Wheat Rust in Asia: Meeting the Challenges with Old and New Technologies

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Abstract
The rust diseases of wheat pose a constant threat to sustainable wheat production and thus food security in Asia. If susceptible wheat cultivars are grown, approximately 60 and 40 million hectares could experience periodic epidemics of leaf rust and stripe rust, respectively. Avoiding major rust epidemics in the region is a complex challenge, given that fewer cultivars are being cultivated over large areas, that several of those cultivars are protected by the same resistance genes, and that there is free movement of new virulent races in most of west and south Asia. Monitoring the evolution and movement of new rust races and diversifying cultivars sown in the region based on genetic information could help reduce losses, provided newly susceptible cultivars are withdrawn quickly. Using race-specific resistance genes in combinations could enhance their longevity. Linked DNA markers show promise for achieving such combinations; however, a national and regional deployment strategy is necessary to achieve long-term success. The most promising long-term control strategy is to breed and deploy cultivars carrying durable resistance based on minor, slow rusting genes with additive effects. CIMMYT studies show that combining 4-5 such genes results in a high level of resistance, comparable to immunity. Traditional genetic and molecular mapping studies have demonstrated high genetic diversity for such minor genes, but significant investment is necessary to identify closely linked markers that could be used effectively in marker-assisted selection. Targeted incorporation of durable resistance genes into mega-cultivars grown in Asia by using a 'single-backcross selected-bulk breeding scheme' is being pursued at CIMMYT with encouraging results.

Media summary
Breeding and deploying wheat cultivars with durable rust resistance should provide a long-term solution to leaf and stripe rust diseases of wheat in Asia.

Key words
Puccinia triticina, Puccinia striiformis, Triticum aestivum, resistance,

Introduction

Of the total area (approximately 215 million hectares) sown to hexaploid and tetraploid wheat (*Triticum aestivum* and *T. turgidum* var. *durum*) worldwide, 44% (95 million hectares) is in Asia. Of this, 62 million ha are located in just three countries: China, India, and Pakistan. However, food security and production stability are of paramount importance in most Asian countries, given that a majority of farmers are poor. The rust diseases of wheat have historically been one of the major biotic production constraints both in Asia and the rest of the world. Stem (or black) rust caused by *Puccinia graminis* has been under control since the semidwarf spring wheats of the green revolution, which are stem rust resistant, occupied most of the area in South and West Asia in the 1960s. Leaf (or brown) rust caused by *Puccinia triticina* and stripe (or yellow) rust caused by *Puccinia striiformis* continue to pose a major threat to wheat production over a large area. Leaf rust and stripe rust could affect production on approximately 60 (63%) and 43 (46%) m ha, respectively, in Asia (Table 1), if susceptible cultivars were grown there.

Although the timely application of fungicides can provide adequate control, their use adds to production costs and they are considered unfriendly to the environment. Growing resistant cultivars is thus the most effective and efficient control strategy, as it has no cost to farmers and is environmentally safe. Significant variation for virulence to specific resistance genes in populations of highly specialized rust fungi, combined with the rapid evolution of new virulence through migration, mutation, or recombination and selection of existing virulences, have complicated and will continue to complicate the control strategy.

In this paper we attempt to look critically at past successes and failures in controlling the rust diseases in Asia and will suggest what could be done better in the future to meet the enormous challenge of protecting over 95 m ha of wheat in Asia.

Table 1. Wheat area[1] in different countries of Asia where the environment favours the development of leaf rust and stripe rust.

		Estimated rust-prone area (%)		Estimated rust-prone area (m ha)	
Region/country	Area (m ha)	Leaf rust	Stripe rust	Leaf rust	Stripe rust
East Asia					
China	24.00	50	40	12.0	9.6
Mongolia	0.26	80	0	0.2	0.0
North Korea	0.08	80	0	0.1	0.0
South Asia					
Bangladesh	0.70	100	0	0.7	0.0
India	26.95	80	35	21.6	9.4
Myanmar	0.10	100	0	0.1	0.0
Nepal	0.64	80	30	0.5	0.2
Pakistan	8.35	80	70	6.7	5.8
West Asia					
Afghanistan	2.08	20	80	0.4	1.7
Iran	5.47	20	80	1.1	4.4
Iraq	1.52	60	30	0.9	0.5
Saudi Arabia	0.45	80	0	0.4	0.0
Syria	1.68	20	80	0.3	1.3
Turkey	9.30	30	80	2.8	7.4
Yemen	0.10	10	100	0.0	0.1
Central Asia					
Kazakhstan	11.00	90	10	9.9	1.1
Kyrgyzstan	0.46	100	0	0.5	0.0
Tajikistan	0.33	100	100	0.3	0.3
Turkmenistan	0.51	100	100	0.5	0.5
Uzbekistan	1.30	80	80	1.0	1.0
Total	95.27	63	46	60.0	43.4

[1] Modified from Aquino et al. (2002).

Defining the problem

The nature of rust fungi

Rust fungi are obligate parasites and must survive on living plants. Survival during the off-season occurs on either self-sown (or voluntary) wheat plants or other grass species that can also be infected. High-input, irrigated agriculture and the existence of cool highlands in different parts of Asia promote the carryover of inoculum between seasons. Moreover, conditions favorable to disease development (i.e., temperature and high humidity leading to dew formation) occur most years throughout the growing season. The presence of races capable of overcoming different resistance genes, or their combinations, has been demonstrated for all three rust fungi; numerous races are now known to occur worldwide. New races may arise through sexual recombination (not known for *P. striiformis*), mutation, or somatic hybridization followed by selection if a new race has a selective advantage. Being an airborne pathogen, new races may also be introduced into a new area through migration. A *P. striiformis* race with virulence for resistance gene *Yr9* recently migrated from the Eastern African highlands to South Asia, through West Asia (Figure 1). This suggests that the entire wheat area in Asia (except China) may comprise a single epidemiologic zone. Therefore, if a new race arises anywhere in this area, given time it could spread throughout the epidemiologic region. It should be noted that the *Yr9* virulence in China evolved independently in 1985 (Figure 1), as many winter wheat cultivars grown in China at that time carried this gene.

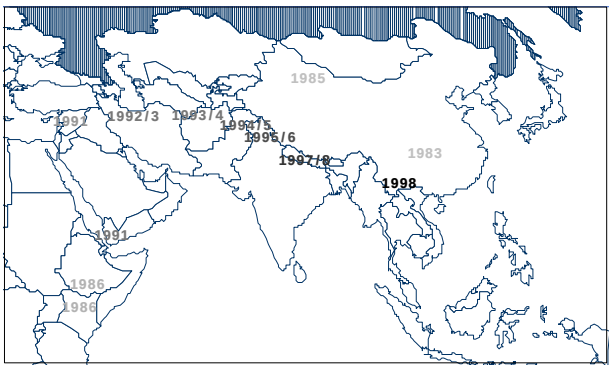

Figure 1. Movement of *Yr9*-virulent race of *Puccinia striiformis* from the East African highlands to the Indian Subcontinent and an independent evolution of Yr9-virulent race in China.

Lack of appropriate races and optimum disease pressure at breeding sites
The probability of identifying resistant parents and resistant progenies is increased through the use of a reliable screening methodology and an environment favorable to disease development. Protocols for screening for resistance to rust diseases are well established (Roelfs et al. 1992). For example, using artificial inoculation with desired races, inclusion of resistant and susceptible check cultivars to assess disease pressure and degree of resistance, and choosing appropriate field sites with reliable environmental conditions, are crucial to making continuous breeding progress. Unfortunately, we have observed that several breeding programs in Asia currently depend on natural epidemics and, as a result, in most years selection is done under insufficient rust pressure or with inappropriate races. Although stem rust is a major disease in Asia historically, it should be noted that practically no one in Asia currently selects wheat for stem rust resistance, as this disease is not considered important at present. This is a precarious situation, as highly virulent *P. graminis* races from Eastern Africa could migrate to Asia as has happened with stripe rust.

Deployment of cultivars with hypersensitive, race-specific resistance genes
With the discoveries of the genetic basis of resistance by Biffen (1905), physiological specialization in rust pathogens by Stakman and Levine (1922), and gene-for-gene interaction by Flor (1956), the utilization of the hypersensitive (race-specific) type of resistance has dominated wheat improvement during the last 50 years. Identifying numerous new sources of resistance genes either in wheat or in related species and genera, and their transfer to wheat through wide hybridization supported the strategy. Utilization of such genes is very attractive, both from the crop cleanliness point of view and because they are simple to incorporate into improved germplasm. Although it must be acknowledged that rusts were controlled effectively through the use of race-specific genes, their use led to “boom-and-bust” production cycles where varieties succumb in a short time to a new virulent race of the pathogen, leading to significant losses. Combining effective race-specific genes is an attractive strategy for increasing the longevity of this resistance but, to be successful, their deployment has to be planned at both the regional and global levels, as the pathogen is known to accumulate virulences for the combined resistance genes in a stepwise manner, if these genes are also deployed singly. Furthermore, when race-specific resistance is used in breeding, a parallel investment is required to monitor the evolution of pathogen and pests, and to continually search for new, effective resistance genes.

In recent years the use of DNA marker-assisted incorporation of multiple race-specific resistance genes has been suggested and is being attempted by a few breeding programs. DNA markers are currently known for only a few resistance genes (Table 2). Unfortunately, the options for selecting such genes is further narrowed down as several genes are now ineffective or the markers are not close enough. Perfect markers are available only for resistance genes located on chromosome segments that were transferred to wheat from alien species or genera. How many such chromosome segments could be put together in a single genotype without having an adverse effect on grain yield and quality remains undetermined.

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Table 2. Race-specific genes for resistance to leaf rust and stripe rust for which linked DNA markers are reported in the literature and also given in McIntosh et al. (2003).

Gene	Ch. location	Marker type	Marker designation	Origin
Leaf rust				
Lr1	5DL	RFLP/SSR	Xpsr567, Xgwm272	*T. aestivum*
Lr9	6BL	STS	J13/1, J13/2	*Ae. umbellulata*
Lr10	1AS	STS	F1.2245, Lr10-6/r2	*T. aestivum*
Lr13	2BS	RFLP/RAPD		*T. aestivum*
Lr19	7DL	STS	STSLr19130	*Th. elongatum*
Lr20	7AL	STS	STS638-L, STS638-R	*T. aestivum*
Lr21	1DL,1DS	STS	KSUD14	*Ae. tauschii*
Lr23	2BS	RFLP/RAPD	*Xtam72-2BS*	*T. aestivum*
Lr24	3DL	SCAR	SC-H5	*Th. elongatum*
Lr25	4BS	RFLP/RAPD		*Secale cereale*
Lr26	1BL-1RS	SCAR	IB267, iag95	*Secale cereale*
Lr27	3BS	RFLP/RAPD		*T. aestivum*
Lr28	4AL	STS	Lr28-01, Lr28-02	*Ae. speltoides*
Lr29	7DS	RFLP/RAPD		*Th. Elongatum*
Lr31	4BL	RFLP/RAPD		*T. aestivum*
Lr35	2B	STS	BCD260F1, 35R2	*Ae. speltoides*
Lr37	2AS, 2NS	PCR, CAPS, SNP	VENTRIUP-LN2, URIC-LN2,	*Ae. ventricosa*
Lr39/Lr41	2DS	SSR	*Xgdm35*	*Ae. tauschii*
Lr47	7AS	CAPS	PS10R, PS10L	*Ae. speltoides*
Lr50	2BL	SSR	*Xgwm382, Xgdm87*	*T. timopheevii*
Stripe rust				
Yr5	2BL	RGAP	*Xwpg17* and *Xwpg18*	*T. spelta album*
Yr9	1BL-1RS	STS	IB267, iag95	*Secale cereale*
Yr10	1BS	RGAP		*T. aestivum*
Yr15	1BS	SSR	Xgwm11	*T. dicoccoides*
Yr17	2AS	PCR, CAPS, SNP	VENTRIUP-LN2, URIC-LN2,	*Ae. ventricosa*
Yr28	4DS	RFLP		*Ae. tauschii*
Yr32	2AL	SSR		*T. aestivum*

The diversity of resistance in Asia can be maintained by growing cultivars that carry different resistance genes. However, there is a general tendency for farmers to grow only one or a few favored cultivars, which, as a result, come to occupy large areas. An example of this is cultivation of varieties 'PBW343' and 'Inqualab 91' on 7 and 6 million hectares in India and Pakistan, respectively. These cultivars are also grown in other countries under different names. Unfortunately, both cultivars are protected from stripe rust by the same resistance gene *Yr27*, for which virulence was detected recently in Asia. Growing fewer cultivars that carry race-specific resistance genes thus leads to greater genetic uniformity and, consequently, greater disease vulnerability.

Strategies to safeguard the wheat crop in Asia

The wheat crop can be protected from rust, or at least the occurrence of epidemics in Asia could be reduced, by emphasizing the following three strategies: 1) regional cooperation in monitoring the evolution and migration of new races of rust fungi, 2) enhanced information on the genetic basis of resistance in important wheat cultivars, and 3) shift towards breeding and deploying wheat cultivars with durable resistance.

1. Regional cooperation in monitoring the evolution and migration of new races of rust fungi

Monitoring the evolution and migration of new, virulent races of *P. triticina* and *P. striiformis* should be a high priority in Asia, especially because 1) the spores of these fungi can move freely over long distances (Fig. 1), 2) several current widely grown cultivars carry race-specific resistance genes, 3) the same cultivars are being grown in more than one country, and 4) the same genes confer resistance to several

cultivars grown in different countries. Political tensions in the region often do not permit scientists in some countries to collaborate and communicate with each other directly and hence the presence of a politically neutral institution such as CIMMYT is important to coordinate such effort. It may also be necessary to test cultivars at hot-spot locations outside the region, as virulence for certain resistance genes may already be present in the pathogen population. Virulence for stripe rust resistance gene *Yr9* was known in China, Eastern Africa, and Central America in the mid 1980s, long before epidemics occurred on cultivars carrying *Yr9* in West and South Asia. Similarly, virulence for gene *Yr27*, present in two leading cultivars in India and Pakistan, was present in Mexico since the mid 1990s. Such testing is useful in determining the cultivars' level of background susceptibility and identifying additional resistant cultivars at a low cost in a short time. This could alert national programs to a forthcoming problem. CIMMYT currently coordinates a rust monitoring network in Asia, Africa and South America. Based on information gathered through the network, CIMMYT predicted that cultivars PBW343 and Inqualab 91 would become susceptible to *Yr27*-virulent races of stripe rust in Asia.

2. Enhanced information on the genetic basis of resistance in important wheat cultivars
Promoting cultivars that have distinct resistance genes can enhance genetic diversity in farmers' fields. Generation of such information often has low priority, as conducting detailed genetic analysis is time consuming and often considered academic. Several Asian countries lack proper greenhouse facilities, trained personnel, or the financial resources needed to conduct such research. A simple technique is to test cultivars as seedlings in the greenhouse with an array of diverse races of known avirulence/virulence combinations and to use the host-pathogen interaction data to postulate the presence of known resistance genes (Singh and Rajaram 1991). Further testing in the field of adult plants with known races at selected sites in Asia, outside the region, and at laboratories in specialist institutions could help determine the effectiveness of such seedling resistance genes and the presence of additional adult-plant resistance. Often this kind of testing, combined with pedigree information, may be enough to enhance knowledge of genetic diversity, but genetic analysis may be necessary to confirm the gene postulations. If DNA markers become available for the most important, useful resistance genes, they could be used for characterizing the presence of such genes in cultivars in a relatively short time and at little cost. Currently the major limitation is the lack of tightly linked DNA markers for several resistance genes and the presence of non-catalogued race-specific genes in wheat, at least for stripe rust resistance. If this strategy is to be applied successfully, significant investment will be needed to generate such markers.

3. Shift towards breeding and deploying cultivars with durable resistance
The phenomenon of the erosion of race-specific resistance genes, or their combinations, has led scientists to look for alternative approaches to resistance management. The multilineal approach promoted by Jensen (1952) and Borlaug (1953) emerged out of frustrations associated with the frequent failure of race-specific genes. Van der Plank (1963) was the first epidemiologist to clearly define the theoretical basis of concepts of resistance. In the late 1960s and 1970s, there was a revival of the concept of general (race-nonspecific) resistance and its application in crop improvement (Caldwell 1968). This approach was advocated for breeding stem rust resistance in wheat by Borlaug (1972), leaf rust resistance by Caldwell (1968) and yellow rust resistance by Johnson (1988). The wide-scale application of such a concept in breeding for leaf rust resistance, commonly known as slow rusting, has dominated in CIMMYT's bread wheat improvement for almost 30 years with major impacts (Marasas et al. 2002). Today, we understand better the genetic basis of durable resistance to rust diseases, and this knowledge is being applied in breeding at least at CIMMYT. The genetic basis of durable resistance to three rusts is described below. We believe that deployment of such resistance will provide a long-term genetic solution to rust control in Asia and other countries.

Sr2 and other minor genes for durable resistance to stem rust
Resistance gene *Sr2*, in addition to other unknown minor genes derived from cultivars Hope and H-44, provided the foundation for durable resistance to stem rust in germplasm from the University of Minnesota in the USA and from Sydney University in Australia, and in the spring wheat germplasm developed by Dr. N.E. Borlaug as part of a program sponsored by the Mexican Government and the Rockefeller Foundation. Cultivar Yaqui 50, released in Mexico during the 1950s, and other *Sr2*-carrying wheats released since then have stabilized the stem rust situation in Mexico and other countries where modern semidwarf wheats were adopted. Changes in stem rust races have not been observed in Mexico in recent years and natural infections are non-existent. Released in 1960 in the Indian Subcontinent and

subsequently grown on millions of hectares, the cultivar Sonalika has also remained resistant. When present alone, the *Sr2* gene confers slow rusting that is not adequate under heavy disease pressure, but does provide adequate resistance in combination with other minor genes. Unfortunately, not much is known about the other genes in the *Sr2* complex and their interactions. Knott (1988) has shown that adequate levels of multigenic resistance to stem rust can be achieved by accumulating approximately five minor genes. In his studies the genes were different from *Sr2*.

Lr34, Lr46 and other minor genes for durable resistance to leaf rust

The South American cultivar Frontana is considered one of the best sources of durable resistance to leaf rust, (Roelfs 1988). The Mexican-Rockefeller Program first used the variety in the 1950s. Later derivatives such as Penjamo 62, Torim 73, and Kalyan/Bluebird showed slow rusting characteristics possibly derived from Frontana. Genetic analysis of Frontana and several CIMMYT wheats possessing excellent slow rusting resistance to leaf rust worldwide has indicated that such adult plant resistance is based on the additive interaction of *Lr34* and two or three additional slow rusting genes (Singh and Rajaram 1992). Leaf rust severity observed in Mexico on most slow rusting cultivars was related to the number of minor genes they carry (Figure 2). When susceptible cultivars display 100% leaf rust severity, cultivars with only *Lr34* display approximately 40% severity; cultivars with *Lr34* and one or two additional minor genes display 10-15% severity; and cultivars with *Lr34* and two or three additional genes display 1-5% severity (Table 3). Leaf rust may increase to unacceptable levels on cultivars carrying only *Lr34*, or *Lr34* and one or two additional genes. However, cultivars with *Lr34* and two or three additional genes show a stable response in all environments tested so far, with final leaf rust ratings lower than 10%. The presence of *Lr34* can be indicated by the presence of leaf tip necrosis in adult plants, which is closely linked with it (Singh 1992a).

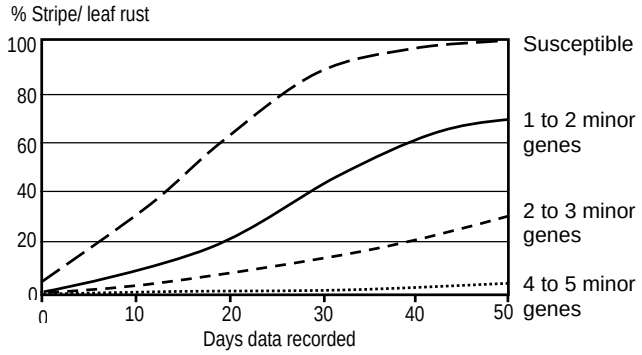

Figure 2. Relationship between the progress of stripe/leaf rust and the number of minor genes present in a wheat cultivar.

Rubiales and Niks (1995) studied the infection process and indicated that slow rusting resistance due to gene *Lr34* was based on a reduced rate of haustorium formation in the early stages of infection, in association with relatively little, or no, cell necrosis. Electron microscopic studies by Alvarez-Zamorano (1995) showed an accumulation of unknown electro-dense substances in the cells of *Lr34* lines near the site where haustorial mother cells try to dissolve the cell wall of mesophyll cells for the formation of haustoria. It appears that the accumulation (cell wall apposition) causes a thickening of cell walls, and reduces the establishment of the haustorial tube. If haustoria are formed, slow mycelial growth may be due to restricted movement of the fungus from one cell to another by a similar phenomenon. Alvarez-Zamorano (1995) also observed structural change in the *Lr34* line leading to invagination, or contraction of the cell wall, which may delay the completion of the infection process. These observations indicate a different mechanism for *Lr34*-based slow rusting compard with hypersensitivity, which is associated with the action of race-specific resistance .

Slow rusting resistance to leaf rust is common in spring wheat germplasm. Our studies have shown that at least 10-12 slow rusting genes are involved in the adult plant resistance of CIMMYT wheats. We have also identified lines, such as Amadina (Table 3), where *Lr34* is absent, but whose level of resistance is high. We therefore believe that durable resistance is feasible even in the absence of *Lr34.* This is the case of variety Pavon 76 (Table 3), where we have identified a new gene *Lr46* for slow rusting on

chromosome 1BL (Singh et al. 1998, William et al. 2003a). Gene *Lr46* also functions in a similar manner to *Lr34* (Martinez et al. 2001).

Table 3. Seedling susceptible bread wheats that carry adult plant resistance to leaf rust in field trials at Mexico and other countries.

Genotype(s)	Usual leaf rust response[1]	Additive genes[2] for resistance
Jupateco 73S	100S(N)	Susceptible (check)
Jupateco 73R	50MSS	*Lr34*
Nacozari 76	30MSS	*Lr34*+ 1 gene
Sonoita 81, Bacanora 88, Rayon 89	20MSS	*Lr34* + 1 or 2 genes
Frontana, Parula, Trap, Tonichi 81	10MSS	*Lr34* + 2 or 3 genes
Chapio, Tukuru, Kukuna, Vivitsi	1MSS	*Lr34* + 3 or 4 genes
Pavon 76, Attila	40MSS	*Lr46* + 1 gene
Amadina	5MSS	4 genes

[1] Leaf rust response evaluated in Mexico has two components: % severity based on the modified Cobb scale (Peterson et al. 1948) and reaction based on Roelfs et al. (1992). The reactions are: MSS = moderately susceptible to susceptible, i.e., medium- to large-sized uredinia without chlorosis or necrosis; S = susceptible, i.e. large uredinia without chlorosis or necrosis; N = necrotic leaves following high leaf rust severity.
[2] Minimum numbers estimated from genetic analysis.

Yr18, Yr29 and other minor genes for durable resistance to stripe rust

Singh (1992b) and McIntosh (1992) indicated that the moderate level of durable adult plant resistance to stripe rust of the CIMMYT-derived US wheat cultivar Anza and winter wheats such as Bezostaja is controlled in part by the *Yr18* gene. This gene is completely linked to the *Lr34* gene. The level of resistance it confers is usually not adequate when present alone. However, combinations of *Yr18* and 3-4 additional slow rusting genes result in adequate resistance levels in most environments (Singh and Rajaram 1994). Cultivars carrying such *Yr18* complexes are listed in Table 4. Genes *Lr34* and *Yr18* occur frequently in germplasm developed at CIMMYT and in various countries. The recently identified slow rusting gene *Yr29* is completely linked to gene *Lr46*, which confers moderate resistance to leaf rust (William et al. 2003a).

Table 4. Seedling susceptible bread wheats that carry adult plant resistance to stripe rust in field trials at Mexico and other countries.

Genotype(s)	Usual stripe rust response[1]	Additive genes for resistance[2]
Jupateco 73S	100MS	Moderately susceptible (check)
Jupateco 73R	50M	*Yr18* + *Yr30*
Parula, Trap, Cook	15M	*Yr18*+ 2 genes
Tonichi 81, Sonoita 81, Yaco	10M	*Yr18* + 2 or 3 genes
Chapio, Tukuru, Kukuna, Vivitsi	1M	*Yr18* + 3 or 4 genes
Pavon 76, Attila	20M	*Yr29* + 2 genes
Amadina	30M	3 genes

[1] Yellow rust response data from Mexico has two components, % severity based on modified Cobb scale (Peterson et al. 1948) and reaction based on Roelfs et al. (1992). The reactions are M = moderately resistant to moderately susceptible, sporulating stripes with necrosis and chlorosis; and S = sporulating stripes without chlorosis or necrosis.
[2] Minimum numbers estimated from genetic analysis.

Because stripe rust can develop systemically, it is different from the other two rusts, where every new pustule develops from a new infection. The epidemiology of stripe rust is also different from that of the other two rusts. Johnson (1988) presented examples of adult plant resistance genes that are race-specific in nature. It is difficult to distinguish such resistances from the resistances conferred by race-nonspecific adult plant resistance genes . Low disease severity to stripe rust is most often associated with at least some reduction in infection type. However, we have observed that in the case of potentially durable slow rusting resistance, the first uredinia to appear are moderately susceptible to susceptible. Subsequent growth of the fungal mycelium causes some chlorosis and necrosis; therefore, the final infection type is usually rated as moderately resistant-moderately susceptible. Durability of such resistance can be expected if the cultivar's low disease severity is due to the additive interaction of several (4 to 5) partially effective genes.

Advances in identifying molecular markers for slow rusting, minor genes
Significant advances have been made in characterizing genes that confer resistance to biotic stresses in several crops, thanks to the use of molecular markers. Although molecular markers have been successfully applied to characterize race-specific resistance genes, finding markers closely associated with durable resistance genes is comparatively more challenging. Because several minor genes are needed in a cultivar to achieve adequate protection under high disease pressure, the mapping populations generated often segregate for a number of these genes as quantitative trait loci (QTLs). Usually populations of doubled haploid (DH) or nearly homozygous recombinant inbred lines (RILs) are used for mapping in wheat. Initial efforts aimed at identifying QTLs for durable rust resistance were documented by Nelson et al. (1997) involving linkage mapping using the ITMI reference population, and by William et al. (1997) using bulked segegant analysis (Michelmore et al. 1991) in a CIMMYT spring wheat cross. PCR-based markers such as microsatellites (SSRs) and amplified fragment length polymorphisms (AFLPs) has made it possible to have more success in characterizing adult resistance genes for a number of diseases including leaf rust and yellow rust. The advantages of using DH or RIL populations is that an identical set of segregating material can be screened under multiple environments, providing more reliable data with which to estimate environmental interactions. At CIMMYT, we have used full linkage mapping as well as bulked segregant analysis with multiple populations to determine the number and genomic locations of genes conferring durable resistance and to identify molecular markers with strong linkages with resistance alleles. Other research groups have also used mapping involving spring and winter wheats to identify minor genes for resistance to the rust diseases.

Populations used in marker application efforts hitherto and the resulting QTLs for adult plant-effective slow rusting resistance genes for leaf rust and yellow rust, as well as their relative effects and genome locations, are given in Table 5. Known slow rusting resistance genes for leaf rust and yellow rust include *Lr34/Yr18*, *Lr46/Yr29*, and *Yr30*. Nelson et al. (1997) using RFLPs first associated molecular markers with *Lr34/Yr18* on the ITMI reference mapping population. The ITMI population exhibited considerable polymorphism as it is derived from the cross of the spring wheat cultivar 'Opata 85' and a synthetic hexaploid wheat. However, these markers did not show polymorphism in several other mapping populations involving spring wheat cultivars at CIMMYT. The availability of a large number of SSR markers with adequate coverage of the wheat genome and other marker systems such as AFLPs has helped to associate markers with genes such as *Lr34/Yr18* (Suenaga et al. 2003). These have subsequently been validated in other populations (William et al. 2003b; Schnubusch et al. 2003). Using molecular markers, we have shown the presence of loci that reduce leaf rust and yellow rust severities simultaneously (William et al. 2003b). The presence of at least some common loci facilitates the accumulation of sufficient minor genes to achieve resistance to both rust diseases. The relative effects of minor genes vary widely, ranging from about 5 to 50% of phenotypic variations (Table 5). The effectiveness of utilizing markers to manipulate these genes depends on finding closely linked or flanking markers for each gene. Because there seems to be a multitude of these resistance genes in the wheat genome, current efforts could concentrate on further characterization of genes with larger effects on more than one disease, so that they can be manipulated and deployed effectively in breeding as soon as possible. Understanding their function and developing cloning strategies for such genes would also enhance their utilization through transformation aimed at overexpression from a single copy or introgression of multiple copies at different genomic location.

Breeding for durable resistance
Breeding for durable resistance based on minor additive genes has been challenging and often slow, for several reasons: 1) a sufficient number of minor genes may not be present in a single source genotype, 2) a source genotype may be poorly adapted, 3) there may be confounding effects from the segregation of both major and minor genes in the population, 4) crossing and selection schemes and population sizes are more suitable for selecting major genes, 5) reliable molecular markers for several minor genes are unavailable, and 6) the cost associated with identifying and utilizing multiple markers, is high. One approach suggested in the literature is to use recurrent selection schemes to accumulate several minor genes in a single genetic background. Such selection schemes have often been of scientific interest rather than actually applied in wheat. Selection for resistance alone will not generate important popular cultivars, unless it is simultaneously combined with other traits such as high yield and quality. However,

such germplasm carrying combinations of minor genes should be very useful in transferring these genes to adapted local cultivars.

Table 5. Quantitative trait loci (QTLs) reported in the literature to reduce wheat leaf rust and stripe rust severities .

	QTL (No.)			Range of effect
Cross	Lr[1]	Yr[2]	Chromosome location	(% R2)
Parula x Siete Cerros[3]	3	-	1BS, 7B, 7DS	10 – 27 (Lr)
Frontana x INIA66[4]	4	4	LR: 1BL*, 3DL, 5B, 7DS*	5 – 46 (Lr)
			YR: 1BL*, 2BL, 3BS, 7DS*	4 – 25 (Yr)
Avocet x Pavon 76[5]	3	5	LR: 1BL*, 4B*, 6A*	7 – 50 (Lr)
			YR: 1BL*, 4B*, 6A*, 6B, 3BS	9 – 35 (Yr)
Avocet x Parula[5]	3	3	LR: 1BL*, 7B, 7DS*	10 – 58 (Lr)
			YR: 1BL*, 3BS, 7B, 7DS*	12 – 64 (Yr)
Avocet x Tonichi 81[5]	3	6	LR: 7DS*, 7D*, 7B? *	10 – 56 (Lr)
			YR: 3BS, 6B, 7B*, 7D*, 7DS*, AFLP[12]	10 – 24 (Yr)
Fuko-kumughi x Oligoculm[6]	2	7	LR: 1BL, 7DS*	15 – 41 (Lr)
			YR: 3BS, 4BL, 4DL, 5BL, 6B, 7BS, 7DS*	2 – 13 (Yr)
Opata 85 x Synthetic wheat[7]	7	5	LR: 2AL, 2B, 2DS, 3BS, 7BL, 7DS*	9 – 33 (Lr)
			YR: 3BS, 3DS, 4DS, 5DS, 7DS*	13 – 20 (Yr)
Camp Remy x Michigan Amber[8]	-	2	YR: 2A, 2B	11 – 46 (Yr)
CD87 x Katepawa[9]	-	3	YR: 1BL, 2DS, 7D	9 –15 (Yr)
Forno x Oberkulmer[10]	7	-	1BS, 2B, 3A, 4B, 4DL, 5DL, 7B	7 – 35 (Lr)
Arina x Forno[11]	8	-	1BS, 2AL, 2DS, 2DL, 4BS, 7BS, 7BL, 7DS	9 – 43 (Lr)

[1]Leaf rust, [2]Stripe (yellow) rust, [3]William et al. (1997), [4]Khairallah et al. (unpublished), [5]William et al. (2003a &b), [6]Suenaga et al. (2003), [7]Nelson et al. (1997) and Singh et al. (2000b), [8]Boukhatem et al. (2002), [9]Bariana et al. (2001), [10]Messmer et al. (2000), [11]Schnurbusch et al. (2003), [12] Not chromosomally located.
* Positive effects in reducing severities of both leaf and stripe rusts.

A successful example of breeding for resistance based on minor genes is the resistance to leaf and stripe rusts in wheat, which took about 30 years of continuous effort at CIMMYT. In the early 1970s, S. Rajaram, influenced by the concepts of slow rusting resistance to leaf rust in wheat advocated by R. Caldwell and partial resistance to late blight of potato demonstrated by J. Niederhauser, made a strategic decision to initiate selection for slow rusting resistance to leaf rust in CIMMYT spring wheat germplasm. In the early phase of breeding he maintained plants and lines in segregating populations that showed 20-30% rust severity with compatible (susceptible) infection types. This strategy led to the release of several successful wheat cultivars, such as Pavon 76 and Nacozari 76, in Mexico and other countries. These slow rusting lines were used heavily in the crossing program and resulted in the wide distribution of minor genes within CIMMYT spring wheat germplasm.

The genetic basis of such resistance started to become clear in the early 1990s. High-yielding lines that combined four or five additive, minor genes for both leaf rust and stripe rust and show near-immune levels of resistance were developed in the 1990s. To achieve this, three or four lines carrying different minor genes were crossed (3-way and 4-way crosses), and plants in large segregating populations were selected under artificially created rust epidemics. Races of pathogens that have virulence for race-specific resistance genes present in the parents were used to create the epidemics (Singh et al. 2000a). The resulting highly resistant lines are now being used in a planned manner to transfer these minor resistance genes to cultivars that are currently grown over large areas but have become susceptible to rust races in Mexico. Based on genetic information on the number of additive, minor genes that must be transferred to achieve the desired level of resistance, the crossing and selection scheme described below was developed

and applied. This strategy has allowed simultaneous transfer not only of resistance genes but also other quantitative traits that increase the yield potential or improve the grain quality of an adapted cultivar.

Incorporation of rust resistance based on additive, minor slow rusting genes into adapted wheat cultivars
To transfer minor genes based resistance into a susceptible adapted cultivar or any selected genotype, we use a single backcross-selected bulk scheme, where the cultivar/genotype is crossed with a group of 8-10 resistance donors (some listed in Tables 3 and 4); 20 spikes of the F_1 plants from each cross are then backcrossed to obtain 400-500 BC_1 seeds. Selection is practiced from the BC_1 generation onwards for resistance and other agronomic features under high rust pressure. Because additive genes are partially dominant, BC_1 plants carrying most of the genes show intermediate resistance and can be selected visually. About 1600 plants per cross are space-grown in the F_2, whereas population sizes of about 1000 plants are maintained in the F_3-F_5 populations. Plants with desirable agronomic features and low to moderate terminal disease severity in early generations (BC_1, F_2 and F_3), and plants with low terminal severities in later generations (F_4 and F_5) are retained. We use a selected-bulk scheme where one spike from each selected plant is harvested and bulked until the F_4 generation. Plants are harvested individually in the F_5. Bulking of selected plants poses no restriction on the number of plants that can be selected in each generation, as harvesting and threshing are quick and inexpensive, and the next generation is derived from a sample of the bulked seed. Because high resistance levels require the presence of 4 to 5 additive genes, the level of homozygosity from the F_4 generation onwards is usually sufficient to identify plants that combine adequate resistance with good agronomic features. Moreover, selecting plants with low terminal disease severities under high disease pressure means that more additive genes may be present in those plants. Selection for seed characteristics is carried out on seeds obtained from individually harvested F_5 plants. Small plots of the F_6 lines are then evaluated for agronomic features and homozygosity of resistance, before conducting yield trials.

Resistant derivatives of several cultivars, including PBW343 and Inqualab 91, were recently developed using the above methodology. In each case we identified derived lines that not only carry high levels of resistance to leaf rust or yellow rust or both, but also show about 5-15% higher yield potential than the original cultivars. We believe this approach to wheat improvement allows us to maintain the characteristics of the original cultivar while improving yield potential and rust resistance. We are taking the improved versions of the original cultivars back to the areas where they were/are grown to achieve long-term control of rusts. It should be noted that having minor gene based resistance in several backgrounds should ease future selection for these resistance genes.

Availability for tightly linked DNA markers in the future can be useful in maintaining and diversifying the combinations of additive slow rusting resistance genes in the wheat germplasm and cultivars. The actual use of multiple markers in breeding strategy at CIMMYT is likely be limited to the characterization and selection of parents to be used in specific crosses as the field screening is very reliable and cost-effective. However, if such genes need to be incorporated in adapted cultivar that contains an effective race-specific resistance gene, then markers are the only option and will be used despite the cost.

Prospects of genetic engineering in rust control
Another area of biotechnology that has potential in wheat cultivar development is the use of genetic engineering approaches. The successful deployment of maize and cotton containing *Bt* genes is well known. Several experiments utilizing other gene constructs such as the coat protein (*Cp*) gene of rice stripe virus introduced into rice (Hayakawa et al. 1992) and *thaumatin-like* protein gene introduced into wheat (Chen et al. 1999) have achieved increased levels of protection from plant pathogens. Although genetically modified wheat has not yet been grown commercially, the area under other genetically modified crops continues to increase. Cloning of resistance genes, especially those that confer durable or broad-spectrum disease resistance, could open new possibilities in wheat improvement. Resistance genes, when cloned and used for transformation, may show over-expression of resistance (Horvath et al. 2002). This strategy could be used to improve the effectiveness of individual slow rusting resistance genes that have minor effects. Also, the insertion of multiple copies of such minor genes could lead to better resistance. However, it is worthwhile mentioning that transformation techniques will need further refining, since at present only a few wheat genotypes can be easily transformed. These genotypes are not the currently grown cultivars, and hence at present any transformed gene will need to be transferred to desired cultivars through traditional or DNA marker-assisted backcrossing and rigorous field-testing.

Conclusion

Rust diseases of wheat can be successfully controlled in Asia through a combination of strategies. Regional cooperation is essential for monitoring the evolution of new races and following their migration path. Enhanced information on the genetic basis of resistance will be necessary to maintain genetic diversity in farmers' fields. Traditional and molecular genetics research to further enhance the understanding of slow rusting resistance based on minor, additive genes should receive high priority in the future. The targeted transfer of durable resistance into widely grown genotypes and the subsequent deployment of their derivatives is an attractive strategy for achieving long-term rust control.

References

Alvarez-Zamorano R (1995) Patogenesis de *Puccinia recondita* Rob. Ex Desm. f. sp. *tritici* y la resistencia en trigo. Ph.D. thesis. Colegio Postgrad., Montecillos, Mexico. 76pp.

Aquino P, Carrion F, Calvo R (2002) Selected wheat statistics. In CIMMYT 2000-2001 World Wheat Overview and Outlook: Developing No-Till Packages for Small-Scale Farmers. (Ed. J. Ekboir) pp. 52-62. (CIMMYT, Mexico DF).

Bariana HS, Hayden MJ, Ahmed NU, Bell JA, Sharp PJ, McIntosh RA (2001) Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Aust. J. Agric. Res. 52, 1247-1255.

Biffen RH (1905) Mendel's law of inheritance and wheat breeding. J. Agric. Sci. 1, 4-48.

Borlaug NE (1953) New approach to the breeding of wheat varieties resistant to *Puccinia graminis tritici*. Phytopathology 43, 467 (Abstr.).

Borlaug NE (1972) A cereal breeder and ex-forester's evaluation of the progress and problems involved in breeding rust resistant forest trees: "Moderator's Summary". Biology of Rust Resistance in Forest Trees. Proc. of a NATO-IUFRO Advanced Study Institute. Aug. 17-24, 1969. USDA Forest Service Misc. Publ. 1221, pp. 615-642.

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.

Caldwell RM (1968) Breeding for general and/or specific plant disease resistance. In Proc. 3rd Int. Wheat Genetics Symp.(Ed. K.W. Finlay and K.W. Shephard) pp. 263-272. (Aust. Acad. Sci., Canberra, Australia).

Chen WP, Chen PD, Liu DJ, Kynast R, Friebe B, Velazhahan R, Muthukrishnan S (1999) Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene. Theor. Appl. Genet. 99, 755-760.

Flor H H (1956) The complementary genic systems in flax and flax rust. Adv. Genet. 8, 29-54.

Hayakawa T, Zhu Y, Itoh K, Kimura Y, Izawa T, Shimamoto K, Toriyama S (1992) Genetically engineered rice resistant to rice stripe virus, an insect-transmitted virus. Proc. Natl. Acad. Sci. 89, 9865-9869.

Horvath H, Rostoks N, Brueggeman R, Steffenson B, von Wettstein D (2003) Genetically engineered stem rust resistance in barley using the *Rpg1* gene. PNAS 100, 364-369.

Jensen N F (1952) Intervarietal diversification in oat breeding. Agron. J. 44, 30-34.

Johnson R (1988) Durable resistance to yellow (stripe) rust in wheat and its implications in plant breeding. In Breeding Strategies for Resistance to the Rusts of Wheat. (Ed. N.W. Simmonds and S. Rajaram) pp. 63-75. (CIMMYT, Mexico).

Knott DR (1988) Using polygenic resistance to breed for stem rust resistance in wheat. In Breeding Strategies for Resistance to the Rusts of Wheat. (Ed. N.W. Simmonds and S. Rajaram) pp. 39-47. (CIMMYT, Mexico).

Marasas, CN, Smale M, Singh RP (2002) The impact of agricultural maintenance research: the case of leaf rust resistance breeding in CIMMYT-related spring bread wheat. CD-ROM Proc. Int. Conf. on Impacts of Agricultural Research and Development, San Jose, Costa Rica, 4-7 Feb. 2002 (CIMMYT, Mexico)

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.

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

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R (2003) Catalogue of gene symbols for wheat. In Proc. 10th Int. Wheat Genetics Symposium (Ed. NE Pogna, M Romano, EA Pogna and G Galterio). Vol.4, pp. 1-34 and associated CD-Rom. (S.I.M.I., via N. Nisco 3/A-00179 Roma, Italy).

Messmer MM, Seyfarth R, Keller M, Schachermayr G, Winzeler M, Zanetti S, Feuillet C, Keller B (2000) Genetic analysis of durable leaf rust resistance in winter wheat. Theor. Appl. Genet. 100, 419-431.

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. 88, 9828-9832.

Nelson JC, Singh RP, Autrique JE, Sorrells ME (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci. 37, 183-285.

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can. J. Res. Sect. C. 26, 496-500.

Roelfs AP (1988) Resistance to leaf rust and stem rust in wheat. In Breeding Strategies for Resistance to the Rusts of Wheat. (Ed. NW Simmonds and S Rajaram). pp. 10-22. (CIMMYT, Mexico).

Roelfs AP, Singh RP and Saari EE (1992) Rust diseases of wheat: concepts and methods of disease management. 81pp. (CIMMYT, Mexico).

Rubiales D, Niks RE (1995) Characterization of *Lr34*, a major gene conferring nonhypersensitive resistance to wheat leaf rust. Plant Dis. 79, 1208-1212.

Schnurbusch T, Paillard S, Schori A, Messmer M, Schachermayr G, Winzeler M, Keller B (2003) Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region. In Proc. 10th Int. Wheat Genetics Symposium (Ed. NE Pogna, M Romano, EA Pogna and G Galterio). Vol.3, pp. 1251-1253. (S.I.M.I., via N. Nisco 3/A-00179 Roma, Italy).

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, Rajaram S (2000a) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. Acta Phytopathlogica Hungarica 35, 133-139.

Singh RP, Kazi-Mujeeb A, Huerta-Espino J (1998) *Lr46*: a gene conferring slow rusting resistance to leaf rust in wheat. Phytopathology 88, 890-894.

Singh RP, Nelson JC, Sorrells ME (2000b) Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Sci. 40, 1148-1155.

Singh RP, Rajaram S (1991) Resistance to *Puccinia recondita* f. sp. *tritici* in 50 Mexican bread wheat cultivars. Crop Sci. 31, 1472-1479.

Singh RP, Rajaram S (1992) Genetics of adult-plant resistance to leaf rust in 'Frontana' and three CIMMYT wheats. Genome 35, 24-31.

Singh RP, Rajaram S (1994) Genetics of adult plant resistance to stripe rust in ten spring bread wheats. Euphytica 72, 1-7.

Stakman EC, Levine MN (1922) Analytical key for the identification of physiologic races of *Puccinia graminis tritici.* (Processed) Div. of Cereal Crops and Dis., U.S. Dept. Agr. and Minn. Agr. Exp. Sta. 7 pp.

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.

Vanderplank JE (1963) “Plant Diseases: Epidemics and Control”. Academic Press New York and London.

William HM, Hoisington D, Singh RP, Gonzalez-de-Leon 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, Ortiz-Islas S, Hoisington D (2003a) Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93, 153-159.

William HM, Singh RP, Huerta-Espino J, Suenaga K, Palacios G, Ortiz-Islas S, Hoisington D (2003b) Characterization of slow rusting genes for resistance to leaf rust and yellow rust in CIMMYT spring wheats. In Proc. 10th Int. Wheat Genetics Symposium (Ed. NE Pogna, M Romano, EA Pogna and G Galterio). Vol.2, pp. 858-860. (S.I.M.I., via N. Nisco 3/A-00179 Roma, Italy).