

## BIOTIC STRESS RESISTANCE IN WHEAT – BREEDING AND GENOMIC SELECTION IMPLICATIONS

E. Todorovska, N. Christov, S. Slavov, P. Christova, D. Vassilev  
AgroBioInstitute, Sofia, Bulgaria  
Correspondence to: Elena Todorovska  
E-mail: elenatod@mail.bg

### ABSTRACT

Leaf and stem rust are among the most important diseases in wheat. The selection of wheat genotypes with combination of non-race-specific genes defining durable resistance over years as well as race specific genes at seedling stage is a task of high importance for breeding programs. Gene pyramiding using conventional method is difficult and time-consuming because it requires simultaneous tests of the same wheat breeding materials with several different rust races before making selection. DNA-based molecular markers have several advantages over the traditional phenotype trait selection. Molecular markers can be used to tag rust resistance genes and further to be used in improvement the efficiency of selection in plant breeding by marker-assisted selection (MAS). MAS is a powerful alternative to facilitate new gene deployment and gene pyramiding for quick release of rust-resistant cultivars. An approach called “genomic selection” and based on the widespread conventional selection with the use of information of the molecular markers will facilitate breeding programmes by providing effective achievement of biotic stress resistance reducing in mean time generation interval and investments in ecological-friendly crop production is reviewed.

**Keywords:** Wheat, Leaf rust, Stem rust, Resistance, Molecular markers, Gene pyramiding, Marker-assisted selection (MAS)

Biotechnol. & Biotechnol. Eq. 2009, **23**(4), 1417-1426

### Introduction

Rice, maize and wheat provide 60% of the energy consumed as food in the world making them the most important food crops. The world consumption of wheat was 687 million tons in 2005. Wheat accounted for 19% of total production among major cereal crops. This was exceeded only by coarse grains and maize. Among the most important diseases in wheat that significantly reduce wheat production are those caused by the rusts (leaf, stem, and stripe) (**Fig. 1**). The rusts of wheat are among the most important plant widespread pathogens that can be found in most areas of the world where wheat is grown.

New pathogenic races are usually developed by mutation and selection for virulence against rust resistance genes in wheat. In recent years, new races of wheat leaf rusts, wheat stripe rust and wheat stem rust have been introduced into wheat growing areas in different continents. These introductions have complicated the efforts of breeders to develop wheat cultivars with durable resistance and have significantly reduced the number of the effective rust resistance genes available at present. This high degree of specificity has made for example durable rust resistance in wheat difficult to achieve because the virulence of wheat rust fungi against wheat resistance genes is highly diverse, resulting in the existence of many different pathogenic races. The specific interactions between resistance genes in wheat and avirulent genes in the pathogen serve as extremely useful markers for characterizing rust populations.

The most efficient, cost-effective and environment-friendly approach to prevent the losses caused by rust epidemics is the BIOTECHNOL. & BIOTECHNOL. EQ. 23/2009/4

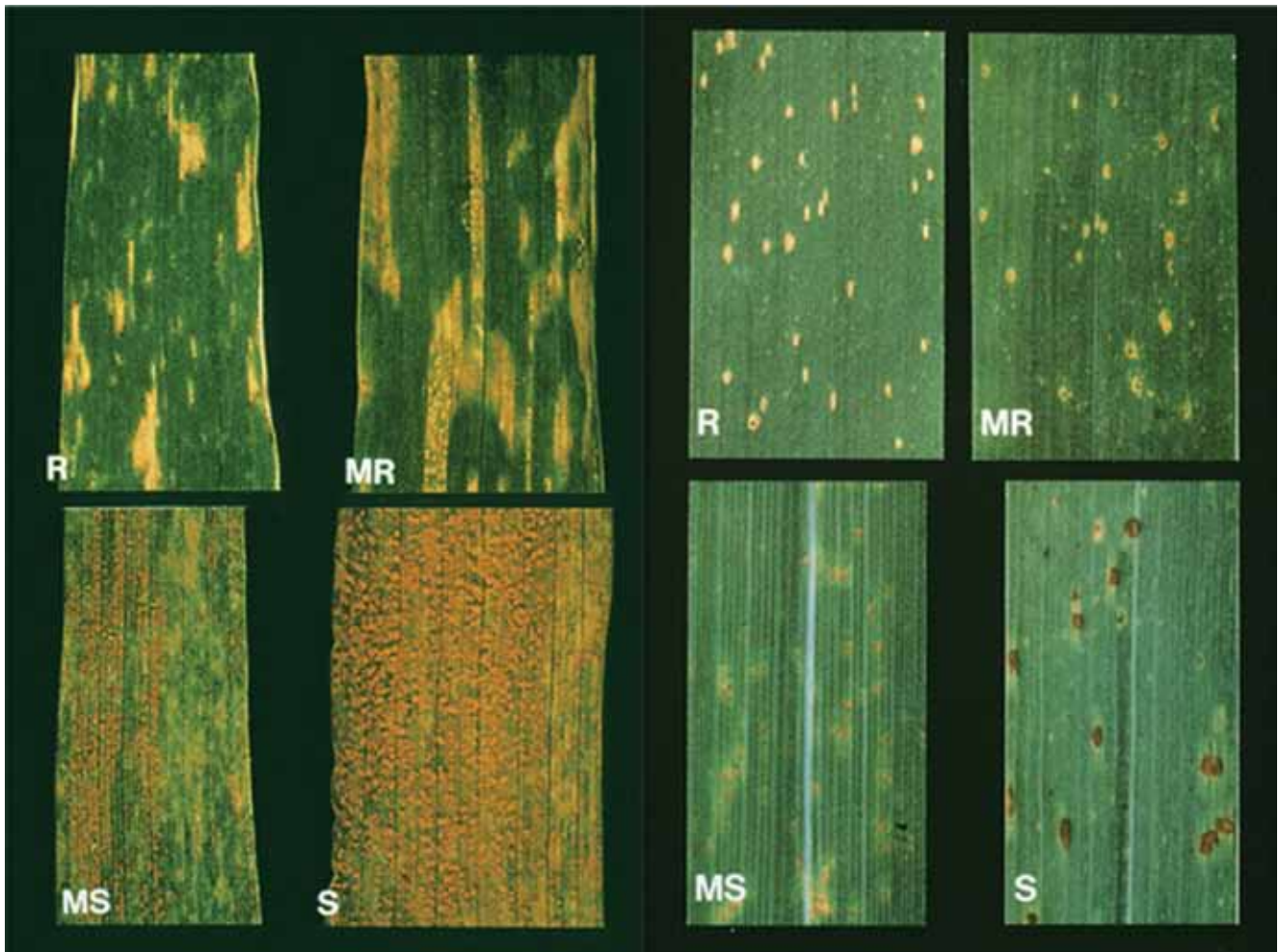
development of genetic resistance to biotic stress. The use of cultivars with single-gene resistance permits the selection of mutations at a single locus to render the resistance effective in a relatively short time. However, due to loss of variation and selection pressure, and evolution, new virulent races of the fungus appear, which increase the need to develop durable resistance. Hence, the use of combinations of resistance genes has been suggested as the best method for genetic control of leaf and other rusts (74). This can be achieved by pyramiding effective resistance genes, but expression of individual resistance genes is difficult to monitor in the field.

With the advent of molecular marker technology now it seems to be possible to solve such complex problems. DNA-based molecular markers have several advantages over the traditional phenotype trait selection. Molecular markers can be used to tag rust resistance genes and further, they can serve for improvement of the efficiency of selection in plant breeding by so called, marker-assisted selection (MAS). The potential benefits of MAS have been widely discussed (2, 63), especially for the solutions that are provided by them to overcome some of the problems faced by classical phenotypic screening approaches in plant breeding programs.

The current review shows that most of the wheat cultivars do not have an adequate level of resistance to leaf rust, indicating the need for incorporating more effective and stem rust resistance genes into the local bread wheat cultivars.

### Leaf and stem rust resistance in wheat

Wheat leaf rust is caused by *Puccinia triticina* Eriks, wheat stem rust by *Puccinia graminis* f. sp. *tritici*, and wheat stripe rust by *Puccinia striiformis* f. sp. *tritici*. Leaf rust occurs more regularly and in more world-wide regions than stem rust or stripe rust of wheat. Yield losses in wheat from *P. triticina*



**Fig. 1.** Relative resistances of wheat to stripe (left) and leaf rust (right). R: resistant; MR: moderately resistant; MS: moderately susceptible; and S: susceptible. Source: Rust Scoring Guide, Research Institute for Plant Protection, Wageningen, Netherlands

infections are usually the result of decreased number of kernels per head and lower kernel weight and it may reach 40% in susceptible cultivars (32). Yield losses caused by the stem rust pathogens in the mid of 20<sup>th</sup> century reached 20-30% in Eastern and Central Europe (95) and many other countries including Australia, China and India (42). The yield losses caused by the most virulent stem rust race Ug99 emerged first in Uganda and after that in Kenya, Ethiopia, Yemen, in Middle East and South Asia and losses were estimated to approximately USD \$ 3 billions (<http://www.seedquest.com/News/releases/2007/january/18117.htm>).

The disease-causing wheat rust fungi are spread in the form of clonally produced dikaryotic urediniospores, which can be dispersed by wind for thousands of kilometers from initial infection sites across different continents and oceans. Epidemics of wheat rusts can occur on a continental scale due to the widespread dispersal of urediniospores (74) Wheat rust fungi are highly specific obligate parasites. Their avirulent genes interact with resistance genes in wheat in a gene-for-gene manner (24, 70).

Rust populations can be characterized by distribution of races and the frequencies of virulence against specific

rust resistance genes on a defined set of wheat differential hosts. The avirulence genes that are present reflect only a small proportion of the total genetic variation found in rust populations, but this variation is subject to intense selection by the resistance genes in commonly grown wheat cultivars. Selectively neutral markers such as isozymes or more recently developed molecular markers, such as random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP), can also be used to characterize and compare rust populations. As the wheat rust fungi are spread easily within and between continents, it is essential to document the genetic changes in rust populations over large geographic areas in order to facilitate the development of rational strategies or durable resistance.

Molecular markers provide opportunities to tag rust resistance genes and as part of MAS to be used in improvement the efficiency of selection in plant breeding. For example, to facilitate breeding for durable resistance to stem rust, molecular markers are useful tools for the development of resistant cultivars and, especially, for pyramiding of resistance genes for several diseases (2).

As a major tool for advanced selection purposes - MAS can be used at an early stage of plant development in order to screen several genes simultaneously. To date more than 120 Lr and Sr genes have been found in wheat. Molecular markers linked to race-specific and slow leaf rusting resistance genes: Lr1, Lr3, Lr9, Lr10, Lr13, Lr19, Lr21, Lr23, Lr24, Lr25, Lr27, Lr28, Lr29, Lr31, Lr34, Lr35, Lr37, Lr39, Lr46, Lr47, Lr50 and Lr51 (9) (<http://maswheat.ucdavis.edu>), have been identified. Molecular markers are also available for some Sr genes such as: Sr9a, Sr24, Sr25, Sr36, Sr 40 (88, 89, 93) (<http://maswheat.ucdavis.edu>).

Some of these are STS markers that can be directly used for MAS, and facilitate the pyramiding of Lr genes (6), while others have the potential to be converted into STS markers. The identified markers are valuable for breeding durable leaf rust resistant cultivars by combining race-specific and race-nonspecific resistance genes.

## **Distribution of leaf and stem rust pathogens and disease cycles**

### **Wheat leaf rust (*Puccinia triticina*)**

Wheat leaf rust is the most common and widely distributed of the three wheat rusts. Extensive annual surveys of wheat leaf rust virulence phenotypes are conducted in both Canada (53) and the US (39). Up to 70 different *P. triticina* races are identified in the US using a standardized set of near-isogenic lines of Thatcher wheat (37). Annually 30-50 races are identified in France, (26), while 10-15 races are detected in Australia (65).

In the US, virulent leaf rust resistance races increase very quickly in response to the widespread use of wheat cultivars with race specific resistance genes. As the leaf rust population is very large it would be expected that random mutations occur in a sufficient number to produce new virulent races. Soft red winter wheats from the southeastern US bearing the leaf rust resistance genes Lr9, Lr11, Lr18, Lr24 and Lr26 are infected by selected races that have virulence against these genes (36). Similarly, races with virulence against Lr3ka, Lr11, Lr24, and Lr26 have been selected by the hard red winter wheat cultivars from the southern Great Plains region of Texas, Oklahoma, and Kansas since the late 1980s (45, 46). Races with virulence against Lr2a and Lr16 have been found in the northern Great Plains, where hard red spring wheats with these genes are grown. Introduction from distant sources of new races with virulence to resistant genes in commonly grown wheat cultivars can also occur. Races with virulence to Lr17, Lr3bg and LrB became common in the southern Great Plains of the US in the mid of 1990s. A combination of the molecular data and the virulence data supported the hypothesis that these races have been introduced to the Great Plains of US, most probably from Mexico or the Pacific Northwest and have not been derived by mutation from previously existing populations (35). In 2001, a new race of leaf rust with high virulence to durum wheat has been detected in northwestern Mexico (81). This

leaf rust race is avirulent against many of the resistance genes present in common hexaploid wheat but it is virulent to most durum wheat cultivars from Europe, South America, Mexico and California. The high degree of virulence and the observed simple sequence repeat (SSR) genetic similarity among the *P. triticina* isolates from the world-wide durum wheat collection suggested that this race might have been introduced to Mexico from other durum-growing areas of the world or might have evolved by mutation and selection in the durum wheat fields of Mexico (41).

The distribution of leaf rust races over continents has been confirmed also by the observation of the new races in Australia and New Zealand which are geographically separated from other major wheat-producing areas of the world. New leaf rust races from other continents have been introduced into these countries numerous times. Two new leaf rust races have been introduced subsequently in 1981 and 1984 in New Zealand and Australia. Additional races obtained by mutation from the original introduction increased rapidly in frequency throughout eastern and western Australia and New Zealand, displacing races that had been common before 1984. Of the six leaf rust races identified in 2003 or 2004 in Australia, all were derived by mutation from the race introduced in 1984. In 2000, a leaf rust race with virulence against Lr24 has been detected in Australia for the first time (64) although wheat cultivars carrying the resistance gene had been grown since 1983. The emergence of the Lr24-virulent race could be explained by mutation and selection from a previously existing race. It is remarkable that Lr24-virulent races were not detected previously in Australia. In the US, virulence against Lr24 appeared within a few years after the introduction of winter wheat cultivars with Lr24 in the southern Great Plains (47). In Australia, almost all released wheat cultivars are highly resistant to leaf rust, and the effective population size of *P. triticina* in which mutation might occur is greatly reduced. The delay of the emergence of Lr24-virulent leaf rust races in Australia might reflect to a reduced probability of mutation to Lr24 virulence in the Australian population. Many of the winter wheats grown in the southern US are susceptible to leaf rust, which allows a very large leaf rust population to survive, creating a reservoir for mutation and selection.

There was evidence for long-distance transfer of *P. triticina* in Europe, since four races accounted for 64% of all isolates in collections from Western Europe (66). Multiple isolates of the same race from different countries have been found to have identical RAPD banding patterns (67) providing further evidence of the long-distance transport of *P. triticina* isolates in Europe. A number of 105 races have been identified in France, Hungary, Italy, Bulgaria and Poland in 1998 (61) with very few races in common between countries. Regional populations of *P. triticina* races can arise within a continent due to use of wheat cultivars with different leaf rust resistance gene composition in different regions. Isolates from Central Europe were found to have high frequencies of virulence against the Lr3, Lr3ka, Lr3bg, and Lr26 genes, whereas most isolates from

Western Europe were avirulent in relation to these genes (38). Cluster analysis of RAPD markers indicated that isolates from Great Britain, Italy, and Spain were more closely related to each other than to isolates from Hungary and Slovakia, which is an indication for the existence of at least two major groups of *P. triticina* in Europe. Isolates from South America had RAPD phenotypes that were nearly identical to those of isolates from western Canada, yet the two populations had different patterns of virulence (38). Similarity of RAPD phenotypes suggest that the Western Canadian and South American isolates of *P. triticina* might have derived from introductions of the same source, presumably from Europe, but the subsequent selection of these introductions by wheats that had different resistance genes might account for the dissimilarities in the virulence of *P. triticina* isolates from Western Canada and South America. Recently, the newly developed locus-specific and co-dominant SSR markers have been extensively used to study the *P. triticina* populations.

*Puccinia triticina* is a macrocyclic and heteroecious rust fungus with five spore stages and two taxonomically unrelated host species: telial such as wheat (hexaploid wheat - *T. aestivum* L., durum wheat - *T. turgidum* ssp. durum, wild emmer - *T. dicoccoides*, domesticated emmer wheat - *T. dicoccon*, triticale, common goatgrass - *Ae. cylindrica* and sometimes *Ae. speltoides*) and alternate, such as meadow rue - *Thalictrum* spp. As heteroecious rust fungus. *P. triticina* has an asexual and sexual life cycle. To complete its sexual life cycle *P. triticina* requires a second, alternate host - *Thalictrum* spp. which the parasite uses to overwinter. In places where *Thalictrum* does not grow, such as Australia and North America the pathogen will only undergo its asexual life cycle and will overwinter as mycelium or uredinia. In this case the disease spreads as windborne urediniospores which give rise the speculation that most probably the adaptations of the North American leaf rust races (virulences) occur only through genetic mutations. The sexual stage of *P. triticina* has been found on *T. speciosissimum* in southern Europe (8, 13, 14, 94). Infections on the alternative host *I. fumaroides* appear to be restricted to a region in Siberia (10). Allen (1) reported in 1932 that flexuous hyphae most commonly extend through stomatal guard cells of the lower leaf and that fertilization typically takes place on the lower leaf surface. *Puccinia triticina* is heterothallic and therefore pycniospores and flexuous hyphae originating from the same pycnium are not sexually compatible. Pycniospores emanate from the tip of the pycnium in a liquid 'nectar' exudate that attracts insects. The pycniospores are disseminated to different pycnia by insects and by movement in water such as dew and rain splashing. *P. triticina* can cycle indefinitely as uredinial infections on telial hosts such as wheat. In the southern Great Plains of the US, leaf rust infections that over-summer on volunteer wheat can serve as reservoirs of inoculum for the autumn-planted winter wheat. In winter wheat, leaf rust can over-winter as mycelial or uredinial infections in areas with suitable temperature conditions (74).

#### Leaf rust resistance genes in wheat

1420

The first world-wide genetic studies of leaf rust resistance in wheat have been conducted about a century ago by wheat researchers. The first one, (48) determined that the wheat cultivars Malakof and Webster had a gene that conditioned leaf rust resistance, later designated as Lr1 and Lr2, respectively (3). Later Soliman et al. in 1964 (85) mapped Lr genes through identification of the chromosomes that carried leaf rust resistance genes Lr1, Lr3 and Lr11. Allelic variation in Lr genes (three alleles at the Lr2 locus) has been firstly demonstrated by Dyck and Samborski in 1968 (20).

Currently there are 60 leaf rust resistance genes with permanent gene designations from Lr1 to Lr 60 (59). These genes have been found and characterized in common hexaploid wheat, tetraploid durum wheat and many diploid wild wheat species.

A significant number of Lr resistance genes originate from *Triticum aestivum* cultivars, but some resistance genes were initially introduced into common wheat cultivars from wild species accessions: *Aegilops umbellulata* – Lr 9, *Aegilops squarrosa* – Lr 21, Lr 22, Lr 32, Lr 39, Lr 40, Lr 41, Lr 42, Lr 43, *Agropyron elongatum* – Lr 19, Lr 24, Lr 29, *Aegilops speltoides* – Lr 28, Lr 35, Lr 36, Lr 47, or *Aegilops ventricosa* – Lr 37.

In hexaploid wheat, leaf rust resistance genes are widely distributed across the genome, being present on nearly everyone of the 42 chromosome arms. Four allelic variants have also been described and mapped to a specific loci on different chromosome arms. Genes Lr2a, Lr2b and Lr2c have been mapped on chromosome 2DS (56) while Lr3a, Lr3ka and Lr3g on chromosome 6BL (27). Genes Lr17a and Lr17b have been mapped to chromosome 2AS (19), and Lr22a and Lr22b on 2DS (78). The extremely tightly linked Lr14a and Lr14b (7BL) have been considered as alleles for all practical purposes by Dyck and Samborski (16).

It is thought that there is a gene-for-gene relationship between the leaf rust pathogen and the wheat host meaning that for a single resistance gene in wheat there is a single gene in the pathogen that makes it either virulent or avirulent on the host (40). Leaf rust resistance in wheat is due to major genes, which can provide adult or seedling resistance, and minor genes. Seedling resistance genes are effective for the whole lifecycle of the plant. Adult plant resistance genes are effective only in adult plants, but have been shown to be an important part of durable leaf rust resistance. Minor genes have effects that are too small to be identified individually, but are thought to be more durable than these of the major genes. They result in fewer, smaller pustules and longer latent periods (40).

Most leaf rust resistance genes determine effective resistance to specific races of *P. triticina*. Race-specific resistance is usually manifested by a hypersensitive response (HR) of rapid cell death that occurs at the interface between fungal haustoria and host cells in the epidermal and mesophyll layers. Different resistance genes determine specific resistance phenotypes or infection types. For instance, the resistance

response of wheat lines with Lr3 is characterized by clearly defined hypersensitive flecks while lines with Lr2a have only very light flecks that are difficult to see. Other race-specific resistance responses, such as those conditioned by wheat with Lr3ka, Lr3bg and Lr11, are manifested by small uredinia surrounded by chlorosis, and lines with Lr16 have small uredinia surrounded by necrosis. Resistance conditioned by these last genes is probably expressed at a later point in the infection and colonization process of *P. tritricina*. Race-specific Lr genes are effective in seedling plants and remain effective in the adult plant stage. However, the resistance conditioned by some genes, such as Lr12, Lr13 and Lr22a, is best expressed in adult plants. In wheat lines that have combinations of resistance genes, the gene with greatest resistant infection type is epistatic to genes with less resistant infection types. To date, three genes that confer race-specific resistance have been cloned: Lr1 and Lr10, originated from common wheat, and Lr21, from *T. tauschii* (11, 23, 29). These genes condition an HR response to isolates of *P. tritricina* that carry matching avirulence genes, and encode proteins with nucleotide binding site-leucine rich repeat (NBS-LRR) regions typical for most disease resistance genes in plants. Not all leaf rust resistance genes determine race-specific resistance. Gene Lr34 was first described as a modifier of adult plant resistance in the cultivar Frontana (22). This gene was found in a number of wheat lines from around the world (21) and was later mapped to chromosome arm 7DS (17). The resistance response conditioned by Lr34 does not involve HR, but instead is characterized by fewer and smaller uredinia with no chlorosis or necrosis on flag leaves of adult plants, often with a decreasing gradient of uredinia density from the leaf base to tip. Lr34 conditions the same resistance response to all isolates of *P. tritricina* that have been tested (i.e. non race-specific resistance), and is associated with non-HR resistance to stripe rust and powdery mildew (87). Gene Lr46 is another adult plant resistance gene originally found in the cultivar Pavon 76. Lr46 confers a resistance response of fewer and smaller uredinia, but with varying amounts of chlorosis in adult plants (84). Genes that confer non-HR resistance will probably differ both in DNA sequence and protein function compared with race-specific, NBS-LRR resistance genes. Genetic aspects of leaf rust resistance in wheat were previously reviewed by Kolmer (34).

### **Wheat stem rust (*Puccinia graminis*)**

Wheat stem rust epidemics occurred regularly from 1900–1955 in North America (33). Heavy epidemics in different years such as 1916, 1935, 1937, 1950–1954 and others caused massive yield losses in wheat. Since then, wheat stem rust has successfully been controlled in North America and most parts of the world by the use of highly resistant wheat cultivars, and by eradication of the barberry (*Berberis vulgaris*) alternate host. This has greatly reduced the number of stem rust races and the effective size of the *P. graminis* population, therefore directly reducing the chances of a virulence mutation occurring in an adapted *P. graminis* genotype. In recent years, relatively few races of wheat stem rust have been found in North

America (60). In the US, six highly discrete virulence groups of *P. graminis* have been described in the asexual population from the Great Plains using 16 host differential single gene lines (75). These virulence groups correlated very highly with patterns of isozyme variation at 13 loci (7). In 1989, a stem rust race that had high virulence against the stem rust resistance gene Rpg1 in barley appeared in the Great Plains region of the US and Canada (77). This race was avirulent to nearly all of the spring and winter wheats grown in North America, yet was virulent to nearly all of the barley cultivars that were tested. The new race differed for virulence against the resistance genes Sr6, Sr12, Sr13, and Sr33 when compared to the previously characterized virulence groups, and was virulent to the cultivar McNair. The stem rust isolates virulent to Rpg1 also differed from other stem rust races in their isozyme genotypes at two loci. It is likely that the stem rust race with virulence to Rpg1 was wind disseminated to the Great Plains region from a sexual population of *P. graminis* in the Pacific Northwest. The stem rust populations in North and South America are speculated to have originated by introductions from the same or similar sources in Europe because there is no clear difference between isolates from these regions for virulence patterns or RADP markers (52). Many wheat cultivars throughout the world have the stem rust resistance gene Sr31, which was introgressed into wheat on a translocated chromosomal fragment from rye. This gene has provided highly effective resistance for many years. Stem rust races with virulence against Sr31 were not detected despite the extensive cultivation of wheat with this gene. In 1999, high levels of stem rust infections caused by a new race of stem rust, Ug99 (also called TTKK) were found in Uganda on wheat genotypes that had the rye translocation and Sr31 (72). Subsequent tests confirmed that a new race of *P. graminis* with virulence against Sr31 had been found in central Africa (Kenya, Ethiopia, Yemen). The new phenotypes were also virulent against Sr38, for which virulence had never been previously detected. In 2004, heavy stem rust infections were observed on International Center for Wheat and Maize Improvement (CIMMYT)-derived lines of wheat in Kenya. The stem rust infections in Kenya were caused by the same race that had virulence against Sr31 and Sr38 originally detected in Uganda. More recently, Ug99 has spread to major wheat production areas of the Middle East, such as Iran, Afghanistan, India, Pakistan, Turkmenistan, Uzbekistan and Kazakhstan (<http://www.fao.org/newsroom/en/news/2008/1000805/>). The possible route of stem rust spread is proposed as East Africa - Middle East - West Asia - South Asia. The stem rust race from central Africa is also virulent to many of the wheats currently grown in Canada and the US. To overcome such infections and their distribution it is essential to develop germplasm that has effective resistance to this dangerous new race in all potentially affected regions.

### **Stem rust pathogen and disease cycles**

The parasitic fungus *Puccinia graminis* f.sp. *tritici*, causing stem rust of wheat is heteroecious which means that two unrelated hosts, such as wheat and barberry, are required to

---

complete its life cycle (<http://www.apsnet.org/education/lessonsPlantPath/StemRustWheat/default.htm>) (42). Wheat is the primary host where the pathogen spends most time, while barberry is the secondary host. The fungus can reproduce itself only in living host plants. The fungus has five types of spores at different developmental stages: pycniospores (spermatia), aeciospores, urediniospores (uredospores), teliospores, and basidiospores (43). The disease cycle starts with the exposure of the new wheat crop to stem rust inoculum. The source of the inoculum is different under different environments. In regions with warm climates, the volunteer plants carry the spores over summer and urediniospores from the infected volunteer wheat are the source of initial inoculum for wheat planted in the next fall to start a disease cycle (42). In the regions with a cold winter, aeciospores are the main source of initial inocula for wheat stem rust. Aeciospores are produced on barberry, the most common alternate host in the United States (<http://www.apsnet.org>) (42).

In wheat, rust infection mainly occurs on stems and leaf sheaths. Within two weeks after inoculation, a brick-red structure, called a rust pustule also known as a uredium containing urediospores, appears at the point of inoculation. In heteroecious rusts, urediospores can reproduce themselves and re-infect wheat multiple times (42), which can lead to severe damage on wheat production. In a later developmental stage, another type of spore called teliospore, which is a black overwintering spore, is produced in telia (12) to conclude the disease cycle of stem rust in wheat and to start a new life cycle in barberry. In the United States, barberry eradication has significantly reduced the number of epidemics for many years. Since common barberry has been largely removed after the 1920s, barberry bushes have not been a major source of disease inoculum (37) (<http://www.ars.usda.gov/Main/docs.htm?docid=10755>). In recent years, barberry plants have reappeared in a few original eradication sites in southeastern Minnesota, and it is still necessary to monitor the barberry's population and avoid rust infection from barberry (37). However, since *P. graminis* can survive in wheat in southern part of USA and Mexico, stem rust epidemics can still occur without the help of barberry. Urediniospores, as the major inoculum source can spread by wind along the coast of the Gulf of Mexico to infect the winter wheat in the central Great Plains, and eventually the spring wheat in the north Great Plains (42). In the southern areas, urediniospores from volunteer wheat plants infect local winter wheat seedlings in the fall to start a new disease cycle.

The development of stem rust in wheat is favored by warm temperature (18-30°C) with high relative humidity. The symptoms of the disease are visualized as erumpent uredia pustules mainly on the stems and leaf sheaths about 7 to 10 days after infection (43). The shape of pustules is oval or spindle-shaped, and the color is reddish brown. The symptoms on resistant plants range from no uredia to small or medium uredia surrounded by chlorosis or necrosis, while the

symptoms on susceptible plants include large uredia with or without chlorosis or necrosis (76).

### **Stem rust resistance genes in wheat**

To date, about fifty stem rust resistance genes have been identified and some of them have been mapped on different chromosomes in wheat and its relatives (57). All these genes are race specific except *Sr2* that has provided durable non-race-specific plant resistance to slow rusting adult (58, 79, 81, 85). Among these resistance genes, some genes deployed in commercial cultivars worldwide were effective individually or in combination with other *Sr* genes until recently. *Sr2*, derived from *Triticum turgidum* and located on chromosome 3BS, has been widely used in the wheat program in CIMMYT, Mexico for global improvement of wheat stem rust resistance and hard winter wheat breeding programs in the USA (85). Moreover, the *Sr2* complex in combination with other resistance genes showed effective protection against Ug99 (79).

### **Prevention of the diseases caused by leaf and stem rust pathogens**

Two main approaches are currently used to prevent the diseases caused by leaf and stem rust. Destruction of previous wheat plants and volunteer wheat, by tillage or herbicides (triazoles and strobilurins), is an important step in prevention of several diseases, including wheat streak mosaic, High Plains virus, barley yellow dwarf, and leaf and stem rusts. Eliminating the 'green bridge' between wheat crops will help prevent mechanisms of carryover from one growing season to the next. Crop rotation is also very helpful at reducing disease carryover because many diseases are host specific and proliferate when the same crop is planted year after year. Most disease cycles can be disrupted by as little as one year of rotation or fallow conditions. Several locations on the Southern High Plains have been 're-infected' from one year to the next by spores moving into the region from distant, more southerly areas where summer survival of rust is more likely. Therefore, even growers who take precautions to eliminate green bridge plants may experience infected fields due to the prolific spreading characteristic of the spores through wind.

One of the only, early prevention methods for avoiding rust diseases or minimizing their impact is to plant a variety with known resistance. Variety resistance is the most economical and environmentally safe method of control. To date more than 120 resistance genes to leaf, stem and stripe rust have been found in wheat. Many of them confer race-specific resistance in a gene-for-gene manner, and several are currently used by breeders to develop new cultivars. However, the resistance provided by these genes can be short-lived as new races of the rust pathogens, continuously evolving and acquire virulence against these genes. For example, varieties, formerly rated as 'resistant', have in recent years, begun to show signs of susceptibility at various locations. In many situations, the varieties remained resistant for only three to four years before showing signs of susceptibility. Large differences in susceptibility exist among wheat varieties. Some of them that

have exhibited resistance to leaf and stripe rust have been released in the breeding programs worldwide. This is not to imply that these varieties will always resist infection in every situation or that other varieties will exhibit susceptibility every time. Considering the high adaptability and rapid distribution of virulent isolates of rust pathogens over long distances the best strategy is to develop cultivars with good levels of protection under high disease pressure. For such purpose several “slow rusting” gene complexes such as Lr34 (19) and Lr46 (84) need to be combined with the race-specific genes. Although Lr34 and Lr46 provide durable and non-specific adult plant resistance their effect is more reduced than that of race-specific genes. Lr34 was recently cloned and it was shown that it is the same gene as Yr18 (resistance to adult plant stripe rust), powdery mildew resistance (*Pm38*) and leaf tip necrosis (*Ltn1*). This gene codes for a putative ABC transporter. A similar tight linkage or pleiotropic effect was observed between Lr46 and Yr29, also a slow rusting gene for stripe rust. Lr34 was first described in cultivar Frontana in 1966. It is located on the short arm of chromosome 7D, close to locus Xgwm295. The resistance phenotype displayed by this gene includes longer latent period, fewer uredina and smaller uredina size. Lr34 is tightly linked to the leaf tip necrosis (LTN) locus, and it is also possible that the LTN phenotype could be a pleiotropic effect of Lr34 itself. The Lr34 gene has been widely used in wheat breeding programs because of its durable resistance to leaf rust, its association with Yr18, a stripe rust resistance gene, and its association with tolerance to Barley yellow dwarf virus infection (55, 79). The combination of Lr34 with other genes, such as Lr12 and/or Lr13, provided durable leaf rust resistant cultivars worldwide (4, 51). In fact most of the identified durable leaf resistant cultivars carry Lr 34 genes, and other race-specific gene(s). The South American cultivar Frontana, which has been regarded as one of the best sources of durable resistance to leaf rust to leaf rust carries Lr 34, Lr13 and LrT3 (21). Chinese Spring, a popular wheat cultivar whose resistance to leaf rust has lasted for about a century in North America (40), carries Lr34, Lr12 (18), and Lr31 genes for leaf rust resistance (83). Adult plant resistance is of high importance in field resistance to leaf rust. For example, other genes such as Lr11, Lr12, Lr13, Lr22b, Lr35, and Lr37 have been reported also as adult plant resistance (APR) genes (37, 53, 62). Winzeler et al. (91) reported that 55% of European wheat cultivars had APR resulting from the activity of quantitative trait loci (QTL) and/or Lr34 that enhances resistance. Sixty out of 105 European wheat cultivars that were tested showed APR in the field (68). These genes could be detected by growing adult plants under controlled conditions and testing them by inoculation or by growing the genotypes in the field and monitoring disease severity in the growth period. Although information about the molecular principles of APR to *P. triticina* is limited, QTL studies in segregating populations have been initiated to characterize APR genes for leaf and stripe rust (*P. striiformis*) in several wheat genotypes (44, 90). Durable rust resistance

may be achieved by pyramiding, that is, accumulating several effective resistance genes in one cultivar (54, 61). The combination of *n* (number of genes) undefeated resistance genes should extend the longevity of each resistance since it would require simultaneous mutations in at least *n* avirulence loci to produce a new virulent pathotype (71).

#### **Marker-Assisted Selection (MAS) and resistance gene isolation as tool for improving the resistance to leaf and stem rust resistance in wheat**

Pyramiding of several genes into one cultivar can be an effective strategy to use resistance genes to enhance durability of wheat resistance to leaf and stem rust (42). Durable resistance may be achieved by combination of several genes encoding partial resistance. Gene pyramiding through conventional methods is difficult and time-consuming because it requires simultaneous tests of the same wheat breeding materials with several different rust races before a selection is made. Usually, it is not feasible for a regular breeding program to maintain all necessary rust races needed for this type of work. Therefore, MAS is a powerful alternative to facilitate new gene deployment and gene pyramiding for quick release of rust-resistant cultivars.

Molecular markers such as STS or SCAR and CAPS are available for leaf rust resistance genes Lr1, Lr9, Lr10, Lr 19, Lr21, Lr24, Lr 25, Lr28, Lr29, Lr34, Lr35, Lr37, Lr39, Lr47 and Lr51. Enzymatic marker (endopeptidase Ep-D1c) for Lr19 has also been developed (92). Microsatellite (SSR) and AFLP markers for some Lr genes such as Lr3bg, Lr18, Lr40, Lr46 and Lr50 have been developed by Purnhauser et al. (73).

Molecular markers are available also for stem resistance genes such as Sr2 (28), Sr 9a (88), Sr22 (31, 69), Sr24 and Sr26 (50), Sr31 (15), Sr36 (5, 89) and Sr39 (25). Some of the markers have been used in MAS, but markers for some of the genes are not diagnostic for the genes and must be improved and markers for other genes are not available. At the present time, the research of stem rust in wheat has focused on identifying more resistance genes to control Ug99. According to the Farm and Ranch Guide report, currently 50% of winter wheat and 70 to 80% of spring wheat used in the USA are susceptible to Ug99. Moreover, 75-80% of the breeding materials are susceptible to Ug99 and most stem rust resistance genes deployed in breeding programs have been overcome by this new fungus ([http://www.farmandranchguide.com/articles/2008/03/13/ag\\_news/production\\_news/pro10.txt](http://www.farmandranchguide.com/articles/2008/03/13/ag_news/production_news/pro10.txt)). Microsatellite marker closely linked to resistance gene Sr40 have been also obtained (93).

To date three genes for leaf rust resistance in wheat Lr1 (11), Lr10 (23) and Lr 21 (29) have been isolated cloned and sequenced. They all have sequences that encode nucleotide binding site (NBS)-leucine-rich repeat (LRR) regions, which are characteristic of disease resistance genes in plants. Molecular description of these genes in wheat provides a unique biological system to study the molecular mechanisms of wheat-pathogen interaction and transduction as well as

the resistance gene function, evolution and diversity. This will allow further manipulation of wheat resistance genes to increase the resistance durability by genetic transformation of wheat.

## Conclusions

Biotic stress resistance emerges as principal selection criteria in contemporary wheat selection objectives. The discussed pathogens (as leaf and stem rust) continue to cause huge losses worldwide in wheat production due to reliance on cultivars with race-specific resistance and the high level of virulence variation in rust pathogens.

The selection of genotypes with combinations of non-race-specific resistance genes defining durable resistance over year as well as race specific genes at seedling stage is a task of prime importance for molecular marker assisted selection.

A new approach called “genomic selection” that is based on the widespread conventional selection with the use of information from the molecular markers will facilitate breeding programmes by providing effective achievement of biotic stress resistance reduction, in mean time generation interval, and also investments in ecological-friendly crop production.

## REFERENCES

1. Allen R.F. (1932) *J. Agric. Res.*, **44**, 733-753.
2. Anderson J.A. (2003) In: *Plant molecular breeding* (H.J. Newbury, Ed.), Blackwell Publ., Boca Raton, 184-215.
3. Ausemus E.R., Harrington J.B., Reitz L.P., Worzella W.W. (1946) *J. Am. Soc. Agron.*, **38**, 1082-1099.
4. Barcellos A.L., Roelfs A.P., de Moraes-Fernandes M.I.B. (2000) *Trop. Plant Dis.*, **84**, 90-94.
5. Bariana H.S., Hayden M.J., Ahmed N.U., Bell J.A., Sharp P.J., McIntosh R.A. (2001) *Aust. J. Agric. Res.*, **52**, 1247-1255.
6. Blaszczyk L., Chelkowski J., Korzun V., Kraic J., Ordon F., Ovesnb J., Purnhauser L., Tar M., Vida G. (2004) *Cellular and molecular biology letters*, **9**, 805-817.
7. Burdon J.J., Roelfs A.P. (1985) *Phytopathology*, **75**, 907-913.
8. Casulli F. and Siniscalco A. (1987) *Thalictrum flavum* L. as an alternate host of *Puccinia recondita* f. sp. *tritici* in southern Italy. In: 7<sup>th</sup> Congress of the Mediterranean Phytopathology Union, Granada, Spain.
9. Chelkowski J. and Stepień J. (2001) *J. Appl. Genet.*, **42**, 117-126.
10. Chester K.S. (1946) *The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat*, *Chronica Botanica*, Waltham.
11. Cloutier S., McCallum B.D., Loutre C., Banks T.W., Wicker T., Feuillet C., Keller B., Jordan M.C. (2007) *Plant Mol. Biol.*, **65**, 93-106.
12. Cummins G.B. and Hiratsuka Y. (1983) *Illustrated Genera of Rust Fungi*, Revised edition, The American phytopathological Society, St. Paul.
13. D'Oliveira B.D. (1940) *Portugal. Revista Agronomica*, **28**, 201-208.
14. D'Oliveira B.D. and Samborski D.J. (1966) In: *Proceedings of the first European Brown Rust Conference* (R.C. Macer, M.S. Wolfe, Eds.), Cambridge, UK, 133-150.
15. Das B.K., Saini A., Bhagwat S.G., Jawali N. (2006) *Plant Breeding*, **125**, 544-549.
16. Dyck P.L. and Samborski D.J. (1970) *Can. J. Genet. Cytol.*, **12**, 689-694.
17. Dyck P.L. (1987) *Genome*, **29**, 467-469.
18. Dyck P.L. (1991) *Crop Sci.*, **31**, 309-311.
19. Dyck P.L. (1977) *Can. J. Genet. Cytol.*, **19**, 711-716.
20. Dyck P.L. and Samborski D.J. (1968) *Can. J. Genet. Cytol.*, **10**, 7-17.
21. Dyck P.L. and Samborski D.J. (1982) *Can. J. Genet. Cytol.*, **24**, 273-283.
22. Dyck P.L., Samborski D.J., Anderson R.G. (1966) *Can. J. Genet. Cytol.*, **8**, 665-671.
23. Feuillet C., Travella S., Stein N., Albar L., Nublat L., Keller B. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 15253-15258.
24. Flor H.H. (1971) *Annu. Rev. Phytopathol.*, **9**, 275-296.
25. Gold J., Harder D., Townley-Smith F., Aung T., Procunier J. (1999) *EJB Elect. J. Biotech.*, **2**, 1-6.
26. Goyeau H., Park R., Schaeffer B., Lannou C. (2006) *Phytopathology*, **96**, 264-273.
27. Haggag M.E.A. and Dyck P.L. (1973) *Can. J. Genet. Cytol.*, **15**, 127-134.
28. Hayden M.J., Kuchel H., Chalmers K.J. (2004) *Theor. Appl. Genet.*, **109**, 1641-1647.
29. Huang L., Brooks S.A., Li W., Fellers J.P., Trick H.N., Gill B.S. (2003) *Genetics*, **164**, 655-664.
30. Jin Y., Singh R.P., Ward R.W., Wangyera R., Kinyua M., Njau P., Fetch T., Pretorius Z.A., Yahyaoui A. (2007) *Plant Disease*, **91**, 1096-1099.
31. Kham R.R., Bariana H.S., Dholakia B.V., Naik S.V., Lagu M.D., Rathjen A.J., Bhavani S., Gupta V.S. (2005) *Theor. Appl. Genet.*, **111**, 846-850.
32. Knott D.R. (1989) *The wheat rust—breeding for resistance*. In: *Monographs on Theoretical and Applied Genetics*, **12**, Springer-Verlag, Berlin.
33. Kolmer J.A. (2001) In: *Stem Rust of Wheat: from Ancient Enemy to Modern Foe* (P. Peterson, Ed.), APS Press, St Paul, 51-82.
34. Kolmer J.A. (1996) *Annu. Rev. Phytopathol.*, **34**, 435-455.
35. Kolmer J.A. (2001) *Can. J. Bot.*, **79**, 917-926.
36. Kolmer J.A. (2002) *Plant Dis.*, **86**, 288-291.



37. Kolmer J.A., Jin Y., Long D.L. (2007) *Australian Journal of Agricultural Research*, **58**, 631-638.
38. Kolmer J.A. and Liu J.Q. (2000) *Phytopathology*, **90**, 427-436.
39. Kolmer J.A., Long D.L., Hughes M.E. (2004) *Plant Dis.*, **88**, 1079-1084.
40. Kolmer J.A. (1996) *Annu. Rev. Phytopathol.*, **34**, 435-455.
41. Kolmer J.A. and Ordonez M.E. (2007) *Phytopathology*, **97**, 1141-1149.
42. Leonard K.J. and Szabo L.J. (2005) *Mol. Plant Pathol.*, **6**, 99-111.
43. Leonard K.J. (2001a) Black stem rust biology and threat to wheat growers, The Central Plant Board Meeting, February 5-8, Lexington, KY.
44. Leonova I.N., Laikova L.I., Popova O.M., Unger O., Borner A., Roder M.S. (2007) *Euphytica*, **155**, 79-86.
45. Long D.L., Leonard K.J., Hughes M.E. (2000) *Plant Dis.*, **84**, 1334-1341.
46. Long D.L., Leonard K.J., Roberts J.J. (1998) *Plant Dis.*, **82**, 1391-1400.
47. Long D.L., Schafer J.F., Roelfs A.P. (1985) *Plant Dis.*, **69**, 343-347.
48. Mains E.B., Leighty C.E., Johnston C.O. (1926) *J. Agric. Res.*, **32**, 931-971.
49. Margo R., Bariana H.S., Dundas I.S., Spielmeyer W., Lawrence G.J., Pryor A.J., Ellis J.G. (2005a) *Theor. Appl. Genet.*, **111**, 496-504.
50. Margo R., Miah H., Lawrence G.J., Wellings C.R., Spielmeyer W., Bariana H.S., McIntosh R.A., Pryor A.J., Ellis J.G. (2005b) *Theor. Appl. Genet.*, **112**, 41-50.
51. Martinez F., Niks R.E., Singh R.P., Rubiales D. (2001) *Hereditas*, **135**, 111-114.
52. McCallum B.D., Roelfs A.P., Szabo L.J., Groth J.V. (1999) *Plant Pathol.*, **48**, 574-581.
53. McCallum B.D. and Seto-Goh P. (2006) *Can. J. Plant Pathol.*, **28**, 566-576.
54. McDonald B.A. and Linde C. (2002) *Annu. Rev. Phytopathol.*, **40**, 349-379.
55. McIntosh R.A. (1992) *Plant Pathol.*, **41**, 523-527.
56. McIntosh R.A. and Baker E.P. (1968) In: 3<sup>rd</sup> International Wheat Genetics Symposium (K.W. Finlay, K.W. Shepard, Eds.), Butterworth, Sydney, Australia, 305-308.
57. McIntosh R.A., Hart G.E., Devos K.M., Gale M.D., Rogers W.J. (1998) In: Proceedings of the 9<sup>th</sup> International Wheat Genetics Symposium 2-7 August (A.E. Slinkard, Ed.) **5** 1-235.
58. McIntosh R.A., Wellings C.R., Park R.F. (1995) Wheat rusts: an atlas of resistance genes.
59. McIntosh R.A., Yamazaki Y., Devos K.M., Dubcovsky J., Rogers W.J., Appels R. (2007) Catalogue of gene symbols for wheat, Supplement, KOMUGI Integrated Wheat Science Database. Available online at <http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp>
60. McVey D.V., Long D.L., Roberts J.J. (2002) *Plant Dis.*, **86**, 568-572.
61. Mesterhazy A., Bartos P., Goyeau H., Niks R.E., Csoz M., Andersen O., Casulli F., Ittu M., Jones E., Manisterski J., Manninger K., Pasquini M., Rubiales D., Schachermayer G., Strzembicka A., Szunics L., Todorova M., Unger O., Vanco B., Vida G., Walther U. (2000) *Agronomie*, **20**, 793-804.
62. Mishra A.N., Kaushal K., Shirsekar G.S., Yadav S.R., Brahma R.N., Pandey H.N. (2005) *Plant Breed.*, **124**, 514-516.
63. Mohan M., Nai S., Bhagwat A., Krishna T.G., Yano M., Bhatia C.R., Sasaki T. (1997) *Mol. Breed.*, **3**, 87-103.
64. Park R.F., Bariana H.S., Wellings C.R., Wallwork H. (2002) *J. Agric. Res.*, **53**, 1069-1076.
65. Park R.F., Burdon J.J., McIntosh R.A. (1995) *Eur. J. Plant Pathol.*, **101**, 613-622.
66. Park R.F. and Felsenstein F.G. (1998) *Plant Pathol.*, **47**, 157-164.
67. Park R.F., Jahoor A., Felsenstein F.G. (2000) *J. Phytopathol.*, **148**, 169-179.
68. Pathan A.K. and Park R.F. (2006) *Euphytica*, **149**, 327-342.
69. Paull J.G., Pallota M.A., Langridge P. (1994) *Theor. Appl. Genet.*, **89**, 1039-1045.
70. Person C. (1959) *Can. J. Bot.*, **37**, 1101-1130.
71. Pink D.A.C. (2002) *Euphytica*, **124**, 227-236.
72. Pretorius Z.A., Singh R.P., Wagoire W.W., Payne T.S. (2000) *Plant Dis.*, **84**, 203.
73. Purnhauser L., Gyulai G., Csoz M., Heszky L., Mesterhazy A. (2000) *Acta Phytopathol. Entomol. Hung.*, **35**(1-4), 31-36.
74. Roelfs A.P. (1989) *Can. J. Plant Pathol.*, **11**, 86-90.
75. Roelfs A.P. and Groth J.V. (1980) *Phytopathology*, **70**, 855-862.
76. Roelfs A.P. and Martens J.W. (1988) *Phytopathology*, **78**, 526-533.
77. Roelfs A.P., McCallum B., McVey D.V., Groth J.V. (1997) *Phytopathology*, **87**, 910-914.
78. Rowland G.C. and Kerber E.R. (1974) *Can. J. Genet. Cytol.*, **16**, 137-144.
79. Singh R.P. (1993) *Plant Dis.*, **77**, 1103-1106.
80. Singh R.P., Hodson D.P., Jin Y., Huerta-Espino J., Kinyua M., Wanyera R., Njau P., Ward R.W. (2006) *CAB Reviews: Perspective in Agriculture, Veterinary Science and Natural Resources*, **54**, 1-13.
81. Singh R.P., Huerta-Espino J., Pfeiffer W., Figueroa-Lopez P. (2004) *Plant Dis.*, **88**, 703-708.

- 
82. Singh R.P., Kinyua M.G., Wanyera R., Njau P., Jin Y., Huerta-Espino J. (2007) Spread of a highly virulent race of *Puccinia Graminis Tritici* in eastern Africa: challenges and opportunities **12**, 51-57.
83. Singh R.P. and McIntosh R.A. (1984) Can. J. Genet. Cytol., **26**, 723-735.
84. Singh R.P., Mujeebkazi A., Huerta-Espino J. (1998) Phytopathology, **88**, 890-894.
85. Soliman A.S., Heyne E.G., Johnston C.O. (1964) Crop Sci., **4**, 246-248.
86. Spielmeyer W., Sharp P.J., Lagudah E.S. (2003) Crop Sci., **43**, 333-336.
87. Spielmeyer W., McIntosh R.A., Kolmer J.A., Lagudah E.S. (2005) Theor. Appl. Genet., **111**, 731-735.
88. Tsilo T.J., Jin Y., Anderson J.A. (2007) Crop Sci., **47**, 2013-2020.
89. Tsilo T.J., Jin Y., Anderson J.A. (2008) Crop Sci., **48**, 253-261.
90. William H.M., Singh R.P., Huerta-Espino J., Palacios G., Suenaga K. (2006) Genome, **49**, 977-990.
91. Winzeler M., Mesterhazy A., Park R.F., Bartos P., Csoz M., H. Goyeau, Ittu M., Jones E., Loschenberger F., Manninger K., Pasquini M., Richter K., Rubiales D., Schachermayr G., Strzembicka A., Trottet M., Unger O., Vida G., Walther U. (2000) Agronomie, **20**, 783-792.
92. Winzeler M., Winzeler H., Keller B. (1995) Plant Breeding, **114**, 24-28.
93. Wu (2003). Molecular mapping of stem rust resistance genes in wheat. A MS Thesis, B. S. Kansas State University.
94. Young H.C. and D'Oliveira B. (1982). A further study of race populations of *Puccinia recondita* f. sp. tritici, Garcia de Orta, Sér. Est. Agron, Lisboa, **9**, 37-52.
95. Zadoks J.C. (1963). Epidemiology of wheat rust in Europe. FAO Plant Protection Bull **13**, 97-108.