all-stage resistance genes (Coram et al. 2008d, 2010) are also given in Table 1. In general, transcripts detected in the seedling low-temperature tests were also significant in the adult-plant high-temperature tests for race specific all-stage resistance.

Based on common and unique transcripts identified in the *Yr* gene-mediated resistances, a dendrogram was constructed to show their relationships (Fig. 2). *Yr5* was more closely related to *Yr17*; *Yr8* was more closely related to *Yr10*; and *Yr18* was more closely related to *Yr39* than to other genes. *Yr29* was more distantly related to all of the other genes. *Yr36* was not included in the dendrograms as none of the transcripts for other genes was significantly changed in expression levels; this could indicate that it utilizes signaling and defense pathways that are very different from those identified for the other genes. Although this hypothesis needs to be tested, the results may be in agreement with the finding by Fu et al. (2009) that *Yr36* is a very old gene that is not present in common wheat cultivars.

Fig. 2 ***Dendrogram showing hierarchical clustering (Euclidean metrics, complete linkage) of shared and unique data from Table 1***



After various comparisons, five genes were clearly identified to be involved in race-specific all-stage resistance controlled by *Yr1*, *Yr5*, *Yr7*, *Yr9* and *Yr15* and only one gene commonly expressed in HTAP resistances mediated by different genes (*Yr18*, *Yr29*, *Yr36* and *Yr39*) (Table 2). The annotation of the five transcripts specific to all-stage resistance provided additional evidence for classic *R*-gene mediated pathways being involved in races-specific resistance. The five genes commonly involved in all-stage resistance included a hydroxyproline-rich glycoprotein, a NB-ARC domain containing protein, a protein kinase and two function-unknown genes. In addition to the separate analysis at the seedling stage (Coram et al. 2010), we found another NB-ARC protein and a protein kinase for defense signaling. Also, the hydroxyproline-rich glycoprotein is involved in cell wall strengthening like the WIR1A protein (Bull et al. 1992). Among the five genes, three were first identified in *Yr5*-mediated all-stage resistance and two were first identified in the *Yr39*-mediated HTAP resistance. The transcript with significant changes across all HTAP resistances is a nonclathrin coat protein. The function of nonclathrin coat protein-related resistance is not clear, but such proteins have been reported to bind to the cytoplasmic dilysine motif of membrane proteins of the early secretory pathway (Harter et al. 1996). The nonclathrin coat protein identified in this study may be involved in transporting antifungal substances across the cell membrane to directly contact *Pst* haustoria or hyphae. The lack of many shared transcripts in HTAP resistance compared to all-stage resistance leads to a hypothesis that diverse genes and biochemical

Table 1 *Shared and unique significantly (changes > 2 fold; P < 0.10) induced transcripts for stripe rust resistance genes conferring race-specific all-stage resistance identified in seedling tests under the low temperature profile (4-20°C) and in adult-plant tests under the high-temperature profile (10-30°C) (bold script) or race non-specific high-temperature adult-plant (HTAP) resistance (bold)*

		Magnitude (fold) of induced expression of resistance gene[b]										
		Race-specific[c]								Race non-specific[d]		
Putative function[a]	Probe ID	Yr1	Yr5	Yr7	Yr8[e]	Yr9	Yr10	Yr15	Yr17	Yr18	Yr29	Yr39
Cell Death												
Senescence-associated protein	Ta.14231.2.S1_x_at											3.5
Defense												
Alternative oxidase	Ta.10549.2.A1_at											5.4
Alternative oxidase	Ta.28112.1	3.4										
Alternative oxidase	Ta.28112.1.S1_at											3.8
Beta-1,3-glucanase	Ta.223.1							2.7	2.5			
Beta-1,3-glucanase	Ta.1174.1.S1_x_at		6.9									7.0
Beta-1,3-glucanase	Ta.21297.1.S1_at		4.8									
Beta-1,3-glucanase	Ta.21354.1.A1_at		2.1									3.6
Beta-1,3-glucanase	Ta.21354.1.A1_x_at		2.4									3.6
Beta-1,3-glucanase	Ta.22427.1	10.2								2.1		
Beta-1,3-glucanase	Ta.22427.1.A1_x_at											5.5
Beta-1,3-glucanase	Ta.22562.1				2.8							
Beta-1,3-glucanase	Ta.26048.1.S1_x_at		3.0									
Beta-1,3-glucanase	TaAffx.119315.2.S1_at		2.6									
Beta-1,3-glucanase	TaAffx.119315.2.S1_x_at		2.3									
Cf2/Cf5 disease resistance protein homolog	Ta.25518.1.S1_at											2.4
Chitinase	Ta.30501.1.S1_at		9.5									
Cold-acclimation induced protein	Ta.351.2.S1_x_at											2.8
Dirigent-like protein	Ta.22687.1				9.2							
ERD1 protein - water-stress induced	Ta.4014.2.S1_at											2.7
Germin-like protein	TaAffx.15880.1.S1_at											2.9
Hydroxyanthranilate hydroxycinnamoyl transferase	Ta.14063.1.S1_at											2.5
Leucine-rich repeat family protein	Ta.4479.2.S1_x_at											2.1
Leucine-rich repeat transmembrane protein kinase	Ta.8590.1.S1_s_at											2.4
MAC/perforin protein	Ta.12913.1									2.7	2.3	
Mitogen-activated protein kinase	Ta.236.1.S1_at											2.4
Mla6 barley powdery mildew resistance protein	Ta.14550.1				2.9							
NADPH-dependent HC-toxin reductase Hm1	Ta.12946.1.S1_at											3.5
NB-ARC domain containing protein	TaAffx.4601.2									2.5		
NB-ARC domain containing protein	TaAffx.103209.1.S1_at											2.6
NBS-LRR disease resistance protein	Ta.25549.1.S1_at											2.7
Pathogen induced WIR1A protein	Ta.13.1	**2.6**/2.3	**2.1**/2.0		**4.5**/4.1		**2.4**/2.1	**2.3**/2.2		3.7		
Pathogen induced WIR1B protein	Ta.97.1											2.3
Pathogen induced WIR1A protein	Ta.97.2.S1_x_at											4.4
Pathogen induced WIR1A protein	Ta.22732.1.S1_s_at											4.8
Pathogen-induced protein WIR1A homolog	Ta.22732.1.S1_x_at		3.6									3.6
Pathogen-induced protein WIR1A homolog	Ta.3133.1							3.8				3.7
Pathogen-induced protein WIR1A homolog	Ta.3133.1.S1_x_at		9.6									5.0
Pathogen-induced secretory protein	Ta.231.1				2.8	2.4	3.0					
Peroxidase	Ta.82.1				3.4		2.5					
Peroxidase	Ta.18497.1					4.8	9.9			3.5		3.0
Peroxidase	Ta.18497.1.S1_at		5.6									
Peroxidase	Ta.21307.1.S1_x_at											6.5
Peroxidase	Ta.22564.1						2.9					
Peroxidase	Ta.24106.1.S1_x_at		3.1									
Phenylalanine ammonia-lyase	Ta.7022.1	**4.6**/3.3										
Phenylalanine ammonia-lyase	Ta.7022.1.S1_at		2.5									3.9

Putative function[a]	Probe ID	Magnitude (fold) of induced expression of resistance gene[b]										
		Race-specific[c]								Race non-specific[d]		
		Yr1	Yr5	Yr7	Yr8[e]	Yr9	Yr10	Yr15	Yr17	Yr18	Yr29	Yr39
Phenylalanine ammonia-lyase	Ta.7022.3	**11.5/10.0**	**10.3**/6.5	**10.3**/6.4								
Phenylalanine ammonia-lyase	TaAffx.131379.1		2.9									
Phenylalanine ammonia-lyase	TaAffx.131379.1.A1_at		5.4									
Phenylalanine ammonia-lyase	TaAffx.92008.1				3.2							
Phenylalanine ammonia-lyase	TaAffx.92008.1.A1_s_at		4.2									3.3
Phenylalanine ammonia-lyase	Ta.20429.1.S1_at											4.6
Phenylalanine ammonia-lyase	Ta.28046.1.A1_at											3.8
Phenylalanine ammonia-lyase	Ta.7022.1.S1_s_at											8.6
Phenylalanine ammonia-lyase	Ta.7022.1.S1_x_at											3.8
Pleiotropic drug resistance/ABC transporter	Ta.6990.1.S1_at											2.4
Pleiotropic drug resistance protein/ABC transporter	Ta.21281.1	5.2										3.7
PR protein 1	Ta.13013.2									3.3		
PR protein 10	Ta.22619.1	**3.2**/2.7			**11.5**/11.2		**3.5**/3.5					
PR protein 10	Ta.22619.1.S1_at		5.3									
PR protein 10	Ta.22619.1.S1_x_at		7.0									
Proline-rich protein	Ta.16599.1.S1_at		3.8									
Protein kinase	Ta.10236.1.A1_at											3.6
Protein kinase	Ta.12007.2.S1_at											2.8
Protein kinase - similar to barley stem rust R protein Rpg1	Ta.10236.2									3.8		
Protein kinase - similar to barley Rpg1	Ta.10236.2.S1_a_at											4.7
Protein kinase - similar to barley Rpg1	Ta.10326.1.S1_at											3.7
Protein kinase - similar to barley Rpg1	Ta.10236.2.S1_x_at											3.3
Putative disease resistance protein	Ta.14786.1				**7.0**/6.0		**4.7**/4.3					
Putative disease resistance protein	Ta.14786.1.S1_at											8.3
Putative disease resistance protein	Ta.22482.1	**7.4**/4.7			**6.8**/6.6							
Putative disease resistance protein	Ta.22482.1.S1_s_at											3.0
Putative latex protein allergen	Ta.9588.2.S1_a_at											4.8
Putative stripe rust resistance protein Yr10	TaAffx.43336.1				2.8							
Putative stripe rust resistance protein Yr10	TaAffx.43336.1.S1_at											2.4
Receptor-like protein kinase	Ta.7017.1.S1_at											2.8
Receptor-like protein kinase	Ta.11135.1									2.6		
Receptor-like protein kinase	Ta.11135.1.S1_at											2.5
Receptor-like protein kinase	TaAffx.111955.1									2.1		
Receptor-like protein kinase	TaAffx.111955.1.S1_at											3.2
Reticuline oxidase	Ta.27350.1										2.5	
Serine/threonine protein kinase	Ta.728.1									2.6		
Serine/threonine protein kinase	Ta.7718.2.S1_a_at											2.2
Strictosidine synthase	TaAffx.56754.1.S1_at											2.2
Thaumatin-like protein	Ta.27762.1.S1_x_at		4.5									
UDP-glycosyltransferase	Ta.30731.1									3.5		
UDP-glucosyl transferase	Ta.8495.1.A1_at											8.3
UDP-glucosyl transferase	TaAffx.23237.1.S1_at											3.3
Energy												
Blue copper-binding protein	Ta.9336.1	**4.9**/4.6	**6.4**/5.1		**10.9**/7.1	3.1	**4.6**/4.1	**5.0**	**5.8**/4.0			
Blue copper-binding protein	Ta.18203.1		2.4					2.5	2.4			
Blue copper-binding protein	Ta.18203.1.S1_at											3.3
Blue copper-binding protein	Ta.5654.1.S1_at											3.2
Blue copper-binding protein	Ta.9336.1.S1_x_at		3.0									
Blue copper-binding protein	TaAffx.55612.1.S1_at											
Cytochrome P450	Ta.8262.1.S1_at		3.3									
Cytochrome P450	Ta.8447.1	**51.3**/16.8										
Cytochrome P450	Ta.8447.2	3.4					4.1					
Cytochrome P450	Ta.8447.1.S1_a_at		5.8									
Cytochrome P450	Ta.8447.1.S1_x_at		8.8									
Cytochrome P450	Ta.29826.1.S1_at		2.7									
Cytochrome P450	TaAffx.109794.1.S1_s_at		7.3									

Putative function[a]	Probe ID	Magnitude (fold) of induced expression of resistance gene[b]										
		Race-specific[c]								Race non-specific[d]		
		Yr1	Yr5	Yr7	Yr8[e]	Yr9	Yr10	Yr15	Yr17	Yr18	Yr29	Yr39
Growth												
Ent-kaurene synthase	Ta.8418.1		**10.4**/6.9		**34.4**/23.8	**8.8**/6.8	**8.7**/8.1	**4.1**/3.5		2.7		5.6
Ent-kaurene synthase	Ta.8418.1.S1_at		3.0									
Gibberellin oxidase	Ta.24934.3		5.6		3.0							
Gibberellin oxidase	Ta.24934.3.S1_at		2.9									
Metabolism												
Acid phosphatase	Ta.21271.1									2.7		
Aspartyl protease	Ta.15123.1.A1_at											2.1
Bifunctional coenzyme A synthase	TaAffx.63502.1									2.4		
Beta-fructofuranosidase	TaAffx.82312.1.S1_s_at		2.2									
Glucosyl hydrolase	TaAffx.9022.1.S1_at											4.7
Prephenate dehydratase	Ta.9122.1.S1_x_at											2.3
Protein phosphatase	TaAffx.16090.1									4.1		
Protein phosphatase	TaAffx.16090.1.S1_at											3.2
Shikimate kinase	Ta.8618.1.S1_at											3.0
SIS domain protein	Ta.4815.1.S1_at		2.4									
UDP-glucose dehydrogenase	Ta.2657.1.S1_x_at		3.1									
Signal transduction												
Ankyrin-like protein	TaAffx.12271.1.S1_at											2.7
Calmodulin-binding heat shock protein	Ta.10168.1		3.9		**2.1**/4.4							
Calmodulin-binding heat shock protein	Ta.10168.1.S1_at											2.1
Calmodulin-binding protein	Ta.7711.1	2.6										
Calmodulin-binding protein	Ta.7711.1.A1_at											4.0
Calmodulin-like protein	TaAffx.128621.1.S1_at											3.5
GTP1/OBG family protein	Ta.16040.1									2.6	2.1	
LRR-containing extracellular glycoprotein	Ta.27314.1		**5.7**/4.6		**5.8**/4.8		**3.1**/2.9			2.4		
LRR-containing extracellular glycoprotein	Ta.27314.1.S1_at		3.5									
Protein kinase	TaAffx.27775.1									2.4		
Secretory protein kinase	TaAffx.52945.3		3.3		2.7			2.6				
Secretory protein kinase	TaAffx.52945.1.S1_at		3.7									
Transcription												
CR4-NOT transcription complex subunit 8 protein	TaAffx.33753.1.S1_at											3.9
Heat-stress transcription factor	TaAffx.120360.1	**5.0**/3.1		**2.3**/2.1	**4.8**/4.2	**3.5**/2.6	**2.3**/2.0	**2.1**/2.1		2.1		
Heat-stress transcription factor	TaAffx.120360.1.A1_at											2.6
Putative WRKY5 protein	TaAffx.80313.1.S1_at											2.0
Zinc finger DNA binding protein	TaAffx.28280.1									2.6	2.1	
Zinc finger POZ domain protein	Ta.19786.1				2.5							
Transport												
Ammonium transporter	Ta.27506.1				12.9							3.0
Ammonium transporter	Ta.27506.1.S1_at											4.0
ATPase	TaAffx.54526.1									2.3		
ATPase	TaAffx.54526.1.S1_at											5.0
Glucose transporter	Ta.12517.1.S1_at		2.3									3.7
Histidine amino acid transporter	Ta.3869.1	**4.3**/3.6										
Histidine amino acid transporter	Ta.3869.1.S1_at											4.8
Histidine amino acid transporter	Ta.12339.1.S1_s_at											2.1
Histidine amino acid transporter	Ta.28479.1				3.4							
Histidine amino acid transporter	Ta.28479.1.S1_at											3.2
Integral membrane protein	Ta.15082.1.S1_at											3.4
Integral membrane protein	Ta.15082.1.S1_x_at											3.1
Integral membrane protein	Ta.29523.1.S1_at											3.8
Integral membrane protein	TaAffx.52897.1.S1_at											3.9

Putative function[a]	Probe ID	Magnitude (fold) of induced expression of resistance gene[b]										
		Race-specific[c]								Race non-specific[d]		
		Yr1	Yr5	Yr7	Yr8[e]	Yr9	Yr10	Yr15	Yr17	Yr18	Yr29	Yr39
Nonclathrin coat protein	Ta.7616.1									3.7		
Phosphate transporter	Ta.10084.1.S1_at											2.5
Potassium transporter	Ta.9064.2.S1_s_at											2.5
Putative membrane protein	Ta.23392.1.S1_at											4.8
Putative peptide transporter	TaAffx.111465.1									2.3		
Sugar transporter	Ta.27329.1.S1_at		2.5									
UDP-galactose transporter	Ta.4921.1				6.2							
UDP-galactose transporter	Ta.4921.1.S1_at											3.2
Unknown												
Hypothetical protein	Ta.954.1.S1_s_at											2.3
Hypothetical protein	Ta.3088.1	**3.5**/3.1		**6.0**/3.5		**3.7**/3.1						
Hypothetical protein	Ta.13991.1.S1_x_at		3.3									3.8
Hypothetical protein	Ta.14129.1.S1_at											3.9
Hypothetical protein	Ta.14231.1.S1_x_at		2.4									
Hypothetical protein	Ta.22669.1.A1_at											3.7
Hypothetical protein	Ta.6155.2.S1_a_at											2.2
Hypothetical protein	Ta.13991.1.S1_x_at		3.3									3.8
Hypothetical protein	TaAffx.7032.1				4.7							
Hypothetical protein	TaAffx.7302.1.S1_at		2.7									
Hypothetical protein	TaAffx.107538.1								3.6	2.3		
Hypothetical protein	TaAffx.110081.1									8.3		
Hypothetical protein	TaAffx.107538.1.S1_x_at		2.8									
Hypothetical protein	TaAffx.110081.1.S1_at		2.7									
Hypothetical protein	TaAffx.110081.1.S1_x_at		2.9									
Hypothetical protein	TaAffx.110250.1.S1_x_at		2.8									
No homology	Ta.520.1.S1_at											3.0
No homology	Ta.424.1	**10.6**/8.6			**4.7**/4.3							
No homology	Ta.3247.1.S1_at		2.1									
No homology	Ta.4747.1			4.5	5.5			4.0				
No homology	Ta.5518.1.S1_at											7.2
No homology	Ta.8254.1.A1_at		3.5									
No homology	Ta.8582.1	4.7								2.1		
No homology	Ta.8582.1.S1_at		4.3									
No homology	Ta.8582.2.S1_a_at		4.5									
No homology	Ta.8582.2.S1_x_at		3.9									
No homology	Ta.11087.1				2.1							
No homology	Ta.11087.2.S1_at		2.1									
No homology	Ta.11087.2.S1_x_at		2.7									
No homology	Ta.12795.1									5.9		
No homology	Ta.15072.1									2.0		
No homology	Ta.16472.1									3.3		
No homology	Ta.19411.1				2.8					2.6		
No homology	Ta.20149.1.S1_at											4.0
No homology	Ta.21236.1.S1_a_at		4.7									
No homology	Ta.21236.3.S1_x_at		5.5									
No homology	Ta.21314.1				2.4		3.5		3.6			
No homology	Ta.21314.1.S1_at		8.3									
No homology	Ta.21314.1.S1_x_at		8.1									
No homology	Ta.21531.1.S1_at											2.7
No homology	Ta.22223.1.S1_at		2.5									
No homology	Ta.22462.1				4.0							
No homology	Ta.22957.1.S1_at		5.9									
No homology	Ta.23271.1				4.4							
No homology	Ta.23271.2			4.5						3.0		
No homology	Ta.24564.1	6.4								2.4		

Putative function[a]	Probe ID	Magnitude (fold) of induced expression of resistance gene[b]										
		Race-specific[c]								Race non-specific[d]		
		Yr1	Yr5	Yr7	Yr8[e]	Yr9	Yr10	Yr15	Yr17	Yr18	Yr29	Yr39
No homology	Ta.24564.1.S1_a_at											2.4
No homology	Ta.24564.1.S1_x_at											2.6
No homology	Ta.24564.3.S1_x_at											2.3
No homology	Ta.27882.1					3.5						
No homology	Ta.29516.1				2.5							
No homology	Ta.29984.1				**3.1**/3.0							
No homology	Ta.29984.1.S1_at											5.1
No homology	Ta.30753.1.A1_at											2.5
No homology	TaAffx.2056.1.A1_at											4.1
No homology	TaAffx.7236.1.S1_at											4.4
No homology	TaAffx.26815.1		3.2					3.3	3.3			
No homology	TaAffx.108203.1.S1_at											3.7
No homology	TaAffx.108939.1.S1_at		3.5									
No homology	TaAffx.109709.1.S1_at		2.6									
No homology	TaAffx.109765.1.S1_at		3.0									
No homology	TaAffx.110215.2.S1_at											5.9
No homology	TaAffx.129395.1									2.4		
No homology	TaAffx.23342.1								6.9			
No homology	TaAffx.27177.1									2.1		
No homology	TaAffx.27177.1.S1_at		2.7									
No homology	TaAffx.27427.1									2.4		
No homology	TaAffx.27427.1.S1_at											2.0
No homology	TaAffx.27956.1.S1_at											2.8
No homology	TaAffx.29213.1				2.4				2.7			
No homology	TaAffx.37517.1									6.0		
No homology	TaAffx.50112.1									2.7		
No homology	TaAffx.52926.1.S1_at		4.1									
No homology	TaAffx.55533.1.S1_at		2.3									2.6
No homology	TaAffx.56501.1.S1_at											4.1
No homology	TaAffx.59551.1									2.5		
No homology	TaAffx.64918.1									4.5		
No homology	TaAffx.82674.1		**4.3**/4.2									
No homology	TaAffx.82674.1.S1_at		2.1									
No homology	TaAffx.84007.1.S1_at											3.1

[a] Functional categories were based on the Munich Information Center for Protein Sequences classifications and putative function shows the best significant BLASTX database hit from HarvEST

[b] Only expression values in fold change >2.0 ($P < 0.10$) of *Pst*-inoculated plants compared with mock-inoculated for each gene are presented. Values are combined for all time points

[c] Inoculations were done at the two-leaf seedling stage and inoculated plants were grown at a low-temperature diurnal cycle (changing from 4°C at 2:00 a.m. to 20°C at 2:00 p.m.). Different races avirulent for these genes were used in inoculation

[d] Inoculation were done at booting and inoculated plants were grown in a high-temperature diurnal cycle (changing from 10°C at 2:00 a.m. to 30°C at 2:00 p.m.). Race PST-78, which is virulent on seedlings of the HTAP resistance gene lines, was used in inoculation. The *Yr36* line was included in the study, but no significantly induced genes were identified

[e] The *Yr8* near-isogenic line (AvSYr8NIL) also has a gene for race non-specific HTAP resistance linked to the race-specific all-stage resistance gene *Yr8*

pathways are used by HTAP resistance controlled by different genes. Together with diverse genes and pathways identified for *Yr39*-mediated HTAP resistance (Coram et al. 2008c), we conclude that highly diverse genes and biochemical pathways are the molecular basis for the race non-specificity and durability of HTAP resistance.

Perspectives

The transcriptomics studies conducted so far have identified genes involved in *Yr5*-mediated all-stage resistance and *Yr39*-mediated HTAP resistance; and common genes involved in all-stage resistance and HTAP resistance mediated by different *Yr* genes. The results have provided some insights for understanding the molecular mechanisms of race-specific resistance compared to race non-specific resistance. In particular, the studies have linked a large number of genes with diverse functions to race non-specificity and durability of HTAP resistance. The data of these studies lead to several hypotheses to be tested and more studies to be conducted for a better understanding of various types of resistance and how to utilize the basic information to achieve more sustainable and better control of stripe rust.

We are currently conducting studies to test a hypothesis that ABC transporter proteins are involved in nonrace specific HTAP resistance. We are in the process of obtaining the full-length sequence for the identified ABC transporter gene, and so far have 5,754 bp of the genomic sequence from the *Yr39* donor, Alpowa, using a PCR based genome walking technique (Clontech GenomeWalker™ Universal Kit, 638904). Thus far, the ABC transporter-like wheat gene has more than 75% identity in genomic sequence to a rice gene, Os01g42410, with the greatest differences occurring in intron regions. Sequence conservation of the ABC transporter gene in Alpowa (*Yr39*) also appears to be high across different wheat cultivars after alignment of the newly acquired sequence with Chinese Spring genomic sequences (cerealsdb.uk.net) failed to detect significant differences, supporting the high up-regulation of the gene in the *Yr18* line presented above, as Chinese Spring has *Yr18* (Krattinger et al. 2009, 2011). Comparison of the ABC transporter gene in Alpowa with *Yr18/Lr34* (Krattinger et al. 2009; 2011) shows that they have low nucleotide sequence similarity (39%), confirming our initial hypothesis that they are different genes. Future goals include obtaining the full-genomic sequence of the gene in addition to the 5′ untranslated region from Alpowa and other wheat cultivars to identify functional domains and single nucleotide polymorphisms (SNPs) potentially influencing HTAP resistance in Alpowa and other wheat cultivars.

The identification of the nine *R*-protein genes involved in *Yr39*-mediated HTAP resistance was initially a surprise to us, as R proteins are largely believed to be involved in recognition of pathogen effectors, leading to race-specific resistance. However, the high number

Table 2 ***Expression level changes for transcripts detected in race-specific all-stage resistances (Yr1, Yr5, Yr7, Yr9 and Yr15) and in race non-specific high-temperature adult-plant (HTAP) resistance (Yr18, Yr29, Yr36 and Yr39)***

Probe ID	Putative function	Function category	Origin	Mean log(2) fold change	P value
Higher expression in all-stage resistance					
Ta.22462.1	No homology	Unknown	*Yr39 Pst*-induced	-2.13	0.000
Ta.6952.1	Hydroxyproline-rich glycoprotein	Defense - cell wall	*Yr5* incomplete isogenicity	-3.43	0.000
TaAffx.103209.1	NB-ARC domain containing protein	Defense - R protein	*Yr39* HTAP-specific	-1.15	0.006
TaAffx.27177.1	No homology	Unknown	*Yr5* HR-specific	-1.28	0.000
TaAffx.27775.1	Protein kinase	Signal transduction	*Yr5* incomplete isogenicity	-2.46	0.000
Higher expression in HTAP resistance					
Ta.7616.1	Nonclathrin coat protein	Transport	*Yr39* Pst-induced	1.11	0.000

of such types of genes leads us to believe that these genes collectively contribute to race non-specificity and therefore durability. Our hypothesis is that when up-regulated by the *Yr39* master gene, these genes serve as secondary master genes regulating other defense or functionally related genes to operate the entire defense machinery against *Pst* infection and growth in the plant tissue. These R proteins may recognize different effectors, which may make it difficult for the fungus to change to non-recognition. In regard to the ABC transporter gene, we are currently obtaining full-length sequences of these *R* genes to characterize them among wheat genotypes and to determine their functions for the HTAP resistance phenotype and their roles of being regulated or regulating in the total network of defense pathways.

With a primary goal of identifying transcripts commonly involved in all-stage resistance or HTAP resistance controlled by different genes, the custom microarray was constructed using genes identified in the *Yr5* and *Yr39* studies to represent those involved in either type of resistance. However, such a cost-saving approach did not allow us to identify transcripts uniquely involved in resistance mediated by individual *Yr* genes. Therefore, we still do not have the majority of the genes identified for all *Yr* genes studied, except for *Yr5* and *Yr39*. Using the Wheat Affymetrix GeneChip, which continues to have new genes added, is still a major high-throughput technique to identify possible genes involved in resistance to stripe rust and other diseases in wheat.

HTAP resistance has two components: temperature sensitivity and development stage dependence. These two components are not always equally required for resistance. Among cultivars with a broad-sense HTAP resistance, which can be determined by a virulent race in a seedling test under low temperatures and in a field or greenhouse test with the same race under high temperatures, resistance in some cultivars is more temperature sensitive whereas others are more plant-stage dependent. In our studies, *Yr39* is more typical of HTAP, where the maximum expression of resistance is in flag leaves and under high-temperatures. In contrast, both *Yr18* and *Yr36* can express resistance even in the seedling stage when under high temperatures (Fu et al. 2009; Krattinger et al. 2009). Thus it is likely that some transcripts involved in HTAP resistance respond more to temperatures, some more to growth stage, and others more or less neutral. In our studies to date this issue has not been addressed. Identification of genes responding to different environmental and growth stage conditions may allow choice of durable resistance genes that are more suited to specific regions in order to more effectively diversify resistance.

If two resistance genes regulating different transcripts (more additive gene action) provide resistance that is more difficult for the pathogen to overcome than those regulating largely shared transcripts (more epistatic gene action), selection of appropriate genes for combining may lead to more durable resistance. Furthermore, the correct combinations of genes may provide higher levels of resistance. In this way, transcriptomics studies of resistance genes will not only provide an understanding of the basic mechanisms of resistance, but will allow for immediate application in selecting resistance genes for precision breeding. The results may be able to tell us which genes are more likely to be durable and which are not. It will also add to the biotechnological tool box for identification of the same or different genes. As the technology is advancing, transcriptomics testing should become less expensive and higher throughput. It will become more feasible to use transcriptomics approaches for identifying genes and developing markers for different types of resistance.

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References

Brueggeman R, Drader T, Kleinhofs A (2006) The barley serine/threonine kinase gene *Rpg1* providing resistance to stem rust belongs to a gene family with five other members encoding kinase domains. Theor Appl Genet 113:1147-1158

Bull J, Mauch F, Hertig C, Rebmann G, Dudler R (1992) Sequence and expression of a wheat gene that encodes a novel protein associated with pathogen defense. Mol Plant-Microbe Interact 5:516-519

Chen XM (2005) Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. Can J Plant Pathol 27:314-337

Chen XM (2011) High-temperature adult-plant resistance, the key for sustainable control of stripe rust. Amer J Plant Sci Biotech 00:000-000 (in press)

Chen XM, Line RF (1992) Identification of stripe rust resistance genes in wheat cultivars used to differentiate North American races of *Puccinia striiformis*. Phytopathology 82:1428-1434

Coram TE, Brown-Guedira G, Chen XM (2008a) Using transcriptomics to understand the wheat genome. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 3. No. 083

Coram TE, Huang XL, Zhan GM, Settles ML, Chen XM (2010) Meta-analysis of transcripts associated with race-specific resistance to stripe rust in wheat demonstrates common induction of blue copper-binding protein, heat-stress transcription factor, pathogen-induced WIR1A protein, and ent-kaurene synthase transcripts. Func Integ Genom 10:383-392

Coram TE, Settles ML, Wang MN, Chen XM (2008b) Surveying expression level polymorphism and single-feature polymorphism in near-isogenic wheat lines differing for the *Yr5* stripe rust resistance locus. Theor Appl Genet 117:401-411

Coram TE, Settles ML, Chen XM (2008c) Transcriptome analysis of high-temperature adult-plant resistance conditioned by *Yr39* during the wheat-*Puccinia striiformis* f. sp. *tritici* interaction. Mol Plant Pathol 9:479-493

Coram TE, Settles ML, Chen XM (2009) Large-scale analysis of antisense transcription in wheat using the Affymetrix GeneChip wheat genome array. BMC Genomics 10:253-264

Coram T, Wang MN, Chen XM (2008d) Transcriptome analysis of the wheat-*Puccinia striiformis* f. sp. *tritici* interaction. Mol Plant Pathol 9:157-169

Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JDG (1998) The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. Plant Cell 10:1915-1926

Fu DL, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen XM, Sela H, Fahima T, Dubcovsky J (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323:1357-1360

Harter C, Pavel J, Coccia F, Draken E, Wegehingel S, Tschochner H, Wieland F (1996) Nonclathrin coat protein gamma, a subunit of coatomer, binds to the cytoplasmic dilysine motif of membrane proteins of the early secretory pathway. Proc Natl Acad Sci USA 93:1902-1906

Johal GS, Briggs SP (1992) Reductase activity encoded by the HM1 disease resistance gene in maize. Science 258:985-987

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360-1363

Krattinger SG, Lagudah ES, Wicker T, Risk JM, Ashton AR, Selter LL, Matsumoto T, Keller B (2011). *Lr34* multi-pathogen resistance ABC transporter: molecular analysis of homoeologous and orthologous genes in hexaploid wheat and other grass species. Plant J 65:392-403

Line RF (2002) Stripe rust of wheat and barley in North America: A restrospective historical review. Annu Rev Phytopathol 40:75-118

Qayoum A, Line RF (1985) High-temperature, adult-plant resistance to stripe rust of wheat. Phytopathology 75:1121-1125

Stubbs RW (1985) Stripe rust. In: Roelfs AP, Bushnell WR (eds) The Cereal Rusts, Vol. 2. Disease, distribution, epidemiology and control. Academic Press, Orlando, FL, pp61-101

Yan GP, Chen XM, Line RF, Wellings CR (2003) Resistance gene analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust. Theor Appl Genet 106:636-643

Unraveling the entry mechanism of oomycete and fungal effector proteins into host cells

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Abstract Oomycetes and fungi facilitate pathogenesis via secretion of effector proteins that have apoplastic and intracellular localizations. These effector proteins have a diverse array of functions that aid in pathogenesis, including modification of defense responses. In the oomycetes, well characterized effector proteins that can translocate into the host cells share a pair of conserved N-terminal motifs known as RXLR and dEER. The RXLR motif has been shown to mediate translocation of the oomycete avirulence proteins Avr1b and Avr3a into host cells. Detailed mutagenesis of the RXLR motif of Avr1b revealed that the motif is tolerant to several amino acid substitutions while retaining functional translocation activity, resulting in the definition of a broadened RXLR-like motif, [R,K,H]X[L/M/I/F/Y/W]X. This motif has been used to identify functional translocation motifs in several fungal effector proteins, AvrL567, Avr2, and AvrLm6. Effectors with both RXLR and RXLR-like motifs bind phosphatidylinositol-3-phosphate (PI-3-P) to mediate translocation via lipid raft mediated endocytosis. Mutations in RXLR or RXLR-like motifs result in loss of phospholipid binding and translocation by effectors. Effector entry into plant cells can be blocked by proteins and inositides that disrupt binding to PI-3-P, suggesting effector-blocking technologies that could be used in agriculturally important plant species.

Keywords

Avirulence gene, biotechnology development, effectors, endocytosis, RXLR-dEER motif, fungi, lipid raft, phosphatidylinositol-3-phosphate (PtdIns-3-P), phospholipids

Phytophthora

Phytophthora comprises a genus in the stramenopiles notorious for their devastating destruction of important crops (Erwin and Ribiera 1996). Though more closely related to diatoms and algae, oomycetes share similar mechanisms of pathogenesis to fungi (Meng et al. 2009). *Phytophthora sojae* accounts for US$1-2 billion of losses to soybean (*Glycine max*) production each year (Tyler 2007). *P. infestans*, the causative agent of the Irish potato famine, is responsible for losses to potato (*Solanum tuberosum*) exceeding US$6 billion dollars yearly (Haverkort et al. 2008). *P. palmivora* is responsible for 10-30% of loss in production of cacao beans annually (Guest 2007). Other oomycetes, such as *P. ramorum*, which causes sudden oak death syndrome on live oaks, are considered a significant threat to California's coastal forests (Rizzo et al. 2002). The genus contains over 106 species that cause significant damage to a large number of diverse plants (Erwin and Ribiero 1996).

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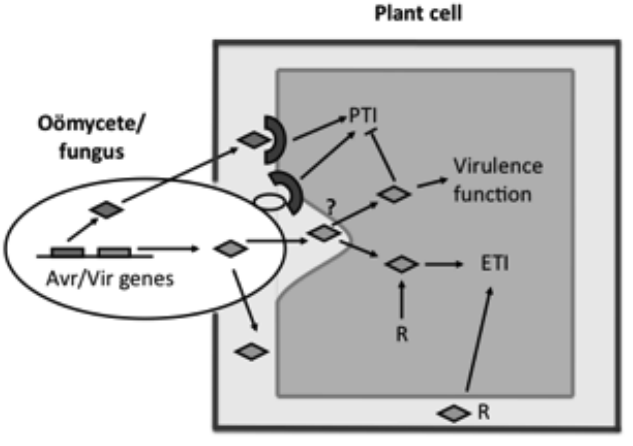
Pathogenesis

The outcome of pathogenesis by *P. sojae* on soybean is often decided in the first 24 hours of infection. In a compatible interaction, oomycete zoospores are attracted to soybean roots via isoflavones (Tyler et al. 1996). Encystment occurs on a root and penetration commences immediately. The germ tube initially transverses between root epidermal cells, into the root cortex (Tyler 2007). By 4 hours, haustoria are abundant along the hyphae, which have generally reached the 4th layer of the cortex (Tyler 2007). By 15 hours hyphae have generally reached the vascular tissue and cells are being penetrated directly (Tyler 2007). After approximately 16-24 hours of biotrophy *P. sojae* switches to a necrotrophic mode (Tyler 2007).

Haustoria form an intimate site of interaction between host plant cells and fungal or oomycete pathogens. Nutrient acquisition from host to pathogen occurs through the haustoria. Haustoria also mediate the delivery of some, possibly many, pathogen-encoded secreted proteins, known as effectors.

Effectors are diverse proteins secreted by many pathogens that modify host physiology to promote infection (Fig. 1). Some effectors remain in the apoplast, whereas others are targeted to the cytoplasm of host cells. Many effectors from bacteria are injected into host cells via the type III secretion machinery (Cornelis 2006). The mechanisms by which fungal and oomycete effectors enter host cells are less well characterized; recent progress in this area will be discussed below. Intracellular effectors such as AvrPtoB from the bacterium *Pseudomonas syringae* pv. *tomato* and ATR1 from the oomycete *Hyaloperonospora arabidopsidis* have the ability to suppress host immune responses

Fig. 1 ***Roles of effector proteins in pathogenesis. Pathogen Associated Molecular Patterns (PAMPs) trigger a defense response that mediates immunity (PAMP triggered immunity, PTI). Effectors, intracellular or extracellular, can be "recognized" directly or indirectly by R gene products to trigger a robust defense response that mediates immunity (effector triggered immunity, ETI). A subset of effectors can suppress PTI and ETI. Others have different functions in virulence***



(Abramovitch et al. 2006; Sohn et al. 2007). Some effectors such as Avr4 and Ecp6 from the fungus *Cladosporum fulvum* play functional roles in the apoplast (van den Burg et al. 2006; de Jonge et al. 2010). Plant defenses can be triggered by commonly occurring microbial molecules (Pathogen Associated Molecules Patterns; PAMPs) such as bacterial flagellin or fungal chitin (Felix et al. 1999; Boller et al. 1995). Defenses can also be triggered by pathogen effectors though the action of NBS-LRR resistance proteins (DeYoung and Innes 2006). In turn, pathogens have evolved effectors that can suppress both PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl 2006). The resulting interplay, or arms race, between pathogen and host to silence or amplify a defense response causes rapid evolution of the pathogen and host molecules involved.

RXLR effector reservoirs

Oomycete effectors are characterized by a conserved N-terminal motif, RXLR-dEER. The RXLR-dEER motif was identified after the sequencing of the genomes of *P. sojae* and *P. ramorum,* and ESTs of *P. infestans,* together with the cloning of the first oomycete avirulence genes, *P. sojae Avr1b-1*, *Hyaloperonospora arabidopsidis ATR13* and *ATR1*, and *P. infestans Avr3a* (Allen et al. 2004; Shan et al. 2004; Armstrong et al. 2005; Rehmany et al. 2005; Randall et al. 2005; Birch et al. 2006; Tyler et al. 2006). Comparison amongst these avirulence proteins and with predicted proteins that shared similarity to Avr1b resulted in the identification of the RXLR and dEER motifs (Rehmany et al. 2005; Birch et al. 2006; Tyler et al. 2006). A Hidden Markov Model (HMM) targeted to the 24 amino acids spanning the RXLR motif was utilized to identify large reservoirs of putative effector proteins in oomycete genomes: 396 and 374 RXLR proteins in the genomes of *P. sojae* and *P. ramorum*, respectively, and 550 and 134 in the recently sequenced *P. infestans* and *H. arabidopsidis* genomes, respectively (Jiang et al. 2008; Haas et al. 2009; Baxter et al. 2010). Subsequently, the RXLR motif was used as an aid to clone additional avirulence genes including *P. sojae Avr3a, Avr3c, Avr1a,* and *Avr4/6*, and *P. infestans Avr2, Avr4, AvrBlb1,* and *AvrBlb2* (Qutob et al. 2009; Dong et al. 2009; Dou et al. 2010; Lokossou et al. 2009; van Poppel et al. 2008; Vleeshouwers et al. 2008; Oh et al. 2009). The dEER (also referred to as EER) motif is also present in these avirulence proteins (except for ATR13) though the sequence, length, and position vary in every avirulence protein.

Oomycete effector protein translocation

The RXLR motif was initially postulated to be a protein translocation motif based on the fact that several avirulence proteins containing this motif, interacted with known intracellular resistance (*R*) gene products (Rehmany et al. 2005; Birch et al. 2006), and that the related *Plasmodium falciparum* PEXEL motif, RXLX[E/D/Q], facilitated translocation of *Plasmodium* effectors into erythrocytes (Marti et al. 2004).

In vivo assays using *P. sojae* Avr1b and *P. infestans* Avr3a demonstrated the physiological relevance of the RXLR and dEER motifs in protein translocation (Whisson et al. 2007; Dou et al. 2008). Alanine substitution mutations of the Avr1b and Avr3a RXLR and dEER motifs resulted in a loss of protein translocation inferred by a loss of the avirulence phenotype conferred by the genes in pathogen transformants (Whisson et al. 2007; Dou et al. 2008). Expression of these RXLR and/or dEER mutant proteins in the cytoplasm of host cells resulted in cell death associated with avirulence implying that the mutations do not affect the interaction of the Avr and R proteins (Whisson et al. 2007; Dou et al. 2008). The N-terminus of Avr3a was sufficient to translocate β-glucuronidase from *P. infestans* transformants into potato cells (Whisson et al. 2008). Even subtle mutations of the RXLR and dEER motifs of Avr3a to KMIK-DDK resulted in loss of translocation (Whisson et al. 2008).

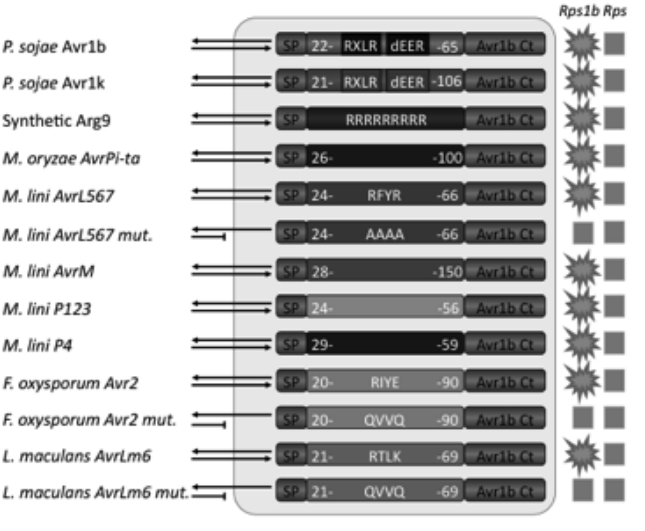
The validation of the RXLR-dEER motif in protein translocation *in vivo* was the first step to understanding the translocation of effector proteins. As a next step, purified Avr1b -GFP fusion proteins expressed in *E.coli,* added to soybean roots exogenously, were found to enter the root cells, revealing that no pathogen-encoded machinery was needed for entry (Dou et al. 2008; Kale et al. 2010). Similar findings were obtained for the oomycete effector proteins Avh331 and Avh5. Mutating the RXLR and/or dEER motifs of these proteins resulted in a loss of translocation of the GFP fusions into soybean cells (Kale et al. 2010). A novel double barrel particle bombardment assay was used to show that pathogen-independent translocation also occurs in soybean leaf cells and onion epidermal cells. Furthermore, full length Avr1k and Avr1b proteins, when applied exogenously, had the ability to enter soybean leaf cells and produce a cell death response in the presence of Rps1k and Rps1b, respectively (Shan et al. 2004; Kale et al. 2010). Interestingly, Avr1b contains two RXLR motifs, one predicted to be functional by HMM and the other not (Dou et al. 2008). Mutation of the non-canonical RXLR motif of Avr1b still resulted in translocation of GFP implying that the surrounding sequences are important for the correct function of the RXLR motif (Dou et al. 2008). Oomycete effector proteins also could translocate into other cell types such as human airway epithelial cells (Kale et al. 2010). That entry was also reliant on the RXLR motif.

Fungal effector protein translocation

In fungi there are no obvious conserved translocation motifs among intracellular effectors comparable to the oomycete RXLR and dEER motifs, except for effectors from powdery mildew fungi (Godrey et al. 2010). Using the double barrel gene gun assay, detailed mutagenesis of the RXLR motif of oomycete effector Avr1b was performed to identify which amino acid substitutions in the motif resulted in loss of translocation activity (Kale et al. 2010). Activity was retained when the first arginine was mutated to lysine or histidine, but not glutamine. The leucine could be substituted with a wide variety of large hydrophobic amino acids, but not alanine or glycine. The second arginine could be substituted with a wide array of amino acids ranging from glycine to glutamine. Based on this analysis of Avr1b, a much broader "RXLR-like" motif, [R,K,H]X[L/M/I/F/Y/W]X, was defined (Kale et al. 2010). Concurrently the N-termini of several known fungal intracellular effectors were fused to the C-terminus of Avr1b and tested using the soybean bombardment re-entry assay (Fig. 2) (Kale et al. 2010). In many cases the N-termini of the fungal effectors possessed cell entry activity. Each of these N-terminal cell entry domains contained RXLR-like motif(s). Mutation of the RXLR-like motifs resulted in identification of functional motifs required for translocation in the bombardment re-entry assay. In the case of *Melampsora lini* AvrL567 the N-terminal motif RFYR was required for translocation (Kale et al. 2010; Rafiqi et al. 2010). The N terminus of *M. lini* AvrM containing several RXLR-like motifs was shown to mediate translocation (Kale et al. 2010; Rafiqi et al. 2010). Rafiqi et al. (2010) were able to narrow down the translocation domain to a region containing three RXLR-like sequences. *Fusarium oxysporum* Avr2 contained one functional motif (RIYER) and one non-functional motif (RMLH) (Kale et al. 2010). *Leptosphaeria maculans* AvrLm6 also contained an RXLR-like motif (RYWT) that mediated translocation (Kale et al. 2010).

A drawback of the high sequence redundancy of the RXLR-like motifs is the large number of false positives produced when the motif is used for bioinformatic searches. Thus experimental assays are essential for identifying which motif (if any) is actually functional in a potential effector protein.

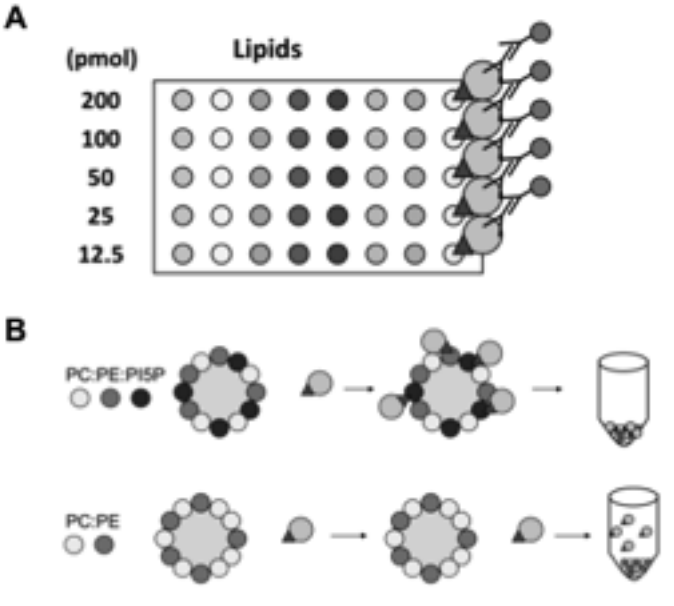
Fig. 2 ***Oomycete and fungal cell entry domains identified by the soybean bombardment re-entry assay. Arrows indicate translocation across the membrane. Cell death occurs in leaf tissue containing Rps1b only when the C-terminal domain of Avr1b re-enters the cells after secretion. When Rps1b is absent (Rps) no cell death occurs. Explosion symbol indicates cell death due to hypersensitive response (HR), blank symbol signifies a lack of cell death***



Phospholipid binding

As described above, several oomycete and fungal effectors can translocate into a variety of cell types without any pathogen-encoded machinery via RXLR or RXLR-like motifs. Phosphatidylinositol-3-phosphate (PtdIns-3-P) has been shown to mediate the entry of these effectors into host cells (Kale et al. 2010). So far a total of 3 oomycete and 3 fungal effector proteins have been found to bind PtdIns-3-P in a lipid filter assay and a liposome-binding assay (Kale et al. 2010) (Fig. 3). In each case, mutation of the functional RXLR or RXLR-like motif required for cell entry also resulted in a loss of PtdIns-3-P binding (Kale e al. 2010). A strong correlation thus exists between cell entry activity and binding of PtdIns-3-P for these effectors.

Fig. 3 ***Assaying lipid binding. A) The lipid filter assay is a useful tool to perform an initial screen against many different lipids. If used appropriately, the assay can provide a semi-quantitative measure of the strength of protein-lipid binding. Protein bound to a lipid(s) is detected through HRP-linked antibodies. B) The liposome binding assay is used to validate a potential interaction. The assay provides greater physiological relevance and confidence for an interaction. When an interaction occurs the bound protein pellets with the liposomes after high speed centrifugation. Protein that does not interact with liposomes stays in the supernatant. Binding to liposomes can be quantitated to obtain dissociation constants***



The presence of PtdIns-3-P on the outer leaflet of plant cells was demonstrated using three specific PtdIns-3-P-binding proteins, 2xFYVE, VAM7p-PX domain, and PEPP1-PH domain fused to a fluorescent protein (GFP and mCherry) (referred to from here as biosensors) (Kutateladze et al. 2001; Lee et al. 2006; Dowler et al. 2000). Incubation of these probes with soybean cells at 4°C, to prevent endocytosis, resulted in binding of the PtdIns-3-P biosensors to the outer leaflet the plasma membranes of the cells (Kale et al. 2010). In contrast, a PtdIns-4-P biosensor did not bind the membranes. The same experiments demonstrated that PtdIns-3-P was also on the surface of human lung epithelial cells, albeit in punctate patterns that differ from the uniform labeling of the plant membranes.

Mechanism of entry

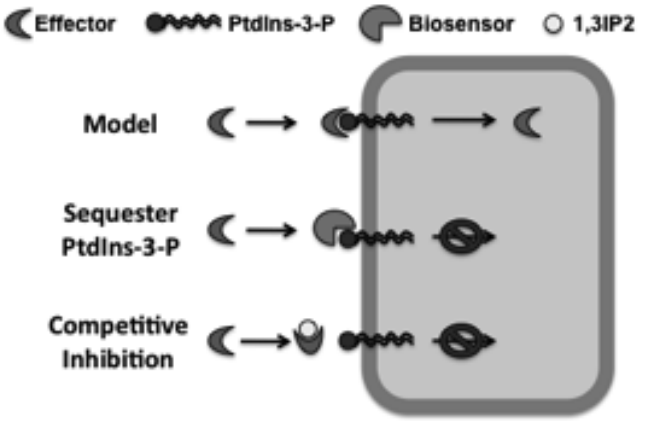
When RXLR effector-GFP fusions are rapidly internalized in human airway epithelial cells, the proteins can be visualized in small vesicle structures (Kale et al. 2010). Similar vesicle structures can be visualized when effector-GFP fusion proteins are incubated with soybean suspension culture cells (Kale et al. 2010). Filipin and nystatin, that disrupt the formation of lipid rafts and thus raft-mediated endocytosis, block entry of the effector-GFP fusions (Kale et al. 2010). Inhibitors of clathrin-mediated endocytosis, macropinocytosis, and flippases did not inhibit internalization. These results indicate that the RXLR and RXLR-like effectors likely bind to PtdIns-3-P found in lipid rafts and this binding results in endocytosis of the effectors.

Blocking effector entry

Effector entry mediated by PtdIns-3-P can be blocked by two strategies (Fig. 4). The first strategy utilizes the biosensor proteins that have a strong affinity to PtdIns-3-P. These biosensor proteins, when pre-incubated with soybean root cells or human airway epithelial cells, prevented entry of Avr1b-GFP and AvrL567-GFP, presumably by sequestering PtdIns-3-P (Kale et al. 2010). The second strategy is based on competitive inhibition of the binding of Avr1b-GFP and AvrL567-GFP to PtdIns-3-P by 1,3-inositol-bisphosphate (1,3IP2). Incubation of Avr1b-GFP and AvrL567-GFP with soybean root cells or human airway epithelial cells in the presence of 500 µM 1,3-inositol-bisphosphate (1,3IP2) prevented internalization of effector proteins (Kale et al. 2010). Liposome binding assays validated that 1,3IP2 could block the binding

of Avr1b-GFP and AvrL567-GFP to PtdIns-3-P (Kale et al. 2010). These experiments confirm that PtdIns-3-P binding is required for cell entry and suggest strategies for blocking the infection of crop plants by fungal and oomycete pathogens that depend on RXLR-like effectors.

Fig. 4 ***Simplified model of effector entry and effector-blocking strategies. Certain oomycete and fungal effectors bind PtdIns-3-P in lipid raft regions on the outer leaflet of plant and animal cells to mediate translocation into host cells. Entry mechanism involves endocytosis. The mechanism of escape from these endosomes is currently unknown. Access to PtdIns-3-P may be blocked through the use of Ptd-3-P-binding biosensor proteins. The binding pocket of the effectors may be occupied by a small molecule such as 1,3IP2, thereby competing against binding by PtdIns-3-P on the membrane***



Cereal rusts perspectives

Although dissimilar phylogenetically, oomycetes and cereal rust pathogens share some remarkably similar mechanisms of pathogenesis as a result of convergent evolution. Both oomycetes and cereal rust pathogens utilize haustoria for nutrient acquisition and, presumably, as a site for effector delivery. At least one effector from a rust fungus, and likely many more, translocate into host plant cells via RXLR-like sequences and PI-3-P. We speculate that inhibiting translocation of effector proteins by targeting PI-3-P and/or RXLR-like sequence motifs may provide broad spectrum protection against diverse fungal and oomycete pathogens, including multiple species of rust fungi. For example, wheat might be protected simultaneously against *P. striiformis*, *P. graminis* f. sp. *tritici* and *P. triticina*.

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References

Abramovitch RB, Janjusevic R, Stebbins CE, Martin GB (2006) Type III effector AvrPtoB requires intrinsic E3 ubiquitin ligase activity to suppress plant cell death and immunity. Proc Natl Acad Sci USA 103:2851-2856

Allen RL, Bittner-Eddy PD, Grenville-Briggs LJ, Meitz JC, Rehmany AP, Rose LE, Beynon JL (2004) Host-parasite coevolutionary conflict between *Arabidopsis* and downy mildew. Science 306:1957-1960

Armstrong MR, Whisson SC, Pritchard L, Bos JIB, Venter E, Avrova AO, Rehmany AP, Böhme U, Brooks K, Cherevach I, Hamlin N, White B, Fraser A, Lord A, Quail MA, Churcher C, Hall N, Berriman M, Huang S, Kamoun S, Beynon JL, Birch PRJ (2005) An ancestral oomycete locus contains late blight avirulence gene Avr3a, encoding a protein that is recognized in the host cytoplasm. Proc Natl Acad Sci USA 102:7766-7771

Baxter L, Tripathy S, Ishaque N, Boot N, Cabral A, Kemen E, Thines M, Ah Fong A, Anderson R, Badejoko W, Bittner-Eddy P, Boore JL, Chibucos MC, Coates M, Dehal P, Delehaunty K, Dong S, Downton P, Dumas B, Fabro G, Fronick C, Fuerstenberg SI, Fulton L, Gaulin E, Govers F, Hughes L, Humphray S, Jiang RHY, Judelson H, Kamoun S, Kyung K, Meijer H, Minx P, Morris P, Nelson J, Phuntumart V, Qutob D, Rehmany A, Rougon-Cardoso A, Ryden P, Torto-Alalibo T, Studholme D, Wang Y, Win J, Wood J, Clifton SW, Rogers J, Van den Ackerveken G, Jones JDG, McDowell JM, Beynon J, Tyler BM (2010) Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome. Science 330:1549-1551

Birch PR, Rehmany AP, Pritchard L, Kamoun S, Beynon JL (2006) Trafficking arms: oomycete effectors enter host plant cells. Trends Microbiol 14:8-11

Boller T (1995) Chemoperception of microbial signals in plant cells. Annu Rev Plant Physiol Plant Mol Biol 46:189-214

Cornelis GR (2006) The type III secretion injectisome. Nature Reviews Microbiol 4:811-825

de Jonge R, van Esse HP, Kombrink A, Shinya T, Desaki Y, Bours R, van der Krol S, Shibuya N, Joosten MHA, Thomma BPHJ (2010) Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. Science 328:953-955

DeYoung BJ, Innes RW (2006) Plant NBS-LRR proteins in pathogen sensing and host defense. Nat Immunol 7:1243-1249

Dou D, Kale SD, Liu TL, Tang Q, Wang X, Arredondo FD, Basnayake S, Whisson S, Drenth A, Maclean D, Tyler BM (2010) Different domains of Phytophthora sojae effector Avr4/6 are recognized by soybean resistance genes Rps4 and Rps6. Mol Plant Microbe Interact 23:425-435

Dou D, Kale SD, Wang X, Jiang RHY, Bruce N, Arredondo FD, Zhang X, Tyler BM (2008) RXLR-mediated entry of *Phytophthora sojae* effector Avr1b into soybean cells does not require pathogen-encoded machinery. Plant Cell 20:1930-1947

Dong S, Qutob D, Tedman-Jones J, Kuflu K, Wang Y, Tyler BM, Gijzen M (2009) The *Phytophthora sojae* avirulence locus *Avr3c* encodes a multi-copy RXLR effector with sequence polymorphisms among pathogen strains. PLos One 4(5):e5556

Dowler S, Currie RA, Campbell DG, Deak M, Kular G, Downes CP, Alessi SR (2000) Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities. Biochem J 351:19-31

Erwin DC, Ribiero OK (1996) Phytophthora diseases worldwide,. APS Press, St. Paul, MN

Felix G, Duran JD, Volko S, Boller T (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J 18:265-276

Godrey D, Böhlenius H, Pedersen C, Zhang Z, Emmersen J, Thordal-Christensen H (2010) Powdery mildew fungal effector candidates share N-terminal Y/F/WxC-motif. BMC Genomics 11:317

Guest D (2007) Black pod: Diverse pathogens with a global impact on cocoa yield. Phytopathology 97:1650-1653

Haas BJ, Kamoun S, Zody MC, Jiang RHY, Handsaker RE, Cano LM, Grabherr M, Kodira CD, Raffaele S, Torto-Alalibo T, Bozkurt TO, Ah-Fong AMV, Alvarado L, Anderson VL, Armstrong MR, Avrova A, Baxter L, Beynon J, Boevink PC, Bollmann SR, Bos JIB, Bulone V, Cai G, Cakir C, Carrington JC, Chawner M, Conti L, Costanzo S, Ewan R, Fahlgren N, Fischbach MA, Fugelstad J, Gilroy EM, Gnerre S, Green PJ, Grenville-Briggs LJ, Griffith J, Grunwald NJ, Horn K, Horner NR, Hu CH, Huitema E, Jeong DH, Jones AME, Jones JDG, Jones R, Karlsson EK, Kunjeti SG, Lamour K, Liu Z, Ma L, MacLean D, Chibucos MC, McDonald H, McWalters J, Meijer HJG, Morgan W, Morris PF, Munro KO, Ospina-Giraldo M, Pinzon A, Pritchard L, Ramsahoye B, Ren Q, Restrepo S, Roy S, Sadanandom A, Savidor A, Schornack S, Schwartz DC, Schumann UD, Schwessinger B, Seyer L, Sharpe T, Silvar C, Song J, Studholme DJ, Syker S, Thines M, van de Vondervoorts PJI, Phuntumart V, Wawra S, Weide S, Win J, Young C, Zhou S, Fry W, Meyers BC, van West P, Ristaino J, Govers F, Birch PRJ, Whisson SC, Judelson HS, Nusbaum C (2009) Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans.* Nature 461:393-398

Haverkort AJ, Boonekamp PM, Hutten R, Jacobsen E, Lotz LAP, Kessel GJT, Visser RGF, van der Vossen EAG (2008) Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. Potato Res 51:47-57

Jiang RHY, Tripathy S, Govers F, Tyler BM (2008) RXLR effector reservoir in two *Phytophthora* species is dominated by a single rapidly evolving super-family with more than 700 members. Proc Natl Acad Sci USA 105:4874-4879

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323-329

Kale SD, Gu B, Capelluto DGS, Dou D, Feldman E, Rumore A, Arredondo FD, Hanlon R, Fudal I, Rouxel T, Lawrence CB, Shan W, Tyler BM (2010) External lipid PI-3-P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. Cell 142:284-295

Kutateladze T, Overduin M (2001) Structural mechanism of endosome docking by the FYVE domain. Science 291:1793-1796

Lee SA, Kovacs J, Stahelin RV, Cheever ML, Overduin M, Setty TG, Burd CG, Cho W, Kutateladze TG (2006) Molecular mechanism of membrane docking by the Vam7p PX domain. J Biol Chem 281:37091-37101

Lokossou AA, Park TH, van Arkel G, Arens M, Ruyter-Spira C, Morales J, Whisson SC, Birch PRJ, Visser RGF, Jacobsen E, van der Vossen EAG (2009) Exploiting knowledge of *R/Avr* genes to rapidly clone a new LZ-NBS-LRR family of late blight resistance genes from potato linkage group IV. Mol Plant Microbe Interact 22:630-641

Marti M, Good RT, Rug M, Knuepfer E, Cowman AF (2004) Targeting malaria virulence and remodeling proteins to the host erythrocyte. Science 306:1930-1933

Meng S, Torto-Alalibo T, Chibucos MC, Tyler BM, Dean RA (2009) Common processes in pathogenesis by fungal and oomycete plant pathogens, described with gene ontology terms. BMC Microbio 9:(Suppl 1):S7

Oh SK, Young C, Lee M, Oliva R, Bozkurt TO, Cano LM, Win J, Bos JI, Liu HY, van Damme M, Morgan W, Choi D, Van der Vossen EA, Cleeshouwers VG, Kamoun S (2009) In planta expression screens of Phytophthora infestans RXLR effectors reveal diverse phenotypes, including activation of the Solanum bulbocastanum disease resistance protein Rpi-blb2. Plant Cell 21:2928-2947

Qutob D, Tedman-Jones J, Dong S, Kuflu K, Pham H, Wang Y, Dou D, Kale SD, Arredondo FD, Tyler BM, Gijzen M (2009) Copy number variation and transcriptional polymorphisms of *Phytophthora sojae* RXLR effector genes Avr1a and Avr3a. PLoS One 4(4):e5066

Rafiqi M, Gan PH, Ravensdale M, Lawrence GJ, Ellis JG, Jones DA, Hardham AR, Dodds PN (2010) Internalization of flax rust avirulence proteins into flax and tobacco cells can occur in the absence of the pathogen. Plant Cell 22:2017-2032

Randall TA, Dwyer RA, Huitema E, Beyer K, Cvitanich C, Kelkar H, Ah Fong AMV, Gates K, Roberts S, Yatzkan E, Gaffney T, Law M, Testa A, Toto-Alalibo T, Zhang M, Zheng, L, Mueller E, Windass J, Binder A, Birch PRJ, Gisi U, Govers F, Gow NA, Mauch F, van West P, Waugh ME, Yu J, Boller T, Kamoun S, Lam, ST, Judelson HS (2005) Large-scale gene discovery in the oomycete *Phytophthora infestans* reveals likely components of phytopathogenicity shared with true fungi. Mol Plant Microbe Interact 18:229-243

Rehmany AP, Gordon A, Rose LE, Allen RL, Armstrong MR, Whisson SC, Kamoun S, Tyler BM, Birch PRJ, Beynon JL (2005). Differential recognition of highly divergent downy mildew avirulence gene alleles by RPP1 resistance genes from two *Arabidopsis* lines. Plant Cell 17:1839-1850

Rizzo DM, Garbelotto M, Davidson JM, Slaughter GW, Koike ST (2002) *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. Plant Dis 86:205-214

Shan W, Cao M, Leung D, Tyler BM (2004) The Avr1b locus of Phytophthora sojae encodes an elicitor and a regulator required for avirulence on soybean plants carrying resistance gene Rps1b. Mol Plant Microbe Interact 17:394-403

Sohn KH, Lei R, Nemri A, Jones JDG (2007) The downy mildew effector proteins ATR1 and ATR13 promote disease susceptibility in *Arabidopsis thaliana*. Plant Cell 19:4077-4090

Tyler BM (2007) Phytophthora sojae: root rot pathogen of soybean and model oomycete. Mol Plant Pathol 8:1-8

Tyler BM, Wu MH, Wang JM, Cheung W, Morris PF (1996) Chemotactic preferences and strain variation in the response of *Phytophthora sojae* zoospores to host isoflavones. Appl Environ Microbiol 62:2811-2817

Tyler BM, Tripathy S, Zhang X, Dehal P, Jiang RHY, Aerts A, Arredondo FD, Baxter L, Bensasson D, Beynon JL, Chapman J, Damasceno CMB, Dorrance AE, Dou D, Dickerman AW, Dubchak IL, Garbelotto M, Gijzen M, Gordon SG, Govers F, Grunwald, NJ, Huang W, Ivors KL, Jones RW, Kamoun S, Krampis K, Lamour KH, Lee MK, McDonal DJ, Medina M, Meijer HJG, Nordberg EK, Maclean DJ, Ospina-Giraldo MD, Morris PF, Phuntumart V, Puntnam NH, Rash S, Rose JKC, Sakihama Y, Salamov AA, Savidor A, Scheuring CF, Smith BM, Sobral BWS, Terry A, Torto-Alalibo TA, Win J, Xu Z, Zhang H, Grigoriev IV, Rokhsar DS, Boore JL (2006) Phytophthora genome sequences uncover evolutionary origins and mechanisms of pathogenesis. Science 313:1261-1266

van den Burg HA, Harrison SJ, Joosten MH, Vervoort J, de Wit PJ (2006) Cladosporium fulvum Avr4 protects fungal cell walls against hydrolysis by plant chitinases accumulating during infection. Mol Plant Microbe Interact 19:1420-1430

van Poppel PMJA, Guo J, van de Vondervoort PJI, Jung MW, Birch PR, Whisson SC, Govers F (2008) The *Phytophthora infestans* avirulence gene Avr4 encodes an RXLR-dEER effector. Mol Plant Microbe Interact 21:1460-1470

Vleeshouwers VGAA, Rietman H, Krenek P, Champouret N, Young C, Oh SK, Wang M, Bouwmeester K, Vosman B, Visser RGF, Jacobsen E, Govers F, Kamoun S, Van der Vossen EAG (2008) Effector genomics accelerates discovery and functional profiling of potato disease resistance and *Phytophthora infestans* avirulence genes. PLoS ONE 3:e2875

Whisson SC, Boevink P C, Moleleki L, Avrova AO, Morales JG, Gilroy EM, Armstrong MR, Grouffaud S, van West P, Chapman S, Hein I, Toth IK, Pritchard L, Birch PRJ (2007) A translocation signal for delivery of oomycete effector proteins into host plant cells. Nature 450:115-119

Investigating rust resistance with the model grass Brachypodium

D. F. Garvin

Abstract

The model plant *Arabidopsis thaliana* has provided unique opportunities to explore and unravel many key biological features of plant biology including disease resistance. However, the inability of rust fungi of the genus *Puccinia* to infect Arabidopsis has prevented its use in exploring grass-rust interactions. The model plant *Brachypodium distachyon* is a member of the same grass subfamily as the principal cool-season grain crops, and can be infected with various *Puccinia* species. We have focused our efforts on establishing Brachypodium as a model for exploring grass - *Puccinia graminis* interactions. Brachypodium can be successfully infected by different *formae speciales* of the stem rust pathogen, including *P. graminis* f. sp. *tritici*. A wide range of response to stem rust occurs in Brachypodium and efforts are underway to decipher the genetic basis for this variation using recombinant inbred populations from parents with differing levels of response. Similarly, induced mutants with compromised stem rust resistance have been identified and are now being employed within a program to understand the molecular biology of stem rust resistance and susceptibility. Our results to date suggest that Brachypodium holds promise as a model plant for advancing our understanding of stem rust resistance.

Keywords

Brachypodium distachyon, leaf rust, mutants, natural variation, *Puccinia brachypodii*, *Puccinia graminis*, *Puccinia striiformis*, stem rust, stripe rust

Introduction

Members of the fungal genus *Puccinia*, including *P. triticina*, *P. graminis*, and *P. striiformis*, cause significant losses in cool-season grain crops in many areas of the world. The ongoing threat from these pathogens which respectively cause leaf rust, stem rust and stripe rust on wheat, has led to widespread and successful breeding for resistance. Nonetheless, the potential for the emergence of new virulent races that overcome existing resistance persists and so maintaining resistance is an ongoing endeavor. Historically, most of the resistance to these pathogens has derived from major race-specific resistance genes. More recently increased attention has been given to identification of race non-specific resistance genes that can provide durable resistance. Both strategies rely upon natural variation in the particular crop. An additional area of interest for securing durable rust resistance is non-host resistance. The rust fungi are specialized to their hosts and thus do not cause disease in non-hosts, and so unraveling the biological basis of non-host resistance may provide novel avenues for controlling the diseases in a manner complimentary to the deployment of race-specific and non-race specific resistance genes. A model system would be particularly useful for research on exploring the to-date amorphously characterized concept of non-host resistance.

Recently, *Brachypodium distachyon* (Brachypodium) has emerged as a *bona fide* model grass. A series of features of this species that are akin to those of Arabidopsis lend themselves well to a model system. For instance, the species is petite and spring habit genotypes can exhibit a rapid life cycle of 2 months under appropriate growth conditions (Garvin et al. 2008). Brachypodium has a small 272 Mb genome, approximately the same average size as a chromosome arm of wheat (The International Brachypodium Initiative 2010). Recently a multitude of genetic and genome resources has become available for Brachypodium including freely available inbred reference genotypes (Vogel et al. 2006, 2009; Filiz et al. 2009) with more being developed from a wide geographic range, recombinant inbred lines, T-DNA tagged mutants (Thole et al. 2009; see also http://brachypodium.pw.usda.gov/), a genetic linkage map (Garvin et al. 2010) with a higher resolution one soon to be released, efficient *Agrobacterium* transformation methods (Vogel and Hill 2008; Alves et al. 2009), BAC libraries and physical maps, and lastly a high quality draft genome sequence (The International Brachypodium Initiative 2010) with several dozen additional genotypes to be resequenced by the US-DOE. Access to these resources has dramatically accelerated research with Brachypodium in recent years.

Importantly, Brachypodium belongs to the large and complex grass subfamily Pooideae, which encompasses the majority of the economically important cool-season grasses including turf, forage and cereal grain crops. While belonging to a monophyletic tribe that is estimated to have diverged from the Triticeae and other important pooid tribes approximately 35 million years ago (The International Brachypodium Initiative 2010), Brachypodium is reported to be a host for a variety of *Puccinia* species

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(Watson and Dallwitz 1992; Draper et al. 2001). Thus, Brachypodium may be a useful experimental portal to increase our biological understanding of grass-rust pathogen interactions. We sought to examine the potential utility of Brachypodium as a model for such research, with a focus on stem rust.

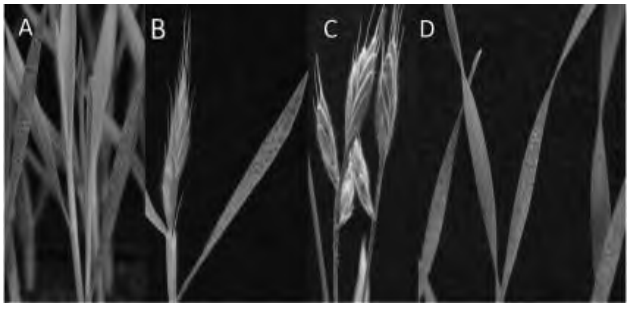
Screening for stem rust isolates that infect Brachypodium

A set of 5 inbred diploid Brachypodium genotypes was employed for an initial screen with a set of *Puccinia* isolates representing eight diverse *Puccinia* species and three different *P. graminis* f. sp. Pots containing the Brachypodium genotypes were spray-inoculated at the one to two leaf stage with the different *Puccinia* isolates and then subjected to an overnight dew period. One of the isolates, *P. graminis* f. sp. *phlei-pratensis*, produced a very small number of sporulating pustules. The result has since been confirmed repeatedly in subsequent inoculations, and mist chamber conditions have gradually been optimized to obtain consistent results (Fig. 1). As well, new isolates since collected from timothy (*Phleum pratense*) have been used to confirm the pathogenicity of *Puccinia graminis* f. sp. *phlei-pratensis* on Brachypodium.

Natural variation for stem rust resistance in Brachypodium

The aforementioned experiments also revealed that there is significant natural variation between Brachypodium genotypes for resistance to *P. graminis* f. sp. *phlei-pratensis*, ranging from nearly immune to significant levels of pustule development, although none of the genotypes so far screened can be considered fully susceptible (Fig. 1). As part of our lab's research with Brachypodium, we are developing or have completed development of a series of recombinants inbred line populations (RILs). Among the first RILs developed is a set derived from a cross between the most and least resistant Brachypodium genotypes. These were evaluated for segregation in stem rust response, with results suggesting both major gene effects and the action of modifiers/minor genes. With the plethora of Brachypodium genomic resources, as well as new genomics tools, we are seeking to further characterize the genes underlying both major stem rust resistance genes and modifiers and minor genes in future studies.

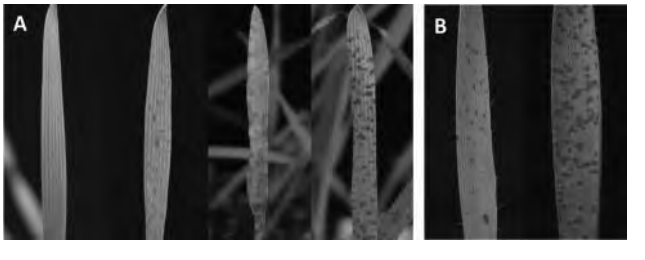
Fig 1 ***Infection of Brachypodium by P. graminis f. sp. phlei-pratensis. A, primary leaves; B, leaf on culm; C, culm; D, differential infection of primary leaves of two different Brachypodium genotypes.***



Infection of Brachypodium by *P. graminis* f. sp. *tritici*

We were interested in determining if Brachypodium could serve as a surrogate for research on stem rust of wheat and barley. Thus, several races and isolates of *P. graminis* f. sp. *tritici* were used in a disease screen of approximately two dozen diverse Brachypodium genotypes at the single leaf and later growth stages. Disease phenotypes varied depending on the race/isolate (Fig. 2), but a small subset of genotypes was identified on which pustule development was pronounced. However, over time plants do mount a defense response and thus no genotypes were considered fully susceptible. Reinoculation of plants approximately 14 days after initial primary leaf inoculations revealed that the correlation between disease development on primary and later developing leaves was imperfect, with some genotypes exhibiting significant disease development on primary, but not on later leaves. We are developing populations involving new genotypes that show the highest levels of stem rust susceptibility to explore its genetic basis.

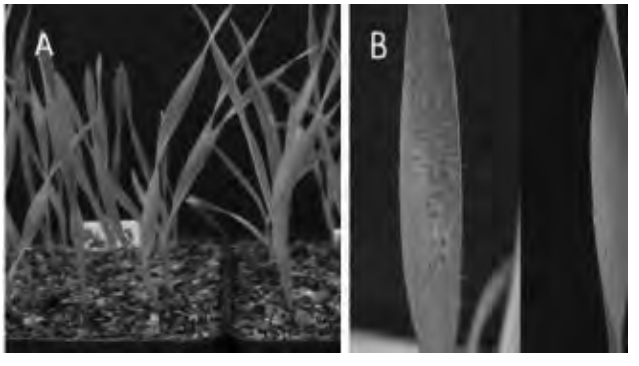
Fig 2 ***Examples of disease phenotypes observed on different Brachypodium genotypes inoculated with a single race of P. graminis f. sp. tritici. A, primary leaves; B, older leaves.***



Mutagenesis as a tool to dissect the stem rust resistance response

Mutagenesis is a potent tool for assisting in the dissection of plant defense response pathways. We have screened both EMS-treated and gamma-irradiated populations of a highly resistant Brachypodium genotype to identify mutants with compromised resistance to *P. graminis* f. sp. *phlei-pratensis*. A number of mutants derived from both treatments were identified with increased susceptibility relative to the wild type parent (Fig. 3). We are now employing these for research to examine the genetic basis of susceptibility to this and other *P. graminis* f. sp., and to generate resources for broader genome analysis of stem rust resistance and susceptibility.

Fig 3 ***Infection of wild type Brachypodium and an induced mutant with compromised resistance to Puccinia graminis f. sp. phlei-pratensis. A, primary leaf inoculations; left, mutant exhibiting significant pustule development; right, wild type plants. B. Disease development on older inoculated leaves of mutant (left) and wild type (right) plants.***



Conclusions

Brachypodium is an attractive model for disease resistance research relevant to wheat, barley, and other cool-season cereal grains. Its petite stature, rapid life cycle, compact but comparable gene content, and evolutionary affinity to these crops, coupled with the fact that it can be infected by *P. graminis*, suggests that it may be a useful surrogate to dissect aspects of stem rust resistance in a manner unattainable in the crop species themselves.

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References

Alves SC, Worland B, Thole V, Snape JW, Bevan MW, Vain P (2009) A protocol for *Agrobacterium*-mediated transformation of *Brachypodium distachyon* community standard line Bd21. Nature Prot 4:638-649

Draper J, Mur LAJ, Jenkins G, Ghosh-Biswas GC, Bablak P, Hasterok R, Routledge APM (2001) *Brachypodium distachyon*. A new model system for functional genomics in grasses. Plant Physiology 127:1539-1555

Filiz E, Ozdemir BS, Budak F, Vogel JP, Tuna M, Budak H (2009) Molecular, morphological, and cytological analysis of diverse *Brachypodium distachyon* inbred lines. Genome 52:876-890

Garvin DF, Gu Y-Q, Hasterok R, Hazen SP, Jenkins G, Mockler TC, Mur LAJ, Vogel JP (2008) Development of genetic and genomic research resources for *Brachypodium distachyon*, a new model system for grass crop research. Crop Sci 48:S69-S84

Garvin DF, McKenzie N, Vogel JP, Mockler TC, Blankenheim ZJ, Wright J, Cheema JJS, Dicks J, Huo N, Hayden DM, Gu Y, Tobias C, Chang JH, Chu A, Trick M, Michael TP, Bevan MW, Snape JW (2010) An SSR-based genetic linkage map of the model grass *Brachypodium distachyon*. Genome 53:1-13

The International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463:763-768

Thole V, Alves SC, Worland B, Bevan MW, Vain P (2009) A protocol for efficiently retrieving and characterizing flanking sequence tags (FSTs) in *Brachypodium distachyon* T-DNA insertional mutants. Nature Prot 4:650-661

Vogel JP, Garvin DF, Leong OM, Hayden DM (2006) *Agrobacterium*-mediated transformation and inbred line development in the model grass *Brachypodium distachyon*. Plant Cell Tiss Org Cult 85:199-211

Vogel J, Hill T (2008) High-efficiency *Agrobacterium*-mediated transformation of *Brachypodium distachyon* inbred line Bd21-3. Plant Cell Rep 27:471-478

Vogel JP, Tuna M, Budak H, Huo N, Gu YQ, Steinwand MA (2009) Development of SSR markers and analysis of diversity in Turkish populations of *Brachypodium distachyon*. BMC Plant Biol 9: Art. 88

Watson L, Dallwitz MJ (1992) The grass genera of the world. CAB International, Wallingford, UK

New tools for wheat genetics and breeding: Genome-wide analysis of SNP variation*

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Abstract

Single nucleotide polymorphism (SNP) is one of the most broadly distributed types of molecular variation in a genome which, along with the availability of cost- and labor-effective genotyping platforms, make it the marker of choice for many crops. Our work is aimed at the development of a dense set of genetically mapped SNP markers for low-cost high-throughput genotyping of wheat germplasm. Next generation sequencing of normalized cDNA libraries was used for developing gene-associated SNPs in polyploid wheat. A total of 7.5 million 454 reads were generated from cDNA libraries of 10 wheat cultivars from US and Australia and processed for discovering SNPs using a bioinformatical pipeline specifically designed for variant discovery in polyploid transcriptomes. A total of 25,000 high-quality SNPs distributed among 14,500 EST contigs were identified. All these SNPs were validated by comparison with RNA-seq data generated from an additional set of 17 U.S. and Australian cultivars. A total of 9,000 genome-wide common SNPs were selected for designing an Illumina iSelect assay. Preliminary testing showed that more than 95% of SNPs produce high-quality genotype calls with up to 70% being polymorphic in a diverse sample of U.S. and Australian cultivars with a minor allele frequency >0.05. The assay is currently being used for studying patterns of genetic diversity in a worldwide collection of wheat cultivars and for developing a high-density SNP map. A long term goal of this initiative is to advance wheat research and breeding by developing genetic and genomic tools for efficient analysis of agronomic traits using high-resolution linkage and association mapping and deploying SNP markers in breeding programs.

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Key words:

High-throughput genotyping; next-generation sequencing; polyploid wheat; Single Nucleotde Polymorphism

Introduction

Single nucleotide polymorphism (SNP) is the major type of intra-specific genetic variation and is widely distributed across genomic regions. A dense set of molecular markers covering the entire genome is a pre-requisite for high-resolution genetic analysis of agronomically important traits and deployment of efficient breeding strategies in crops (Breseghello and Sorrells 2006; Yu and Buckler 2006; Wang et al. 2007). Although, a wide variety of molecular markers is available (Vos et al. 1995; Gupta et al. 1999; Akbari et al. 2006; Flavell et al. 1998), those based on SNPs are the most efficient genotyping tool (Rostoks et al. 2006; Hyten et al. 2009; Chao et al. 2010).

Next-generation sequencing technologies capable of generating up to gigabases of sequence data have made SNP discovery a routine procedure for any organism. Massively parallel pyrosequencing technology has been successfully used to detect SNPs in maize and *Eucalyptus* transcriptomes (Barbazuk et al. 2007; Novaes et al. 2008) and in reduced representation genomic libraries of maize and cattle (van Orsouw et al. 2007; Van Tassell et al. 2008). DNA sequence capture approaches have been applied for targeted enrichment and sequencing of selected genomic regions for variant discovery in human and maize genomes (Albert et al. 2007; Porreca et al. 2007; Gnirke et al. 2009). New sequencing technologies make feasible the discovery of thousands of SNPs in domesticated crop species with low levels of genetic diversity (Choi et al. 2007).

Technical advances facilitating SNP discovery have been paralleled by the development of cost- and labor-efficient high-throughput genotyping technologies capable of genotyping thousands of individuals at thousands of SNP sites. A large variety of genotyping systems satisfying these requirements are now available (Syvänen et al. 2005). For example, the Illumina BeadArray platform combined with the GoldenGate assay is able to generate genotype data for several thousand polymorphic sites in 96 individuals in a single reaction (Oliphant et al. 2002). Molecular Inversion Probe (MIP) technology (Hardenbol et al. 2005) and the Illumina Infinium assay (Steemers and Gunderson 2007) can be used to genotype tens of thousands to hundreds of thousands of SNPs in a large number of individuals.

Compared to other important crops, SNP-based assays for wheat genotyping have only recently become available (Akhunov et al. 2009; Chao et al. 2010). Polyploidy and the low level of polymorphism in cultivated germplasm were the major challenges for SNP discovery in wheat and required the development of labor-intensive and expensive experimental approaches (Akhunov et al. 2010). However, in recent years a marked change has come with the availability of next-generation sequencing technologies. These technologies enable multiple large scale SNP discovery efforts (wheatgenomics.plantpath.ksu.edu/IWSWG/) that use the power of next-generation sequencing of genomic DNA and transcriptomes from multiple wheat cultivars for SNP detection.

Here, we present the development of gene-associated SNPs by large-scale transcriptome sequencing of a diverse panel of wheat cultivars from the U.S. and Australia. These SNPs were used to design a 9,000-plex SNP assay based on the Illumina Infinium platform. The goal of this initiative was to develop bioinformatical procedures for SNP calling in next-generation sequence data generated for polyploid transcriptomes, and to test the utility of the Infinium platform for high-throughput genotyping of polyploid wheat cultivars.

cDNA preparation and normalization

RNA samples were extracted using the RNeasy Plant Mini Kit (QIAGEN). Concentration and purity of total RNA was checked on a Nanodrop Spectrophotometer. The RNA integrity was evaluated on a Bioanalyzer (Agilent) and by standard formaldehyde agarose gel electrophoresis. cDNA libraries for sequencing using Illumina GAII$_x$ and HiSeq2000 platforms were prepared according to manufacturer's instructions (Illumina Corp., San Diego), while cDNA libraries for 454 sequencing were prepared as follows. First-strand cDNA synthesis was performed according to SMART cDNA synthesis technology (Clontech Laboratories, Inc.) using 3' SMART CDS modified Primer II A primer (5'-AAG CAG TGG TAT CAA CGC AGA GTA CTT TTG T(9) C T(10) VN-3') and SuperScript III reverse transcriptase (Invitrogen). Double-stranded cDNA was amplified by long-distance (LD) PCR using the Advantage 2 PCR Enzyme System (Clontech Laboratories, Inc). Amplification was performed on a thermal cycler (Applied Biosystem) with the following PCR parameters: 1 cycle at 95˚C (1 min); 16 cycles at 95˚C (15 s), 65˚C (30 s), 68˚C (6 min); and 4˚C (45 min) (optional). Double-stranded cDNA was checked on 1.1% agarose/EtBr gels in 1XTAE buffer and purified with the QIAquick PCR Purification Kit (QIAGEN). The double-stranded cDNA was normalized using the TRIMMER cDNA Normalization Kit (EVROGEN), which is based on a unique DSN (duplex-specific nuclease) normalization technology and is specially developed for normalization of cDNA enriched with full-length sequences. The efficiency of normalization was examined by determining the abundance of two highly expressed transcripts before and after normalization using quantitative Real-Time PCR. All quantitative PCR experiments were performed on an iCycler Real-Time PCR system (BioRad Laboratories) using IQ SYBR Green Supermix (BioRad Laboratories). The PCR conditions were 1 cycle at 95ºC (5 min); 40 cycles at 95ºC (15 s), 55ºC (15 s), 72ºC (50 s); followed by the melting curve program.

Data processing and analysis

Custom Perl scripts were used for quality trimming of RNA-seq data generated using Illumina GAII$_x$ and HiSeq2000 platforms. The program Lucy was used for removal of adaptor sequences and quality trimming of 454 sequence reads (Li and Chou 2004). For each wheat line, a reference cDNA sequence was built using MIRA software (Chevreux et al. 2004). Each reference set was then consecutively used for read mapping using Mosaik software (bioinformatics.bc.edu/marthlab/Mosaik). SNP discovery was performed using the Bayesian algorithm implemented in the GigaBayes software. An additional post-processing filtering step was applied to select high-quality SNPs, which removed all variable sites having alleles covered by less than 3 reads in the alignments. This filter was based on the results of empirical validation by Sanger re-sequencing of randomly selected SNP-harboring gene fragments. SNPs and their flanking sequences were extracted and used for comparison with RNA-seq data generated for an additional set of 17 U.S. and Australian cultivars. This analysis allowed the frequency of discovered SNPs in U.S. and Australian cultivars to be estimated more precisely.

Design of 9,000-plex SNP assay

Several criteria were applied for selecting 9,000 SNPs for an Infinium iSelect assay design. First, repetitive elements in reference sequences were detected by comparing them with the TREP (http://wheat.pw.usda.gov/ITMI/Repeats/) and GIRI (www.girinst.org) databases. Second, the distribution of cDNA contigs used for SNP discovery across the wheat genome was inferred by comparing them with the genomes of Brachypodium and rice using the blastn program. Third, SNPs were ranked according to their minor allele frequency (MAF) in the discovery panel and their

distribution between US and Australian populations. Priority was given to SNPs that showed high MAF and were shared by U.S. and Australian cultivars. Finally, SNPs and their flanking sequences were submitted to Illumina for processing by the Assay Design Tool which generates scores for each SNP varying from 0 to 1. SNPs with scores above 0.6 and having a high probability to be converted into a successful genotyping assay were selected.

Results and Discussion

Transcriptome sequencing and SNP discovery

Transcriptomes of a diverse set of 27 wheat lines including Chinese Spring and cultivars from U.S. and Australia were sequenced using normalized cDNA libraries prepared from RNA isolated from multiple tissues collected at different developmental stages or subjected to different stress treatments. A complete list of the cultivars sequenced can be found on the project website: http://wheatgenomics.plantpath.ksu.edu/snp/. Ten cultivars were sequenced using Roche 454 technology to generate nearly 7 million reads (Table 1) and 17 cultivars were sequenced using the GAII and HiSeq2000 platforms to produce almost 500 million Illumina reads.

The primary challenge for SNP discovery in the hexaploid wheat genome is to distinguish divergence among the wheat sub-genomes from variation between wheat lines. Failure to filter out divergent sites can significantly inflate the false SNP discovery rate. Hence, we developed procedures for effective separation of true inter-cultivar variants from genome-specific mutations differentiating the wheat sub-genomes and applied this approach to discover a genome-wide distributed set of genic SNPs (Fig. 1).

Fig. 1 *SNP discovery workflow*

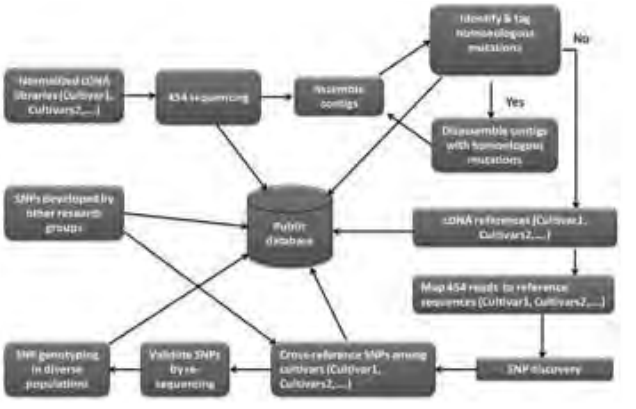

Table 1 *454 transcriptome data generated for 10 wheat cultivars*

Wheat line	Origin	No. of reads generated	Total bases, bp	No. reference contigs	Total contig length, bp
Excalibur	Australia	1,547,934	565,519,405	116,984	95,773,580
Kukri	Australia	1,706, 047	605,719,594	118,506	98,089,478
RAC875	Australia	1,650,614	600,811,369	121,193	98,547,050
Bobwhite	USA	1,303,861	491,025650	67,788	42,695,800
CAP7	USA	284,070	100,314,837	13,321	5,612,611
CAP8	USA	188,601	64,914,768	10,017	4,336,540
CAP11	USA	170,792	59,922,739	9,200	3,959,549
CAP12	USA	191,450	63,713265	9,222	3,848,399
Jagger	USA	80,699	17,718,199	11,060	3, 677,900
Chinese Spring	China	98,219	21,285,912		
Total		**7,222, 287**	**2,590,945, 738**	**477,291**	**356,540, 907**

The preparation of reference sequences for read mapping using the MIRA assembler and 454 sequence data resulted in 477,291 cDNA contigs with an average length of 750 bp (Table 1). Sequential mapping of all 7,222,287 reads to each set of cultivar-specific cDNA contigs using MOZAIK software was followed by SNP discovery using the Bayesian algorithm implemented in the GigaBayes program. The minimum number of reads representing each SNP allele in alignment was considered as the most critical parameter for the discovery of true SNPs, and was adjusted using empirical data obtained by Sanger re-sequencing of 96 gene fragments. Filtering SNPs for alleles covered by at least three reads in the alignments resulted in an 85-90% validation rate. A total of 81,688 SNPs were discovered in all possible pair-wise comparisons between 10 cultivars which, after removing redundant SNPs overlapping among different pair-wise comparisons, resulted in a set of about 25,000 unique high-quality SNPs (Table 2).

All SNPs were deposited into a searchable MySQL database that is available at http://wheatgenomics.plantpath.ksu.edu/snp/. In the current version of the database, users can obtain the list of SNPs polymorphic between any two given wheat cultivars used for SNP discovery and search for SNPs by sequence similarity using the BLASTN program. The output is user-adjustable and contains wheat deletion-bin map data, the best BLASTN and BLASTP hits in the NCBI database along with the description of these hits. In the output of the BLASTN search, users can also obtain SNP genotypes of cultivars included into the discovery panel.

9,000 SNP iSelect BeadChip

The utility of SNPs for genotyping a broad range of wheat cultivars strongly depends on the distribution of SNP alleles among populations. SNP alleles shared between diverse populations will have a high likelihood of being polymorphic in a broad range of populations. We used Illumina RNA-seq data obtained for an additional 17 U.S. and Australian cultivars to assess the frequency and distribution of SNPs discovered by 454 transcriptome sequencing in 10 cultivars. The estimated fraction of SNPs shared between U.S. and Australian wheat cultivars was 45%. These SNPs were primarily targeted for inclusion into the genotyping assay design. In addition, 849 SNPs from the first 1536-plex wheat oligo pool assay (Chao et al. 2010) were also included in the custom 9K iSelect design.

The Infinium iSelect BeadChip was tested using a diverse panel of wheat cultivars including breeding lines and parents of critical mapping populations (US CAP, ITMI and MAGIC). Preliminary results suggest that out of 9,000 attempted bead types nearly 95% produce scorable data. After quality filtering based on the clustering patterns, we obtained about 8,000 SNP assays generating high quality data. In a panel of 181 diverse wheat cultivars from U.S. and Australia 70% of SNP assays could be scored as polymorphic with minor allele frequency >0.05. We are currently performing detailed analyses of genotyping data with the goal of developing SNP calling algorithms with increased sensitivity and accuracy. The 9000 SNP iSelect BeadChip will be used for genotyping a large worldwide collection of wheat cultivars and the development of a high-density SNP-based genetic map.

Table 2 ***Number of SNPs identified in pair-wise comparisons***

	Excalibur	Bobwhite	Jagger	Kukri	RAC875	CAP 7	CAP 8	CAP 11	CAP 12
Ch. Spring	1037	902	341	1,167	1,293	122	95	93	76
Excalibur		5,757	798	16,238	14,817	188	194	147	131
Bobwhite			617	4,939	5,954	299	351	264	286
Jagger				817	856	70	63	53	47
Kukri					17,553	158	173	132	91
RAC875						182	192	138	121
CAP 7							1,087	925	680
CAP 8								852	809
CAP 11									583

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References

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome Theor Appl Genet 113:1409-1420

Akhunov ED, Akhunova AR, Anderson, OD, Anderson, JA, Blake, N, Clegg, MT, Coleman-Derr, D, Conley, EJ, Crossman, CC, Deal, KR, Dubcovsky, J, Gill, BS, Gu, YQ, Hadam, J, Heo, HY, Huo, N, Lazo, GR, Luo, MC, Ma, YQ, Matthews, DE, McGuire, PE, Morrell, P, Qualset, CO, Renfro, J, Tabanao, D, Talbert, LE, Tian, C, Toleno, D, Warburton, M, You, FM, Zhang, W, Dvorak, J (2010) Nucleotide diversity maps reveal variation in diversity among wheat genomes and chromosomes BMC Genomics 11:702

Akhunov ED, C Nicolet, J Dvorak (2009) Single nucleotide polymorphism genotyping in polyploid wheat with Illumina GoldenGate assay. Theor Appl Genet 119:507-517

Albert TJ, Molla MN, Muzny DM, Nazareth L, Wheeler D, Song X, Richmond TA, Middle CM, Rodesch MJ, Packard CJ, Weinstock GM, Gibbs RA (2007) Direct selection of human genomic loci by microarray hybridization. Nat Methods 4:903-905

Barbazuk WB, Emrich SJ, Chen HD, Li L, Schnable PS (2007) SNP discovery via 454 transcriptome sequencing. Plant J 51:910-918

Breseghello F, Sorrells ME (2006a) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172:1165-1177

Chao, S., Dubcovsky, J., Dvorak, J., Luo, M.C., Baenziger, S.P., Matnyazov, R., Clark, D.R., Talbert, L.E., Anderson, J.A., Dreisigacker, S., Glover, K., Chen, J., Campbell, K., Bruckner, P.L., Rudd, J.C., Haley, S., Carver, B.F., Perry, S., Sorrells, M.E., Akhunov, E.D. (2010) Population- and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (Triticum aestivum L.). BMC Genomics 11(1):727

Choi IY, Hyten DL, Matukumalli LK, Song Q, Chaky JM, Quigley CV, Chase K, Lark KG, Reiter RS, Yoon MS, Hwang EY, Yi SI, Young ND, Shoemaker RC, van Tassell CP, Specht JE, Cregan PB (2007) A soybean transcript map: gene distribution, haplotype and single-nucleotide polymorphism analysis. Genetics 176:685-696

Chevreux B, Pfisterer T, Drescher B, Driesel AJ, Müller WE, Wetter T, Suhai S (2004) Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. Genome Res 2004 Jun;14(6):1147-59. Epub 2004 May 12

Flavell AJ, Knox MR, Pearce SR, Ellis TH (1998) Retrotransposon-based insertion polymorphisms (RBIP) for high throughput marker analysis. Plant J 16:643-650

Gnirke A, Melnikov A, Maguire J, Rogov P, LeProust EM, Brockman W, Fennell T, Giannoukos G, Fisher S, Russ C, Gabriel S, Jaffe DB, Lander ES, Nusbaum C (2009) Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nat Biotechnol 27:182-189

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breeding 118:369-390

Hardenbol P, Yu F, Belmont J, MacKenzie J, Bruckner C, Brundage T, Boudreau A, Chow S, Eberle J, Erbilgin A, Falkowski M, Fitzgerald R, Ghose S, Iartchouk O, Jain M, Karlin-Neumann G, Lu X, Miao X, Moore B, Moorhead M, Namsaraev E, Pasternak S, Prakash E, Tran K, Wang Z, Jones HB, Davis RW, Willis TD, Gibbs RA (2005) Highly multiplexed molecular inversion probe genotyping: Over 10,000 targeted SNPs genotyped in a single tube assay. Genome Res 15:269-275

Hyten DL, Song Q, Choi IY, Yoon MS, Cregan PB (2008) High-throughput genotyping with the GoldenGate assay in the complex genome of soybean. Theor Appl Genet 116:945-952

Li S, Chou HH. (2004) LUCY2: an interactive DNA sequence quality trimming and vector removal tool. Bioinformatics 20:2865-2866

Novaes E, Drost DR, Farmerie WG, Pappas GJ Jr, Grattapaglia D, Sederoff RR, Kirst M (2008) High-throughput gene and SNP discovery in Eucalyptus grandis, an uncharacterized genome. BMC Genomics 9:312

Oliphant A, Barker DL, Stuelpnagel JR, Chee MS (2002) BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping. Biotechniques Suppl:56-58

Rostoks N, Ramsay L, MacKenzie K, Cardle L, Bhat PR, Roose ML, Svensson JT, Stein N, Varshney RK, Marshall DF, Graner A, Close TJ, Waugh R (2006) Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. Proc Natl Acad Sci USA 103:18656-18661

Steemers FJ, Gunderson KL (2007) Whole genome genotyping technologies on the BeadArray platform. Biotechnol J 2:41-49

Syvänen AC (2005) Toward genome-wide SNP genotyping. Nat Genet 37 Suppl:S5-1Van Orsouw NJ, Hogers RC, Janssen A, Yalcin F, Snoeijers S, Verstege E, Schneiders H, van der Poel H, van Oeveren J, Verstegen H, van Eijk MJ (2007) Complexity reduction of polymorphic sequences (CRoPS): a novel approach for large-scale polymorphism discovery in complex genomes. PLoS ONE 2:e1172

Van Tassell CP, Smith TP, Matukumalli LK, Taylor JF, Schnabel RD, Lawley CT, Haudenschild CD, Moore SS, Warren WC, Sonstegard TS (2008) SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. Nat Methods 5:247-252

Vos P, Hogers R, Bleeker M, Reijans M, Lee Th van der, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407-4414

Wang J, Chapman SC, Bonnett DG, Rebetzke GJ, Crouch J (2007) Application of population genetic theory and simulation models to efficiently pyramid multiple genes via marker-assisted selection. Crop Sci 47:582-590

Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol 17:155-160

Porreca GJ, Zhang K, Li JB, Xie B, Austin D, Vassallo SL, LeProust EM, Peck BJ, Emig CJ, Dahl F, Gao Y, Church GM, Shendure J (2007) Multiplex amplification of large sets of human exons. Nat Methods 4:931-936

High yielding CIMMYT spring wheats with resistance to Ug99 and other rusts developed through targeted breeding*

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Abstract

Targeted breeding to develop high yielding wheat germplasm resistant to Ug99 and other rusts initiated at CIMMYT in 2006. Ug99 resistant materials, especially those with adult plant resistance (APR), were used in crossing. F_3 and F_4 populations from simple, BC_1 and top crosses were grown for two generations under high rust pressures at Njoro, Kenya in a Mexico-Kenya shuttle breeding scheme. Parallel populations were also grown in Mexico for comparison. Approximately 5,000 advanced lines were tested for grain yield performance at Ciudad Obregon, Mexico in 2009/10 season, and phenotyped for resistance to Ug99 and other rusts. The 728 retained lines were evaluated for grain yield performance in five environments during the 2010/11 season in Mexico. About 68% of the 728 lines had near-immune (16.5% entries) to adequate APR to Ug99. An additional 13.6% lines carried one of the six (*Sr25, Sr26, SrTmp, SrHuw234, SrSha7,* and an unidentified gene) race-specific resistance genes often in combination with APR gene *Sr2.* About 80% entries were highly resistant to yellow rust in Kenya and Mexico, and 90% entries to leaf rust in Mexico. Yield distribution of lines derived from Mexico-Kenya shuttle breeding was similar to lines selected only in Mexico. Sufficient lines with >5% superior yields than the Mexican checks varieties in 2 years testing were identified. Our results indicate that targeted crossing and shuttle breeding are powerful tools for a simultaneous improvement of grain yield potential and resistance to rusts.

Keywords

Durable resistance, Leaf rust, Stem rust, Stripe rust, *Puccinia graminis tritici*, *Puccinia triticina, Puccinia striiformis,* Shuttle breeding, *Triticum aestivum*

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*This is an unedited original draft. A revised version will appear in the workshop proceedings published later this year.

Introduction

Wheat production worldwide should increase about 2% annually to meet the projected demands due to population and prosperity growth. Rust diseases continue to pose major threats to destabilize wheat production especially affecting livelihoods of small subsistence farmers. Races belonging to the Ug99 lineage of stem (black) rust fungus *Puccinia graminis* f. sp. *tritici*, and aggressive, high temperature adapted *Yr27* virulent yellow (stripe) rust fungus *P. striiformis* f. sp. *tritici* were recognized as major threats to wheat production worldwide (Singh et al. 2008; Hovmøller et al. 2010). Various sources of race-specific and complex adult-plant resistance were identified by testing CIMMYT and other wheat germplasm to Ug99 group of races in Njoro, Kenya, which initiated in 2005 (Njau et al. 2010, Singh et al. 2008). Ug99 resistant wheat varieties with race-specific resistance genes, or with moderate levels of APR, were released in various countries using the resistance information from Kenya and Ethiopia (Joshi et al. 2011).

A targeted breeding program was initiated at CIMMYT in 2006 to develop wheat germplasm that has >5% yield advantage over the current popular varieties and resistant to Ug99 and other rusts, including *Yr27*-virulent races of yellow rust fungus. Simple, top and single back-cross derived hybrid populations were selected at two Mexican field sites, Ciudad Obregon and Toluca, and Njoro in Kenya. The two generations per season 'Mexico-Kenya shuttle breeding' scheme (Singh et al. 2011) allows simultaneous selection for grain yield, agronomic characteristics and resistance to all three rusts. Parallel populations were also selected in Mexico (Cd. Obregon-Toluca) shuttle scheme for comparison. Characterization of advanced lines, derived from crosses made in 2006 and selected through the Mexico shuttle and Mexico-Kenya shuttle scheme, was done for grain yield performance and related traits in 2009/10 and 2010/11 crop seasons in Mexico, and resistance to Ug99 and other rusts in 2010 and 2011 in Kenya and Mexico. Results are summarized in the following sections.

Grain yield performance of advanced wheat lines derived from crosses made in 2006 in Ciudad Obregon 2009/10

A total of 4,956 entries were tested in 1st year grain yield performance trials at Cd. Obregon during 2009/10 on raised beds with 5 irrigations. The distribution of the yield performance of lines, expressed as percentage of the checks mean, is shown in Figure 1. A total of 1,030 (20.8%) entries had yield potential of 100% or above compared to checks ('Roelfs F2007' and 'Waxwing')

mean. Also significant to note is that 445 (9%) lines had yield potential between 105-120% of the checks mean. Lines derived from parallel populations undergone selections in Mexico shuttle and Mexico-Kenya shuttle had similar yield distributions (Figure 2) indicating no apparent negative or positive effects of selecting in Kenya in the employed shuttle breeding scheme.

Fig. 1 ***Grain yield performance of 4,956 new bread wheat lines expressed as percentage over the mean performance of checks (Roelfs F2007 and Waxwing) on raised beds with 5 irrigations during 2009/10 crop season at Ciudad Obregon, Mexico***

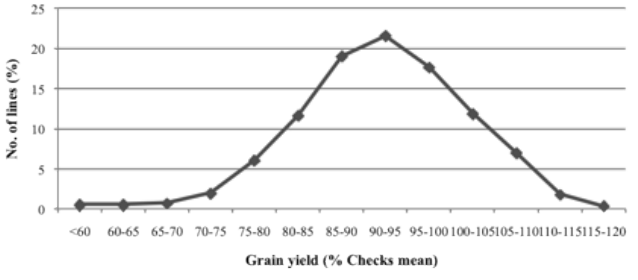
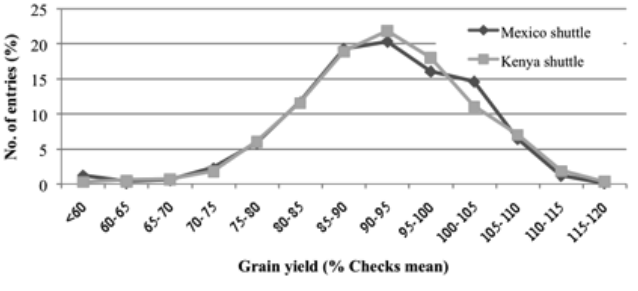


Fig. 2 ***Comparison of grain yield performance of 3,903 Mexico shuttle and 1,053 Mexico-Kenya shuttle breeding derived new bread wheat lines in Ciudad Obregon, Mexico 2009/10 on raised bed planting with five irrigationsFig. 3 Grain yield performances (Ciudad Obregon, Mexico 2009/10) of all 728 bread wheat lines with 298 Ug99 adult plant resistant (near-immune resistant and resistant) lines retained for expanded grain yield performance evaluations in 2010/11***



The 1,258 lines, retained in Cd. Obregon, were phenotyped in El Batan and Toluca in Mexico, and Njoro in Kenya in 2010 for various traits including leaf rust, yellow rust, stem rust, and end-use quality. Two-seasons, 2010-off and main, Ug99 stem rust data were available for lines derived from Mexico-Kenya shuttle and a majority of lines had similar responses when tested in 2010-main season. Based on all data, 728 entries were retained for multi-environment trials during 2010/11 season in Cd. Obregon and simultaneous seed multiplication for international distribution. The 728 lines were derived from 322 different crosses and capture a broad range of parental diversity. Their heading varied from 73-102 days, maturity from 121-142 days, and height from 87-120 cm in Obregon during 2009/10. Correlation coefficients (r) between grain yield (expressed as % checks means) and heading, maturity and height of the retained lines were 0.348, 0.418 and 0.312, respectively.

Resistance to Ug99 and other rusts

Establishment and growth of wheat crop was excellent during the 2010-main season at Njoro, Kenya. Stem rust build-up in nurseries was also excellent and the susceptible checks were dead when final disease data were recorded. Also stem rust pressure was high during the 2010-off-season; only Mexico-Kenya shuttle breeding lines were evaluated and 188 lines were part of the 728 retained for extended yield performance testing during 2010/11 crop season in Cd. Obregon, Mexico. Stem rust responses of these 728 lines are summarized in Table 1. A total of 298 (41%) lines had high levels of adult plant resistance in the near-immune (16.5%) or resistant (24.5%) categories. An additional 27.3% lines carried good adult plant resistance in the resistant-moderately resistant category. A total of 13.6% entries carried race-specific resistance genes that were effective to Ug99 group of races. *SrTmp* and *SrSha7* were often present with *Sr2* and other slow rusting adult plant resistance genes and therefore the final disease severities of the retained lines were low. *Sr26* was also present in a few retained lines. Only 9.7% retained entries had unsatisfactory levels of resistance or were susceptible but kept due to other desirable traits.

Grain yield performance of lines with high level of APR to Ug99

Comparison of the grain yield performance of all 728 advanced lines and the fraction of 298 advanced lines that have high levels (near-immune and resistant categories) of adult plant resistance to Ug99 race of stem rust fungus is shown in Figure 3. Although the frequencies of APR lines were somewhat lower in high-yielding classes, presence of sufficient lines indicated that it is possible to simultaneously select high yield potential and APR to stem rust.

Table 1 Responses of 728 entries to stem rust at Njoro, Kenya during 2010 main-season (Susceptible check Cacuke had dried out following 100% stem rust severity when data recorded and PBW343 displayed 60-70% stem rust severity)

Adult plant resistance	Stem rust	Entries			Entries	
Category	severity (%)	No.	%	R-genes	No.	%
Near-Immune Resistant	1	120	16.5	Sr25	17	2.3
Resistant	5-10	178	24.5	Sr26	9	1.2
Resistant- Mod. Res.	15-20	199	27.3	SrTmp	49	6.7
Moderately Resistant	30	63	8.7	SrHuw234	1	0.1
				SrSha7	19	2.6
Mod. Res.- Mod. Sus.	40	34	4.7	SrUnknown	5	0.7
Moderately Susceptible	50-60	27	3.7			
Mod. Sus.- Susceptible	70-80	5	0.7			
Susceptible	90-100	2	0.3			

Fig. 3 *Grain yield performances (Ciudad Obregon, Mexico 2009/10) of all 728 bread wheat lines with 298 Ug99 adult plant resistant (near-immune resistant and resistant) lines retained for expanded grain yield performance evaluations in 2010/11*

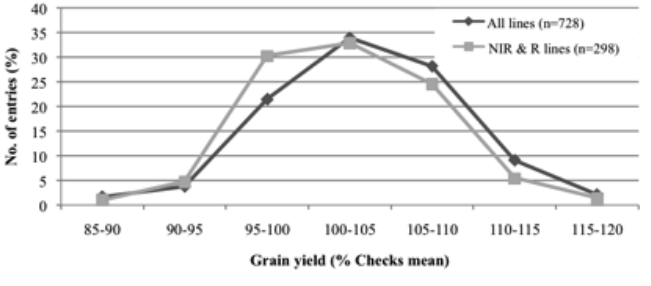
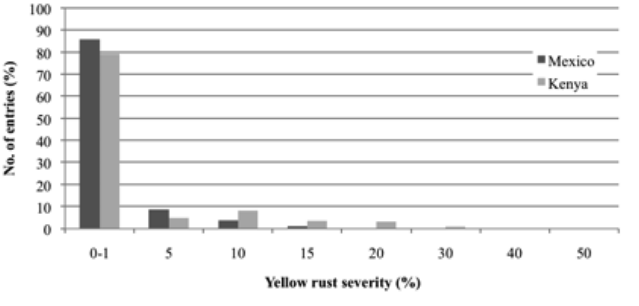


Fig. 4 *Yellow rust responses of 728 bread wheats at Toluca, Mexico and Njoro, Kenya during 2010 (Note: data recorded when susceptible checks had necrotic leaves following 100% rust severity; and 594 or 82% entries displayed 0-5% severity a both sites)*



Resistance to yellow rust and leaf rust

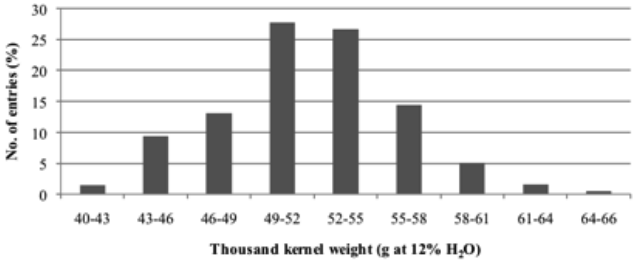
Severe yellow rust epidemic occurred at both Toluca, Mexico and Njoro, Kenya during 2010. As shown in Figure 4, about 80% retained lines had 0 or 1% severities to *Yr27* and *Yr31* virulent races present in the nurseries. Approximately 82% entries displayed 0 to 5% severities at both sites. This is also a major shift in resistance to yellow rust in bread wheat germplasm and increases the possibility of finding new superior yielding wheat lines that can displace the yellow rust susceptible popular varieties in various countries.

A majority of the retained entries were highly resistant to leaf rust based on 2010 and 2010/11 field data from El Batan and Cd. Obregon, respectively in Mexico where disease pressures were severe. Approximately 93% retained lines had 0 to 5% severities. Approximately half of the retained entries displayed compatible seedling reactions to *P. triticina* race MBJ/SP, one of the predominant field races, and showed high level of adult plant resistance with severities ranging between 0 to 5% in field trial. Frequency of *Lr16* has increased in recent materials as several lines have Waxwing lineage. However, this leaf rust resistance gene alone works like a slow rusting resistance gene with leaf rust severity on adult plants reaching to 70-80% when present alone. Lines carrying *Lr16* must also possess 2-3 slow rusting resistance genes to bring disease severity to zero to 5% (Singh and Huerta-Espino 1995).

Grain characteristics and end-use quality

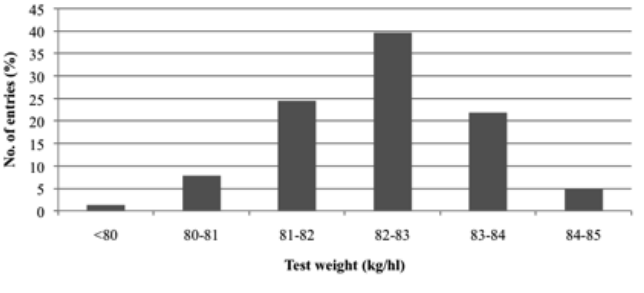
Distribution of kernel weight of 728 entries is shown in Figure 5. It ranged from 40.4 to 65.8 g and 76% entries had >49 g TKW. This represents a major change in kernel weight and lines with larger kernels now dominate the improved wheat germplasm. Correlation coefficient (r) of -0.029 between grain yield and kernel weight indicates independent genetic control of the two traits.

Fig. 5 ***Kernel weight (2009/10 grain) of 728 entries retained for multi-environment yield performance trials in Ciudad Obregon, Mexico during 2010/11 crop season (Note: TKW of 'PBW343' = 44-45 g)***



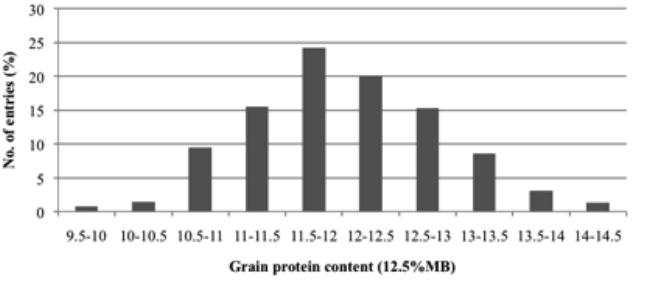
also increasing and over 66% entries had test weights of 82-85 kg/hl. Approximately 5% entries had test weights of 84-85 kg/hl, which is unusual in bread wheat where in the past test weights of 80-82 kg/hl were common and rarely went above 82 kg/hl. Correlation coefficient (r) of 0.029 between grain weight and test weight indicated that high test weight and increased kernel weight were independent traits. Grain yield and test weight had slight negative correlation coefficient of -0.141 indicating the high yield and high test weight can be simultaneously selected.

Fig. 6 ***Test weight (2009/10 grain) of 728 entries retained for multi-environment yield performance trials in Ciudad Obregon, Mexico during 2010/11 crop season***



The grain protein content of the 728 lines showed highly quantitative variation and ranged from 9.5 to 14.5%MB and about half of the entries had 12% or higher protein content (Figure 7). As expected grain yield and protein content showed significant but low negative correlation (r = -0.253) indicating that at least some high yielding lines also possessed high protein content in the grain.

Fig. 7 ***Grain protein content (2009/10 grain) of 728 entries retained for multi-environment yield performance trials in Ciudad Obregon during 2010/11 crop season***



The loaf volume and end user type characteristics of the 728 lines are shown in Figures 8 and 9, respectively. Approximately 60% of the entries had acceptable to excellent loaf volumes of higher than 700 ml (Figure 8) and approximately half of the entries had appropriate quality characteristics to make leavened as well as various types of flat bread (user-types 1a, 1b and 2a in Figure 9). Approximately 20% additional entries belonging to user-type 2b, 3a and 3b have adequate characteristics to make flat breads of various types. Only 3% entries had soft grain (user-type 4a and 4b) and have utility in making some products, such as biscuits with user-type 4b. About 26% entries with tenacious characteristics (user-type 5) were retained due to their high yield or other desirable traits. Loaf volume showed small but significant negative correlation (r = -0.242) with grain yield. It is therefore possible to indentify high-yielding lines with good leavened and flat bread making quality characteristics.

Fig. 8 Loaf volumes (2009/10 grain) of 728 entries retained for multi-environment yield performance trials in Ciudad Obregon during 2010/11 crop season

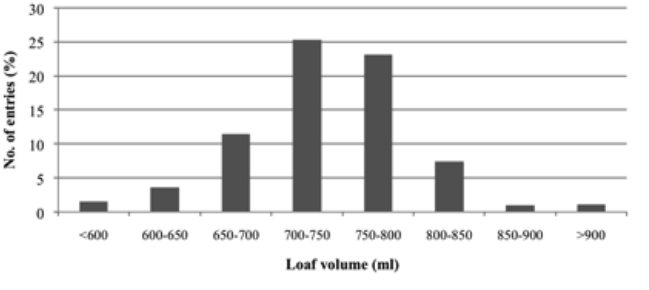
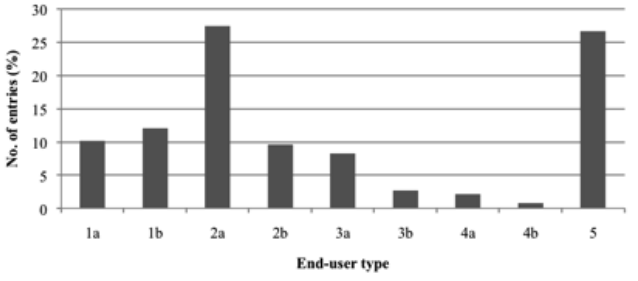


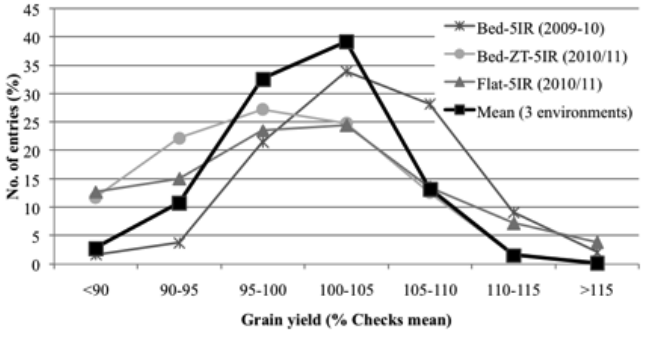
Fig. 9 End-user quality characteristics (2009/10 grain) of 728 entries retained for multi-environment yield performance trials in Ciudad Obregon during 2010/11 crop season



Grain yield performance of advanced lines in 2nd year evaluation in Ciudad Obregon during 2010/11 crop season

Grain yield performance results were available for replicated yield trials grown under four environments in Ciudad Obregon, Mexico when paper was written. Trials grown under late sown (3 months delayed planting), heat stressed environment will be available in early June. Performance of entries relative to checks mean for raised bed-5 irrigations under zero-tillage and flat-5 irrigations during 2010/11 is presented along with the results of raised bed-5 irrigations during 2009/10 and the mean performance in the three environments (Figure 10). Roelfs F2007 and Waxwing were used as checks during 2009/10. Waxwing was replaced with Mexican variety 'RSM Norman F2008' in 2010/11 trials. Approximately 15% (108 entries) yielded 5-15% higher than the checks means during two years of testing in the three optimally irrigated environments. Approximately 40% (285 entries) additional entries also had the same or 5% higher yields than checks mean (Figure 10).

Fig. 10 Grain yield performance of 728 advanced lines evaluated in replicated yield trials grown at Ciudad Obregon, Mexico with five irrigations in 2009/10 (raised beds), 2010/11 (raised beds, zero-tillage and flat planting systems) and mean performance in three environments



Ninety (12%) entries had similar or up to 15% higher yields than the best yielding check Roelfs F2007 in yield trials grown under intermediate drought stress created by providing only two irrigations, 1st irrigation after sowing and the 2nd irrigation about 50 days later at early boot stage (data not presented). Similarly 79 (11%) entries yielded 5-60% higher than the best yielding check 'Vorobey' in trials grown under high drought stress (about 180 mm of water) created through drip irrigation (data not presented). Under high drought stress coefficient of variation was higher and best performing entries often were earlier maturing than the checks. It was also common to find entries that completely collapsed in contrast to entries that yielded >2 t/ha.

Conclusion and future outlook

Breeding and growing high-yielding wheats that have complex adult-plant resistance to Ug99 and derivative races are considered to be the best strategy in reducing risks of major losses in various countries of Africa, Asia and Latin America where CIMMYT derived spring wheat germplasm is adapted. Significant progress in developing new resistant wheat germplasm that has potential to displace current popular varieties is evident from the results obtained so far. The best lines will be distributed for yield performance testing in various countries in 2011/12 season through the international wheat improvement network. Entries found to be better performers will then be promoted by the National Programs in 'National Performance Trials' and seed multiplication.

The Mexico-Kenya shuttle breeding scheme seems to be an effective strategy to shift the segregating populations towards resistance. About 6,500 advanced lines derived from the targeted crosses made in 2007 were evaluated for stem rust in Njoro, Kenya during 2011-off season and are under yield performance tests in Ciudad Obregon, Mexico during 2010/11 crop season. Lines with high yields and Ug99 resistance will be evaluated again for Ug99 resistance in 2011-main season at Njoro and characterized for various agronomic, quality and disease resistance traits. The 3rd group of advanced lines from crosses made in 2008 will be ready for stem rust resistance characterization and 1st year of yield performance testing in 2011/12 crop season. These materials should ensure a constant supply of new Ug99 resistant, high-yielding materials in coming years.

Table 2 *Some of the new high yielding lines that have shown high levels of resistance to Ug99 when tested in Njoro, Kenya during 2010*

		Stem rust resistance
Cross Name	**GID**	**Category[1]/genes**
CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7	6178401	APR_NIR
SAUAL/YANAC//SAUAL	6178534	*Sr26*
PRL/2*PASTOR*2//FH6-1-7	6178556	APR_R
TUKURU//BAV92/RAYON*2/3/JUCHI	6178193	APR_R
UP2338*2/KKTS*2//YANAC	6178243	APR_NIR
FRNCLN/ROLF07	6177828	APR_R
PFAU/SERI.1B//AMAD/3/WAXWING/4/BABAX/LR42//BABAX*2/3/KURUKU	6178972	*SrTmp*
BECARD/KACHU	6174889	APR_R
ALTAR_84/AE.SQ._(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/MILAN/S87230//BAV92	6174903	APR_NIR
TRCH/HUIRIVIS #1	6177148	APR_NIR
ROLF07/MUU	6177327	APR_NIR
BECARD/AKURI	6177394	APR_R
KBIRD//WBLL1*2/KURUKU	6177447	APR_R
KINGBIRD #1//INQALAB 91*2/TUKURU	6177598	APR_NIR
UP2338*2/VIVITSI/3/FRET2/TUKURU//FRET2/4/MISR_1	6177947	*Sr25*
KFA/2*KACHU	6179218	APR_NIR
KIRITATI//HUW234+LR34/PRINIA	6085804	*SrHuw234*
SITE/MO//PASTOR/3/TILHI/4/WAXWING/KIRITATI	6175662	APR_NIR
ATTILA*2/PBW65//MURGA	6175744	APR_NIR
ROLF07/4/BOW/NKT//CBRD/3/CBRD/5/FRET2/TUKURU//FRET2	6176013	APR_R
ROLF07*2/4/CROC_1/AE.SQ._(205)//BORL95/3/2*MILAN	6176024	APR_NIR
WBLL1*2/TUKURU*2/4/CROC_1/AE.SQ._(205)//BORL95/3/2*MILAN	6176056	APR_R
CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/HAR311	6178369	*SrSha7*
PBW343*2/KUKUNA*2//FRTL/PIFED	6179225	APR_NIR
WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQ._(213)//PGO/4/HUITES	6176355	APR_R
KACHU/4/CROC_1/AE.SQ._(205)//BORL95/3/2*MILAN/5/KACHU	6176372	APR_R
WBLL1/4/BOW/NKT//CBRD/3/CBRD/5/WBLL1*2/TUKURU	6178888	APR_RMR
PFAU/WEAVER*2/4/BOW/NKT//CBRD/3/CBRD	6176689	*SrCbrd*
WBLL1*2/BRAMBLING/5/BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	6177024	*SrTmp*
WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	6174877	APR_R
ATTILA*2/PBW65//KBIRD	6177080	APR_R
ROLF07/KINGBIRD #1	6177342	APR_NIR
WBLL1*2/KUKUNA//AKURI #1	6179077	APR_R
NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU	6175063	APR_R
MURGA/3/GAN/AE.SQUARROSA_(408)//2*OASIS/5*BORL95/4/FRANCOLIN #1	6177930	APR_R
TACUPETO_F2001/6/CNDO/R143//ENTE/MEXI_2/3/AE._SQ._(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07	6179272	APR_NIR

[1] Resistance category as described in Table 1; APR = adult plant resistance, NIR = near-immune, R = resistant, R-MR = resistant-moderately resistant.

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References

Hovmøller MS, Walter S, Justesen AF (2010) Escalating Threat of Wheat Rusts. Science 329:369

Joshi AK, Azab M, Mosaad M, Moselhy M, Osmanzai M, Gelalcha S, Bedada G, Bhatta MR, Hakim A, Malakar PK, Haque ME, Tiwari TP, Majid A, Kamali MRJ, Bishaw Z, Singh RP, Payne T, Braun HJ *(2011)* Delivering rust resistant wheat to farmers: a step towards increased food security. Euphytica 179:187–196

Njau PN, Jin Y, Huerta-Espino J, Keller B, Singh RP (2010) Identification and evaluation of sources of resistance to stem rust race Ug99 in wheat. Plant Dis 94:413-419

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward RW (2008) Will Stem Rust Destroy the World's Wheat Crop? Advances in Agronomy 98:271-309

Singh RP, Huerta-Espino J (1995) Inheritance of seedling and adult plant resistance to leaf rust in wheat cultivars Ciano 79 and Papago 86. Plant Dis 79:35-38

Singh RP, Huerta-Espino J, Bhavani S, Herrera-Foessel SA, Singh D, Singh PK, Velu G, Mason RE, Jin Y, Njau P, Crossa J (2011) Race non-specific resistance to rust diseases in CIMMYT spring wheats. Euphytica 179:175–186

Breeding for Ug99 Resistance at ICARDA: Results from Regional Trials*

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Abstract

Wheat is the most important stable crop in Central and West Asia and North Africa (CWANA) region. The emergence of Ug99 stem rust race capable of destroying most of the CWANA wheat cultivars posed serious threat to wheat production in the region. The International Center for Agricultural Research in the Dry Areas (ICARDA) has made tremendous efforts in developing Ug99 resistant wheat germplasm, and since 2009 has distributed five stem rust resistant spring bread wheat yield trials composed of 24 wheat genotypes each to the National Agricultural Research Systems (NARS) in CWANA region to assist them in identifying stable, high yielding wheat genotypes suited to their environments. Most of the distributed genotypes showed high level of resistance to Ug99 infection at Njoro, Kenya and Debre Zeit, Ethiopia and other know Ug99 hotspots in the region. Analysis of grain yield of these multilocation trials revealed that in addition to Ug99 resistance a number of lines performed as good as or significantly better than the national check. A number of the evaluated lines ranked among the top-yielding group across many sites implying that some of the lines are widely adapted. The high yielding and Ug99 resistant genotypes identified in this study are recommend for use as direct release to replace currently Ug99 susceptible varieties by the respective NARS and/or to be used as parents in wheat improvement programs by NARS collaborators and international wheat scientific community.

International center for Agricultural Research in the Dry Areas (ICARDA[1]); Ethiopian Institute for Agricultural Research (EIAR[2]); Kenya Agricultural Research Institute (KARI[3])

*This is an unedited original draft. A revised version will appear in the workshop proceedings published later this year.

Breeding for stripe rust resistance in the U.K. and Europe

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Abstract Wheat breeding in the U.K. is primarily the domain of private industry. The high input agricultural system means that most diseases are controlled by fungicide application. However, many wheat breeders still consider it important to breed for resistance. Of the three rust diseases affecting wheat, stripe rust is the most common in the U.K. and northern Europe. Traditionally resistance breeding has utilised major, race-specific resistance genes as these are easy to screen for in a multi-trait selection program. However, in recent years breeders have expressed a greater interest in partial, adult plant resistance (APR). To maximise the effective use of APR it is necessary to have an understanding of the genetic diversity underpinning stripe rust resistance within the U.K. winter wheat gene pool. Through a number of genetic mapping and characterisation projects we have undertaken to address this question. The results from these projects will be presented.

Keywords

Adult plant resistance, hypersensitive cell death, race-specific, wheat, yellow rust

Introduction

In a world economy faced with global food insecurity the demand for increased agricultural production has never been greater. Along with rice and maize wheat provides a substantial proportion of the calorific intake of the human population, either directly or through livestock feed (http://faostat.fao.org). Wheat production within the U.K. and across most of Europe follows a high input regime and wheat yields in the range of 10-12 tonnes per hectare are not uncommon.

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While many fungal diseases of wheat can be controlled through chemical application U.K. wheat breeders still consider it financially beneficial to breed for resistance. Stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) is of particular concern within the U.K. and northern regions of Europe (Boyd 2005). The first genetic characterisation of disease resistance in wheat was to stripe rust in the cv. Rivet, work undertaken at the former Plant Breeding Institute in Cambridge. (Biffin 1905). Since then many genes effective against stripe rust have been identified (Catalogue of Gene Symbols for Wheat, at http://shigen.lab.nig.ac.jp/wheat/komugi/genes/symbolClasslist.jsp). However, many major stripe rust resistance genes deployed in wheat cultivars have proven to be race-specific, having a relatively limited effective life (Bayles et al. 2001). In contrast, some wheat cultivars grown extensively for many years retain a good level of resistance, including the European cultivars Cappelle Desprez (Johnson 1983), Camp Remy (Boukhatem et al. 2002; Mallard et al. 2005) and Alcedo (Meinel 1997). These sources of resistance are often referred to as durable. The term 'Durable Resistance' was coined by Dr. Roy Johnson and describes resistance, which has remained effective in a cultivar during its widespread cultivation, over a long period of time, in an environment favorable to the disease (Johnson and Law 1975).

Deployment of stripe rust resistance in U.K. wheat cultivars

Many major stripe rust resistance genes have been deployed in U.K. cultivars. However, over and over again the release of a new resistant cultivar was quickly followed by the break-down of that resistance (Table 1). After the break-down of the race-specific resistance gene *Yr17* in 1996 U.K. breeders began to express a greater interest in those sources of resistance identified as "durable". However, many forms of resistance considered as durable often express a partial, growth stage-specific phenotype referred to as adult plant resistance (APR) and are less easy to define and follow within a traditional wheat breeding program (Boyd 2006).

Table 1 *Break-down of race-specific stripe rust resistance genes used in U.K. wheat cultivars*

Year released	Cultivar	Year of resistance break-down	Resistance genes
1993	Brigadier	1996	*Yr9,Yr17*
1987	Hornet	1988	*Yr2,Yr6,Yr9*
1983	Stetson	1983	*Yr1,Yr9*
1975	Clement	1975	*Yr2,Yr9*
1971	Talent	1972	*Yr7*
1968	Maris Ranger	1969	*Yr3a,Yr4a,Yr6*
1964	Rothwell Purdix	1966	*Yr1,*(*Yr2,Yr6*)
Unknown	Heines VII	1955	*Yr2*

Table 2 *Characterisation of stripe rust resistances in U.K. wheat cultivars on the U.K. 2010 Recommended List*

Cultivar	HGCA rating[1]	Effective seedling resistance	Seedling R gene
KWS-Sterling	9.0	unknown	-
Panorama	8.9	yes	Same as in Solstice
Zebedee	8.9	yes	unknown
Warrior	8.9	unknown	-
Scout	8.9	yes	unknown
Claire	8.6	no	-
Beluga	8.6	unknown	-
Cassius	8.8	no	-
Istabraq	8.8	yes	unknown
Alchemy	8.7	yes	unknown
Gladiator	8.9	no	-
Xi 19	9.0	yes	unknown

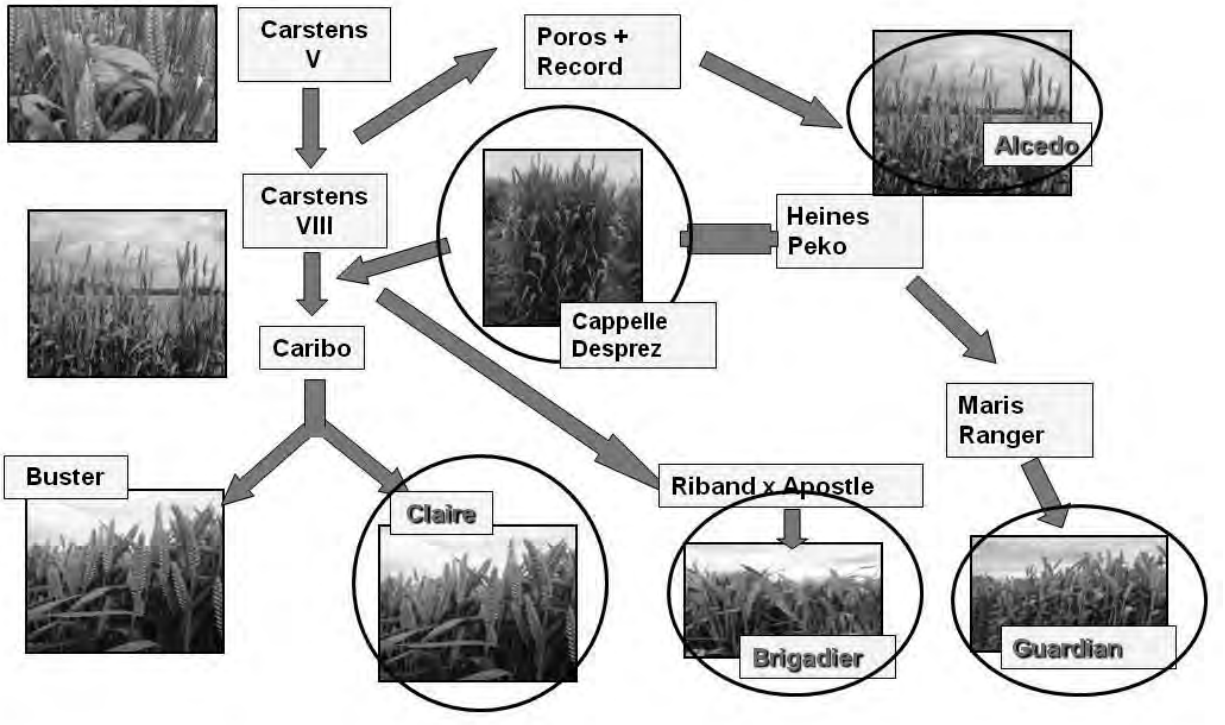
[1]1-9 scale with 9 indicating no symptoms

Despite the rapid loss of resistance experienced in many U.K. wheat cultivars there are still cultivars on the current U.K. Recommended List that express complete stripe rust resistance (Table 2). However, where seedling resistance is expressed it is not known whether resistance is conferred by individual and as yet undefeated resistance genes, or is due to a combination of resistance genes, that while ineffective alone, are not matched by a virulent pathotype in the *P. s.* f. sp. *tritici* population. Where there is no effective seedling resistance, but effective field resistance, again the genetics underlying this resistance is generally undefined.

Genetic characterisation of stripe rust resistance in U.K. wheat cultivars

Over the years we have worked with a number of U.K. breeders to define the genetic diversity underlying stripe rust resistance within the U.K. winter wheat gene pool. This has resulted in a snap shot of the genes for stripe rust resistance present in U.K. cultivars, and an indication as to the origin of these resistance genes (Fig. 1). Primarily these studies have concentrated on the genetic characterisation of resistance as expressed under field conditions, i.e. APR. In addition to the genetic characterisation of stripe rust resistance we have used microscopy in an attempt to define the effect of individual resistance genes, examining the development of the pathogen *in planta* and the cellular response of the plant to pathogen invasion.

Fig. 1 *Pedigree relationships between U.K. winter wheat cultivars. Ringed cultivars are those on which stripe rust resistance studies are being undertaken*

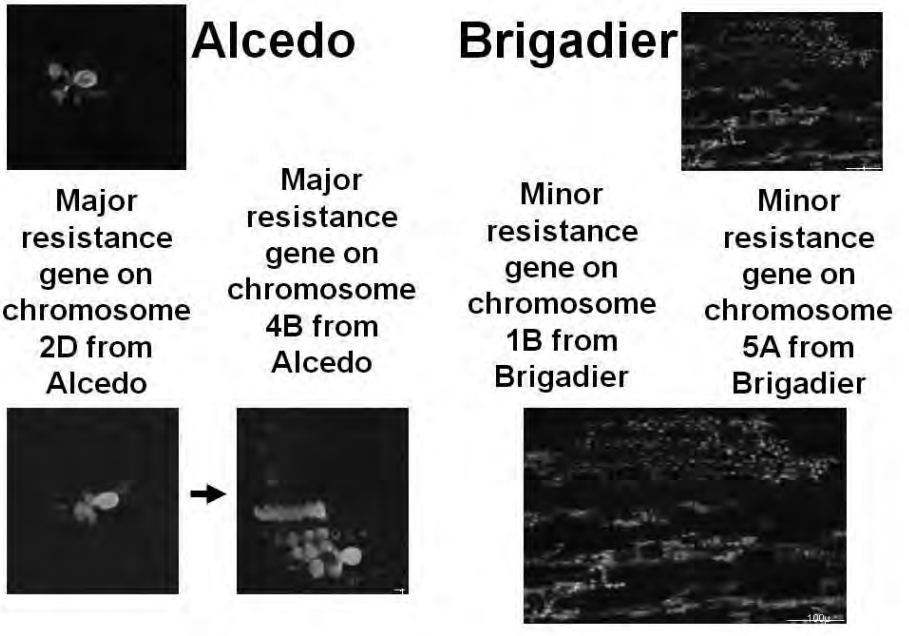


The bread wheat cv. Alcedo expresses complete field stripe rust resistance (Meinel 1997). This resistance remained effective when Alcedo was grown extensively in Germany and Eastern Europe between 1975 and 1989, occupying 47% of the wheat area at its peak in 1981. Alcedo was introduced into U.K. wheat breeding in the 1970's, with the first cultivar derived from Alcedo, cv. Apostle, being released in 1980. However breeders did not know to what extent the resistance in Alcedo was present in current European cultivars, or whether Alcedo represented an unused source of stripe rust resistance. Alcedo was crossed to the high yielding U.K. feed wheat cv. Brigadier, which expressed complete stripe rust resistance when released in 1993 due to the seedling resistance genes *Yr9* and *Yr17*. In 1996 the resistance in Brigadier was overcome, following the appearance of a pathotype of *P. s.* f. sp. *tritici* virulent for both *Yr9* and *Yr17* (Bayles 2001).

Two major genes for stripe rust resistance were identified in Alcedo, one on the long arm of chromosome 2D and the other on 4BL (Fig. 2; Jagger et al. 2011a). In addition, two small-effect resistance loci were identified in Brigadier. Subsequent examination of a number of stripe rust resistant cultivars indicated that the chromosome 2D locus was common within U.K. pedigrees, whereas the 4B gene was poorly represented (S. Berry, Limagrain, unpublished data). Among the cultivars found to carry the 2D gene was the very popular biscuit wheat cv. Claire (L. Boyd unpublished data). Claire was released in 1999 with complete field resistance to stripe rust, and was grown extensively for a number of years. It was also used extensively by wheat breeders as a parent in many crosses.

Fig. 2 *Phenotype and chromosomal location of stripe rust resistances identified in cultivars Alcedo and Brigadier*

Fig. 3 *Microscopic phenotypes of stripe rust resistances identified in cultivars Alcedo and Brigadier. The microscopic phenotypes seen in the parental cvs Alcedo and Brigadier are shown along with the phenotypes of the individual stripe rust resistance loci indentified in cv. Alcedo on chromosomes 2D and 4B, and in cv. Brigadier on chromosomes 1B and 5A, which confer similar phenotypes*



Microscopic examination of stripe rust resistance

The seedling, race-specific resistance gene *Yr1* is associated with a strong plant cell death response (Bozkurt et al. 2010). In the South African cv. Kariega APR was located on chromosomes 7D (probably APR gene *Yr18*) and 2B (Ramburan et al. 2004). Whereas the 2B APR is associated with extensive plant cell death, *Yr18* is not (Moldenhauer et al. 2006, 2008). Microscopic examination of *P. s.* f. sp. *tritici* infection in near-isogenic lines of Brigadier carrying either the Alcedo chromosome 2D and/or the 4B APR genes indicated that both resistances operated through a rapid plant cell death mechanism (Fig. 3; Jagger et al. 2011b).

In 2006 significant stripe rust infection was detected on cv. Claire in New Zealand. In 2010 stripe rust infection was reported on Claire in the U.K. (David Feuerhelm, Syngenta, pers comm). It has yet to be confirmed, but the most likely candidate for the broken resistance is the chromosome 2D locus derived from Alcedo. If this holds true then the extensive deployment of the 2D Alcedo resistance in U.K. cultivars does not bode well for many of the cultivars listed in Table 2 and reported as exhibiting good stripe rust resistance.

These studies only serve to highlight the importance of a good understanding of the genetic diversity underlying resistance, both seedling and adult plant within the gene pool in which breeders make crosses and selections. This knowledge enables breeders to make more informed crosses that effectively utilise the full diversity of resistance available to them, and to know if and when new sources of resistance need to be introduced into their adapted wheat genotypes.

References

Bayles RA (2001) Yellow Rust of Wheat — the UK Experience. Bericht über die 52. Tagung 2001 der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Ōsterreichs BAL Gumpenstein, pp57-59 (20–22th November 2001)

Biffin RH (1905) Mendel's law of inheritance and wheat breeding. J Agric Sci:4-48

Boukhatem N, Baret PV, Mingeot D, Jacquemin JM (2002) Quantitative trait loci for resistance against yellow rust in two wheat-derived recombinant inbred line populations. Theor Appl Genet 104:111-118

Boyd LA (2005) Centenary review: Can Robigus defeat and old enemy?-Yellow rust of wheat. J Agric Sci 143:233-243

Boyd LA (2006) Perspective - can the durability of resistance be predicted? J Science of Food and Agric 86:2523-2526

Bozkurt O, McGrann G, Unver T, MacCormack R, Boyd LA, Akkaya MS (2010) Cellular and transcriptional responses of wheat during compatible and incompatible race-specific interactions with *Puccinia striiformis* f. sp. *tritici.* Mol Plant Patholol 11:625-640

Jagger LJ, Newell C, Berry ST, MacCormack R, Boyd LA (2011a) The genetic characterisation of stripe rust resistance in the German wheat cultivar Alcedo. Theor Appl Genet 122:723-733

Jagger LJ, Newell C, Berry ST, MacCormack R, Boyd LA (2011b) Histopathology provides a phenotype by which to characterise stripe rust resistance genes in wheat. Plant Pathology Doi: 10.1111/j.1365-3059.2011.02436.x.

Johnson R (1983) Genetic background of durable resistance. In: Lamberti F, Waller JM, van de Graff NA (eds) Durable resistance in crops. Plenum Press, London, p 5-24.

Johnson R, Law CN (1975) Genetic control of durable resistance to yellow rust (*Puccinia striiformis*) in the wheat cultivar Hybride de Berseé. Ann Appl Biol 81:385-391

Mallard S, Gaudet D, Aldeia A, Abelard C, Besnard AL, Sourdille P, Dedryver (2005) Genetic analysis of durable resistance to yellow rust in bread wheat. Theor Appl Genet 110:1401-1409

Meinel A (1997) Breeding aspects of durable adult plant resistance against airborne pathogens in wheat. COST Action 817 Conference: Approaches to improving disease resistance to meet future needs: Airborne pathogens in wheat and barley. 11–13th November, Prague, Czech Republic. pp 52–55. Published by The Research Institute of Crop Production, Praha-Ruzyně.

Moldenhauer J, Moerschbacher BM, van der Westhuizen AJ (2006) Histological investigation of stripe rust (*Puccinia striiformis* f.sp. *tritici*) development in resistant and susceptible wheat cultivars. Plant Pathology 55:469-474

Moldenhauer J, Pretorius ZA, Moerschbacher BM, Prins R, van der Westhuizen AJ (2008) Histopathology and PR-protein markers provide insight into adult plant resistance to stripe rust of wheat. Mol Plant Pathol 9:561-569

Ramburan VP, Pretorius ZA, Boyd LA, Smith PH, Boshoff WHP, Prins R (2004) A genetic analysis of adult plant resistance to stripe rust in the wheat cultivar Kariega. Theor Appl Genet 108:1426-1433

Multiple rust resistance and gene additivity in wheat: Lessons from multi-location case studies in cultivars Parula and Saar

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Abstract

Partial race non-specific resistance to rust diseases and powdery mildew in wheat is valuable in providing cultivars with durable resistance. Partial resistance is inherited as a quantitative trait, and the success of breeding for this type of resistance is based on the ability of resistance genes to complement each other and provide offspring with increased levels of resistance. The objective of the present study was to investigate the additivity of rust resistance genes in two mapping populations that segregated for resistance at the *Lr34* and *Lr46* loci: 'Avocet x Parula' and 'Avocet x Saar'. The former also segregated for a third resistance gene on chromosome 7BL from Parula, designated *LrP*. The populations were tested for resistance to leaf rust, stripe rust and powdery mildew in multiple environments across Mexico, the Southern Cone of South America, Norway and China. *Lr34* showed consistent effects on resistance to all three diseases in both populations and across all environments. *LrP* also showed consistent effects on leaf rust in all environments where it was tested, and contributed additively to improved resistance when combined with other genes. *Lr46* conferred resistance to all three diseases, but the magnitude of effect was always less than that for *Lr34*. Combinations of *Lr34* and *Lr46* resulted in only slight improvements in resistance over *Lr34* alone. We conclude that partial resistance genes with different mechanisms need to be combined to achieve a stable resistance performance across environments.

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Keywords

AMMI model, *Blumeria graminis* f. sp. *tritici,* disease resistance; genotype x environment interaction, GxE, *Puccinia triticina, P. striiformis*.

Introduction

The short durability of commonly used race specific resistance genes to cereal rusts and powdery mildew has created an increased interest in partial and race non-specific resistance. Race non-specific resistance to rust diseases in cereals is usually associated with susceptibility at the seedling stage but retarded and therefore slower disease development in adult plants. Hence, it is often referred to as adult plant resistance (APR) or slow rusting resistance. Partial resistance is inherited in a quantitative manner, and breeding experience has shown that near-immune levels of resistance to leaf rust (caused by *Puccinia triticina*) and stripe rust (caused by *P. striiformis*) can be achieved by combining several genes for partial resistance into the same cultivar (Singh et al. 2000). Additive gene effects are often attributed to the combination of resistance genes that act through different resistance mechanisms, and hence complement each other to increase the overall level of resistance. For successful resistance breeding, it is of importance to identify the optimal gene combinations that provide the best complementary resistance for target environments.

One well-characterized gene for partial rust resistance is *Lr34/Yr18/Pm38* on chromosome arm 7DS, which confers resistance not only to leaf rust (Dyck et al. 1966), but also stripe rust (Singh 1992b) and powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*) (Spielmeyer et al. 2005; Lillemo et al. 2008). The gene is associated with a premature senescence of the leaf tip, commonly referred to as leaf tip necrosis (LTN), which can be used as a phenotypic marker in resistance breeding (Singh 1992a). *Lr34* was recently cloned and shown to encode an ABC transporter of the ABCG (formerly pleiotropic drug resistance, PDR) subfamily (Krattinger et al. 2009). The *Lr46/Yr29/Pm39* gene on 1BL is another example of a LTN-associated resistance gene that confers partial resistance to multiple biotrophic pathogens (William et al. 2003; Singh et al. 1998; Rosewarne et al. 2006; Lillemo et al. 2008).

The CIMMYT-derived bread wheat line Saar confers high levels of resistance to leaf rust, stripe rust and powdery mildew based on *Lr34* and *Lr46* in combination with other, more pathogen-specific partial resistance genes (Lillemo et al. 2008). Parula is an old

line developed at CIMMYT that combines both *Lr34* and *Lr46* with a third gene for partial leaf rust resistance, designated *LrP*, on 7BL (William et al. 1997; William et al. 2007; Herrera-Foessel et al. 2009).

The main objective of the present study was to investigate the additivity of the above rust resistance genes across multiple environments based on the 'Avocet x Parula' and 'Avocet x Saar' mapping populations.

Materials and methods

Avocet x Parula population

The parental lines and 139 F_6-derived recombinant inbred lines (RILs) from the cross between Avocet-S and Parula were tested in nine field trials for leaf rust resistance and three field trials for stripe rust resistance in Mexico and the Southern Cone of America, and for powdery mildew resistance at two locations in Norway. Details of the trial environments are given in Table 1.

The Avocet x Parula population was briefly described by William et al. (2007), and a full account of the QTL mapping including all test environments will be published elsewhere. For the present study, the following flanking markers were used to tag individual resistance genes: *1BATP, gwm259* and *P33M59_1* for *Lr46* on 1BL, *P41M49_2, pRP1M61_1* and *P36M60_2* for *LrP* on 7BL, and *barc92* and *csLV34* for *Lr34* on 7DS.

Avocet x Saar population

The Avocet x Saar mapping population is described in Lillemo et al. (2008). The parents and 113 RILs were tested for leaf rust and stripe rust reactions in four field trials each, across various locations in Mexico and China. In addition, the powdery mildew data from five environments in Norway and China (Lillemo et al. 2008) were included for comparison. Details of the trial environments are given in Table 2.

Phenotypic effects of QTL in the Avocet x Saar population were calculated based on the following flanking markers: *hbe248* and *csLV46G22* for *Lr46* on 1BL, *gwm251* and *gwm149* for the powdery mildew resistance QTL on 4BL, *wmc475b* and *wmc256* for the rust resistance QTL on 6AL, and *gwm1220* and *swm10* for *Lr34* on 7DS.

Table 1 Field testing environments for the Avoc et x Parula population

Location	Country	Year	Trait	Acronym	Longitude	Latitude	Altitude
Marcos Juarez	Argentina	2006	Leaf rust	LR_MJ06	32°42′S	62°07′W	110 m
Marcos Juarez	Argentina	2007	Leaf rust	LR_MJ07	32°42′S	62°07′W	110 m
Passo Fundo	Brazil	2007	Leaf rust	LR_PF07	28°15′S	52°25′W	684 m
Chillán	Chile	2008	Leaf rust	LR_Ch08	36°31′S	71°55′W	217 m
La Estanzuela	Uruguay	2005	Leaf rust	LR_LE05	32°40′S	57°41′W	50 m
La Estanzuela	Uruguay	2006	Leaf rust	LR_LE06	32°40′S	57°41′W	50 m
La Estanzuela	Uruguay	2007	Leaf rust	LR_LE07	32°40′S	57°41′W	50 m
Cd. Obregon	Mexico	1997	Leaf rust	LR_Y97	27°21′N	109°56′W	38 m
Cd. Obregon	Mexico	1998	Leaf rust	LR_Y98	27°21′N	109°56′W	38 m
Chillán	Chile	2008	Stripe rust	YR_Ch08	36°31′S	71°55′W	217 m
Toluca	Mexico	1996	Stripe rust	YR_MV96	19°16′N	99°51′W	2640 m
Toluca	Mexico	1997	Stripe rust	YR_MV97	19°16′N	99°51′W	2640 m
Hamar	Norway	2006	Powdery mildew	PM_St06	60°44′N	11°06′E	153 m
Ås	Norway	2006	Powdery mildew	PM_Vb06	59°40′N	10°46′E	90 m

Table 2 *Field testing environments for the Avocet x Saar population*

Location	Country	Year	Trait	Acronym	Latitude	Longitude	Altitude
Baoding, Hebei	China	2007	Leaf rust	LR_BD07	38°52′N	115°28′E	19 m
Xinxiang, Henan	China	2007	Leaf rust	LR_XX07	35°17′N	113°55′E	111 m
Cd. Obregon	Mexico	2005	Leaf rust	LR_Y05	27°21′N	109°56′W	38 m
El Batan	Mexico	2006	Leaf rust	LR_BV06	19°31′N	98°50′W	2249 m
Beijing	China	2006	Stripe rust	YR_Bj06	39°57′N	116°19′E	33 m
Tianshui, Gansu	China	2007	Stripe rust	YR_GS07	34°34′N	105°43′E	1377 m
Toluca	Mexico	2005	Stripe rust	YR_MV05	19°16′N	99°51′W	2640 m
Toluca	Mexico	2006	Stripe rust	YR_MV06	19°16′N	99°51′W	2640 m
Beijing	China	2005	Powdery mildew	PM_Bj05	39°57′N	116°19′E	33 m
Hamar	Norway	2005	Powdery mildew	PM_St05	60°44′N	11°06′E	153 m
Hamar	Norway	2006	Powdery mildew	PM_St06	60°44′N	11°06′E	153 m
Ås	Norway	2005	Powdery mildew	PM_Vb05	59°40′N	10°46′E	90 m
Ås	Norway	2006	Powdery mildew	PM_Vb06	59°40′N	10°46′E	90 m

Statistical analysis

Simple and Composite Interval Mapping were conducted with the software PLABQTL v. 1.2 (Utz and Melchinger 2003) in order to localize the most important QTL for the three diseases in the two mapping populations, and to identify closely linked and flanking markers for the QTL of interest.

Simple trait correlations were calculated by the PROC CORR procedure in SAS (SAS Institute Inc., v 9.2). Calculations of phenotypic effects of individual QTL and QTL combinations and tests of statistical significance were conducted by the PROC GLM procedure in SAS. Insight into genotype x environment (GxE) interactions was gained by applying the additive main effects and multiplicative interaction (AMMI) model to the disease data of the two mapping populations. Briefly, this analysis is done in two steps by first conducting an ordinary analysis of variance (ANOVA) and then conducting a principal component analysis (PCA) on the residuals after removing the genotype and environment main effects from the data matrix (Gauch 1992). The GxE interaction can then be visualized by plotting the first and second order PCA scores of genotypes and environments against each other in biplots. The AMMI analysis was done with the SAS code developed by the biometrics and statistics unit at CIMMYT, Mexico (Burgueño et al. 2001).

Results

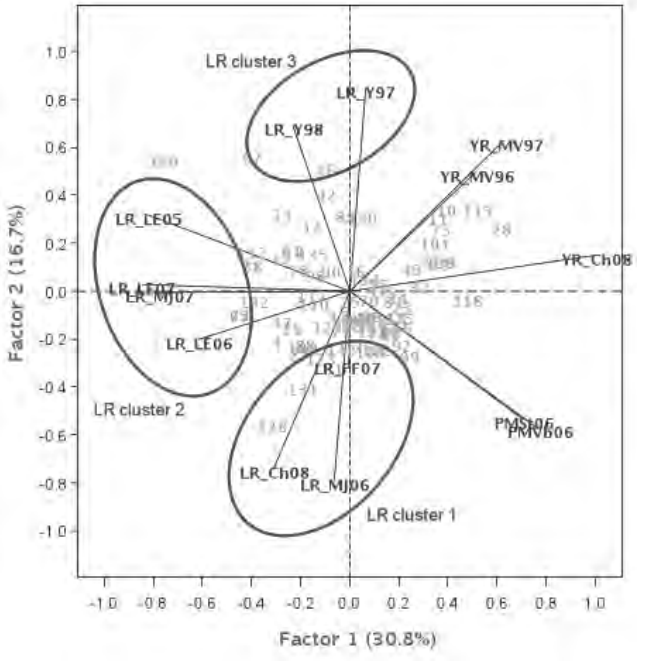
Avocet x Parula population

The mean severities for leaf rust, stripe rust and powdery mildew in the Avocet x Parula population showed strong associations, with correlation coefficients of 0.59, 0.47 and 0.60 for leaf rust *vs.* stripe rust, leaf rust *vs*. powdery mildew and stripe rust *vs.* powdery mildew, respectively. This indicated that at least part of the resistance to each of the three diseases was under common genetic control.

The *Lr34* locus on chromosome 7DS showed significant effects against all three diseases in QTL mapping. The *LrP* locus on 7BL was detected as the second major QTL in this population, but was significant only for leaf rust. The *Lr46* locus on 1BL was detected only in some of the leaf rust and stripe rust trials. Avocet contributed two minor QTL with resistance to leaf rust on 6AL, and stripe rust and powdery mildew resistance on 4BL. The effects of these QTL were very small compared to *Lr34* and *LrP* in this population and were not considered further for this analysis.

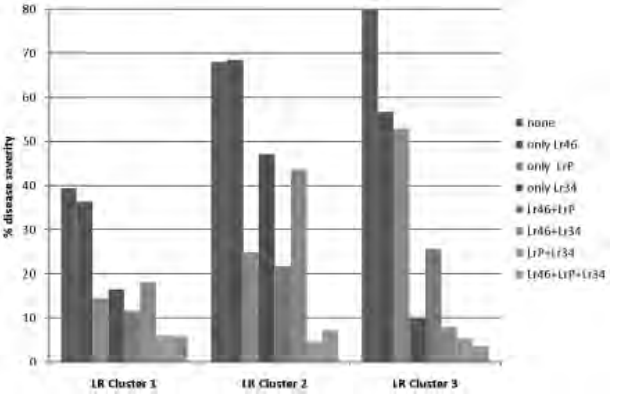
AMMI analysis was conducted to gain insight into possible GxE interactions. The first two principal components clearly differentiated the three diseases and further separated the leaf rust environments into three clusters (Fig. 1).

Fig. 1 ***Biplot of the first two principal components from the AMMI analysis of the Avocet x Parula disease data. The genotype vectors of individual RILs are represented by numbers and environment vectors are shown as arrows and identified by the acronyms listed in Table 1. Factor 1 differentiates the environments according to the phenotypic effects of LrP with strong effects in the leaf rust environments to the left, moderate effects in the leaf rust environments in the middle and no effect in the stripe rust and powdery mildew environments to the right. Factor 2 reflects differential effects of Lr34, with strong effects in the environments at the top and relatively weaker effects in the environments in the lower part of the diagram. The three clusters of leaf rust environments are marked with circles***



QTL analysis of the genotype scores of the principal components revealed that the first component explained a differential effect of *LrP* whereas the second component explained a differential effect of *Lr34*. Mean disease severities of contrasting maker alleles at the *Lr46*, *LrP* and *Lr34* resistance loci, and their different combinations are given in Table 3, and visualized for the three clusters of leaf rust environments in Fig. 2.

Fig. 2 ***Phenotypic effects of Lr46, LrP and Lr34 gene combinations on leaf rust severity for the three groups of leaf rust environments in the Avocet x Parula population***



In cluster 1 (Passo Fundo, Brazil; Chillán, Chile; Marcos Juarez, Argentina 2006), *LrP* and *Lr34* showed effects of similar magnitude and both genes were capable of reducing the leaf rust severity to less than a half when occurring alone, and down to less than a quarter when occurring in combination. Although a tendency for reduced severity was observed at some sites during some years, *Lr46* did not show any significant effect in these environments, either alone or in combination with any other gene. The overall disease levels in these environments were lower than for those that grouped into the other two clusters.

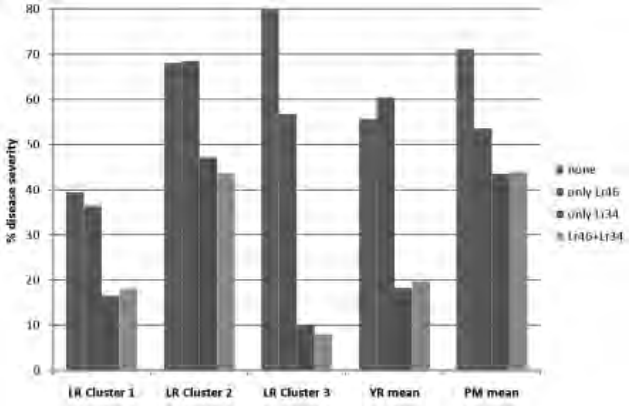
The leaf rust environments in cluster 2 (La Estanzuela, Uruguay, 3 years; Marcos Juarez 2007) were characterized by a strong effect of *LrP* and only a moderate effect of *Lr34*. In these environments, the combination of *LrP* and *Lr34* reduced the severity from almost 70% to only 5%, whereas the effect of *Lr46* was not significant either when alone or in combination with *LrP* or *Lr34* (Fig. 2).

The third cluster of leaf rust environments involved the two trials at Cd. Obregon, Mexico. Here, the effect of *LrP* was only moderate whereas *Lr34* showed a strong effect and reduced the severity from 80% to only 10% when used alone. *Lr46* contributed significant improvements in resistance, both alone and in combination with the other two genes. The triple combination of *Lr46*, *LrP* and *Lr34* reduced the severity to less than 5%.

Table 3 *Mean disease severities of contrasting alleles of single genes and gene combinations in the Avocet x Parula population, for individual test environments with leaf rust, stripe rust and powdery mildew. See Table 1 for location codes*

Main effects of individual gene:			Leaf rust									Stripe rust			Powdery mildew	
			Cluster 1			Cluster 2				Cluster 3						
	Allele	n	MJ06	Ch08	PF07	LE06	MJ07	LE07	LE05	Y98	Y97	Ch08	MV96	MV97	St06	Vb06
lr46	Avocet	64	21.9	23.0	12.3	32.8	32.2	29.5	46.8	36.6	40.5	40.1	40.4	43.7	56.4	55.2
Lr46	Parula	53	23.1	16.3	6.3	33.7	23.6	27.1	42.9	22.4	24.0	38.5	33.4	42.5	49.2	50.8
			n.s.	n.s	*	n.s.	n.s.	n.s.	n.s.	**	**	n.s.	n.s.	n.s.	n.s.	n.s.
lrP	Avocet	56	36.6	36.2	17.8	53.4	54.8	52.7	72.8	49.1	46.3	38.4	42.6	49.2	57.8	56.7
LrP	Parula	68	14.3	8.1	4.8	16.7	8.0	7.3	22.6	17.0	24.4	39.7	34.0	40.0	49.5	51.9
			**	**	**	**	**	**	**	**	**	n.s.	n.s.	n.s.	*	n.s.
lr34	Avocet	62	33.2	27.8	15.5	43.2	39.4	39.5	63.4	54.4	58.3	55.9	58.0	69.5	64.7	64.1
Lr34	Parula	61	16.2	15.6	3.6	25.0	17.0	29.6	29.3	8.0	7.6	20.0	16.0	17.3	39.4	41.6
			**	**	**	**	**	**	**	**	**	**	**	**	**	**
Gene combination:																
none		15	41.2	52.2	25.0	54.3	76.7	58.6	82.7	84.7	75.5	45.3	58.3	63.3	75.0	67.1
only *Lr46*		9	58.9	30.0	20.3	60.0	63.3	64.3	86.4	56.7	56.7	54.4	60.0	66.7	49.7	57.2
only *LrP*		10	19.1	10.0	14.5	24.8	13.1	18.2	43.8	39.0	67.0	84.0	76.0	84.0	71.6	70.7
only *Lr34*		9	18.4	26.1	4.8	54.4	37.2	42.8	54.4	9.1	10.8	21.9	14.6	18.3	43.2	43.8
Lr46+LrP		15	16.4	12.2	6.1	31.1	10.4	11.8	33.4	22.0	29.2	51.3	46.3	66.7	57.0	60.8
Lr46+Lr34		8	20.8	30.0	3.4	43.1	31.3	43.8	56.6	7.5	8.2	19.7	14.4	25.0	45.0	43.0
LrP+Lr34		19	10.7	6.9	0.6	3.4	4.7	1.6	8.4	5.2	5.5	17.4	13.8	11.6	32.4	37.9
Lr46+LrP+Lr34		13	11.2	4.9	1.6	9.7	4.8	2.8	11.5	3.8	3.5	22.9	14.2	16.2	40.9	40.2
Parental mean:																
Avocet			80	99	80	90	90	65	99	100	95	40	100	100	75	63
Parula			0	10	1	1	0	1	0	5	1	5	1	1	33	46

Fig. 3 ***Phenotypic effects of Lr46 and Lr34 gene combinations on leaf rust, stripe rust and powdery mildew severities in the Avocet x Parula population***



Lr34 showed a strong effect in reducing the severity of both stripe rust and powdery mildew whereas *Lr46* mainly contributed resistance to powdery mildew (Fig. 3, Table 3). For both stripe rust and powdery mildew, the *Lr46*+*Lr34* combination did not improve resistance compared to the effect of *Lr34* alone (Fig. 3). Although *LrP* was associated with small reductions in stripe rust and powdery mildew severities in some environments, the effects were significant only at one site for powdery mildew (Table 3).

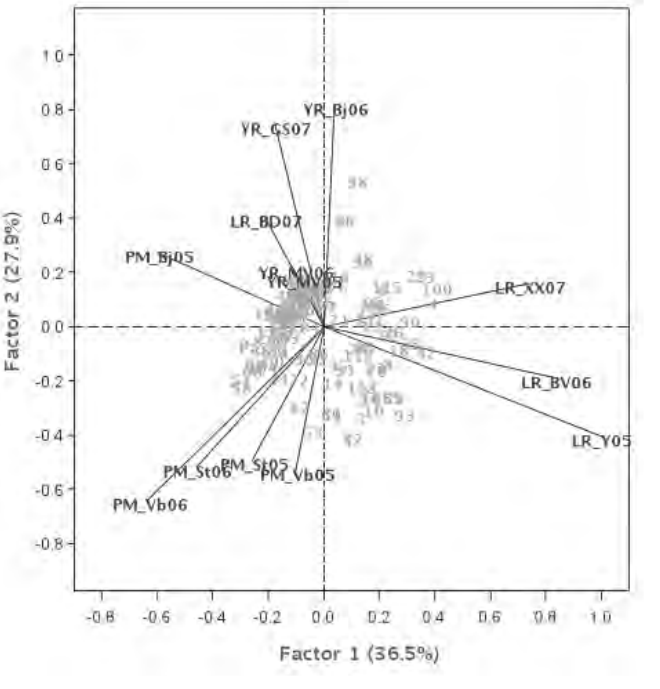
Avocet x Saar population

The Avocet x Saar population also showed significant correlations among the mean disease severities for leaf rust, stripe rust and powdery mildew, with correlation coefficients of 0.81, 0.72 and 0.65 for leaf rust *vs.* stripe rust, leaf rust *vs*. powdery mildew and stripe rust *vs.* powdery mildew, respectively.

The QTL analysis detected a major QTL at the *Lr34* locus on chromosome 7DS with significant effects on the three diseases in all environments. A QTL at the *Lr46* locus on 1BL contributed resistance to all three diseases, but was not significant in all test environments. A QTL for leaf rust and stripe rust resistance from Avocet was detected close to the *Sr26* gene in the centromeric region of 6AL. This QTL did not contribute resistance to powdery mildew. A QTL for powdery mildew resistance from Avocet was present on chromosome 4BL. This QTL also showed a minor contribution to leaf rust resistance, but only in the Mexican environments. The overall effect of these two resistance loci from Avocet was larger in Avocet x Saar than in Avocet x Parula.

The first two principal components of the AMMI analysis separated the test environments into three clusters mostly according to the disease traits (Fig. 4). Important exceptions from this pattern were the leaf rust trial at Baoding, China, and the powdery mildew trial at Beijing, China, which showed more similarity with stripe rust environments.

Fig. 4 ***Biplot of the first two principal components from the AMMI analysis of the Avocet x Saar disease data. The genotype vectors of individual RILs are represented by numbers whereas the environment vectors are shown as arrows and identified by the acronyms listed in Table 2. Factor 1 reflects differential effects of Lr34, with strongest effects in the environments in the right hand side of the diagram. Factor 2 reflects differential effects of the 4BL QTL, which showed significant contribution to resistance in the environments in the lower part of the diagram***



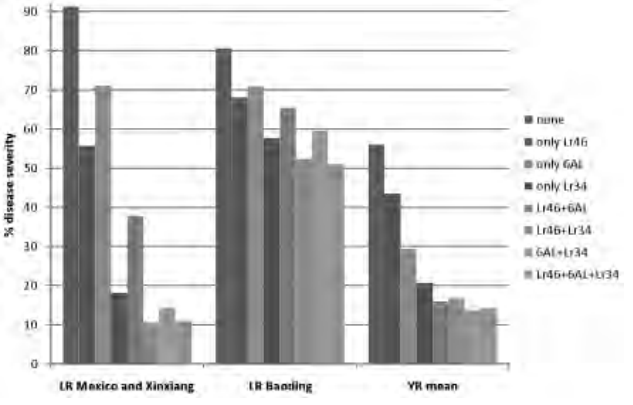
QTL analysis of the genotypic scores of the first principal component showed that it differentiated the environments according to the phenotypic effect of *Lr34* with strongest contribution to resistance in the environments to the right in the diagram. The second component was explained by differential effects of the 4BL QTL, which showed significant contributions to resistance in the environments in the lower part of the diagram.

Table 4 *Mean disease severities of contrasting alleles of single genes and gene combinations in the Avocet x Saar population, for individual test environments with leaf rust, stripe rust and powdery mildew. See Table 2 for location codes*

Main effects of individual gene:	Allele	n	Leaf rust				Stripe rust				Powdery mildew				
			Y05	BV06	XX07	BD07	Bj06	GS07	MV05	MV06	Bj05	St05	St06	Vb05	Vb06
lr46	Avocet	53	50.4	54.2	48.6	67.2	21.6	18.0	51.3	42.9	28.6	54.0	48.8	56.0	51.3
Lr46	Saar	47	19.8	35.4	37.1	59.8	11.9	13.0	38.8	30.7	24.7	34.3	32.7	34.8	35.1
			**	**	*	**	*	n.s.	**	**	*	**	**	**	**
4BL QTL	Saar	59	43.5	51.0	46.4	64.3	17.2	15.9	50.1	42.3	29.9	55.3	53.1	57.3	57.1
4BL QTL	Avocet	41	25.4	38.0	38.7	62.0	18.0	16.1	39.4	30.5	21.6	29.0	23.4	29.3	24.8
			**	*	n.s.	n.s.	n.s.	n.s.	**	**	**	**	**	**	**
6AL QTL	Saar	49	47.6	53.1	52.9	66.9	24.4	22.3	53.5	45.0	27.6	47.9	44.2	49.8	45.4
6AL QTL	Avocet	26	20.4	29.7	30.5	58.5	4.9	6.3	30.4	20.8	23.0	37.8	33.1	35.2	35.0
			**	**	**	*	**	**	**	**	*	*	*	*	n.s.
lr34	Avocet	45	65.9	73.8	71.7	72.5	29.4	25.2	58.9	50.3	31.5	57.6	52.1	62.9	52.2
Lr34	Saar	52	8.4	18.9	18.0	54.9	6.3	6.8	32.8	24.8	22.0	32.8	31.5	30.3	35.4
			**	**	**	**	**	**	**	**	**	**	**	**	**
Gene combination:															
none		16	97.8	95.0	81.3	80.6	46.2	39.2	74.1	65.0	31.9	69.5	60.3	77.2	59.7
only *Lr46*		11	40.0	60.0	66.9	68.0	31.0	29.1	60.0	53.6	31.3	44.0	48.3	49.4	46.6
only 6AL		4	76.3	67.5	69.4	70.8	12.1	12.7	52.5	40.0	30.2	77.3	75.6	85.0	69.4
only *Lr34*		14	10.9	21.1	22.5	57.7	7.8	7.4	38.0	29.6	23.3	32.6	33.0	31.0	35.1
Lr46+6AL		5	19.5	43.0	51.2	65.3	4.8	6.1	31.0	22.0	25.3	33.7	24.1	35.6	28.2
Lr46+*Lr34*		8	4.4	11.9	14.9	52.3	4.3	7.5	31.3	24.4	21.8	29.8	27.3	28.3	34.4
6AL+Lr34		10	8.2	22.0	12.6	59.5	1.9	3.8	29.5	20.0	25.4	38.3	38.0	33.8	44.0
Lr46+6AL+*Lr34*		10	4.3	17.2	11.5	51.0	4.0	5.7	28.0	19.5	18.8	30.0	28.1	25.6	28.6
Parental mean:															
Avocet			100	100	92	100	88	70	98	95	48	83	82	90	76
Saar			1	1	5	48	0	0	8	10	11	6	6	3	5

The phenotypic effects of the most important resistance genes in the different test environments of the Avocet x Saar populations are listed in Table 4, and general effects of the *Lr46*, *Lr34* and 6AL gene combinations on leaf rust and stripe rust are visualized in Fig. 5.

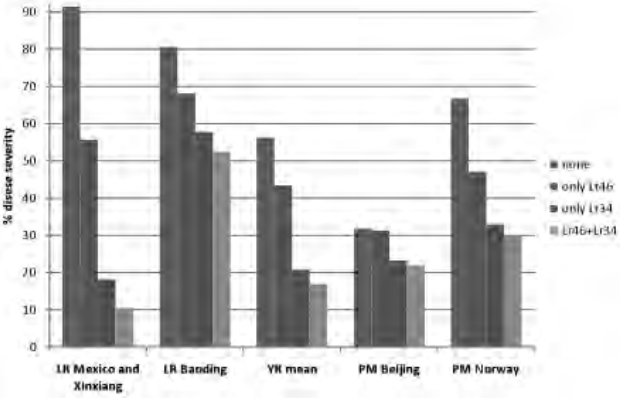
Fig. 5 ***Phenotypic effects of different combinations of*** *Lr46, Lr34* ***and the 6AL QTL on leaf rust and stripe rust in the Avocet*** *x* ***Saar population***



It is evident that the leaf rust trial at Baoding, which grouped together with stripe rust environments, could be characterized by much weaker effects of the different resistance genes compared with the other leaf rust environments. This environment also had a much higher overall disease level than the other leaf rust environments, and the resistant parent Saar was scored with an average severity of 48%. The 6AL QTL showed significant effect in all leaf rust and stripe rust environments, and contributed additively to resistance when combined with *Lr46* or *Lr34* in all environments except the leaf rust trial at Baoding.

In contrast to the Avocet x Parula population, the *Lr46* gene contributed resistance to all three diseases in Avocet x Saar, but the effect was negligible in the powdery mildew trial in Beijing (Fig. 6). *Lr46* contributed additive resistance when combined with *Lr34* in the leaf rust trials in Mexico and Xinxiang, China, but the additive effect was negligible in the leaf rust trial at Baoding, and for stripe rust and powdery mildew (Fig. 6.). *Lr34*, on the other hand, was always associated with larger reductions in disease severity than *Lr46*, and adding *Lr34* to *Lr46* always resulted in improved resistance.

Fig. 6 ***Phenotypic effects of*** *Lr46* ***and*** *Lr34* ***gene combinations on leaf rust, stripe rust and powdery mildew in the Avocet*** *x* ***Saar population***



Discussion

Additivity of Lr34 and Lr46

Multi-environment testing of the Avocet x Parula and Avocet x Saar populations against leaf rust, stripe rust and powdery mildew in very diverse environments made it possible to study the environmental stability of the *Lr34* and *Lr46* resistances against three different biotrophic pathogens and across two genetic backgrounds.

The most significant observation from this study was the consistent contribution to resistance shown by *Lr34* across all test environments for all three diseases in the two mapping populations. Although the effectiveness of *Lr34* varied with environments (discussed below) it always contributed a moderate to high level of resistance and is thus highly valuable in resistance breeding. Another important implication is that the resistant parents showed relatively stable performance across environments (except the Baoding leaf rust trial) even though the individual genes varied in their effects. This highlights the importance of combining diverse minor genes to achieve resistance stability.

Consistent with previous studies (Lillemo et al. 2008; Martinez et al. 2001), the effect of *Lr46* was always weaker than the effect of *Lr34*, and its contribution to resistance was significant only in some of the test environments for leaf rust, stripe rust and powdery mildew. The main effects of *Lr46* and its contribution to additive resistance when combined with *Lr34* were stronger in Avocet x Saar than Avocet x Parula, which

can be attributed to the different genetic backgrounds of the resistant parents. This is the most likely explanation for the contrasting effects of this gene in the two populations tested at the same trial locations in Norway in 2006.

Lr34 and *Lr46* are both associated with leaf tip necrosis (Singh 1992a; Rosewarne et al. 2006) and are characterized by very similar disease resistance phenotypes with increased latent period and decreased infection efficiency and uredinium size in adult plants (Martinez et al. 2001; Rubiales and Niks 1995). Although there is no indication of sequence homology of the two resistance genes at the DNA level (Krattinger et al. 2009; Lagudah 2011), they might act through the same defence response pathways and thus not complement each other to the extent expected based on their individual additive effects. The ongoing work to clone *Lr46* and to elucidate its molecular function will ultimately answer this question, but for resistance breeding it suffices to say that the combination of *Lr34* and *Lr46* will improve resistance beyond the effect of *Lr34* only in some environments and/or genetic backgrounds.

The partial resistance gene LrP

The resistance gene *LrP* from Parula contributed significantly to reduced leaf rust infections on the Avocet x Parula population in all test environments. This gene for partial resistance in adult plants is likely the same as the one detected at the same chromosomal location in the CIMMYT wheat Weebill 1 (Zhang et al. 2008b), and is closely linked to *Lr14b* on chromosome 7BL (Herrera et al. unpublished). The race specific seedling resistance gene *Lr14b* is practically ineffective everywhere, therefore the adult plant resistance of *LrP* is most likely located at a different locus on 7BL. *LrP* contributed additive resistance when combined with *Lr34* and/or *Lr46* in all leaf rust environments of the Avocet x Parula population, and represents a valuable source to diversify the genetic basis of partial and race non-specific resistance to leaf rust in wheat.

Environmental impact on the expression of partial resistance

Although both *Lr34* and *LrP* contributed resistance to leaf rust across a wide diversity of environments in the present study, the magnitudes of the resistance conferred by these two genes varied among environments. This was reported previously when the *Lr34* resistance in cv. Brambling was more effective in Mexico than in Minnesota (Zhang et al. 2008a), and *LrP* on the contrary contributed better resistance in Minnesota than in Mexico (Zhang et al. 2008b). Zhang et al. (2008b) attributed these differences to temperature and concluded that *LrP* in Weebill 1 conditions better resistance at high temperature whereas the opposite was shown for *Lr34*. This is somewhat contradictory to the experience in Mexico where *LrP* in Parula seems to work better in cooler seasons (R.P. Singh pers comm).

It is well documented that *Lr34* conditions higher resistance at low temperature (Singh and Huerta-Espino 2003; Singh D et al. 2007), and the presence of this race non-specific resistance gene can even be detected at the seedling stage when seedlings are exposed to low temperatures (Drijepondt and Pretorius 1989; Rubiales and Niks 1995). The high effectiveness of the *Lr34* resistance in Mexico reported in previous studies (Lillemo et al. 2008; Zhang et al. 2008a) was also confirmed in the two mapping populations used here. The study also confirmed a contrasting environmental response of the *LrP* gene, which was more effective in the Southern Cone than in Mexico. These contrasting effects can possibly be attributed to both differences in day length and temperature. Analysis of temperature and other climatic data from the test environments could perhaps shed more light on the relationship between environmental conditions and the expression of adult plant resistance in the two mapping populations.

Conclusions

This study confirmed the high levels of partial and race non-specific resistance to leaf rust, stripe rust and powdery mildew conferred by *Lr34* across two genetic backgrounds and a wide diversity of environments. The other LTN-associated partial resistance gene *Lr46* also contributed resistance to all three biotrophic diseases, but at a lower magnitude and resulted in only slightly improved resistance when combined with *Lr34*. The *LrP* gene from Parula showed effects in all leaf rust environments and contributed additively when combined with *Lr34* and/or *Lr46*. Keeping *Lr46* and *LrP* in gene combinations is recommended for reducing leaf rust severity.

Acknowledgements

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References

Burgueño J, Crossa J, Vargas M (2001) SAS programs for graphing GE and GGE biplots. Biometrics and Statistics Unit, CIMMYT, Mexico DF, Mexico. Available at http://apps.cimmyt.org/biometrics/biplots.exe

Drijepondt SC, Pretorius ZA (1989) Greenhouse evaluation of adult-plant resistance conferred by the gene *Lr34* to leaf rust of wheat. Plant Dis 73:669-671

Dyck PL, Samborski DJ, Anderson RG (1966) Inheritance of adult-plant leaf rust resistance derived from common wheat varieties Exchange and Frontana. Can J Genet Cytol 8:665-671

Gauch HG (1992) Statistical analysis of regional yield trials: AMMI analysis of factorial designs. Elsevier, Amsterdam

Herrera-Foessel SA, Singh RP, Huerta-Espino J, Lagudah ES (2009) Characterization and mapping of a gene component for durable leaf rust resistance in chromosome arm 7BL. Phytopathology 99:S53-S53

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360-1363

Lagudah E (2011) Molecular genetics of race non-specific rust resistance in wheat. Euphytica 179:81-91

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjørnstad Å (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor Appl Genet 116:1155-1166

Martinez F, Niks RE, Singh RP, Rubiales D (2001) Characterization of *Lr46*, a gene conferring partial resistance to wheat leaf rust. Hereditas 135:111-114

Rosewarne GM, Singh RP, Huerta-Espino J, William HM, Bouchet S, Cloutier S, McFadden H, Lagudah ES (2006) Leaf tip necrosis, molecular markers and β1-proteasome subunits associated with the slow rusting resistance genes *Lr46/Yr29*. Theor Appl Genet 112:500-508

Rubiales D, Niks RE (1995) Characterization of *Lr34*, a major gene conferring nonhypersensitive resistance to wheat leaf rust. Plant Dis 79:1208-1212

Singh D, Park RF, McIntosh RA (2007) Characterisation of wheat leaf rust resistance gene *Lr34* in Australian wheats using components of resistance and the linked molecular marker csLV34. Austr J Agric Res 58:1106-1114

Singh RP (1992a) Association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. Crop Sci 32:874-878

Singh RP (1992b) Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835-838

Singh RP, Huerta-Espino J (2003) Effect of leaf rust resistance gene *Lr34* on components of slow rusting at seven growth stages in wheat. Euphytica 129:371-376

Singh RP, Huerta-Espino J, Rajaram S (2000) Achieving near-immunity to leaf rust and stripe rust in wheat by combining slow rusting resistance genes. Acta Phytopathologica et Entomologica Hungarica 35:133-139

Singh RP, Mujeeb-Kazi A, Huerta-Espino J (1998) *Lr46*: A gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathology 88:890-894

Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES (2005) Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor Appl Genet 111:731-735

Utz HF, Melchinger AE (2003) PLABQTL: A computer program to map QTL, Version 1.2. Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany

William HM, Hoisington D, Singh RP, Gonzalez deLeon D (1997) Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. Genome 40:253-260

William HM, Singh RP, Huerta-Espino J, Rosewarne G (2007) Characterization of genes for durable resistance to leaf rust and yellow rust in CIMMYT spring wheats. In: Buck HT, Nisi JE, Salomon N (eds) Developments in Plant Breeding Vol 12 Wheat production in stressed environments, pp 65-70, Springer, Dordrecht, The Netherlands

William M, Singh RP, Huerta-Espino J, Islas SO, Hoisington D (2003) Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93:153-159

Zhang JX, Singh RP, Kohner JA, Huerta-Espino J, Jin Y, Anderson JA (2008a) Genetics of leaf rust resistance in Brambling wheat. Plant Dis 92:1111-1118

Zhang JX, Singh RP, Kolmer JA, Huerta-Espino J, Jin Y, Anderson JA (2008b) Inheritance of leaf rust resistance in the CIMMYT wheat Weebill 1. Crop Sci 48:1037-1047

Canadian initiatives in breeding for stem rust resistance to race Ug99 and variants

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Abstract

With the first Kenya germplasm screening report for resistance to the Ug99 (race TTKSK) in 2005, Canadian wheat breeding programs were quick to initiate crosses with the Canadian cultivars Peace and AC Cadillac. With a 2008 initiative by Agriculture and Agri-Food Canada (AAFC) to actively integrate resistance to Ug99, targeted funding has allowed wheat breeding programs across Canada to aggressively pursue this goal. Access to the Kenya stem rust nurseries has been important in identifying existing resistant cultivars and breeding populations where resistance was already segregating. A level 3, bio-containment facility is currently under construction to safely use Ug99 in Canada to determine the response of genetic materials to TTKSK and its variants. Resistance already existing within breeding programs has been emphasized in developing new breeding populations; germplasm from other programs has been utilized and novel genetics are being introduced. In addition, marker development through collaboration with geneticists has yielded new tools to assist in managing gene complexes. Efforts to understand the current basis of stem rust resistance in Canadian wheat is underway to ensure a diverse array of stem rust genes is developed to control Ug99 and future variants.

Introduction

The initial opportunity to test Canadian wheat varieties for resistance to Ug99 in 2005 demonstrated the general susceptibility of Canadian wheat varieties with the important exceptions of AC Cadillac (DePauw et al. 1998) and Peace spring wheat and Napoleon (Humphreys et al. 2010) durum wheat (Table 1). An assessment of the situation by DePauw et al. (2009) revealed that:

- The area seeded to these varieties was very small (<2% area for each)
- New varieties representing recent gains in productivity traits and disease and insect resistance were susceptible to Ug99
- Existing Ug99 resistance lacked diversity of origin
- Many other sources of resistance were in backgrounds with low productivity
- Arrival of Ug99, or a race with similar capabilities, into North America was considered a likely eventuality

The Canadian wheat research community was very responsive to the importance of these concerns. Agriculture and Agri-Food Canada (AAFC) supported a 5-year, $13 million project which started in 2008 and included significant funding directed to wheat breeding. A summary of progress in breeding resistant cultivars for Canada is reported.

Resistant cultivars

Of the nine currently registered Canadian wheat varieties that are resistant or intermediately resistant to Ug99, six are hard red spring types and three are durum (Tables 1 and 2). The cultivar AC Splendor, which lacks *Lr34*, and its parent Roblin, which has *Lr34*, likely share other factors that confer resistance to Ug99. The other four spring wheat varieties all have BW553 = Seln 70-3524/8*Neepawa in their parentage. BW553 was an elite line developed to introduce *Bt10* for resistance to common bunt (caused by *Tilletia tritici* (Bjerk.) R. Wolff and *T. laevis* Kuhn in Rabenh.) into the CWRS background. Hiebert et al. (2011) recently reported the presence of *SrCad* which is tightly linked to *Bt10* and confers low stem rust severity when combined with *Lr34*; both of these genes are in AC Cadillac and Peace. Although, *SrCad* is present in AC Taber, AC Karma and AC Crystal (Hiebert et al. 2011), their Kenya 2005 field ratings were 40 MR, 40 MS and 80 S, respectively. These varieties do not have *Lr34*.

Kenya nursery screening results

The stem rust nursery operated by Kenya Agricultural Research Institute in Njoro has enabled breeding progress in developing new wheat cultivars with resistance to Ug99 that would otherwise not be possible. The participation by Canadian wheat scientists in this nursery is summarized in Table 3. Increased funding has allowed the nursery entry numbers to rise significantly. Infection levels at this nursery varied from year to year as is indicated by the large changes in percentage of entries that were scored as 10 MR or less. Thus repeated testing over years is required to confirm preliminary observations.

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Table 1 ***Canadian wheat cultivars and unregistered advanced lines that are resistant or intermediately resistant to Ug99 and its variants. Genotypes in bold have*** *SrCad* ***which is closely linked with*** *Bt10*

Cultivar	Parentage[1]	Year of registration	Class[2]
Roblin	BW15/BW38//BW40/RL4353	1986	CWRS
AC Splendor	Laura/RL4596//Roblin/BW107	1996	CWRS
AC Cadillac	Pacific*3/**BW553**	1996	CWRS
5700PR	N91-3051/AC Foremost = HY320*5/**BW553**// HY320*6/7424-BW5B4	2000	CPSR
Peace	BW165= Pacific*3/**BW553**/RL4660	2002	CWRS
HY694	97M27/AC Vista= HY344/Losprout 'S'//HY358*3/**BW553**	2011	CPSR
AC Melita	Medora/Lloyd	1994	CWAD
AC Napoleon	Vic/DT384//DT471	1999	CWAD
Commander	W9260-BK03/AC Navigator//AC Pathfinder	2004	CWAD
BW711	Pacific*3/**BW553**	Declined	CWRS
BW796	**AC_Cadillac**/8405-JC3C//AC_Elsa	Supported	CWRS
PT571	BW191= Laura/**RL4596**//Roblin/BW107/W93105	Declined	CWRS
HW341	BW275/Sunmist//Snowbird	Declined	CWHW
GP069	HY459/Alsen//Snowhite475= HY398/AC Karma//AC Vista	Declined	CWGP

[1] BW553 and RL4596 *Bt10* derived from the same source: i.e. Seln 70-3524

[2] CWRS, Canada Western Red Spring, CWAD; Canada Western Amber Durum; CPSR, Canada Prairie Spring Red; CWHW, Canada Western Hard White; CWGP, Canada Western General Purpose

Current status of breeding for resistance

The specific end-use quality market classes that characterize the majority of the Canadian wheat marketing system has demanded multiple introductions of Ug99 stem rust resistance into each wheat class. This challenge must be met without sacrificing recent breeding improvements in yield, insect resistance to wheat midge (*Sitodiplosis mosellana* Géhin) and sawfly (*Cephus cinctus* Norton), and resistance to leaf rust (*Puccinia triticina* Eriks.) and Fusarium head blight (*Fusarium graminearum* Schwabe). Breeding efforts to incorporate resistance to Ug99 may be separated into three categories: use of existing resistance, use of resistance from advanced lines from other breeding programs, and use of new germplasm. These divisions are demonstrated by examples of populations currently under development (Table 4).

All Canadian hexaploid breeding programs have utilized the AC Cadillac and Peace resistance (*Lr34* + *SrCad*) with strategic crosses being made since 2006. SNP and SSR markers exist for *Lr34* + *SrCad*, respectively, which offer reliable and rapid indirect selection for this gene combination. The expression of *Bt10* which confers resistance to common bunt is also being used to indirectly select for *SrCad* as recombination between these two genes is very low. Roblin has *Lr34*, but lacks *SrCad* whereas AC Splendor lacks both genes; however, their Kenya field reactions were moderately resistant. Roblin and AC Splendor have susceptible seedling reactions to TTKSK; whereas AC Cadillac and Peace are resistant (Fetch unpublished). Cultivars such as CDC Teal and AC Elsa carry *Lr34* but have susceptible seedling and moderately susceptible field reactions to Ug99. This suggests additional resistance factors in Roblin and AC Splendor conferring Ug99 resistance that differs from AC Cadillac and Peace. These factors are unknown, but crosses between AC Splendor and Peace and AC Cadillac were made to determine what the other factors may be.

In 2009, Fetch identified an F_3 selection from the cross RL6071/Webster which contained both *Sr30* and a gene (*SrWeb*) effective against Ug99. Although *Sr30* is not effective against Ug99, it has not been widely used in Canadian breeding programs and will serve to broaden the genetic basis for stem rust resistance.

The hard white breeding line HW341 (BW275/Sunmist//Snowbird) has been used in crosses since 2009 for the CWRS market class and much earlier for the CWHW market class. Sunmist, an Australian cultivar, contains the stem rust genes *Sr12*, *Sr13*, and *Sr30* (Park 2007). *Sr13* is somewhat effective against Ug99 and is also relatively unused in Canadian bread wheat. However, the stem rust reaction of HW341 is much lower than would be expected from *Sr13* alone, suggesting additional resistance genes are present.

Table 2 *Summary of stem rust reactions on selected Canadian cultivars from Kenya Ug99 nurseries, 2005-2010*

	Year of evaluation, severity and reaction[1]					
Cultivar	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Peace	5R	5R	1R	1R	1MR	0
AC Cadillac	5R	5RMR	1R	1R	1MR	0
Pasqua	20MS			5I		
AC Cora	15MS	15MS		20I		
AC Splendor	10MR	30MS	1R	5RMR		
AC Intrepid	60MS	50S	5R	15MS		
CDC Bounty		10MS	5R	10S		
CDC Osler		5MS	1R	2S		
PT571			1R			0
Roblin			5R	5I		
Neepawa		20I	5R	15S		
BW553		20I	1R	5MR		
AC Morse	10MR	20I	1RMR	10MR	1R	
AC Melita	5MR	15I	5R	1R	1R	
Napoleon	10MS	10RMR	1R	1R	1R	
Plenty	10MR			30MR	5R	
Kyle		40I	1R	20MR	5I	
Commander			1R	10R	1R	
AC Avonlea	40S	50I	30I	40I	5MR	
Nursery infection level	med	med	low	high	med	med

[1] R, resistant; MR, moderately resistant; RMR, resistant to moderately resistant; I, moderately resistant to moderately susceptible; MS, moderately susceptible; S, susceptible

Durum breeding efforts have utilized resistance from AC Napoleon and Commander as well as from introductions to diversify resistance. The resistance genes in the introduced lines are not characterized. Advanced generation lines are now being tested and include resistance to TTKST along with improved performance traits relative to the currently registered cultivars that are resistant to TTKST. The Canadian durum breeding programs have genotypes in pre-registration testing that have shown good resistance.

Identified resistance and strategies

Recently, breeding lines produced between 1983 and 1996 with either *Sr22* or *Sr26* were tested in the 2010-11 off-season Kenya nursery and evaluated with genetic markers associated with these genes. Several breeding lines putatively containing *Sr22* and *Sr26* had good field resistance to TTKST (Table 5). For the *Sr22* lines, there was complete agreement between the resistant field reactions and three molecular markers (*cfa2123, cssu22, wmc633*) (Periyannan et al. 2011) of which *cssu22* was found to work very well and was diagnostic. However, for the *Sr26* lines, the marker *Sr26#43/BE518379* was not diagnostic in this germplasm. These resistant elite lines will be used in future crosses to diversify the basis for resistance.

Table 3 *Summary of Canadian spring hexaploid and durum wheat breeding lines with resistant or moderately resistant reactions to stem rust in the Kenya Ug99 stem rust nurseries, 2005-2010*

Year	No of lines	No 10 MR or less ratings			
		Hexaploid	Durum	Total	%
2005	76	9	-	9	12
2006	185	26	5	31	17
2007	298	73	49	122	41
2008	579	27	16	42	7
2009	1927	364	205	569	30
2010	1917	290	123	413	22

Table 4 *Sources of resistance to Ug99 and variants currently used in Canadian wheat breeding programs and the generation of the experimental lines resulting from hybridization*

Market class	Source of resistance	Gene postulation and/or DNA markers	Generations
CW[1] Amber Durum	Commander	*Sr9e*, *Sr13*, and *Sr14* and other unknown genes	F_2 to F_8
	Napoleon	*Sr9e*, *Sr13*, and *Sr14* and other unknown genes	F_2 to F_8
CW Red Spring	AC Cadillac	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_{10}, DH
	Peace	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	DH3, F_1 to F_{10}
	B0371AC41&AD007	*Sr2, Sr24*, *Lr34*, Syn Hex, others	F_1 to F_4
	B0071D&01AC08	*Sr2, Lr34*, others, Syn Hex?	F_1 to F_4, DH
	HW341	*Sr2, Sr12*, *Sr13*, *Sr30*, *Lr34* in parents	F_1 to F_4
	Webster selection	*Sr30, SrWeb*	F_1-F_2
CW Hard White	AC Cadillac	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_2
	SrCad/Bt10 Parental Lines	*SrCad* linked to *Bt10*, *Lr34*,	F_1 to F_{10}, DH
	HW341	*Sr2, Sr12*, *Sr13*, *Sr30*, *Lr34* in parents	F_1 to F_{10}, DH
	Webster selection	*Sr30, SrWeb*	F_1-F_4
	Peace	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	DH3, F_1 to F_8
CW Soft White Spring	Peace	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_4, DH
	Lang	*Sr24*, *Sr36*	F_1 to F_3
	CID394092	*SrSha7*	F_1
	CID428593	*Sr-synth*	F_1
	Pavon 76 S	*Sr2, Sr9*, *Sr30*	F_4 to F_{26}
	Others	*Sr26, Srweb, Sr27, Sr32, Sr39, Sr40*	F_2 to F_3
Canada Prairie Red Spring	AC Cadillac	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_5
	Peace	*SrCad* linked to *Bt10*, *Lr34*, *Sr2*	F_1 to F_5
	HY694	*SrCad* linked to *Bt10*	F_1 to F_5
	Webster selection	*Sr30, SrWeb*	F_1-F_4, DH
	HY697	*SrCad* linked to *Bt10*, *Lr34*	
CW Red Winter	Peace	*SrCad* linked to *Bt10*, *Lr34*	F_1 to F_3, DH
	Others	*Sr2, Sr22*, *Sr24*, *Sr29*, *Sr33*, *Sr35*, *Sr36*, *SrTmp*	F_1 to F_8, DH

[1]Canada Western

Table 5 ***Kenya Ug99 off-season nursery stem rust reactions on breeding lines likely having*** *Sr22* ***or*** *Sr26****. Lines in "bold type" were those lines that were stem rust resistant***

		Stem rust reaction at date of observation			cssu22 allele
Variety/ Line	**Parentage**	**2/4/11**	**7/4/11**	**13/4/11**	**bp**
RL 4314 Benito	Neepawa/3/CT433*4//Manitou/CI7090	5M	5M	20MS	355
RL 4352.1 Columbus	Neepawa*6/RL4137	TM	5MS	5MS	355
RL 4376 Katepwa	Neepawa*6/CT244/3/Neepawa*6//CI8154/2*Frocor	1M	5M	15M	355
Putative *Sr22* lines[1]					
RL 4505	Benito*6/RL5432	TR	1M	5MS	**237**
RL 4552	Benito*6/Sr22//Benito*6/Lr21	TR	TR	10RMR	**237**
RL 4615	Columbus*6/3/Napayo*4//Thatcher*6/RL5289/4/Columbus*6//Benito*5/RL5432	TR	TR	15RMR	**237**
RL 4672	Columbus*6/PI58548//Columbus*6/Sr22	5MR	10MR	20RMR	**237**
RL 4759	SD3005/BW135	10MR	10MR	30RMR	**237**
RL 4575	Columbus*6/3/Napayo*4//Thatcher*6/PI58548/4/Columbus*6//Benito*5/RL5432	20MS	20MS	40M	355
RL 4579	Columbus*6//Benito*5/RL5432	15MS	20MSS	30MSS	355
RL 4749	Roblin/BW135	15MSS	15M	20MSS	355
RL 4782	Sharp/BW90I//RL4596/BW135	20M	20MSS	50MSS	355
RL 4783	Sharp/BW90I//RL4596/BW135	30MSS	40MSS	60MSS	355
Putative *Sr26* lines					
RL 4554	Columbus*6/3/Napayo*4//Thatcher*6/PI58548/4/Columbus*6//Thatcher*6/Eagle	5MR	5MR	10RMR	355
RL 4700	Roblin/BW108	TR	TR	10RMR	355
RL 4711	BW83/ND585//BW108	5MR	5MR	10RMR	355
RL 4576	Columbus*6/3/Napayo*4//Thatcher*6/RL5289/4/Columbus*6/Thatcher*6/Eagle	15MS	20MS	40M	355
RL 4706	Roblin/ND604//BW108	30MS	30MS	30MSS	355
RL 4707	Roblin/ND604//BW108	20MS	20MS	20MSS	355
RL 4748	BW108/Roblin*2//Erik	30MSS	30MSS	50MSS	355
Susceptible control		40-50S	70S	100S	
Sr26	Eagle	TR	10MR	10RMR	

[1]Typical *Sr22* reaction to Ug99 is 10-20 MR (Fetch, personal communication). A line thought to be RL5244 and used as a control was not correct

R.E. Knox has been investigating the role of *Sr2* in Ug99 resistance and has started evaluating parents with markers for *Sr2* and is attempting to validate the markers in three breeding populations. The populations possess AC Cadillac as a parent and lines with the AC Cadillac molecular variant for the *X3B028F08* marker will be evaluated for improved resistance to Ug99.

Association mapping was performed on a global set of 94 durum wheat cultivars and breeding lines using both field (Njoro, Kenya; 2007, 2008, 2009) and seedling response data. Two microsatellite markers were identified that were significantly associated with seedling resistance and field severity and infection response in all three years of field testing. *Xgwm617-6A* was significant and located near *Sr13*. *Sr13* is effective against race TTKSK (Simons et al. 2011) and is known to be derived from the cultivated emmer variety Khapli. Of the 94 durum cultivars and breeding lines used in the association mapping panel, 32 were postulated to carry *Sr13* and a high frequency of these were Canadian varieties and breeding lines. Based on molecular analysis of breeding material from the Crop Development Centre (University of Saskatchewan) durum program, we postulate that over 80% are carriers of *Sr13*. *Xcfd48* was also highly significant, and has previously been mapped to the long arm of chromosome 1B. *Sr14*, also derived from Khapli, provides an intermediate resistance to TTKS and has been localized to 1B. However, we are not certain if *Xcfd48* is detecting variation at *Sr14*. Based on molecular haplotyping, only a few Canadian varieties are postulated to carry *Sr14*. Two genomic regions on group 5 chromosomes were identified that were strongly associated with field resistance over all three years of field testing, but not associated with seedling resistance, suggesting they may be novel adult plant resistance factors. We are currently performing marker assisted selection for all of these regions in relevant germplasm pools.

Conclusion

An increasingly diverse pool of TTKSK resistant germplasm coupled with effective nursery screening, rapid accumulation of genetic information, and discovery of genetic markers are the tools being applied to develop resistance to Ug99. The effort to develop resistance to race Ug99 should result in resistant cultivars with competitive agronomic performance before this race group arrives in North America. This breeding effort should also provide a better understanding of the genetics of stem rust resistance, and identify additional resistance genes, further diversifying the genetics of stem rust resistance in Canadian wheat cultivars.

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References

DePauw RM, Fetch T, Hiebert CW, Humphrey, DG, Knox RE, Pozniak CJ, Thomas JB, Singh AK, Graf R, Randhawa HS, Fox SL, Brown PD, Clarke FR, Clarke JM (2009) Sources of resistance to stem rust race Ug99 and its variants in Canadian wheat germplasm. In: R.A. McIntosh (ed) Borlaug Global Rust Initiative (BGRI) Technical Workshop, CIMMYT, Ciudad Obregon, Mexico, 17-20 March, 2009, pp171-178

DePauw RM, Thomas JB, Knox RE, Clarke JM, Fernandez MR, McCaig TN, McLeod JG (1998) AC Cadillac hard red spring wheat. Can J Plant Sci 78:459-462

Hiebert CW, Fetch TG, Zegeye, T (2010) Genetics and mapping of stem rust resistance to Ug99 in the wheat cultivar Webster. Theor Appl Genet 121:65-69

Hiebert CW, Fetch TG, Zegeye T, Thomas JB, Somers DJ, Humphreys DG, McCallum BD, Cloutier S, Singh D, Knott DR (2011) Genetics and mapping of seedling resistance to Ug99 stem rust in Canadian wheat cultivars 'Peace' and 'AC Cadillac'. Theor Appl Genet 122:143-149

Humphreys DG, Townley-Smith TF, Leisle D, McCallum B, Gaudet D, Gilbert J, Menzies J (2010) Napoleon Amber durum wheat. Can J Plant Sci 90:863-867

Park RF (2007) Stem rust of wheat in Australia. Aust J Agric Res 58:558-566

Periyannan S, Bansal U, Bariana HS, Pumphrey M, Lagudah ES (2011) A robust molecular marker for the detection of shortened introgressed segment carrying the stem rust resistance gene *Sr22* in common wheat. Theor Appl Genet 122:1-7

Simons K, Abate Z, Chao S, Zhang W, Rouse M, Jin Y, Elias E, Dubcovsky J (2011) Genetic mapping of stem rust resistance gene *Sr13* in tetraploid wheat *Triticum turgidum* ssp. *durum*.Theor Appl Genet 122:649-658

Highly expressed RPG1 protein in a five-copy *Rpg1*-transgenic barley line results in susceptibility to stem rust

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Abstract

The stem rust resistance gene *Rpg1* has provided durable resistance in barley for over 60 years in the Upper Midwest region of the United States. In previous research, a susceptible barley cultivar Golden Promise was transformed into a resistant cultivar with a single copy of *Rpg1*. Other transgenic lines with different copy numbers exhibited different infection phenotypes. Thus, to investigate the relationship between *Rpg1* transgene copy number and level of stem rust resistance, we selected five transgenic lines containing different copy numbers of *Rpg1* and tested them for stability of transgene inheritance, level of *Rpg1* transcript and protein expression, and also degradation of RPG1 protein upon stem rust infection. Southern blot transgene copy number estimation revealed unstable transgene inheritance over several generations in a five copy T_0 line. All transgenic lines exhibited higher transcription and protein levels than cultivar Morex (the cultivar from which *Rpg1* was cloned), but the relationship between these factors and copy number was not linear. Western blot assays of RPG1 protein degradation after challenge by avirulent stem rust pathotype MCCF revealed rapid degradation between 20-28 hours post-inoculation in all transgenic lines, except the susceptible five-copy line G04-288. The failure of RPG1 protein to degrade rapidly in line G04-288 resulted in susceptibility to stem rust.

Key words

Disease resistance; *Puccinia graminis*; transgenic

Abbreviations

cv. - cultivar: *Pgt - Puccinia graminis* f. sp. *tritici*;; IT - infection type; IR - infection response

Introduction

Stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*), has been a major biotic constraint to barley production in the United States and caused a number of major epidemics prior to 1940 (Roelfs 1978). Since 1942, losses caused by stem rust have been minimal due to the release of barley cultivars with the stem rust resistance gene *Rpg1* (Roelfs 1978). *Rpg1* was the first stem rust resistance gene identified in barley and remains the principal source of stem rust resistance in nearly every malting barley cultivar in the northern Great Plains region of North America. *Rpg1* encodes a receptor kinase-like protein with two tandem protein kinase domains (Brueggeman et al. 2002). Rostoks et al. (2004) found that *Rpg1* is transcribed at relatively uniform and low levels in almost all organs of barley and at all developmental stages, but in leaf epidermis the *Rpg1* transcript level was up to 30 times higher than in whole leaves. Nirmala et al. (2006) found the protein was present mainly in the cytosol, but also on the plasma membrane and intracellular membranes. Upon infection with avirulent stem rust pathotypes, the RPG1 protein disappeared to an undetectable level in barley seedlings. RPG1 degradation is correlated with, but is not alone sufficient to confer disease resistance (Nirmala et al. 2007). A recent study revealed that the phosphorylation of RPG1 protein occurred within five minutes after inoculation with urediniospores of avirulent stem rust pathotypes, suggesting that it may have a role in the very early response against stem rust infection (Nirmala et al. 2010).

Horvath et al. (2003) developed transgenic stem rust resistant barley lines by transferring *Rpg1* from barley cultivar (cv.) Morex into the susceptible cv. Golden Promise by *Agrobacterium*-mediated transformation. Initial transformation yielded 42 primary transgenic (T_0) plants, and 21 T_0 plants gave T_1 progeny with highly resistant or resistant reactions (Horvath et al. 2003). Transgenic line H228.2c, containing a single copy *Rpg1* insertion, showed a 3:1 segregation ratio for resistance : susceptibility among the T_1 progeny, indicating the sufficiency of one *Rpg1* copy for resistance. Interestingly, transgenic line H228.19, with five copies of *Rpg1*, showed a wide range of different infection types among the T_1 progeny (Horvath et al. 2003). T_1 transgenic lines were increased over several successive generations (to T_2, T_3, T_4 and T_5) and evaluated for adult plant resistance in the field (B. Steffenson unpublished). Transgenic lines with different *Rpg1* copy numbers continued to exhibit different stem rust phenotypes at the adult plant stage. One particularly intriguing result was that one transgene copy of *Rpg1* was sufficient to confer a high level of stem rust resistance, whereas five transgene copies resulted in susceptibility at both the seedling and adult plant stages (Horvath et al. 2003; B. Steffenson unpublished).

To investigate the relationship between *Rpg1* transgene copy number and stem rust resistance level, we selected five transgenic lines containing different copy numbers of *Rpg1* and tested them for stability of transgene

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inheritance, level of *Rpg1* mRNA transcript, level of RPG1 protein expression, and degradation of RPG1 protein upon stem rust infection.

Results and discussion

Transgenic lines selection and their stem rust phenotypes

For this study, a total of five T_5 transgenic lines (G04-271, G04-273, G04-288, G04-266, and G03-210) were selected based on their reported transgene copy number and their disease phenotypes against stem rust pathotype *Pgt*-MCCF (Table 1). Lines G04-271, G04-273 and G04-288 shared the same 5-copy T_0 ancestor (H228.19), whereas line G04-266 was derived from a 2-copy T_0 line (H228.5) and line G03-210 from a 1-copy T_0 ancestor (H228.2c) (Horvath et al. 2003). Barley cvs. Morex (CIho15773) with *Rpg1* and Golden Promise (PI343079) with no known resistance gene were included as the controls.

In seedling stage phenotyping tests, transgenic lines G04-271, G04-266, and G03-210 exhibited very low ITs (00;) with no sporulating uredinia. Line G04-273 exhibited slightly higher ITs (i.e. sporulating "1" type uredinia) than G04-271, G04-266 and G03-210. Of note, these lines exhibited phenotypes that were more resistant than Morex, the cv. from which *Rpg1* was cloned. Interestingly, line G04-288 exhibited high ITs (3 with range of 1 to 3) to stem rust even though it presumably contained five copies of *Rpg1*.

Adult stage phenotyping tests were conducted from year 2004 to 2006 in the field. Transgenic lines G04-271, G04-266, and G03-210 exhibited severity scores of 0% and highly resistant infection responses (IRs) as no sporulating uredinia were observed. Line G04-273 exhibited a slightly higher rust severity (3% to 4%) and more compatible IRs (MS) in 2004 and 2006. Line G04-288 was susceptible to moderately susceptible, exhibiting stem rust severities ranging from 30 to 35%.

Transgene copy numbers as assessed by Southern blot analysis

Genomic DNA from individual plants of each line were extracted and analyzed using the *Rpg1*-specific probe. *Rpg1* copy numbers were estimated by Southern blot analysis (Fig. 1). Three lines (G04-288, G04-266 and G03-120) retained the same copy numbers (five, two, and one, respectively) as their T_0 ancestors. The other two lines (G04-271 and G04-273) showed only one copy of *Rpg1*, although they came from the same five-copy T_0 plant. This result demonstrated the instability of *Rpg1* transmission across generations in this lineage. No strong correlation was found between *Rpg1* copy number and stem rust phenotype (Fig. 1 and Table 1).

Table 1 ***Reaction of Golden Promise*** *Rpg1*-***transformed lines and controls to wheat stem rust pathotype*** *Pgt*-***MCCF at the seedling stage and adult plant stage***

Line or control	Adult stage			Seedling stage	
	Stem rust severity (0-100% scale)[1] and infection response (IR)[2]			Infection types (ITs) (0-4 scale) [3]	
				2007/2008/2009 (T_5) [4]	
	2003 (T_2) [4]	2004 (T_4) [4]	2006 (T_4) [4]	Most Common	Range
G04-271	0 HR	0 HR	0 HR	00;	0 to 00;
G04-273	0 HR	3 MS-MR	4 MS-S	0;1	00; to 20;
G04-288	30 MS-S	30 S-MS	35 S	3	1 to 3
G04-266	0 HR	0 HR	0 HR	00;	0 to 00;
G03-210	0 HR	--[5]	--[5]	00;	00; to 0;
Morex	0 HR	0 HR	0 HR	12	0;1 to 3-
Golden Promise	25 MS-S	20 S-MS	20 MS-S	3	2 to 33+

[1] Visual percentage of stem and leaf sheath tissue covered by uredinia

[2] Based on the size and type of uredinia following the description of Roelfs et al. (1992), where R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible. An additional category of highly resistant (HR) was added for "fleck" reactions, i.e. obvious stem rust infection sites with no sporulation

[3] Based on the size and type of uredinia observed according to the 0-4 scale (Stakman et al. 1962) and modified by Miller and Lambert (1955) for barley. For each accession, the one or two most common ITs observed are given in addition to the range of ITs observed (lowest and highest types)

[4] Year experiment was conducted with transgenic generation in parentheses

[5] Not tested

Rpg1 transcript levels

Quantitative real-time PCR was used to measure *Rpg1* mRNA levels in the transgenic lines. Mean values of three samples from each line were used to estimate *Rpg1* transcript levels (shown in Fig. 2) in comparison with Golden Promise (0%) and Morex (100%). All transgenic lines had higher levels of *Rpg1* transcript than Morex. The association between *Rpg1* transgene copy number and transcript level was not particularly strong in this small sample of transgenic lines. Yet given that the five-copy line G04-288 had a much higher *Rpg1* transcript level than the other lines, it appears that higher copy numbers generally have a positive effect on transcript level. Variation in transcript level was not strongly correlated with stem rust resistance (Table 1 and Fig. 2). This was especially true for line G04-288, which exhibited the highest *Rpg1* mRNA transcript level, but showed moderately susceptible to susceptible reactions to stem rust.

RPG1 protein levels

Using quantitative ELISA, RPG1 protein expression levels were assayed in 10-day old seedlings of the transgenic lines (Fig. 3). ELISA revealed that the RPG1 protein was expressed in all transgenic lines. The five-copy transgenic line G04-288 exhibited RPG1 protein levels nearly 3x higher than cv. Morex and about 2x higher than other transgenic lines. Compared to the quantitative real-time PCR result, line G04-288 exhibited both the highest transcript and protein level; however, the protein levels assayed by ELISA were not proportional with the transcript levels. Also, differences in RPG1 protein levels were not strongly correlated with stem rust resistance (Table 1 and Fig. 3). This was especially true for line G04-288, which had the highest RPG1 protein level, but was susceptible to stem rust.

Fig. 1 ***Southern blot analysis of*** *Rpg1* ***copy number in Golden Promise*** *Rpg1*-***transformed lines. Control cv. Morex showed one band and cv. Golden Promise showed no band. The estimated*** *Rpg1* ***copy numbers for transgenic lines are given at the bottom of each lane***

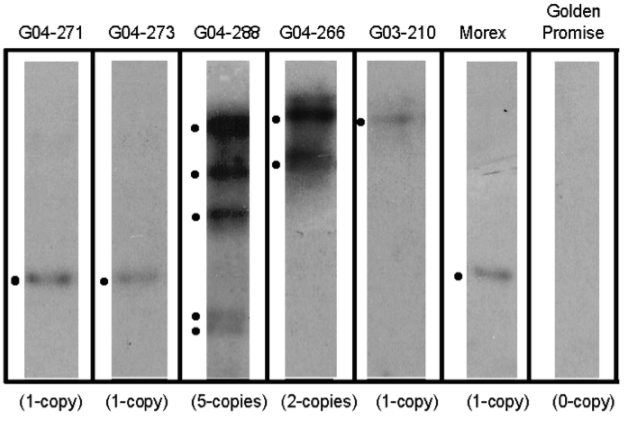


Fig. 2 *Rpg1* ***transcript levels in Golden Promise*** *Rpg1*-***transformed lines containing different copy numbers.*** *Rpg1* ***mRNA levels were normalized against GAPDH as a reference gene. The*** *Rpg1* ***mRNA level in Morex was considered as 100% and all other transgenic lines were compared with Morex mRNA level. Numbers given above each column are mean*** *Rpg1* ***mRNA values (as a percentage of Morex) from three individual plants per line. Error bars represent standard deviations***

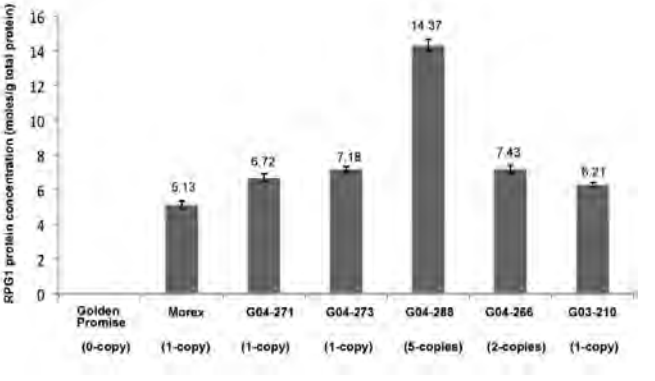
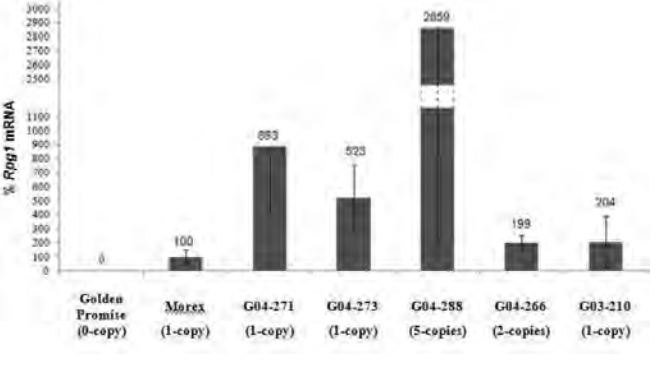


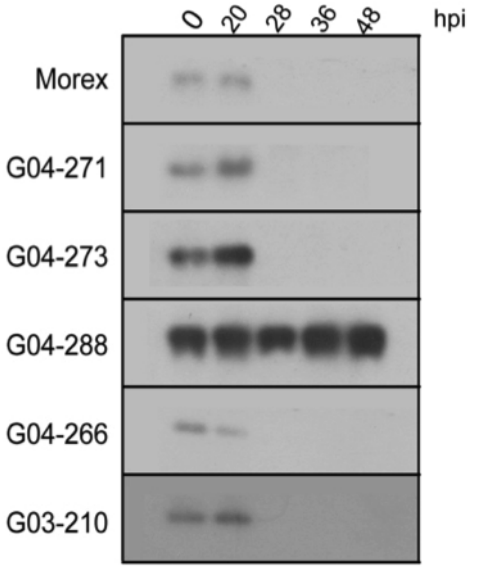
Fig. 3 ***Results of ELISA for RPG1 protein expression in 10-day old Golden Promise*** *Rpg1*-***transformed lines. RPG1 protein concentration was measured as moles per gram of total protein. For each barley line, three plants were sampled individually and the mean RPG1 protein levels are given above the bars. Error bars represent standard deviations***



Degradation of RPG1 protein

Western blot analysis was used to assay the level of RPG1 protein up to 48 hr post-inoculation (Fig. 4). Western blots showed that in all the resistant transgenic lines and Morex control, RPG1 protein was degraded to undetectable levels between 20 and 28 hr post-inoculation. However, in the moderately susceptible to susceptible line G04-288, RPG1 protein remained at high levels, even 48 hr after inoculation. Thus, the susceptibility of line G04-288 appears to be due to the failure of RPG1 to degrade.

Fig. 4 ***Western blot assays for RPG1 protein after infection by wheat stem rust pathotype*** *Pgt*-***MCCF in Golden Promise*** *Rpg1*-***transformed lines. The first leaves were inoculated and for each time point the inoculated tissue from three plants was pooled for protein samples. Time point 0 was sampled immediately prior to inoculation. Other time points were 20 hr, 28 hr, 36 hr, and 48 hr post-inoculation (hpi)***



Conclusion

Five transgenic barley lines with different numbers of copies of *Rpg1* were investigated for stem rust resistance, stability of transgene inheritance over several generations, level of *Rpg1* mRNA transcript, level of RPG1 protein expression, and degradation of RPG1 protein upon stem rust infection. Southern blot analysis for transgene copy number estimation revealed the instability of transgene inheritance over several generations in some lines (i.e. lines G04-271 and G04-273 were reduced from 5 copies at T_0 to only 1 copy at T_5), but stable inheritance in others (i.e. lines G03-210, G04-266 and G04-288 retained 1 copy, 2 copies and 5 copies, respectively, from T_0 to T_5). Moreover, *Rpg1* transgene copy numbers were not strongly correlated with transcription and protein levels. Upon stem rust infection, all transgenic lines exhibited RPG1 protein degradation between 20-28 hr post-inoculation. One possible mechanism proposed by Nirmala et al. (2007) is that, upon infection by the stem rust pathogen, the RPG1 protein recognizes the pathogen elicitors either directly or indirectly and then RPG1 is degraded to an undetectable level. For susceptible line G04-288, RPG1 protein levels remained high even at 48 hr post inoculation, suggesting that the failure of RPG1 protein to degrade rapidly resulted in susceptibility to stem rust. For *Rpg1*-transformation in barley, our study demonstrated that introducing one copy can achieve a high level of stem rust resistance both at seedling and adult plant stages, while over-expressing *Rpg1* in high copy number transgenic lines can have a negative effect on resistance. Studying the molecular mechanisms during host-pathogen interactions is of great importance for successfully developing disease resistant transformants.

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References

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci U SA 99:9328-9333

Horvath H, Rostoks N, Brueggeman R, Steffenson B, von Wettstein D, Kleinhofs A (2003) Genetically engineered stem rust resistance in barley using the *Rpg1* gene. Proc Natl Acad Sci USA 100:364-369

Miller JD, Lambert JW (1955) Variability and inheritance of reaction of barley to race 15B of stem rust. Agron J 47:373-377

Nirmala J, Brueggeman R, Maier C, Clay C, Rostoks N, Kannangara CG, von Wettstein D, Steffenson BJ Kleinhofs A (2006) Subcellular localization and functions of the barley stem rust resistance receptor-like serine/threonine-specific protein kinase Rpg1. Proc Natl Acad Sci USA 103:7518-7523

Nirmala J, Dahl S, Steffenson BJ, Kannangara CG, von Wettstein D, Chen X, Kleinhofs A (2007) Proteolysis of the barley receptor-like protein kinase RPG1 by a proteasome pathway is correlated with *Rpg1*-mediated stem rust resistance. Proc Natl Acad Sci USA 104:10276-10281

Nirmala J, Drader T, Chen X, Steffenson B, Kleinhofs A (2010) Stem rust spores elicit rapid RPG1 phosphorylation. Mol Plant Microbe Interact 23:1635-1642

Roelfs AP (1978) Estimated losses caused by rust in small grain cereals in the United States - 1918-76, USDA Misc Publ no 1363

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: Concepts and methods of disease management. CIMMYT, Mexico

Rostoks N, Steffenson BJ, Kleinhofs A (2004) Structure and expression of the barley stem rust resistance gene *Rpg1* messenger RNA. Physiol Mol Plant Pathol 64:91-101

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiological races of *Puccinia graminis* f. sp. *tritici*.. US Dept Agric ASR E6 17 53pp

Fungicide strategies for managing the Ug99 stem rust threat - Australian and international perspectives

R.W. Rainbow

Abstract

Activities in readiness for exotic variants of *Puccinia graminis* f. sp. *tritici* (*Pgt*), race Ug99 being undertaken in Australia include significant work in surveillance and pre-breeding for cultivar resistance. Should Ug99 become established in Australia, management could potentially include the use of chemical (fungicide) applications. It is however unlikely that sufficient chemical stockpiles will be available in the event of a significant national epidemic. The challenges of looming pesticide regulations in Europe and the imminent changes in application regulation in Australia, present a need for continued industry-wide discussion on the implementation of the most appropriate risk mitigation technologies required to provide sustainable cereal rust management for growers. These risks provide significant resolve to continue the delivery of effective plant breeding programs to deliver long term sustainable solutions to a range of pests and pathogens including *Pgt*. Continued fungicide resistance surveillance of cereal rust pathogens is required internationally. Investment in new fungicide-active research and development, including efficacy research of existing chemistries under modified use patterns, is needed to mitigate environmental risks and spray drift. This paper presents the most recent observations on Australian fungicide control options for *Pgt*, fungicide contingencies for epidemic management and incursion of Ug99, and current global and Australian regulatory threats to fungicide use.

Keywords

Fungicide resistance, fungicide regulation, pesticide regulation, *Puccinia graminis tritici*

Introduction

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), has afflicted the Australian wheat industry since early settlement with the first reported incidence in 1799 (Waterhouse 1929). It was also suggested by Watson (1981) that it may have been present before European colonization, surviving on susceptible native grasses.

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The population structure of *Pgt* over the past 85 years has been strongly influenced by exotic introductions in 1925, 1954, and 1969, subsequent random mutations to virulence, and selection of genotypes with virulence matching resistance genes in cultivars (Park 2007). Following the 1973 *Pgt* epidemic in southern Australia which cost Australian grain growers an equivalent value today of AUS$2.27 billion (Park 2009), a National Wheat Rust Control Program was initiated that has evolved today to become the Australian Cereal Rust Control Program (ACRCP) (McIntosh 2007).

The global threat and spread of exotic *Pgt* pathotypes such as Ug99 internationally is significant (Singh et al. 2006). These threats are also ever present for Australia, which is currently under threat of existing *Pgt* pathotypes (Park and Wellings 2010) following above average rainfall and widespread green-bridging over the past several months. Investing in collaborative research to develop genetic, cultural and pesticide solutions is an important part of Australia's grains industry crop protection strategy. Issues currently facing Australia include cereal rust management, and putting in place measures to address the threat of exotic incursions of pests and pathogens such as race Ug99 and its derivatives. The ACRCP research partners collaborate in the Durable Rust Resistance in Wheat project (Anon 2011a). This paper reviews the most recent Australian fungicide control options for stem rust, fungicide contingencies for epidemic management and incursion of Ug99, and current global and Australian regulatory threats to fungicide use. Investing in the combined research efforts to develop genetic, cultural and pesticide solutions is an important part of the Grains Research and Development Corporation – Australia (GRDC) strategy to manage crop protection issues in the Australian grains industry, such as cereal rust management including stem rust (Rainbow 2007).

Economic benefit of rust control

Murray and Brennan (2009) estimated losses from stem rust in wheat of AUS$8 million annually with potential losses of up to AUS$478 million. The report stated that the combined estimated annual losses from stem rust, leaf rust and yellow rust in wheat of AUS$147 million could potentially be up to AUS$1.67 billion. They also estimated that breeding for stem rust resistance contributes to reducing losses of AUS$438 million, cultural green bridge management contributes AUS$24 million, and fungicide intervention currently contributes AUS$8 million in reduced losses, whereas fungicide use in Australia currently contributes to reducing losses from yellow rust of AUS$359 million annually.

A review of the GRDC's seventeen year investment in the ACRCP is estimated to have delivered a net present value of AUS$632 million in 2006/07 and a benefit cost ratio of nearly 23 to 1 (Anon 2007a). These high returns supports grain grower views that the ACRCP is one of the most important research investments made by the Australian grains industry.

Ug99 contingency planning in Australia

The possibility of exotic incursions of Ug99 and related races via wind borne rust spore transmission from southern Africa has been well documented (see Brown and Hovmøller 2002). In addition the risk of incursion from international travellers to Australia (Wright 2011) will be influenced by the actual pathotype, subsequent random mutations to virulence, and selection of genotypes with virulence matching the resistance genes in cultivars (Park 2007).

The Australian organisation responsible for coordinating exotic pest incursion response plans, Plant Heath Australia, has developed a large number of industry contingency plans for incursion response. A Ug99 *Pgt* incursion response plan endorsed by the Australian grains industry developed by Park (2009) states that should Ug99 be detected in Australia, it is unlikely eradication or containment will be technically feasible. Spores are readily spread by wind and the time required to survey and definitively differentiate this pathotype from existing *Pgt* pathotypes is likely to be too long to consider either option. Based on surveys of the stem rust responses of Australian wheat cultivar deliveries in 2005/06 (Anon 2007b) cited in Park (2009), currently 28% of wheat cultivars in Australia are rated as moderately susceptible (MS) to susceptible (S) to current endemic *Pgt* pathotypes. If race Ug99, or a derivative, were to become established, up to 60% of cultivars would change in response rating from MS to S. It is likely that further mutations would also occur, potentially affecting other effective resistance genes in use in breeding programs in Australia.

Previous experience in Australia has shown that cereal rust pathogens have the potential to spread rapidly following an incursion or mutation (Park 2009). There are a number of examples of rust pathotypes spreading in wheat in Australia, including *Pgt* (Zwer et al. 1992), *P. triticina* (Park et al. 1995) and *P. striiformis* (Wellings 2007). These pathotypes dispersed across much of the Australian continent in as little as 12 months, and in many cases, with subsequent dispersal to New Zealand. These situations have demonstrated quite clearly that once the threshold of detection is reached, eradication is unlikely to be successful (Park expo 2009).

Park (2009) states that surveillance of cereal rusts in Australia is linked to the Global Cereal Rust Management System through the Australian Cereal Rust Control Program (ACRCP) and, providing funding for this program is maintained at current levels, ACRCP will detect any new pathotypes of *Pgt* that establish in Australia. Preparedness activities for race Ug99 being undertaken in Australia include significant work being carried out on surveillance and pre-breeding for resistance. Should Ug99 become established, management could potentially include the use of chemical (fungicide) application. It is however unlikely sufficient chemical stockpiles will be available in the event of a significant national epidemic.

Inoculum levels and pathotype diversity in *Pgt* have declined in all wheat-growing regions of Australia since the mid-1970s, most likely as a consequence of the use of cultivars with effective resistance combinations to stem rust in most wheat breeding programs (Park 2007). However, significant areas of the southern and northern regions of Australia are sown to cultivars carrying *Sr24* or *Sr36*, which would be vulnerable to currently known variants of Ug99 (Park 2009). The presence of such variants in the country does not necessarily mean that an epidemic is imminent, as weather conditions also play a significant role. Around 70% of the wheat area in W.A. in 2005-06 was either MS or S to the endemic *Pgt* pathotypes (Park 2009), but no epidemic ensued.

Should Ug99 become established in Australia, extensive communication within the grains industry will be required to encourage the use of resistant cultivars. With the current threat of endemic rusts for the 2011 season detailed by Park and Wellings (2010), the ACRCP Consultative Committee, with financial support of the GRDC, have launched a National campaign titled "The Rust Bust" (Cay 2011) to raise industry awareness of endemic rust threats to growers. This campaign also provides a vehicle for communicating exotic rust threats such as Ug99 and barley stripe rust, which are currently not in Australia. If an incursion does occur, additional resources will be required to ensure that sufficient quantities of seed of resistant cultivars are available for sowing in the years following initial detection of a new rust or new pathotype (Park 2009).

Fungicide control of Ug99

A major outcome of the ACRCP investment has been a reduction in the use of fungicides in Australia due to the availability of resistant varieties to growers. In Australia fungicides are used as pre-plant treatments mostly with susceptible varieties, as an early insurance policy, and as foliar sprays if an outbreak occurs. When varieties that are resistant from the seedling stage are planted, fungicides are generally not applied.

Fungicidal control of cereal rust diseases has increased in recent years as they have become significantly cheaper (<AUS$8/ha). Application efficiency of fungicides has improved significantly as a result of investment in GPS-guided pesticide application infrastructure and new spray nozzle technology for aerial and ground application. The main fungicide group (triazoles or Group 3 demethylation inhibitors - DMIs) used in Australia have proven quite effective and there have been no signs of resistance to date (Oliver, unpublished data 2010). Also, the alternative mode of action strobilurin fungicides (Group 11 quinone outside inhibitors – QoIs) are now becoming more widely available for use in Australia. This offers industry scope for more effective control of a range of cereal foliar diseases including cereal rusts (Rainbow 2007).

Research outcomes on fungicide efficacy on stem rust caused by Ug99 in Kenya reported by Wanyera et al. (2009) suggest that a number of triazole and strobilurin/triazole fungicide mixtures can be used effectively in reducing rust severity and increasing the yield of susceptible wheat cultivars in stem rust-prone areas. Until as recently in 2010 fungicides have not been used extensively in Australia to control stem rust in commercial wheat crops. Relatively few studies have examined the efficacy of fungicides in controlling stem rust, but Mayfield (1985) and Loughman et al. (2005) demonstrated that fungicide use for control of stem rust can be economic. Loughman et al. (2005) found that tebuconazole was more effective than flutriafol or triadimefon in reducing stem rust, and in improving yield and grain quality where previously uncontrolled losses were up to 45%. However, the timing of application was important in optimising efficacy.

Although there are a number of registration gaps due to insufficient recent disease events to generate efficacy data in natural field infection situations, a number of fungicides are currently registered in Australia for stem rust control. Following the first significant wide-scale stem rust incident in Australia since the 1970s, a suite of comprehensive fungicide control experiments, funded by the GRDC as an emerging issue, were conducted across Southern Australia in 2010-11 by Poole (2011). The results showed that fungicides can be employed successfully to control stem rust in wheat, but timing in relation to disease development is crucial. Poole (2011) found that in susceptible cultivars, the application of fungicide must be made at a very early stage of disease development, preferably prophylactically, as fungicide activity was limited where the disease was already established in the stem at application. In these cases cultivar resistance was far more effective in defence against this disease than fungicide application (Poole 2011).

A key research finding by Poole (2011) was that control of *Pgt* with fungicides is very rate-sensitive, particularly where single active ingredients of DMI triazole fungicides, such as tebuconazole (Folicur®), epoxiconazole (Opus®) and propiconazole (Tilt®) were applied; all showed a sharp fall off in activity when label rates for *Pgt* were reduced. Formulated mixtures used in this research, which in most cases apply more total active ingredient and which are generally more expensive, also showed reduced activity at lower dose rates; however, the fall off in activity was less pronounced. Propiconazole gave significantly poorer stem rust control than the other fungicides tested at full label rates. Prothioconazole, the partner triazole to tebuconazole in Prosaro®, was particularly strong on stem rust, making Prosaro® one of the most cost effective fungicides for control of this disease. The QoIs strobilurin formulations azoxystrobin/cyproconazole (Amistra Xtra® 800 ml/ha), pyraclostrobin/poxiconazole (Opera® 1,000 ml/ha) provided good stem rust control at high rates. The lower cost of high (label) rate applications of DMIs tebuconazole (Folicur® 290ml/ha), epoxiconzole (Opus ® 500ml/ha) and tebuconazole/ prothioconazole (Prosaro® 150ml/ha plus adjuvant and 300ml/ha plus adjuvant rate) granted these products better cost benefit ratios. The results of this research will be used to bridge Australian registration gaps for fungicides providing efficacy for control of stem rust, including epoxiconazole (Opus ®) and azoxystrobin/ cyproconazole (Amistra Xtra®).

A key problem identified by Park (2009) with fungicidal control of rust diseases in broad-acre crops like wheat in Australia was that chemical suppliers want to be able to predict fungicide requirements in any given year up to 6 months in advance, as they do not want to hold large stockpiles of active ingredient. It is unlikely Australia would have sufficient stockpiles of fungicide in the country should a major stem rust epidemic occur (Bodnurak pers comm).

Regulation risks for fungicides

Fungicides offer a potential solution for stem rust management if an incursion or epidemic occurs, particularly if cultivars carry partial resistance. The problems of fungicide supply and distribution will present challenges in situations where epidemics occur, and Australia experienced this with cereal fungicides and particularly pulse fungicides in 2010 (Bodnaruk pers comm). An emerging issue for both the strategic and tactical use of fungicides is the increasing regulation of their use due to both environmental and human toxicity concerns.

In 2010, the Australian Pesticides and Veterinary Medicines Authority (APVMA) issued an operational notice that covers new registration application and label requirements in relation to spray drift management effective from 1 March 2010 (Anon 2010). This notice is the result of an extensive review of chemical spray drift in Australia and proposes increased regulation of spray drift buffer zones which will require a change to coarse or very coarse spray quality to reduce spray drift risk. It will affect fungicide efficacy, which is optimised with fine to medium droplet size, and will also significantly impact on where fungicides can be used in relation to sensitive areas including public areas, waterways, rivers, lakes and native vegetation. As a result of this legislation, all pesticides including fungicides in Australia will eventually come under this review. While the impact on the size of new buffer zones for fungicides is not yet clear, the outcomes of the spray drift review have proposed buffer zones of 300 m and 700 m for phenoxy herbicide application for ground-based and aerial applications, respectively.

In the European Union (EU) there have been recent changes to Directive 91/414/EEC which have seen a shift from risk-based to hazard-based assessment (Anon 2011b). Under Directive 91/414/EEC, the hazard associated with using any chemical, such as a pesticide, is its inherent ability to cause adverse health or environmental effects. The risk that such a chemical will cause these adverse effects is dependent on the way it is used in practice and this risk assessment takes into account both the hazards of the chemical and exposure to it. In January 2009, the European Parliament voted for a regulation to replace Directive 91/414/EEC. Among the changes, new hazard based 'cut-off' criteria will be introduced and as a result active ingredients will lose approval if they have any of the following properties; cause DNA mutations (mutagenic); cause cancers (carcinogenic); disrupt endocrines or are toxic for reproduction; are a persistent organic pollutant; are persistent, bioaccumulating and toxic or are '*very persistent and very bioaccumulating*'. A number of pesticides currently used in Europe that do not meet these criteria will be withdrawn. These include a number of currently approved EU Annex 1 DMI triazole fungicides that are now due for de-registration. The New EU regulations include a provision that an active ingredient (AI) shall only be approved if *"it is not considered to have endocrine disrupting properties that may cause adverse effects in humans [or] on non-target organisms."*

AIs most likely to be eliminated by hazard criteria based on endocrine disruption risks (note expiry of existing approval in brackets) include key cereal fungicides epoxiconazole (2018), and tebuconazole (2018). However, these products are covered by a derogation allowing a 5 year approval if there are no alternatives to combat a serious danger to plant health, noting that this may be unlikely for cereal rusts. EU member states have agreed that fenbuconazole, fluquinconazole and cyproconazole will be voluntarily withdrawn for registered use by 31 December 2011.

The United States Environmental Protection Agency (EPA) has issued orders for a Endocrine Disruptor Screening Program (EDSP) (Anon 2011c) including the fungicides propiconazole, tebuconazole and triadimefon for Tier-1 screening in the EDSP. The EPA state that a variety of chemicals were found to disrupt the endocrine systems of animals in laboratory studies, and compelling evidence shows that endocrine systems of certain fish and wildlife have been affected by chemical contaminants, resulting in developmental and reproductive problems. Based on these findings and other evidence, Congress passed the Food Quality Protection Act and the Safe Drinking Water Act Amendments in 1996 requiring that EPA screen pesticide chemicals for their potential to produce effects similar to those produced by female hormones (estrogen) in humans and giving EPA the authority to screen certain other chemicals and to include other endocrine effects. Based on recommendations from an Advisory Committee, EPA has now expanded the EDSP to include male hormones (androgens) and the thyroid system, and to include effects on fish and wildlife.

These international regulations are likely to affect the review and ultimate registration and use of pesticides in Australia including the use of fungicides. The current Australian Government's policy on chemical regulation (Anon 2011d) is to "*Deliver a more efficient way to review chemicals of concern – using the most up-to-date international research more effectively and making it easier for the APVMA to initiate that process; Put the onus on chemical companies to prove their products remain safe at regular intervals – bringing Australia into line with most regulators in the United States and Europe; Move to ensure that if chemicals are banned in a comparable market overseas, trigger a process for review of domestic use of the chemical; Allow the science used in approvals in comparable markets overseas to be considered in applications for Australian use; Deliver a risk management framework to match the level of regulation with the level of risk and focus resources on the higher risk products. This will also allow approvals of new, safer chemicals to be fast-tracked*". The outcomes of this process are likely to result in reduced grower access to pesticides currently available based on risk and hazard criteria.

The impacts of these regulations require significant investment by pesticide registrants, research organisations and the agricultural industry to either provide a scientific basis for continued access to AIs under review, or develop new pesticide or non-chemical solutions such as genetic or cultural management options. By not taking into consideration threats or not investing in continued access to these technologies, threats to agricultural industries will increase, including managing rust epidemics and incursions of new pathotypes, such as Ug99 in the grains industry.

Conclusions

The threat of Ug99 incursion continues to loom over the Australian and international grains industries. While this threat exists, the challenges of encouraging growers to plant varieties resistant to current endemic *Pgt* pathotypes must continue. Currently high inoculum levels following unusually wet summer conditions in south-eastern Australia constitute a threat for the 2011-12 crop, and if there is a continuation of widespread plantings of susceptible varieties Australia's preparedness for chemical intervention may be put to the test.

Nationally coordinated communication programs to raise grower awareness of the risks and solutions for stem rust management are in place through ACRCP Consultative Committee initiatives, such as "The Rust Bust", that are supported by the GRDC and the wider cereal breeding community. The looming challenges of pesticide regulation in Europe and the imminent application regulation changes in Australia present a need for continued industry-wide discussion on the most appropriate risk mitigation technologies required for sustainable cereal rust management for growers. These risks provide significant resolve to continue the delivery of effective plant breeding programs for long term sustainable solutions for a range of pests and pathogens, including *Pgt*. Continued fungicide resistance surveillance for cereal rusts is required internationally, as well as increased investment in the development of new fungicide actives. Efficacy research of existing chemistries under modified use patterns to mitigate environmental risks and spray drift is also required. The GRDC is supporting these research efforts in Australia and welcomes increased linkages with international stakeholders to tackle this issue as efficiently as possible.

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References

Anon (2007a) An economic analysis of the Australian Cereal Rust Control Program GRDC Impact Assessment Report Series. Grains Research and Development Corporation, Canberra, Australia

Anon (2007b) Confidential Australian Wheat Board wheat receival figures for 2005-06. Grains Research and Development Corporation, Canberra, Australia

Anon (2011a) http://wheatrust.cornell.edu/

Anon (2011b) http://ec.europa.eu/food/plant/protection/index_en.htm

Anon (2011c) http://www.epa.gov/endo/; http://www.epa.gov/endo/pubs/final_list_frn_041509.pdf

Anon (2011d) http://www.alp.org.au/federal-government/news/better-regulation-of-chemicals/

Brown JKM, Hovmøller MS (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297:537-541

Cay B (2011) http://www.rustbust.com.au/
McIntosh RA (2007) From Farrer to the Australian Cereal Rust Control Program. *Austr J Agric Re* 58:550-557
Park RF (2007) Stem rust of wheat in Australia. *Austr J Agric Res* 58:558-566
Loughman R, Jayasena K, Majewski J (2005) Yield loss and fungicide control of stem rust of wheat. Austr J Agric Res 56:91-96
Mayfield AH (1985) Efficacies of fungicides for control of stem rust of wheat. Austr J Agric Res 25:440-443
Murray GM, Brennan JP (2009) The current and potential costs from diseases of wheat in Australia. Grains Research and Development Corporation, Canberra, Australia
Park RF (2009) Australia's preparedness for Ug99 - A review of the Australian grains industry's ability to respond to the arrival of stem rust of wheat (Puccinia graminis f. sp. tritici) pathotype Ug99. Industry Biosecurity Plan for the Grains Industry, Plant Health Australia, Canberra, Australia
Park R, Wellings C (2010) Cereal Rust Report Season 2010: Cereal Rust Situation Update, Late Spring 2010, Vol 8, Issue 8, November 2010. http://sydney.edu.au/agriculture/documents/pbi/cereal_rust_report_2010_vol_8_8.pdf
Poole N (2011) Evaluation of different foliar fungicides for the control of stem rust (*Puccinia graminis* f.sp. *tritici*) in wheat. Interim Disease Data Report. GRDC Internal Report Project SFS00017, Grains Research and Development Corporation, Canberra, Australia
Rainbow R (2007) GRDC Cereal Rust Management Implementation Plan, Confidential Report. Grains Research and Development Corporation, Canberra, Australia
Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P, Ward RW (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 54:1-13
Wanyera R, Macharia JK, Kilonzo SM, Kamundia JW (2009) Foliar fungicides to control wheat stem rust, race TTKS (Ug99), in Kenya. Plant Dis 93:929-932
Waterhouse WL (1929) Australian rust studies. I. *Proc Linn Soc NSW,* 54:615-680
Watson IA (1981) Wheat and its rust parasites in Australia. In: Evans LT, Peacock WJ (eds) Wheat science - Today and tomorrow.) pp129-147, Cambridge University Press, Cambridge, U.K.
Wright DG (2011) Defining the plant pathogen incursion risk posed by international travellers. GRDC project final report NPB00010. Grains Research and Development Corporation, Canberra, Australia
Zwer PZ, Park RF, McIntosh RA (1992) Wheat stem rust in Australia 1969–1985. Austr J Agric Res 43:399-431

Release of durable rust resistant wheat varieties in certain wheat districts of Ethiopia

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The appearance of Ug99 in the late 1990s prompted collaborative global research to develop wheat varieties with durable resistance, especially in the interests of resource-poor farmers in the developing world. These collaborations from 2005, led to durably rust resistant/tolerant wheat varieties in 2009. Five such varieties originating from CIMMYT/ Mexico were rapidly tested in Ethiopia with the participation of wheat farmers. Participatory evaluation of these and earlier released varieties resulted in approval of several lines that were preferred by farmers. Farmers' selection criteria were the driving force for the official release of two CIMMYT lines in May 2010. A yellow rust epidemic in August 2010 prompted promotion and pre-scaling up of new varieties, Danda'a and Kakaba, and the previously released, but less promoted variety Digalu. Danda'a and Digalu are suitable for high moisture highland areas and were favored by farmers in several districts. Kakaba is an early maturing variety suitable for mid-altitude and lowland areas. A yellow rust epidemic in 2010 caused heavy losses to long serving varieties such as Kubsa and Galama; Digalu, Danda'a and Kakaba, on the other hand, were resistant to both yellow rust and stem rust. The potential value of these varieties on farmers' fields were recognized by other farmers, extension experts in different *woredas* (districts) and seed producers raising the demand for seed for the 2011/12 season. This encouraged seed enterprises, private seed producers and some research centers to produce pre-basic and basic seed during the 2011 off-season (Jan-April). Farmer to farmer dissemination of seeds has also been effective and successful well ahead of the June 2011 planting time. This paper will describe the promotion and scaling-up of the three varieties.

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Delivering quality seeds

Usha Barwale Zehr

Wheat farmers in India typically replace planting material every four years. With the disease challenges currently faced and emerging, it is critical to have resistant varieties available for planting by farmers. At a minimum these must perform at least as well as the most popular varieties in each of the five key wheat growing zones. With the threat of Ug99, much discussion has focused on adoption of resistant varieties. It is important that the new varieties also be resistant to current local races of other rust pathogens. Standards for wheat seed quality are prescribed. Consequently, the seed replacement rate of wheat is low, and getting new resistant varieties in place is a major challenge. It is also important to look at simple cost-effective tests for establishing genetic purity. While several varieties have been identified as resistant to Ug99 and yellow rust, on farm acceptance is low. The quantities of seed of resistant varieties needed over the next five years, and their quality standards, will be discussed.

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Looking for a needle in a haystack: Screening of the International Stem Rust Nursery in Kenya for new sources of resistance in spring wheat expo landraces

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Abstract

Identification and deployment of new sources of resistance in adapted wheat varieties is essential to ensure food security under the current threat from race Ug99 of the stem rust pathogen and its variants. In an effort to diversify the genetic base of stem rust resistance, especially to Ug99, common bread wheat landraces from the USDA-ARS National Small Grains Collection are being evaluated in the International Stem Rust Nursery at Njoro, Kenya. Since 2007, nearly 3,000 spring common wheat landraces have been tested and potentially new sources of resistance were identified. Several approaches including association analysis and bi-parental populations are being used to characterize the resistance in accessions that showed consistent resistance in the nursery. To date, 165 landraces have been identified with some degree of resistance when compared to susceptible checks in multiple field seasons in Kenya. Two accessions from Iran and one from Montenegro were crossed to the susceptible line LMPG-6 to determine the inheritance of resistance. Preliminary genetic analyses based on F_3 family segregation in the seedling stage suggest that the resistance in each Iranian landrace is conferred by a single dominant gene. Segregation of resistance in F_3 families of a PI 362698-1 (Montenegro) cross suggested complex, multigenic inheritance. Preliminary association analysis, based on data collected from a subgroup of accessions, identified genomic locations on chromosomes 2B and 5B associated with resistance. Further analyses using additional resistant accessions, statistical models and disease indices are under investigation to validate these expo associations.

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Keywords

Genome-wide association analysis, germplasm collections, *Puccinia graminis f. sp. tritici,* Ug99

Introduction

The successful use of host resistance to manage wheat rust depends on constant pathogen monitoring and on the release of varieties with new and diverse sources of resistance. The detection of Ug99 and its variants in East Africa revived memories of past stem rust epidemics, such as that caused by race 15-B in the U.S.A. in the 1950s, and the devastating yield losses that can arise from growing susceptible wheat varieties in rust conducive environments (Rodenhiser and Moore 1951). Identifying and deploying new sources of resistance is extremely important to prevent future stem rust epidemics.

Germplasm collections are valuable sources of disease resistance in wheat, including leaf rust (Kolmer et al. 2007) and stem rust (Bansal et al. 2008; Nazari et al. 2008). Wild relatives of wheat have offered genes for resistance to various diseases, including the rusts. However, introgressing these genes without also introducing agronomically undesirable traits can be difficult to achieve without utilizing multiple backcrosses to the recurrent parent or size reductions of alien segments using chromosome engineering techniques (Niu et al. 2011). The use of landraces as donors of new sources of resistance should enable easier incorporation of resistance with less problems caused by linkage drag.

The National Small Grain Collection (NSGC) is part of the United States Department of Agriculture - Agricultural Research Service (USDA-ARS) National Plant Germplasm System (NPGS). The NSGC maintains, evaluates, and distributes accessions of small grains, including wheat (*Triticum* spp.), barley (*Hordeum* spp.), oat (*Avena* spp.), rice (*Oryza* spp.), rye (*Secale* spp.), triticale (X *Triticosecale*), and various wild relatives. The NSGC holds 60,662 wheat accessions and of these 44,451 are common or bread wheat. A total of 9,814 of these are spring habit landraces.

Beginning in 2005 entries from the NSGC were included in the International Stem Rust Nursery at the Kenya Agricultural Research Institute in Njoro, Kenya, as part of the overall efforts among USDA-ARS scientists to discover and deploy resistance to Ug99 in U.S. wheat and barley. Landrace evaluations began in 2007. Since then, nearly 3,000 spring habit landraces have been screened for resistance in the field against Ug99 and its variants. In this paper we describe the methodology used to screen the landrace collection in an effort to find new sources of resistance that can be deployed in a new generation of stem rust resistant varieties.

Pre-screening

The NSGC accessions evaluated in this study comprised over 9,000 spring common wheat landraces from 62 countries. Because screening space in the Kenya nursery is limited, not all 9,000 accessions could be screened. To reduce the number of accessions for testing in Kenya, selection criteria were developed to choose entries more likely to be resistant to Ug99 and its variants. Approximately the first 1,000 selections were based on geographic origin (Bonman et al. 2007), adult plant resistance to a composite of North American races, or chosen at random.

After these initial 1,000 accessions were tested, all spring habit landrace accessions were pre-screened as seedlings in greenhouse tests using the local race QFCS. Accessions showing seedling resistance to QFCS were chosen for testing. As information on Ug99 responses became available, we came to know that some genes are effective to both Ug99 and North American races (e.g. *SrTmp*) whereas others (e.g. *Sr28* and *Sr42*) are uniquely effective against Ug99 and its variants. Thus, the most effective future selection strategy will be to use a selection strategy based on the information obtained from previous screening results. For example, if other traits are associated with Ug99 resistance (NSGC descriptors or geographic origin) such information could be used as a basis for choosing accessions for testing.

Field screening with race Ug99

Field evaluations in Kenya were performed in two 1m row plots of each accession. Wheat varieties susceptible to Ug99 were grown adjacent to the experimental plots and inoculated before heading with urediniospores increased on lines with *Sr31*, and also *Sr24* in later seasons. Disease reactions were rated as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) and disease severities were recorded as percentages of stem affected. Ratings were recorded at least twice per season. Plant growth stage data were also recorded in most cases. Accessions showing resistance in one season were re-tested in the following season, and also screened at the seedling stage with race TTKSK (Ug99) in greenhouse tests at the USDA Cereal Disease Laboratory. To date, field results are available for seven of eight seasons and 165 accessions have shown resistance in more than one season. Resistant accessions were screened with genetic markers diagnostic for *Sr2* (csSr2) and *Sr36* (WMC477). Only six and nine resistant accessions were positive for the markers for *Sr2* and *Sr36*, respectively. In addition, seven of the resistant accessions were positive for the functional allele of *Lr34* (csLv34). Accessions positive for *Sr36* and *Sr2* are likely the products of plant breeding and thus are not landraces. Since the classification of landrace status is subjective, this result is not surprising.

Mining field nursery data for new sources of resistance against Ug99 and variants

Genome-wide association analysis

Genome-wide association analyses were recently conducted in wheat to identify markers and genes associated with different traits, including disease resistance (Tomasini et al. 2007; Maccaferri et al. 2010), grain quality (Breseghello and Sorrels 2006) and yield (Kraakman et al. 2004). Association, or linkage disequilibrium, mapping describes statistical relationships between phenotypic and genotypic variation in collections of diverse germplasm (Hendrick 1987; Gutpa et al. 2005; Ingvarsson and Street 2011). To facilitate the identification of genomic regions associated with resistance to Ug99 in spring wheat landraces, a subgroup of 708 accessions representing 54 countries were screened with the Diversity Array Technology (DArT) platform. A total of 832 polymorphic markers were identified. Preliminary analysis using a mixed model accounting for two levels of population structure (based on principal component assignments and allele sharing relationships) identified a chromosomal region on chromosome 2B significantly associated with categorical stem rust infection responses from field experiments (p-value with no correction for multiple test 0.00003, false discovery rate (FDR) Q-value 0.0071). The mixed model analysis has been shown to limit the detection of false signals by the confounding factor of population structure (Yu et al., 2006). Thus, we interpret these preliminary findings as evidence that the genetic variation at the locus on 2B is correlated with the variation in stem rust field ratings because the locus is directly involved in the resistance response. An additional chromosomal region on 5B showed association with stem rust field ratings, although results were not significant using the mixed model (p-value with no correction for multiple test 0.0013, FDR Q-value 0.12). The detected associations are correlative evidence for putative marker-trait relationships. Additional work is in progress to further test these preliminary results (Newcomb et al. 2010).

Genetic studies

Single plant selections from three landrace accessions, that were resistant in multiple field tests in Kenya, and also in seedling greenhouse tests using TTKSK, were used for inheritance studies. Two of these landraces, PI 626573 and PI 623181, showed seedling infection types (IT) of 2 to 3 and 2, respectively, and had field severity ratings varying between trace and 30% and infection responses from MR-MS to MR. These accessions, originally collected in Iran, were deposited in the NSGC in 1997. The third accession, PI 362698 collected in Montenegro in 1971, had a seedling infection type of ;1 to ;1+ to TTKSK, field severity ratings ranging from 0 to 10%, and field infection responses ranging over R, MR, MR-MS and MS. Single plant selections from these accessions were crossed to the stem rust susceptible line LMPG-6 to generate mapping populations. F_3 families from each cross were evaluated as seedlings in a University of Minnesota biosafety facility (BSL3). The single plant selection of the accession PI 362698 (PI 362698-1) had seedling ITs that varied from 0; to 31; (a mesothetic or 'X' reaction). Out of 107 F_3 families assayed, a total of 58 did not exhibit resistance. The level of resistance varied both within and among families exhibiting resistance with no families exhibiting the parental low IT. The F_3 family segregation ratios did not fit postulated Mendelian ratios for 1, 2, or 3 dominant gene models. Thus, the seedling resistance against TTKSK in PI 362698-1 is complex and the low number of resistant plants suggests recessive genes may be involved. Modifier genes and segregation distortion cannot be ruled out at this point as playing a role in the complexity of the observed resistance segregation. Since resistance was rarely detected both within and among families, the possibility exists that PI 362698-1 is a partial amphiploid and the resistance is conferred by segregation of unpaired chromosomes. However, since the level of resistance varied among resistant families, the evidence suggests a more complex scenario. Currently, the F_3 families are being advanced in single seed descent to develop a mapping population. F_6 recombinant inbred lines (RILs) and parents will be evaluated in Kenya during the off season nursery of 2012. In addition, since PI 362698-1 was positive for the *Lr34* functional allele, screening of RILs with the *Lr34* marker will allow identification of lines that do not carry *Lr34*. A new population can then be developed using such RILs to study the resistance in the absence of the *Lr34*. The segregation ratios of F_3 families derived from crosses of the two Iranian landraces and a susceptible genotype fitted 1:2:1 (homozygous resistant: segregating: homozygous susceptible) ratios indicating that resistance in each was controlled by a single gene (Table 1). Given the common origin and similar low infection types it is possible the same gene is present in both lines. The lines will be intercrossed to conduct an allelism test. Two different approaches were chosen for resistance mapping based on the available DNA from the F_2 plants that were progeny tested for rust response. For the PI 626573-2 population, sufficient F2 DNA is available to allow an F_2 bulk segregant analysis (BSA), whereas F_5 recombinant inbred lines will be genotyped for the PI 623181-1 population. For the F_2 BSA approach, parental lines and bulks of 10 resistant and 10 susceptible F_2 plants will be evaluated with up to 1,600 SSR markers. Polymorphic markers will be evaluated in advanced lines. For the F_5 evaluation approach, 900 SSR markers will be used to screen the parents and polymorphic markers will be used to screen the entire population. Polymorphic markers from both approaches will be used to develop genetic maps and localize the resistance gene(s).

Table 1 ***F_3 family segregation in two crosses of stem rust resistant Iranian landraces and susceptible line LMPG-6 tested with race Ug99 at the seedling stage***

	Observed ratio		
F_3 population	**(HR : Seg.: HS)**	**$Chi^2_{1:2:1}$**	**P-value**
PI 626573/ LMPG-6	37:77:42	0.35	0.84
PI 623181-1/ LMPG-6	21:34:16	0.83	0.66

Conclusion

Screening of the NSGC landraces in the Stem Rust Nursery in Njoro, Kenya, allowed the identification of resistance sources that may have not been previously utilized in breeding programs or deployed in common wheat cultivars. PI 626573-2, PI 326181-1 and PI362698-1 may provide new stem rust resistance genes to broaden the genetic basis of resistance in common wheat against TTKSK and related races. Results from association mapping will be complementary to the bi-parental mapping population work. By pursuing both approaches, we hope to obtain a more complete understanding of the genetics of Ug99 resistance in landraces. Moreover, the association mapping approach has the potential to explain the genetics of complex resistance (i.e. gene interactions) by making use of recently developed algorithms.

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References

Bansal UK, Singh D, Miah H, Park RF, Bariana HS (2008) Revisiting old landraces of wheat for stem rust resistance. In: Appels R; Eastwood R; Lagudah E; Langridge P; Mackay M; McIntyre L; Sharp P (eds) Proc 11th Int Wheat Genet Symp, University of Sydney Press NSW, Australia, Vol 1:188-190

Bonman JM, Bockelman HE, Jin Y, Hijmans R, Gironella AIN (2007) Geographic distribution of stem rust resistance in wheat landraces. Crop Sci 47:1955-1963

Breseghello F, Sorrells MS (2006) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172:1165-1177

Gutpa P, Rustgi S, Kulwal P (2005) Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Mol Biol 57:461-485

Hendrick PW (1987) Gametic disequilibrium measures: Proceed with caution. Genetics 117:331-341Ingvarsson PK,Street NR (2011) Association genetics of complex traits in plants. New Phytol 189:909-922

Kolmer JA, Oelke LM, Liu JQ (2007) Genetics of leaf rust resistance in three Americano landrace-derived wheat cultivars from Uruguay. Plant Breeding 126:152-157

Kraakman ATW, Rients EN, Petra MM, Van den Berg M, Stam P (2004) Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. Genetics 168:435-446

Maccaferri M, Sanguineti MC, Mantovani P, Demontis A, Massi A, Ammar, KK, Kolmer JA, Czembor JH, Ezrati S, Tuberosa R (2010) Association mapping of leaf rust response in durum wheat. Mol Breed 26:189-228

Nazari K, Amini A, Yahyaoui A, Singh R (2008) Characterization of seedling and adult-plant resistance to stem rust race Ug99 in Iranian bread wheat landraces. International Conference on Wheat Stem Rust Ug99 – A threat to Food Security. November 6-8, 2008, *http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/UG99/7Ug99_Conference_Papers.pdf*

Newcomb M, Acevedo M, Bockelman HE, Goates B, Jackson EW, Jin Y, Kilian A, Njau P, Singh D, Wanynera R, Bonman JM (2010) Association mapping of stem rust resistance to race lineage 'Ug99' in spring wheat landraces. Proc Plant Animal Genomes XIX Conf, abstr, *http://www.intl-pag.org/19/abstracts/P05c_PAGXIX_333.html*

Niu Z, Klindworth D, Friesen TL, Chao S, Jin Y, Cai X, Xu SS (2011) Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics 187:1011-1021

Rodenheiser HA, Moore EG (1951) The new stem rust threat. Science 113:3

Yu J, Pressoir G, Briggs WH, Vroh BI, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics 38:203-208

Deployment of rust resistant wheat varieties: Successes and challenges in Afghanistan

S. Kugbei and A. Habibi

Abstract While stripe (yellow) rust remains the major disease of wheat in Afghanistan, recent detection of the virulent race Ug99 of the stem rust pathogen in some neighboring countries has heightened concern about protecting Afghanistan's wheat crop against the potential devastating effects of this new race. To effectively combat the threat of Ug99 and other rusts, established principles of wheat rust control involving rapid variety release, seed multiplication and distribution have been followed. Based on these principles, Afghanistan is pursuing an integrated approach that includes variety development, accelerated seed multiplication, disease management and extension to mitigate wheat rusts. Part of this approach has been the importation and rapid multiplication of 151.5 tonnes of seed of the resistant variety Misr-1 from Egypt (also released in Afghanistan as Moqawim-09). This is expected to cover a substantial proportion of the irrigated wheat area within a few years while efforts are made to increase genetic diversity by releasing more new wheat rust resistant varieties and delivering certified seed to farmers. The disease management and extension components of the integrated approach are yet to be fully implemented. Obvious challenges being confronted in attempting to render these efforts more successful and long lasting, include replacement of currently popular but susceptible varieties, and to increase genetic diversity in rust resistance, create adequate awareness about Ug99 without causing panic in farming communities, and to convince ordinary farmers to buy certified seed at cost-covering but affordable prices.

Keywords

Afghanistan, rust resistance, seed multiplication, stem rust, stripe rust, Ug99

Introduction and background

Cereals are the most important food crops in Afghanistan and wheat makes up 80% of the country's cereal production. The area under wheat cultivation is estimated at 2.5 million hectares (ha), of which 1.2 million ha are irrigated. With wheat serving as the leading staple and strategic crop and occupying a vast proportion of the total cultivated land, any significant reduction in yield due to disease could have a severe effect on food security in a country that experiences frequent deficits in production and significant annual import requirements depending on seasonal weather conditions.

The three wheat rusts, stem (or black), leaf (or brown) and stripe (or yellow), have been observed to varying extents in Afghanistan over the years (Lal 1973), but stripe rust has been the most serious disease of wheat in the country (Torabi and Nazari 1998; Osmanzai et al. 2008), and is believed to cause significant crop losses in years of disease severity although the precise level of loss has not been estimated due to the lack of disease or surveillance or assessment. However, losses of up to 40% have been caused by stripe rust in other countries, with some fields completely destroyed (Mumtaz et al. 2009). Both stripe rust and leaf rust have been particularly serious in cool and wet seasons, such as during 2005 and 2009. Environmental conditions favored the development of stripe rust in 2009 and several reports were received of serious outbreaks, especially in Bamyan, Kabul, Laghman and Nangahar provinces.

A study by CIMMY) in 2007 revealed that all varieties under cultivation were susceptible to the stripe rust strains present in Afghanistan. The wide virulence of races was confirmed by the susceptibility of most of a set of near-isogenic lines (NILs) tested by Osmanzai et al. (2008).

Recent fears of the new virulent race Ug99 of the stem rust pathogen and its apparent detection in some neighboring countries (MacKenzie 2008; Nazari et al. 2009; Osborn and Bishaw 2009) heightened fears and concern about protecting the national wheat crop. Hot spot screening of wheat varieties and breeding lines from countries at immediate risk, including Iran, Pakistan and Tajikistan, has indicated Ug99 susceptibility of essentially all varieties that are currently grown. Similarly, all existing Afghan varieties submitted by CIMMYT and ICARDA for screening in East Africa during 2009 were susceptible.

To combat the threat of Ug99 and other rusts, Osborn and Bishaw (2009) outlined some basic principles for rapid variety release, seed multiplication and distribution in developing countries. In accordance with these principles, Afghanistan has adopted an integrated approach to include variety development, accelerated seed multiplication, disease management and extension for mitigating wheat rusts. This approach is expected to guide the fast development and release of new resistant varieties, rapid multiplication and availability of quality seed, effective use of other disease management practices such as fungicide application, relevant agronomic practices, and communication / coordination of rust-related information, particularly

through the national extension network. While some new varieties have been released and others potentially developed, a fast track measure was implemented by importing early generation seed of resistant varieties for further multiplication and distribution nationwide. Both these measures have been fairly successful and possible because of existing variety screening capacity and an organized seed production system already available in the country. Capacities for disease management and extension components of the integrated approach remain limited, but are expected to become stronger in the near future.

Apart from the recent disease preparedness efforts necessitated by fear of race Ug99, there have been no other significant disease management activities in the country despite the historical importance of stripe rust in particular. This paper will summarize the progress of ongoing wheat rust mitigation measures and other important areas that will require attention and will highlight key challenges along the way.

Wheat varieties grown in Afghanistan

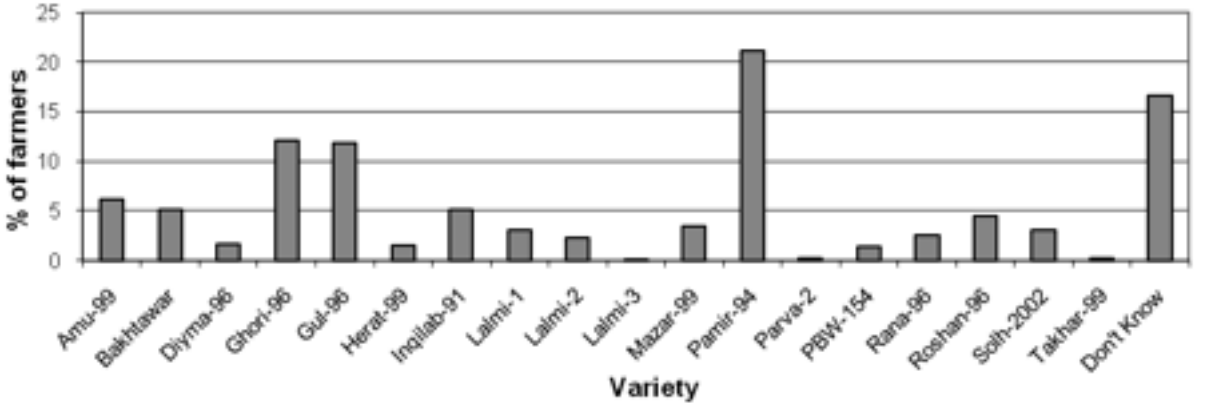
There is currently no formal wheat breeding taking place in Afghanistan. Advanced breeding lines from International Agricultural Research Centers notably CIMMYT and ICARDA, and from neighbouring countries, such as Pakistan, India and Turkey, are tested for adaptation, and those with the best performance are released as new varieties. Up to 55% of the irrigated wheat area in the country is known to be covered by such improved varieties (Tunwar 2004). Since 1994, a total of 28 improved wheat varieties have been officially released and registered in Afghanistan as summarized in Table 1.

Table 1 ***Improved wheat varieties released in Afghanistan since 1994***

No	Year of release	Variety name	Origin	Main characteristics[1]
1	1994	Pamir-94	CIMMYT/Turkey	Facultative bw with winter hardiness
2	1995	Bhaktawar-92/Kauz	Pakistan	Facultative bw for lower elevations
3	1995	PBW-154	India	Facultative bw of wide adaptation
4	1996	Gul-96	CIMMYT/Turkey	Facultative bw with winter hardiness
5	1996	Rana-96	CIMMYT/ICARDA	Facultative bw for cold/mild winters
6	1996	Takhar-96	CIMMYT	Facultative bw for mild winters
7	1996	Roshan-96	CIMMYT/ICARDA	Facultative bw of wide adaptability
8	1996	Ghori-96	CIMMYT/ICARDA	Medium duration bw for rainfed areas
9	1996	Daima-96	CIMMYT	Early maturing bw for rainfed areas
10	1999	Amu-99	CIMMYT/ICARDA	Facultative bw of wide adaptability
11	1999	Herat-99	CIMMYT	Facultative bw of wide adaptability
12	1999	Mazar-99	CIMMYT	Facultative bw of wide adaptability
13	2000	Lalmi-1	CIMMYT/ICARDA	Facultative bw for rainfed areas
14	2000	Lalmi-2	CIMMYT/ICARDA	Facultative bw for rainfed areas
15	2000	Lalmi-3	CIMMYT/ICARDA	Facultative bw for rainfed areas
16	2002	Solh-02	CIMMYT	Facultative bw with winter hardiness
17	2003	Parva-2	CIMMYT	Early maturing dw/wide adaptability
18	2007	Ariana-07	CIMMYT	Facultative bw for fall/spring sowing
19	2007	Darulaman-07	CIMMYT	Facultative bw for fall/spring sowing
20	2008	Durokhshan-08	CIMMYT	Facultative bw for fall/spring sowing
21	2008	Shesham Bagh-08	CIMMYT	Facultative bw for fall/spring sowing
22	2009	Moqawim-09	CIMMYT	Facultative bw for fall/spring sowing
23	2009	Baghlan-09	CIMMYT	Facultative bw for fall/spring sowing
24	2009	Kushan-09	CIMMYT	Facultative bw for fall/spring sowing
25	2010	Afghan-1	France	Long duration winter bw
26	2010	Afghan-2	France	Long duration winter bw
27	2010	Afghan-3	France	Long duration winter bw
28	2010	Chonte-10	CIMMYT	Facultative bw for two seasons

[1]bw, bread wheat; dw, durum wheat

Fig. 1 *Improved varieties grown by farmers (n = 1,866*



Many of the released varieties are facultative breads wheat suitable for cultivation in irrigated conditions, among which a few are adapted to cold winter conditions in high elevation areas. Five of the improved varieties are adapted to rain-fed conditions and one is a durum. A survey in 2007 (Fig. 1) showed that most of these varieties are widely distributed among farmers (Kugbei and Shahab 2010).

Besides improved varieties, there are many traditional or local varieties that are grown throughout the country. For some of these, it is not clear whether they are truly traditional varieties or older improved varieties that have local names.

Survey results illustrated in Fig. 2 show that for both improved and local varieties, the farmers rank high yield potential, earliness (days to maturity) and bread-making quality as the most desirable attributes in choosing varieties; significant but less attention is given to tolerance to insect pests and diseases (particularly stripe rust) (Kugbei and Shahab 2010). This finding has important implications for the introduction of new varieties, which should not only possess rust resistance, but must have other important attributes desired by the farmers.

One of the few attempts to assess the rust responses of wheat under cultivation in Afghanistan was a study conducted by CIMMYT at two locations in 2007 indicating that almost all existing wheat varieties were susceptible to stripe rust (Table 2). In addition, the ineffectiveness of a number of resistance genes in near-isogenic lines (NILs) tested at four locations during 2005, 2006 and 2007 in another CIMMYT study also showed that races of the stripe rust pathogen in Afghanistan were widely virulent (Osmanzai et al. 2008).

Fig. 2 *Farmers' ranking of improved and local varieties*

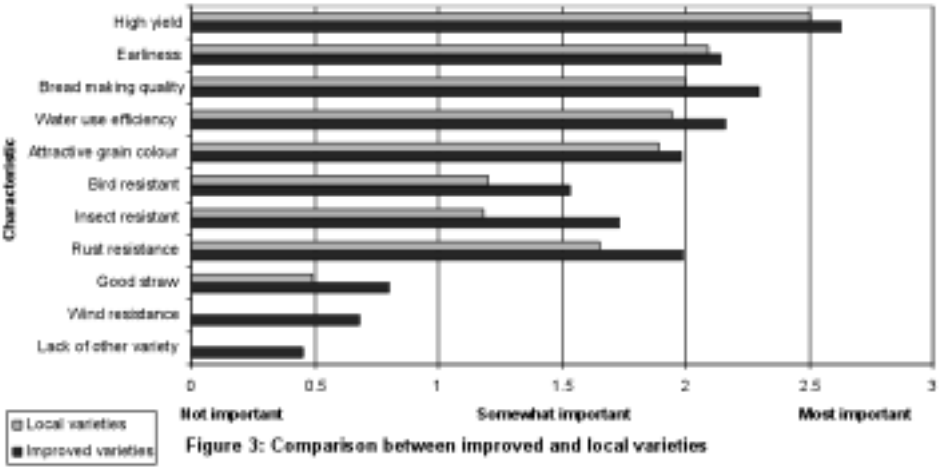


Figure 3: Comparison between improved and local varieties

Table 2 Yellow rust and leaf rust severities (%) and reactions[1] on wheat varieties in Afghanistan, 2007

Variety	Yellow rust (Darulaman, Kabul)		Leaf rust (Herat)
	June 3	June 13	
Parva-2 (Durum)	0	0	40 S
Kauz (Bakhtawar)	0	40 MS	40 S
PBW154	10 MS	20 MR	Tr
Lalmi-3	40 S	40 S	5R
Lalmi-1	5 MR	5 MR	5R
Takhar-96	100 S	100 S	–
Daima-96	60 S	100 S	–
Mazar-99	5 MR	20 MR	Tr
Rana-96	Tr	Tr	–
Ghori-96	20 S	40 S	10 MR
Roshan-96	80 S	100 S	–
Amu-99	100 S	100 S	–
Gul-96	0	5 MR	80 S
Herat-99	80 S	100 S	5R
Pamir-94	0	Tr	0
Solh-02	Tr	10 MR	–
Darulaman-07	0	Tr	–
Ariana-07	0	–	–

[1]S, susceptible; MS, moderately susceptible; MR, moderately resistant; R, resistant; Tr, traces.

Results of screening carried out in East Africa during 2009 indicated that all 21 varieties released up to 2008 were somewhat susceptible to *Pgt* race Ug99 emphasizing an an urgent need for introduction of stem rust resistant varieties and gradual withdrawal of the older susceptible ones. Consequently, three new varieties already in adaptation trials and found to be resistant to race Ug99 and other rusts were released in 2009, followed by another in 2010. One of the varieties released in 2009 was Moqawim-09, also released in Egypt as Misr-1. This variety has the stem rust resistance gene *Sr25* which remains effective against Ug99. Among recently released varieties, Baghlan-09 has APR to stem rust and Chonte-10 has APR genes Sr2+. These varieties should also offer much needed genetic diversity in resistance.

An integrated approach for combating wheat rusts

An integrated approach, including variety development, accelerated seed multiplication, disease management, and extension, is necessary in Afghanistan for combating the threat of wheat rusts including race Ug99. This should guide the fast development and release of new resistant varieties, rapid multiplication and availability of quality seed, effective use of other disease management practices such as fungicide application, relevant agronomic practices, and communication / coordination of rust-related information through national extension services. This will be a multi-stakeholders approach. Given the wide range of institutional involvement that involves agricultural researchers, plant protection services, the national seed association and seed enterprises, public sector seed production services, seed quality certification services, agricultural extension services, farmers' cooperatives, and donor agencies. Stakeholder contingency planning workshops to raise awareness and mobilize support will be an important feature of the integrated approach.

Host resistance will remain the primary factor in the management of wheat rusts for which Afghanistan will continue in the foreseeable future to rely on and source adapted cultivars, and experimental and breeding lines from international research centers, mainly CIMMYT and ICARDA. Once new rust resistant varieties are released, foundation, registered and certified seed should be multiplied as fast as possible so that sufficient quantities of seed will be available to farmers for commercial production. Accelerated seed multiplication has so far been the most comprehensive rust mitigation and preparedness measure adopted in Afghanistan as elaborated in the next sections of this paper.

Closely linked with new varieties and seed multiplication is disease management, especially in the absence of adequate resistance or when such resistance breaks down. Fungicide application may be necessary as an interim or emergency measure to provide acceptable control of rusts in hot spots. In Afghanistan, a pilot stripe rust control project is being considered which would involve spraying up to 2,200 ha of wheat seed plots with fungicide (Folicur) in three provinces (Kabul, Nangahar and Balkh). Such an operation can only be effective and economical when based on thorough field surveillance, and when applications are early and timely. Besides chemical control in the field, there are other possible rust mitigation measures, including eradication of volunteer wheat plants from the previous summer to prevent 'green-bridging', treating seed with fungicides to prevent early season infections of stripe rust and leaf rust (but not stem rust), adjustment of planting time to minimize the risk of early infections, and growing alternative crops (crop rotation) to break the disease cycle and reduce potential infestation.

Disease monitoring, surveillance and reporting through the extension services are vital missing functions in Afghanistan. Formal reports or information on rust incidence are limited and not up to date. In order to make informed decisions and take appropriate action, the development and spread of wheat rusts and pathogen races should be effectively monitored and relevant information communicated to the wheat growing community including researchers, farmers, and other stakeholders. While information-sharing is essential, adequate capacity should be developed for proper identification and verification of particular diseases to ensure that accurate and reliable information is disseminated and in the right manner.

Seed multiplication and distribution systems

Organized seed production and distribution is recent in Afghanistan. Considerable progress has been made within a relatively short time with the support of two European Union-funded seed projects implemented jointly by FAO and the Ministry of Agriculture, Irrigation and Livestock (MAIL). A notable achievement is rapid growth of the private seed sector which began with 8 small-scale pilot enterprises in 2004 and has now expanded to 95 enterprises in 28 of the 34 provinces. These enterprises have all become registered members of a National Seed Association. The Afghanistan National Seed Organization (ANSOR) established in 2008 serves as an umbrella organization for the private seed sector. In addition, a National Seed Policy adopted in 2005, defines four official seed classes (breeder, foundation, registered and certified) and specifies the respective roles of the public and private sectors in the seed multiplication process, with all classes except certified seed being produced by the public sector. Breeder seed is produced by stations of the Agricultural Research Institute of Afghanistan (ARIA), foundation and registered seed by stations of the state run Improved Seed Enterprise (ISE), and certified seed is produced by private enterprises of ANSOR. A seed law was enacted in 2009 and a National Seed Board is now in place for coordinating all seed industry functions.

Out of a total annual wheat seed requirement of about 300,000 tonnes, certified seed accounted for only 18,000 tonnes in 2010, but is projected to reach 30,000 tonnes in 2011 - equivalent to a 10% replacement rate and considered an ideal injection level of certified seed, a level recommended for a wide range of developing countries (Osborn and Bishaw 2009). Since 2002, almost all quality seed has been distributed each year to farmers at subsidized rates. MAIL took the leading role in seed distribution and coordination in the past two years and may continue doing so in the foreseeable future with the aim of widely promoting the attributes of certified seed among farmers and encouraging them to buy directly from the private sector.

Strategic seed multiplication of rust resistant wheat varieties

Recent fears of *Pgt* race Ug99 have raised serious concerns about vulnerability of the nation's wheat crop, which already suffers significant losses from stripe rust in years of severe disease incidence. Fortunately, the existence of a functional seed system, including a growing private sector, provides a good opportunity for responding quickly to the race Ug99 threat by enabling rapid seed multiplication of new resistant varieties and making them available for distribution to farmers across Afghanistan.

For example, as part of the preparedness for a rust outbreak, 1.5 tonnes of early generation seed of Moqawim-09 (Misr-1) were imported from Egypt in 2009 for rapid multiplication. Although received and planted late in the season, a combined total of 26.9 tonnes of seed of three classes were produced in 2010 (5 tonnes breeder seed, 8.2 tonnes foundation seed and 13.7 tonnes registered seed) in addition to 14 tonnes of the other resistant varieties. On the basis of the favorable performance of Misr-1 in the first year, a further 150 tonnes of Registered Seed were imported by the U.S. Government in 2010, and was distributed in all regions of 19 provinces and among 55 enterprises as shown in Table 3.

Table 3 *Allocation of Ug99 resistant seed for 2010/11 sowing*

Region	Province	Seed allocation (tonnes)	No of recipient enterprises	No of contract growers
Northern	Balkh	16	6	93
	Samangan	2	1	5
	Faryab	4	2	12
	Jozjan	2	1	8
	Saripul	2	1	5
Sub total		26	11	115
Central	Kabul	7	2	20
	Parwan	6	2	14
	Kapisa	4	2	12
	Logar	3	2	8
Sub total		20	8	54
North-Eastern	Takhar	8	3	50
	Baghlan	19	7	90
	Kunduz	22	6	80
	Badakhshan	2	2	30
Sub total		51	18	250
Western	Herat	23	6	130
Sub total		23	6	130
Southern	Helmand	5	2	15
	Kandahar	5	2	10
Sub total		10	4	25
Eastern	Jalalabad	16	6	75
	Laghman	2	1	15
	Kunar	2	1	16
Sub total		20	8	106
Grand total		**150**	**55**	**680**

A theoretical calculation in Table 4 shows how fast and to what extent the new rust resistant variety could spread in the country assuming a multiplication factor of 30 and a recommended seeding rate of 100 kg/ha. The results indicate that with the injection of 150 tonnes into the seed system in 2010 it is possible by the fall of 2012 to have about 50% of the total national wheat seed requirement accounted for by new rust resistant varieties covering almost the entire irrigated wheat area in the country.

Besides Moqawim-09, foundation seed is being multiplied of other recently released varieties such as Baghlan-09 and Chonte-10. In addition, efforts are ongoing to increase genetic diversity by releasing more resistant varieties in the near future, multiplying seed fast and making it available as widely as possible.

Table 4 *Potential seed production and area coverage with Moqawim-09 at a seeding rate of 100 kg/ha*

Year	Seed sown/ produced (tonnes)	Cultivated area (ha)
2010 (injection)	150	1,500
2011	4,500	45,000
2012	135,000	1,350,000

Promoting the use of rust resistant wheat seed

The fact that up to 50% of Afghanistan's irrigated area has been covered by improved varieties over time during the long period of conflict and limited capacity of the national extension service is an indication that Afghan farmers are responsive to the introduction of new varieties that possess superior attributes and bring significant net benefit. While most farmers are aware of yield losses from stripe rust, only a small proportion may have heard about the threats and potential damaging effects of Ug99 stem rust. The success of any extension message in this regard would be judged by the ability to alert farmers adequately while avoiding unnecessary panic.

In Afghanistan, the obvious initial point of contact for the promotion of new rust resistant varieties would be the seed-producing enterprises and their contract growers in farming communities, who receive the first stocks of new seed for further multiplication. The main vehicles by which these private enterprises would demonstrate the features of new varieties are on-farm demonstrations and farmers' field days. These events are effective as they often bring together the key stakeholders, including local government leaders and officials, regional MAIL staff, NGOs, enterprise members and contract growers, university/vocational staff and students and farmers. During 2010, a field day was organized by each of 23 enterprises and attended by up to 4,000 participants, most of whom were small farmers (FAO 2010). Participation is expected to increase in the 34 field days planned for 2011 (FAO 2011), which will include significant demonstrations of rust resistant varieties particularly Moqawim-09.

Apart from the MAIL extension service which is currently limited in capacity there are other ongoing extension efforts, including activities organized by the private seed enterprises, NGOs and other development agencies. A key to effective awareness creation about rust resistance and the availability of new varieties and seed will require synergy from close collaboration and coordination of the various extension undertakings throughout the country.

Challenges in promoting the use rust resistant wheat seed

Despite the obvious potential benefits, there are several challenges involved in producing varieties with rust resistance, making them widely available to Afghan farmers, and in convincing them to adopt the new seeds. Amongst key challenges are the following:

1. Although Afghan farmers show responsiveness to new varieties, the process of adoption is gradual and takes time. Therefore, striking a good balance between creating adequate awareness about Ug99 and avoiding a panic amongst farmers will be critical.
2. Rust resistance of new varieties by itself will not be sufficient but should come in combination with other attributes such as enhanced yield potential, earliness and suitability of wheat flour for bread making. Only new varieties having such combined merits would be relatively easy to promote, but still challenging since the farming communities have been using, and are accustomed to, other improved varieties for many years.
3. Assuming a rapid adoption rate, the large-scale seed injection of one variety (Moqawim-09) would soon result in genetic uniformity across large areas thus creating ideal conditions for future epidemics in the event of a breakdown in resistance. The introduction of appropriate genetic diversity through rapid seed multiplication and distribution in combination with vigorous extension are immediate challenges for the wheat sector.
4. Direct sales and purchase of certified seed of existing varieties is already a problem and it is not likely this pattern will change significantly with the introduction of new rust resistant varieties. The choice between faster diffusion through subsidized certified seed and uncertain seed sales at cost-covering prices will be crucial. However, a strategic protection of the nation's wheat crop from the threat of Ug99 through some form of subsidized seed distribution would seem justified.
5. Identifying new rust resistant varieties for rainfed areas and replacing local varieties already in the hands of farmers will be a slow and difficult process.
6. Moving certified seed over long distances especially to remote locations has proved difficult in the prevailing uncertain security situations although the spread of seed production enterprises nationwide has somewhat eased this problem.

Conclusions

On the whole, the integrated approach being implemented has the potential of making new rust resistant wheat varieties more readily available to Afghan farmers. This will be achieved by establishing a close link between the source of new varieties and an accelerated seed multiplication system involving a network of 95 small-scale private seed enterprises

throughout the country. This will be done in line with established principles according to which stakeholder contingency planning is used in defining appropriate roles and responsibilities as well as relevant resources for rapid variety release, seed multiplication and distribution to counter the threat of wheat rusts. The importation into Afghanistan of a large quantity of early generation seed of a resistant variety already released in the country has fitted well into this concept and made a significant difference with the promise of a greater impact within a relatively short time. However, there is need for care and caution in reducing the genetic diversity and thereby increasing the risk of more damaging epidemics in the future. It is hoped that while the variety development and seed multiplication components take hold, there will be sufficient time to strengthen the other less developed disease management and extension components of the integrated approach such that eventual seed diffusion becomes smoother and much more efficient. Finally, the success of the integrated approach will very much depend on coordination, planning and information sharing among the wide range of stakeholders from agricultural research, plant protection services, the national seed association and seed enterprises, public sector seed production services, seed quality certification services, agricultural extension services, farmers' cooperatives, NGOs and donor agencies.

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References

FAO (2010) Trust Fund Programme Progress Report (July – December 2010) for Variety and
Seed Industry Development (Project GCP/AFG/045/EC). FAO, Kabul, Afghanistan
FAO (2011) Trust Fund Programme Annual Work Plan 2011 for Variety and Seed Industry
Development (Project GCP/AFG/045/EC). FAO, Kabul, Afghanistan
Kugbei S, Shahab S (2010) Efficiency of wheat seed production and scope for crop
diversification in Afghanistan. FAO, Rome, Italy
Lal SB (1973) Plant diseases in Afghanistan. Publication 2, Plant Protection Association of
Afghanistan, Kabul, Afghanistan
MacKenzie D (2008) Killer wheat fungus threatens starvation for millions. New Scientist
2647:14-15
Mumtaz S, Khan IA, Ali S, Zeb B, Iqbal A, Shah Z, Swati ZA (2009) Development of RAPD
based markers for wheat rust resistance gene cluster (Lr37-Sr38-Yr17) derived from
Triticum ventricosum L. African J Biotech 8:1188-1192
Nazari K, Mafi M, Yahyaoui A, Singh RP, Park RF (2009) Detection of wheat stem rust
(Puccinia graminis f. sp. tritici) race TTKSK (Ug99) in Iran. Plant Dis 93:317
Osborn T, Bishaw Z (2009) Principles for rapid variety release, seed multiplication and
distribution in developing countries to counter the threat of wheat rust. In: McIntosh R (ed)
Proc Oral Papers and Posters, 2009 Technical Workshop, BGRI, Cd Obregón, Sonora,
Mexico, pp179-188
Osmanzai M, Sharma RC, Ghanizada G, Ahmadzada Z (2008) Wheat rusts in Afghanistan – an
assessment of occurrence and cultivar resistance. Karana Scientific and Professional Publication of MAIL, Kabul 38:52-54
Torabi M, Nazari K (1998) Seedling and adult plant resistance to yellow rust in Iranian bread
wheats. Euphytica 100:52-54
Tunwar N (2004) End of assignment report: GCP/AFG/018/EC and GCP/AFG/025/GER
projects. FAO, Kabul, Afghanistan