University of Alberta

Genetic studies of adult-plant resistance to leaf and stripe rusts in wheat

by

Alireza Navabi 

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To Khatereh and Mahan

with thanks

Abstract

Leaf rust (LR), caused by *Puccinia triticina* Eriks., and stripe rust (YR), caused by *P. striiformis* Westend., are important diseases of wheat (*Triticum aestivum* L. em. Thell). Genetic studies were conducted to determine the numbers and modes of action of genes involved in high levels of adult-plant resistance (APR), to study the quantitative trait loci (QTLs) involved, and the phenotypic association between APR to LR and YR.

The F_1 crosses, F_2 populations, and F_3 and F_5 single seed-descent (SSD) lines of a one-way diallel cross among 5 APR genotypes and a susceptible genotype, Avocet-*YrA*, were developed in the greenhouse at the University of Alberta. Field-evaluations were carried out at the CIMMYT research stations at Ciudad Obregon and Toluca, Mexico under artificial epidemics of LR and YR, respectively. APR to LR and YR tended to be incompletely dominant in susceptible × resistant crosses and appeared to be based on the interaction of *Lr34/Yr18* in addition to at least 2 and 3 additive genes, respectively. Segregation for disease severity levels, higher than the parents, was detected in all F_2 populations and F_5 SSD lines derived from resistant-parent intercrosses, indicating that some additive genes were different. Additive gene effects were found to be more important than non-additive gene effects in the inheritance of APR to LR and YR in the evaluated material.

A recombinant inbred line population of a cross between the Australian APR cultivar, Cook and susceptible genotype, Avocet-*YrA* was phenotyped at several locations in Canada and Mexico under artificial epidemics of LR or YR and genotyped using DNA markers. At least 3 and 4 QTLs, accounting for up to 43% of the phenotypic variation, were identified with significant effects on LR and YR, respectively. In accordance with

high phenotypic associations between APR to LR and YR, some of the identified QTLs appeared to be pleiotropic for LR and YR across tests. Although a QTL was identified on chromosome 7D with significant effects on LR and YR at some locations, it was not possible to refine the location of *Lr34/Yr18* because of the scarcity of markers in this region. The temperature-sensitive YR resistance gene *YrCK* significantly contributed to resistance to both diseases indicating that this locus is also pleiotropic for APR to LR and YR.

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List of symbols and abbreviations

APR	Adult-plant resistance
AFLP	Amplified fragment length polymorphism
ARCBD	Augmented randomized complete block design
AUDPC	Area under the disease progress curve
b	Coefficient of regression
χ^2	Chi square
CIMMYT	International Wheat and Maize Improvement Center
cv.	Cultivar
GCA	General combining ability
h^2_{bs}	Broad-sense heritability
h^2_{ns}	Narrow-sense heritability
*Lr_*	Leaf rust resistance gene
LRS	Likelihood ratio statistic
Ltn	Leaf tip necrosis
masl	Meters above see level
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
r	Coefficient of correlation
R^2	Coefficient of determination
rAUDPC	Relative area under the disease progress curve
RCBD	Randomized complete block design
rDS	Relative disease severity
RIL	Recombinant inbred line
σ^2_g	Genotypic variance
σ^2_{ge}	Genotype × environment variance
σ^2_{ph}	Phenotypic variance
SCA	Specific combining ability
*Sr_*	Stem rust resistance gene
SSD	Single seed-descent
*Yr_*	Yellow (stripe) rust resistance gene

Chapter 1

Resistance in wheat-rust pathosystems: A review of literature

1.1 Introduction

Stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici* henceforth referred to as *P. striiformis*), leaf rust (caused by *P. triticina* Eriks.), and stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks.) are important diseases of wheat (*Triticum aestivum* L. em. Thell.) throughout the world. The magnitude of yield losses due to rust diseases is highly influenced by the growth stage of wheat when the disease occurs and by the severity of epidemics. Roelfs *et al.* (1992) estimated losses of up to 30% due to leaf rust while losses due to stem and stripe rusts were estimated around 50% and up to 100% in extreme conditions for susceptible varieties in farmer's field. Importance of rust diseases is mainly due to their wide distribution throughout the world, their ability to cause serious yield losses, and the fact that the pathogens are genetically variable, and capable of overcoming resistance of the host. Among disease caused by approximately 5000 species of rust fungi (Alexopoulos *et al.*, 1996), the three rust diseases of wheat are the most important because of the global importance of their host, wheat, which is historically, socially, and economically an important food crop in many countries. Knott (1989) linked the significance of rust diseases of wheat with the importance of wheat, when he stated:

> "Several thousands of rust species attack a wide range of higher plants. A number of them cause serious economic losses in crops, but the three wheat rusts are no doubt the most important of them, because they attack wheat, the world's most important crop".

On a worldwide basis, wheat is one of the most important food crops as it contributes to supplying nearly 55% of the consumed carbohydrates in the world. According to recent statistics (FAO; http:\\www.fao.org) wheat is grown on an area of over 200 million hectares with total annual production of around 600 million tons. Data from the same source indicate that during the period of 40 years from 1961 to 2001, there has only been little change in the harvested wheat area in the world (around 4%), while the total wheat production has increased 260%. Though much of this increase has been

through improved agricultural practices, breeding of improved wheat varieties no doubt accounts for a substantial portion of increase in the production (Marshall *et al.*, 2001). The gains from breeding for disease resistance are likely to have been at least as important as the gains from breeding for increased yield (Byerlee and Moya, 1993).

Growing resistant cultivars is 'the most favored method of control' of wheat rust diseases in many countries. However, in some parts of the world, where farmers are not able to afford the cost of chemicals and/or cultural practices or such practices are practically unmanageable, resistance is considered 'the only method of control'. Early in the 20th century, following rediscovery of Mendel's laws, the basis of resistance to rust diseases was developed (Biffen, 1905). Flor (1947) described the genetic interdependence of the host and pathogen in a gene-for-gene relationship. This concept had a great impact on breeding for disease resistance. Several rust resistance genes have been identified (McIntosh *et al.*, 1998) and used in breeding for resistance. Most of the rust resistance genes however have remained effective for only a short period and were overcome by races of the pathogen with added new virulence. Alternatively, wheat breeders have identified sources of rust resistance that have remained effective for longer periods of time and against different races of pathogen. Overall, two types of resistance have been described in the wheat-rust pathosystems. On the one hand there is race-specific resistance, characterized by a hypersensitive response in a gene-for-gene relationship, and on the other hand there is race non-specific resistance, characterized as slow-rusting (Caldwell, 1968), partial resistance (Parlevliet and Van Ommeren, 1975), or adult-plant resistance (APR). However, not all APR genes are race non-specific (Roelfs *et al.*, 1992)

The concept of durable resistance (Johnson and Law, 1975; Johnson, 1983) was defined as a type of resistance that remains effective during its prolonged and widespread use in an environment favorable to disease. McIntosh (1992b) defined the characteristics that are associated with durable rust resistance. These characteristics are that they are more likely to be of the adult plant, rather than seedling type, that more than a single gene acting additively conditions them, and that they are not associated with genes conferring hypersensitive response. Durable rust resistance has become the major objective of many wheat breeding programs, as it is no doubt an essential component of sustainable systems of wheat in agriculture (Stuthman, 2002).

The objective of this chapter is to review the concept of resistance in wheat-rust pathosystems, with an emphasis on potential sources of durable resistance.

1.2 Wheat

Several species with different ploidy levels are grouped in the genus *Triticum*. However, the hexaploid species, *T. aestivum*, commercially known as bread wheat, followed by the tetraploid species, *T. turgidum* L., commercially known as durum wheat, are the two most commonly cultivated species of this genus. Hexaploid wheat (*2n = 6x = 42*) is a segmental alloploid containing three distinct, but genetically related (homoeologous) genomes, A, B, and D (Gupta *et al.*, 1999). The A and B genomes of *T. aestivum* are contributed by the tetraploid species (*2n = 4x = 28*), *T. turgidum*, while the D genome has been contributed by a diploid species (*2n = 2x = 14*), *Triticum tauschii* (Coss.) Schmalh. (Feldman, 1976). Despite its hexaploid nature, cultivated bread wheat behaves as a diploid species in gamete formation and sexual reproduction. This has been achieved through the process of diploidization during the evolution and domestication of wheat. Diploid behavior of hexaploid wheat is controlled mainly by a single gene (*Ph1*) located on the chromosome arm 5BL that is responsible for suppressing the pairing of the homoeologous chromosomes (Kimber and Sears, 1987). Inheritance studies in hexaploid wheat, therefore, follow the assumptions of disomic inheritance, as for any diploid species.

Influenced by polyploidy and extensive duplications in the genome, hexaploid wheat has a large genome size. The haploid DNA content of the bread wheat genome is approximately 1.7×10^{10} bp (Arumugathan and Earle, 1991) with an average of 810 Mb per chromosome (Gupta *et al.*, 1999).

1.3 Rust diseases of wheat

The causal agents of all three rust diseases of wheat (Figure 1-1) belong to the genus *Puccinia* in the order *Uredinales* of the phylum *Basidiomycota* (Alexopoulos *et al.*, 1996). They differ in morphology, life cycle, and optimal growth conditions.

1.3.1 Leaf rust

Leaf rust is the most widely distributed and the most common of all three wheat rusts (Roelfs *et al.*, 1992). On a worldwide basis, it probably causes more losses than the two other wheat rusts (Samborski, 1985).

The causal agent of wheat leaf rust was previously known as *P. recondita* Roberge ex Desmaz. f. sp. *tritici* (Eriks. & E. Henn.) D.M. Henderson (http://www.apsnet.org). Savile (1984) used the name *P. triticina* for the leaf rust with *Thalictrum* spp. as pycnial-aecial host. Ainkster *et al.* (1997) agreed to the usage of this name. In their study of a collection of leaf rusts, they concluded that the leaf rusts that have *Thalictrum* spp. as the pycnial-aecial host belong in a species apart from the rusts with pycnial-aecial hosts on the *Boraginaceae* which are included under *P. recondita*. The two groups are different in DNA content and spore morphology (Savile, 1984), as well as infection structure morphology (Swertz, 1994). Moreover, d'Oliveira and Samborski (1966) demonstrated that members of the two groups do not hybridize. The name *P. triticina* is currently the generally accepted scientific name for the leaf rust pathogen of wheat.

1.3.2 Stripe rust

Stripe rust proliferates best at relatively lower temperatures especially when the night temperature is cold (Roelfs *et al.*, 1992). Stripe rust is distributed in cooler wheat growing areas of the world in the temperate climate or in the tropical areas where wheat is grown in the cool and moist season. Stripe rust can be a damaging disease in severe epidemics. However, its lower optimum temperature for growth limits it as a major disease in many wheat-growing areas in the world.

The causal agent of stripe rust underwent a few name changes. It was first described in 1827 in Europe as *Uredo glumarum* J.C. Schmidt. Later on, based on telial growth of the fungus, it was named as *P. glumarum* by Eriksson and Hennings (Stubbs, 1985). Cummins and Stevenson (1956) changed the name to the currently accepted name, which is *P. striiformis*.

Unlike stem and leaf rusts, there is no known alternate host for the stripe rust pathogen and only uredial and telial stages are known (Stubbs, 1985). Eriksson and

Hennings (reviewed in Line, 2002) reported that teliospores and basidiospores were formed each year and that the teliospores could germinate and produce basidiospores. The attempt to use basidiospores to infect wheat failed and there is no report of another host that basidiospores would infect. Winter survival and carry-over of the pathogen into the next season are most likely through dormant mycelium and the uredial stage. Sanford and Broadfoot (1929) reported that stripe rust could survive at least some winters on grasses in Alberta, Canada. Biffen (1908) had previously observed that the rust might survive the winter in the uredial stage in England. Hungerford (1923) reported that stripe rust survived the summer as dormant mycelium in the leaves of wild grasses. Sporulation was diminished later in the summer and began again in the fall.

1.3.3 Stem rust

Stem rust is the most damaging of all three wheat rusts (Knott, 1989), caused by *P. graminis*. The damage caused by wheat stem rust can be more spectacular than any other cereal disease (Roelfs, 1985). Due to its seriousness, it was once the most feared disease in most wheat growing areas of the world (Roelf *et al.*, 1992). However, it has largely been controlled in many areas by earlier maturing genotypes and resistance based on single genes with established durability (McIntosh, 1998). Resistant cultivars play an important role in the control of stem rust. The most commonly used resistance gene since 1940 is the APR gene *Sr2*, originally derived from the cv. Hope.

1.4 Rust fungi life cycle

Rust fungi of wheat have complex life cycles (Figure 1-2) that may include up to five spore stages. They are basidial, pycnial, aecial, uredial, and telial stages. In the case of *P. striiformis*, however no alternate host is known and only uredia and telia exist. Based on the ploidy level and the number of nuclei, a complete life cycle of rust fungi can be divided into three major phases: haploid (n), dikaryon (n+n), and diploid (2n).

The haploid phase (n) starts with the formation of basidiospores developed and released after the teliospore germinates. These haploid spores are wind-borne to susceptible species of the alternate hosts, *Berberis* and *Mahonia* spp. for stem rust and

Thalictrum spp. for leaf rust. After infection of the alternate host, mostly through the upper surface of the leaf, pycnia develop in approximately 5 days. Pycniospores are haploid unicellular spores that function as one type of gamete and fuse with the receptive hyphae that function as the other gamete.

Following fertilization, the dikaryotic phase (n+n) of the life cycle starts and aeciospores develop in the aecia, usually on the lower leaf surface of the alternate host. They are dikaryotic and are generally wind-borne over long distances. Aeciospores germinate on wheat in the presence of free water. Following germ-tube formation, an appressorium develops over the host stoma, and penetration takes place through the stoma by a penetration peg. Urediospores develop in the uredia, which are formed after successful infection of the wheat plant. Large numbers of urediospores are produced over a period of several weeks. The uredial cycle repeats every 14-20 days. Urediospores are wind-borne to other wheat plants and germinate in the presence of free-water. An appressorium forms over the host stoma following the germ-tube formation. The formation of appressoria is well documented in the case of leaf rust (Bender *et al.*, 2000) and stem rust (Collins *et al.*, 2001). However, the report by Broers and Lopez-Atilano (1996) is the only report of appressoria formation in the *P. striiformis* on susceptible genotypes. They also reported that quantitative resistance to stripe rust was associated with significant reductions in the frequency of appressoria formation.

Teliospores develop in telia later as the host plant matures. Mature teliospores are blackish-brown, diploid, two-celled, and resistant to environmental extremes. Telia function as the over-wintering stage of the fungus in climates with freezing temperatures.

The teliospores survive in the winter and germinate following a process of weathering, alternating freezing and thawing, and drying and wetting. Basidia develop as a result of teliospore germination, during which meiosis occurs and the haploid nuclei migrate into the basidia.

1.4.1 Development of new virulence

The causal agents of the rust diseases of wheat are obligate and highly specialized parasites (Parlevliet, 2002) in which new races develop readily and against which many race-specific resistance genes occur. Many rust resistance genes are therefore short-lived

and become ineffective as new races develop in the pathogen population. The combined effects of gene/genotype flow and spontaneous mutation (McDonald and Linde, 2002) can influence development of new races with added new virulences in the pathogen population. Gene or genotype flow is the process in which particular alleles (genes) or individuals (genotypes) are exchanged among geographically separated populations. This exchange may happen through sexual or somatic recombination (gene flow) or migration of the spores of new genotypes into an area (genotype flow). The initial introduction of *P. striiformis* in Australia in 1979 is a good example of genotype flow through long-distance migration of the pathogen. Moreover, Hovmoller *et al.* (2002) provided molecular evidence for long distance migration of *P. striiformis* in North-west Europe that together with low diversity in the host species may cause the resistance genes to become ineffective on a continental scale.

Gene flow may be influenced by sexual or somatic recombination. For sexual recombination, the pathogen needs to undergo gamete formation and fertilization that takes place on the alternate host in the pycnia. The presence of the alternate host is therefore essential for sexual recombination to occur. This is restricted to leaf and stem rusts with known alternate hosts. In the case of stripe rust with no known alternate host, there is no evidence for the existence of sexual recombination.

Wright and Lennard (1980) demonstrated that somatic recombination of the whole nuclei during germ-tube fusion can result in development of new pathotypes. In their study, mixing of urediospores of two pathotypes of *P. striiformis* resulted in a new pathotype. However, there is no evidence that this may also occur spontaneously in nature (Steele *et al.*, 2001).

Spontaneous mutation in a step-wise manner seems to be the major cause of development of new virulences in the pathogen population. In Australia, following the initial introduction of a single pathotype of *P. striiformis* in 1979, Wellings and McIntosh (1990) detected more than 20 derivative pathotypes. Steele *et al.* (2001), based on the molecular data, provided evidence that genetic variability in the Australian and New Zealand stripe rust populations has been caused by stepwise mutations.

1.5 Disease control

Although the three rust diseases of wheat seem to have been threatening wheat farmers since the very beginning of agriculture, disease control based on knowledge of the biology of the disease and breeding for resistance started in the late 1800s and early 1900s (Roelfs, 1985). During the 20th century, extensive attempts were made to reduce the losses caused by rust diseases. These attempts can be classified into three major groups i.e., chemical control, cultural practices, and resistance. Chemical control of rust diseases of wheat is economically costly, environmentally unsafe, and often practically unmanageable. Moreover, most available fungicides provide inadequate control on susceptible cultivars (Roelfs *et al.*, 1992). Cultural practices such as planting early maturing cultivars, early planting dates, and removing the "green bridge" (Zadoks and Bouwman, 1985) by controlling the off-season growth and volunteer plants, may enhance and prolong the effectiveness of resistance genes and longevity of chemicals, but they provide only partial control of the endogenous rust inocula in a certain area. Moreover, they may not be manageable in most areas, as they require cooperation of all farmers a relatively large area.

Eradication of the alternate host is also a form of cultural management of rust. The first use of the eradication of alternate host was in France, even before science showed a relationship between stem rust and barberry. For stem rust, eradication of barberry was an effective strategy for reducing the frequency of epidemics in North America (Roelfs, 1982) where the alternate host played an important role in stem rust epidemiology. However, it was less helpful than initially thought, because stem rust continued to over-winter in southern states and Mexico, and migrated northwards every season.

Eradication of the alternate host in North America did delay the onset of stem rust and reduced the level of initial inocula. It also reduced the number of pathogen phenotypes by eliminating the sexual stage of the pathogen. Consequently, it may have increased durability of resistance genes. However, eradication of alternate hosts can be costly and is not feasible in many areas. Moreover, it should be noted that sexual recombination could only generate new combinations of virulence that already exist in

the population. New virulence is introduced into a population either by mutation or migration from other areas.

Amongst the control measures, the use of resistant cultivars is the most efficient method for controlling rust diseases of wheat (Roelfs *et al.*, 1992; Kolmer, 1996; Knott, 1989). Breeding for resistant varieties is an economical activity that reduces costs to farmers by reducing or eliminating the need for chemical control, and additional cultural practices. Sayre *et al.* (1998) demonstrated that progress in protecting yield potential through genetic resistance to leaf rust in Yaqui Valley, in state of Sonora in Mexico has been approximately three times as great as progress in advancing yield potential. Nonetheless, resistance in many cases seems to be effective for only a short period, therefore becoming ineffective as new races of the pathogen with added new virulences become established in the pathogen population. The failure of resistance genes over a short period of time has led to a cycle known as the "boom-and-bust cycle" (Roelfs *et al.*, 1992). In this cycle, the release of a new variety and its rapid distribution in an area can be followed by appearance of new virulent races in the pathogen population and a consequent break down of resistance. Breeding will then shift towards finding new sources of resistance genes. For most of the identified rust resistance genes, there is virulence somewhere in the world, and there is no reason to assume that any resistance gene may remain effective forever. For this reason, many breeding programs are becoming interested in alternate types of resistance with increased promises of long-lasting resistance.

1.6 The host-pathogen interaction

Following rediscovery of the Mendelian laws, Biffen (1905) reported that rust resistance, wheat and *P. striiformis* in this case, is an inherited trait that followed the rules of Mendelian genetics. It was later demonstrated by Stakman and associates (Stakman and Piemeisal, 1917), studying *P. graminis*, that resistance in plants can be overcome by variants of the pathogen. Flor (1942) established the basis of gene-for-gene relationships and provided evidence for interdependence of host and pathogen. Flor (1942) demonstrated that a genotype of flax was resistant to a particular genotype of *Melampsora lini* only if it carried a dominant resistance gene corresponding to a

dominant avirulence gene in the pathogen. The gene-for-gene relationship has been extensively studied in the wheat-rust pathosystems. The infection type produced when a wheat genotype is infected with a genotype of rust is the product of the interaction between two genetic systems. In a single resistance gene interaction, a low infection type is only observed when a wheat genotype with a resistance gene is infected by a rust genotype with the corresponding avirulence gene.

1.6.1 Race-specificity in gene-for-gene relationships

Race-specific resistance is defined as a type of resistance that interacts differentially with pathogen races (Johnson, 1984). Many of the catalogued genes for rust resistance (McIntosh *et al.*, 1998) follow the gene-for-gene relationship. The phenotypic expression of these genes is a hypersensitive response, characterized by a low infection type (Figure 1-4). Rave-specific resistance genes often confer high levels of resistance that remain effective throughout the life of the plant. However, such resistance may become ineffective as new races with added new virulence develop in the pathogen population.

The molecular theory basis of the gene-for-gene relationship envisions that the products of resistance genes serve as receptors for specific elicitors produced by the pathogen, either directly or indirectly through expression of avirulence genes. This specific receptor-elicitor recognition event, subsequently elicits a cascade of defensive responses, resulting in a resistant phenotype (Cook, 1998). The resistance phenotype therefore results from several gene products working together. These include genes involved in pathogen recognition, genes that are involved in signal transduction pathways and genes that are involved in the disease resistance response (Beynon, 1997). Genes that are involved in recognition of the pathogen encode proteins that can recognize the elicitor of the pathogen. A majority of the so-far isolated and sequenced resistance genes encode for a group of proteins that are essentially similar in a range of plants. This similarity involves a leucine-rich repeat (LRR) domain (Collins *et al.*, 2001; Mago *et al.*, 1999; Meyers *et al.*, 2002; Spielmeyer *et al.*, 2000; Wang *et al.*, 1998).

The process of recognition in a gene-for-gene relationship results from a specific receptor-elicitor interaction at the molecular level. This specific interaction has been

described as a "key-lock combination" in plant pathology textbooks. Any structural change in the specific elicitor of the pathogen results in the failure of the host in recognition of the invasion of the pathogen. Such structural changes may take place in the pathogen elicitor molecule(s), as discussed earlier, through spontaneous mutation or new genotypes may develop in the pathogen population through gene/genotype flow.

1.6.2 Interactions other than gene-for-gene relationship

There are reports of cases where the host-pathogen interaction differs from the classical one-to-one relationship, as described in Flor's gene-for-gene concept. Kolmer (1996) presented cases where the interaction of wheat and *P. triticina* differed from the one-to-one relationship. The leaf rust resistance gene *Lr2* with three alleles (*Lr2a*, *Lr2b*, and *Lr2c*) correspond with a single avirulence gene in the pathogen for the avirulence phenotype. An independent dominant gene in the pathogen however differentially inhibits expression of virulence (Dyck and Samborski, 1974). Moreover, the dominance relationships of the avirulence/virulence genes in the pathogen and resistance genes may be dependent on the respective genotype (Kolmer and Dyck, 1994). An inhibitor gene in cv. Prelude that retards the resistance expressed by the gene *Lr3* and the two complementary genes conditioning virulence to *Lr3bg* (Haggag and Dyck, 1973; Haggag *et al.*, 1973) are other cases in which the host-pathogen interaction differs from a one-to-one relationship.

Johnson (1984), in a critical analysis of durable resistance, argued that genes that follow the gene-for-gene relationship are not the only type of resistance genes. There is much resistance in the host-pathogen interaction that cannot be described by this theory. Examples were provided by Johnson in the case of *P. striiformis* and *P. hordei* Otth. Even in the cases where a gene-for-gene relationship has been demonstrated, this only accounts for a part of the observed resistance. There are sources of resistance such as non-hypersensitive APR, slow-rusting, and partial resistance that cannot be explained by the gene-for-gene theory. These mechanisms provide some levels of resistance that are expressed in compatible interactions of host and pathogen. Compatible interaction indicates that either the host does not carry any race-specific resistance gene or the

pathogen carries virulence genes for every resistance gene in the host. Such resistance must be the result of mechanisms other than the gene-for-gene relationship.

During the past few decades the objectives of many wheat breeding programs have been directed towards such resistance mechanisms as the sources of gene-for-gene type resistance in many cases have failed to remain effective for a long period of time.

1.6.3 Slow rusting, partial, and adult-plant resistance

The concept of slow-rusting was first defined by Caldwell (1968). This type of resistance was characterized in the wheat cv. Knox, as an interaction between wheat genotype and the rust pathogen in which the infection process had slowed down through a barrier in one or more phases of this process. Slow rusting under field conditions may result from longer latent period, fewer and smaller uredia, resistance genes that function only at certain stages of growth, and environment-resistance gene interactions (Roelfs *et al.*, 1992). Slow-rusting is a relative measure against a susceptible check. Reduced terminal disease severity and the area under the disease progress curve (AUDPC) have been used in breeding programs and genetic studies to evaluate wheat genotypes for slow-rusting. Slow-rusting is considered to be controlled by race nonspecific resistance genes and is expected to be more durable.

Parlevliet and Van Ommeren (1975) defined the term partial resistance in barley-leaf rust as a phenomenon in which a relatively small diseased leaf area is accompanied by a high infection type. Small diseased leaf area is caused by relatively lower rate of multiplication of the pathogen due to a longer latent period, lower infection ratio, smaller and fewer uredia, and a reduced sporulation period. These components of partial resistance were shown to be pleiotropic and governed by polygenes (Parlevliet, 1978). Furthermore, the high infection type is assumed to avoid hypersensitivity genes that are often highly race-specific. Partial resistance and hypersensitive response seemed to be of a completely different nature. Partial resistance seems to be based on a pre-haustorial resistance mechanism without cell collapse, whereas hypersensitive response is based on a post-haustorial mechanism that involves cell collapse (Niks, 1983). Partial resistance was assumed to be more durable compared to resistance conditioned by single major resistance genes (Parlevliet, 1993).

Some sources of nonspecific resistance have been characterized by a complete or incomplete resistance in the adult plant stage, despite their seedling compatibility. Most of the rust resistance genes that are effective in the seedling stage are effective in the adult plant stage, but they frequently do not provide long-lasting resistance (Kolmer, 1996). On the other hand, there are resistance genes that confer smaller diseased leaf areas in the adult stage, accompanied by a compatible interaction in the seedling stage. This type of rust resistance, defined as APR, has been characterized in many spring and winter wheats (Bariana *et al.*, 2001; Bariana and McIntosh, 1995; Bjarko and Line, 1988; Broers and Jacobs, 1989; Chen and Line, 1995b; Johnson, 1983; Jacobs and Broers, 1989; Kolmer and Liu, 2001; Knott, 1997; Lee and Shaner, 1985; Milus and Line, 1986; Messmer *et al.*, 2000; Singh and Rajaram, 1994; Singh *et al.*, 2001; Wagoire *et al.*, 1998; Zhang *et al.*, 2001). Non-hypersensitive APR is one of the known features likely to be associated with durable rust resistance (McIntosh, 1992b).

Conceptually, the terms slow-rusting and partial resistance share common characteristics, in that they are both based upon a compatible host-pathogen interaction, despite a reduced terminal disease severity. In the case of stem and leaf rusts of wheat, phenotypic expression of some sources of adult-plant resistance resembles the definition of slow-rusting and partial resistance. In most cases, a reduced diseased area is accompanied by a susceptible to moderately susceptible infection type. In the case of stripe rust, however, some levels of reduction in the infection type in the adult plant stage seem to be associated with APR. Therefore, there has been some controversy over the attainability of durable partial or slow-rusting resistance using APR genes. Broers (1993) concluded that the prevalence of APR genes, including *Yr18*, in wheat impede accumulation of partial resistance to stripe rust. Danial (1993) reported that although partial resistance against stripe rust does exist in wheat, combinations of sufficiently high levels of resistance together with high infection types is so rare that partial resistance to stripe rust does not seem to be a promising approach for breeders. On the other hand, Singh *et al.* (2001) related the reduction of infection type in stripe rust APR genotypes to the systematic development of stripe rust and not to an active hypersensitive response by the host. They stated that in the case of potentially durable resistant genotypes, the first uredia to appear are moderately susceptible to susceptible (compatible) and reduction in

the infection type is not due to an active hypersensitive response, but due to the subsequent systematic growth of mycelia in the leaf inter-veins that causes some chlorosis and necrosis.

For the purpose of this review and the research presented in this thesis, the term adult-plant resistance is preferred as the seedling and adult plant tests seem to have become a practical tool in many wheat breeding programs to divide wheat germplasm for potential race non-specific resistance.

1.7 Temperature effect and adult-plant resistance

Rust resistance has been reported in some studies as being conditioned by temperature sensitive genes. Sharp and associates (Lewellen and Sharp, 1968; Lewellen *et al.*, 1967; Sharp and Volin, 1970) were probably among the first scientists to study the temperature sensitive rust resistance genes. They concluded that this type of resistance to stripe rust was being conditioned by genes with minor additive effects. These genes could be accumulated through crossing and selection for higher levels of resistance. They stated that such genes were race non-specific. Stubbs (1977) confirmed the nonspecificity of the minor temperature sensitive genes with additive effects.

Line and associates (reviewed in Line, 2002) described different types of stripe rust resistance and characterized high-temperature adult-plant resistance (HTAPR). Seedlings of HTAPR wheat genotypes are susceptible at both low and high temperatures. Adult plants are susceptible at low temperatures, but resistant at high temperatures. They also demonstrated that flag leaves are more resistant than the lower leaves. The expression of HTAPR was demonstrated to start at the jointing stage becoming stronger at the later stages of growth. HTAPR seems to be different from the temperature sensitive stripe rust resistance genes reported by Sharp and associates which are expressed at all growth stages (Line, 2002). Milus and Line (1986) demonstrated that HTAPR genes are responsible in protecting the cultivars, Nugaines and Crest, which have remained resistant for a long period of time in North Western United States.

Park *et al.* (1992) reported a temperature sensitive stripe rust resistance gene in the Australian cv. Cook. This temperature sensitive response was characterized by a low infection type in post-inoculation temperature conditions of 24°C instead of usual

temperature conditions of 17°C. Bariana *et al.* (2001), located the cv. Cook-derived temperature-sensitive resistance gene in chromosome arm 2DS of the cv. Sunco. This gene was temporarily designated *YrCK* and is known to contribute in part to the adult plant field resistance of the cv. Cook.

Temperature sensitivity of leaf rust resistance genes was discussed in a review by Kolmer (1996). He highlighted the work by Dyck and Johnson (1983) in which responses of leaf rust resistance genes in a set of the Thatcher isogenic lines were tested with four different isolates at different temperatures. Although there were evident differences among responses of genes at different temperatures, they concluded that the temperature sensitivities of leaf rust resistance genes were highly isolate dependent. Drijepondt and Pretorius (1989) and Drijepondt *et al.*, (1991) reported that *Lr34* was more effective at lower temperatures. McIntosh *et al.* (2001) also reported observations that leaf rust severities in some lines with *Lr34* may reach 60-70% in the rising spring temperatures in Australia, while the same lines in cooler conditions in Canada and northern Mexico develop lower levels of leaf rust. They related this to higher effectiveness of *Lr34* at lower temperatures.

1.8 Inheritance of adult plant rust resistance

Numerous scientists have studied inheritance of APR to stripe rust during the past few decades. Line (2002) summarized the results of inheritance studies of stripe rust HTAPR in North Western United States. These studies (Chen and Line, 1995a; Chen and Line, 1995b; Milus and Line 1986) were done with periodic quantitative measurements of disease intensity, using quantitative techniques for genetic analysis. HTAPR was partially recessive with the number of genes ranging from 1 to 3. It was also mentioned that genes for HTAPR in wheat genotypes were different and that there was a possibility to combine HTAPR genes in order to achieve higher levels of resistance or to combine HTAPR with seedling resistance genes. In other genetic investigations on sources of adult-plant resistance, studies on the inheritance of either AUDPC, latent period, or terminal disease severity (Bariana *et al.*, 2001; Zhang *et al.*, 2001; Wagoire *et al.*, 1998; Bariana and McIntosh, 1995; Singh and Rajaram, 1994) have produced similar results. These workers have also reported additive involvement of few to several genes with

disease severities being continuously distributed in crosses of APR and susceptible genotypes. Additive gene effects were often important contributor in the inheritance of adult-plant stripe rust resistance and known to be relatively highly heritable in several studies (Ghanadha *et al.*, 1995; Krupinsky and Sharp, 1978; Wagoire *et al.*, 1998). The APR gene *Yr18* contributes in overall resistance in many widely grown cultivars. Acceptable levels of adult-plant stripe rust resistance may be achieved by combinations of *Yr18* and 2-3 additional genes (McIntosh *et al.*, 2001; Singh and Rajaram, 1994).

Kolmer (1996) reviewed inheritance studies of APR to leaf rust. Most genetic studies of APR to leaf rust (Bjarko and Line, 1988; Broers and Jacobs, 1989; Jacobs and Broers, 1989; Lee and Shaner, 1985; Singh and Rajaram, 1992) have resulted in similar conclusions, with involvement of 2-3 genes with small effects and relatively high heritability estimates. The gene *Lr34* is a major contributor to high levels of APR to leaf rust. Singh *et al.* (1998) located the *Lr46* gene on the chromosome arm 1BL of Pavon 76, a Mexican cultivar with prolonged leaf rust resistance. The leaf rust severity of some lines with *Lr34* or *Lr46* alone may reach 50-60%. Additive interaction of these genes with 2-3 minor genes resulted in higher levels of resistance (Singh *et al.*, 2000).

The leaf rust race-specific APR gene *Lr13*, singly or in combination with *Lr34*, was also known to contribute to high levels of durable leaf rust APR (German and Kolmer, 1992) in some areas. However, *Lr13* has been ineffective in many locations such as southern America and northern Mexico (Singh and Rajaram, 1992) and has recently become ineffective in some areas in North America. In the Canadian leaf rust survey in 2001 *Lr13* was found to be ineffective against 61 isolates of 65 isolates tested (Dr. B. D. McCallum, personal communication, 2002). Also in the United States in 2000, *Lr13* did not provide effective resistance, either singly or in lines with other genes (Cereal Rusts Bulletin, 2000; http://www.cdl.umn.edu/crb/crbupd.html). Despite its ineffectiveness in many locations, *Lr13* gene in combination with other genes seems to have provided effective resistance in Australia for at least 30 years (McIntosh *et al.*, 2001).

In the case of stem rust, an acceptable level of resistance is available in wheat, provided by the stem rust APR gene *Sr2*. This gene is known as the basis of durable stem rust resistance in many wheat cultivars throughout the world (Roelfs *et al.*, 1992; McIntosh *et al.*, 2001). The *Sr2* gene is associated (McIntosh *et al.*, 1998) with pseudo-

black chaff (Figure 1-3) and occurs very frequently in commercial wheat cultivars, especially in the germplasm produced and distributed by CIMMYT. It should also be noted that the *Sr13* gene is common in some North American durum wheats. The gene *Sr26*, despite its apparent association with yield loss, has remained effective in some Australian cultivars for a long time (McIntosh *et al.*, 2001). The gene *Sr31* has also been an effective gene providing high levels of resistance especially in many CIMMYT derived cultivars throughout the world. Virulence against *Sr31*, however, was found in Uganda in 1999 for the first time (Pretorius *et al.*, 2000).

1.8.1 *Lr34/Yr18*

The tightly linked leaf rust resistance gene *Lr34* and stripe rust resistance gene *Yr18* are present in many widely grown wheat cultivars. This linkage accounts for a significant portion of overall resistance to stripe and leaf rusts in many areas (Kolmer, 1996; McIntosh, 2001). The leaf rust APR gene *Lr34* was first identified in an introduction from China by Dyck (1977). It was found to be associated with chromosome arm 7DS (Dyck, 1987). Piech and Supryn (1978) also located a gene on the chromosome 7D of Chinese Spring wheat. Dyck (1991) assumed that it must be the same gene as *Lr34*. Dyck (1991) noticed that sources of *Lr34* show chlorosis and necrosis at the tips and along the sides of leaves. This phenotype was found to be controlled by a single locus (*Ltn*) tightly linked or pleiotropic with *Lr34* (Singh, 1993) and has been used as a morphological marker for the *Lr34* gene. It is also known that *Lr34* is linked to, or pleiotropic with the stripe rust APR gene *Yr18* (Singh, 1992; McIntosh, 1992). Furthermore, *Lr34/Yr18* was linked to *Bdv1* gene for slow yellowing response to barley yellow dwarf virus (Singh, 1993) and is also known to enhance resistance to stem rust (Dyck, 1987; Dyck, 1993; Kerber and Aung, 1999). Dyck *et al.* (1994) indicated that either *Lr34* may be located on two different chromosomes or there might be two different genes with very similar phenotypic expressions and association with leaf tip necrosis. They also suggested that *Lr34* might have been translocated onto another chromosome. Cytogenetic evidence supported this assumption (Kolmer, 1996).

The mechanism of resistance conferred by the gene *Lr34* seems to be different from seedling resistance genes and from the other leaf rust APR gene *Lr13*. Rubiales and

Niks (1995) studied the effect of *Lr34* on leaf rust development compared with that of *Lr13* in Thatcher background. They showed that *Lr34* increased the latent period and decreased the infection frequency, especially at low temperature. The effect was more obvious in the adult plant stage with reduced rate of haustorium formation due to a lower rate of hyphal development, with no or relatively little plant cell necrosis. The main difference of *Lr34* from *Lr13* was that the latter was associated with chlorosis at the macroscopic level and cell necrosis at the microscopic level.

Elahinia and Tewari (2000) studied calcium mobilization resulting from infection of the flag leaves of susceptible and *Yr18*-gene near-isogenic lines in Thatcher background, using scanning electron microscopy in conjunction with energy-dispensive X-ray microanalysis. Calcium-rich crystals of various shapes were frequently present on the pustules and on the infected leaf areas in the resistant line (Figure 1-5a and b). More calcium was mobilized in infected areas of the *Yr18* line (Figure 1-5b) than in the susceptible one (Figure 1-5a). Moreover, the levels of calcium present in the non-infected areas of both resistant and susceptible hosts, and in the infected areas of the susceptible host were nearly the same. In a separate study, Navabi and Tewari (unpublished) used the susceptible and *Yr18*-isogenic lines in an Avocet background to verify whether calcium mobilization and crystal formation is regulated by the gene *Yr18*. Results were different from that observed for the Thatcher isogenic lines. There was no evidence of crystal formation on the pustules (Figure 1-5c and d) and the level of calcium mobilization seemed not to be significantly different between the susceptible and *Yr18*-isogenic lines. Therefore, it seems very unlikely that the calcium-rich crystal formation was influenced by the *Yr18* gene. Whether *Yr18* gene interacting with other genetic factors in Thatcher background caused these phenotype or the two Thatcher isogenic lines were also different for gene(s) other than *Yr18* needs to be further investigated.

Although the *Lr34/Yr18* gene(s) are known to contribute to overall resistance in many cultivars throughout the world, the levels of resistance that they confer may not be acceptable in some genetic backgrounds. Leaf or stripe rust severities of some sources of *Lr34/Yr18* in some areas can be as high as 50-60% (Singh and Gupta, 1992; McIntosh *et al.*, 2001, Ma and Singh, 1996). An acceptable level of leaf or stripe rust resistance has been shown to be the result of additive interaction of the *Lr34/Yr18* with at least 2-4

genes (Singh and Rajaram, 1992; Singh and Rajaram, 1994; McIntosh *et al.*, 2001). It has been demonstrated by Singh *et al.* (2000) that incorporating additional genes into *Lr34/Yr18* background may result in stripe and leaf rust APR levels, as high as being close to immunity.

1.8.2 *Lr46/Yr29*

Singh *et al.* (1998), using a partially developed chromosome substitution line analysis, located the APR gene *Lr46* on chromosome arm 1BL of the cv. Pavon 76. They demonstrated that *Lr46* provides a degree of slow-rusting, which may reach up to 60% when deployed alone, but can result in high levels of resistance (5-10% leaf rust) when deployed in combination with another additive gene in Pavon-76. McIntosh *et al.* (2001) reported that lines with *Lr46* alone are not attractive options for use by wheat breeders in Australia. It was later reported that *Lr46* was completely linked with a stripe rust APR gene designated *Yr29* (Singh *et al*, 2001).

Martinez *et al.* (2001) compared the leaf rust resistance phenotype conferred by *Lr46* gene with that of the *Lr34*. They reported that *Lr46* resembled the phenotypic expression of *Lr34* and partial resistant genotypes. *Lr46* increased the latency period, increased the early aborted infection units with no association with host cell necrosis, and decreased the colony size. They concluded that the effect of *Lr46* was comparable with that of *Lr34* except that in the seedling stage *Lr46* had a weaker effect.

1.9 Quantitative trait loci analysis of adult-plant rust resistance

Most genetic studies of APR to rust diseases in wheat have reported a quantitative inheritance with the additive involvement of few to several genes. Oligo- to polygenic inheritance of APR makes it difficult for researchers to study the inheritance of APR using conventional techniques, especially when the number of expected genes is higher than 3-4. Alternatively, quantitative trait loci analysis techniques have provided researchers with a powerful tool to study complex traits (Young, 1996).

Researchers have used QTL analysis to characterize quantitative durable rust resistance. William *et al.* (1997) identified a QTL on the chromosome arm 7BL with strong effect on leaf rust severity in a recombinant inbred line population derived from a

cross between Parula (resistant) and Siete-Cerros (susceptible). This QTL was suggested to be homoeoallelic to the *Lr34* gene. Messmer *et al.* (2000) identified 8 QTLs associated with leaf rust resistance in the Swiss cv. Forno. Although *Lr34* was not involved, some of the QTLs were associated with leaf tip necrosis. Bariana *et al.* (2001) located at least four QTLs associated with stripe rust resistance in a CD87/Katepwa population; two QTLs were contributed by CD87. Based on pedigree information and chromosomal location of the QTLs, they concluded that these loci most probably correspond to the *Yr18* and *Yr29*. They also mapped a QTL on the chromosome arm 2DS of Katepwa, temporarily designated it as *YrKat*, and another one on chromosome arm 2DS of cv. Sunco, temporarily designated as *YrCK*. The gene *YrCK* is believed to be the temperature-sensitive stripe rust resistance gene contributed by the cv. Cook (Park *et al.*, 1992). The genetic relationship of *YrKat* and *YrCK* is under study (Dr. H. S. Bariana, 2002, personal communication). Singh *et al.* (2000) used QTL analysis to map the *Yr28* gene in a population of recombinant inbred lines developed from a cross between synthetic wheat and the cv. Opata 85. In the same study a genomic region was identified on the chromosome arm 7DS, assumed to be the *Yr18* gene. Boukhatem *et al.* (2002) identified 2 and 5 QTLs with significant effects on stripe rust in two populations of wheat. They reported that the centromeric region of chromosome 2B and the telomeric regions of chromosome arms 2AL and 7DS of the durable cv. Camp Remy were involved in quantitative stripe rust resistance. Suanaga *et al.* (unpublished; personal communication with Dr. R. P. Singh, 2002) identified two QTLs that reduced leaf rust severity and up to eleven and seven QTLs that could have influenced stripe rust severity and infection type, respectively. They mapped a QTL on 7DS of a Japanese cv. Fukuho-komugi. This QTL was common for leaf and stripe rusts and was thought to represent *Lr34/Yr18*. They were also able to detect other QTLs on chromosome arms 1BL and 3BS, representing the genes *Lr46* and *Yr30*, respectively.

There are several advantages in application of QTL analysis as a tool for genetic studies, compared to conventional genetic analysis techniques. Most classical genetic analysis techniques are based upon the pre assumptions of no linkage, no epistatic effects, and the equal genotypic effects of the segregating factors, whereas QTL analysis is free of such assumptions. With QTL mapping, the role of each locus in genetically complex

traits can be described. A fine linkage map of markers provides a framework for an analysis of the QTLs in genetic populations.

1.10 Conclusions from the literature

Two types of resistance in the wheat-rust pathosystems were discussed in this review. On the one hand, race-specific resistance was defined in a gene-for-gene relationship, often characterized by a hypersensitive response at the seedling stage that remains effective throughout the life of the plant. This type of resistance is often conditioned by a single or few major genes. On the other hand, race non-specific resistance was discussed that is characterized in some wheat germplasm as slow-rusting, partial resistance, or APR. This type of resistance has frequently been reported to be conditioned by few to several genes with additive effects.

Unlike seedling resistance genes, the resistance conditioned by some APR genes is more likely to be race non-specific. APR genes conferring non-hypersensitive rust resistance are known in many wheat germplasms. In the case of stem rust, the APR gene *Sr2* in combination with other genes has provided adequate resistance in many widely grown cultivars and has remained effective for more than 40 years. The gene *Lr34* that is tightly linked with, or is pleiotropic for stripe rust APR gene *Yr18* is present in many cultivars. Moreover, the leaf rust APR gene *Lr46* is also linked with, or pleiotropic for stripe rust resistance gene *Yr29*. These resistance genes confer some level of resistance in adult stage. Many sources of *Lr34/Yr18* and *Lr46/Yr29* have remained effective for many years, but it has been shown in many studies that in some genetic backgrounds the level of resistance may not be adequate. In an additive interaction with a few minor genes, *Lr34/Yr18* or *Lr46/Yr29* may provide acceptable levels of resistance. Such resistance is likely to be durable as it is conditioned by a few additive genes of non-hypersensitive response.

1.11 Objectives of the study

The development of wheat cultivars that have durable resistance to rust diseases has been a long-standing objective of wheat breeders. Currently, stem rust seems to be highly under control by earlier maturing genotypes and resistance based on genes with

established durability, e.g., *Sr2*, *Sr26*, or gene combinations involving two to several resistance genes (McIntosh, 1998). In the case of leaf and stripe rusts also, sources of resistance with established durability are known. Leaf and stripe rust APR genes *Lr34/Yr18* and *Lr46/Yr29* contribute to leaf and stripe rust resistance in a wide range of wheat genotypes. However, as discussed earlier, the level of resistance may not be adequate in some backgrounds. Additional genes with minor additive effects have been shown to be present in wheat germplasm that in combination with *Lr34/Yr18* and/or *Lr46/Yr29* genes may provide acceptable levels of protection. The challenge, therefore, is to incorporate *Lr34/Yr18* and/or *Lr46/Yr29* with additional additive genes in high yielding wheat backgrounds.

The objectives of the investigations presented in this thesis were to:

1. Determine the number and mode of action of the effective genetic factors and to study the combining abilities and gene effects for APR to leaf and stripe rusts in five spring wheat genotypes.
2. Study the association of APR to stripe and leaf rusts and to explore possibilities of breeding for co-improvement in the levels of nonspecific resistance to both diseases.
3. Study the QTLs involved in APR to leaf and stripe rusts in the Australian wheat cultivar, Cook, and to determine the stability of the identified QTLs across locations and against both diseases.

The results of these investigations should contribute to a better understanding of nonspecific leaf and stripe rust resistances, and assist wheat breeders in designing strategies for rust resistance breeding.

1.12 Figures

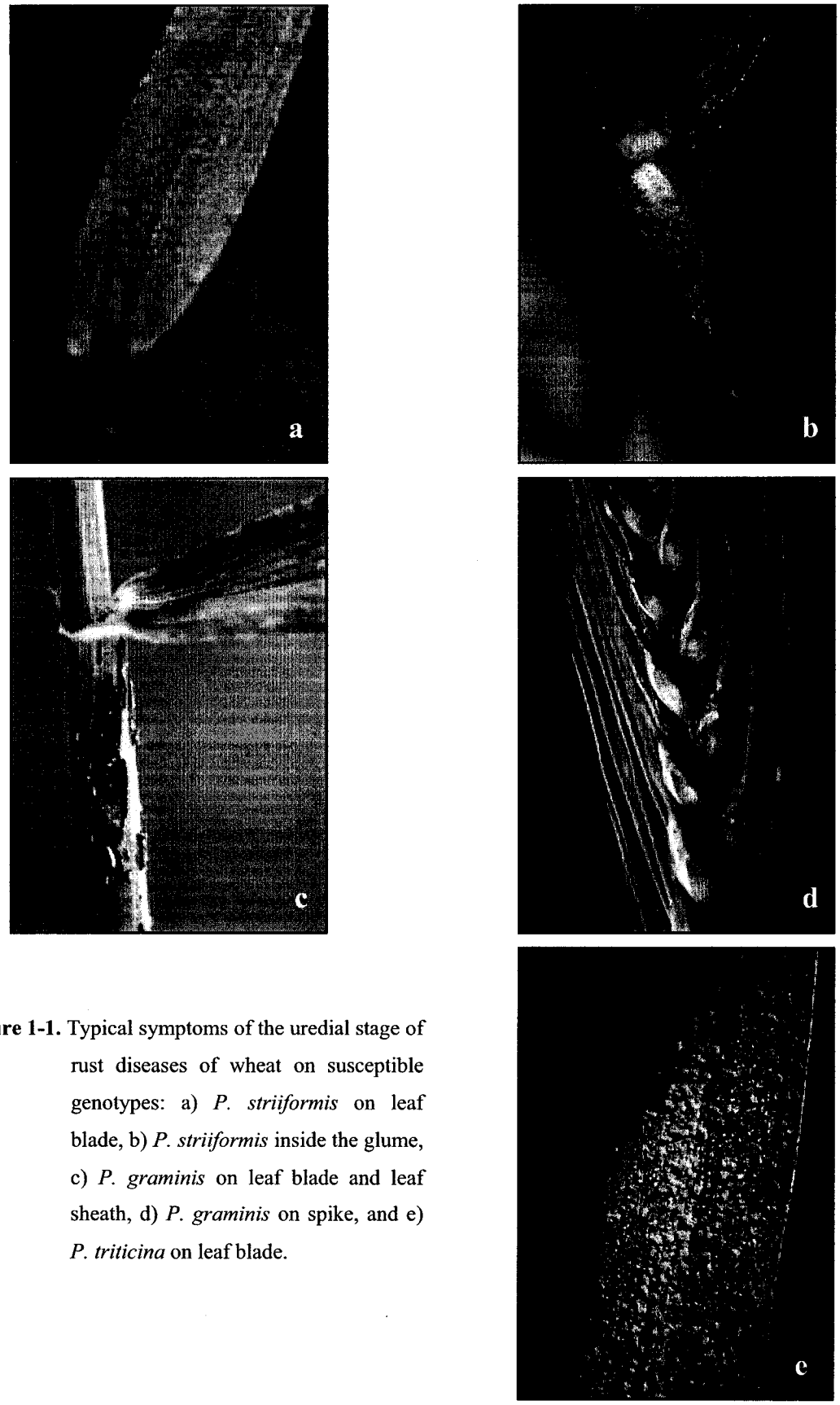


Figure 1-1. Typical symptoms of the uredial stage of rust diseases of wheat on susceptible genotypes: a) *P. striiformis* on leaf blade, b) *P. striiformis* inside the glume, c) *P. graminis* on leaf blade and leaf sheath, d) *P. graminis* on spike, and e) *P. triticina* on leaf blade.

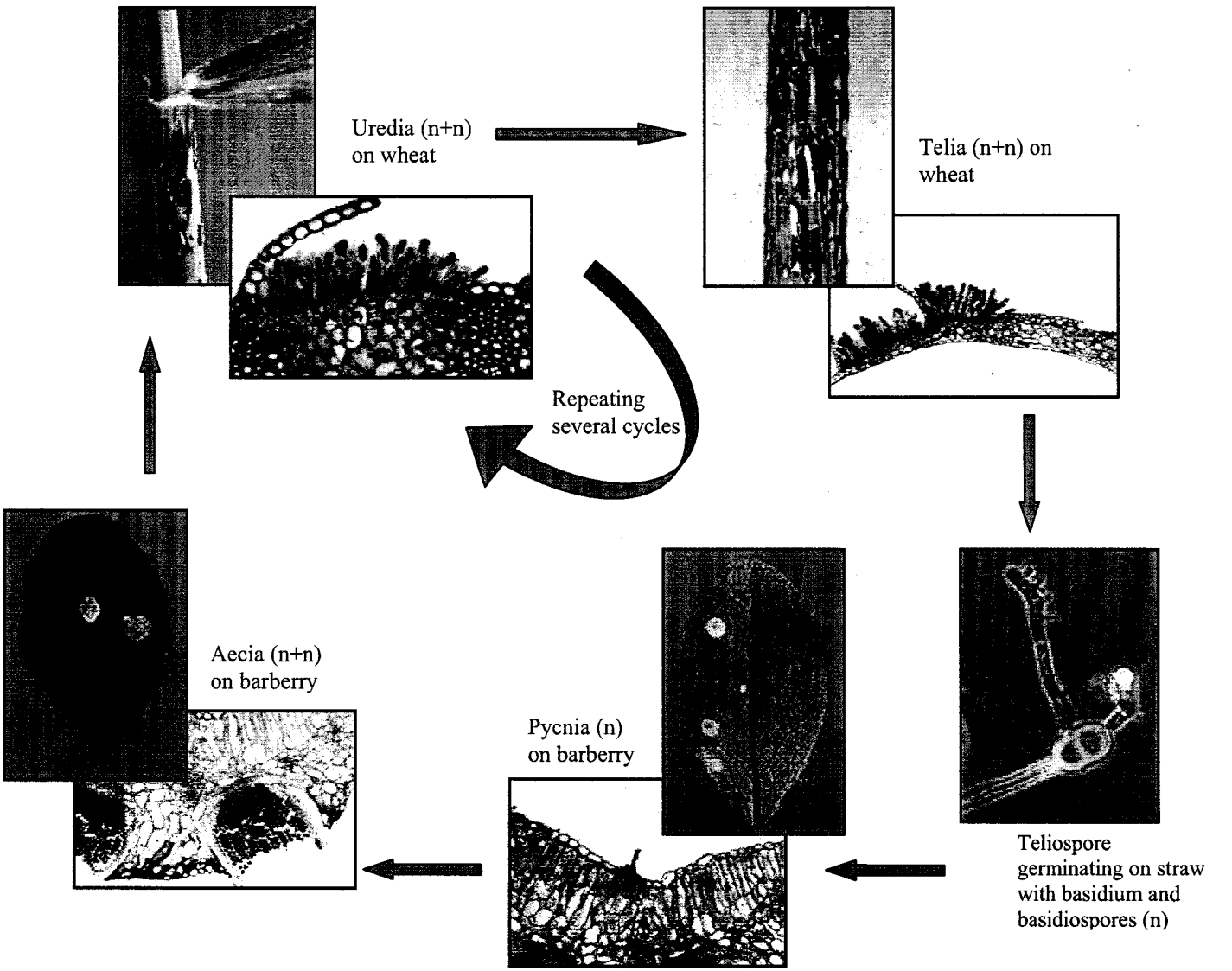


Figure 1-2. Life cycle of *P. graminis*, as a typical life cycle of rust fungi, with five spore stages (see the text for detailed description of the life cycle).

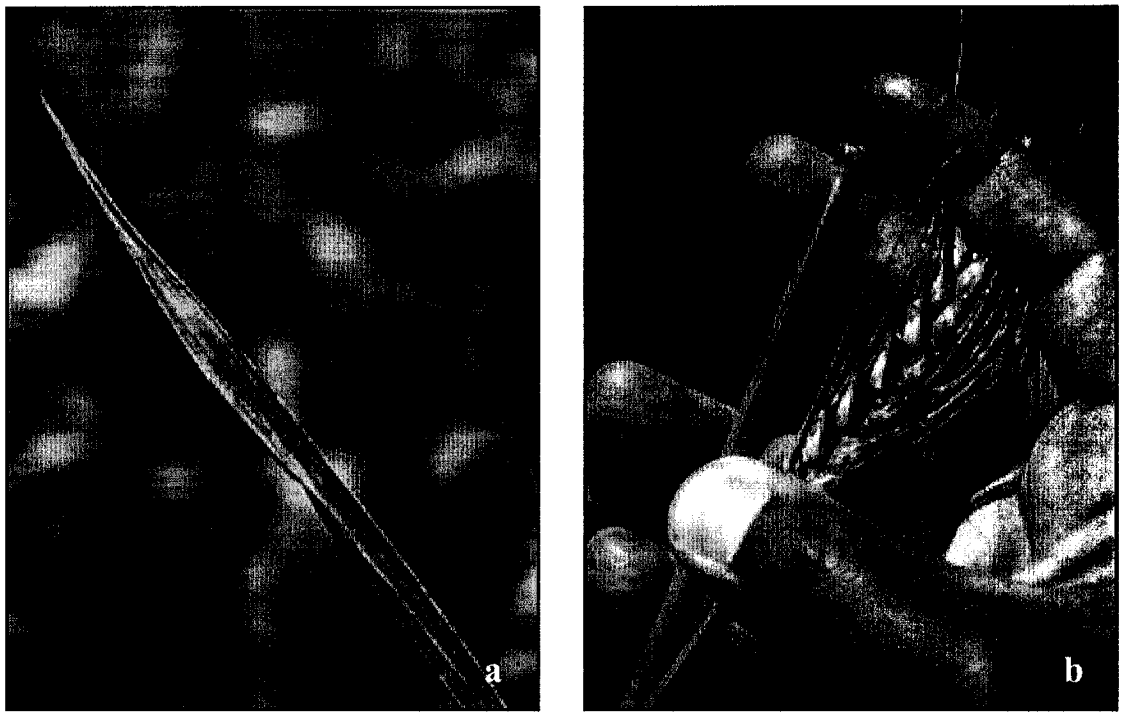
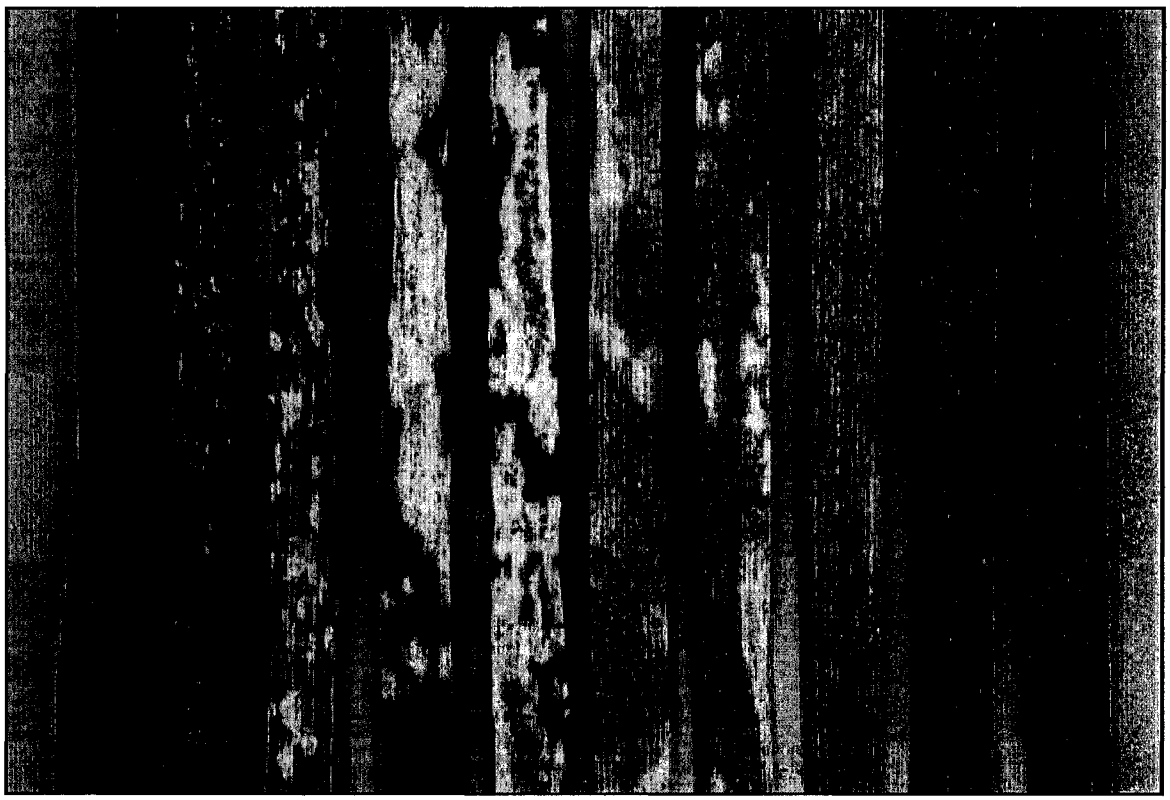
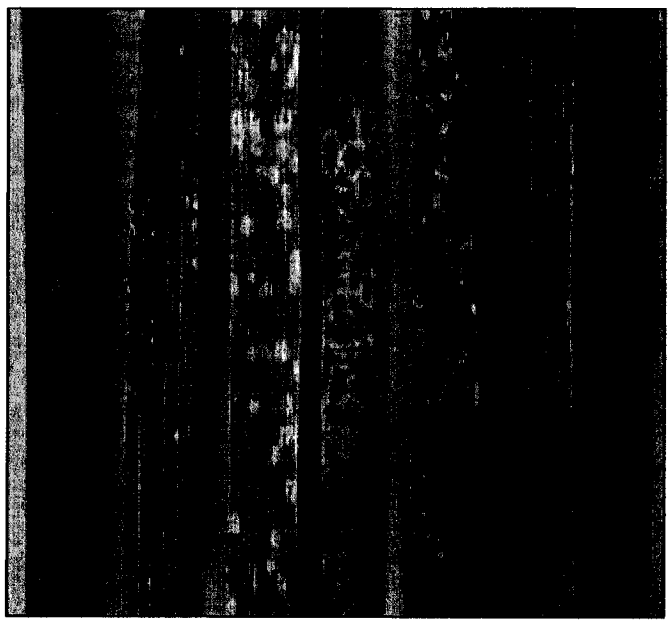


Figure 1-3. Leaf tip necrosis (a), known to be associated with adult plant leaf and stripe rust resistance gene(s) *Lr34/Yr18*, and pseudo black-chaff (b), known to be associated with adult plant stem rust resistance gene *Sr2*.



0 1 2 3 4 5 6 7 8 9



0 ; 1 2 X 3 4

Figure 1-4. Seedling infection types of the stripe rust (top) and leaf rust (bottom) diseases of wheat (courtesy of Dr. R. P. Singh, CIMMYT, Mexico). A 0-9 scale is used for stripe rust and 0-4 scale is used for leaf rust infection type in CIMMYT, Mexico.

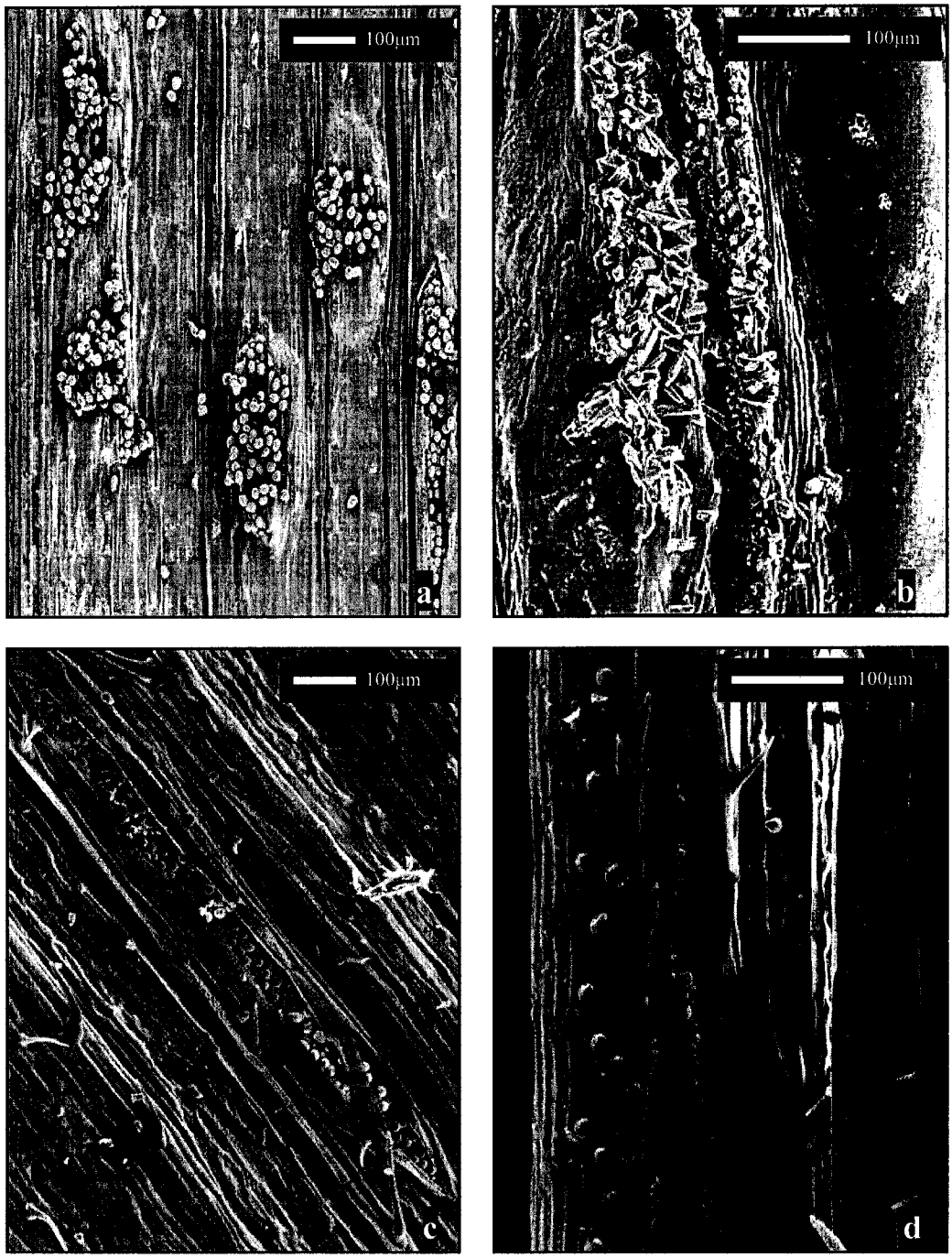


Figure 1-5. Scanning electron microscopy of wheat isogenic lines infected with stripe rust: a and b are Thatcher S, and Thatcher+*Yr18* (courtesy Elahinia and Tewari), c and d are Avocet S and Avocet + *Yr18*, respectively. Note the abundant calcium-rich crystals in Thatcher+*Yr18* isogenic line (b).

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Chapter 2

Inheritance of high levels of adult-plant resistance to stripe rust in five spring wheat genotypes[1]

2.1 Introduction

Stripe rust, caused by *Puccinia striiformis* Westend., is an important fungal disease of wheat in many countries (Roelfs *et al.,* 1992). Genetic resistance is the most effective, economical, and environment-friendly method of controlling stripe rust. Almost 30 genes that confer resistance to stripe rust in wheat are catalogued so far (McIntosh *et al.*, 1998). Most of these resistance genes are expressed in the seedling growth stage and are effective throughout the life of the plant. These genes are recognized by a characteristic hypersensitive response. However, high genetic variation and the ability of the pathogen to evolve into new races with added new virulences have always been major limiting factors in successful long-term control of stripe rust when race-specific resistance genes are deployed. The break-down of such race-specific stripe rust resistance genes as a consequence of the pathogen acquiring new virulence, has frequently been reported (Chen *et al.*, 2002; Bayles *et al.*, 2000; Danial *et al.*, 1995), even in the cases with deployment of race-specific resistance genes in combination (Johnson, 1988). On the other hand, adult-plant resistance (APR) has been described in several spring and winter wheat genotypes (Bariana *et al.*, 2001; Zhang *et al.*, 2001; Wagoire *et al.*, 1998; Bariana and McIntosh, 1995; Singh and Rajaram, 1994; Milus and Line, 1986; Johnson, 1980). APR is characterized as a type of interaction between the host and pathogen in which the adult plant is partially resistant, despite seedling compatibility.

McIntosh (1992b) indicated that the characteristics that are associated with durable (Johnson and Law, 1975) rust resistance are: that they are more likely to be of

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the adult plant, rather than seedling type, that they are conditioned by more than a single gene acting additively, and that they are not associated with genes conferring hypersensitive response. Stripe rust resistance gene *Yr18*, located in chromosome arm 7DS (Singh, 1992b; McIntosh, 1992a) is a major contributor to durable APR to stripe rust. Under high disease pressure however, the level of stripe rust severity of lines with *Yr18* alone may reach 50-60% (Ma and Singh, 1996), which is not acceptable in breeding programs. Incorporating additional genetic factors in *Yr18* background may result in higher levels of resistance (Singh *et al.*, 2000).

The parental genotypes studied were the outcome of a breeding project at the International Wheat and Maize Improvement Center (CIMMYT), designed to combine *Yr18* with other additive APR genes to achieve high levels of APR in high-yielding wheat germplasm (Singh *et al.*, 2000). The objective of the present study was to determine the number and mode of action of the effective genetic factors and to study the combining abilities and gene effects for APR to stripe rust in a one-way diallel cross involving five of these APR spring wheat genotypes and a susceptible genotype, Avocet-*YrA*.

2.2 Materials and Methods

2.2.1 Plant material and population development

Five spring wheat genotypes were chosen for a genetic study based on their APR to stripe rust in Mexico, Ecuador, Kenya, and Iran, despite their susceptibility in the seedling growth stage to Mexican race Mex96-11 and Iranian race 134E134A$^+$ of *P. striiformis* (Table 2-1). One-way diallel crosses were made among the five resistant genotypes and a susceptible genotype, Avocet-*YrA*, in the greenhouse at the University of Alberta, Canada. All fifteen F_1 crosses were made by hand-emasculation and artificial pollination (Figure 2-2) using the Approach Method (Allan, 1980). From each cross, 90-150 F_1 seeds were harvested. Ten F_1 seeds from each cross were grown in a greenhouse in 15 cm diameter pots filled with a Metromix® soil mixture. The greenhouse was maintained at 20/15 day/night temperature, and 16 h photoperiod regime. F_1 plants were self-fertilized to produce F_2 seeds that were harvested from individual plants in each cross. F_3 lines of the susceptible × resistant crosses were harvested from F_2 single plants

that were planted in 15 cm pots in the greenhouse. Furthermore, the F_4 single plant-derived F_5 lines of the diallel cross were developed by advancing segregating generations using the single seed-descent (SSD) method (Figure 2-1). For this purpose, 200 F_2 seeds from each cross were planted in root containers (25 × 25 mm wide and 140 mm deep) containing Metromix (Figure 2-3). Root containers were kept in packages of 72 in irrigation trays. Water and fertilizer were applied by filling the irrigation trays containing the packages of root containers for two hours. Frequency of irrigation was reduced when plants were established so that the length of growing cycle was reduced to 100-120 days, and as a result, most plants produced a single tiller with 5-6 seeds per spike. The subsequent F_3 generation was planted as described for the F_2. From each single plant in each generation, three seeds were harvested, two of which were kept as reserve seeds and one seed was planted to achieve the next generation. F_4 seeds were space-planted at the University of Alberta Edmonton Research Station in the summer of 2000 and F_5 SSD lines were obtained by harvesting single F_4 plants.

Population development was done in the absence of disease and no selection pressure was applied for any trait. Seeds were given 72 hours cold treatment at 4°C and soaked in water 12 hours prior to planting in order to overcome any potential seed dormancy and to achieve uniform germination. However, some lines were unintentionally lost due to lack of germination or extremely late heading that occurred in the segregating generations and thus failed to produce seed. This caused some unwanted selection pressure that reduced the number of lines obtained in some crosses, especially those involving Cocnoos. For genetic-analysis, it was assumed that the unwanted selection pressure was randomly distributed.

2.2.2 Experimental procedure

Field evaluations were carried out at the CIMMYT Research Station near Toluca (state of Mexico) in the highlands of central Mexico (19° N, 2640 masl), during 2000 and 2001 crop seasons. Toluca Station has a favorable environment for stripe rust development. Parental lines, F_1 crosses, F_2 populations and F_3 lines were evaluated in 2000 (Figure 2-4 and 2-5). The F_1 experiment comprised fifteen F_1 crosses and six parental lines planted in a three-replication randomized complete block design. F_1

experimental units comprising 15 plants were grown in 1 m long plots on the top of 75 cm wide raised beds in two rows, 20 cm apart. The F_2 experiment included fifteen F_2 populations and six parental lines arranged in a randomized complete block design with three replicates. Each F_2 experimental unit comprised 120 space-sown plants at 10-15 cm spacing on top of the beds (as described for the F_1) 2.5 m long (Figure 2-5). About 80 seeds of each of the 624 F_3 lines, 118-130 from each susceptible × resistant cross, were planted in a plot size the same as for the F_1 experiment, with parental lines repeated after every 10 lines as checks.

During the summer of 2001, the 6 parents, 564 F_3 lines derived from the five susceptible × resistant crosses, 68-155 lines from each cross, 1810 F_5 lines derived from all 15 crosses, 75-142 lines from each cross, and 10 check genotypes with different stripe rust responses were included in the 2001 trial. Lines were planted in an augmented randomized complete block design (ARCBD) as described by Scott and Milliken (1993), each block comprised of 200 experimental units. One set of check genotypes were randomly planted in each block. Plot size and seed density was as described for the F_3 experiment in 2000.

2.2.3 Artificial epidemics and stripe rust race

In all field experiments, one row of the susceptible spreader cultivar, Morocco, was planted on one side of the plots in the pathways and around the experiments (Figure 2-4). Artificial inoculation was done with a single race of *P. striiformis*, MEX96-11, virulent on *Yr 2, (3), 6, 7, 9, 27,* and *A*, four weeks after sowing. For this purpose fresh urediniospores suspended in a lightweight mineral oil (Soltrol®-170), were sprayed on the spreader rows. The parental genotypes were tested earlier for seedling infection type to race MEX-96-11 at CIMMYT and all displayed a high seedling infection type (Table 2-1; data provided by Dr. R. P. Singh, 1998). Therefore, this race was suitable for the study of APR.

2.2.4 Stripe rust assessment

Stripe rust severity of the F_1 crosses, F_3 lines, and F_5 lines and F_2 single plants were rated following the modified Cobb's Scale (Peterson *et al.,* 1948). Based on the

experience at Toluca Research Station (Singh and Rajaram, 1994) disease rating was done twice. The first rating was done when the susceptible parent, Avocet-*YrA*, showed 80 to 100% severity, whereas the second rating was done two weeks later when rust had dried out the leaves of Avocet-*YrA*. Disease severity in F_1, F_3, and F_5 experiments were assessed based on the average rust severity on the flag and penultimate leaves in the plot. An overall disease severity was visually estimated for each plot. In the F_2 experiment, however, all single plants of the parents and F_2 populations were evaluated for rust severity. Several flag and penultimate leaves of each single plant were evaluated and an average rust severity was estimated for each plant.

2.2.5 Genetic analysis

2.2.5.1. Qualitative analysis

For segregation analysis, single plants in each F_2 population derived from susceptible × resistant crosses were classified into three groups: 1) parental type resistant (PTR): those with disease severity equal or less than that of the resistant parent, 2) parental type susceptible (PTS): those with disease severity equal or more than that of the susceptible parent, and 3) others. In F_3 and F_5 of the susceptible × resistant crosses, however, lines were classified into four groups: 1) homozygous for the parental type resistance (HPTR), 2) homozygous for the parental type susceptibility (HPTS), 3) segregating or homozygous for disease severity levels higher than that of the resistant parent but less than that of the susceptible parent (SegI), and 4) segregating with at least one plant having disease severity similar to the susceptible parent's response (SegS). Chi-square analyses were carried out to test the distribution of observed phenotypic frequencies against those expected for segregation models. Frequency distributions of the disease severity of the F_2 plants and F_5 lines of the resistant parent intercrosses were used to study the similarity of the segregating factors.

2.2.5.2. Quantitative analysis

Quantitative genetic analysis of the diallel cross was done on the plot mean values of the disease severities for F_1 crosses, F_2 populations, and F_5 lines. The treatment residuals were not normally distributed as determined by the Proc UNIVARIATE

procedure (SAS Institute, Cary, NC). Percent mean disease severity of each experimental unit was therefore, transformed by the arcsine of the square root to normalize the scale. To test the null hypothesis that there were no genotypic differences among the parents, F_1 crosses and F_2 populations, a one way analysis of variance (Steel *et al.*, 1997) was performed using the Proc GLM procedure (SAS Institute, Cary, NC) while the genotype sum of squares was broken into three components, parents (P), crosses (C), and P *vs.* C. Analyses of variance for the F_3 and F_5 lines in augmented designs were done separately using the Proc GLM and Proc MIXED procedures (SAS Institute, Cary, NC) as described by Scott and Milliken (1993) with minor modifications to break the genotype sum of squares into four components, parents (P), crosses (C), checks (Ck), and P *vs.* C, and also to estimate the nested effect of line(genotype). The analyses of variance for F_3 and F_5 can be modeled as:

$$X_{ijk} = \mu + b_i + g_j + l_k(g_j) + e_{ijk}$$

where: X_{ijk} = observed value of the experimental unit, μ = mean, b_i = the block effect, g_j = the genotype effect, $l_k(g_j)$ = the nested effect of SSD line in genotype and e_{ijk} = error.

Combining ability analyses were conducted separately for F_1, F_2, and F_5 diallel following the Model I, Method IV of Griffing (1956), with genotypes treated as fixed effects. Diallel Analysis and Simulation Software by Burrow and Coors (1994) was used to estimate the general combining ability (GCA) and specific combining ability (SCA) effects and the components of variance. The model used in the combining ability analysis of F_1 and F_2 was:

$$X_{ijk} = \mu + g_i + g_j + s_{ij} + b_k + e_{ijk}$$

where: X_{ijk} = observed average value of the experimental unit for F_1 and F_2, μ = population mean, g_i = GCA effect for parent i, g_j = GCA effect for parent j, s_{ij} = SCA for parents i and j, b_k = rep (block) effect for block k, and e_{ijk} = error. The model in the above software assumes that epistasis and genotype × environment interaction effects are not significant. Parents were not included in the combining ability analysis to obtain unbiased estimates of GCA and SCA parameters (Das and Griffey, 1994; Singh *et al.*, 1992). For the F_5 diallel, the GCA and SCA effects were estimated using the mean stripe rust severities of the F_5 SSD lines derived from each cross and were tested using the

mean square of the nested effect of line in genotype in the ANOVA. In order to estimate the relative importance of GCA and SCA to determine the progeny performance, as suggested by Baker (1978) the ratio $[2\sigma^2_g/(2\sigma^2_g + \sigma^2_s)]$ was computed. Narrow-sense heritability of the disease severity was estimated for each generation from the estimated components of variance, as the ratio of additive variance to the sum of genotypic and environmental variance. Mean stripe rust severities of F_1 crosses and F_2 populations, and least square mean of the severities of F_3 and F_5 lines were computed using MEANS and LSMEANS statements, respectively, in the Proc GLM procedure with Tukey's standardized range test used to test the hypothesis of the similarity of the means in F_1 and F_2 diallels (SAS institute, Cary, NC). Parental genotypes and crosses were classified based on the mean stripe rust severities of the parental lines, F_1 crosses, F_2 populations, and least square means of the F_5 SSD lines. For this reason a cluster analysis was performed using the average linkage method in the Proc CLUSTER procedure, and a dendrogram was developed using the TREE statement (SAS Institute, Cary, NC).

Minimum numbers of additive APR genes in the resistant parents were estimated based on the quantitative data in susceptible × resistant crosses in F_1, F_2 and F_5 generations according to the formula proposed by Mather and Jinks (1982):

$$N = [d]^2 / V_A$$

where: N is the minimum number of effective additive genes, d is the additive component of the gene action, and V_A is the additive genetic variance. Minimum numbers of additive genes in F_5 generation were also estimated according to the formula proposed by Bjarko and Line (1988):

$$N = (GR)^2 / 4.27\ [(V_{F5} - V_{P1} + V_{P2})\ / 2]$$

where: N is the minimum number of effective additive genes, GR is the genotypic range, V_{F5} is variance of F_5 SSD lines, and V_{P1}, and V_{P2} are variance of resistant and susceptible parents, respectively. The formulae are based on the assumptions that resistance genes that segregate in a cross are in only one parent, segregating resistance genes are not linked, all resistance genes have equal effects, additive × dominance and genotype × environment effects do not exist, and dominance is equal at all loci.

2.3 Results

The mean terminal stripe rust severities of the parental genotypes, F_1 crosses, F_2 populations and least square means of the F_3 and F_5 SSD lines, and 10 check-genotypes are presented in Table 2-2. Although the stripe rust severities of the resistant parental lines were slightly higher in the second year, all five of them displayed very low levels (between about 1 to 10%) of stripe rust severity, which was significantly less than the 100% severity of the susceptible parent, Avocet-*YrA*. Simorgh was the most resistant parent in all experiments in both years. Though the relative disease severity levels among the other APR parental genotypes and crosses varied slightly between years and trials, high levels of correlation were found between severity scored in different generations, ranging from $r = 0.95$ ($P < 0.01$) to $r = 0.99$ ($P < 0.01$). Parental genotypes and crosses were classified using a cluster analysis (Figure 2-6), based on the F_1, F_2, and F_5 average values. From this the susceptible parent, Avocet-*YrA* was grouped individually (cluster S in Figure 2-6), with the highest levels of stripe rust severities in all experiments. All resistant parents and resistant intercrosses were grouped in one cluster (cluster R in Figure 2-6) all with very low mean stripe rust severities. Susceptible × resistant crosses except Avocet-*YrA* × Simorgh were grouped in one cluster (cluster SR in Figure 2-6), all with intermediate stripe rust severities, significantly higher than those of the resistant parents (Table 2-2). Avocet-*YrA* × Simorgh with significantly ($P < 0.05$) less stripe rust severity than the other susceptible × resistant crosses and Saar × Parastoo with higher stripe rust severity than other resistant intercrosses grouped together in a two-member cluster (cluster SRR in Figure 2-6).

2.3.1 Segregation analysis:

2.3.1.1. Susceptible × resistant crosses

Continuous distributions of disease severity were observed in all F_2, F_3 and F_5 generations of susceptible × resistant crosses (Figure 2-7 as an example). Frequency distributions were significantly skewed with population means shifting from the mid-parental values towards the resistant parents (Table 2-7). All susceptible × resistant F_1 crosses displayed an intermediate stripe rust severity (Table 2-2) suggesting that the APR in the evaluated genotypes tends to be incompletely dominant. In all susceptible ×

resistant crosses only a few of the F_2 plants (Table 2-4), F_3 (Table 2-5), and F_5 (Table 2-6) lines resembled the disease levels of the resistant or susceptible parents. These were all indications of quantitative inheritance of APR to stripe rust in the evaluated genotypes, and resistance genes having additive effects.

The frequencies of PTR and PTS plants in the F_2 populations of susceptible × resistant crosses suggested a polygenic inheritance of resistance and the presence of a minimum of 4 independent resistance genes with additive effects in each of the resistant parents (Table 2-3). Segregation of F_3 lines that were classified into four groups, *viz.* HPTR, HPTS, SegI and SegS, also suggested a polygenic inheritance, with segregation of 4 independent genes in all crosses in 2000 and 3-4 independent genes in 2001 (Table 2-3).

The population sizes of F_3 lines that were tested were relatively small for testing 3 and 4 gene based segregation ratios. Therefore, F_5 SSD lines from these crosses were also evaluated in 2001 to verify the F_2 and F_3 results. The distribution of F_5 lines in four phenotypic groups in each of the susceptible × resistant crosses were also in accordance with segregation of a minimum of 4 independent genes having additive effects (Table 2-3). The overall results of segregation analyses indicated that APR to stripe rust in the five resistant parents was quantitatively inherited and a minimum of 4 genes having additive effects conferred resistance in each parent.

2.3.1.2. Resistant × resistant crosses

Although none of the F_2 plants derived from the resistant-parent intercrosses were as susceptible as Avocet-*YrA*, single F_2 plants with stripe rust severity levels higher than those of parental lines were observed in all F_2 populations (Figure 2-8). Disease severity as high as 50% was observed in F_2 populations derived from Saar × Parastoo and Simorgh × Parastoo (Table 2-4). F_5 lines of the resistant-parents intercrosses were also evaluated to verify the F_2 results. Segregation for stripe rust severities was again observed in F_5 populations of the resistant × resistant crosses, with few lines having disease severities as high as 60% in crosses of Saar × Homa and Saar × Parastoo, and in crosses of Simorgh × Cocnoos and Homa × Parastoo (Table 2-6). This indicated that although all resistant parental lines probably have at least one gene in common, some of

the additive genes in each of the parents are different. Some crosses like Saar × Simorgh, or Simorgh × Homa displayed less segregation for disease severity levels (Tables 2-4 and 2-6). Considering that on the average each parent had two common genes for stripe rust resistance and they differed by two additive genes then as many as twelve different additive genes could be present in the parents.

2.3.2 Quantitative analysis

The analysis of variance showed highly significant ($P < 0.01$) differences among parents, F_1 crosses, F_2, F_3, and F_5 populations (Table 2-8). From the F_3 lines, only susceptible × resistant derivatives were evaluated. The parent *vs.* F_1 crosses effect was significant ($P < 0.05$) and parent *vs.* F_2 population and parent *vs.* F_3 susceptible × resistant cross effects were highly significant. This indicated significant deviations of the F_1 crosses, F_2 and F_3 populations from their mid-parental values. This effect was not significant for the F_5 lines. Non-significant deviation of population means from mid-parental values in F_5 was probably due to the reduced dominance effect in the F_5 associated with an increased homozygosity. GCA effects were highly significant for F_1s, F_2s and significant for F_5s, while SCA effects were significant only in F_1 and F_2 experiments (Table 2-8). The components of variance ratios were 0.95, 0.97, and 0.93 for F_1, F_2, and F_5, respectively (Table 2-9), and indicated that GCA was of major importance in predicting the progeny performance. The importance of GCA was also evident based on the relatively high correlations between parental means and GCA effects ($r = 0.95$, $P < 0.01$, for F_1; $r = 0.97$, $P < 0.01$, for F_2; and $r = 0.97$, $P < 0.01$, for F_5). Narrow-sense heritability estimates (Table 2-9) were also relatively high, 0.91, 0.96, and 0.88 for F_1 crosses, F_2 populations, and F_5 SSD lines, respectively.

GCA effects of parents and SCA effects of crosses are given in Table 2-10. The F_1 crosses, F_2 populations, and F_5 lines involving parents with negative GCA effects are more resistant than the average of the respective generations. However, a positive value indicates greater susceptibility. All APR parents had negative GCA effects. Simorgh had the greatest negative GCA effects in all generations that were highly significant in F_1 and F_2 generations and significant in F_5. This indicated that the most resistant F_1 crosses, F_2 populations, or F_5 lines on average had Simorgh as one parent. Homa had significant to

highly significant negative GCA effect. Saar had significant GCA effects in F_1 and F_2, but not in F_5, while Cocnoos had a significant and negative GCA effect in F_5 and a highly significant negative GCA effect in F_2.

SCA effects were significant in F_1 and highly significant in F_2 generations, but not significant in F_5. In crosses with the susceptible parent, the cross Simorgh × Avocet-*YrA* had the greatest negative SCA in F_1 (SCA effect = -17.85) and in F_2 (SCA effect = -4.79) generations. This indicated that Simorgh contributed the greatest reduction in disease severity when crossed with the susceptible parent. In resistant parent intercrosses, the cross Saar × Cocnoos had significant to highly significant negative SCA effects across generations (SCA effect = -5.76, for F_1, -3.51 for F_2, and -4.06 for F_5).

The numbers of effective additive genes that were estimated from the quantitative analysis are presented in Table 2-11. Based on the two formulae used in different generations the estimates for the minimum numbers of additive genes ranged from 3.3 to 8.4. The numbers estimated in the F_1 and F_5 generations were comparable with those estimated from segregation analysis (Table 2-3). The numbers of genes estimated from the F_2 data were evidently higher than those estimated by analysis of subsequent generational segregation.

2.4 Discussion

The resistant parental genotypes chosen for this research showed high levels of APR, despite high infection type to Mexican stripe rust race Mex96-11 in the seedling stage. Compatible interaction of race Mex96-11 with the parental genotypes in the seedling indicated that this race was suitable for the study of APR.

Compatible host-pathogen interaction accompanied by a reduced infected leaf area is a known feature of partial resistance (Parlevliet and Ommeren, 1975) and slow-rusting (Caldwell *et al.*, 1970). In other rust pathosystems of wheat, sources of APR have been referred to as slow-rusting or partially resistant (Kolmer and Liu, 2001; Kolmer, 1996), because a reduced terminal rust severity is associated with a moderately susceptible to susceptible infection type. In the case of stripe rust however, some levels of reduction in infection type in the adult plant stage seem to be associated with APR. For this reason, in the case of stripe rust resistance, researchers have differentiated

among partial resistance, slow-rusting and APR. Broers (1993) concluded that the prevalence of APR genes in wheat impedes accumulation of partial resistance to stripe rust. Danial (1993) reported that the combination of sufficient high levels of resistance together with a high infection type is rare in the wheat-stripe rust pathosystem. Singh *et al.* (2001), on the other hand, related the reduction of infection type of stripe rust APR genotypes to the systemic development of stripe rust. They stated that in the case of potentially durable resistant genotypes, the first uredinia to appear cause a moderately susceptible to susceptible reaction and reduction in the infection type is due to the subsequent systemic growth of the mycelia in the leaf inter-veins that causes some chlorosis and necrosis. In this research the histological interaction between the host and pathogen was considered compatible, as had been determined by the seedling test. Infection type was, therefore, not included in the genetic analysis to avoid any possible confounding effects of necrosis and chlorosis caused by fungal mycelial growth.

Comparison of stripe rust severities of the five resistant parents with their respective F_1 hybrids with the susceptible parent and the mid-parental values indicated that APR to stripe rust tends to be incompletely dominant. Segregation results indicated that the APR in these resistant parents was controlled by a minimum of 4 genes having additive effects. Because all resistant parents expressed leaf tip necrosis that is known to be either pleiotropic or linked to *Yr18* (Singh, 1992 a and b) It was evident than *Yr18* was one of the identified genes in each parent. The skewness of the frequency distributions of the F_5 lines and the shift of F_1 values and F_2 and F_5 population means from the mid-parental values towards the resistant parent in susceptible × resistant crosses was probably due to the greater effect of at least one of the additive genes, probably *Yr18*, than other genetic factors. Singh *et al.* (2001) designated the APR gene, *Yr29*, in CIMMYT germplasm, located in chromosome 1BL of Pavon-76. It is likely that Saar and Simorgh carry *Yr29* in addition to *Yr18*, since they are both three-way cross-derivatives having Sonoita-81 as one of the parents. Sonoita-81 shares common parentage with Pavon-76 (coefficient of parentage = 0.48; extracted from IWIS; Fox *et al.*, 1997).

The numbers of genes estimated for the F_3 generation in 2000 and 2001 were slightly different. This inconsistency may have been caused by several factors. The F_3 lines that were tested in 2001 were not identical to those tested in 2000, as the amount of

available seed was limiting. Lack of exact congruity between the F_3 results may also be due to the smaller population sizes within the F_3 lines that could be inadequate for testing of four gene ratios (Steel *et al.*, 1997). The expected frequency of lines that are homozygous for four independent loci in the F_3 generation is one in 256. Gene number estimates based on the F_5 results are therefore more reliable as the expected frequency of HPTR or HPTS lines, homozygous at all loci, increases to 3.7% in the case of four gene segregation ratio. The number of effective genes estimated from the quantitative analyses of F_1 and F_5 were comparable with those estimated in segregation analysis. The numbers estimated in the F_2 generation were greater than the estimates in other generations and those estimated in segregation analysis. It should be noted that the estimates of the number of genes were made under the assumption that all additive genes have equal effects. However, it appears that *Yr18* has a bigger effect as compared to other additive genes. Overall, it can be concluded that the high levels of adult-plant resistance to stripe rust in the five resistant parents included in this study is conditioned by *Yr18* and a minimum of 3 additional genes having additive effects. *Yr29* is likely to be one of the additive genes in Saar and Simorgh. However, this hypothesis needs to be further investigated.

Quantitative analysis pointed to additive gene effects as being the major contributor in the inheritance of terminal stripe rust severity. The preponderance of GCA effects, as demonstrated by the components of variance ratio, showed that the additive gene effects were more important than non-additive gene effects in the evaluated genotypes. The estimates of narrow-sense heritabilities were also relatively high and were in agreement with the importance of additive gene effects. Results are consistent with those of Ghannadha *et al.* (1995), Krupinsky and Sharp (1978), and Wagoire *et al.* (1998), who reported the importance of additive gene effects in the inheritance of latent period, infection type and coefficient of infection to stripe rust.

The importance of additive gene effects suggests that the most resistant progeny may be derived from crosses with genotypes with greatest negative GCA. The GCA and SCA effects were slightly inconsistent across generations. Das and Griffey (1994) stated that the inconsistency of GCA effects across generations could have resulted from segregation and recombination of resistance genes. Combining ability of more advanced

generations therefore, seems to be more reliable. Simorgh was the most resistant parent in all experiments with the greatest negative GCA in F_1 and F_2. Cocnoos also had a high negative GCA effect and can be considered a good general combiner for enhancement of APR to stripe rust.

The significance of parent *vs.* crosses effects indicated a deviation of F_1 and F_2 means from mid-parent values, which is an indication of presence of non-additive gene effects, including dominance. This effect was not significant in F_5, probably due to increased homozygosity. Additionally, the significance of SCA effects in F_1 and F_2 may be an indication of the presence of non-additive gene effects. Wagoire *et al.* (1998) reported the presence of significant dominance and epistasis gene effects, while additive gene effects were major components in the inheritance of coefficient of infection to stripe rust. Chen and Line (1995a) also reported significant dominance and epistasis effects in the inheritance of high-temperature adult-plant resistance in Druchamp wheat. Vanderplank (1984) argued that reports of non-additive gene effects in genetic studies of quantitative disease resistance must be treated with uncertainty, as the quantitative analysis of disease resistance is always associated with an overestimate of non-additive and an underestimate of additive gene effects. The reason is that the estimated mid-parental values are based on a visual scale of infected leaf area and there are always less than the actual mid-parental values. The significant non-additive gene effects that were estimated in the present research can be explained, at least in part, by the possible overestimation of the non-additive gene effects due to the visual scale.

Although all resistant parents carry the *Yr18* gene, there were plants/lines with 50-60% stripe rust severities among the F_2 and F_5 derivatives of the resistant-parent intercrosses. Therefore, some of the additive genes in each resistant parent were different. Probably, of the 4 additive genes present in each parent, at least one or more likely two, could be different between parents. Those with 50-60% stripe rust severity in resistant-parent intercrosses were probably segregants with *Yr18* alone, resulted from genetic recombination. This is in accordance with moderate effect of *Yr18* under high disease pressure when present alone (Ma and Singh, 1996). This is also of interest for breeding purposes and demonstrates that resistance levels of genotypes with *Yr18* can be improved by selection for additional genes.

Results demonstrate that an acceptable level of APR to stripe rust can be achieved by combining a few additive genes. This is in agreement with the findings of Wallwork and Johnson (1984), Bariana and McIntosh (1995), Singh *et al.* (2000), Singh and Rajaram (1994), and Bariana *et al.* (2001). Considering the relatively small number of genes required and the high heritability of APR to stripe rust, genetic gain in breeding for APR to stripe rust should be easily achieved. However, as Bariana and McIntosh (1995) have pointed out heavy selection pressure for high resistance levels in early generations may reduce the frequency of heterozygotes that are capable of giving progenies with higher resistance. It is also necessary to carry out selection under epidemics created by races with virulence for seedling resistance genes that may act epistatically and mask the effects of APR genes.

Numerous authors (Johnson and Law, 1975; Quayoum and Line, 1985; McIntosh, 1992b; Singh and Rajaram, 1994; Bariana and McIntosh, 1995; Chen and Line, 1995b; Bariana *et al.*, 2001; Zhang *et al.,* 2001) have reported that durable sources of stripe rust resistance in both winter and spring wheats involve additive interactions of a few APR genes. The parental genotypes tested in the present study appeared to carry packages of *Yr18* and additional genes with additive effects. It is therefore expected that the high levels of *Yr18* gene based APR of the parental genotypes included in this study should be durable.

2.5 Tables and figures

Table 2-1. Parentage, seedling and adult plant responses of parental genotypes to *P. striiformis* in Mexico, Ecuador, Kenya, and Iran (unpublished data from CIMMYT provided by Dr. R. P. Singh, 1998).

Genotype	Parentage Cross and selection history	Seedling infection type [a]	
		Isolate MEX96-11	Race 134E134A+
Avocet-*YrA*	WW191/WW15//EGT	9	-
Saar	Sni/Trap#1//Bav92 CG25-099Y-099M-4Y-2M-3Y-0B	7-8	7
Simorgh	Sni/Yaco//Bav92 CG29-099Y-099M-10Y-2M-3Y-0B	7-8	6
Homa	HD2281/Yaco/3/Kauz*2/Trap//Kauz CG54-099Y-099M-10Y-3M-5Y-0B	7-8	7
Parastoo	Pvn//Car422/Ana/3/Kauz*2/Trap//Kauz CG62-099Y-099M-3Y-3M-3Y-0B	7-8	8
Cocnoos	Trap#1/PBW65//Star CG99-099Y-099M-3Y-7M-1Y-0B	7-8	7
Bolani	Local susceptible check in Iran	-	9

		Adult plant response [b]				
		Mexico 1997	Mexico 1998	Ecuador 1998	Kenya 1998	Iran 1998
Avocet-*YrA*	WW191/WW15//EGT	100	100	100	100	-
Saar	Sni/Trap#1//Bav92 CG25-099Y-099M-4Y-2M-3Y-0B	1	5	5	1	5
Simorgh	Sni/Yaco//Bav92 CG29-099Y-099M-10Y-2M-3Y-0B	0	1	1	1	1
Homa	HD2281/Yaco/3/Kauz*2/Trap//Kauz CG54-099Y-099M-10Y-3M-5Y-0B	0	5	1	1	5
Parastoo	Pvn//Car422/Ana/3/Kauz*2/Trap//Kauz CG62-099Y-099M-3Y-3M-3Y-0B	10	10	10	15	10
Cocnoos	Trap#1/PBW65//Star CG99-099Y-099M-3Y-7M-1Y-0B	1	5	5	5	5
Bolani	Local susceptible check in Iran	-	-	-	-	100

[a] Seedling infection types are based on a 0-9 scale (McNeal *et al.*, 1971) in which infection types 7, 8, and 9 are considered compatible or susceptible. Isolate Mex96-11 is the common stripe rust race in Mexico and 134E134A+ is an Iranian race.

[b] Adult plant responses are terminal stripe rust severities (0-100%) recorded based on the modified Cobb's Scale (Peterson *et al.*, 1948).

Table 2-2. Mean stripe rust severities of parents, F_1 crosses and F_2 populations, and least square means of F_3 and F_5 lines derived from 6-parent diallel cross along with check genotypes.

Genotype	Means 2000		Least square means 2001	
	F_1	F_2	F_3	F_5
parents				
Avocet-*YrA*	100.00[a] a[b]	100.00 a	100.00	99.97
Saar	4.99 cdef	4.29 efgh	10.53	9.22
Simorgh	0.11 f	1.00 i	1.18	2.57
Homa	4.99 cdef	3.92 fgh	8.98	7.66
Parastoo	2.00 def	4.50 efgh	4.27	10.06
Cocnoos	4.99 cdef	3.69 gh	5.38	5.35
susceptible × resistant crosses				
Avocet-*YrA* × Saar	46.50 b	41.12 b	46.04	49.22
Avocet-*YrA* × Simorgh	13.01 c	25.76 c	38.05	35.61
Avocet-*YrA* × Homa	36.59 b	34.69 b	40.13	36.45
Avocet-*YrA* × Parastoo	53.35 b	37.35 b	43.60	40.86
Avocet-*YrA* × Cocnoos	53.35 b	40.44 b	46.49	44.80
resistant × resistant crosses				
Saar × Simorgh	1.17 ef	3.06 hi	-	12.38
Saar × Homa	2.00 def	7.57 ef	-	14.92
Saar × Parastoo	9.59 cd	16.43 d	-	26.58
Saar × Cocnoos	3.33 cdef	6.60 efg	-	10.95
Simorgh × Homa	1.00 ef	2.36 hi	-	6.72
Simorgh × Parastoo	2.00 def	7.51 ef	-	9.43
Simorgh × Cocnoos	1.00 ef	3.90 fgh	-	8.99
Homa × Parastoo	6.48 cde	8.16 e	-	10.19
Homa × Cocnoos	4.99 cdef	7.05 efg	-	10.51
Parastoo × Cocnoos	8.15 cde	7.38 efg	-	6.68
check genotypes				
Avocet S	-	-	99.39	100.00
Avocet+*Yr18*	-	-	55.24	52.58
Thatcher S	-	-	70.20	66.03
Thatcher+*Yr18*	-	-	8.72	7.40
Jupateco S	-	-	80.69	68.06
Jupateco+*Yr18*	-	-	35.12	27.77
AC-Nanda	-	-	16.36	13.98
Cook	-	-	8.72	9.87
Saar	-	-	9.83	12.27
Cocnoos	-	-	6.20	6.09

[a] Field stripe rust data based on modified Cobb's Scale (Peterson *et al.*, 1948).

[b] Means followed by common letters within generations do not differ significantly based on Tukey's standardized test on arcsine transformed data ($P < 0.05$). Range test is not practical for least square means.

Table 2-3. Frequency distribution of F_2 plants, F_3 and F_5 lines derived from crosses between the susceptible Avocet-*YrA* and five adult plant resistant wheat genotypes inoculated with *P. striiformis*.

Generation/year tested	Avocet-*YrA* crossed with	No. of plants with reaction			Tested [g] for segregation of	χ^2	P value
		PTR[a]	PTS[b]	Others			
F_2/2000	Saar	0	2	217	4 genes	2.38	>0.25
	Simorgh	3	2	272	4 genes	4.20	> 0.05
	Homa	1	3	258	4 genes	3.83	> 0.10
	Parastoo	0	3	242	4 genes	5.32	> 0.05
	Cocnoos	0	3	225	4 genes	5.89	> 0.05
		No. of lines with reaction					
		HPTR[c]	HPTS[d]	SegI[e]	SegS[f]		
F_3/2000	Saar	0	0	92	38	4 genes	2.15	>0.25
	Simorgh	0	0	102	28	4 genes	1.95	>0.50
	Homa	1	1	95	33	4 genes	0.98	>0.75
	Parastoo	1	1	86	30	4 genes	1.29	>0.50
	Cocnoos	0	0	88	32	4 genes	1.08	>0.75
F_3/2001	Saar	0	0	88	67	3 genes	5.10	>0.10
	Simorgh	1	0	81	30	4 genes	1.35	>0.50
	Homa	3	0	42	23	3 genes	5.73	>0.10
	Parastoo	1	2	80	31	4 genes	6.58	>0.05
	Cocnoos	0	0	83	32	4 genes	1.33	>0.50
F_5/2001	Saar	1	3	99	19	4 genes	3.47	>0.25
	Simorgh	9	4	91	17	4 genes	4.89	>0.10
	Homa	4	2	96	20	4 genes	1.86	>0.50
	Parastoo	2	6	104	14	4 genes	2.96	>0.25
	Cocnoos	1	4	105	9	4 genes	7.68	>0.05

[a] Parental type resistant.
[b] Parental type susceptible.
[c] Homozygous for the parental type or higher resistance (homozygous for all resistance alleles).
[d] Homozygous for the parental type or higher susceptibility (homozygous lacking all resistance alleles).
[e] Either segregating or homozygous for disease levels higher that that of the resistant parent but less than that of the susceptible parent (homozygous for at least one resistance allele).
[f] Segregating with disease levels reaching the susceptible parent response (heterozygous for at least one locus and homozygous for susceptibility alleles at other loci).
[g] The ratios used in F_2 were 0.0039 PTR : 0.0039 PTS : 0.9921 Seg for the segregation of 4 genes. The ratios used in F_3 were 0.0156 HPTR : 0.0156 HPTS : 0.5625 SegI : 0.4062 SegS for the segregation of 3 genes, and 0.0039 HPTR : 0.0039 HPTS : 0.7421 SegI : 0.2500 SegS for the segregation of 4 genes, as adjusted for population size (Singh and Rajaram, 1992). The ratios used in F_5 were 0.0367 HPTR : 0.0367 HPTS : 0.7853 SegI : 0.1414 SegS for the segregation of 4 genes.

Table 2-4. Frequency distribution of disease severity of F_2 plants derived from diallel cross when inoculated with *P. striiformis*.

Cross	No. of plants with stripe rust severity[a]												Total
	1	5	10	20	30	40	50	60	70	80	90	100	
Susceptible×Resistant													
Avocet-*YrA* × Saar	0	0	26	50	29	27	22	14	16	22	11	2	219
Avocet-*YrA* × Simorgh	5	40	62	62	39	16	14	10	10	12	4	0	274
Avocet-*YrA* × Homa	1	8	23	90	46	20	19	12	13	17	8	3	260
Avocet-*YrA* × Parastoo	0	3	25	59	56	28	21	9	16	17	7	3	244
Avocet-*YrA* × Cocnoos	0	1	9	50	59	25	25	15	17	22	1	3	227
Resistant×Resistant													
Saar × Simorgh	165	97	17	3	0	0	0	0	0	0	0	0	282
Saar × Homa	65	126	56	21	15	3	0	0	0	0	0	0	286
Saar × Parastoo	4	36	70	84	32	10	6	0	0	0	0	0	242
Saar × Cocnoos	59	108	70	22	2	0	0	0	0	0	0	0	261
Simorgh × Homa	196	80	5	1	0	0	0	0	0	0	0	0	282
Simorgh × Parastoo	87	84	44	42	6	2	1	0	0	0	0	0	266
Simorgh × Cocnoos	144	80	30	11	1	0	0	0	0	0	0	0	266
Homa × Parastoo	57	95	73	45	5	1	0	0	0	0	0	0	276
Homa × Cocnoos	84	104	56	24	11	2	0	0	0	0	0	0	281
Parastoo × Cocnoos	51	101	82	31	3	0	0	0	0	0	0	0	268

[a] Stripe rust severities based on modified Cobb's Scale (Peterson *et al.*, 1948) recorded when plants of the susceptible parent, Avocet-*YrA*, displayed 100% rust.

Table 2-5. Frequency distribution of disease severity of F_3 lines derived from susceptible × resistant crosses when inoculated with *P. striiformis*.

Year	Avocet-*YrA* crossed with	No. of lines with stripe rust severity[a]												Total
		1	5	10	20	30	40	50	60	70	80	90	100	
2000														
	Saar	0	0	3	12	16	28	22	31	13	5	0	0	130
	Simorgh	1	9	16	22	17	18	18	23	5	1	0	0	130
	Homa	0	1	10	29	18	14	27	19	9	2	0	1	130
	Parastoo	0	1	0	9	13	24	20	33	14	2	1	1	118
	Cocnoos	0	0	3	5	15	35	17	25	19	1	0	0	120
2001														
	Saar	0	0	5	12	26	34	32	27	15	1	3	0	155
	Simorgh	0	3	5	18	26	19	16	10	9	3	2	1	112
	Homa	0	1	4	9	18	10	4	8	7	6	1	0	68
	Parastoo	0	1	3	13	23	23	18	16	9	6	1	0	114
	Cocnoos	0	0	0	12	17	31	17	25	12	0	1	0	115

[a] Stripe rust severities based on modified Cobb's Scale (Peterson *et al.*, 1948) recorded when plants of the susceptible parent, Avocet-*YrA*, displayed about 100% rust.

Table 2-6. Frequency distribution of disease severities of F_5 lines derived from diallel cross when inoculated with *P. striiformis*.

Cross	Lines with stripe rust severity[a]												Total
	1	5	10	20	30	40	50	60	70	80	90	100	
Susceptible×Resistant													
Avocet-*YrA* × Saar	0	1	1	16	20	26	11	26	9	6	2	4	122
Avocet-*YrA* × Simorgh	10	5	11	21	13	11	16	14	12	2	1	5	121
Avocet-*YrA* × Homa	0	6	15	18	17	16	11	22	12	1	2	2	122
Avocet-*YrA* × Parastoo	0	0	3	25	31	19	14	16	11	2	2	3	126
Avocet-*YrA* × Cocnoos	0	1	3	15	25	29	16	13	11	2	0	4	119
Resistant×Resistant													
Saar × Simorgh	18	43	31	39	9	1	1	0	0	0	0	0	142
Saar × Homa	11	30	24	33	17	11	5	3	0	0	0	0	134
Saar × Parastoo	0	1	8	56	33	15	13	5	0	0	0	0	131
Saar × Cocnoos	10	30	29	46	7	2	0	0	0	0	0	0	124
Simorgh × Homa	30	48	15	14	7	1	0	0	0	0	0	0	115
Simorgh × Parastoo	27	26	30	41	12	1	1	0	0	0	0	0	138
Simorgh × Cocnoos	7	29	16	16	3	0	3	1	0	0	0	0	75
Homa × Parastoo	25	37	29	36	1	2	1	2	0	0	0	0	133
Homa × Cocnoos	12	42	14	20	7	2	0	0	0	0	0	0	97
Parastoo × Cocnoos	6	51	35	19	0	0	0	0	0	0	0	0	111

[a] Stripe rust severities based on modified Cobb's Scale (Peterson *et al.*, 1948) recorded when plants of the susceptible parent, Avocet-*YrA*, displayed about 100% rust.

Table 2-7. Disease severity statistics of F_5 line populations derived from diallel cross when inoculated with *P. striiformis*.

Cross	Stripe rust severity[a]						
	P1	P2	Mid-parent	Mean	Std. deviation	Range	Skewness[b]
Susceptible × resistant							
Avocet-*YrA* × Saar	100	9.0±3.0	54.5	47.0	21.1	95	0.43±0.21
Avocet-*YrA* × Simorgh	100	2.6±2.0	51.3	38.0	26.3	100	0.43±0.22
Avocet-*YrA* × Homa	100	8.0±2.5	54.0	40.1	22.6	95	0.35±0.21
Avocet-*YrA* × Parastoo	100	11.0±3.1	55.5	41.8	20.5	90	0.77±0.21
Avocet-*YrA* × Cocnoos	100	6.2±2.5	53.1	43.2	19.7	100	0.74±0.22
Resistant × resistant							
Saar × Simorgh	9.0±3.0	2.6±2.0	5.3	11.2	9.1	52	1.66±0.20
Saar × Homa	9.0±3.0	8.0±2.5	8.5	17.7	14.5	59	1.04±0.20
Saar × Parastoo	9.0±3.0	11.0±3.1	9.0	28.0	13.2	65	0.84±0.21
Saar × Cocnoos	9.0±3.0	6.2±2.5	7.6	12.7	8.3	39	0.79±0.21
Simorgh × Homa	2.6±2.0	8.0±2.5	5.3	7.9	8.1	39	1.80±0.22
Simorgh × Parastoo	2.6±2.0	11.0±3.1	6.8	11.6	9.3	49	1.12±0.20
Simorgh × Cocnoos	2.6±2.0	6.2±2.5	4.4	11.7	11.8	59	2.36±0.27
Homa × Parastoo	8.0±2.5	11.0±3.1	9.5	10.5	10.0	59	2.38±0.21
Homa × Cocnoos	8.0±2.5	6.2±2.5	7.1	10.3	9.0	39	1.42±0.24
Parastoo × Cocnoos	11±3.1	6.2±2.5	8.6	8.4	4.9	19	0.93±0.22

[a] Stripe rust severities of parentals (±standard deviation) and populations are based on modified Cobb's Scale (Peterson *et al.*, 1948) recorded when plants of the susceptible parent, Avocet-*YrA*, displayed about 100% rust.

[b] Skewness is presented as the estimated value of skewness ± standard error of skewness.

Table 2-8. Mean squares for the arcsine transformed stripe rust severities of parents, F_1 crosses, F_2 populations, and F_5 SSD lines derived from a 6-parent diallel cross, and F_3 of susceptible × resistant crosses.

SV	2000		
		F_1	F_2
	df	MS	MS
Block	2	0.021**	0.001
Genotypes (G)	20	0.403**	0.668**
Parents (P)	5	0.997**	1.149**
P vs. C	1	0.035*	0.896**
Crosses (C)	14	0.217**	0.480**
GCA	5	0.575**	1.271**
SCA	9	0.019*	0.042 **
C × B	28	0.005	< 0.001
Error	40	0.004	0.004

	2001			
	F_3		F_5	
	df	MS	df	MS
Block	2	0.036	9	0.006
Genotypes (G)	20	0.545**	30	1.420**
Parents (P)	5	0.890**	5	1.400**
P vs. C	1	0.693**	1	0.007
Crosses (C)	4	0.060	14	0.624**
GCA	-	-	5	0.095*
SCA	-	-	9	0.003
Checks	9	0.730**	9	1.628**
Line(genotype)	557	0.053	1777	0.039**
Error	31	0.038	102	0.003

*, ** significant at $P < 0.05$ and $P < 0.01$, respectively.
GCA = general combining ability.
SCA = specific combining ability.

Table 2-9. Estimates of components of variance, components of variance ratio, and heritability of terminal stripe rust severities of F_1, F_2 and F_5.

Component	Generation		
	F_1	F_2	F_5
GCA (σ^2_g)	0.040	0.026	0.022
SCA (σ^2_s)	0.004	0.002	0.003
Additive	0.090	0.052	0.089
Dominance	0.004	0.001	0.012
$[2\sigma^2_g/(2\sigma^2_g + \sigma^2_s)]$	0.952	0.972	0.938
h^2_{ns}	0.918	0.965	0.881

GCA = general combining ability.
SCA = specific combining ability.
h^2_{ns} Narrow-sense heritability.

Table 2-10. Estimates of general (on diagonal) and specific combining abilities for terminal stripe rust severity.

F_1	Avocet-*YrA*	Saar	Simorgh	Homa	Parastoo	Cocnoos
Avocet-*YrA*	30.36**	4.23	-17.85**	-2.51	6.90*	9.23**
Saar		-4.30*	5.48*	-2.18	-1.77	-5.76*
Simorgh			-15.56**	7.73**	1.81	2.81
Homa				-7.56**	-1.85	-1.18
Parastoo					-0.30	-5.10
Cocnoos						-2.64

F_2	Avocet-*YrA*	Saar	Simorgh	Homa	Parastoo	Cocnoos
Avocet-*YrA*	24.05**	2.51*	-4.79**	-0.10	-1.78	4.17**
Saar		-2.09*	-1.34	-1.16	3.51**	-3.51**
Simorgh			-10.13**	1.66	2.59*	1.88
Homa				-5.81**	-1.07	0.68
Parastoo					-1.56	-3.23**
Cocnoos						-4.43**

F_5	Avocet-*YrA*	Saar	Simorgh	Homa	Parastoo	Cocnoos
Avocet-*YrA*	24.10**	-0.68	-0.58	-0.02	-1.79	3.08**
Saar		0.89	-4.12**	0.94	7.92**	-4.06**
Simorgh			-8.26*	0.13	0.44	4.11**
Homa				-6.73*	-2.24	1.18
Parastoo					-3.26	-4.32**
Cocnoos						-6.74*

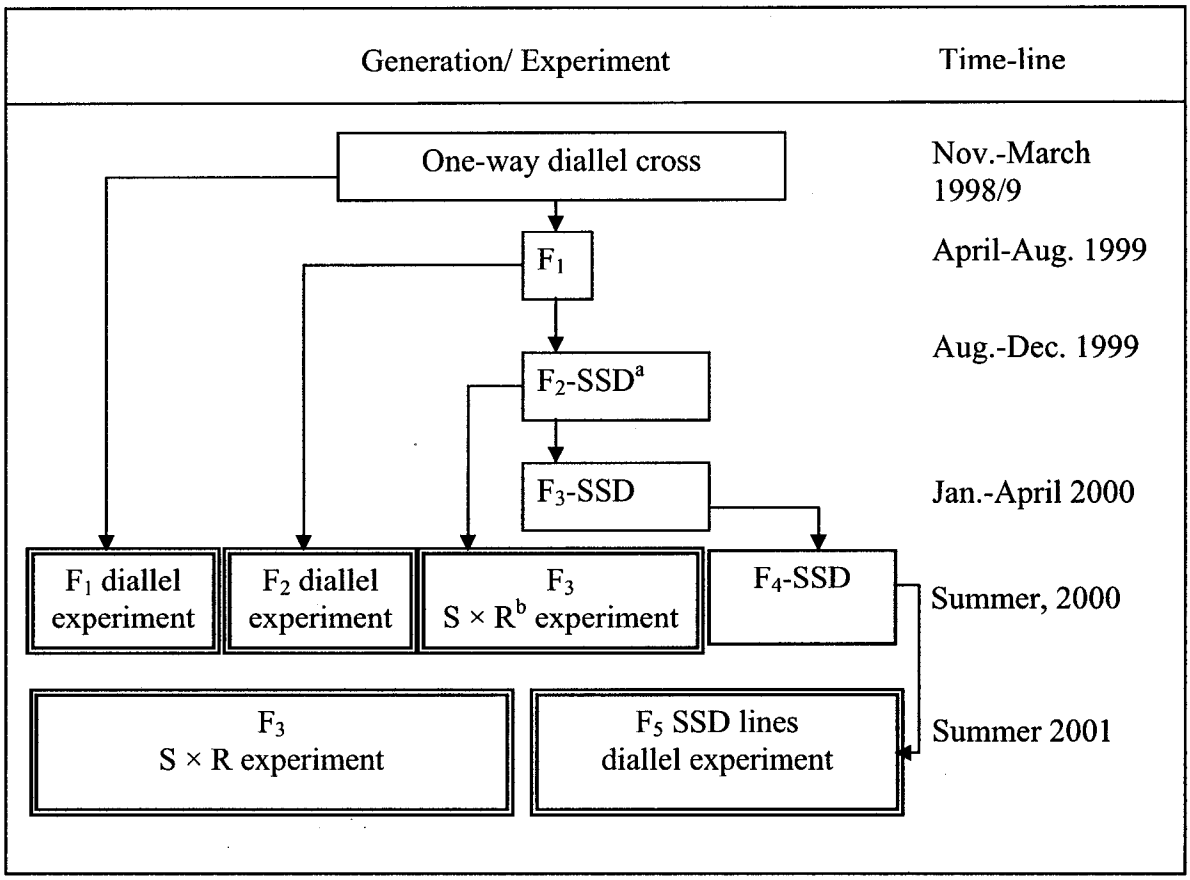
*, **, significantly different from zero at $P < 0.05$ and $P < 0.01$, respectively.

Table 2-11. Minimum numbers of genes estimated for the adult plant stripe rust resistance in parental genotypes.

Avocet-*YrA* crossed with	Number of genes in			
	F_1[a]	F_2[a]	F_5[a]	F_5[b]
Saar	4.9	7.9	3.7	4.7
Simorgh	5.4	8.4	4.2	3.3
Homa	4.9	7.9	3.7	4.1
Parastoo	5.2	7.8	3.5	4.5
Cocnoos	4.9	8.0	3.9	6.1

[a] Number of genes were estimated according to Mather and Jinks (1982).
[b] Numbers of genes were estimated according to Bjarko and Line (1988).

Figure 2-1. Diagram of the development and field evaluation of genetic populations. Those framed with double lines were field-evaluated under artificial stripe rust epidemics in Toluca, Mexico.



[a] Single-seed descent.
[b] Susceptible × resistant crosses.

Figure 2-2. Emasculation of the female genotype (left), pollination using the approach method (middle), and F_1 seed set (right).

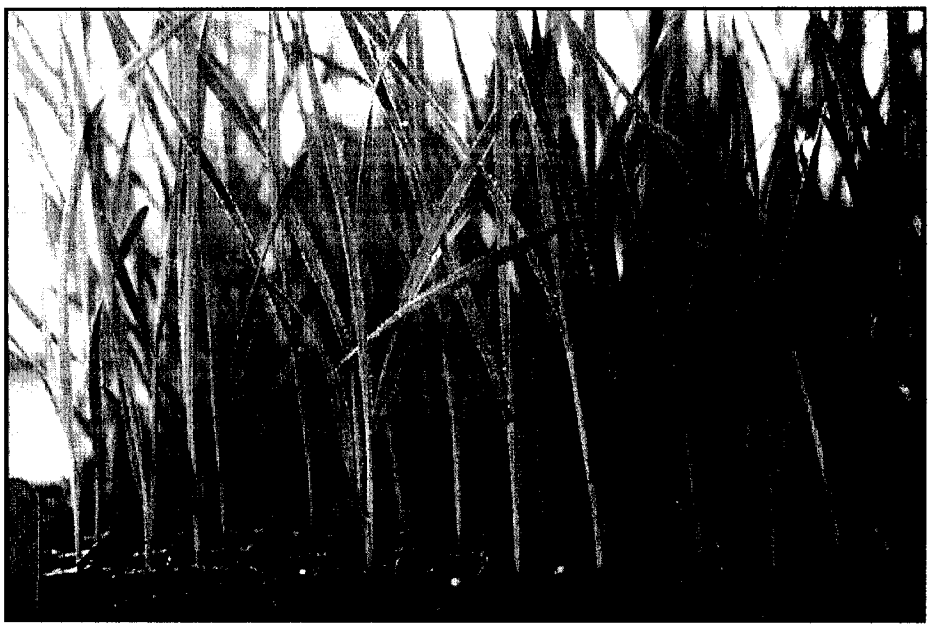


Figure 2-3. Single-seed descent populations of the crosses growing in root containers. See text for details.

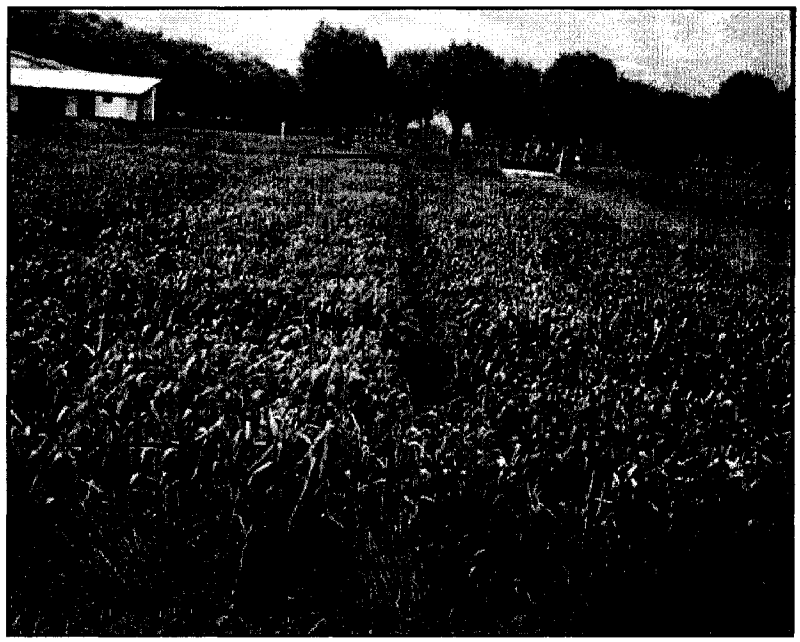


Figure 2-4. Field evaluation of parental genotypes, F_1 crosses, F_3, and F_5 SSD lines in Toluca, MX, under artificial stripe rust epidemics. Black arrows indicate the spreader rows inoculated with stripe rust.

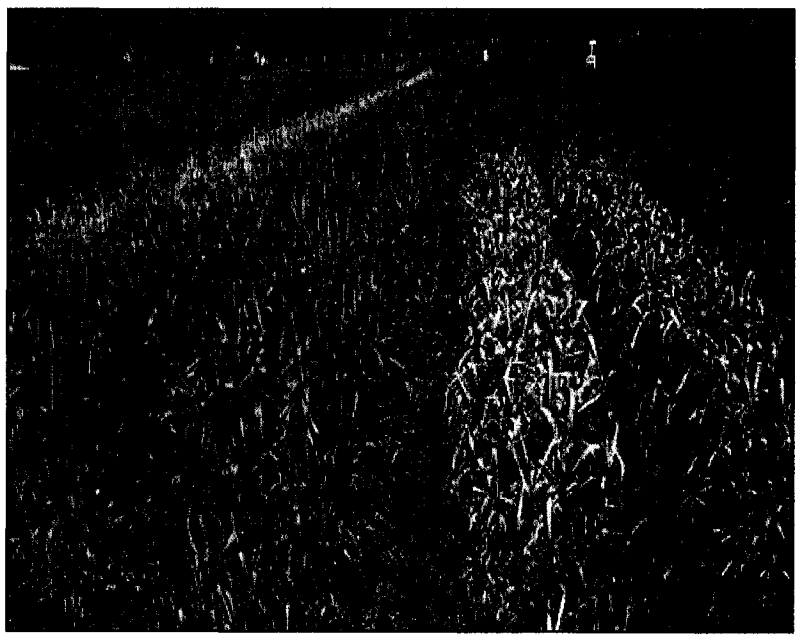


Figure 2-5. Field evaluation of parental genotypes and F_2 populations in Toluca, MX. The resistant genotype Simorgh (on the left) compared with the susceptible genotype Avocet-*YrA* (on the right).

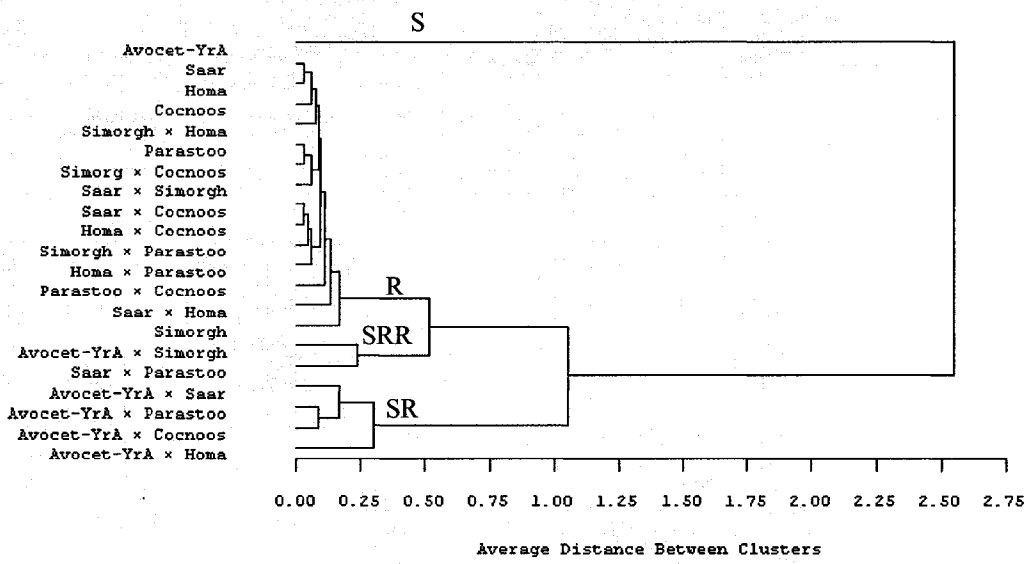


Figure 2-6. Clustering of parental genotypes and crosses based on the average stripe rust reaction in F_1, F_2, and F_5 experiments. S, R, SRR, and SR represent cluster groups - see text.

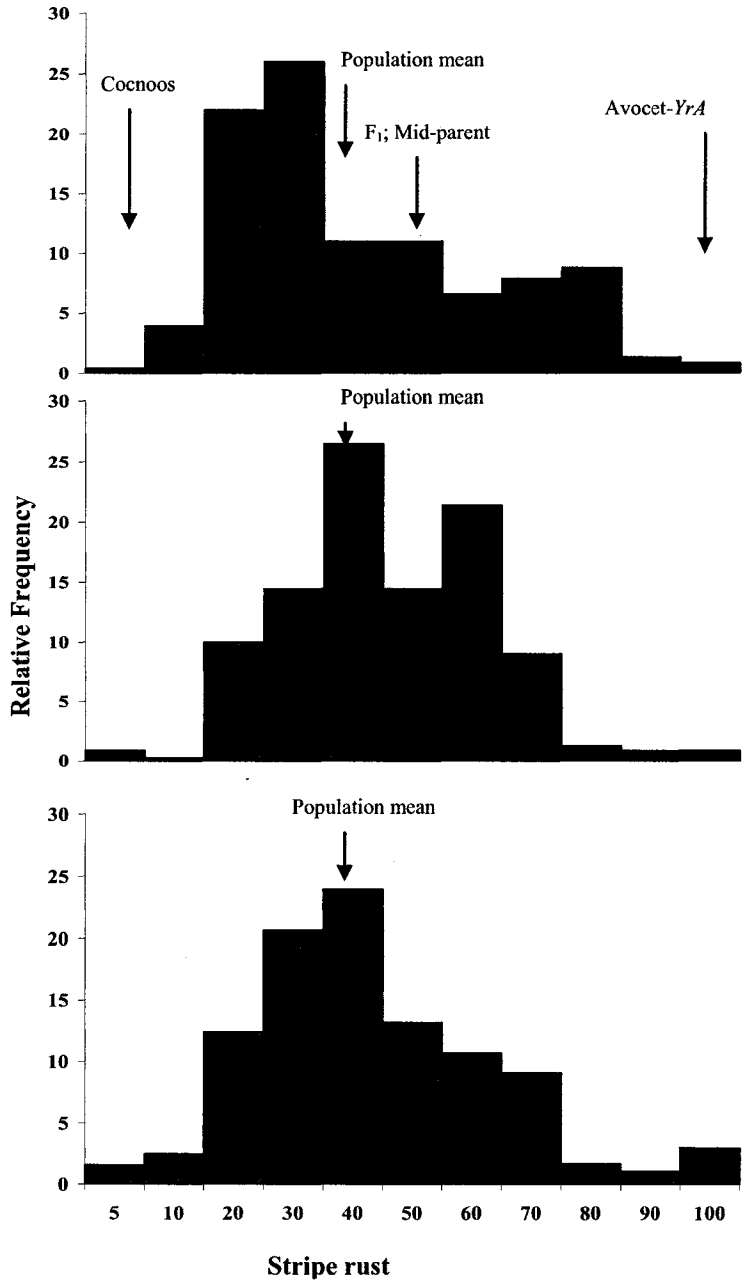


Figure 2-7. Relative frequency distribution of stripe rust severity of F_2 population (top), F_3 lines (middle), and F_5 SSD lines (bottom) derived from Avocet-*YrA* × Cocnoos cross.

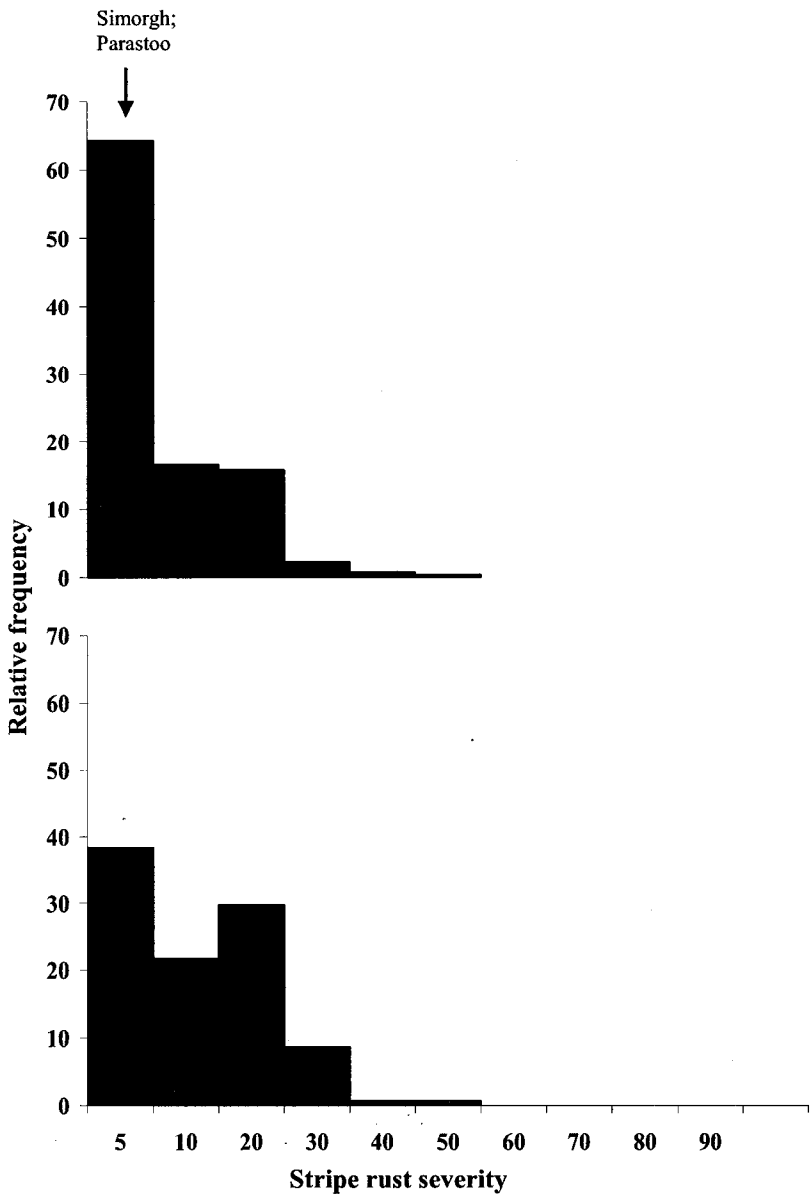


Figure 2-8. Relative frequency distribution of stripe rust severity of F_2 population (top) and F_5 SSD lines (bottom) derived from Simorgh × Parastoo cross.

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Chapter 3

Inheritance of adult-plant resistance to leaf rust in five spring wheat genotypes[1]

3.1 Introduction

Leaf rust, caused by *Puccinia triticina* Eriks., is the most widely distributed and the most common of all three wheat rusts (Roelfs *et al.*, 1992). On a worldwide basis, it probably causes more damage than the other wheat rusts (Samborski, 1984). In a study by Khan *et al.* (1997) for leaf rust severities of up to approximately 20%, every 1% of the flag leaf covered by rust pustules caused a reduction of 1% in grain yield in winter wheat cultivars commonly grown in Mississippi. However, losses to leaf rust are highly influenced by the growth stage at disease on set (Roelfs *et al.*, 1992). Chemical control of leaf rust is potentially hazardous to the environment, often practically unmanageable, and economically costly. Genetic resistance is the most economical and preferable method of reducing yield loss due to leaf rust (Kolmer, 1996). Most of the catalogued leaf rust resistance genes (McIntosh *et al.*, 1998) confer race-specific hypersensitive responses that remain effective for a short period of time, becoming ineffective, as races with added new virulence develop in the pathogen population.

Substantial economic benefits are likely to be associated with the development of race non-specific resistance to leaf rust in many wheat-producing areas, especially in areas where farmers change cultivars slowly (Smale *et al.*, 1998). Race nonspecific adult-plant resistance (APR) to leaf rust, often referred to as slow rusting (Caldwell, 1968) or partial resistance (Parlevliet, 1988) is characterized as a type of interaction between host plant and pathogen in which plants show less disease severity compared to the susceptible check despite compatibility, or susceptible infection types. Several studies report the inheritance of such resistance to leaf rust (Gavinlertvatana and Wilcoxon, 1978; Bjarko and Line, 1988; Broers and Jacobs, 1989; Jacobs and Broers, 1989; Lee and

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Shaner, 1985; Das *et al.*, 1992; Singh and Huerta-Espino, 1995; Messmer *et al.*, 2000; Singh *et al.*, 2001a; Kolmer and Liu, 2001). Genetic studies based on either latent period or the area under the leaf rust progress curve often have shown that APR to leaf rust is under oligogenic or polygenic control of additive genes with relatively high heritability (Lee and Shaner, 1985; Bjarko and Line, 1988; Broers and Jacobs, 1989; Jacobs and Broers, 1989; Das *et al.*, 1992; Shaner *et al.*, 1997).

The primary focus of wheat breeding should be to achieve durable resistance (Johnson and Law, 1975). Such resistance often involves race nonspecific APR genes. Attempts have been made in several wheat breeding programs to accumulate additive genes into high-yielding wheat background to achieve higher levels of race non-specific resistance. Resistant parental genotypes studied in the stripe rust (*P. striiformis* Westend.) resistance inheritance study (Chapter 2) were developed at the International Maize and Wheat Improvement Center (CIMMYT), selected for high seedling infection type accompanied by low stripe rust and leaf rust severities in the adult stage. Selection was carried out under alternate stripe and leaf rust epidemics at different locations in Mexico (Singh *et al.*, 2000).

The objectives of the present research were 1) to determine the number and mode of action of genes that confer high levels of APR to leaf rust in five wheat genotypes, and 2) to study the combining abilities and gene effects involved in such resistance.

3.2 Materials and methods

3.2.1 Plant material

Five APR genotypes and the susceptible genotype, Avocet-*YrA*, were intercrossed in a one-way diallel mating design. Parental genotypes, F_1 crosses, F_3 lines, and F_5 single seed descent (SSD) lines derived from the diallel were developed. Methods used to develop the populations were described in Chapter 2.

3.2.2 Test sites

Field evaluations of the genetic populations were carried out at two CIMMYT Research Stations: El-Batan (Mexico State) in the highlands of central Mexico (19°N, 2249 *masl*) near Mexico City during the 2000 crop season, which starts in mid-May, and

Ciudad Obregon (Sonora State) in Northwestern Mexico (22°N, 7 masl) during the winter 2000-01 crop season, starting in late November. Both sites have favorable environments for leaf rust development.

3.2.3 Experimental procedure

During the summer 2000 crop season, 6 parental lines, fifteen F_1 crosses, and fifteen F_2 populations derived from the diallel cross, and 52-80 F_3 lines from the susceptible × resistant crosses were evaluated at the CIMMYT Research Station at El-Batan. The F_1 diallel experiment, comprised of 6 parental lines and fifteen F_1 crosses, was planted in a three-replication randomized complete block design (RCBD). Fifteen seeds were planted in each experimental unit on a double row bed, 1 m long and 70 cm wide. F_2 diallel experiment comprised of 6 parental lines and 15 F_2 populations derived from the diallel cross. One-hundred-twenty seeds were space-sown, about 10 cm apart, in each experimental unit on two double row beds, 3 m long and 75 cm wide. F_3 lines of the susceptible × resistant crosses were planted in plots of similar size to the F_1 experiment, with parental lines repeated as checks after every 10 lines. In each F_3 plot, 3 g of seed was planted to obtain at least 60 plants per plot.

During the winter 2000-2001 the 6 parental lines, a total of 564 F_3 lines from the susceptible × resistant crosses (66-155 lines from each cross) and 1802 F_5 lines from the diallel cross (76-142 lines from each cross) along with 10 check genotypes with different leaf rust responses were evaluated at the CIMMYT Research Station at Ciudad Obregon. An augmented randomized complete block design (ARCBD) as described by Scott and Milliken (1993) was used for planting the experiment. F_3 and F_5 lines and the parental genotypes were planted along with check genotypes that were replicated throughout the experiment. Every 200 experimental units were considered as one block, which was comprised of 190 experimental F_3 or F_5 lines, and 10 check genotypes that were randomly planted in each block. Plot size and seed density were the same as in the F_3 experiment in El-Batan.

3.2.4 Artificial epidemics and leaf rust race

In all field experiments, one row of the highly susceptible cultivar, Morocco, was planted as spreader on one side of the plots in the middle of the 0.5 m wide pathways and around the field. Artificial inoculation was carried out with the Mexican *P. triticina* race MCJ/SP by spraying the spreader rows with urediniospores suspended in a light-weight mineral oil (Soltrol®-170) about four and six weeks after planting at El-Batan and Cd. Obregon, respectively. Fresh inoculum was obtained by multiplying urediniospores on the susceptible cv. Morocco in the greenhouse. The nomenclature of this race is based on Singh (1991) with the following avirulence/virulence formula:

Lr2a,2b,2c,3ka,9,16,19,21,24,25,28,29,30,32,33/1,3,3bg,10,11,12,13,14a,14b, 15,17,18,20,22b,23,26,27+31.

Because all wheat parents included in the study showed high seedling infection types with race MCJ/SP, this race was considered suitable for the study of APR to leaf rust.

3.2.5 Leaf rust assessment

Leaf rust severities were visually assessed following the Modified Cobb's Scale (Peterson *et al.*, 1948). The F_1 diallel experiment planted during 2000 was rated five times during the disease cycle on the following dates: July 24 and 31, and August 8, 15, and 22. The area under the disease progress curve (AUDPC) of each experimental unit was estimated using the following formula:

$$\text{AUDPC} = \Sigma\,[(x_i + x_{i+1})/2](t_{i+1} - t_i)$$

where x_i = leaf rust severity on the ith date and $t_i = i$th day. Relative area under the disease progress curve (rAUDPC) was then estimated for each experimental unit in the F_1 diallel experiment as the ratio of AUDPC of the experimental unit to the average AUDPC of the susceptible genotype, Avocet-*YrA*. F_2 single plants, and F_3 and F_5 lines were rated twice. Based on the practice used in CIMMYT (Singh and Rajaram, 1992), the first rating was done when the leaf rust severity of the susceptible parent, Avocet-*YrA*, reached 80-100% and the second rating was done 10-15 days later when the leaves of the susceptible parent had dried out due to leaf rust infection. Leaf rust severities of the F_1, F_3 and F_5 lines were assessed as the average rust severities on the flag and penultimate leaves. For

this purpose, several leaves were examined and an average leaf rust severity estimate was recorded for each plot. In the F_2 diallel experiment, however, all single plants in each plot were rated for disease severity. Flag and penultimate leaves of the two most advanced tillers of each plant were observed to determine the average severity estimate of each F_2 plant. Average leaf rust severity for each F_2 plot was then computed.

3.2.6 Genetic analysis

3.2.6.1. Qualitative analysis

For segregation analysis, the F_3 lines of the susceptible × resistant crosses were classified into four phenotypic groups, i.e., homozygous for the parental type resistance (HPTR), homozygous for the parental type or higher susceptibility (HTPS), either segregating or homozygous for disease levels higher than that of the resistant parent but less than that of the susceptible parent (SegI), and segregating for disease levels reaching the susceptible parent (SegS). The F_5 SSD lines were classified into three groups: HPTR, HPTS and others. HPTR lines in F_3 or F_5 were assumed to be derivatives of the respective F_2 or F_4 single plants homozygous for all resistance alleles; HPTS lines were similarly assumed to be the derivatives of the F_2 or F_4 single plants homozygous for all susceptibility alleles; SegI lines were assumed to be the derivatives of the F_2 or F_4 single plants homozygous for at least one resistance allele; and SegS lines were assumed to be the derivatives of the F_2 or F_4 single plants heterozygous for resistance alleles for at least one locus and homozygous for susceptibility alleles at all other loci. Chi-square analyses were carried out to test the distribution of the observed phenotypic frequencies against those expected for the segregation models. Frequency distributions of leaf rust severities of the F_5 SSD lines derived from the crosses of resistant parents were used for studying the similarity of the resistance alleles.

3.2.6.2.Quantitative analysis

Quantitative analyses were performed on the mean values of the terminal leaf rust severities of experimental units, separately for F_1, F_2 and F_5 diallel experiments, and for susceptible × resistant crosses in the F_3 generation. The residuals for treatments were not normally distributed as determined by the Proc UNIVARIATE procedure (SAS Institute,

Cary, NC). Percent mean disease severity of each plot was, therefore, transformed by the arcsine of the square root to normalize the scale.

For the F_1 diallel experiment a repeated-measure multivariate analysis of variance (MANOVA) was conducted, using the REPEATED statement in the Proc GLM procedure (SAS Institute, Cary, NC). Moreover, analysis of variance was performed for the rAUDPC in the F_1 diallel experiment using the Proc GLM (SAS Institute, Cary, NC). Single degree of freedom contrasts were performed using the CONTRAST statement (SAS Institute, Cary, NC) to compare parents *vs.* crosses, resistant parent *vs.* resistant × resistant crosses, resistant parent *vs.* susceptible × resistant crosses, and susceptible parent *vs.* susceptible × resistant crosses. Analyses of variance and combining ability analyses were performed for the terminal leaf rust severities separately for the F_1, F_2 and F_5 diallel experiments following Griffing (1956) and components of variance and narrow-sense heritabilities were estimated. Diallel Analysis and Simulation Software by Burrow and Coors (1994) were used to estimate the general combining ability (GCA) and specific combining ability (SCA) effects and the components of variance as described in Chapter 2.

Mean terminal leaf rust severities of genotypes were computed for the F_1 and F_2 diallel using the MEANS statement in the Proc GLM procedure (SAS Institute, Cary, NC). However, as the F_3 and F_5 experiments were unbalanced, least square means were computed for each genotype, using the LSMEANS statement in the Proc GLM procedure (SAS Institute, Cary, NC). Parental lines and crosses were classified, on the basis of mean terminal leaf rust severities in the F_1 and F_2 diallel experiments, and least square means in F_5 diallel experiment. For this reason a cluster analysis was performed using the average linkage method in the Proc CLUSTER procedure, and a dendrogram was developed using the TREE statement (SAS Institute, Cary, NC).

Minimum numbers of additive genes in the resistant parents were estimated from the F_5 data following the method of Mather and Jinks (1982):

$$N = [d]^2 / V_A$$

where: N is the minimum number of effective additive genes, d is the additive component of the gene action, and V_A is the additive genetic variance. Minimum numbers

of additive genes were also estimated according to the formula proposed by Bjarko and Line (1988), using data from the F_5 experiment:

$$N = (GR)^2 / 4.27 [(V_{F5} - V_{P1} + V_{P2}) / 2]$$

where: N is the minimum number of effective additive genes, *GR* is the genotypic range, V_{F5} is the variance of the F_5 SSD lines, and V_{P1}, and V_{P2} are variances of the resistant and susceptible parents, respectively. The formula proposed by Wright (1968) was also used to estimate the minimum number of genes:

$$N = (P_s - P_r)^2 \times [1.5 - 2h (1 - h)] / 8 (V_{F2} - V_E)$$

where P_r and P_s are the mean parental values of the resistant and susceptible parents, respectively, $h = (F_1 - P_r) / (P_s - P_r)$, V_{F2} is the variance of F_2 and V_E is the environmental variance. The formulae are based on the assumptions that resistance genes that segregate in a cross are in only one parent, segregating resistance genes are not linked, all resistance genes have equal effects, additive × dominance and genotype × environment effects do not exist, and dominance is equal at all loci.

3.3 Results

Terminal leaf rust severities of parental genotypes, F_1 crosses, F_2 populations and least-square means of the leaf rust severities of F_3 and F_5 lines are presented in Table 3-1. Least square means are basically the genotypic means adjusted for other effects in the experimental design (SAS Institute, Cary, NC). All resistant parents displayed low levels of terminal leaf rust severity, whereas the susceptible parent exhibited a high severity. Although the leaf rust severities of the parents varied slightly in generation-trials, the correlations (r) between severity scores for different trials were high and ranged between 0.93 ($P < 0.01$) and 0.97 ($P < 0.01$). The parents and the crosses in different generations were classified into 3 major groups by cluster analysis (Figure 3-1). Resistant parents and intercrosses between the resistant parents with very low terminal leaf rust severities in all generations were grouped into one cluster (R cluster in Figure 3-1). Except for the Avocet-*YrA* × Parastoo cross, all other susceptible × resistant crosses grouped together in a second cluster (SR cluster in Figure 3-1) exhibiting intermediate levels of leaf rust severity, and the susceptible parent Avocet-*YrA* formed a single-genotypic group (S cluster in Figure 3-1) with maximum leaf rust severity in all experiments. The cross

Avocet-*YrA* × Parastoo did not group with other susceptible × resistant crosses, mainly because of its low leaf rust severity, especially in the F_1 diallel experiment, where severity was significantly ($P < 0.05$) less than for other susceptible × resistant crosses.

Analysis of variance for the relative area under the leaf rust progress curve and repeated measure analysis of variance for the progress of leaf rust over time in the F_1 diallel experiment is presented in Table 3-2. The effect of genotype was highly significant ($P < 0.001$) for single measurements over time and for the rAUDPC. The parents *vs.* cross effect, resistant parent *vs.* susceptible parent, and susceptible parent *vs.* susceptible × resistant crosses effects were highly significant for both single measurements and for rAUDPC. In single measurements over time the effect of time and the interaction effect of time × genotypes were highly significant. Leaf rust progress over time is presented in Figure 3-2. The susceptible parent, Avocet-*YrA*, reached 100% severity by the third recording, resistant parents and resistant × resistant crosses had very low disease severities with terminal disease severities significantly less than susceptible × resistant crosses and the susceptible parent. Susceptible × resistant crosses had intermediate levels of leaf rust severity with average terminal severities ranging between 12 and 34%.

3.3.1 Segregation analysis

3.3.1.1. Susceptible × resistant crosses

All F_1 susceptible × resistant crosses displayed intermediate levels of leaf rust severity (Table 3-1). The frequency distributions of leaf rust severity of the F_3 (Table 3-3) and F_5 lines (Table 3-4), derived from the susceptible × resistant crosses showed continuous variation with few lines resembling the leaf rust severities of the resistant and susceptible parents (Figure 3-3). The frequency distributions were significantly skewed (Table 3-5), with population means shifting from the mid-parental values towards the resistant parent.

The frequency of the 4 and 3 phenotypic groups in the F_3 and F_5 lines of the susceptible × resistant crosses are displayed in Table 3-6. F_3 lines tested in the two locations were not identical. The ratios from both testing sites were in agreement with the inheritance of at least 3 independent genes with additive effects in all resistant parents.

The cross Avocet-*YrA* × Homa, however gave very low probability estimates at Ciudad Obregon experiment. The F_5 lines of the susceptible × resistant crosses were also evaluated to verify the F_3 results. The hypothesis of the segregation of a minimum of 3 independent genes having additive effects was again the most likely explanation in all susceptible × resistant crosses (Table 3-6), with Avocet-*YrA* × Homa cross giving the lowest probability among all crosses, due to the higher frequency of HPTR lines.

3.3.1.2. Resistant-parent intercrosses

The F_5 lines of the resistant-parent intercrosses were tested at Ciudad Obregon to study the similarity/diversity of the resistance genes in the parents. Although none of the F_5 lines had severities as high as the susceptible parent, Avocet-*YrA*, a few lines with leaf rust severities evidently higher than those of the resistant parents were found (Table 3-4). In crosses of Saar with Homa, Parastoo and Cocnoos (Figure 3-4) and the cross Homa × Cocnoos, few lines had disease severities as high as 50-70%. This indicated that although parental lines have at least one gene in common, the other additive genes were different. Crosses of Simorgh and Homa with Parastoo (Figure 3-4) displayed less transgressive segregation for disease severity indicating perhaps two additive genes were common in the parents of the respective crosses.

3.3.2 Quantitative analysis

Analyses of variance for the terminal leaf rust severities in all experiments are given in Table 3-7. Only the susceptible × resistant crosses were included in the F_3 experiment. In other experiments, all crosses in the diallel were tested. The differences among the parental lines, F_1 crosses, F_2 populations, F_3, and F_5 lines were highly significant. The parents *vs.* F_1 crosses, F_2 populations, F_3, and F_5 lines were highly significant indicating deviations of F_1 crosses and F_2, F_3, and F_5 population means from mid-parental values. The differences among the check genotypes tested in the F_3 and F_5 experiments at Ciudad Obregon were also highly significant. GCA mean squares were highly significant in the F_1, F_2, and F_5 diallels, while SCA mean squares were only significant in F_1 and F_2 diallel experiments. Components of variance ratios were 0.69, 0.94, and 0.98 for the F_1, F_2, and F_5 diallel experiments, respectively (Table 3-8). The

components of variance ratio estimated for the F_1 diallel experiment was relatively smaller than those estimated for the F_2 and F_5 diallel experiments. This was due to greater SCA estimates in the F_1 experiment. The relatively high estimates for the components of variance ratios indicated that GCA was of major importance in predicting the progeny performance. The greater importance of GCA effects was also evident based on the relatively high correlations between parental means and GCA effects ($r = 0.89$, $P < 0.05$ for F_1; $r = 0.94$, $P < 0.01$ for F_2; and $r = 0.97$, $P < 0.01$ for F_5). Narrow-sense heritability estimates were 0.67, 0.92, and 0.96 for F_1, F_2 and F_5 diallel experiments, respectively (Table 3-8). Similar to the component of variance ratios, the heritability estimate for F_1 diallel was relatively smaller than those for the F_2 and F_5, probably due to the greater dominance variance estimated in the F_1 experiment.

Estimates of GCA effects for parents and SCA for the crosses are given in Table 3-9. The F_1 crosses, F_2 populations and F_5 lines involving parents with negative GCA effects are more resistant than the average of the respective generations, while significant positive GCA indicates greater susceptibility. Parastoo had greater negative GCAs in all experiments that were significant in F_1 and F_5, and highly significant in F_2. This indicated that the most resistant F_1 crosses, F_2 populations or F_5 lines on average had Parastoo as one parent. The relative values of GCA effects were slightly inconsistent across the generations.

The SCA of the F_1 crosses, F_2 populations and F_5 lines are presented in Table 3-9. In crosses involving the susceptible parent, Parastoo had the greatest negative SCA effect in all generations (SCA = -12.11, $P < 0.01$ for F_1; SCA = -7.74, $P < 0.01$ for F_2; and SCA = -4.30, $P < 0.01$ for F_5). This indicated that Parastoo had the greatest reduction in average terminal leaf rust severity when crossed with the susceptible parent. In the resistant parent intercrosses the cross Saar × Cocnoos had significant and highly significant negative SCA effects in F_2 and F_5, respectively (SCA = -3.24, $P < 0.05$ for F_2; and SCA = -2.52, $P < 0.01$ for F_5). As with the GCA effects, a slight inconsistency in SCA effects of the crosses was observed across generations.

Estimates of the minimum numbers of additive genes in the resistant parental lines based on the data on F_5 lines from the susceptible × resistant crosses are presented in Table 3-10. The estimates based on the formula proposed by Wright (1968) appeared

to be smaller that the other two formulas, ranging between 2.17 and 3.09. Estimates based on Mather and Jinks (1982) and Bjarko and Line (1988) were similar ranging 2.18 to 4.02.

3.4 Discussion

The resistant parents included in the study were selected at CIMMYT, Mexico for seedling susceptibility, characterized by a high infection type, accompanied by low adult plant leaf rust severity to common Mexican races of *P. triticina*. Furthermore, they all displayed moderately susceptible to susceptible infection types in field evaluations indicating the compatible nature of the host-pathogen interaction, a known feature of partial (Parlevliet, 1988) or slow-rusting resistance (Caldwell, 1968).

F_1 crosses showed intermediate terminal leaf rust severities, which were significantly higher than those of the resistant parents. This indicated that APR to leaf rust in the evaluated materials was incompletely dominant. Segregation results indicated the presence of at least three genes having additive effects in each resistant parent. The distribution of lines in the cross Avocet-*YrA* × Homa gave significantly higher Chi-square values in F_3 and F_5 tests at Ciudad Obregon. This was due to a higher frequency of HPTR lines. The frequencies did not fit the expected segregation ratios for either 2 or 3 genes. This may have resulted from experimental error or it may be possible that Homa has 4 genes and when the parental genotypes were homozygous for three resistance alleles, the resistance phenotype of the lines was indistinguishable with HPTR in some cases. The same situation could also occur for Parastoo. In both cases, the frequencies of HPTS lines were lower in both the F_3 and F_5 generations. In addition, there was less SegS lines in the F_3 generation. When quantitative methods of analysis were used, the estimates of the numbers of additive genes ranged between 2.17 to 4.02. These numbers are comparable with those estimated by the Mendelian method of segregation analysis.

As mentioned in Chapter 2, all resistant parents carried leaf tip necrosis, which is known to be either pleiotropic or tightly linked to genes *Lr34* and *Yr18* for APR to leaf rust and stripe rust, respectively (Singh, 1992a, 1992b). Therefore, it was concluded that *Lr34* was present in all resistant parents. The frequency distributions of leaf rust severity in F_3 and F_5 lines derived from susceptible × resistant crosses were skewed, with

population means shifting towards the resistant parent. This may have resulted from the larger effect of *Lr34* than the other two additive genes in each cross. Based on the coefficients of parentage (estimated by IWIS; Fox *et al.*, 1997), it was indicated in Chapter 2 that stripe rust resistance gene *Yr29* could be present in Saar and Simorgh as Mexican cv. Pavon 76 was one of their ancestors. Because *Yr29* is tightly linked with leaf rust resistance gene *Lr46* (Singh *et al.*, 2001b), *Lr46* is probably the second leaf rust resistance gene be present in Saar and Simorgh.

Although gene *Lr34* is common in all resistant parents, some of the F_5 SSD lines derived from intercrosses of resistant parents had disease severities considerably higher than their parental responses. This indicated that some of the additive genes in the parents were non-allelic and therefore they can be accumulated in breeding lines to further enhance the level of resistance. The transgression for the maximum infection level varied among the resistant × resistant crosses. The highest levels of leaf rust severity (50-70%) in the F_5 generation were found for the cross Saar × Cocnoos, whereas the cross Homa × Parastoo gave rise to lines with no more than 10% leaf rust severities. Saar and Cocnoos also share less common parentage based on the pedigree information and seem to carry common genes additional to *Lr34*. Similarly, it appears that up to at least 8 different additive genes in addition to *Lr34* were present among the 5 parents. Homa and Parastoo seem to carry two genes in common. They share Kauz*2/Trap//Kauz as a common parent.

The F_5 SSD lines in the resistant-parent intercrosses with terminal leaf rust severities as high as 60% are probably segregants with *Lr34* alone. This further indicated that a single additive leaf rust APR gene does not provide an acceptable level of resistance. This was in accordance with the reports of Singh and Gupta (1992) and McIntosh (1992) who reported leaf rust severities of up to 50-60% when *Lr34* was deployed alone. A further example involves gene *Lr46*. Singh *et al.* (1998) reported leaf rust severities of up to 60% when *Lr46* gene was present alone.

The effect of time of rating was highly significant in the repeated measure analysis of variance. Several authors (Das *et al.*, 1992, Singh and Rajaram, 1992; Bjarko and Line, 1988) estimated the AUDPC based on repeated disease ratings over time. Jeger and Viljanen-Rollinson (2001) concluded that an estimation of AUDPC based on two

data points provides an equivalent amount of information as from repeated ratings. In the F_1 diallel experiment there was a highly significant correlation ($r = 0.99$, $P < 0.001$) between the AUDPC and/or rAUDPC with the terminal disease rating. It was therefore, concluded that the terminal leaf rust severities could be as informative as AUDPC for the purpose of quantitative genetic analysis.

Additive gene effects were more important than non-additive gene effects in the inheritance of APR to leaf rust as indicated by the quantitative analysis in this study. The GCA, which represents the average performance of a genotype and is mainly reflective of the additive gene effects, was significant in all generations. Although the SCA was also found to be significant in F_1 and F_2, the mean squares for SCA were much smaller than the mean squares for GCA. Moreover, the components of variance ratio, proposed by Baker (1978), were 0.67 for F_1 and close to unity for F_2 and F_5. The conclusion that additive gene effects contributed the most in the inheritance of APR to leaf rust is congruent with previous studies (Das *et al.*, 1992; Shaner *et al.*, 1997; Messmer *et al.*, 2000).

In addition to the additive gene effects, there was evidence for the presence of significant non-additive gene effects. The model that is used in the combining ability analysis assumes that epistasis and genotype × environment effects are negligible. Therefore, any non-additive effect is referred to as dominance. The significant parent *vs.* crosses effect in the ANOVA for all generations indicated a consistent significant deviation of F_1 crosses and F_2 and F_5 population mean values from mid-parental values. The frequency distributions of the leaf rust severities of the susceptible × resistant crosses were also skewed. Moreover, significant SCA effects in F_1 and F_2 and smaller components of variance ratio estimated for F_1 pointed to the presence of non-additive gene effects. Messmer *et al.* (2000) reported the presence of significant dominance effects in the inheritance of APR to leaf rust in wheat.

Because leaf rust severity assessment was based on a visual scale, there could be overestimation of non-additive effects. The application of a quantitative genetic approach in the study of gene effects was argued by Vanderplank (1984) for having underestimates of additive variance and overestimates of non-additive variance, mainly because the actual mid-parental values between estimated infected leaf areas of the parental value are

often higher than the mathematical estimates of the mid-parental values. Therefore, the significant non-additive variance estimated in this analysis was attributed, at least in part, to overestimation of such effects due to the visual scale that was used.

A high level of APR to leaf rust in the resistant parents included in this study was conferred by a few genes with additive effects. Results were in agreement with the findings of Singh *et al.* (1998), Bjarko and Line, (1988), Broers and Jacobs (1989), Kuhn *et al.* (1980), Singh and Rajaram (1992), and Singh *et al.* (2001b), who all reported that APR to leaf rust was based on the additive interaction of a few genes. The resistances of some widely grown cultivars carrying *Lr34* and additional genes having additive effects have remained effective for a long period of time (Singh and Rajaram, 1992). The high levels of resistance in the resistant parents included in this study is therefore expected to be durable.

Transgressive segregation giving rise to lines with higher levels of resistance than those of parents was observed in some of the intercrosses of resistant parents. Broers and Jacobs (1989), Lee and Shaner (1985), and Das *et al.* (1993) reported transgressive segregation for latent period and receptivity of leaf rust response in wheat. Incorporating the additive genes from different sources, therefore, will be possible through intercrossing APR genotypes carrying different additive genes. It has been demonstrated by Singh *et al.* (2000) that incorporating these additive genes in high yielding cultivars by the means of top-crossing or limited back crossing may result in resistance levels approaching immunity while maintaining or even increasing the actual yield potential.

The estimates of narrow-sense heritability ranged from 0.67 to 0.97 among generations. This indicated that the terminal leaf rust severity was moderately to highly heritable. This was in accordance with the results of Shaner *et al.* (1997), Das *et al.* (1992, 1993) and Kuhn *et al.* (1980) who reported moderate to high heritabilities for the latent period and areas under the leaf rust progress curve. Kuhn *et al.* (1980) suggested that the selection could be done for a longer latent period because this trait was more heritable than pustule size. Kolmer and Liu (2001) however, recommended the use actual disease response, as latent period may not always be predictive of APR to leaf rust. It can be concluded from this study that breeding for APR is possible by selection for low disease severity at the critical stages of growth. Selection must be done using the

epidemics of *P. triticina* with virulence for any known or unknown seedling resistance gene that may act epistatically to mask the effect of APR. High selection pressure for very low leaf rust severities should be avoided in the early segregating generations to avoid deletion of heterozygous plants capable of giving progenies with higher levels of resistance.

3.5 Tables and figures

Table 3-1. Mean terminal leaf rust severities of parents, F_1 crosses and F_2 populations, and least square means of F_3 and F_5 lines derived from the 6-parent diallel cross along with check genotypes.

Genotype	2000				2001	
	F_1		F_2		F_3	F_5
Parents						
Avocet-*YrA*	100.00[a]	a[b]	100.00	a	81.27	99.84
Saar	5.00	efg	1.98	fg	6.47	12.97
Simorgh	10.00	def	4.46	def	6.47	10.91
Homa	1.00	g	3.11	defg	6.93	6.51
Parastoo	1.00	g	1.00	g	1.97	5.46
Cocnoos	3.34	fg	1.00	g	1.52	0.80
Susceptible × resistant crosses						
Avocet-*YrA* × Saar	19.31	cd	30.88	b	40.41	46.01
Avocet-*YrA* × Simorgh	26.52	bc	28.49	b	44.98	45.39
Avocet-*YrA* × Homa	38.32	b	18.51	c	35.87	36.71
Avocet-*YrA* × Parastoo	6.49	efg	12.21	c	34.01	24.19
Avocet-*YrA* × Cocnoos	38.18	b	33.32	b	44.29	40.34
Resistant × resistant crosses						
Saar × Simorgh	10.00	def	6.54	d	-	11.43
Saar × Homa	3.34	fg	2.68	efg	-	10.77
Saar × Parastoo	6.49	efg	3.83	def	-	8.74
Saar × Cocnoos	6.49	efg	5.95	de	-	10.70
Simorgh × Homa	10.00	efg	6.56	d	-	7.88
Simorgh × Parastoo	5.00	efg	2.50	efg	-	5.94
Simorgh × Cocnoos	6.49	efg	6.07	de	-	9.28
Homa × Parastoo	1.00	g	1.11	g	-	2.39
Homa × Cocnoos	13.24	de	4.86	def	-	5.95
Parastoo × Cocnoos	5.00	efg	3.56	defg	-	2.77
Check genotypes						
Avocet-*YrA*	-		-		83.20	98.92
Avocet+*Yr18*	-		-		10.74	10.58
Thatcher	-		-		75.67	79.47
Thatcher+*Yr18*	-		-		1.12	1.06
Jupateco S	-		-		99.44	99.99
Jupateco+*Yr18*	-		-		26.43	30.20
AC-Nanda	-		-		38.01	32.48
Cook	-		-		18.83	13.97
Saar	-		-		11.54	10.83
Cocnoos	-		-		1.87	2.09

[a] Field leaf rust severity estimates based on the modified Cobb's Scale (Peterson *et al.*, 1948).

[b] Means followed by letters in common within generations do not differ significantly based on Tukey's standardized test on arcsine transformed data ($P < 0.05$). Range test is not practical for least square means.

Table 3-2. Repeated measures analysis of variance for leaf rust severity and simple ANOVA for the relative area under the disease progress curve (rAUDPC) of parental genotypes and F_1 crosses derived from diallel.

Sources of variation[a]	df	Mean square[b]	
		leaf rust severity[c]	rAUDPC
Replication (R)	2	< 0.001	< 0.001
Genotype (G)	20	1.038**	0.156**
P *vs.* C	1	0.409**	0.098**
RP *vs.* RR	1	0.011	< 0.001
RP *vs.* SR	1	2.910**	0.333**
SP *vs.* SR	1	8.343**	1.445**
Time (T)	4	0.392**	-
T × R	8	0.003	-
T × G	80	0.034**	-
R × G	40	0.006	0.001
Error(T)	160	0.001	-

[a] P = parents, C = Crosses, RP = resistant parents, RR = resistant × resistant crosses, SR = susceptible × resistant crosses, and SP = susceptible parents.
[b] **, significant at $P < 0.01$.
[c] Within subject effects in repeated measure analysis were tested according to Greenhouse-Geisser tests (SAS Institute, Cary, NC).

Table 3-3. Frequency distribution of disease severities of F_3 lines derived from susceptible × resistant crosses when inoculated with *P. triticina*.

Location	Avocet-*YrA* crossed with	No. of lines with leaf rust severity[a]												Total
		1	5	10	20	30	40	50	60	70	80	90	100	
El-Batan														
	Saar	0	2	8	10	11	11	8	8	7	5	1	1	72
	Simorgh	0	0	0	7	8	11	11	5	15	8	7	1	73
	Homa	0	2	1	13	13	17	7	7	4	3	5	1	73
	Parastoo	3	2	1	8	22	15	6	6	3	2	3	1	72
	Cocnoos	0	0	0	4	3	17	12	8	4	6	5	0	59
Cd. Obregon														
	Saar	1	1	2	20	32	26	23	17	18	3	8	4	155
	Simorgh	1	1	1	16	28	15	14	17	10	6	2	1	112
	Homa	0	6	4	18	11	8	8	8	4	1	0	0	68
	Parastoo	3	2	9	28	31	11	9	10	6	3	0	0	112
	Cocnoos	1	1	1	18	19	22	24	18	7	3	1	0	115

[a] Leaf rust severities (terminal) estimates are based on the modified Cobb's Scale (Peterson *et al.*, 1948).

Table 3-4. Frequency distributions of disease severities of F_5 lines derived from the diallel when inoculated with *P. triticina.*

Cross	Lines with leaf rust severity[a]												Total
	1	5	10	20	30	40	50	60	70	80	90	100	
Susceptible × Resistant													
Avocet-*YrA* × Saar	1	4	8	32	6	14	13	11	14	6	8	6	123
Avocet-*YrA* × Simorgh	3	7	8	20	15	20	8	13	4	9	7	6	120
Avocet-*YrA* × Homa	3	21	7	20	15	16	14	6	12	3	3	2	122
Avocet-*YrA* × Parastoo	14	8	15	34	11	9	7	10	7	3	3	2	123
Avocet-*YrA* × Cocnoos	4	0	2	24	20	20	11	15	4	9	5	2	116
Resistant × Resistant													
Saar × Simorgh	3	38	41	51	9	0	0	0	0	0	0	0	142
Saar × Homa	27	22	16	42	15	7	2	3	0	0	0	0	134
Saar × Parastoo	49	11	14	29	16	7	2	1	0	0	0	0	129
Saar × Cocnoos	21	25	20	39	11	1	1	0	1	0	0	0	119
Simorgh × Homa	28	45	23	20	1	0	0	0	0	0	0	0	117
Simorgh × Parastoo	45	54	29	10	0	0	0	0	0	0	0	0	138
Simorgh × Cocnoos	18	24	10	19	4	1	0	0	0	0	0	0	76
Homa × Parastoo	83	44	6	0	0	0	0	0	0	0	0	0	133
Homa × Cocnoos	31	35	14	14	2	2	1	0	0	0	0	0	99
Parastoo × Cocnoos	52	31	18	7	1	0	0	0	0	0	0	0	111

[a] Leaf rust severities (terminal) estimates based on the modified Cobb's Scale (Peterson *et al.*, 1948).

Table 3-5. Leaf rust severity statistics of F_5 populations derived from the diallel when inoculated with *P. triticina*.

Cross	Leaf rust severity[a]						
	P1	P2	Mid-parent	Mean	Std. deviation	Range	Skewness[b]
Susceptible × Resistant							
Avocet-*YrA* × Saar	100	14±4.4	57	44.76	28.13	99	0.36±0.21
Avocet-*YrA* × Simorgh	100	10±3.1	55	42.98	27.92	99	0.48±0.22
Avocet-*YrA* × Homa	100	6±2.0	53	34.94	25.34	99	0.57±0.21
Avocet-*YrA* × Parastoo	100	6.4±5.8	53.2	29.83	25.53	99	0.94±0.21
Avocet-*YrA* × Cocnoos	100	1±1.6	50.5	42.79	23.80	99	0.49±0.22
Resistant × Resistant							
Saar × Simorgh	14±4.4	10±3.1	12	12.35	7.00	29	0.78±0.20
Saar × Homa	14±4.4	6±2.0	10	15.31	13.63	59	1.20±0.20
Saar × Parastoo	14±4.4	6.4	10.2	13.05	13.53	59	1.06±0.21
Saar × Cocnoos	14±4.4	1±1.6	7.5	12.82	11.05	69	1.75±0.22
Simorgh × Homa	10±3.1	6±2.0	8	7.42	6.16	29	1.13±0.22
Simorgh × Parastoo	10±3.1	6.4±5.8	8.2	5.65	4.68	19	1.20±0.20
Simorgh × Cocnoos	10±3.1	1±1.6	5.5	9.64	8.77	39	1.22±0.27
Homa × Parastoo	6±2.0	6.4±5.8	6.2	2.73	2.45	9	1.25±0.21
Homa × Cocnoos	6±2.0	1±1.6	3.5	7.79	8.93	49	2.42±0.24
Parastoo × Cocnoos	6.4±5.8	1±1.6	3.25	4.93	5.25	29	1.90±0.23

[a] Mean terminal leaf rust severities of parentals (±standard deviation) and populations are based on modified Cobb's Scale (Peterson *et al.*, 1948).

[b] Skewness is presented as the estimated value of skewness ± standard error of skewness.

Table 3-6. Frequency distribution of F_3 and F_5 lines derived from crosses between the susceptible parent Avocet-*YrA* and five adult plant resistant parents.

Generation/ tested in	Avocet-*YrA* crossed with	Lines (No.) with reaction				Tested [e] for segregation of	χ^2	P value
		HPTR[a]	HPTS[b]	SegI[c]	SegS[d]			
F_3/El-Batan	Saar	1	1	39	37	3 genes	1.51	>0.50
	Simorgh	1	2	33	34	3 genes	2.87	>0.25
	Homa	3	1	51	25	3 genes	5.03	>0.10
	Parastoo	3	1	44	20	3 genes	6.50	>0.05
	Cocnoos	0	0	26	26	3 genes	3.11	>0.25
F_3/Cd. Obregon	Saar	3	3	93	56	3 genes	1.43	>0.50
	Simorgh	3	1	60	48	3 genes	1.49	>0.50
	Homa	4	1	43	18	3 genes	12.06	<0.05
	Parastoo	5	1	71	35	3 genes	9.65	>0.01
	Cocnoos	4	1	58	52	3 genes	4.34	>0.10
		No. of lines with reaction						
		HPTR	HPTS	Others				
F_5/Cd. Obregon	Saar	6	10	107		3 genes	2.00	>0.25
	Simorgh	14	11	95		3 genes	1.89	>0.25
	Homa	20	5	97		3 genes	12.25	<0.05
	Parastoo	15	5	103		3 genes	4.87	>0.10
	Cocnoos	4	8	104		3 genes	4.22	>0.10

[a] Homozygous for the parental type resistance (homozygous for all resistance alleles).
[b] Homozygous for the parental type or higher susceptibility (homozygous lacking all resistance alleles).
[c] Either segregating or homozygous for disease levels higher that that of the resistant parent but less than that of the susceptible parent (homozygous for at least one resistance allele).
[d] Segregating with disease levels reaching the susceptible parent response (heterozygous for at least one locus and homozygous for susceptibility alleles at other loci).
[e] The ratio used in F_3 was 0.0156 HPTR : 0.0156 HPTS : 0.5625 SegI : 0.4062 SegS expected for the segregation of 3 additive genes. The ratio used in F_5 was 0.0837 HPTR : 0.0837 HPTS : 0.8327 Seg for the segregation of three genes.

Table 3-7. Mean squares for the arcsine transformed terminal leaf rust severity of parents, F_1 crosses, F_2 populations, and F_5 SSD lines derived from a 6-parent diallel cross, and F_3 of susceptible × resistant crosses.

SV	Generation		
		F_1	F_2
	df	MS	MS
Block (B)	2	< 0.001	< 0.001
Genotypes (G)	20	0.309**	0.317**
Parents (P)	5	0.979**	1.020**
P *vs.* C	1	0.074**	0.073**
Crosses (C)	14	0.086**	0.083**
GCA	5	0.187**	0.221**
SCA	9	0.031**	0.007**
C × B	28	0.004	0.001
Error	40	0.002	< 0.001

	Generation			
	F_3		F_5	
	df	MS	df	MS
Block	2	0.036	9	0.008
Genotypes (G)	20	0.545**	30	1.850**
Parents (P)	5	0.479**	5	1.450**
P *vs.* C	1	0.693**	1	0.132**
Crosses (C)	4	0.382**	14	0.535**
GCA	-	-	5	0.116**
SCA	-	-	9	0.001
Checks	9	0.854**	9	2.554**
Line(genotype)	557	0.053	1765	0.048**
Error	31	0.066	105	0.005

*, ** significant at $P < 0.05$ and $P < 0.01$, respectively.
GCA = general combining ability.
SCA = specific combining ability.

Table 3-8. Estimates of components of variance, components of variance ratio, and heritability of terminal leaf rust severities of F_1, F_2 and F_5.

	Generation		
Component	F_1	F_2	F_5
GCA (σ^2_g)	0.010	0.018	0.028
SCA (σ^2_s)	0.009	0.002	0.001
Additive	0.026	0.035	0.111
Dominance	0.009	0.002	0.004
$[2\sigma^2_g/(2\sigma^2_g + \sigma^2_s)]$	0.690	0.947	0.981
h^2_{ns}	0.674	0.921	0.965

GCA = General combining ability.
SCA = Specific combining ability.
h^2_{ns} = Narrow-sense heritability.

Table 3-9. Estimates of general (on diagonal) and specific combining ability for the terminal leaf rust severity.

F_1	Avocet-*YrA*	Saar	Simorgh	Homa	Parastoo	Cocnoos
Avocet-*YrA*	16.00**	-4.45	-0.61	9.05**	-12.11**	8.13*
Saar		-4.75*	3.46	-4.86	8.63**	-2.78
Simorgh			-1.91	-1.36	4.13	-5.61*
Homa				0.08	-1.86	-0.95
Parastoo					-10.41*	1.21
Cocnoos						1.00
F_2	Avocet-*YrA*	Saar	Simorgh	Homa	Parastoo	Cocnoos
Avocet-*YrA*	16.94**	4.25*	1.79	-4.05*	-7.74**	5.75**
Saar		-1.47	-1.78	-1.54	2.31	-3.24*
Simorgh			-1.36	2.38	0.83	-3.22*
Homa				-5.46**	3.54*	-0.33
Parastoo					-8.14**	1.05
Cocnoos						-0.50
F_5	Avocet-*YrA*	Saar	Simorgh	Homa	Parastoo	Cocnoos
Avocet-*YrA*	24.88*	0.04	3.33**	-2.20*	-4.30**	3.12**
Saar		0.68	-3.03**	2.41*	3.10**	-2.52**
Simorgh			-4.38	-0.45	0.80	-0.64
Homa				-6.89	0.30	-0.05
Parastoo					-9.91*	0.09
Cocnoos						-4.37

* , ** , Significantly different from zero at $P < 0.05$ and $P < 0.01$, respectively.

Table 3- 10. Minimum numbers of effective additive genes estimated for susceptible × resistant crosses.

Avocet-*YrA* crossed with	Number of genes estimated based on		
	Mather and Jinks (1982)	Bjarko and Line (1988)	Wright (1968)
Saar	2.95	2.18	2.19
Simorgh	3.23	2.42	2.50
Homa	3.53	3.20	2.17
Parastoo	3.50	3.21	3.09
Cocnoos	3.92	4.02	2.50

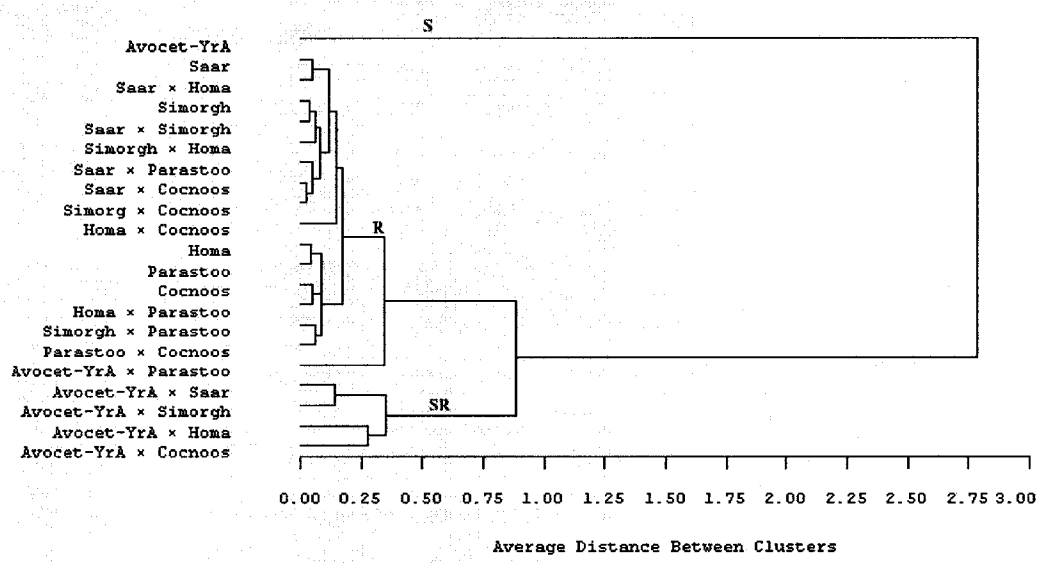

Figure 3-1. Clustering of parental genotypes and crosses based on leaf rust reaction in F_1, F_2, and F_5 experiments. S, R, and SR represent cluster groups – see text.

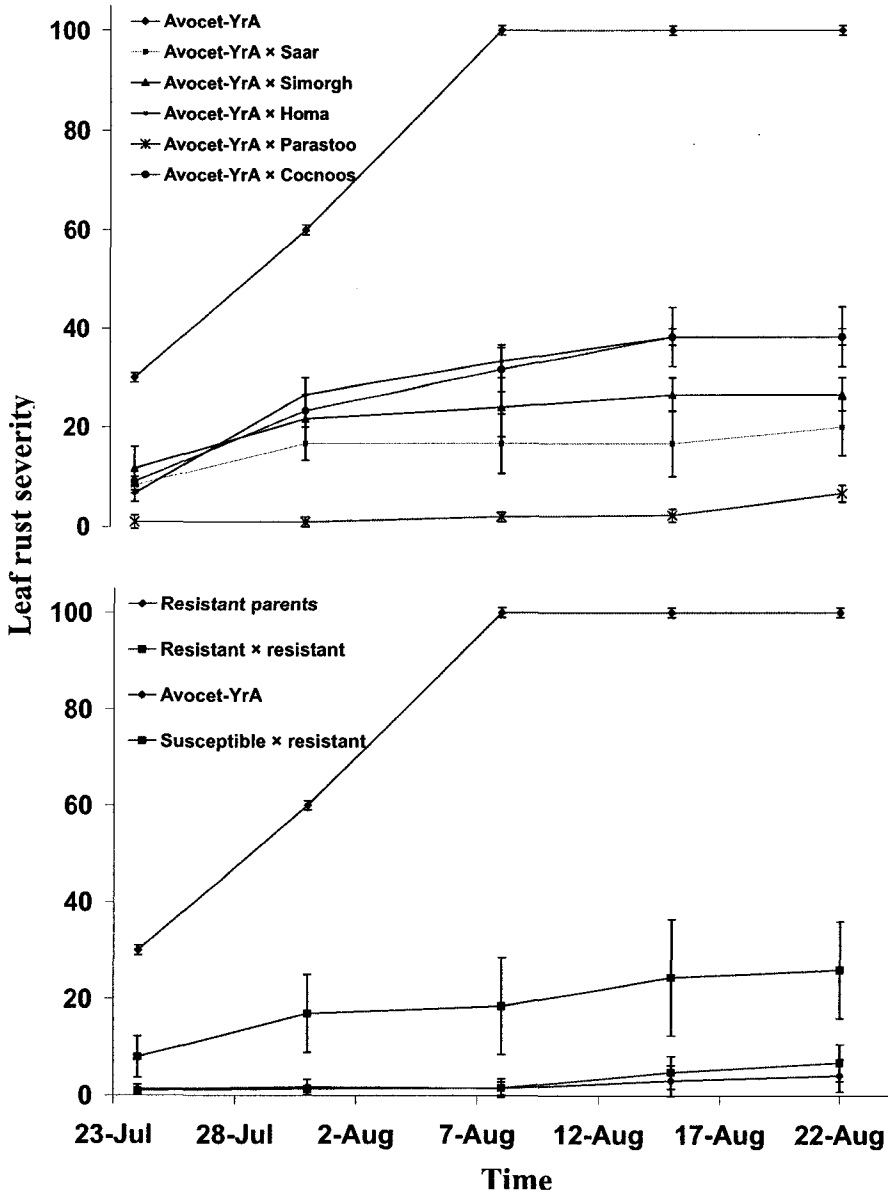

Figure 3-2. Leaf rust progress in the F_1 generation of the susceptible × resistant crosses (top) and average of susceptible × resistant, and resistant × resistant crosses, and resistant parents (bottom) in comparison with the susceptible parent, Avocet-*YrA*.

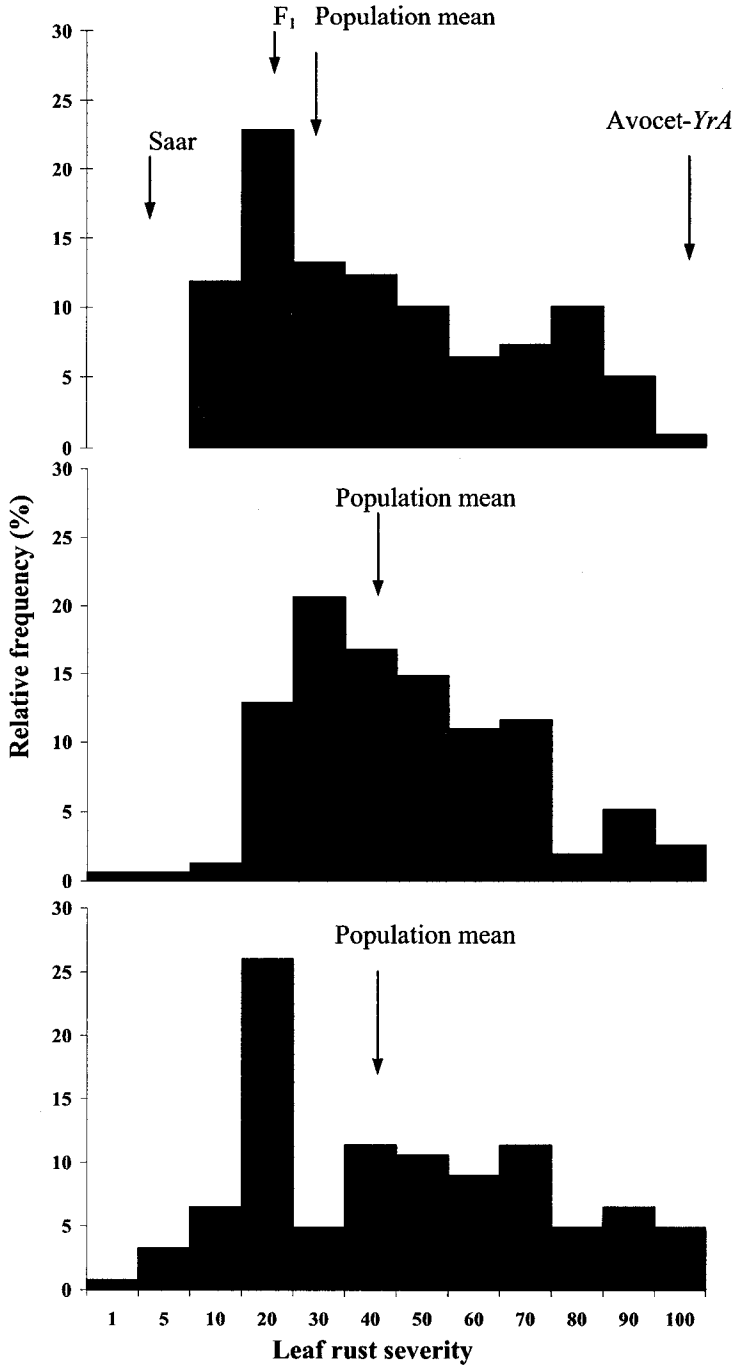

Figure 3-3. Relative frequency distribution of leaf rust severity of F_2 population (top), F_3 (Middle), and F_5 SSD lines derived from Avocet-*YrA* × Saar cross.

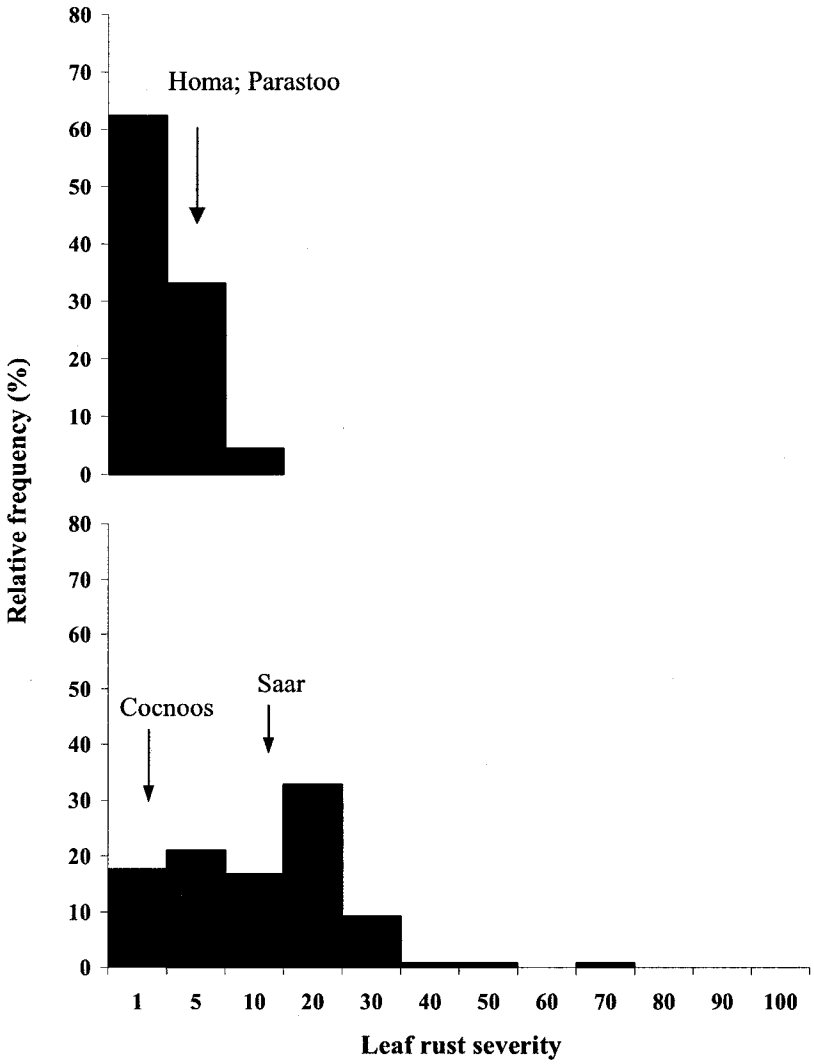

Figure 3-4. Frequency distribution of leaf rust severity of F_5 SSD lines derived from resistant intercrosses: Homa × Parastoo (top) and Saar × Cocnoos (bottom).

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Chapter 4

Association of adult-plant resistance between leaf and stripe rusts in wheat[1]

4.1 Introduction

Leaf rust, caused by *Puccinia triticina* Eriks., and stripe rust, caused by *P. striiformis* Westend., are common fungal diseases of wheat in many areas of the world. Several genes, which confer resistance to leaf and stripe rusts have been catalogued (McIntosh *et al.*, 1998) and deployed in wheat breeding. Many of these resistance genes are race-specific and induce hypersensitive responses in response to infection. Most of the known race-specific resistance genes provide high levels of resistance that may become ineffective in a short period of time, as races with new virulence evolve in the pathogen population. However, race non-specific adult-plant resistance (APR) introduced into some widely grown cultivars has remained effective for a long period of time. Unlike race-specific resistance that is often controlled by a single major gene, durable (Johnson and Law, 1975) APR is known to be inherited quantitatively. As shown in earlier studies (Bariana *et al.*, 2001; Singh *et al.*, 2000; Bariana and McIntosh, 1995; Singh and Rajaram, 1992) and through results presented in Chapters 2 and 3, additive interaction of a few genes can result in high levels of APR to leaf and stripe rusts. Therefore, the utilization of durable APR should be the goal of wheat breeding programs throughout the world for achieving a sustainable control of rust diseases.

Closely linked, or pleiotropic, genes involved in resistance to leaf and stripe rusts should interest wheat breeders as it would simplify the selection process. McIntosh (1992a) and Singh (1992b) reported close genetic linkage between genes *Lr34* and *Yr18* that confer APR to leaf rust and stripe rust, respectively. Leaf rust APR gene *Lr46* also is

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known to be closely linked or pleiotropic with the stripe rust APR gene *Yr29* (Singh *et al.*, 2001a). None of these genes provide adequate levels of resistance to either leaf or stripe rust when deployed alone. Leaf or stripe rust terminal severities of genotypes with *Lr34*, *Lr46*, or *Yr18* alone could reach 50-60% under high rust pressure (McIntosh, 1992b; Singh and Gupta, 1992; Ma and Singh, 1996; Singh *et al.*, 1998; also Chapters 2. and 3). However, the level of resistance can reach as high as being close to immunity when one of these linked genes is deployed in combination with 2-3 additional minor genes (Singh *et al.*, 2000).

The objective of this study was to determine the phenotypic association between the expressions of APR to leaf and stripe rusts in various F_5 populations derived from a one-way diallel cross between one susceptible and five resistant spring wheat genotypes. These F_5 lines were segregating for different combinations of *Lr34/Yr18* and other additive genes that conferred high levels of resistance to both leaf and stripe rusts (results presented in Chapters 2 and 3).

4.2 Materials and methods

Two sets of an identical nursery of 1792 F_5 single seed descent (SSD) lines, obtained from a one-way diallel cross among five stripe and leaf rust APR parents and a susceptible parent were evaluated together with the parents and other checks under artificially created epidemics of stripe and leaf rusts at CIMMYT's research stations at Toluca (State of Mexico, Mexico) and Ciudad Obregon (State of Sonora, Mexico), respectively. Methods used in the development of genetic populations, stripe and leaf rust pathogen races, phenotypic grouping and field assessment were described in Chapters 2 and 3. In order to study the phenotypic associations of leaf and stripe rust responses of lines, two-way comparisons of the leaf and stripe rust phenotypic classifications were made. Also boxed-plot analysis (SPSS Inc., Chicago, Illinois) was performed to generate summary plots for each susceptible × resistant cross to study the leaf rust severities of F_5 lines in groups of stripe rust severities. Linear regression analyses were performed, separately for each susceptible × resistant cross, and for all susceptible × resistant crosses, with leaf rust severity treated as the dependent variable and stripe rust severity as the independent variable. Coefficients of determination (R^2 value) were computed for

each regression analysis. F_5 lines of the resistant × resistant crosses were classified in two-way tables to present the frequency of leaf rust severities of the lines in three groups of stripe rust severities; 0-15, 20-40, and 50-70 (modified Cobb's Scale; Peterson *et al.*, 1948). The presence (homozygous and segregating) and absence, and the extent of leaf tip necrosis were recorded for each F_5 line of the susceptible × resistant crosses between the milk and dough stages in the leaf rust nursery at Ciudad Obregon. For this purpose, a 0-4 scale (Figure 4-1) was used, with 0 representing lines without leaf tip necrosis, 1 representing lines with faint necrosis, and 4 representing lines with necrosis extending to more than one half of individual leaves. The frequency distributions of the presence and absence of the leaf tip necrosis were tested for congruency with ratios expected for the segregation of 1 and 2 genes through Chi-square analysis. In order to test the null-hypothesis that there were no differences between F_5 lines for mean rust severities in the presence or absence of the leaf tip necrosis, one-way analysis of variance was performed separately for each susceptible × resistant cross. Statistical analyses were done by SPSS, version 11.0.

4.3 Results

Two-way tables of the combined frequencies of the F_5 lines from susceptible × resistant crosses in each phenotypic group of stripe and leaf rusts are presented in Table 4-1. In all crosses there was an absence of F_5 lines with HPTR response for one rust disease and an HPTS response for the other. This indicated that some common genes could be involved in APR to leaf and stripe rusts. Most of the F_5 lines that were classified as HPTR for stripe rust reaction were also classified in a similar group for leaf rust reaction. There were however exceptions in crosses of Avocet-*YrA* with Simorgh, Homa, and Cocnoos that stripe rust-HPTR lines were classified in a segregating class with intermediate severity for leaf rust.

In order to study the relationship between the severities of the two diseases, box plots (Figure 4-2) were generated separately for each susceptible × resistant cross and for the data collected for F_5 lines in all susceptible × resistant crosses. These are the summarized plots based on the median, quartiles, and extreme values. Each box represents the inter-quartile range, which contains 50% of values. The whiskers are lines

that extend from the box to the highest and lowest values, excluding outliers. The solid line across the box indicates the median. There was a positive trend in all plots indicating that in general F_5 lines with higher stripe rust severities had higher leaf rust severities and *vice versa*. There are, however a few exceptions in some crosses that resulted in some deviations from this trend. In F_5 lines derived from Avocet-*YrA* × Simorgh cross, for example, there were three lines that were rated 0 for stripe rust had leaf rust severities ranging from 0 to 60%. Coefficient of regression of leaf rust severity over stripe rust severity was positive and highly significant ($P < 0.01$) with R^2 ranging from 0.27 to 0.50 (Figure 4-2).

Two-way tables of the combined frequency of F_5 lines in different stripe and leaf rust severity classes along with the stripe and leaf rust severities of the parental lines of resistant-parent intercrosses are summarized in Table 4.2. In most resistant intercrosses, except crosses of Saar with Homa and Parastoo, the majority of F_5 lines (56 to 87%) with low stripe rust severities (0-15%) had low leaf rust severities as well. In crosses of Saar with Homa and Parastoo, higher frequencies of lines with intermediate (20-40%) stripe rust severities that expressed low (0-15%) leaf rust severity were observed.

F_5 lines in the susceptible × resistant crosses were tested for the presence and absence, and the extent of leaf tip necrosis. The susceptible parent Avocet-*YrA* lacked leaf tip necrosis whereas all resistant parents had some expression (class 1 or 2). Some of the F_5 lines expressed severe leaf tip necrosis which extended to 30-50% of the leaves (class 4). As an example, the frequency distribution of the extent of leaf tip necrosis in Avocet-*YrA* × Saar cross is presented in Figure 4-1. Frequency distributions for the presence or absence of leaf tip necrosis in susceptible × resistant crosses (Table 4-3) indicated that it was conditioned by 1 gene in Saar and Simorgh and by 2 genes in Homa, Parastoo, and Cocnoos. Mean leaf and stripe rust severities of F_5 lines with leaf tip necrosis were significantly ($P < 0.01$) less than the means of the lines without leaf tip necrosis (Table 4-4).

4.4 Discussion

APR to leaf and stripe rusts were closely associated in the wheat genotypes included in this study. Genetic studies of the parental lines (Chapters 2 and 3)

demonstrated that linked genes *Lr34* and *Yr18* and at least three and two additional genes that have additive effects conditioned resistance to stripe rust and leaf rust, respectively. In all crosses, a majority of the lines with low stripe rust severities also had low leaf rust severities. There was a positive and highly significant ($P < 0.01$) linear trend in leaf rust severities of the lines tested at Ciudad Obregon in relation to their stripe rust severities at Toluca. Regression analyses indicated that stripe rust severity accounted for 27 to 50% of the phenotypic variation in leaf rust severity of the F_5 lines from susceptible × resistant crosses. These observations indicated the genetic linkages, or pleiotropic effects, of at least some genes involved in APR to stripe and leaf rusts. The lack of a much higher correlation could be due to two reasons: 1) It was estimated in inheritance studies (Chapters 2 and 3) that more genes were involved in resistance to stripe rust compared to leaf rust indicating that not all genes conferred resistance to both diseases, and 2) the effects of the linked genes could be different for the two diseases as known for *Lr34* and *Yr18*, where the effect of the former in reducing leaf rust severity is much higher than the effect of the later in reducing stripe rust severity (McIntosh *et al.*, 1992b).

Genetic linkage or pleiotropic effects of APR to leaf and stripe rusts is known in wheat. Leaf rust resistance gene *Lr34* was reported to be tightly linked or pleiotropic with stripe rust resistance gene *Yr18* (Singh, 1992b; McIntosh, 1992a). Similarly, leaf rust resistance gene *Lr46* (Singh *et al.*, 1998) was also found to be linked to or pleiotropic with the stripe rust resistance gene *Yr29* (Singh *et al.*, 2001a; Bariana *et al* 2001). Singh *et al.* (2001b) stated that at least 10-12 additional APR genes having minor to intermediate but additive effects should be present in a set of CIMMYT germplasm and showed through QTL mapping that additional chromosomal regions different from those carrying the *Lr34/Yr18* and *Lr46/Yr29* were involved in resistance to both rusts (Singh *et al.* 2001a). Although the combined leaf and stripe rust analyses supports this, it also appears that linkage, or pleiotropism, of the APR genes does not occur in every instance. A few of the lines derived from some susceptible × resistant crosses (crosses with Simorgh and Parastoo, Figure 4-2) showed very low stripe rust severities at Toluca but exhibited up to 60% leaf rust severity at Ciudad Obregon (Figure 4-2). Similarly, a few such F_5 lines also occurred in the resistant-parent intercrosses (Table 4-2). These lines were presume to be those involving combinations of *Yr18/Lr34* with additive genes that

were effective against stripe rust but not against leaf rust. The presence of such lines resulted in a few extreme outliers in some populations, deviating from linear regression and causing smaller R^2 values.

As expected, lines with leaf tip necrosis appeared to have significantly ($P < 0.01$) less leaf and stripe rust severities compared to the lines lacking it (Table 4-4). Lines with leaf tip necrosis on average had 30.5 and 20.8 percent less leaf and stripe rust severities, respectively. Leaf tip necrosis is known to be associated with *Lr34* (Dyck, 1991; Singh, 1992a) and *Yr18* (Singh, 1992b). Assuming that *Lr34/Yr18* is absent in lines without leaf tip necrosis, genes other than *Lr34/Yr18* must have contributed 40 and 43 percent in leaf and stripe rust disease severity reduction effects, respectively, given the fact that leaf and stripe rusts reached 100% in the susceptible parent, Avocet-*YrA*. In the lines without leaf tip necrosis there were also positive trends ($b = 0.50$, $P < 0.01$, $R^2 = 0.23$) in the association of leaf and stripe rust severities which is in agreement with the conclusion that was made earlier that some additive genes other than *Lr34/Yr18* are also either linked or pleiotropic.

The presence and absence of leaf tip necrosis appeared to be controlled by 1 gene in Saar and Simorgh and 2 genes in Homa, Parastoo, and Cocnoos (Table 4-3). Variation in the extent of leaf tip necrosis can be an indication of the presence of a few modifier genes that influenced the extent of the expression. A single gene, located on the short arm of the chromosome 7D and designated as *Ltn*, was originally known to control the leaf tip necrosis (Singh, 1992a). Dyck *et al.* (1994) indicated that *Lr34* may be either located on two different chromosomes or there might be two different genes with very similar phenotypic expressions and association with leaf tip necrosis. They also suggested that *Lr34* might have been translocated onto another chromosome. Cytogenetic evidence further supported this assumption (Kolmer, 1996). Messmer *et al.* (2000) indicated that leaf tip necrosis in the winter wheat 'Forno' is probably neither monogenic nor located on chromosome 7D. They found multiple quantitative trait loci contributing to leaf tip necrosis and concluded that at least two genes with additive effects are responsible for its expression in 'Forno'. Based on observations in this study and findings of Dyck *et al.* (1994) and Messmer *et al.* (2000), it seems that leaf tip necrosis is not only associated with *Lr34/Yr18* but also with other APR genes. Mexican wheat cv. Pavon 76, known to

carry genes *Lr46/Yr29* and additional minor, additive genes for resistance to both leaf and stripe rusts (Singh *et al.*, 1998, Singh *et al.*, 2001), also displays some leaf tip necrosis.

Singh and Huerta-Espino (1997) believed that APR and leaf tip necrosis are likely to have resulted from pleiotropism rather than linkage of genes and hypothesized that leaf tip necrosis is a secondary feature resulting from mechanisms involved in APR conditioned by *Lr34*. They assumed that APR to leaf rust may involve the production of a toxic metabolite, which, when accumulated in high concentrations, may induce leaf tip necrosis. Alternatively, Messmer *et al.* (2000) hypothesized that the mechanisms involved in the expression of leaf tip necrosis may cause physiological changes in the leaves, which could disturb the infection processes of a range of pathogens. The later hypothesis can be supported by the fact that *Lr34/Yr18* is linked to *Bdv1* which confers a slow yellowing response to barley yellow dwarf virus (Singh, 1993) and enhances resistance to stem rust (Dyck, 1987; Dyck, 1993; Kerber and Aung, 1999). If the above assumption is true, a single mechanism could be involved in multiple-rust or even multiple disease resistance. Future biochemical investigations of the mechanisms involved in the expression of leaf tip necrosis in relation to APR may lead to exploring new avenues towards breeding for multiple disease resistance.

From breeding point of view, linked/pleiotropic leaf and stripe rust resistance genes have several advantages. First, genetic gain for increasing the level of resistance to both diseases can be achieved by selecting for either one of them. Therefore, if a breeding program that is selecting only for resistance to one of the two diseases because of the lack of facilities or suitable environmental conditions during certain years for the other disease, improvement in the levels of resistance to both diseases can be achieved. Secondly, when a seedling resistance gene for one disease is present and masks the effect of APR genes for that disease, selecting for APR for the other disease should result in indirect selection of the APR genes for both diseases in the progenies.

Improving the level of APR seems to be possible by incorporating as many as possible additive genes. Some breeding programs have been doing this by inter-crossing genotypes with different levels of APR (Singh, 2001b) simply by selecting for an acceptable level of resistance that must be reached in order for a variety to be approved for commercial release. Results of the present study indicated that it is possible to

improve the level of APR to leaf and stripe rust at the same time. In the F_5 SSD line populations of the resistant-parent intercrosses, more than 90% of the lines with less than 15% stripe rust severities also had less than 15% leaf rust severities. Therefore, it can be concluded that a close genetic association often exists between phenotypic expression of APR to leaf and stripe rusts and exploitation of the association can be a reliable and useful tool in wheat breeding.

4.5 Tables and figures

Table 4-1. Combined frequencies of stripe and leaf rust phenotypic classes of F_5 SSD lines derived from the Avocet-*YrA* × resistant crosses and tested in the field.

Avocet-*YrA* crossed with	Leaf rust phenotypic class	Stripe rust phenotypic class			Total
		HPTR[a]	HPTS[b]	Seg[c]	
Saar	HPTR	1	0	5	6
	HPTS	0	0	10	10
	Seg	0	3	103	106
	Total	1	3	118	122
Simorgh	HPTR	7	0	7	14
	HPTS	0	3	8	11
	Seg	2	1	92	95
	Total	9	4	107	120
Homa	HPTR	2	0	18	20
	HPTS	0	2	3	5
	Seg	2	0	95	97
	Total	4	2	116	122
Parastoo	HPTR	2	0	13	15
	HPTS	0	1	4	5
	Seg	0	5	98	103
	Total	2	6	115	123
Cocnoos	HPTR	0	0	4	4
	HPTS	0	1	7	8
	Seg	1	3	100	104
	Total	1	4	111	116

[a] Homozygous for the parental type resistance (homozygous for all resistance alleles).
[b] Homozygous for the parental type or higher susceptibility (homozygous lacking all resistance alleles).
[c] Segregating or homozygous with disease severities less than that of the susceptible and more than that of the resistant parent.

Table 4-2. Average leaf and stripe rust severities of parental lines and frequencies of F_5 SSD lines of resistant × resistant crosses in groups of disease severity classes.

Cross	Leaf rust severity [a]		Stripe rust severity [a]		Leaf rust severity [a] range	Frequency of lines with stripe rust severity [a] range		
	P_1	P_2	P_1	P_2		0-15	20-40	50-70
Saar × Simorgh	14	10	9	2.6	0-15	95	13	1
					20-40	16	17	0
					50-70	0	0	0
Saar × Homa	14	6	9	8	0-15	56	28	0
					20-40	20	17	8
					50-70	1	4	0
Saar × Parastoo	14	6.4	9	11	0-15	20	56	10
					20-40	4	29	7
					50-70	0	2	1
Saar × Cocnoos	14	1	9	6.2	0-15	67	17	0
					20-40	14	19	0
					50-70	0	0	1
Simorgh × Homa	10	6	2.6	8	0-15	90	13	0
					20-40	11	0	1
					50-70	0	0	0
Simorgh × Parastoo	10	6.4	2.6	11	0-15	108	24	1
					20-40	0	5	0
					50-70	0	0	0
Simorgh × Cocnoos	10	1	2.6	6.2	0-15	52	6	2
					20-40	10	3	2
					50-70	0	0	0
Homa × Parastoo	6	6.4	8	11	0-15	111	20	2
					20-40	0	0	0
					50-70	0	0	0
Homa × Cocnoos	6	1	8	6.2	0-15	74	12	0
					20-40	3	6	0
					50-70	1	0	0
Parastoo × Cocnoos	6.4	1	11	6.2	0-15	95	9	0
					20-40	4	0	0
					50-70	0	0	0

[a] Leaf and stripe rust severities estimates follow the modified Cobb's Scale (Peterson *et al.*, 1948) and are presented as the average severity of parental lines.

Table 4-3. Frequencies of presence and absence of leaf tip necrosis in F_5 SSD lines derived from susceptible × resistant crosses.

Avocet-*YrA* crossed with	Leaf tip necrosis		Tested [a] for the segregation of	χ^2	P-Value
	Present	Absent			
Saar	80	45	1 gene	3.05	> 0.05
Simorgh	70	52	1 gene	0.06	> 0.90
Homa	96	23	2 genes	0.96	> 0.25
Parastoo	106	19	2 genes	1.16	> 0.10
Cocnoos	92	25	2 genes	0.38	> 0.50

[a] The ratios used were 0.5625 present: 0.4375 absent for segregation of one gene and 0.8086 present: 0.1914 absent for segregation of two genes.

Table 4-4. Average leaf and stripe rust severities of susceptible × resistant F_5 SSD lines in the presence and absence of leaf tip necrosis.

Avocet-*YrA* crossed with	Leaf tip necrosis	Average rust severity [a]	
		P. triticina	*P. striiformis*
Saar	Absent	63.05 a	58.50 a
	Present	35.62 b	41.52 b
Simorgh	Absent	62.74 a	49.53 a
	Present	27.36 b	29.36 b
Homa	Absent	58.10 a	56.48 a
	Present	28.35 b	35.47 b
Parastoo	Absent	67.78 a	69.44 a
	Present	23.32 b	37.24 b
Cocnoos	Absent	53.47 a	52.32 a
	Present	37.56 b	38.64 b

[a] Values in each column followed by different letters are significantly different for each cross according to F-test in one way ANOVA ($P < 0.01$).

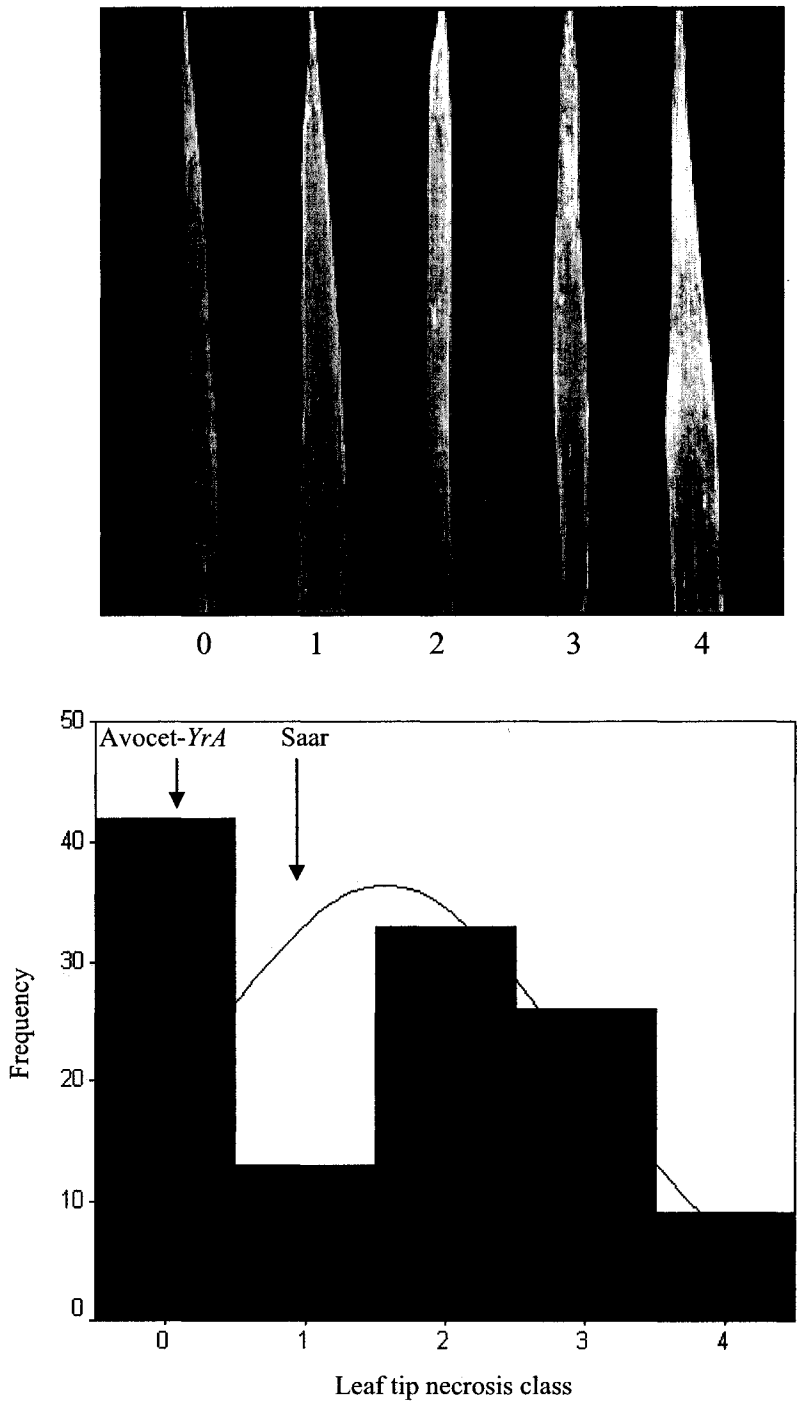

Figure 4-1. Upper: Extent of leaf tip necrosis recorded on a 0-4 scale with 0 representing lines without leaf tip necrosis and 4 representing lines with the highest extent of leaf tip necrosis. Lower: Frequency distribution of F3 lines of Avocet-*YrA* × Saar in leaf tip necrosis phenotypic classes.

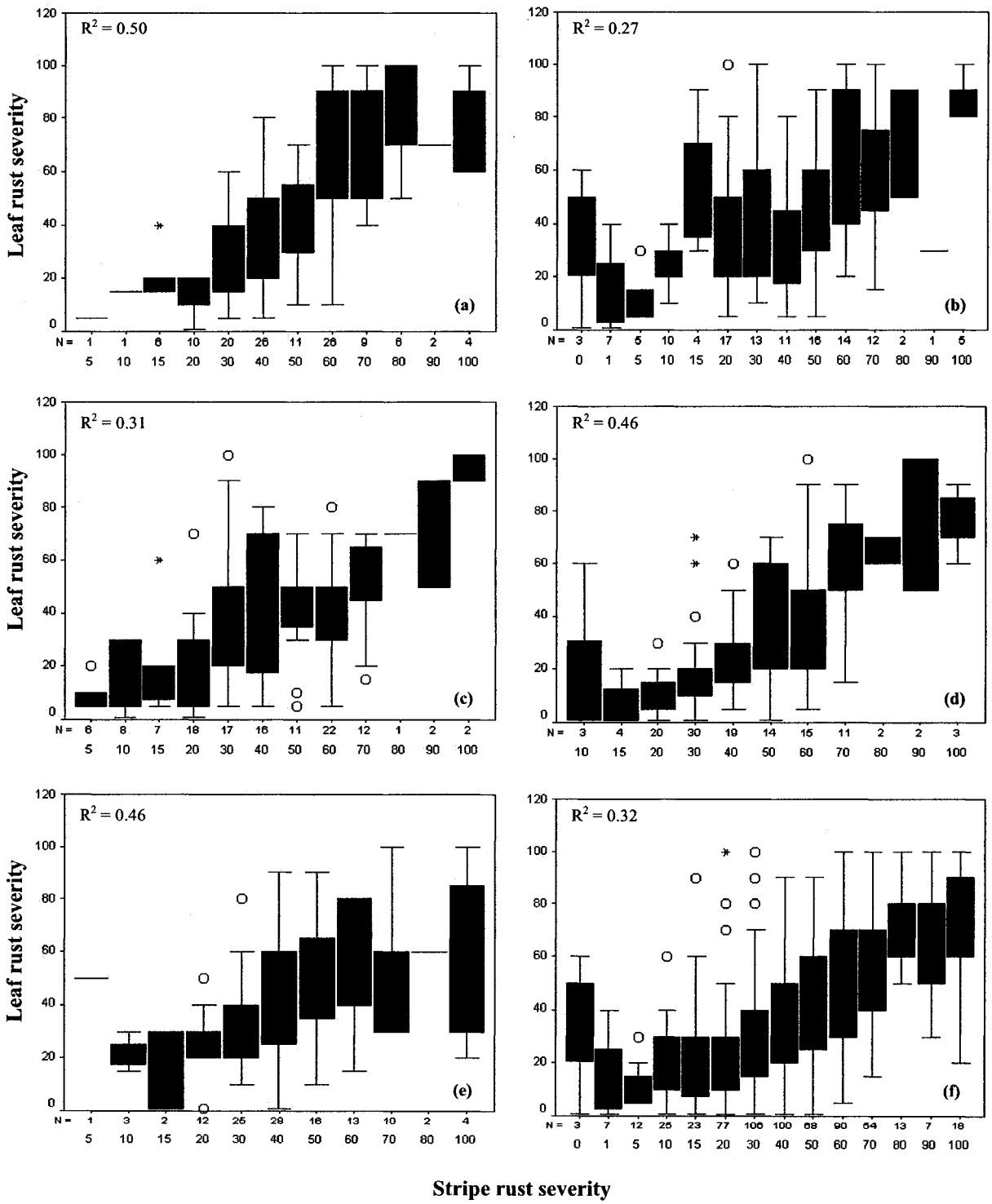

Figure 4-2. Box plots representing the leaf rust severities of F_5 SSD lines in each group of stripe rust severities for crosses of Avocet-*YrA* with Saar (a), Simorgh (b), Homa (c), Parastoo (d), Cocnoss (e) and all susceptible × resistant crosses (f). * and ○ represent extremes and outliers in each group, respectively.

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Chapter 5

Quantitative trait loci analysis of associated adult-plant resistance to leaf and stripe rusts in the Australian wheat cultivar, Cook[1]

5.1 Introduction

Durable resistance to leaf rust (*Puccinia triticina* Eriks.) and stripe rust (*P. striiformis* Westend.) in wheat is likely to be associated with adult-plant resistance (APR) genes (McIntosh, 1992b). Inheritance of APR has been studied extensively for leaf rust (Gavinlertvatana and Wilcoxon, 1978; Bjarko and Line, 1988a; Broers and Jacobs, 1989; Jacobs and Broers, 1989; Lee and Shaner, 1985; Singh and Rajaram, 1992; Das *et al.*, 1992; Messmer *et al.*, 2000; Kolmer and Liu, 2001; Chapter 3) and stripe rust (Bariana *et al.*, 2001; Zhang *et al.*, 2001; Wagoire *et al.*, 1998; Bariana and McIntosh, 1995; Chen and Line, 1995a; Singh and Rajaram, 1994; Milus and Line, 1986; Johnson, 1980; Chapter 2). Most studies have reported a quantitative inheritance of APR with the involvement of a few genes with additive effects (Kolmer, 1996). Leaf and stripe rust resistance gene(s) *Lr34/Yr18* are key contributors to the APR in several widely grown cultivars. In addition, involvement of several additive minor genes has been reported in wheat germplasm (Singh *et al.*, 2000; Wallwork and Johnson, 1984; Bariana and McIntosh, 1995). Additive interaction of *Lr34/Yr18* with a few minor genes has been reported to result in high levels of nonspecific resistance (Singh *et al.*, 2000).

The genetic study of durable rust resistance is a challenging task, mainly because of its complex and quantitative inheritance. Application of quantitative trait loci (QTL) analysis provides a powerful tool for studying complex traits, including quantitative disease resistance (Young, 1996). With QTL mapping, the role of each locus in genetically complex traits can be described. A fine linkage map of markers provides a framework for an analysis of the QTLs. Researchers have used QTL analysis to characterize quantitative rust resistance (Bariana *et al.*, 2001; Boukhatem *et al.*, 2002;

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Messmer *et al.*, 2000; Suanaga *et al.*, unpublished). Multiple QTLs with small effects have been reported to contribute to high levels of APR to stripe and leaf rusts.

A population of 148 recombinant inbred lines (RILs) was used in this study. This population was developed from a cross between an Australian durable leaf and stripe rust resistant cv., Cook, and susceptible genotype, Avocet-*YrA*, at the International Wheat and Maize Improvement Center (CIMMYT) in Mexico. The advantages of RILs for detecting QTLs have been shown by Austin and Lee (1996). The RILs undergo multiple cycles of meiosis before homozygosity is reached. Consequently, linked genes have a greater probability of recombination and their pleiotropic effects can be detected (Burr and Burr, 1991). This effect increases the efficacy of testing differences between genotypic classes.

The objective of the present investigation was to study the genetic basis of quantitative APR in the cv. Cook, using QTL analysis.

5.2 Materials and methods

5.2.1 Plant material

A population of 148 F_4-derived F_6 recombinant inbred lines (RILs) of a cross between the cv. Cook, and Avocet-*YrA* was provided by Dr. R. P. Singh CIMMYT, Mexico. Cook (Timgalen/Condor sib.//Condor) is an Australian stripe and leaf rust resistant spring wheat cultivar, known to carry the leaf rust resistance genes *Lr3a* (Cereal Disease Laboratory web-site: http://www.cdl.umn.edu/index.htm) and *Lr34* (McIntosh *et al.*, 1998), the stripe rust resistance gene *Yr18* (McIntosh *et al.*, 1998), and the stripe rust temperature-sensitive resistance gene *YrCK* (Park *et al.*, 1992; Bariana *et al.*, 2001). Avocet-*YrA* (WW119/WW15//EGT) is a reselection from the Australian cv., Avocet, which carries the leaf rust resistance genes *Lr10* and *Lr13* (McIntosh *et al.*, 1998) and none of the known stripe rust resistance genes (Dr. R. P. Singh, 2002, Personal communication). These race-specific genes are ineffective to current populations of leaf rust pathogen in Mexico, Canada, and many other countries. The coefficient of parentage between the two parents, as determined by the International Wheat Information System (IWIS; Fox *et al.*, 1997) is 0.38. This signifies the highest theoretical genetic similarity of parental genotypes, based on the pedigree information.

5.2.2 Test sites

The population of RILs and the parental genotypes were field evaluated at several locations in Mexico and Canada. Evaluations for the stripe rust responses were carried out at CIMMYT Research Station near Toluca, State of Mexico, in the highlands of central Mexico (19°N, 2640 *masl*), during 1998 and 2000, and in Creston Valley, in south-eastern British Colombia (BC), Canada, during 2001 and 2002. Evaluations for leaf rust responses were carried out at Ciudad Obregon, State of Sonora, in Northwestern Mexico (22°N, 7 *masl*) during the winter 1998 crop season, in Glenlea, Manitoba (MB), Canada, during 2000-2002 crop seasons, and at the Edmonton Research Station in Edmonton, Alberta (AB), Canada, during 2000 and 2002. All field experimental sites have favorable environments for the respective diseases. However, the data collected in the stripe rust experiment at Creston, BC, 2001, and the leaf rust experiment at Edmonton, AB, 2000 were not included in the analysis because the rust severity of the susceptible parent, Avocet-*YrA*, was not high enough, due to dry seasons, and was not uniformly spread throughout the experiments.

5.2.3 Experimental layout

Each leaf or stripe rust experiment in Glenlea, MB, Creston, BC, and Edmonton, AB was planted in a randomized complete block design with two replications. Each experimental unit in Glenlea, MB comprised a double-row plot, 95 cm long. In Creston, BC and Edmonton, AB, however, each experimental unit was a hill-plot, with 0.5 m between hill-plots. Each experimental unit in Mexico was a single 1 m plot grown on a 75 cm wide raised bed with two rows, 20 cm apart.

5.2.4 Disease epidemics and pathogen races

In all field experiments one row of a fully susceptible spreader, cv. Morocco, was planted on one side of the plots and around the experiment. For artificial inoculation of the field experiments, pathogen races were selected with matching virulence for the known race-specific resistance genes present in the parental genotypes, including the APR gene *Lr13* (Table 5-1). The leaf rust experiment at Ciudad Obregon, Mexico, was artificially inoculated with *P. triticina* race MCJ/SP. Uridiospores of this race was

multiplyed on the susceptible variety, Morocco, in the greenhouse. The nomenclature of this race is according to Singh (1991). Leaf rust experiments in Canada were artificially inoculated with *P. triticina* race MBDS. Virulence formula and nomenclature of this race is according to McCallum and Goh (2002). Field inoculation of stripe rust experiments in Mexico was done with a single isolate of *P. striiformis*, MEX96-11. Except for the stripe rust experiment in Creston, BC, in all other experiments spreader rows were artificially inoculated, once or twice with fresh urediniospores, suspended in a light-weight mineral oil (Soltrol® 170).

The experiment in Creston, BC, grown in a farmer's field in 2002 was naturally infected with stripe rust. A field collection of *P. striiformis* from the susceptible parent, Avocet-*YrA*, designated SRC02UA, was tested for virulence or avirulence on a set of *Yr*-gene carrying near-isogenic lines in the seedling and adult plant stages. For this purpose two-week old seedlings of the Avocet near-isogenic lines were inoculated with fresh urediniospores, collected from the infected leaves of the susceptible parent, Avocet-*YrA*. Urediniospores were suspended in light-weight mineral oil (Soltrol® 170) and sprayed on the seedlings. After 48 h in humid chambers in a cold room at 7°C, the inoculated seedlings were transferred to a growth chamber set at 18/12°C day/night temperature with 12 h photoperiod. Infection types were recorded 14 days after inoculation following a 0-9 scale (McNeal *et al.*, 1971). Adult plant responses of the Avocet isogenic lines were also recorded in a nursery of these lines that was planted in the same field. Stripe rust isolate SRC02UA appeared to carry virulence for resistance genes *Yr 6, 7, 8, 9, 11, 27,* and *A* (Table 5-2).

5.2.5 Phenotypic assessment

Leaf and stripe rust severities were visually assessed following the modified Cobb's Scale (Peterson *et al.*, 1948). Several flag and penultimate leaves from each experimental unit were assessed and an average rust severity was recorded per experimental unit. The leaf rust experiments in Glenlea, MB were rated 5 times, each year, at 4-5 day intervals. The area under the leaf rust progress curve (AUDPC) was then computed for each experimental unit, using the formula:

$$\text{AUDPC} = \Sigma\,[(x_i + x_{i+1})/2](t_{i+1} - t_i)$$

where x_i = leaf rust severity on the ith day and t_i = ith day. Relative area under the disease progress curve (rAUDPC) was then determined for each experimental unit as the ratio of the AUDPC of the unit to the average AUDPC of the susceptible parent, Avocet-*YrA*. In other experiments each experimental unit was rated twice. The first rating was done when the rust severity of the susceptible parent, Avocet-*YrA*, reached 80-100% and the second rating was done 10-15 days later when the leaves of the susceptible parent had dried out due to rust infection. The rust severity of Avocet-*YrA* ranged between 80-100% across the experiments. Therefore, to make the data comparable across the experiments, the relative disease severities (rDS) of all experimental units in all locations were computed as the ratio of the rust severity of the unit to the average rust severity of the susceptible parent, Avocet-*YrA*, in that experiment.

The presence or absence of the leaf tip necrosis, a morphological trait, known to be associated with *Lr34/Yr18* genes, was recorded after anthesis in all leaf rust field experiments.

5.2.6 Screening for the stripe rust temperature-sensitive gene *YrCK*

Seedling tests were carried out to test the RIL population for the temperature-sensitive gene *YrCK*, located in the chromosome arm 2DS (Bariana *et al.*, 2001). For this, two sets of the population were grown in a growth chamber set at 18/12°C day/night temperature with 12 h photoperiod, each in a two-replication completely randomized design. Inoculation was done 12 days after sowing, using a urediniospore suspension of stripe rust isolate SRC02UA (Table 5-2) in Soltrol® 170 that was sprayed onto the seedlings. Inoculated seedlings were then kept in humid chambers in a cold room set at 7°C for 48 h. The two sets of lines were then transferred to growth chambers, one set at 18/12°C and the other set at 24/15°C day/night temperatures, both with 12 h photoperiods. Stripe rust infection types were scored 14 days after inoculation on the 0-9 scale (McNeal *et al.*, 1971). For mapping purposes scores 7-9 were considered susceptible (Avocet-*YrA* phenotype), whereas scores 0-3 were considered resistant (Cook phenotype).

5.2.7 Phenotypic data analysis

For the single-replication experiments, the numbers of effective genes were estimated using segregation analysis. For this reason RILs were classified into three groups: 1) homozygous for the parental type resistance (HPTR), 2) homozygous for the parental type susceptibility (HPTS), and 3) others. Chi-square analyses were carried out to test the distribution of observed phenotypic frequencies against those expected for segregation models. For the experiments with replication, analyses of variance (Steel *et al.*, 1997) were performed. Leaf rust experiments at Glenlea, MB with multiple disease ratings were also analyzed using a repeated-measure multivariate analysis of variance (MANOVA) to test the effect of time of recording and the interaction of time with other effects in the experiment. For this, the REPEATED statement in the Proc GLM procedure (SAS Institute, Cary, NC) was used. The analysis of variance for rAUDPC for single years and the combined analysis of variance for three years of data were performed, using Proc GLM procedure (SAS Institute, Cary, NC).

Components of variance and the broad-sense heritabilities were estimated from on the analysis of variance, using the following formulae, adopted from Fehr (1987):

$$\sigma^2_g = (MS_l - MS_e) / ry$$

$$\sigma^2_{ph} = (MS_l) / ry$$

$$\sigma^2_{ge} = (MS_{gy} - MS_e) / r$$

$$h^2_{bs} = \sigma^2_g / \sigma^2_p$$

where: σ^2_g, σ^2_{ph}, and σ^2_{ge} are the components of genotypic, phenotypic, and genotype by environmental variances, MS_l, MS_e, and MS_{gy} are the mean squares of RILs, RILs × year, and error, estimated in the ANOVA, r and y are the numbers of replications and years, respectively, and h^2_{bs} is the broad-sense heritability.

The minimum numbers of additive genes were estimated using Wright's (1968) formula with modification for the level of inbreeding of the progenies (Cockerham, 1983) as follow:

$$n = (GR)^2 / 4.27\ (\sigma^2_g)$$

where: n is the minimum number of effective additive genes, GR is the genotypic range, and σ^2_g is the genotypic variance. The formula assumes that the segregating resistance genes come from one parent, they are not linked, they have equal effects, additive ×

dominance and genotype × environment effects do not exist, and dominance is equal at all loci.

Coefficients of simple correlation between leaf and stripe rust severities or AUDPC estimates in different locations were computed using the Proc CORR procedure (SAS Institute, Cary, NC) and scatter-plots were generated.

5.2.8 DNA extraction

Genomic DNA from all 148 RILs and parental lines was extracted from two week-old plants grown in a growth chamber. For this purpose 0.3 g leaf tissue was harvested from each RIL and immediately frozen in liquid nitrogen. Frozen leaf tissues were then lyophilized for 72 h at -60°C. A FastDNA® kit from BIO-101 (Catalog No. 6540-400) was used for DNA extraction, following the protocol provided by the manufacturer.

5.2.9 AFLP analysis

The amplified fragment length polymorphism (AFLP) method developed by Vos *et al.* (1995) was followed (Figure 5-1) with minor modifications. Genomic DNA (1 µg) was digested with the restriction endonucleases *PstI* and *MseI*. Double-stranded *PstI* and *MseI* adaptors were then ligated to the restriction sites. Pre-amplification was performed using primers specific for the *PstI* and *MseI* adaptors including one selective nucleotide, followed by selective amplification using similar primers with 3 selective nucleotides. Pre-amplification PCR was performed in a volume of 25 µl in GeneAmp®-9700 thermocycler (Applied Biosystems, Foster City, CA). The reaction mixture contained 0.56 µM of each pre-amplification primer, 0.2 mM of each deoxynucleotide, 1.6 mM $MgCl_2$, 1 unit *Taq* polymerase, and 4 µl of the ligated DNA. Pre-amplification PCR consisted of 25 cycles at 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min. Selective amplification PCR was performed in a volume of 20 µl. The reaction mixture contained 0.25 µM of *MseI*-selective primer, 0.1 µM of *PstI*-selective primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 0.75 units *Taq* polymerase, and 2 µl of 1:5 diluted pre-amplification PCR products. Selective amplification PCR consisted of 1 cycle at 94°C for 30 s, 65°C for 1 min, and 72°C for 1.5 min, followed by 9 cycles over which the

annealing temperature was decreased by 1°C per cycle with final steps of 23 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min. Nucleotide sequences of the *MseI* and *PstI* primers and double stranded adaptors are given in Table 5-3.

The selectively amplified PCR products were mixed with 7 µl of the Formamide Loading Buffer: consisting of 98% formamide, 10mM EDTA, and 0.005% each of xylene cyanol and bromophenol blue and were denatured for 4 min at 95°C. Five µl of each sample were size-fractionated through a pre-heated (50°C) 6% denaturing polyacrylamide gel (0.4 mm thick) using an Awl separation system electrophoresis unit, model S3S (Protsmouth, NH, USA) for 3 h at 65 W. In order to estimate the fragment size, two molecular weight ladders, 20 bp molecular ruler (BioRad®, CA, USA) and 25 bp DNA ladder (Invitrogene®) were included in each gel. The top reservoir buffer was 1X TBE, while the bottom reservoir buffer was 2/3 X TBE, 1 M sodium acetate. Adding sodium acetate to the bottom reservoir during electrophoresis cause the mobility of small fragments to be retarded as they approach the bottom of the gel (Sheen and Seed, 1988). A Silver-Sequence® Kit (Promega, Madison, WI, USA) was used to visualize the DNA fingerprints, following the protocol provided by the manufacturer.

5.2.10 Bulked-segregant analysis

Bulked-segregant analysis (Michelmore *et al.* 1991) was used to target the genomic regions associated with the leaf and stripe rust resistances. For each disease, two bulk DNA samples were constructed using equal amounts of DNA from 7 parental-type susceptible and 7 parental-type resistant RILs for each disease using the phenotypic assessments from field experiments of 1998 and 2000. Forty-eight AFLP primer combinations were screened on the parents and the four bulk DNA samples, from which 22 primer combinations (Table 5-4) generated bands that were polymorphic, not only between parental genotypes, but also between pairs of DNA bulk of either one or both diseases (Figure 5-2). AFLP primer pairs with the desired polymorphisms were used to fingerprint the full set of RILs.

5.2.11 Microsatellite analysis

Nine microsatellite loci from chromosome 7D (Table 5-5) were selected from the previously mapped microsatellite markers of wheat (Röder *et al.*, 1998) as anchor markers to identify linkage groups associated with this chromosome, which is the genomic location of leaf and stripe rust resistance genes *Lr34/Yr18* (McIntosh *et al.*, 1998). The microsatellite PCR reaction was performed in a 20 µl volume. The reaction mixture contained 250 nM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1 unit *Taq*-polymerase, and 50 ng of template DNA. After 3 min at 94°C, 45 cycles were performed forl min at 94°C, 1 min at either 50, 55, or 60°C (depending on the individual microsatellite primer pairs, Table 5-5), 2 min at 72°C, and a final extension step of 10 min at 72°C. Size-fractionation and visualization of PCR products were performed as described for the AFLPs, except for the electrophoresis time that was 2 h for the microsatellites.

5.2.12 Partial linkage map

The RIL population was screened, using the AFLP primers revealing polymorphism between parental genotypes and at least one pair of bulk DNA samples (Figure 5-3a) and microsatellites primer pairs polymorphic between parents (Figure 5-3b). Polymorphic amplified DNA fragments detected by AFLP primer pairs were scored separately from top to bottom of the sequencing gel. The nomenclature of the AFLP markers was based on the primer pairs as designated by KeyGene®, listed at the following web address: http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.htm, and the relative molecular weight. For example the AFLP marker locus M60/P41-250 represents an AFLP fragment, with relative molecular weight of 250 b, resulting from selective amplification with primers M60 and P41. Microsatellite loci were designated according to Röder *et al.* (1998). In total, 157 polymorphic markers were scored for the RILs population. A partial linkage map was constructed using Map Manager QTXb17 (Manly *et al.*, 2001). The Kosambi Mapping Function (Kosambi, 1944; Lander *et al.*, 1987) was used with the threshold value of P = 0.0001. For this purpose the 'make linkage groups' function was used. Furthermore, the 'ripple' function was used to improve the order of

loci in a linkage group by testing alternative orders created by local permutations of the locus order.

5.2.13 Quantitative trait loci analysis

Quantitative trait loci (QTL) analysis was performed using single marker regression and composite interval mapping in MapManager QTXb17. Average rAUDPC or rDS of the RILs in each location were computed and entered along with the mapping data. The 'marker regression' function (P = 0.01) was used to find single marker loci associated with each set of quantitative data. The locus with the highest likelihood ratio statistic (LRS) for each set of quantitative data was added to the background. Composite interval mapping was applied using the 'interval mapping' function. The locus in the background of each set of quantitative data was used as a control for other QTLs. For each linkage group carrying a significant QTL, the confidence interval was estimated by bootstrap re-sampling, and interval map figures and histograms representing the confidence interval of peak LRS values were generated. The LRS thresholds for declaring suggestive, significant ($P < 0.05$) and highly significant ($P < 0.01$) QTL effects were calculated for each set of quantitative data and each linkage group using the 'quick test' function. This function provided an approximation to a permutation test according to the method of Piepho (2001).

5.3 Results

5.3.1 Phenotypic analysis

The leaf and stripe rust severities or AUDPC of the parental geneotypes are presented in Table 5-6. The leaf and stripe rust APR cultivar, Cook, had much lower disease severities or AUDPC levels compared to the susceptible genotype, Avocet-*YrA*. The level of leaf or stripe rust severity of Cook was less than 10% across all the experiments, while the susceptible parent, Avocet-*YrA*, reached between 80 and 100% severities in each experiment. Population mean values at most locations/years were smaller than the mid-parental values, except the AUDPC in Glenlea, 2002 and Edmonton, 2002. There were high levels of association between stripe rust severities of RILs at different locations and years, determined by the coefficients of correlation that

were highly significant ($P < 0.01$) for all pairs (Figure 5-4). Likewise, the leaf rust AUDPC of the RILs appeared to be highly associated across the three years of evaluations at Glenlea, MB (Figure 5-5). Coefficients of correlation were always highly significant ($P < 0.01$). Moreover, high levels of association were also found between the average stripe and leaf rust severities across locations and years, with highly significant ($P < 0.01$) coefficients of correlations (Figure 5-6).

Frequency distribution of rDS and rAUDPC of RILs followed a continuous variation for both diseases in all locations/years. In the case of stripe rust rDS (Figure 5-7) only a few lines exhibited disease levels similar to respective resistant and susceptible parents. The frequency distribution of leaf rust rAUDPC at Glenlea, with two peaks (Figure 5-8) seemed to be different from the others. Some transgressive segregation was also observed in some experiments (Figure 5-7 and 5-8), with few lines showing leaf or stripe rust severities or AUDPC values less than the resistant parent or more than the susceptible parent. The frequencies of HPTR and HPTS lines in the stripe rust experiments at Toluca in 1998 and 2000 indicated the segregation of 3 genes with additive effects (Table 5-7). In the case of leaf rust, however, the segregation ratios conformed to the segregation of two genes at Ciudad Obregon in 1998 and three genes at Edmonton, 2002 experiment (Table 5-7).

Repeated measure-analysis of variance of multiple leaf rust severities of the RILs at Glenlea, MB during the three years of field evaluations are presented in Table 5-8. The effects of RILs were highly significant ($P < 0.01$) during all three years of evaluations. The effects of the time of scoring, time × replication, and time × RILs were also highly significant ($P < 0.01$). There was a highly significant ($P < 0.01$) correlation between the last leaf rust severities in Glenlea, MB, and AUDPC ($r = 0.86$, $P < 0.01$) and rAUDPC ($r = 0.95$, $P < 0.01$). The leaf rust rAUDPC was, therefore, used in the genetic and QTL analyses because it provided a scale that is comparable across years. Analysis of variance of the leaf rust rAUDPC for the three years of field evaluations and the combined analysis of variance are presented in Table 5-9. In all three years, the effects of replication and RILs were highly significant ($P < 0.01$). In the combined analysis of variance the effects of year, replications in years, RILs, and RILs × year were also highly significant ($P < 0.01$).

Components of variance for the leaf rust rAUDPC, estimated for each year of the study and for the combined analysis are presented in Table 5-10. The broad-sense heritability estimates for the leaf rust rAUDPC were 0.96, 0.95, and 0.97 estimated for the experiments in Glenlea in 2000, 2001, and 2002, respectively, and 0.94 for the combined analysis. The estimates of the minimum numbers of effective factors controlling the leaf rust rAUDPC for the three years of study at Glenlea, MB were 3.04, 3.55, and 2.19 for 2000, 2001, and 2002, respectively, and 2.77 for the combined analysis.

5.3.2 Partial linkage map

Using Kosambi mapping function (Kosambi, 1944; Lander *et al.*, 1987) at a threshold value of P = 0.0001 in MapManager QTX, a partial linkage map was constructed based on the segregating AFLP and microsatellite data from 148 RILs. From 48 AFLP primer pairs, screened in bulked-segregant analysis, 22 (45%) identified at least one band that was polymorphic between parents and either one or both sets of bulk DNA samples. These AFLP markers and microsatellite markers of chromosome 7D were screened on the 148 RILs. From a total of 157 AFLP and microsatellite markers, scored on the population, 36 markers remained unlinked and 121 markers were mapped onto 17 linkage groups (LKG1-LKG17 in Figure 5-9), with a minimum of two markers per linkage group. This partial linkage map covered a map distance of 1084 cM, with an average of 63.7 cM per linkage group. One of the linkage groups (LKG1) carried two microsatellite markers, previously mapped on chromosome 7D, and was assumed to represent a portion of this chromosome. There were other microsatellites of chromosome 7D that either remained unlinked or mapped independently in a short linkage group (LKG10 in Figure 5-9). A hypothetical linkage map of chromosome 7D was constructed (Figure 5-10) based on the selected marker loci from LKG1 and LKG10 that was assumed to be parts of the chromosome 7D. In this hypothetical linkage map the microsatellite marker loci *Xgwm*635-7D and *Xgwm*295-7D were previously mapped on the short arm of 7D, while *Xgwm*37-7D was mapped on the long arm (Röder *et al.*, 1998). The loci representing *YrCK* and leaf tip necrosis did not group with any of the 157 molecular markers and remained unlinked.

5.3.3 Single marker QTL analysis

5.3.3.1.Stripe rust resistance

Four sets of data were used for QTL analysis of APR to stripe rust i.e., stripe rust rDS of RILs from Toluca experiments in 1998 and 2000, Creston experiment in 2002, and the average stripe rust rDS over three sets of data. Single marker QTL analysis, using 'marker regression' function in the MapManager QTX identified at least 4 marker loci in chromosome 7D and one marker locus in LKG13 with significant effects on stripe rust rDS (Table 5-11). The *YrCK* locus also had significant effects, contributing 13-19% in the phenotypic variance of the stripe rust rDS. Among the AFLP markers, locus M61/P33-235 of LKG13 had the greatest effect in the experiments in Toluca compared to other loci and appeared to be effective and highly significant ($P < 0.001$) in all three experiments and on the combined data. From the marker loci in 7D, the AFLP marker locus M48/P41-190 had highly significant effect in all three experiments and on average. The effects of the other three marker loci were not consistently significant in all experiments. AFLP marker loci M48/P41-190 and M49/P33-280 have a genetic distance of 15.4 cM on 7D and most probably are associated with another QTL for APR to stripe rust. Likewise, AFLP marker loci M59/P41-215 and M59/P36-165 are closely linked (genetic distance = 1.5 cM) and likely associated with one QTL. Therefore, the loci in Table 5-11 represent 4 QTLs with significant effects on stripe rust resistance. In multiple regression analyses, 33 to 39% of the phenotypic variance of the stripe rust rDS of the RILs was accounted for by the multiple QTLs identified in this research.

Table 5-12 represents the average stripe rust rDS of the RILs in resistant and susceptible allelic groups for each locus and the genotypic effects of the loci flanking the QTLs detected in regression analysis. Except for the AFLP marker locus M48/P41-190 all other markers are in repulsion phase with the APR genomic regions. All loci detected by regression analysis seemed to have significant to highly significant genotypic effects on stripe rust rDS.

5.3.3.2. Leaf rust resistance

For the single marker QTL analysis of the leaf rust resistance, five sets of data were used i.e., rAUDPC of RILs from Glenlea, MB, 2000, 2001, and 2002, the average

rAUDPC of the RILs over the three years, and the leaf rust rDS of the RILs from Cuidad Obregon in 1998. Single marker QTL analysis using 'marker regression' function in the MapManager QTX identified 3-4 marker loci with significant to highly significant effects for each set of data (Table 5-13). The *YrCK* locus appeared to be consistently effective on leaf rust rAUDPC or rDS in all experiments. AFLP marker locus M61/P33-235 located in LKG13 that was significant in all stripe rust experiments also had highly significant effects in all leaf rust experiments. Two marker loci from 7D, one from each QTL that was identified in the stripe rust analysis, had significant effects on leaf rust severity at Cuidad Obregon experiment in 1998, but the effect of these two QTLs were not significant on rAUDPC of the RILs in leaf rust experiments at Glenlea. Another QTL was identified on LKG10, flanking with AFLP marker M49/P41-240, with significant to highly significant effects on leaf rust rAUDPC in all experiments. In multiple regression analysis, significant QTLs accounted for 33 to 43% of the phenotypic variance of leaf rust rAUDPC or rDS of the RILs.

The Average rAUDPC of the RILs at Glenlea, MB and the leaf rust rDS in Ciudad Obregon in resistant and susceptible parent allelic groups and the genotypic effects of each QTL are presented in Table 5-14. The *YrCK* locus and the AFLP marker locus M61/P33-235 on LKG13 had highly significant effects, consistently effective in all experiments, with relative genotypic effects ranging between -0.20 and -0.34. These loci had highly significant effects in all stripe rust experiments as well. Another highly significant QTL effect was found, flanking with AFLP marker locus, M60/P41-250, on LKG4 in all experiments at Glenlea. The relative genotypic effects of this QTL ranged between -0.18 and -0.26. The effect of this QTL was not significant on leaf rust rDS at Ciudad Obregon-experiment. The genotypic effects of the two QTLs on 7D (-0.31 and -0.40) were highly significant in the Ciudad Obregon experiment. The effects of the QTL on LKG10 were somewhat smaller than those of QTLs (0.14, $P < 0.05$ to 0.19, $P < 0.05$).

5.3.4 Composite interval mapping

Contours of LRS values and the levels of confidence intervals for linkage groups carrying significant QTLs are presented in Figures 5-10, 5-11, and 5-12. Figure 5-10 represents the hypothetical linkage map of chromosome 7D. A QTL with significant

effects on the stripe rust rDS in all experiments and the leaf rust rDS at Ciudad Obregon and Edmonton experiments was identified in this linkage map. The AFLP marker loci M49/P33-280 was the closest to the peak LRS and confidence interval values in all experiments, except the stripe rust experiment in Creston, BC, where the two tightly linked AFLP marker loci M59/P41-215 and M59/P36-165 (genetic distance = 1.5 cM) were closest to the peak LRS value. Another QTL was identified in LKG13, flanking with AFLP marker locus M61/P33-235 (Figure 5-11), located at the peak of the LRS values and confidence intervals. This QTL was effective against both leaf and stripe rusts and across experiments, with LRS values ranging between significant and highly significant. This was in accordance with results obtained in the single marker QTL analysis. Another QTL was identified, flanking the AFLP marker locus M60/P41-250 on LKG4 (Figure 5-12). This QTL was specifically effective against leaf rust in Glenlea, MB, with no significant effect on stripe rust and leaf rust experiments in other locations.

5.4 Discussion

5.4.1 Inheritance of adult-plant resistance

Durable APR to leaf and stripe rusts in the Australian wheat cultivar, Cook, was quantitatively inherited. Leaf rust resistance gene *Lr34* that is tightly linked or pleiotropic with stripe rust resistance gene *Yr18* (McIntosh, 1992a; Singh, 1992b) is known to be present in the cv. Cook (McIntosh *et al.*, 1998). Based on the genetic analysis it was concluded that in addition to *Lr34/Yr18* at least 1-2 and 2 genes with minor additive effects contribute to APR to leaf rust and stripe rust in cv. Cook. Additive inheritance of APR with involvement of a few minor additive genes is well documented for leaf rust (Kuhn *et al.*, 1980; Lee and Shaner, 1985; Bjarko and Line, 1988b; Broers and Jacobs, 1989; Jacobs and Broers, 1989; Das *et al.*, 1993; Das *et al.*, 1992; Singh and Rajaram, 1992; Shaner *et al.*, 1997; Messmer *et al.*, 2000; Singh *et al.*, 2000) and stripe rust (Wallwork and Johnson, 1984; Bariana and McIntosh, 1995; Singh *et al.*, 2000; Singh and Rajaram, 1994; Bariana *et al.*, 2001). This type of *Lr34/Yr18* based oligo- or polygenic APR is expected to be durable (Johnson, 1980; McIntosh, 1992b).

APRs to leaf and stripe rusts were found to be highly heritable. In this research, relatively high heritability estimates were obtained for APR to leaf rust. Several authors

(Shaner *et al.*, 1997; Das *et al.*, 1992; Das *et al.*, 1993; and Kuhn *et al.*, 1980; and results presented in Chapter 3) have reported moderately high- to high-heritability estimates for APR to leaf rust based on either latent periods or areas under leaf rust progress curve. High heritability of APR to stripe rust has also been documented by Ghanadha *et al.* (1995), Krupinsky and Sharp (1978), and Wagoire *et al.* (1998) and was demonstrated through the results presented in Chapter 2.

5.4.2 QTL analysis of adult-plant resistance

In constructing the partial genetic linkage map, a bulked segregant analysis (Michelmore *et al.*, 1991) was used to target the genomic regions with significant effects on leaf and stripe rust resistance. However, from the polymorphic primer pairs that were identified through the bulked segregant analysis, not only the bands polymorphic between the bulks, but also any polymorphic band between parents were scored. The mapped region of the linkage map, covered 1084 cM, probably less than one-third of the wheat genome, was limiting. However, the application of bulked segregant analysis helped to target some of the QTLs affecting response to leaf and stripe rusts. Integrating phenotypic data for both leaf and stripe rusts from several locations, permitted a study of specificity of QTLs for pathogens, races and locations.

Seven marker loci and the *YrCK* locus were detected with significant effects on either or both stripe and leaf rusts. Five marker loci and *YrCK* showed significant association with stripe rust severities across the experiments. Based on the linkage map, however, it was concluded that these probably represent 4 QTLs (Table 5-11). These QTLs were effective across all stripe rust experiments as determined by highly significant genotypic effects of each QTL across the experiments. In the case of leaf rust, six QTLs were identified each with significant effects in at least in one location. At each location at least 3 QTLs contributed significantly to leaf rust resistance. Involvement of several QTLs in the inheritance of quantitative APR to leaf and stripe rusts were consistent with results published by Bariana *et al.* (2001), Boukhatem *et al.* (2002), Singh *et al.* (2000), Nelson *et al.* (1997), Messmer *et al.* (2000) and Suenaga *et al.* (unpublished; personal communication with Dr. R. P. Singh). The genotypic effects of the loci were different and varied across the experiments (Tables 5-12).

Involvement of multiple loci in APR to leaf and stripe rusts is also in accordance with the results obtained in classical genetic studies. However, based on the percent phenotypic variance explained in this study it was assumed that there must be more QTLs involved in the inheritance of APR to leaf and stripe rusts in the cv. Cook that could not be detected in QTL analysis. A broader coverage of the genome with more markers may have enabled detection of additional QTLs. Therefore, the number of QTLs involved must even more exceed the minimum number of genes estimated in the inheritance study. It should be noted that the estimates of the numbers of genes were made under the assumption that additive genes are not linked and have equal effects. The genotypic effects determined in QTL analysis varied considerably among the QTLs at different locations. Therefore, the assumption of equal gene effects was not met. It was concluded that the APR to leaf and stripe rusts in cv. Cook is a complex trait, conditioned by several QTLs with small but different genotypic effects.

Assuming that *Lr34/Yr18* is the major contributor to APR in the resistant parent (McIntosh *et al.*, 1998), in this study attempted to target this gene by selecting a set of previously mapped microsatellites of wheat (Röder *et al.*, 1998) as anchor markers for mapping chromosome 7D. The map of 7D (Figure 5-10) constructed on the basis of published positions of microsatellites on this chromosome and selected linked AFLPs carried a QTL with significant effects on leaf and stripe rust resistance in some locations. However, a significant association involving this chromosome region was not detected at Glenlea, MB (Tabla 5-13). Moreover, this QTL was less effective than expected from the previous studies of *Lr34/Yr18* (Bariana *et al.*, 2001; Suenaga *et al.*, unpublished). It was therefore, assumed that the present QTL identified in this linkage group does not represent the gene(s) *Lr34/Yr18*, but possibly represents a genomic region close to *Lr34/Yr18*. The reason for this limited resolution may be the scarcity of markers on the 7D due to lack of sufficient polymorphism obtained from this chromosome.

Mapping the same set of microsatellites, Suenaga *et al.* (unpublished) found the microsatellite marker *Xgwm*295-7D to be the closest 7DS marker to a QTL assumed to represent *Lr34/Yr18*. In the single marker QTL analysis in the present study, this locus did not show a significant association with leaf and stripe rust resistances. However, it was mapped close (about 20 cM) to the QTL flanking with the AFLP marker locus

M49/P33-280 in 7D, with significant effects on leaf and stripe rust resistance in some experiments. As mentioned above, this QTL may not be close enough to the genomic region of *Lr34/Yr18*. The population used in the study by Suenaga *et al.* was a doubled-haploid population. Lack of significant association of *Xgwm*295-7D with leaf and stripe rust resistance in the present study may have been influenced by the higher frequency of recombination in the RIL populations compared to doubled-haploid populations, caused by crossing over in the segregating generations. Additionally, it is possible that the inversion of the genomic region carrying this marker in Cook have caused the *Xgwm*295-7D to be mapped at a greater distance from *Lr34/Yr18* gene(s), resulting in no association with leaf and stripe rust resistances. Furthermore, the genomic region carrying *Lr34/Yr18* was reported to be translocated to another genomic region in some wheat germplasm (Dyck *et al.*, 1994). It is possible that this translocation does not include the *Xgwm*295-7D locus. While possible, there is no cytological evidence determining whether Cook carries such a translocation.

5.4.3 Race- and pathogen- non specificity of some QTLs

The highly significant association between leaf and stripe rust severities in this study indicated the involvement of same genomic regions in the inheritance of APR to leaf and stripe rusts. This was in accordance with the results presented in Chapter 4 and the previously published reports of the linkage or pleiotropic association of leaf and stripe rusts APR genes *Lr34* and *Yr18* (McIntosh 1992a; Singh, 1992b), and *Lr46* and *Yr29* (Singh *et al.*, 2001). The QTL associated with the AFLP marker locus M61/P33-235 was consistently effective in all stripe and leaf rust experiments. Likewise, the *YrCK* locus was significantly effective in imparting resistance in all leaf and stripe rust experiments.

Race- and pathogen- nonspecificity was not the case for every locus. The QTL associated with the AFLP marker M49/P33-280 was effective in all stripe rust experiments and in leaf rust experiment at Ciudad Obregon, but not effective in leaf rust experiments at Glenlea, MB. The QTL flanking with the AFLP marker locus M60/P41-250 appeared as a race-specific locus, significantly effective in all leaf rust experiments in Glenlea, MB against race MBDS across three years of evaluation, but not effective in other leaf and stripe rust experiments. This specificity may have been caused by

environmental factors. Comparing the leaf rust experimental sites, Glenlea, MB is a summer planted location about latitude 50°N with a longer photoperiod during the growing season, as compared to Ciudad Obregon, which is a winter sown location in the Northern Mexico. Singh *et al.* (2000) also reported the APR conditioned by the gene *Yr28* to be sensitive to temperature and other environmental factors.

Results support findings of pleiotropic effects of some QTLs for leaf and stripe rust resistance, but also indicated that the pleiotropism is not a rule for every leaf or stripe rust resistance QTL. Pleiotropism or linkage between leaf and stripe rust APR genes has been reported for *Lr34* and *Yr18* (McIntosh, 1992a; Singh, 1992b) and more recently for *Lr46* and *Yr29* (Singh *et al.*, 2001). The genomic region of *Lr34/Yr18* is also known to be associated with a non-suppressor gene for stem-rust resistance (Dyck, 1987) and slow yellowing response to barley yellow dwarf virus (Singh, 1993). Singh *et al.* (2000) pointed to the coincidence of genes for different foliar diseases and predicted that the APR does not involve specific recognition of the pathogen, but possibly involves some more general cellular mechanisms of inhibiting invasion by the pathogen.

5.4.4 Leaf tip necrosis and adult-plant resistance

Leaf tip necrosis is known to be associated with *Lr34/Yr18* (Dyck, 1991; Singh, 1992a) and is reported to be inherited as a single Mendelian locus (Singh, 1992a; William *et al.*, 1997). Frequency distribution of leaf tip necrosis phenotype followed the segregation of one gene (tested for the hypothesis of 0.562 present : 0.4375 absent; χ^2 = 0.91, P > 0.25). However, as presented in Chapter 4 there is evidence that in some genetic backgrounds leaf tip necrosis is under an oligogenic control and that the extent of this phenotype may be controlled by modifier genes. The presence and absence of the leaf tip necrosis were first integrated in the mapping data as alternative genotypes of one locus. There was no association with any marker. However, it accounted for 51 to 80% of the phenotypic variance in the relative leaf and stripe rust severities, with genotypic effects ranging from -0.27 to -0.69 of the rDS across the experiments. William *et al.* (1997) and Messmer *et al.* (2000) were also not able to find linkage between leaf tip necrosis, as a single Mendelian locus with markers. Messmer *et al.*, (2000) reported that multiple QTLs were involved in the leaf tip necrosis phenotype. It was also predicted in Chapter 4 that

other minor additive genes, involved in APR, could also be associated with leaf tip necrosis phenotype. By integrating the leaf tip necrosis as a phenotypic rather than a genotypic data, at least 4 independent QTLs were identified to be associated with leaf tip necrosis. Three of these QTLs coincided with QTLs identified for APR to leaf and stripe rusts. This was in accordance with the results of Messmer *et al.* (2000) who reported association of multiple QTLs with leaf tip necrosis. Multiple QTL inheritance of leaf tip necrosis can govern the hypothesis made in Chapter 4 that leaf tip necrosis is pleiotropic with not only *Lr34/Yr18* but also with additional additive genes conditioning the mechanisms involved in APR to leaf and stripe rusts.

5.4.5 Stripe rust temperature-sensitive gene *YrCK*

Park *et al.* (1992) first reported the presence of a temperature-sensitive stripe rust resistance gene in cv. Cook. This temperature sensitive response was characterized by a low infection type obtained in post-inoculation temperature conditions of 24°C instead of the usual temperature conditions of 17°C. Bariana *et al.* (2001) placed the cv. Cook-derived temperature-sensitive resistance gene in chromosome arm 2DS of the cv. Sunco. It was temporarily designated *YrCK*. The present RIL population was screened for segregation of this gene in the seedling stage. Frequencies followed the segregation of a single Mendelian locus ($\chi^2 = 1.2$, $P > 0.25$). This locus was integrated into the mapping data, but did not show a significant linkage with any of the 157 molecular markers or leaf tip necrosis. However, it accounted for 13-19% of the phenotypic variation of the stripe rust rDS (Table 5-11) and 9-13% of the phenotypic variation of the leaf rust rAUDPC (Table 5-13) in different experiments. Infection types of the RILs, scored at the high temperature were also integrated into the mapping data as a quantitative trait. None of the QTLs found in this study contributed significantly to low infection type at the higher temperature, except for a locus on 7DS associated with *Xgwm*295-7D and M48P41-190 that had a small effect ($R^2 = 0.06$; LRS = 6.5).

Temperature-sensitivity of APR genes has been reported for stripe rust (Qayoum and Line, 1985) and leaf rust (Drijepondt and Pretorius, 1989) responses. In the case of stripe rust, sources of high-temperature adult plant resistant genotypes are known as being susceptible at lower temperatures and resistant at higher temperatures in the field

condition (Line, 2002). Gene *Lr34* is known to be more effective at lower temperatures (McIntosh *et al.*, 2001). In addition to *YrCK*, Bariana *et al.* (2001) identified a QTL (temporarily designated *YrKat*), in a different population. The QTL was also located in 2DS. Whether *YrKat* and *YrCK* are the same gene is to be determined. Results of the present study indicated that the temperature-sensitive gene, *YrCK*, as characterized at the seedling stage, does contribute to overall leaf and stripe rust resistance in adult stage.

5.5 Conclusions

APR to leaf and stripe rusts in cv. Cook appeared to be a complex trait, being controlled by several QTLs with small effects. At least 3 and 4 QTLs, accounting for up to 43% of the phenotypic variation, were identified with significant effects on leaf and stripe rusts, respectively. In accordance to high phenotypic associations between APR to leaf and stripe rusts, some of the identified QTLs appeared to be pleiotropic to both diseases across tests. Although a QTL was identified on chromosome 7D with significant effects on leaf and stripe rusts in some locations, it was not possible to refine the location of *Lr34/Yr18* because of the scarcity of markers in this region. In addition to *Lr34/Yr18*, temperature-sensitive resistance gene *YrCK* significantly contributed to resistance to both diseases indicating that this locus is also pleiotropic for APR to both diseases. The genomic region of some of the identified QTLs remained to be determined. The number of QTLs contributing to APR is likely to be more than identified. Constructing a more detailed linkage map through screening more markers would result in a more accurate characterization of the QTLs involved in leaf and stripe rust resistance. Some QTLs appeared to function as race- and pathogen- nonspecific loci, but nonspecificity does not seem to be the rule for every APR QTL.

5.6 Tables and figures

Table 5-1. Locations and races of leaf and stripe rusts used for the field evaluations.

Location	Disease	Year(s) tested	Race/Isolate	Virulent for
Toluca, MX, Mexico	stripe rust	1998, 2000	MEX96-11	*Yr2, (3), 6, 7, 9, 27,* and *A*
Creston, BC, Canada	stripe rust	2002	Natural infection of SRC02UA	*Yr6, 7, 8, 9, 11, 12, 27, and A*
Cd. Obregon, Sonora, Mexico	leaf rust	1998	MCJ/SP	*Lr1, (3), (3bg), 10, 11, 12, 13, 14a, 14b, 15, 17, 18, 20, 22b, 23, 26, 27+31, 37*
Glenlea, MB, Canada	leaf rust	2000, 2001, 2002	MBDS	*LR 1, 3, 10, 13, 14a, 17, and B*
Edmonton, AB, Canada	leaf rust	2002	MBDS	*LR 1, 3, 10, 13, 14a, 17, and B*

Table 5-2. Seedling and adult plant responses of a set of stripe rust resistance-gene isogenic lines when inoculated with the stripe rust isolate SRC02UA.

Avocet isogenic lines	*Yr*-gene	Stripe rust reaction in	
		Seedling [a]	Adult plant [b]
YR1/6*AOC	*Yr1*	1	5R
YR5/6*AOC	*Yr5*	2	5R
YR6/6*AOC	*Yr6*	8	100S
YR7/6*AOC	*Yr7*	8	100S
YR8/6*AOC	*Yr8*	9	-
YR9/6*AOC	*Yr9*	8	100S
YR10/6*AOC	*Yr10*	2	5R
YR11/3*AOC	*Yr11*	9	100S
YR15/6*AOC	*Yr15*	2	5R
YR18/3*AOC	*Yr18*	8	50S
YR24/3*AOC	*Yr24*	3	20MR
YR26/3*AOC	*Yr26*	3	5R
YRSP/3*AOC	*YrSP*	2	5R
YR27/3*AOC	*Yr27*	7	90S

[a] Seedling infection types are based on 0-9 scale (McNeal *et al.*, 1971) in which infection types 7, 8, and 9 are considered susceptible.
[b] Adult plant responses recorded on the modified Cobb's Scale (Peterson *et al.*, 1948).
- Not tested.

Table 5-3. Primers and adaptors used in the AFLP analysis and their sequences.

Primer / adaptor	Sequence [a]
MseI adaptor	5′GACGATGAGTCCTGAG 3′ TACTCAGGACTCAT
PstI adaptor	CCTACGCAGTCTACGAG 3′ ACGTGGATGCGTCAG 5′
Pre-amplification primers	
M-C	5′ GATGAGTCCTGAGTAAC
P-A	5′ GACTGCGTAGGTGCAGA
Selective amplification primers	
MseI primers	
M47	5′ GATGAGTCCTGAGTAACAA
M48	5′ GATGAGTCCTGAGTAACAC
M49	5′ GATGAGTCCTGAGTAACAG
M50	5′ GATGAGTCCTGAGTAACAT
M55	5′ GATGAGTCCTGAGTAACGA
M56	5′ GATGAGTCCTGAGTAACGC
M57	5′ GATGAGTCCTGAGTAACGG
M58	5′ GATGAGTCCTGAGTAACGT
M59	5′ GATGAGTCCTGAGTAACTA
M60	5′ GATGAGTCCTGAGTAACTC
M61	5′ GATGAGTCCTGAGTAACTG
M62	5′ GATGAGTCCTGAGTAACTT
PstI primers	
P33	5′ GACTGCGTAGGTGCAGAAG
P36	5′ GACTGCGTAGGTGCAGACC
P37	5′ GACTGCGTAGGTGCAGACG
P41	5′ GACTGCGTAGGTGCAGAGG

[a] The underlined nucleotides in each primer represents the selective site of the primer.

Table 5-4. AFLP primers used for genotyping the RIL population of cross Avocet-*YrA* × Cook.

Primer combination		No. of bands polymorphic between	
		parents	parents and bulks
M47	P33	9	0
M48	P33	5	1
M49	P33	7	1
M50	P33	14	1
M55	P33	8	2
M56	P33	9	1
M57	P33	7	0
M58	P33	3	1
M59	P33	4	0
M60	P33	15	1
M61	P33	18	1
M62	P33	11	1
M47	P36	10	2
M48	P36	12	0
M49	P36	11	0
M50	P36	4	0
M55	P36	5	0
M56	P36	7	2
M57	P36	11	0
M58	P36	6	0
M59	P36	9	1
M60	P36	8	0
M61	P36	-	0
M62	P36	10	0
M47	P37	9	0
M48	P37	9	2
M49	P37	6	1
M50	P37	10	0
M55	P37	14	0
M56	P37	5	0
M57	P37	5	0
M58	P37	2	0
M59	P37	10	3
M60	P37	7	0
M61	P37	13	0
M62	P37	8	0
M47	P41	7	0
M48	P41	20	4
M49	P41	18	1
M50	P41	5	1
M55	P41	12	1
M56	P41	10	0
M57	P41	10	1
M58	P41	11	0
M59	P41	8	3
M60	P41	7	1
M61	P41	9	1
M62	P41	11	0

Table 5-5. Primer combinations used for the mapping microsatellite loci of chromosome 7D of wheat (adopted from Röder *et al.*, 1998).

Locus	Left primer	Right primer	Aneal. temp
*Xgwm*37-7D	ACT TCA TTG TTG ATC TTG CAT G	CGA CGA ATT CCC AGC TAA AC	60
*Xgwm*44-7D	GTT GAG CTT TTC AGT TCG GC	ACT GGC ATC CAC TGA GCT G	60
*Xgwm*111-7D	TCT GTA GGC TCT CTC CGA CTG	ACC TGA TCA GAT CCC ACT CG	55
*Xgwm*121-7D	TCC TCT ACA AAC AAA CAC AC	CTC GCA ACT AGA GGT GTA TG	50
*Xgwm*295-7D	GTG AAG CAG ACC CAC AAC AC	GAC GGC TGC GAC GTA GAG	60
*Xgwm*350-7D	ACC TCA TCC ACA TGT TCT ACG	GCA TGG ATA GGA CGC CC	55
*Xgwm*428-7D	CGA GGC AGC GAG GAT TT	TTC TCC ACT AGC CCC GC	60
*Xgwm*437-7D	GAT CAA GAC TTT TGT ATC TCT C	GAT GTC CAA CAG TTA GCT TA	50
*Xgwm*635-7D	TTC CTC ACT GTA AGG GCG TT	CAG CCT TAG CCT TGG CG	60

Table 5-6. Leaf and stripe rust severities of parental genotypes and some statistics for the Avocet × Cook RIL population at different locations.

Parent or parameter	Stripe rust severity [a]			Leaf rust severity		Leaf rust AUDPC		
	Toluca 1998	Toluca 2000	Creston 2002	Obregon 1998	Edmonton 2002	Glenlea 2000	Glenlea 2001	Glenlea 2002
Avocet-*YrA*	90	90	80	100	80	610.0	658.7	875.0
Cook	5	1	10	5	10	124.7	69.2	114.0
Mid-parent	42.5	45.5	45.0	52.2	45.0	367.3	363.9	494.6
RILs mean	41.2	35.1	31.9	39.5	45.5	429.5	356.1	597.3
Std. deviation	24.9	24.7	22.6	37.58	22.6	193.5	231.5	305.8

[a] Rust severities are based on modified Cobb's Scale (Peterson *et al.*, 1948) recorded when the severities on Avocet-*YrA* reached 80-100%.

Table 5-7. Distribution of 148 RILs derived from Avocet-*YrA* × Cook inoculated with stripe rust and leaf rust at different locations and seasons.

Testing site / year tested	No. of lines with reaction			Tested [c] for segregation of	χ^2	P-value
	PTR[a]	PTS[b]	Others			
Stripe rust						
Toluca / 1998	12	14	122	3 genes	0.23	> 0.95
Toluca / 2000	12	10	126	3 genes	0.54	> 0.90
Leaf rust						
Cd. Obregon / 1998	20	27	101	2 genes	3.52	> 0.25
Edmonton / 2002	12	21	115	3 genes	6.5	> 0.05

[a] Parental type resistant.
[b] Parental type susceptible.
[c] The ratios used were 0.1914 PTR : 0.1914 PTS : 0.6173 others for the segregation of two genes and 0.0837 PTR : 0.0837 PTS : 0.8327 others for the segregation of three genes.

Table 5-8. Repeated measure-analysis of variance for leaf rust severities of the RIL population, rated four times during the leaf rust epidemics for 3 years.

SV	df	Mean square[a]		
		2000	2001	2002
Replication (R)	1	2781.6**	2456.7**	3846.3**
RIL (L)	149	2792.1**	3620.9**	5033.6**
Error	149	124.29	189.4	143.7
Time (T)	3	177848.6**	79376.3**	19695.7**
T × R	3	1495.3**	966.7**	281.8**
T × L	447	193.2**	215.24**	43.6**
Error(T)	447	48.8	36.75	24.4

** Significant at $P < 0.01$.
[a] Within subject effects in repeated measure analysis were tested according to Greenhouse-Geisser tests (SAS Institute, Cary, NC).

Table 5-9. Analysis of variance of leaf rust rAUDPC of the RILs population, tested under leaf rust epidemics during 3 years trials at Glenlea, MB.

SV	df	Mean square			
		2000	2001	2002	Combined
Year (Y)	2	-	-	-	2.25**
Replication (R)	1	0.22**	0.15**	0.21**	-
R(Y)	3	-	-	-	0.19**
RIL (L)	149	0.20**	0.24**	0.24**	0.61**
L × Y	295	-	-		0.03**
L × R	149	< 0.01	0.01	< 0.01	-
Error	442	-	-	-	0.01

** Significant at $P < 0.01$.

Table 5-10. Components of variance, broad-sense heritability, and minimum number of segregating additive genes, estimated for the inheritance of rAUDPC of RILs tested under leaf rust epidemics during 3 years at Glenlea, MB.

Statistic	Estimates in			
	2000	2001	2002	Combined
Error variance (σ^2_e)	< 0.01	0.01	0.01	0.01
Genotype × year variance (σ^2_{ge})	-	-	-	0.01
Genotypic variance (σ^2_g)	0.09	0.11	0.11	0.09
Phenotypic variance (σ^2_{ph})	0.10	0.12	0.12	0.10
Heritability (h^2_{bs})	0.96	0.95	0.97	0.94
Number of genes (n)	3.04	3.55	2.19	2.77

Table 5-11. Likelihood ratio statistic and estimated variance explained by the loci as determined by additive single marker regression model for the terminal stripe rust severities at Toluca, Mexico and Creston, BC.

QTL	Locus	Toluca / 1998			Toluca / 2000			Creston / 2002			Average		
		LRS	%	P	LRS	%	P	LRS	%	P	LRS	%	P
1	M59/P41-215	6.8	5	0.008	6.5	5	0.01	16.1	11	<0.001	11	8	<0.001
	M59/P36-165	6.8	5	0.009	6.0	4	0.01	16.5	11	<0.001	10.5	7	0.001
2	M49/P33-280	8.2	6	0.004	5.5	4	0.01	6.0	4	0.01	8.3	6	0.003
	M48/P41-190	7.3	5	0.006	7.9	6	0.004	10	7	0.001	11	8	<0.001
3	M61/P33-235	15.4	13	<0.001	10.9	9	<0.001	15.2	13	<0.001	16.1	14	<0.001
4	*YrCK*	18.3	13	<0.001	27.3	18	<0.001	24.3	16	<0.001	29.3	19	<0.001
Phenotypic variance explained simultaneously (%)			33			34			36			39	

Table 5-12. Genotypic effects of the marker loci with significant reducing effects on average stripe rust severities as percentages of the susceptible parent, Avocet-*YrA*.

Locus	Allelic group[a]	Toluca 1998	Toluca 2000	Creston 2002	Average
M59/P41-215	C0	0.36	0.30	0.28	0.30
	A1	0.53	0.52	0.57	0.55
Relative genotypic effect[b]		-0.17**	-0.22**	-0.29***	-0.25***
M59/P36-165	C0	0.36	0.31	0.29	0.32
	A1	0.57	0.49	0.58	0.55
Relative genotypic effect		-0.21***	-0.18**	-0.29***	-0.23***
M49/P33-280	C0	0.33	0.29	0.29	0.30
	A1	0.61	0.53	0.59	0.59
Relative genotypic effect		-0.28***	-0.24***	-0.30***	-0.29***
M48/P41-190	A0	0.54	0.47	0.58	0.53
	C1	0.37	0.32	0.29	0.32
Relative genotypic effect		0.17**	0.15*	0.29***	0.21***
M61/P33-235	C0	0.30	0.31	0.29	0.30
	A1	0.60	0.51	0.55	0.55
Relative genotypic effect		-0.30***	-0.20**	-0.26***	-0.25***
YrCK	A0	0.50	0.48	0.50	0.50
	C1	0.29	0.25	0.22	0.25
Relative genotypic effect		0.21***	0.23**	0.28***	0.25***

[a] 0 and 1 are absence and presence of the marker loci, respectively. A and C represent the allelic groups similar with the parental genotypes, Avocet-*YrA* and Cook.

[b] *, **, and *** are significant genotypic effects at 0.05, 0.01 and 0.001 probability, respectively, as determined by ANOVA.

Table 5-13. Likelihood ratio statistic and estimated variance explained by the marker loci as determined by additive single marker regression model for the rAUDPC in three years at the Glenlea Research Station and one year at Ciudad Obregon.

QTL	Locus	rAUDPC in												rDS in		
		Glenlea / 2000			Glenlea / 2001			Glenlea / 2002			Glenlea / Average			Cd. Obregon / 1998		
		LRS	%	P	LRS	%	P	LRS	%	P	LRS	%	P	LRS	%	P
1	M59/P41-215	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	7.6	5	0.005
2	M49/P33-280	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	9.5	7	0.002
3	M60/P41-250	6.8	5	0.009	*ns*	*ns*	*ns*	11.4	8	< 0.001	7.7	6	0.050	*ns*	*ns*	*ns*
4	M49/P41-240	3.9	3	0.04	7.1	6	0.007	10.1	7	0.001	7.4	6	0.006	8.7	7	0.003
5	M61/P33-235	8.6	8	0.003	7.8	7	0.005	9.5	8	0.002	10.1	9	0.001	11	9	< 0.001
6	*YrCK*	16.2	11	<0.001	18.4	13	<0.001	14.0	10	<0.001	18.2	12	<0.001	13.5	9	<0.001
Phenotypic variance explained simultaneously (%)			33			34			34			35			43	

Table 5-14. Genotypic effects of the marker loci with significant reducing effects on average leaf rust rAUDPC at Glenlea during 2000-2002 and on relative leaf rust severities in Cuidad Obregon in 1998.

		rAUDPC in				rDS in
Locus	Allelic group[a]	Glenlea 2000	Glenlea 2001	Glenlea 2002	Glenlea Average	Cd. Obregon 1998
M59/P41-215	A0	-[b]	-	-	-	0.26
	C1	-	-	-	-	0.58
Relative genotypic effect [b]		*ns*	*ns*	*ns*	*ns*	-0.32***
M49/P33-280	C0	-	-	-	-	0.25
	A1	-	-	-	-	0.65
Relative genotypic effect		*ns*	*ns*	*ns*	*ns*	-0.40***
M60/P41-250	C0	0.66	0.49	0.62	0.58	-
	A1	0.76	0.67	0.88	0.77	-
Relative genotypic effect		-0.10*	-0.18*	-0.26**	-0.19*	*ns*
M49/P41-240	A0	0.80	0.63	0.79	0.75	0.51
	C1	0.61	0.48	0.65	0.57	0.29
Relative genotypic effect		0.19*	0.15*	0.14*	0.18*	0.22*
M61/P33-235	C0	0.60	0.45	0.61	0.54	0.26
	A1	0.80	0.67	0.82	0.76	0.60
Relative genotypic effect		-0.20*	-0.22**	-0.21**	-0.22***	-0.34***
YrCK	A0	0.80	0.68	0.83	0.77	0.48
	C1	0.52	0.37	0.51	0.46	0.24
Relative genotypic effect		0.28**	0.31**	0.32**	0.31**	0.24**

[a] 0 and 1 are absence and presence of the marker loci, respectively. A and C represent the allelic groups similar with the parental genotypes, Avocet-*YrA* and Cook.

[b] *, **, and *** are significant genotypic effects at 0.05, 0.01 and 0.001 probability, respectively, as determined by ANOVA.

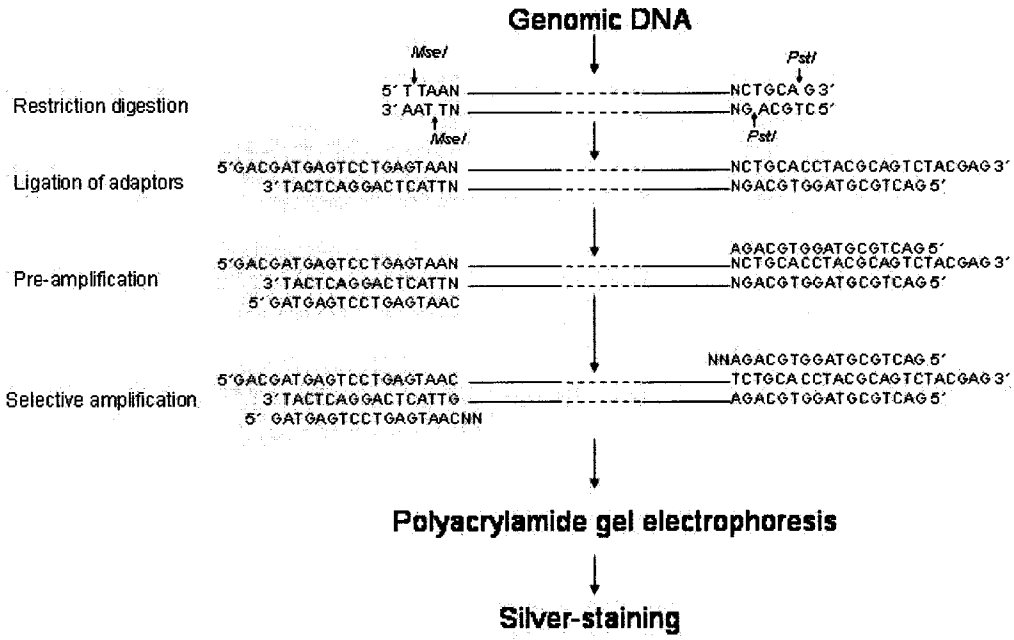


Figure 5-1. Schematic diagram of the steps involved in amplified fragment length polymorphism (AFLP), with nucleotide sequences of adaptors and primers used in this research.

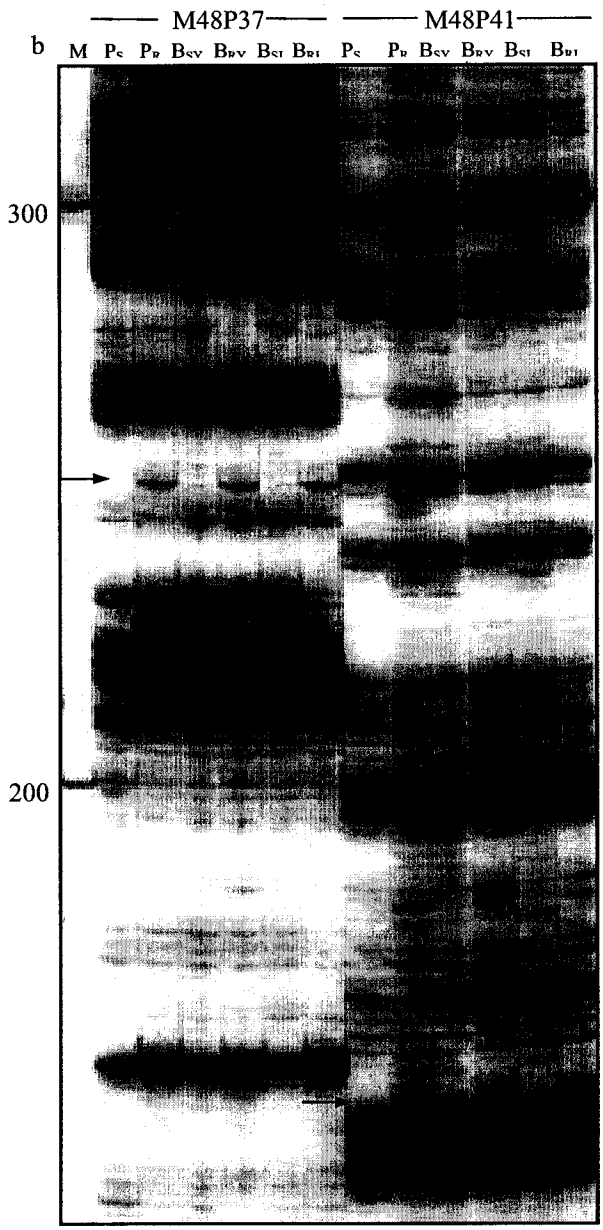


Figure 5-2. Bulked segregant analysis using AFLP. M is 100 bp ladder, P_S is susceptible parent, P_R is resistant parent (Cook), B_{SY} is bulk DNA of 7 RILs susceptible to stripe rust, B_{RY} is bulk DNA of 7 RILs resistant to stripe rust, B_{SL} is bulk DNA of 7 RILs susceptible to leaf rust, and B_{RL} is bulk DNA of 7 RILs resistant to leaf rust. Arrows show 2 polymorphic bands.

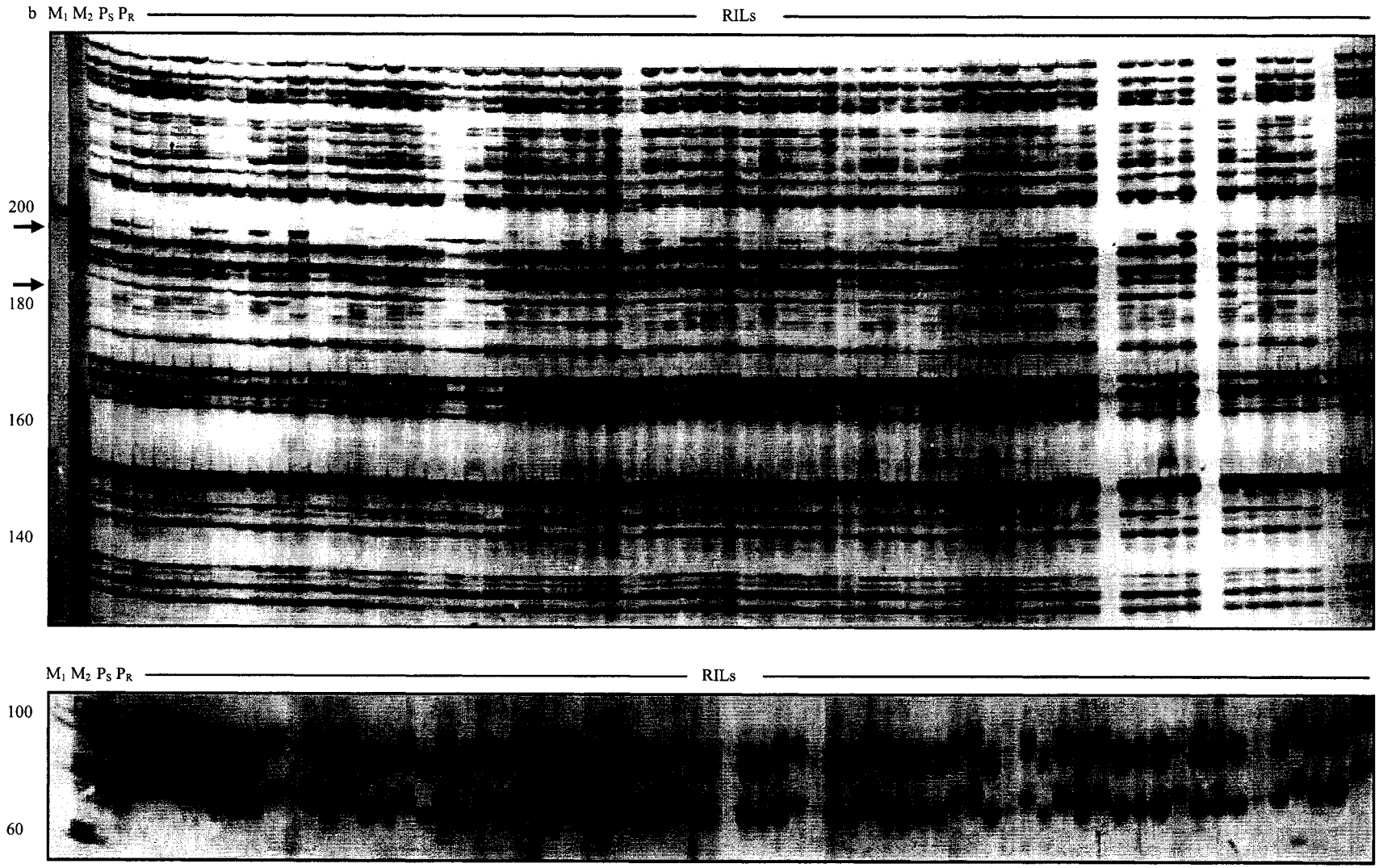


Figure 5-3. Genotyping of parents and RIL population using AFLP (top) and microsatellite (bottom) markers. Denatured polyacrylamide gel electrophoresis using sequencing gel units was used for size-fractionation of fragments and silver-staining was used to visualize the fingerprints. M_1 and M_2 are 100 bp and 20 bp DNA ladders, respectively, P_S is susceptible parent (Avocet-*YrA*), and P_R is resistant parent (Cook). Segregating polymorphic bands were used in constructing a partial linkage map. Arrows indicationg two of the polymorphic AFLPs.

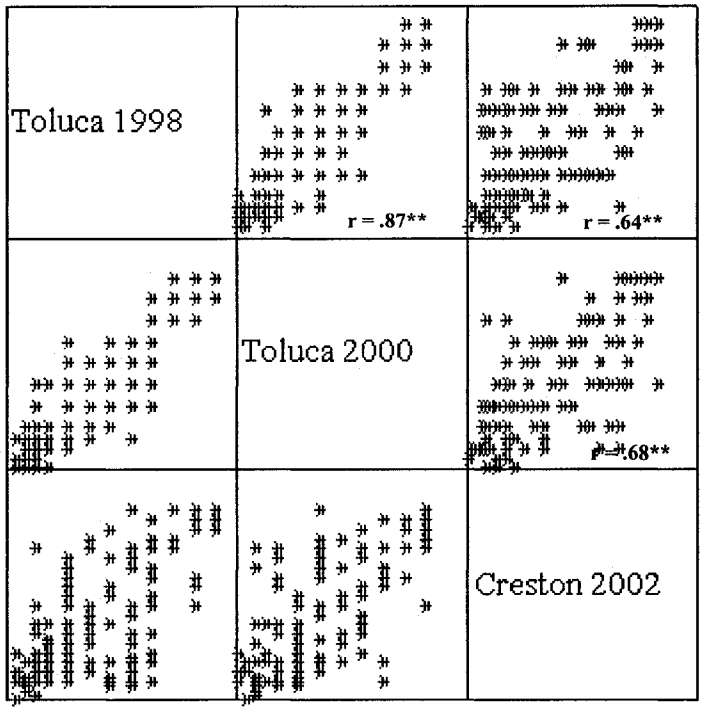


Figure 5-4. Multiple scatter-plot representing the association among stripe rust severities of RILs in Toluca 1998 and 2000 and Creston 2002 experiments.

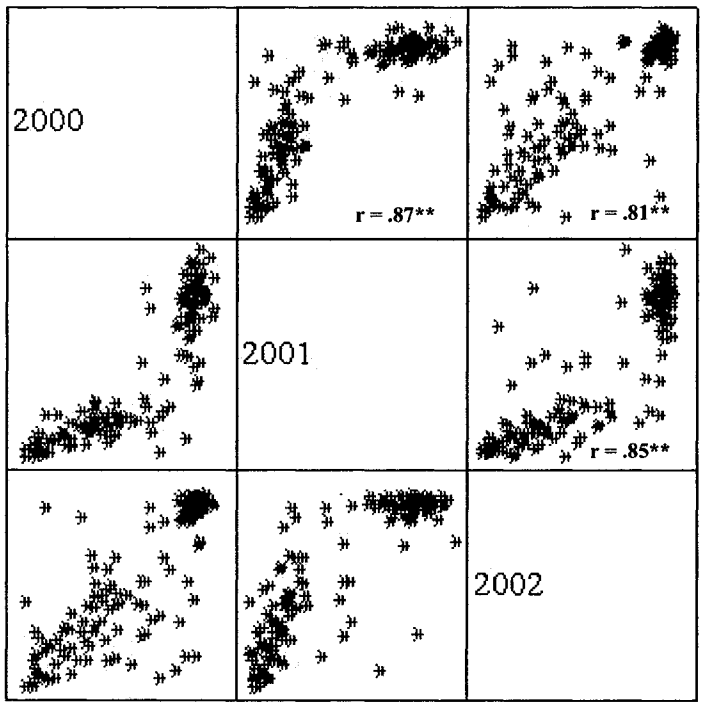


Figure 5-5. Multiple scatter-plot representing the association among leaf rust AUDPC of RILs in three years of field evaluation in Glenlea, MB.

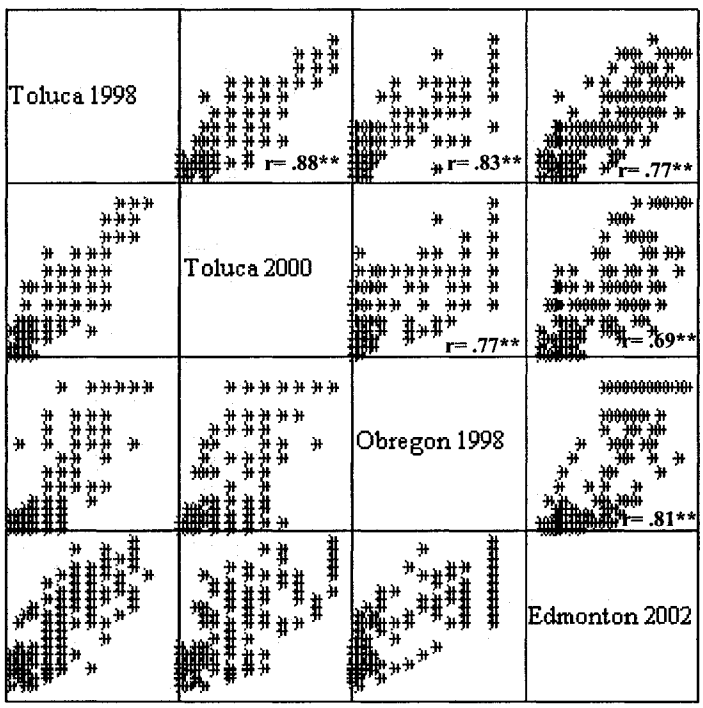


Figure 5-6. Multiple scatter-plot representing the association between stripe and leaf rust severities of RILs in different locations and years. Toluca 1998 and 2000 experiments were infected with stripe rust, while Obregon 1998 and Edmonton 2002 experiments were infected with leaf rust.

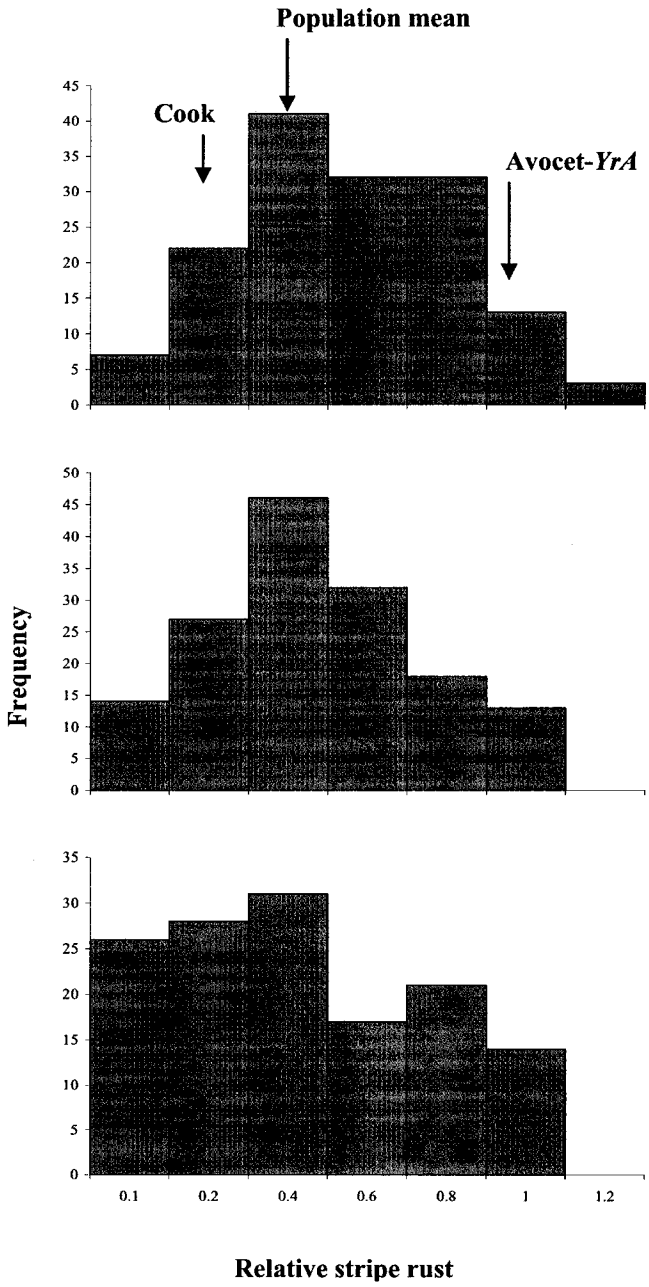


Figure 5-7. Frequency distribution of relative stripe rust severities of RILs at Toluca, 2000 (top), Toluca, 2002 (middle), and Creston, 2002 (bottom).

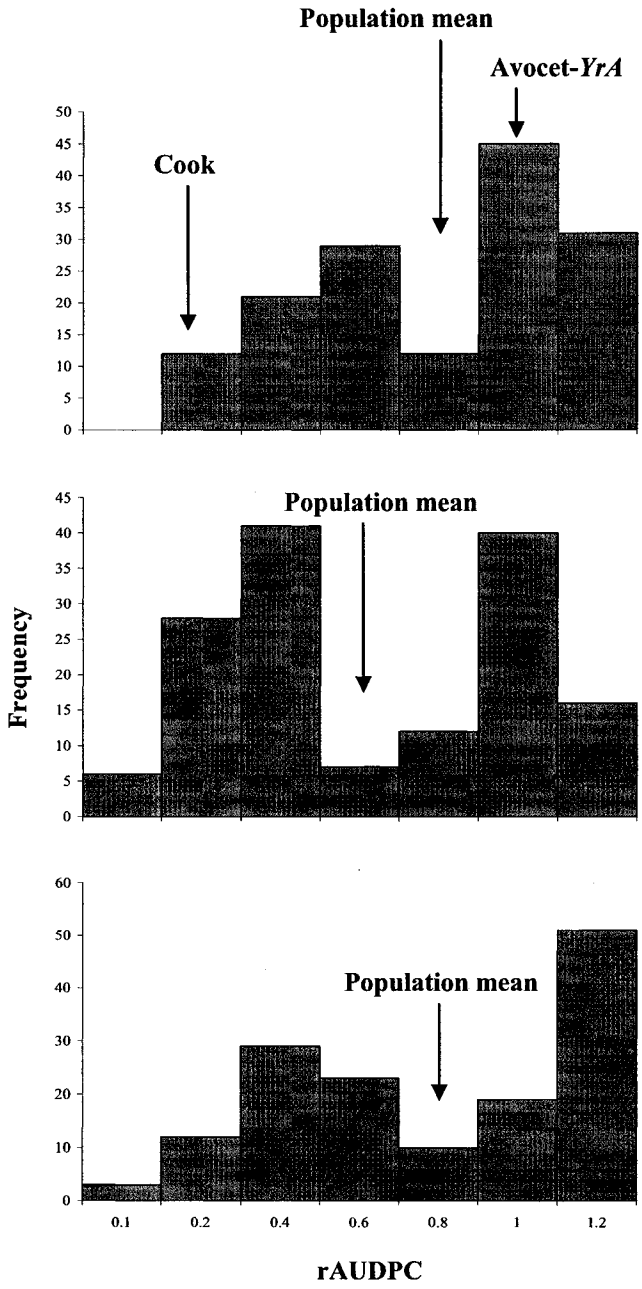
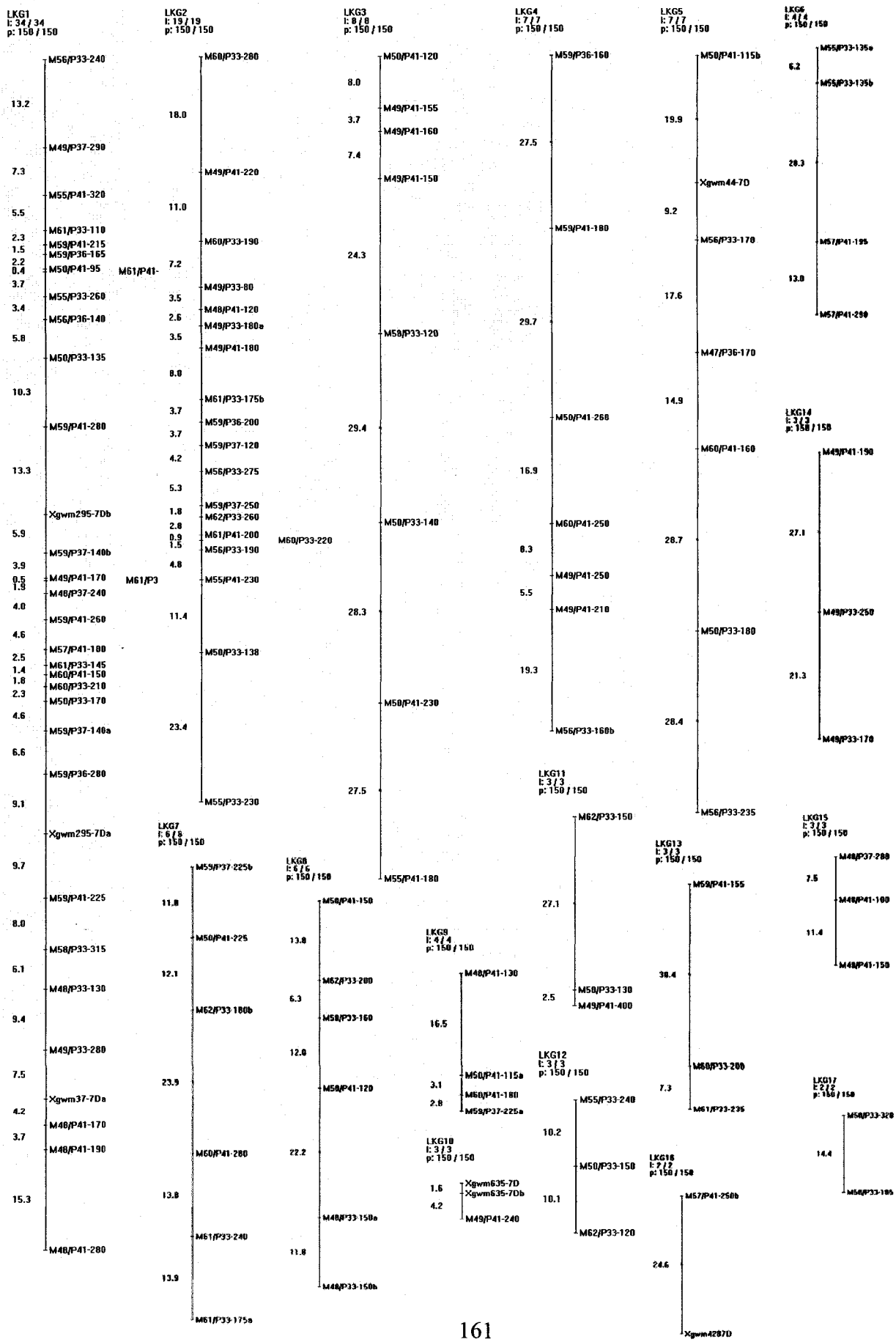


Figure 5-8. Frequency distribution of rAUDPC of leaf rust at Glenlea during 2000 (top), 2001 (middle), and 2002 (bottom).

Figure 5-9. Partial linkage map constructed using segregating AFLP and microsatellite data from 148 RILs. Kosambi mapping function was used with threshold value set at P = 0.0001.



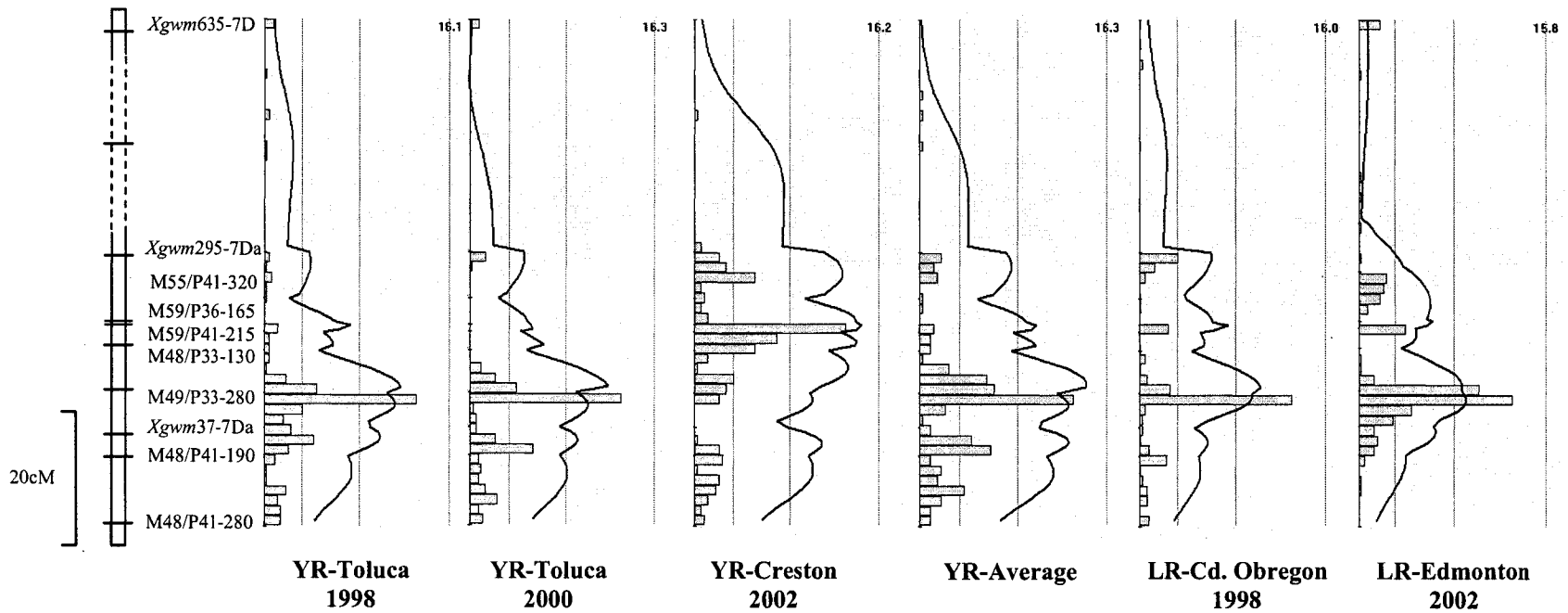


Figure 5-10. Hypothetical linkage map of chromosome 7D and contours of LRS (black line) obtained by composite interval mapping of relative stripe (YR) and leaf rust (LR) severities at different locations. Yellow bars represent the estimated confidence interval by bootstrap resampling in Mapmanager QTX. Green lines in each graph, from left to right, represent the suggestive, significant ($P < 0.05$), and highly significant ($P < 0.01$) threshold LRS values, respectively.

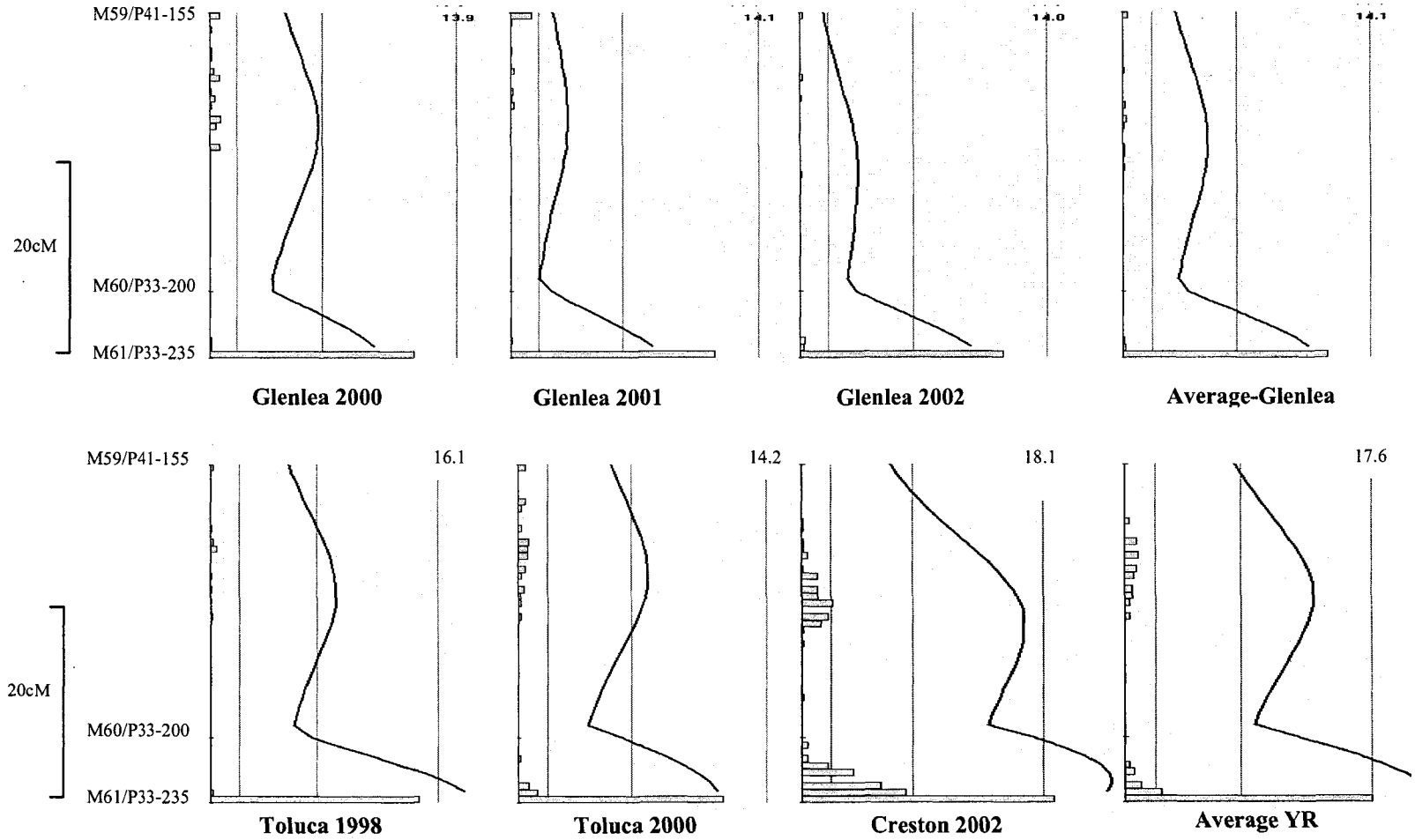


Figure 5-11. Contours of LRS (black line) obtained by composite interval mapping for LKG13 that carries a QTL with significant effect on the leaf rust rAUDPC Glenlea, MB during three years of field evaluation, and significant to highly significant effects on stripe rust rDS at different locations. Yellow bars represent the estimated confidence interval by bootstrap resampling in Mapmanager QTX. Green lines in each graph, from left to right, represent suggestive, significant ($P < 0.05$), and highly significant ($P < 0.01$) threshold LRS values, respectively.

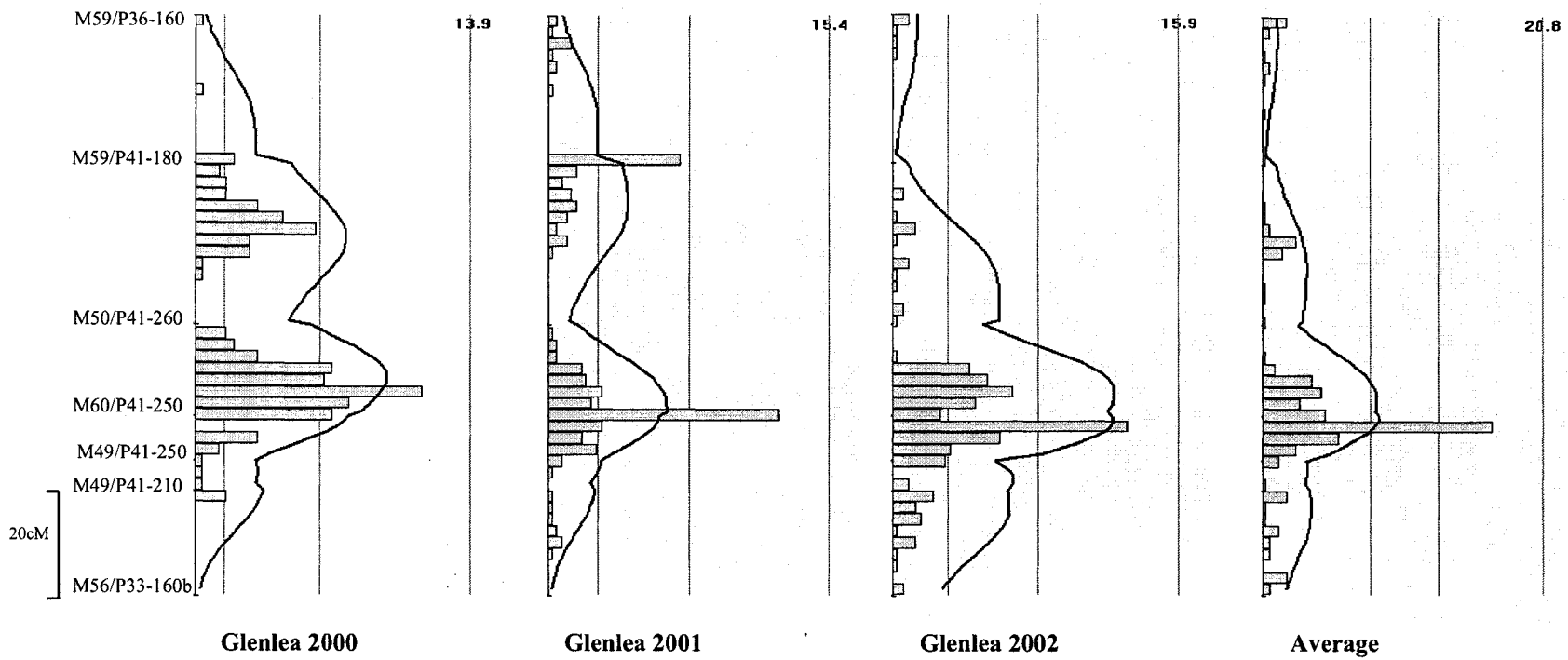


Figure 5-12. Contours of LRS (black line) obtained by composite interval mapping for LKG4 that carries a QTL with significant effect on the leaf rust rAUDPC at Glenlea during three years of field evaluation. Yellow bars represent the estimated confidence interval by bootstrap resampling in Mapmanager QTX. Green lines in each graph, from left to right, represent suggestive, significant ($P < 0.05$), and highly significant ($P < 0.01$) threshold LRS values, respectively.

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Chapter 6

General discussion, summary of results, and implications in wheat breeding

6.1 Introduction

The development of wheat cultivars that have durable resistance to rust diseases has been a long-standing objective of wheat breeders. Durable disease resistance is defined as "a type of resistance that remains effective during its prolonged and widespread use in an environment favorable to disease" (Johnson, 1984). Stuthman (2002) discussed the link between durability of disease resistance in plants and sustainable agriculture. He defined the two major indicators of sustainability for any agricultural system as being 'the stewardship of natural resources' and 'the impact on the gap between haves and have-nots'. It was argued in Stuthman's paper that non-durable disease resistance fails to satisfy these two major criteria of sustainability. Resistance genes, being a part of germplasm, are natural resources that when defeated by the pathogen are consumed excessively. Moreover, the eventual possibility of contamination of the natural resources by movement of fungicides to non-target organisms and/or areas is another example of how non-durable resistance may fail to meet the first criterion. Furthermore, the defeat of resistance genes may lead to major disease epidemics that are costly to human beings and may widen the resource gap and this is how non-durable resistance may fail to meet the second criterion. Smale *et al.* (1998) described the breeding for durable rust resistance as an example of 'productivity maintenance research' and argued that substantial economic benefits are associated with deployment of such resistance in many wheat producing areas, especially in areas where farmers change cultivars slowly because of delays in cultivar release, poorly developed seed markets, and other economic factors.

Durable resistance to stripe rust (caused by *Puccinia striiformis*) and leaf rust (caused by *P. triticina*) in wheat is more likely to be associated with non-hypersensitive adult-plant resistance (APR) genes (McIntosh, 1992b). During the past few decades, attempts have been made in some wheat breeding programs, including that at the

International Wheat and Maize Improvement Center (CIMMYT) to develop wheat lines with combinations of a few non-hypersensitive leaf and stripe rust resistance genes. Several genes of this type have been identified in a range of wheat genotypes. The gene linkages *Lr34/Yr18* and *Lr46/Yr29* on chromosome arms 7DS and 1BL, respectively, are two examples of this type of resistance that have so far been characterized. These genes confer non-hypersensitive APR, often described as slow-rusting or partial resistance. The levels of resistance conferred by them however may not be adequate in some genetic backgrounds. It has been shown that additional genes are present in wheat germplasm, and in an additive interaction with either *Lr34/Yr18* or *Lr46/Yr29*, may provide high levels of resistance that cannot be distinguished from lines with hypersensitive response (Singh *et al.*, 2000).

The objectives of the investigations presented in this thesis were to determine the number and mode of action of the genes involved and the heritability of APR to leaf and stripe rusts in five wheat genotypes developed in the CIMMYT Wheat Program. Furthermore, the associations of the phenotypic expressions of resistance to leaf and stripe rusts were investigated in the populations developed. Studying the quantitative trait loci (QTLs) involved in APR and stability of such QTLs across locations were other objectives of these investigations. The results obtained are summarized and further discussed in this chapter.

6.2 Inheritance of adult-plant resistance

Five resistant CIMMYT spring bread wheats chosen for genetic analysis carried high levels of APR to leaf and stripe rusts at several locations globally despite their seedling susceptibility to Mexican races of leaf and stripe rusts. These genotypes were derived from a breeding project at the CIMMYT Wheat Program, which attempted to incorporate as many such genes as possible in high-yielding wheat background possessing *Lr34/Yr18* (Singh *et al.*, 2000). The resultant lines had high levels of APR that were even more resistant than their parents, in some cases resistant levels were close to immunity. Inheritance studies of these lines were done using a Mendelian segregation analysis, as well as techniques of quantitative genetics. In addition, QTL analysis was

used to study the genetic basis of durable APR to leaf and stripe rusts in the Australian cv. Cook.

6.2.1 Effective factors

Genetic studies of APR in five wheat genotypes demonstrated that APR is a complex trait with involvement of at least 4 and 3 genes in conditioning high levels of resistance to stripe and leaf rusts, respectively. Based on the pedigree information and presence of leaf-tip necrosis in all APR genotypes, *Lr34/Yr18* was common in all resistant genotypes. Moreover, *Lr46/Yr29* is likely to be present in some of the APR parents of this study. The estimates of the numbers of effective factors in segregation analyses were conservative as the population size was limited. The numbers estimated for stripe rust, when quantitative genetics techniques were used were higher than estimates based on Mendelian segregation analysis, up to 6 genes in some cases. The numbers estimated for leaf rust resistance in the quantitative techniques were comparable with the numbers estimated in Mendelian segregation analysis. Involvement of multiple genes with additive effects is in accordance with previously published works (Bariana and McIntosh, 1995; Singh and Rajaram, 1992; Singh and Rajaram, 1994; Bariana *et al.*, 2001; Bjarko and Line, 1988; Messmer *et al.*, 2000). The present results demonstrated that combinations of additive genes, without involvement of hypersensitive resistance genes, achieved high levels of resistance that even approached immunity.

Although the *Lr34/Yr18* linkage was common in all resistant parents, among the F_2 single plants and F_5 SSD lines derived from the resistant-parent intercrosses, there were plants/lines with 50-60% stripe or leaf rust. This indicated that, other than *Lr34/Yr18*, at least one or more likely two, other additive genes were non-allelic. It was estimated that up to 8 and 12 different additive genes, in addition to *Lr34/Yr18*, were present among the 5 parents for APR to leaf rust and stripe rust, respectively. This is of significant interest for breeding purposes and indicates the possibility of incorporating such genes in new breeding lines in order to achieve higher levels of resistance. It can be concluded that even in cases of genotypes with resistance levels close to immunity, genetic variability does exist among the additive genes, and incorporating even more

additive genes through intercrossing of APR genotypes would be feasible, provided selection tools are available.

6.2.2 Gene effect

Additive gene effects were found as being the major contributors in the inheritance of APR to leaf and stripe rusts in these germplasms. Although the specific combining ability (SCA) was also significant, general combining ability (GCA) was found to be the major component of variation among the crosses as suggested by the components of variance ratio $[2\sigma^2_g/(2\sigma^2_g + \sigma^2_s)]$. The importance of additive gene effects was congruent to previous studies (Das *et al.*, 1992; Shaner *et al*, 1997; Messmer *et al.*, 2000; Wagoire *et al*, 1998; Chen and Line, 1995). However, some levels of significant non-additive gene effects were also estimated in some generations/trials for both leaf and stripe rusts. Wagoire *et al.*, (1998) and Chen and Line (1995) reported significant non-additive gene effects in the inheritance of APR to stripe rust. Similarly, Messmer *et al.* (2000) reported significant dominance effects in the inheritance of APR to leaf rust. In quantitative genetics, any deviation form mid-parental value is described as a non-additive gene effect. Vanderplank (1984) argued that because of the evaluation scales used in quantitative disease resistance studies, such studies are often associated with overestimation of non-additive and underestimation of additive gene effects. The significant non-additive gene effects were therefore attributed, at least in part, to overestimation of such effects. The estimates of the marrow-sense heritability of APR to both diseases were relatively high, ranging from 0.88 to 0.96 for stripe rust and 0.67 to 0.96 for leaf rust.

6.2.3 QTL analysis

QTLs involved in APR to leaf and stripe rusts were studied in a RIL population of a cross between the Australian wheat cultivar Cook and Avocet-*YrA*. This population was phenotyped at several locations in Canada and Mexico under leaf or stripe rust epidemics. A QTL analysis approach was used in addition to the classical techniques of segregation analysis and quantitative genetics. Based on Mendelian and quantitative genetics approaches, APR was conditioned by *Lr34/Yr18* in addition to at least 1-2 and 2 more

genes with additive effects for leaf and stripe rusts, respectively. Estimates of the number of additive genes controlling the area under the leaf rust progress curve in quantitative analysis ranged between 2.19 and 3.55, with high estimates of broad-sense heritability ($h^2_{bs} > 0.94$). Single marker QTL analysis and composite interval mapping identified at least 4 QTLs in this population, significantly effective across all stripe rust experiments. In the case of leaf rust, six QTLs were found with significant effect in at least one experiment. In each experiment, at least 3 QTLs were found to be significantly effective against leaf rust. Involvement of various QTLs in the inheritance of APR to leaf and stripe rusts was consistent with previously published works (Bariana *et al.*, 2001; Boukhatem *et al.*, 2002; Singh *et al.*, 2001; Nelson *et al.*, 1997; Messmer *et al.*, 2000).

Assuming that *Lr34/Yr18* is present in the cv. Cook (McIntosh *et al.*, 1998), a set of microsatellite markers for chromosome 7D (Röder *et al.*, 1998) was used as anchor markers in QTL mapping analysis. Using these anchor markers and some of the linked AFLP markers, a hypothetical linkage map of chromosome 7D was constructed. A QTL was detected in this linkage map. It was most closely associated with AFLP marker locus M49/P33-280, and gave significant effects at some locations. However, the effect of this QTL was found to be less than expected for *Lr34/Yr18*. Moreover, it was not effective in all locations, as expected from *Lr34/Yr18*. It was therefore assumed that the QTL identified in this study was not *Lr34/Yr18*, but possibly represented a genomic region close to genomic site of *Lr34/Yr18*. Scarcity of polymorphism in 7D may have been the reason it was not possible to refine the location of *Lr34/Yr18* in this research.

In another study at CIMMYT (Suenaga *et al.*, unpublished; personal communication to Dr. R. P. Singh) a QTL, believed to be the *Lr34/Yr18* gene, was identified on 7DS. The microsatellite marker *Xgwm*295-7D was the closest marker to this QTL. In the single marker QTL analysis in the present study, *Xgwm*295-7D was not significantly associated with leaf and stripe rust resistances. However, it mapped about 20 cM away from QTL associated with the AFLP marker locus M49/P33-280.

The QTLs identified in this study accounted for up to 43% of the phenotypic variations in leaf or stripe rust responses of the RILs. It was therefore, assumed that more QTLs must be involved in conditioning APR to both rusts. A broader coverage of the genome with more markers would probably result in identification of more QTLs. With

this assumption, the actual number of effective factors should be more than the estimates based on classical genetic techniques. Messmer *et al.* (2000) also detected more QTLs than the estimated number of segregating factors. It should be noted that classical genetic techniques are based on pre-assumptions of equal genotypic effects of the effective factors with no involvement of linkage or epistasis. With QTL analysis, on the other hand, the effect of each QTL is described independently. Moreover, QTL analysis is capable of detecting linked loci or loci with epistatic effects. In QTL analysis, the magnitude of the genotypic effects of the QTLs were evidently different and therefore, the assumption of equal effects of effective factors was not met. Genes with different genotypic effects may explain the difference between estimates of the number of genes based on the classical genetics and QTL analysis.

6.2.4 Leaf tip necrosis

Leaf tip necrosis is a known morphological marker for *Lr34/Yr18* (Dyck, 1991; Singh, 1992a). In the studies presented in this thesis leaf tip necrosis was also found to be clearly associated with APR to leaf and stripe rusts. The presence and absence of leaf tip necrosis was controlled by 1-2 genes in the classical genetic analysis of CIMMYT-derived resistant parents. In the QTL analysis, when presence and absence of leaf tip necrosis were incorporated as alternative genotypes of one locus, it accounted for 51-80% of the phenotypic variation in the leaf and stripe rust severities. Alternatively, when leaf tip necrosis was dealt with as a phenotypic trait, it was found to be associated with multiple QTLs. Most of these QTLs coincided with the QTLs identified for the leaf rust and stripe rust responses. Messmer *et al.* (2000) also reported association of multiple QTLs with leaf tip necrosis. It seems that leaf tip necrosis is not only pleiotropic with *Lr34/Yr18*, but also with other additive genes, conditioning mechanisms involved in APR to leaf and stripe rusts.

6.2.5 Temperature-sensitive stripe rust resistance gene in Cook

Bariana *et al.* (2001) mapped a QTL on chromosome arm 2DS of cv. Sunco, temporarily designated as *YrCK*. The gene *YrCK* is believed to be a temperature-sensitive stripe rust resistance gene contributed by the cv. Cook, parent of Sunco. They also

mapped another QTL on the chromosome arm 2DS, temporarily designated as *YrKat*, associated with APR to stripe rust in cv. Katepwa. In this research the RIL population was screened for the gene *YrCK*, contributed by cv. Cook. This gene appeared to significantly contribute to reduction in stripe and leaf rust severities in all experiments. It was concluded that in addition to temperature-sensitive response to stripe rust, this locus must be pleiotropic or linked to a gene with additive contribution to APR to leaf rust.

6.3 Pathogen-nonspecific resistance

Multi-location testing of the RILs under leaf or stripe rust epidemics enabled to study the pathogen-specificity of the QTLs and their effectiveness across locations. APRs to leaf and stripe rusts were found to be closely associated in the materials studied in the research presented here. Genetically linked, or pleiotropic, effects of some APR genes for leaf and stripe rusts is known in wheat. Leaf rust resistance gene *Lr34* was reported to be tightly linked or pleiotropic with stripe rust resistance gene *Yr18* (Singh, 1992b; McIntosh, 1992a). Similarly, leaf rust resistance gene *Lr46* was also found to be linked to, or pleiotropic with, the stripe rust resistance gene *Yr29* (Singh *et al.*, 2001a; Bariana *et al.*, 2001). The present results supported those of Singh *et al.* (2001a) who identified genomic regions other than *Lr34/Yr18* and *Lr46/Yr29* involved in resistance to both rusts, but they also indicated that linkage or pleiotropism did not apply to every APR gene. This was also supported by analysis of the effectiveness of QTLs in multi-location testing. Some of the genomic regions that were identified in QTL analysis appeared to be nonspecific for leaf and stripe rusts across locations, but some were specifically effective against either of the two and only in some locations. Overall, results indicated that co-improvement in the level of APR to both leaf and stripe rusts should be feasible.

6.4 Implications in breeding for rust resistance

Breeding strategies for achieving the ultimate goal of longer-lasting resistance fall into two groups *viz.* strategies based on the major hypersensitive resistance genes and strategies based on additive non-hypersensitive resistance genes. Gene pyramiding is the process of combining resistance genes into an individual variety. McDonald and Linde (2002), based on a 'scale of evolutionary risk ranking assessment', proposed pyramiding

of major genes as the breeding strategy for rust diseases with asexual life cycles. This includes stripe rust, and also leaf or stem rusts in areas with no alternate hosts. However, Johnson (1988) demonstrated that in a climate favorable to stripe rust in the UK, pyramiding as many as 4 race-specific resistance genes was not a successful breeding strategy. Resistance-gene pyramiding in combination with anticipatory breeding (McIntosh and Brown, 1997), which is the process of predicting and breeding for upcoming pathotypes may reduce the risk of major epidemics. This strategy requires monitoring of the virulence in an extensive race survey in a wide area, a strong genetics program to identify new sources of resistance, a functional breeding program to incorporate new resistance genes, and an efficient seed-production and distribution system that is able to replace cultivars, if required. Such breeding strategies may be more limited in the areas of the world where farmers change cultivars slowly, and where adjacent countries support different disease control strategies.

On the other hand, in pathosystems like wheat-leaf and -stripe rusts with long experience with non-durable race-specific hypersensitive resistance genes, quantitative APR of non-hypersensitive type offers excellent alternative approaches for plant breeders. This can be supported by the knowledge that resistance in many durably resistant cultivars appears to be based on a few to several quantitative non-hypersensitive type genes.

Knowledge of the inheritance of APR is beneficial in designing appropriate strategies for breeding. APR to stripe and leaf rusts is a complex trait with quantitative involvement of a few to several genes with small additive effects. Therefore, breeding for APR due to its greater complexity, is not as simple as breeding for hypersensitive resistance, which is controlled by single genes. However, breeding for a complex trait is a common task in plant breeding, as most of the traits like yield and several types of stress resistance are of a complex nature. A population breeding approach with intercrossing and recurrent selection in different generations would result in accumulation of many additive genes of small effects. In addition to sources of *Lr34/Yr18* and *Lr46/Yr29*, wheat genotypes with different degrees of APR can provide the appropriate genetic variation to be used in crossing for such a population-breeding program. Recurrent selection would require screening under epidemics of appropriate races of the pathogen, with virulence as

wide as possible (Parlevliet, 1993), to overcome the epistatic effects of single hypersensitive resistance genes with large effects. In segregating generations, heavy selection pressure for high levels of field resistance in early generations should be avoided (Bariana and McIntosh, 1995) as it may eliminate plants/lines that are capable of giving higher levels of resistance in later generations, when homozygosity is achieved. Moreover, as plants/lines with most of the hypersensitive resistance genes can be identified at the seedling stage, they can be removed from the population by screening the breeding lines with the same race as will be used in the field. Like any other quantitative trait, APR is influenced by environmental factors. Multi-location testing of breeding lines would ensure the stability of resistance across locations, although it does not guarantee the race non-specificity of resistance genes.

Genetic linkage or pleiotropism between leaf and stripe rust APR genetic factors should facilitate breeding for both diseases at the same time. It is also of interest for breeding purposes that some sources of APR to leaf and stripe rusts are associated with resistance to other diseases. The linkage of *Lr34/Yr18* was reported to also be linked to *Bdv1* gene for slow yellowing response to barley yellow dwarf virus (Singh, 1993) and is known to also enhance resistance to stem rust (Dyck, 1987; Dyck, 1993; Kerber and Aung, 1999). Linkage or pleiotropism of this type should facilitate breeding for multiple-rust or even multiple-disease resistance. It should also be noted that breeding for rust resistance is only one of the many objectives of breeder. Therefore, these attempts should accordingly be incorporated into breeding for other objectives in the programs. There are examples of successful breeding for quantitative leaf and stripe rusts resistance. Singh *et al.* (2000) incorporated high levels of quantitative APR into high yielding cultivars by the means of intercrossing, top-crossing and/or limited back crossing while maintaining or even increasing actual yield potential.

Knowledge of the QTLs involved in quantitative resistance may assist plant breeders in designing their optimal breeding strategies. The relative contribution of each QTL to the total variation, the level of interaction with other QTLs, the stability of QTLs in different locations, linkage of QTLs to other traits, pleiotropic effects of the QTLs, and race- or pathogen-specificity of QTLs are examples of the knowledge that can be provided through QTL analysis (Lindhout, 2002). In the research presented in Chapter 5,

few QTLs with significant effects were found with a range of contributions to overall resistance across locations. Some of these QTLs were found as being consistently effective across locations, while some were found as being effective only in some locations. Few QTLs were found with pleiotropic effects for both leaf and stripe rusts.

Marker-assisted selection (MAS) is un ultimate goal of QTL analysis in plant breeding. There are a few examples of successful applications of MAS for QTLs in breeding for quantitative disease resistance in crops (Willcox *et al.*, 2002; Robert *et al.*, 2001; Toojinda *et al.*, 1998). There are advantages and disadvantages of MAS for breeding for quantitative rust resistance. Using markers in screening may increase the breeding efficiency and shorten the breeding cycle. Moreover, in the case of breeding for rust resistance, these techniques may facilitate combining major resistance genes with APR genes, which is difficult, if not impossible, in conventional breeding because of the epistatic effects of the major resistance genes that mask the effects of minor APR genes. On the other hand, the high cost of the analyses of DNA markers and the fact that such high investments do not easily pay off sould limit these techniques from extensive use, especially in crops like wheat with low seed price (Lindhout, 2002). Moreover, with MAS there is a risk of narrowing the resistance down to a minimum number of genes (Wolfe, 1993), because in any MAS project only a limited number of markers can be selected for. Resistance responses of the lines with assembled QTLs through marker-assisted selection need to be verified in disease nurseries.

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