Host-pathogen studies of wheat leaf rust resistance in *Triticum turgidum*

by

Ronelle Barnard

Dissertation submitted in fulfilment of the requirements for the degree Magister Scientiae in the Faculty of Natural and Agricultural Sciences, Department of Plant Sciences – Genetics – at the University of the Free State, Bloemfontein

May 2003

Supervisor:

Prof. Z.A. Pretorius

Co-supervisor

Dr. C.D. Viljoen

Ek verklaar dat die verhandeling wat hierby vir die graad MSc. aan die Universiteit van die Vrystaat deur my ingedien word, my selfstandige werk is en nie voorheen deur my vir 'n graad aan 'n ander universiteit / fakulteit ingedien is nie. Ek doen voorts afstand van outeursreg in die verhandeling ten gunste van die Universiteit van die Vrystaat.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

%	percentage
AFLP	Amplified Fragment Length Polymorphisms
AP	abortive penetration
APR	adult-plant resistance
ASSV	aborted substomatal vesicle
ASSVN	aborted substomatal vesicle with necrosis
BC	back cross
bp	base pair
C	colonies/chlorosis
cm	centimeter
CN	colonies with necrosis
CS	sporulating colonies
CSN	sporulating colonies with necrosis
CTAB	cetyltrimethylammonium bromide
d.p.i.	days post-inoculation
DAF	DNA Amplified fingerprinting
DNA	deoxyribonucleic acid
e.g.	for example
EA	early abortion
EAN	early abortion with necrosis
EDTA	ethylenediamin tetraacetic acid
et al.	and others
F	forma
FDA	fluorescein diacetate
g	gram
h	hour
HCN	host cell necrosis
HI	hypersensitive index
HMC	haustorium mother cell
HR	hypersensitivity response
i.e.	that is
IT	infection type
krpm	kilo revolutions per minute
l	litre
Lr	leaf rust resistance gene
M	molar
m	metre
ml	milliliter
n	nano
N	necrosis
NaCl	sodium chloride

NPA	non-penetrating appressorium
NSA	non-stomatal appressorium
°C	degrees of Celcius
P_0	parent
PCD	programmed cell death
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphisms
s	second
SDS	sodium dodecyl sulphate
sp.	specialis
Sr	stem rust resistance gene
ssp.	subspecies
SSR	simple sequence repeats
TAE	Tris acetic acid EDTA
Tris-HCl	Tris(hydrocymethyl)aminomethane hycrochloric acid
U	unit
UFS	University of the Free State
UV	ultra violet
var	variety
Yr	yellow rust resistance gene
μ	micro
π	pi
X^2	chi-square

ACKNOWLEDGEMENTS

I would like to express my sincere gratefulness to the following people and institutions that helped me to through this study:

Prof. Z.A. Pretorius for opening the door to wheat research for me. I am grateful for all the time, advice and guidance I received from you and am honoured to have worked with you,

Dr. Chris Viljoen who introduced me to the wonders of AFLPs and taught me people skills,

Cornel Bender, Zelda van der Linde, Wilmarie Kriel, Elizma Koen and Juan-Marié Bower who helped wherever they could and had the patience to explain technicalities, computer analysis and microscope work,

The former departments of Botany and Genetics and Plant Pathology for the opportunity and facilities to undertake this study and the NRF for financial support,

The University of Stellenbosch for supplying the UFS with plant material used in this study,

My family, in-laws and friends, old and young, who understood,

My parents who prized knowledge above all,

My husband, Roderick, for love and support – and forcing me to finish the thesis,

And to God. Thank you!

CHAPTER 1

AN OVERVIEW OF WHEAT RUST DISEASES WITH EMPHASIS ON *PUCCINIA TRITICINA*

Wheat is an important part of the diet of people all over the world, including South Africans. Therefore, diseases resulting in the loss of yield and quality have a significant impact on agriculture and the economy in general. Wheat can be infected by a wide range of pathogens, with rust fungi being particularly damaging (Wiese, 1987). When infected by rust pathogens, photosynthesis and water transport in the plant are affected leading to production losses. By understanding the biology of the pathogen, the genetics of the host, and the interaction between them, rust-resistant cultivars can be bred, infections are thus prevented and losses minimized. In this chapter rust diseases of wheat are reviewed with special emphasis on the leaf rust pathogen, *Puccinia triticina,* and measures to control it.

1.1 HOSTS

1.1.1 Classification

The genus *Triticum* belongs to the grass family Poaceae, subfamily Pooidae and the tribe *Triticeae* (Burger, 1995). The tribe *Triticeae* contains four major cereals – barley, rye, wheat and triticale (a hybrid of wheat and rye). The term "wheat" refers to all the cultivated species of the genus *Triticum* and "wild wheat" is the non-domesticated species unsuitable for commercial cultivation.

Attempts to classify the *Triticeae* have often been made, but none of the proposed classifications has been universally accepted. In recent times the

originally seperated genus *Aegilops* has been incorporated into *Triticum* (Morrison, 1993). Acoording to Lupton (1987) Dorofeev and Korovina (1979) divided the *Triticeae* into climate regions, but mostly species of *Triticum* are classified by the presence of four different types of wheat genomes, A, B, D and G (Table 1.1).

1.1.2 Morphology

The genus *Triticum* is characterized by erect monocot plants with parallel veined leaves. Leaves consist of three parts: the sheath, which envelops the culm, the blade that extends from it, and the collar and ligulae located at the junction of the sheath and the blade (Gibbs Russell *et al.,* 1990).

The inflorescence, or spike, is a collection of sessile flowers on a central axis. In wheat, three florets form one flower or spikelet. A floret consists of one pair of bracts, the lemma and palea, which conceal a single delicate lodicule, one or two pistils, and three stamens (Gibbs Russell *et al.,* 1990). The size, number of spikelet parts and number of florets differ between species and cultivars. The length of the awns is an indication of the plant breeding age of a specific wheat species. Modern wheat, *T. aestivum* (Figure 1.1), has shorter awns, while more primitive genotypes that have not been extensively subjected to plant breeding, e.g. *T. durum* (Figure 1.2), have long, sharp awns.

Wheat is semi-resistant to drought. It has specialized chlorophyllous cells around each main vascular bundle acting as a single, conspicuous sheath of

starch rich cells with abundant chloroplasts (Gibbs Russell *et al.,* 1990; Salisbury and Ross, 1992). This type of anatomy (Kranz-anatomy) enables plants to use a mechanism of carbon dioxide transport associated with C4 photosynthesis. The ratio of water loss to carbon dioxide absorption is favourably low with this photosynthetic pathway (Mauseth, 1991).

1.1.3 The evolution of wheat

Wheat has been cultivated by humans for several millennia, and these cultivated species have been subjected to breeding procedures. This influenced the natural evolution of cultivated species of *Triticum* (Harlan, 1981).

It is thought that *Triticum* originated from a diploid species with an A-genome. This species, probably *T. monococcum*, was fertilized by an unknown species – probably *T. searsii* (Starr and Taggart, 1992) or *Aegilops* (Lupton, 1987) – with genomic constitution 2n = 14 BB to produce offspring (*T. turgidum*), 2n = 28, AABB. When *T. tauschii* (2n = 14, DD) and *T. turgidum* crossed, bread wheat, *T. aestivum* (AABBDD) was obtained. *T. aestivum* (*aestivum* meaning "of summer") was earlier known as *Triticum sativum* (Lam.) or *Triticum vulgare* (Vill.) (Knott, 1989; Starr and Taggart, 1992).

Using monosomic lines, developed by Sears (1954) in crosses with tetraploid wheat, it has been possible to identify the D-genome. The identification of the A and B genome chromosomes was made possible by the development of

ditelosomic lines where a particular pair of chromosomes has been replaced by telocentric chromosomes (Knott, 1989).

Originally the genus *Triticum* included only those species containing the A-genome. New genome symbols for the polyploid species have been proposed, but as in the case of *Aegilops*, no agreement has yet been reached (Knott, 1989).

Within the tribe *Triticeae* the genus *Aegilops* is closely related to cultivated wheat (Badaeva *et al.*, 1996). Knott (1989) suggested that the B-genome has been donated to *Triticum* by the goat grasses (*Aegilops*). The status of *Aegilops* as a separate genus has been disputed and the incorporation of *Aegilops* into the genus *Triticum* is not universally accepted (Lupton, 1987). *Aegilops speltoides* has not only been suggested as donor of the B-genome, but also as donor of the G-genome (Lupton, 1987).

1.2 RUST PATHOGENS OF WHEAT

The fungal genus *Puccinia* is responsible for rust diseases of many host plants and belongs to the phylum Dikaryomycota, subphylum Basidiomycotina, class Teliomycetes and order Uredinales. One hundred and fifty genera, containing 6000 species, cause rust and approximately half of these belong to *Puccinia* (Kendrick, 1992).

The rust fungi are obligate, biotrophic parasites of vascular plants and are often host specific, thus being restricted to one family, genus or even a single

species. Rust fungi are often heteroecious, meaning that they have more than one host – like wheat (or other closely related monocots), and alternate hosts, e.g. *Thalictrum* spp., *Anchusa* spp. and *Berberis vulgaris* (Knott, 1989; Roelfs *et al.*, 1992). Considering wheat stem rust in the northern hemisphere, infection occurs early in spring on young leaves of the alternate host (*Berberis vulgaris*). Nectar-producing spermagonia develop on the surfaces of leaves and insects attracted to the nectar carry the spermatia to receptive hyphae. Thus a process of recombination of genetic material, analogous to pollination in plants, takes place. Aeciospores (spores adapted for dispersion) form on the alternate host and after distribution infect wheat plants. These spores can penetrate the true hosts (wheat or other *Triticum* and monocot species), but the alternate hosts are immune to infection of the aeciospores (Knott, 1989). In wheat dicariotic infection occurs and rust pustules, containing urediniospores, form. Urediniospores are produced in abundance and are dependent on the wind for distribution.

All three rust diseases specific to wheat are caused by *Puccinia* species. They are *P. triticina* (previously *P. recondita* f. sp. *tritici*), *P. graminis* f. sp. *tritici,* the pathogen responsible for stem rust, and *P. striiformis* f. sp. *tritici*, the causal agent of yellow (stripe) rust. These rusts differ in their life cycles, morphology and environmental conditions required for successful pathogenesis (Knott, 1989).

1.2.1 Leaf rust

The name first assigned to the fungus causing wheat leaf rust was *Puccinia rubigo-vera*. This was changed to *P. triticina* Eriks. following studies on specialization (Dickson, 1956) and again changed to *P. recondita*. *P. recondita* was the name of the rye leaf rust pathogen, with the type attacking wheat as a specialized form (Anikster *et al.*, 1997). Wheat leaf rust was therefore given the name *P. recondita* Rob. ex Desm. f. sp. *tritici* Eriks. (Knott, 1989). Two groups can be distinguished within the forma specialis *tritici*. Pathogens from the first group originated from cultivated wheat and the wild emmer wheats, whereas those in the second group had their origin from wild wheat and rye (Anikster *et al.*, 1997). After extensive tests it became evident that wheat leaf rust was an independent species and the name was changed back to *P. triticina* (d'Oliveira and Samborski, 1966; Markovà and Urban, 1977; Anikster *et al.*, 1997). Hence, *P. triticina* is at present considered the appropriate name for the causal agent responsible for leaf rust (also known as brown rust or red rust) of *Triticum* spp. *Thalictrum* spp., *Anchusa* spp., *Clematis* spp. and *Isopyrum fumariodides* have been listed as alternate hosts for this pathogen (Roelfs *et al.,* 1992; http://www.crl.umn.edu.tritname.html).

P. triticina is a biotrophic, airborne pathogen and is predominant where wheat matures late (Wiese, 1987). Infections occur in moderate, humid conditions, require temperatures ranging between 10 and 30 °C with an optimum between 15 and 22 °C. *P. triticina* has been considered the most important of all the wheat rust pathogens due to crop losses resulting from its worldwide occurrence (Wahl *et al.*, 1984). According to Trench *et al.* (1992) and Roelfs

et al. (1992) losses of 5 % to 10 % are common during epidemics, but yield losses as high as 40 % to 78 % have been reported (Samborski and Peturson, 1960; Dubin and Torres, 1981; Singh, 1999; Boshoff *et al.*, 2002a). Even resistant cultivars infected by leaf rust have shown losses ranging between 12 % and 28 % (Samborski and Peturson, 1960).

The first symptom of infection is a flecking of the adaxial leaf surface. These flecks turn into isolated, circular, brownish red rust pustules, usually only on the upper leaf surface. Under extreme circumstances pustules may occur on both the upper and lower surfaces. Pustules give rise to urediniospores which re-infect susceptible plants. When plants have some degree of resistance, or when conditions become unfavourable, dark blotches containing teliospores occur on the abaxial epidermis of the necrotic leaves. However, teliospore production in *P. triticina* is not very abundant compared to other rusts (Knott, 1989). Stress conditions, such as drought or other infections, can increase teliospore production. The spore production rate can also rise if the environmental conditions for fungal development are optimized (Knott, 1989).

Usually only the leaf lamina, but in more severe circumstances the stems and leaf sheaths are infected if the conditions are favourable and the cultivar is susceptible. Photosynthesis is inhibited as the chlorophyll-containing cells are destroyed by fungal growth which eventually leads to necrosis of leaf tissue. Yield losses are caused by the reduction of number and weight of kernels per inflorescence (Knott, 1989). The reason for yield reduction can be attributed to various factors. Rusts increase transpiration and respiration and are also

responsible for the export of assimilates from leaves. Rust pathogens can also reduce plant vigour and root growth (Gooding and Davies, 1997). The method of determining the mass of 1 000 grains is a reliable indication of yield loss due to leaf rust infection (Pretorius and Kemp, 1988). Using this method a 10.4 % reduction in 1000-grain mass due to leaf rust infection of Thatcher was indicated (Kloppers and Pretorius, 1995a). In a more recent study, the application of fungicides reduced the severity of leaf rust infection by up to 84 % (Boshoff, 2000; Boshoff *et al.*, 2002b).

Puccinia triticina is represented by different races, also called pathotypes, containing different combinations of avirulence and virulence genes. These races have traditionally been determined using a set of host lines, each with a different resistance genotype. By infecting this set with pure cultures of the leaf rust fungus, races can be differentiated according to the pattern of resistance and susceptibility. More recently molecular techniques have been employed to characterize pathogenic variability (Kolmer, 1996). The races are produced mainly by mutation and sexual recombination (Knott, 1989). Craigie (1927) was the first to describe the sexual cycle of stem rust, and thus focused attention on the importance of this phase in creating pathogenic variation. Shortly thereafter, Newton *et al.* (1930) selfed and crossed several stem rust races, producing offspring that differed from both parental strains.

Distinctly more rust races were found in an area where a sexual cycle exists than in an asexual population (Roelfs and Groth, 1980). The sexual cycle of rusts is therefore an important source of new combinations of genes for

virulence wherever the alternate host occurs. No evidence exists that sexual cycles are completed for any of the wheat rusts in South Africa.

The virulence of *P. triticina* is generally more diverse than in stem or stripe rust. The reason for the diversity has been attributed to the population size within seasons and the survival of more inoculum between wheat crops (Schafer and Roelfs, 1985). The virulence also differs between geographical regions (McIntosh *et al.*, 1995). Virulence markers that describe genetic variation in plant pathogens exist, but there are isolates with identical molecular construct, but highly different virulence (Kolmer *et al.*, 1995). However, correlations between virulence phenotypes and molecular composition have been found, but polymorphisms could be small between diverse virulence phenotypes (Kolmer *et al.*, 1995).

1.2.2 Stem rust

Puccinia graminis Pers. f. sp. *tritici* Eriks. and Henn. causes stem rust of wheat. Other hosts include barley, rye, oat, wild barley and *Agropyron distichum* (Trench *et al.,* 1992). Although *P. graminis* f. sp. *avenae* (specific to oat and related grasses) and *P. graminis* f. sp. *secalis* (specific to rye and related grasses) are able to infect wheat, little or no pustules are produced (Knott, 1989). For infection of stem rust, temperatures warmer (20 – 30 °C) than the optimum for leaf rust are needed (Lupton, 1987).

Stem rust symptoms are generally similar to those of leaf rust. Orange-red, long, often diamond shaped pustules form on stems and both sides of leaves

of susceptible cultivars. Leaf sheaths, spikes and awns are also infected (Knott, 1989). These pustules produce urediniospores. Sporulation occurs on both epidermi, but more severely on the abaxial epidermis. Black teliospores form at the end of the season, hence the name "black" stem rust. Stem rust is an extremely damaging disease of wheat. When pustules burst open, the infected areas are torn and appear tattered and ragged. Similar symptoms are observed on other infected areas. Losses are due to a decrease in photosynthetic area, damage of the flag leafs, shrunken grains, poor seed set, disruption of water and nutrient transport, and stem breakage (Lupton, 1987).

1.2.3 Stripe rust

The causal agent of stripe (yellow) rust is *Puccinia striiformis* Westend. f. sp. *tritici* (Knott, 1989). This pathogen requires relatively cool temperatures (lower than 20 °C) for optimum infection and growth. Therefore, winter wheat is in far greater danger of epidemics caused by this pathogen than spring types grown in moderate temperatures. As is the case with leaf and stem rust, humid conditions are essential for spore germination and infection.

Symptoms of bright yellow to orange stripes on the leaves and other infected parts of the plant are observed. The stripe rust fungus is systemically dispersed through the veins. Whole plants, including developing kernels, are attacked by stripe rust. Primary losses result from defoliation and shrivelling of the kernels. Losses of up to 84 % have been reported (Knott, 1989; Murray *et al.*, 1994). What makes this disease probably more dangerous than either leaf or stem rust is that less than half of South African cultivars tested in a

recent survey had adult plant resistance to stripe rust and only about 10 % possessed seedling resistance (Boshoff, 2000; Boshoff *et al.*, 2002b). Even the epidemiology is different from the other two rusts. Only the asexual stage of stripe rust has been found. Basidiospores are produced, but no alternate host has as yet been identified (Knott, 1989).

1.3 DISEASE CONTROL

Due to losses in yield and quality farmers have tried to control rust infections for centuries. The French noted in the 1600's that the occurrence of stem rust was more severe when wheat was grown in the presence of *Berberis vulgaris*, and passed a law to eradicate barberry. America followed this initiative in the 20 th century and barberry has been eradicated to such an extent that it is no longer important in the occurrence of stem rust epidemics on the continent (Knott, 1989).

Where short and long season wheats are grown in the same area, infected mature plants can infect the seedlings of the new season. This can be countered by delaying new plantings. In areas where the rust inoculum arrives late, early planting can ensure that plants reach maturity before rust becomes epidemic (Roelfs, 1985).

Fungicides have been commonly used for protecting susceptible cultivars, but foliar fungicides are expensive and often the cost of spraying exceeds the market value of the crop (Stevens, 1974). Except for the cost of the chemical

itself, additional application equipment is required, making it more difficult for developing countries to afford. In addition, chemicals may be environmentally unfriendly, especially based on the current trend of an increasing concern over environmental issues. One well-timed spray may be effective, but depending on the type and the growing season of the plant, the amount of inoculum and climatic conditions, more applications are usually required (Knott, 1989). Seven fungicides, all belonging to the triazole group, are registered for leaf rust control in South Africa (Nel *et al.*, 1999).

An alternative way of controlling fungal infections is the breeding and use of resistant wheat cultivars where infection is terminated early in the infection process, or where partial symptom development does not impact significantly on yield. However, the rate at which the pathogen overcomes leaf rust resistance (*Lr*) genes forces scientists to search for new genes or to deploy existing genes in new combinations.

1.3.1 Breeding for resistance

Although the existence of rusts on wheat has been recognized since Biblical times, it has only been divided into the three wheat rust groups in the late 1800's and breeding for resistance was initiated in the early nineteen hundreds. To breed for resistance, a suitable wheat cultivar, containing most of the superior traits, is crossed with a suitable resistant donor. Due to different perspectives and approaches, rust resistance is a broad concept and includes terms such as seedling resistance, adult plant resistance (McIntosh *et al.*, 1995), the combination of genes (Kloppers and Pretorius, 1997), slow

rusting (Wilson and Shaner, 1987), durable resistance (Johnson, 1981) and tolerance (Schafer, 1971). The aim of breeding cultivars resistant to leaf rust is to obtain one that would be resistant for at least its commercial life span (Knott, 1989; Bender *et al.*, 2000).

In most cultivars the use of hypersensitive resistance genes was an economical, but not effective way of controlling rust diseases (Nelson, 1978). This type of resistance is characterised by a necrotic response to infection, low infection type, and non-durability (Parlevliet, 1988). Due to its clear phenotype and simple inheritance, hypersensitive resistance is easy to manage in breeding programmes, specifically in backcrossing and many breeders have therefore relied on this type of resistance. With the exception of only a few *Lr*-genes, all have been overcome by new pathogen races. The latter has led to the search of alternative resistance genes (Nelson, 1978).

In order to identify physiologic races within rust fungi, backcrossed lines with single genes for resistance are used to phenotypically differentiate isolates of the parasite (Samborski and Dyck, 1982). These lines are reared as seedling plants and each reaction pattern is considered typical of a particular race (Dyck *et al.*, 1966). With an array of appropriate races, the breeder can now initiate a resistance programme in which suitable donor lines and selection protocols are identified.

A number of factors must be overcome when breeding not only for resistance against leaf rust, but wheat in general. These factors include incompatibility of

genomes, infertility, susceptibility to other pathogens, environmental factors, suppressors and linked genes (Klug and Cummings, 1994; Gaines *et al.*, 1996; Brown-Guedira *et al.*, 1997).

1.3.1.1 Incompatibility

The wheat family consists of taxa with different ploidy levels. Some of the genomes are incompatible and a cross between such species will not produce any progeny. Aneuploidy, which refers to plants that do not have the normal chromosome number or multiple chromosomes (Knott, 1989), can also play a roll in incompatibility between potential parents. Since wheat is a polyploid, many aneuploids are viable and fertile (Sears, 1954).

1.3.1.2 Infertility

In wide crosses it is often found that the seeds are non-viable and they can only be saved by embryo rescue (Knott, 1989). Even if these seeds produce mature F_1's, the adult plants sometimes are sterile (Brown-Guedira *et al.*, 1997).

1.3.1.3 Environmental factors

When breeding wheat in controlled environments, plants are grown at optimum conditions which often differ from field situations, specifically with regard to expression of adult plant rust resistance (Dyck, 1987; Gaines *et al.*, 1996; Barnard, 1999a). By definition phenotype is influenced by both genotype and the environment (Klug and Cummings, 1994), but because of

the polygenic nature of many characters, the environment largely influences their expression (Gaines *et al.*, 1996; Barnard, 1999a).

1.3.1.4 Suppressors

Suppressor genes prevent the expression of resistance genes (Klug and Cummings, 1994). When cultivars having the resistance genes are nullisomic to chromosomes or the chromosome arm containing the suppressor, resistant plants are obtained. Such a suppressor gene in wheat is located on wheat chromosome 7D (Kerber and Green, 1980). This suppressor inhibits the expression of stem rust resistance genes and might also suppress leaf rust resistance genes (Dyck, 1987). If a plant has a resistance gene that is a non-suppressing allele of the genes on this chromosome, the plant will be resistant (Dyck, 1987).

1.3.1.5 Wild wheat species as sources of resistance genes

The number of genes responsible for resistance in cultivated wheat is limited (Knott, 1989). *Triticum* species related to wheat and known for their resistance to leaf rust, can be used as donors of resistance genes in the breeding process (Knott, 1989). These donors include lines from *T. turgidum* and *T. timopheevii*, as well as species from other grass families (Knott, 1989). High levels of resistance have been identified in *T. monococcum*, (Kerber and Dyck, 1973), *T. speltoides* (Dvorak, 1977) and *T. timopheevii* (Knott and Dvorak, 1976).

Certain accessions of *T. monococcum* are non-hosts to leaf rust (Niks and Dekens, 1991) and would theoretically be useful in breeding for resistance. Almost all accessions are resistant, and show no external symptoms of infection. It was hoped that resistance obtained from such donors would be durable, but the contrary has often been demonstrated (Knott, 1989). Thus, resistance derived from alien species has often been overcome by virulent races and such resistance is not necessarily durable (Knott, 1989; McIntosh *et al.*, 1995).

Because of genome incompatibility, infertility and unwanted traits, breeding is often time consuming and laborious. The transfer of major genes is relatively uncomplicated as it is detected earlier and is easier to measure while polygenic resistance is difficult to transfer and measure (Knott, 1989). For the successful transfer of resistance genes from wild species, an intact gene, the chromosome, or the segment of chromosome of the allele, must be incorporated in the hybrid's genome. To be successful the alien gene should also be expressed in the same way in the wheat genome than in the donor (Lupton, 1987).

1.4 RESISTANCE TO LEAF RUST

Wheat and wheat rust co-evolved for millennia. When wheat developed resistance to a pathogen, the rusts had to mutate in order to survive. Plants with resistance genes enabling it to withstand infection show no symptoms, or less symptoms than susceptible plants (Knott, 1989). Based on genotype and

phenotype several types of rust resistance have been recognised and catalogued.

1.4.1 Seedling and adult-plant resistance

Some plants have resistance against leaf rust expressed from the first-leaf stage onwards (Dyck *et al.*, 1966). Since the identification of rust races is done on seedling plants, the genetic behaviour of most genes for seedling resistance has been investigated and is well understood. According to Dyck *et al.* (1966) there are three reasons why it is difficult to investigate adult-plant resistance (APR). Firstly, the presence of genes responsible for seedling resistance can mask expression of APR. Secondly, the effect of modifying genes has an impact on the behaviour of APR genes and, lastly, genes and modifiers are both sensitive to environmental changes.

Once the chromosomal location of single leaf rust resistance (*Lr*) gene has been determined, it receives a designated number (Table 1.2). At present 40 *Lr* genes for seedling resistance and 10 *Lr* genes for APR have been numbered (McIntosh *et al.*, 1995; http://www.crl.umn.edu/ res_gene/wlr.html). The genes for seedling resistance are *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr3bg, Lr3ka, Lr9, Lr10, Lr11, Lr14a, Lr14b, Lr15, Lr16, Lr17, Lr18, Lr19, Lr20, Lr21, Lr23, Lr24. Lr25, Lr26, Lr27. Lr28, Lr29, Lr30, Lr31, Lr32, Lr33, Lr36, Lr38, Lr39, Lr40, Lr41, Lr42, Lr43, Lr44, Lr45 and Lr47* (Craven, 2002). The following temporarily assigned *Lr* genes are also expressed in primary wheat leaves: *LrEch, LrH, LrLC, LrA, LrB, LrD, LrMo, LrTm* and *LrTr* (McIntosh *et al.*, 1995; http://www.crl.umn.edu/res_gene/wlr.html). Adult plant or field

resistance (Dyck and Kerber, 1981) is expressed by the following numbered genes: *Lr12, Lr13 Lr22a, Lr22b, Lr34, Lr35, Lr37, Lr46, Lr48 and Lr49*, and temporary designations *Lrl, LrJ, LrK, LrL, LrAP, LrM, LrN, LrO, LrT3, LrTrp1* and *LrTrp2* (McIntosh *et al.*, 1995; http://www.crl.umn.edu/ res_gene/wlr.html).

1.4.2 Specific resistance

Specific resistance (resistance against a specific race of the pathogen) can be readily overcome by mutations in the pathogen (Gilchrist, 1998). Breeders have therefore tried to introduce more durable, race non-specific resistance using appropriate sources. Specific resistance includes seedling and APR. The hypersensitive response is frequently associated with specific resistance (Parlevliet, 1988; Gilchrist, 1998).

1.4.3 Hypersensitive response

Stakman (1915) defined the term hypersensitivity or hypersensitive response, observing the interaction between *Puccinia* spp. and non-hosts. He then hypothesized that the rapidity of the cell death must indicate a form of resistance. The hypersensitive reaction or response (HR) occurs when the leaf cells or tissue surrounding an infection site die rapidly upon pathogen invasion (Heath, 1976). Hypersensitive resistance is phenotypically associated with a low infection type, susceptibility, partial expression and non-durability (Parlevliet, 1988; Gilchrist, 1998).

For biotrophic parasites non-specific defences are suppressed and HR only occurs in resistant hosts (Parlevliet, 1988; Heath, 1998). Resistance against

the pathogen can occur before, during or after infection by the pathogen (Prusky *et al.*, 1980). In studies by Goodman and Novacky (1994) it was found that although infection and penetration in susceptible and HR-resistant hosts are identical, fungal development thereafter differs. In susceptible hosts the fungal growth is rapid with no immediate effect on the host cells, but in resistant hosts a rapid death of cells closest to the infection site is observed. This indicates that HR on its own is not the primary resistance mechanism, but rather the phenotypical result of another, or series of defence responses.

The hypersensitive response is not a single phenomenon with a single role in resistance (Heath, 1976), therefore cell deaths caused by HR require the active metabolism of living cells. The hypersensitive response is a characteristic phenotype of programmed cell death (PCD) and other induced resistances like local and systemic acquired resistance (Graham and Graham, 1999). The production of biochemical compounds such as phytoalexins, hydrolytic enzymes, pathogenesis–related proteins, protease inhibitors and the deposition of lignin and callose into the plant cell wall are known to contribute to resistance (Graham and Graham, 1999). When infected, transcription and translation are suppressed in susceptible cells, but increased in resistant cells containing HR-genes. Transcription stops when programmed cell death begins. This process is specific to the hypersensitive cell death process (Mould and Heath, 1999).

Apoptosis (dying of the host cells) deprives the pathogen of nutrients and water and can thus terminate the life of biotrophic pathogens (Richael and

Gilchrist, 1999). Although the HR has been proposed to stop fungal growth and kill the pathogen in the process, not all cells in contact with the fungus die immediately (Silverman, 1959; Skipp *et al.*, 1974). Temperature also plays a role and by raising incubation temperature, a plant known for HR-resistance can turn susceptible (Zimmer and Schafer, 1961). Saprophytic fungi live on dead tissue, and the hypersensitive reaction might slow, but will not terminate these pathogens. Therefore host cell death may contribute to a limitation in fungal growth, and /or lead to partial resistance.

1.4.4 Horizontal and vertical resistance

The terms horizontal (lateral) resistance and vertical (perpendicular) resistance were introduced by Vanderplank in 1963. Vertical resistance (synonym: race-specific resistance) describes a variety that is resistant to certain races of a pathogen, but susceptible to others. Horizontal resistance (synonym: race-non-specific resistance) was defined as an evenly spread resistance against all races of a pathogen. The definition for horizontal resistance was considered impractical by many scientists and was redefined by Nelson (1978) as a resistance that reduces the infection rate.

1.4.5 Tolerance

Tolerance is a condition in which a plant endures disease without severe loss in quality or yield (Schafer, 1971). Examples of true tolerance are rare as it requires extensive field testing of varieties under disease and disease-free conditions.

1.4.6 Slow rusting

Slow rusting is considered the effect of an incompatible interaction between plant and fungus during different stages of pathogenesis (Kulkarni and Chopra, 1980). In comparison with race-specific resistance, slow rusting appears to be more durable (Kuhn *et al.*, 1978). All incomplete resistances to rusts, including resistance with intermediate infection types, result in slow-rusting (Parlevliet, 1988).

1.4.7 Partial resistance

Partial resistance is a condition where susceptible plants render a lower infection rate than expected from its infection type. It is usually the result of recessive genes with small effects, is durable and lacks race-specific characteristics (Parlevliet, 1988; Craven, 2002). Partial resistance and slow rusting are often considered synonyms.

1.4.8 Durable resistance

Historically cultivars with polygenic resistance have been more durable than those with monogenic resistance (McIntosh, 1992). Durable resistance is recognised when the cultivar containing it is extensively grown on a commercial scale under favourable epidemic conditions for a long time (Johnson, 1979; Johnson, 1981). This resistance, which is not a hypersensitive response, is more likely to be expressed in adult plants than in seedlings (McIntosh, 1992).

1.4.9 Resistance genes

Similar to all traits, resistance is the result of single or multiple genes (Young, 1996). When only one gene is responsible for resistance it is called monogenic, while two genes or more are called oligogenic and polygenic. Single genes tend to be more effective in the short term, but in general are short-lived (Bender *et al.,* 1997). Theoretically a combination of resistance genes should result in more durable resistance. Evidence in this regard was provided by Singh and Rajaram (1995) who combined major and minor linked genes. A disadvantage is that polygenes often require modifiers or interactions amongst each other to produce resistance (Dyck *et al.,* 1966).

The currently named leaf rust (*Lr*), stem rust (*Sr*) and yellow rust (*Yr*) resistance genes are listed in Tables 1.2 – 1.4. These genes are single and mostly dominant (McIntosh *et al.*, 1995). Chromosomal locations of some of the genes have been assigned either through monosomic analysis or by observations of intervarietal chromosome substitution series.

The inheritance and dominance of *Lr* genes can differ between cultivars and what might be a dominant gene in one might be expressed as a recessive gene in another (Pretorius *et al.,* 1995).

1.4.10 Gene interaction

The nature of resistance obtained from interacting genes is usually complex and based on the additive interaction of a few or several genes having minor to intermediate effects (Knott and Yadav, 1993; Singh and Rajaram, 1995).

The additive effect of gene combinations has been reported to be larger than that of the single genes (Luig and Rajaram, 1972; Sharp *et al.,* 1976; Samborski and Dyck, 1982). Cases where no enhanced resistance was obtained from resistance gene combinations were also described (Bender *et al.,* 1997; Bender *et al.,* 2000). It was noted that in some cases at least two *Lr* genes had to be present for the expression of resistance (Singh and McIntosh, 1984), indicating the functioning of classical complementary genes.

1.5 ANALYSIS OF RESISTANCE

The nature, chromosome location and expression of resistance genes can be studied through a wide range of techniques, including screening, inheritance studies, cytogenetics, molecular techniques and histology.

1.5.1 Cytogenetic analysis of resistance

Cytogenetics is the genetic analysis of cells, more particularly the nucleus. To understand chromosomal separation, the meiotic divisions of both the parents and the progeny have to be studied. During meiosis the DNA-strings wind up and form chromosomes. They duplicate and separate during the two phases of meiosis and the end result is four genetically different haploid cells. The amount of genetic material per cell is reduced and the four haploid cells do not necessarily contain the same amount (base pairs) of genetic material. Many genes reside on a single chromosome. Unless separated by crossovers, alleles present at the many loci on each chromosome segregate as a unit during gamete formation. Recombinant gametes resulting from crossing over

enhance genetic variability within species and serve as the basis for constructing chromosomal maps (Klug and Cummings, 1994).

Single genes segregate in Mendelian ratios and response groups can be identified by the plant's phenotype. Incomplete dominance or co-dominance can influence the phenotype of individuals and can make it more difficult to determine the genetic constitution (Klug and Cummings, 1994).

Triticum species have different genomes (A, B, D and G) which are present in different ploidy levels. There are many theories about how polyploids originated, firstly through complete non-disjunction at meiosis, followed by the formation of diploid instead of haploid gametes or it could arise when germ cells duplicated their DNA, but failed to divide (Starr and Taggart, 1992). Another theory states that two different plants with different genomes produce infertile offspring and by doubling the genome, the offspring become fertile. Speciation occurs when polyploidy is followed by successful hybridization. Most hybrids are sterile because they have different numbers or types of chromosomes. This usually prevents homologous pairing at meiosis, but if polyploidy happens to occur in the hybrid's germ cells, the extra set of chromosomes can pair with the original ones at meiosis, and viable gametes are formed (Lupton, 1987).

Polyploids do not usually have normal meiotic division (Lupton, 1987). Self-fertilization is therefore common. When a polyploid recombines genetically with a polyploid of a different level, yet another polyploid-level and genetic

construct is obtained. With an uneven chromosome number, aneuploidy occurs where chromosomes are distributed unequally between daughter cells (Lupton, 1987; Klug and Cummings, 1994).

Although diploid plants with aneuploidy loss are expected to be infertile, *Triticum* has multiple sets of genomes and the expected infertile aneuploids (which have a gain or loss in chromosomes) are frequently fertile (Knott, 1989). This would suggest that the occurrence of multiple genomes compensates for the gains and losses of chromosomes.

Sometimes a resistance gene is carried on one arm of a chromosome, while the susceptibility gene is carried on the other. When no mutation has occurred and both genes are present, the effects of these opposing genes often results in a net effect of a neutral chromosome. Allelic variants in either set can shift the balance to either side of susceptibility or resistance (Lupton, 1987). A polyploid plant has a better chance to maintain a chromosome during aneuploidy (loss) than a diploid. If the net result of a chromosome is neutral, gain or loss aneuploidy will not matter.

1.5.2 Molecular markers and techniques to analyze resistance

Molecular markers are used to detect the presence or absence of a locus in a segregating population (Young, 1999). The identification of molecular markers associated with specific traits, like drought or pest resistance, is important since it enables breeders to select for these and other traits at the seedling stage based on genotype (Transley *et al.*, 1989). Furthermore, genetic

diversity of germplasm collections can be assessed through the analysis of pedigree records and molecular markers (Hongtrakul *et al.*, 1997). Multiclonal plants can be obtained, where individuals have the same ideal characteristics, but remain polymorphic (Cervera *et al.*, 1996).

Different techniques are used to detect molecular markers and to analyse the effect and nature of resistance against stem, leaf and yellow rust. These techniques include DNA sequencing, DAF (DNA Amplified Fingerprinting), isozymes, RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), micro-satellites or simple sequence repeats (SSRs) and AFLP (Amplified Fragment Length Polymorphism). RFLPs have been widely used in systematic studies, but the process is laborious, expensive and has few loci detected per assay. The polymerase chain reaction (PCR) provides the foundation for DNA amplification for RAPDs, DAFs, SSRs and AFLPs (Cho *et al.*, 1996). Using the RAPD-technique a large number of markers are usually obtained per assay (Dedryver *et al.*, 1996). This technique is easier to use than RFLPs and the markers are usually dominant. Because of the sensitivity of the PCR reaction, this technique is not as reproducible as RFLPs (Hill *et al.*, 1996). Micro satellites have the ability to produce co-dominant markers and although easy and inexpensive to perform, require the development of primers. It is clear that there is a need for a reliable marker technique. The AFLP- technique, although not as inexpensive as RAPDs, produces more data points per assay than any other fingerprinting technique and is highly reproducible.

1.5.2.1 AFLP (Amplified Fragment Length Polymorphisms)

AFLP'S is a DNA fingerprinting technique developed by Zabeau (1993) at Keygene N.V. in Wageningen, Netherlands. It is a rapid and efficient method for the production of DNA fingerprints and genetic maps. The AFLP technique consists of three main steps: digestion of genomic DNA with two restriction enzymes, ligation of adapter oligonucleotides to the restriction ends, and selection of fragments by two successive PCR-based amplification steps using primers complementary to the adapter oligonucleotides having one to three selective nucleotides.

AFLPs represent a combination of RFLPs and RAPDs, but make use of selective instead of random primers to detect restriction fragments. It is able to detect polymorphisms with higher efficiency than RAPDs and isozymes (Cervera *et al.*, 1996 and Fuentes *et al.*, 1999). Results obtained by AFLPs are also more repeatable than RAPDs (Jones *et al.*, 1997) because of the highly specific annealing of the primers to the complementary adapter oligonucleotides and can be used for genome mapping (Mackill *et al.*, 1996). AFLP markers are usually dominant, but can also be or co-dominant (Cervera *et al.*, 1996).

AFLPs render many markers per assay (Vos *et al.*, 1995). Increasing or decreasing the number of selective bases or changing base composition can manipulate the number and different types of fragments obtained. The average number of polymorphic fragments per primer combination ranges from 4.2 – 19.25 (Hongtrakul *et al.*, 1997; Fuentes *et al.*, 1999).

1.5.3 Histology of resistance

Histopathology is the study of pathogen infection structure differentiation within host plant tissues. It can be done successfully with the use of an epifluorescence microscope and/or a phase contrast microscope (Kloppers, 1994). Electron microscopy can be used for studies of pathogen behaviour on the leaf surface, or within tissues when leaf fracturing techniques are used (Jacobs *et al.*, 2002).

Histological studies on interactions between plants and rust fungi have demonstrated that several mechanisms of resistance can be discerned (Heath, 1981). Two main types occur, namely prehaustorial and posthaustorial. Prehaustorial resistance is expressed, as its name implies, before a haustorium forms, while posthaustorial resistance refers to the termination of the fungal structure after the first haustorium had formed (Heath, 1982).

Prehaustorial resistance is assumed to be long lasting due to the absence of compatibility between the host and pathogen. Usually the fungus develops normal haustorium mother cells, but a papilla is induced at the site of cell wall penetration. Prehaustorial resistance is common in non-host interactions (Heath, 1981). Posthaustorial resistance is not considered long lasting and is typically associated with HR which ensures that the cell containing the haustorium dies (Niks and Dekens, 1991).

Despite the simplification of fungal abortion at pre- and posthaustorial stages, rust structures can be terminated by the plant's defence mechanisms at any of the infection stages (Niks, 1982). The termination of fungal growth can be classified as prestomatal exclusion, abortive penetration, early abortion or restriction of colony formation. The first three are examples of prehaustorial resistance while the latter is posthaustorial.

Prestomatal termination occurs when the spores fail to germinate, when they produce germ tubes but no appressorium is formed, or when a non-stomatal appressorium is formed (Jacobs, 1989). Teng and Bowen (1985) defined germination as the transformation of a mature spore from a dormant to an active state. In order to germinate, spores need moisture and favourable temperatures. Once germinated the fungus must penetrate the leaf surface through a stomatal opening. An appressorium is formed on the stomatal opening (Littlefield and Heath, 1979). When the appressorium is formed away from the stomatal opening it is called a non-stomatal appressorium.

Abortive penetration is classified as sporelings that did not develop beyond the substomatal vesicle phase, or when a non-penetrating appressorium is formed (Parlevliet and Kievit, 1986). Out of a stomatal appressorium an infection peg that penetrates the stomatal aperture is produced (Kloppers, 1994). Inside the leaf a substomatal vesicle forms. This vesicle produces a primary infection hypha that grows towards the host cell (Roelfs *et al.*, 1992).

Haustorium mother cells are formed when the fungus makes contact with the host cells (Roelfs *et al.*, 1992). During an early abortion fungal growth is, according to definition, considered aborted when less than six haustorium mother cells had formed (Niks, 1983). When six or more haustorium mother cells form, it is considered a colony.

1.6 CONCLUSIONS

From reviewing the literature it is clear that rust pathogens of wheat are highly specialised and adaptable organisms. Their ability to specialise in races and overcome resistance genes confront wheat breeders with an ongoing battle against these devastating pathogens. With scientific investigations of host resistance, i.e. new or unused sources, phenotyping, and breeding and selection techniques, progress in rust control is possible. It is hoped that this study will add to that objective.

Figure 1.1. Spikes of *T. aestivum* (SST55) with shorter awns.

Figure 1.2. Spikes of a *T. durum* plant with long, sharp awns (http://www.ibiblio.org/herbmed/pictures/sf-z-o6.html).


Photo Henriette Kress

Table 1.1. Classification of *Triticum* according to various systems

Taxonomic treatment according to: van Slageren (1994)	Kimber and Sears (1984)	Mac Key (1975)
Section *Monococcun* Dumort		**Section *Monococca* Flaksb.**
Triticum monococcum L.		*Triticum monococcum* L.
ssp. *monococcum*	*Triticum monococcum* L.	ssp. *monococcum*
ssp. aegilopoides (Link) Thell	*Triticum monococcum* L.	ssp. *boeticum* (Boiss.) A. Love D. Love
		var. *aegilopoides* (Link) MacKey
		var. *thaoudar* (Reut.) Percival
Triticum urartu Tumanian ex Gandilyan	*Triticum monococcum* L.	*Triticum urartu* Tum
Section *Dicoccoidea* Flaksb.		**Section Dicoccoidea Flaksb.**
Triticum turgidum L.		*Triticum turgidum* (L.) Thell.
ssp. *turgidum*	*Triticum turgidum* L.	ssp. *turgidum*
		conv. *turgidum*
ssp. *durum* (Desf.) Husn.	*Triticum turgidum* L.	conv. *durum* (Desf.)
		conv. *turancium* (Jakubz. MacKey)
ssp. *polonicum*	*Triticum turgidum* L.	conv. *polonicum* (L.) MacKey
ssp. *carthlicum* (Nevski) A,Love & D. Love	*Triticum turgidum* L.	ssp. *carthlicum* (Nevski) A. Love & D. Love
ssp. *dicoccum* Schrank ex Schubler	*Triticum turgidum* L.	ssp. *dicoccum* (Schrank ex Schubler) Thell.
		ssp. *georgicum* (Dekapr. & Menabde) MacKey
ssp. *paleocolchicum* (Menabde) A. Love & D. Love		
ssp. *turanicum* (Jakubz.) A. Love & D. Love		
ssp. dicoccoides (Koern. Ex Aschers. & Graebn.) Thell.	*Triticum turgidum* L.	ssp. *dicoccoides* (Korn.) Thell.
Triticum timopheevii (Zhuk.) Zhuk.		*Triticum timopheevii* (Zhuk.)
ssp. *timopheevii*	*Triticum timopheevii* (Zhuk.) Zhuk.	ssp. *timopheevii*
ssp. *armeniacum* (Jakubz.) MacKey	*Triticum timopheevii* (Zhuk.) Zhuk.	ssp. *armeniacum* (Jakubz.) MacKey
Section *Triticum*		**Section *Speltoidea* Flaksb.**
Triticum aestivum L.		*Triticum aestivum* (L.) Thell.
ssp. *aestivum*	*Triticum aestivum* L.	ssp. *aestivum*
ssp. *compactum* (Host) MacKey	*Triticum aestivum* L.	ssp. *compactum* (Host) MacKey
ssp. *macha* (Dekapr. & Menabde) MacKey	*Triticum aestivum* L.	ssp. *macha* (Dekapr. & Menabde) MacKey
ssp. *spelta* (L.) Thell.	*Triticum aestivum* L.	ssp. *spelta* (L.) Thell.
ssp. *sphaerococcum* (Percival) MacKey	*Triticum aestivum* L.	ssp. *sphaerococcum* (Percival) MacKey
Triticum zhukovskyi Menabde & Ericzjan	*Triticum zhukovskyi* Men. & Ericzjan	*Triticum zhykovskyi* Menabde & Ericzjan

(http://www.ksu.edu/wgrc/taxonomy.taxtrit.html).

Table 1.2. Wheat leaf rust resistance genes

Lr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Remarks	Reference
1	5DL		Malakof	O;	I	RL6003		Ausemus *et al*. (1946)
2	2DS							Ausemus *et al*. (1946)
2a	2DS		Webster	0;,;1	I,MR	RL6016		Dyck and Samborski (1974)
2b	2DS		Carina	;1,;1+	R,MR	RL6019		Dyck and Samborski (1974)
2c	2DS		Brevit	;IN,23	MR-R	RL 6047		Dyck and Samborski (1974)
3	6B							Ausemus *et al*. (1946)
3a	6BL		Democrat	;C,23	R,MR	RL6002		Browder (1980)
3bg	6BL		Bage	;C,23	MR-MS	RL 6042		Haggag and Dyck (1973)
3ka	6BL		Klein Aniversario	;C,12C	MR-MS	RL6007		Haggag and Dyck (1973)
9	6BL		*T. umbellulatum*	O;	I	RL6010		Soliman *et al*. (1963)
10	IAS		Lee	;,2	R-MS	RL6004		Choudhuri (1958)
11	2A		Hussar	Y	MR	RL6053	test at 18 °C	Soliman, *et al*. (1964)
12	4BS		Exchange		R	RL6011	adult-plant resistance	Dyck, *et al*. (1966)
13	2BS		Frontana		R	Manitou	test at 30 °C	Dyck, *et al*. (1966)
14	7BL							McIntosh *et al.* (1967); Law and Wolfe (1966)
14a	7BL		Hope	X	MS	RL6013	test at 18 °C	Dyck and Samborski (1970)
14b	7BL		Bowie	X	MS	RL6006		Dyck and Samborski (1970)
15	2DS		Kenya 1-12 E-19-J	;C	R	RL6052		Luig and McIntosh (1968)
16	2BS	*Sr*23	Exchange	,1 N	MS-MR	RL6005		Dyck and Samborski (1968a)
17	2AS		Klein Lucero	;1+,0;	MR-MS	RL6008		Dyck and Samborski (1968a)
18	5BL		*T. timopheevi*	2+2-	MS	RL6009	test at 18 °C	Dyck and Samborski (1968a)
19	7DL	*Sr*25	*A. elongatum*	0;	R	RL6040		Sharma and Knott (1966); Browder (1972)
20	7AL		Thew	O.	R	Thew		Browder (1972)
21	1DL		*T.tauschii*		I	RL6043		Rowland and Kerber (1974)
22a	2DS		Thatcher	-	MR	RL6044	adult-plant resistance	Rowland and Kerber (1974)
22b	2DS		*T. tauschii*	-	R	Thatcher	adult-plant resistance	Dyck (1979)
23	2BS		Gabo	1;, 23	MR,MS	RL 6012	test at 25 °C	McIntosh and Dyck (1975)
24	3DL	*Sr*24	*A. elongatum*	0;	R	RL6064		Browder (1973b); McIntosh *et al*. (1976)
25	4AB		Rosen rye	;N	I	Transec		Driscoll and Anderson (1967); McIntosh (1988)
26	IBL	*Sr*31;*Yr*9	Imperial rye	0;, ;1	MR	RL6078		Mettin *et al*. (1973), McIntosh (1988)
27	3BS	*Sr*2, *Lr*31	Gatcher	X-	MR	Gatcher	Functional only with *Lr*31	Singh and McIntosh (1984)
28	4AL		*T. speltoides*	0;	I	RL6079		McIntosh *et al*. (1982)
29	7DS		*A. elongatum*	;1 N	R	RL6080		Sears (1977); McIntosh (1988)
30	4BL		Terenzio	123	R	RL6049		Dyck and Kerber (1981)
31	4BS		Gatcher	X-	MR	Gatcher	Functional only with *Lr*27	Singh and McIntosh (1984)

Lr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Remarks	Reference
32	3D		*T. tauschii*	;1+	MR	RL5497-1		Kerber (1987)
33	1BL	*Lr*44	PI58458	1	MR	RL6057		Dyck *et al*. (1987)
34	7D	*Yr*18	Terenizo	12C	MR-MS	RL6058	test at 10 °C	Dyck (1987)
35	2B		*T. speltoides*	-	?	RL5711	Linked to stem rust resistance	Kerber and Dyck (1990)
36	6BS		*T. speltoides*	01N	?	ER84018		Kerber and Dyck (1990)
37	2AS	*Sr*38,	*T. ventricosa*	12Y	I	RL6081	test at 18 °C	Bariana (1991); Bariana and McIntosh (1993)
38	2AL		*A. intermedium*	?	?	RL6097		Friebe *et al*. (1992)
39	2DS		*T. tauschii*	?	?	KS86NGRCO2		Raupp (www.crl.umn)
40	1D		*T. tauschii*	?	?	KS89WGRCO7		Raupp (www.crl.umn)
41	1D		*T. tauschii*	?	?	KS90WGRC10		Cox *et al*. (1994)
42	1D		*T. tauschii*			WGRC11		Cox *et al*. (1994)
43	7D		*T. tauschii*			WGR16		Cox *et al*. (1994)
44	1BL	*Lr*33	*T. aestivum* spelta 7831	;, 3C	MR	RL6147		Dyck and Sykes (1993)
45	2AS		rye			RL6144		McIntosh (www.crl.umn)
46	1BL		Pavon 76			Lalbahadur (*Lr*1)		Singh and Huerta-Espin (www.crl.umn)
47	7AS		*T. speltoides*			KS 90H450		Dubcovsky *et al*. (www.crl.umn)

Temporary designations of *Lr* genes

Lr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Remarks	Reference
19d			*Thinopyrum distichum*					Marais, GF (www.crl.umn)
B			Brevit	2,;		RL6051		Dyck and Samborski (1968b)
Ech			Exchange	;1+		RL6014		Samborski and Dyck (1976)
H			Harrier	;1				Unpublished (www.crl.umn)
HeIV		*Lr*12	Regina					Bartos, P (www.crl.umn)
I			CSP 44				adult-plant resistance	Shiwani (www.crl.umn)
J			CSP 44				adult-plant resistance	Shiwani (www.crl.umn)
K			Oxley				adult-plant resistance	Shiwani (www.crl.umn)
L			CPAN 1235				adult-plant resistance	Shiwani (www.crl.umn)
LC			Little Club	1C	?	Little Club		Ali *et a* l. (www.crl.umn)
LrA	2Ds		*T. tauschii*	0;		RL5683		Innes, RL (www.crl.umn)
LrAPR			KS91WGRC12				adult-plant resistance	Kloppers, FJ and Pretorius, ZA (www.crl.umn)
LrB	5D		*T. tauschii*	;1		RL5688		Innes, RL (www.crl.umn)
LrC			*T. tauschii*	2	R	RL5782-1	unexpressed in 6X seedlings	Innes, RL (www.crl.umn)
LrD			*T. tauschii*	0;		RL5788	unexpressed in 6X	Innes, RL (www.crl.umn)

Lr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Remarks	Reference
Lrv			G-516 (Favorit)					Ittu, M. *et al.* (www.crl.umn)
M			CPan1235				adult-plant resistance	Shiwani (www.crl.umn)
M marks			Trorysa					Bartos, P (www.crl.umn)
Mo			Morocco	0	?	Morocco	recessive	Ali, I *et al*. (www.crl.umn)
N			VL 404				adult-plant resistance	Shiwani (www.crl.umn)
O			VL 404				adult-plant resistance	Shiwani (www.crl.umn)
T3			Terenizo	-	S-MS	Tc*Lr*T3	adult-plant resistance	Dyck and Samborski (1982)
Tm	6A		*T. monococcum*	0;		Ks92WG		Hussian T (www.crl.umn)
Tr			*T. triunciale*					Aghaee-Sarbarzeh , M. *et al*. (www.crl.umn)
Trp-1			Torepi				adult-plant resistance	Barcellos, AL (www.crl.umn)
Trp-2			Torepi				adult-plant resistance	Barcellos, AL (www.crl.umn)
VPM	7DL		VPM1					Worland *et al*. (1988)
W								Dyck and Jedel (1989)

(McIntosh *et al*., 1995; http://www.crl.umn.edu/res_gene/wlr.html).

Table 1.3. Wheat stem rust resistance genes

Sr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Remarks	Reference
1							See *Sr*9d	
2	3BS		*Triticum turgidum*	-	S	CnS(Hope3B)	Few uredinia	Ausemus *et al*. (1946); Knott (1968)
5	6DS	*Sr*42	Reliance	0, ; 1	I	ISr5-Ra		Ausemus *et al*. (1946); Sears *et al*. (1957)
6	2DS		Red Egyptian	O;, X	R	ISr6Ra	Test at 18 °C	Knott and Anderson (1956)
7								Knott and Anderson (1956)
7a	4BL		Kenya117A	2C	MR	Line G sel		Loegering and Sears (1966)
7b	4BL		Marquis	2+-	MS	ISr7b-Ra		Loegering and Sears (1966)
8								Knott and Anderson (1956)
8a	6AS		Red Egyptian	2+-	MS	ISr8-Ra		Loegering and Sears (1966)
8b	6AS		Barleta Benvenuto	X	MR	Barleta		Singh and McIntosh (1986)
9								Knott and Anderson (1956)
9a	2BL		Red Egyptian	2-, 2+3	MR, MS	ISr9a-Ra		Knott and Anderson (1956); Green *et al*. (1960)
9b	2BL		Kenya117A	2, 23	MR	W2691Sr9b		Green *et al*. (1960)
9d	2BL		Hope	;2-	MR	ISr9d Ra		Knott (1966)
9e	2BL		*T. turgidum*	;, ;1+	R	Vernstein		McIntosh and Luig (1973a)
9f	2BL		Chinese Spring	2	?	Chinese		Loegering (1975)
9g	2BL	*Yr*7	Lee	2-	MR	CnSSr9g		McIntosh and Luig (1973a)
10			Egypt NA95	X-N	MR	W2691Sr10		Knott and Anderson (1956)
11	6BL		Lee	;1=C, 2	R-MR	ISrl1-Ra		Knott and Anderson (1956)
12	3BS		Thatcher	;1+, X	I-R	BtSr12Tc	Test at 18 °C	Sheen and Snyder (1964)
13	6AL		*T. turgidum*	2+	MR-MS	W2691Srl3	Test at 25 °C	Knott (1962)
14	1BL		*T. turgidum*	;1CN, 13CN	MS	Line A sel		Knott (1962)
15	7AL		Norka	;1CN, X-CN	MS-S	W2691Srl5	Test at 18 °C	Watson and Luig (1966)
16	2BL		Thatcher	2-, 2+	MS	ISr16-Ra		Loegering and Sears (1966)
17	7BL		*T. turgidum*	;1-N	R	CS (Hope7B)	Test at 18 °C	McIntosh *et al*. (1976); McIntosh (1988)
18	1D		Marquis	; I	I	LCSr18Mq		Baker *et al*. (1970)
19	2BS		Marquis	1	R	LCSr19Mq		Anderson *et al*. (1971)
20	2BL		Marquis	2	MS	LC		Anderson *et al*. (1971)
21	2AL		*T. monococcum*	0;	R	Einkorn		The (1973)
22	7AL		*T. monococcum*	22-	MR	SwSr22T.B.		The (1973)
23	2BS	*Lr*16	Exchange	23C	MS	Exchange		McIntosh and Luig (1973b)
24	3DL	*Lr*24	*Agropyron elongatum*	2+-	MR-MS	BtSr24Agt		McIntosh *et al*. (1976)
25	7DL	*Lr*19	*A. elongatum*	2	MS-S	LCSr25Ars		McIntosh *et al*. (1976)
26	6AL		*A. elongatum*	;2-	MR	Eagle		Knott (1961); McIntosh *et al*. (1976)
27	3A		*Secalis cereale* (Imperial)	0;	I	W2691Sr27		Acosta (1962); McIntosh (1988)
28	2BL		Kota	0,. 0:	I	W2691Sr28K		McIntosh (1978)

Sr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Remarks	Reference
29	6DL		Etiole de Choisy	2-, 23	MS	PusaSr29Edc		Dyck and Kerber (1977)
30	5DL		Webster	2-, 2+	MS	BtSr30Wst		Knott and McIntosh (1978)
31	1BL	*Lr*26, *Yr*9	*S. cereale* (Imperial)	02-	R	Line		Zeller (1973); McIntosh (1988)
32	2A, 2B		*T. speltoides*	2-	MR	ER5155		McIntosh (1988)
33	1DL		*T. tauschii*	2-	MR	TetraCanthat		Kerber and Dyck (1979); McIntosh (1988)
34	2A,2B	*Yr*8	*T. comosa*	23CN	MR	Compair		McIntosh *et al*. (1982)
35	3AL		*T. monococcum*	0; I	I	Mq(2)5xG291		McIntosh *et al*. (1984)
36	2BS		*T. timopheevi*	0;, X-	I, Trace S	W2691SrTt-1		McIntosh (1988)
37	4AL		*T. timopheevi*	0;	I	W2691SrTt-2	Off-type plants common	McIntosh (1988)
38	2AS	*Lr*37,*Yr*17	*T. ventricosa*	;1	MS	VPM1	Test at 18 °C	Bariana (1991); Bariana and McIntosh (1993)
39	2B	*Lr*35	*T. speltoides*	2-	-	RL5711		Kerber and Dyck (1990)
40	2BS		*T. araraticum*	-	-	RL6087		Dyck (1992)
41	4D		Waldron		?	WDR-B1		Williams (1993)
42	6D	*Sr*5	Norin 10					Kim, N-S (www.crl.umn)
43	7D		*Agropyron elongatum*			KS10-2		Kibirige-Sebunya and Knott (1983)
44	7DS							Friebe *et al*. (1993)
45	1DS		*T. tauschii*			RL5289		Marrais (1992)

Temporary designations for *Sr* genes

Sr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Remarks	Reference
;			Frn//Ky58/Nth	;2	R, MS	8N122		Unpublished (www.crl.umn)
Agi			*A. intermedium*	;2	R	A.		Unpublished (www.crl.umn)
dp-2			*T. turgidum* (Golden Ball)	2	MR	Media Ap9d		Unpublished (www.crl.umn)
Em			Entrelargo de Montijo					McIntosh, RA (www.crl.umn)
Gt			Gamut	2+	MS	BtSrGtGt		Unpublished (www.crl.umn)
H			H-44	13, 23C	MS	H44 deriv.		Unpublished (www.crl.umn)
Kt-2	2BL		Kota	2	MS	Line AE sel		Unpublished (www.crl.umn)
LC			Little Club	;1+	?	Little Club		Unpublished (www.crl.umn)
M			*T. turgidum* (Maruccos)	X	?	Maruccos		Unpublished (www.crl.umn)
McN			McNair 701	;2-	?	McNair 701		Unpublished (www.crl.umn)
MqX			Marquis	23	MS	PdSrXMq		Unpublished (www.crl.umn)
Pl			*T. turgidum* (Peliss)	;1	?	Peliss		Unpublished (www.crl.umn)
Pt			*T. turgidum*	2-	?	Petterson		Unpublished (www.crl.umn)
A	1D		*T. taushii*	;1	MR	RL5778		Innes (www.crl.umn)
D	1D		*T. taushii*					McIntosh, RA (www.crl.umn)

Sr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Remarks	Reference
X	1D		*T. taushii*					McIntosh, RA (www.crl.umn)
Satu			*Satu triticale*					McIntosh, RA (www.crl.umn)
Tmp	4B		Triumph 64	2-, 23	MS	Triumph 64		Unpublished (www.crl.umn)
Tt-3			*T. timopheevi*	1+C I-	I-R	Fed *2/SrTt-3		Unpublished (www.crl.umn)
U	2D		Red Egyptian	X-CN	?	CnSSrURE		Unpublished (www.crl.umn)
Wld 1			Waldron	2, 2+	R-MS	BtSrWldWld		Unpublished (www.crl.umn)
Wst-2			Webster	2	MR	LCSrWst2Ws		Unpublished (www.crl.umn)
Zdar	1B		Zdar			Zdar		Bartos, P and Kosner, J (www.crl.umn)
A	2D		Coteau	0;	R			Williams, ND (www.crl.umn)
B	2BL		Coteau	2	MS			Williams, ND (www.crl.umn)
C	2B		Len	2	MS			Williams, ND (www.crl.umn)

(McIntosh *et al*., 1995; http://www.crl.umn.edu/res_gene/wsr.html).

Table 1.4. Wheat yellow/stripe rust resistance genes

Yr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Other genes in Tester	Remarks	Reference
1	2A		Chinese 166	0;	1	Chinese 166			Zadoks (1961); Lupton and Macer (1962)
2	7B		Heines VII	0; - 2	4	Heines VII	HVII		Lupton and Macer (1962)
3a	1B		Vilmorin 23	;	2	Vilmorin 23	V23		Lupton and Macer (1962)
3b	1B		Hybrid 46	;	2	Hybrid 46	4b, H46		Lupton and Macer (1962)
3c	1B		Minister	;	2	Minister	Min		Lupton and Macer (1962)
4a	6B		Capelle-Desprez	;	2	Capelle-Desprez	3a,16		Lupton and Macer (1962)
4b	6B		Hybrid 46	0;	1	Hybrid 46	3b, H46		Lupton and Macer (1962)
5	2BL		*Triticum spelta album*	0;	1	*T. spelta album*			Macer (1966)
6	7BS		Heines Kolben	; - N;	4	Heines Kolben	2, HK		Macer (1966)
7	2BL	*Sr*9g	*lumillo durum*	;N	2	Lee	Le1, Le2		Macer (1966)
8	2D	*Sr*34	*T. comosa*	0; - ;	1	Compair	Com		Riley *et al*. (1968)
9	1BL	*Sr*31	Imperial rye	0;	1	Riebesel 47/51			Macer (1975)
10	1BS		Moro	1	1	Moro	Mor		Macer (1975)
11			Joss Chambier	sus		Joss Chambier		adult-plant resistance	Priestley (1978); McIntosh (1988)
12			Caribo	sus		Mega		adult-plant resistance	Priestley (1978); McIntosh (1988)
13			Ibis	sus		Maris Huntsman		adult-plant resistance	Priestley (1978); McIntosh (1988)
14			Falco	sus		Maris Bilbo		adult-plant resistance	Priestley (1978); McIntosh (1988)
15	1BL		Dippes Triumph	0;	1	*T. dicoccoides* G-25	?		Gerechter-Amitai *et al*. (1989)
16	2DS		Capelle-Desprez	-	3	Capelle-Desprez	3a, 4a	adult-plant resistance	Worland and Law (1986)
17	2AS	*Lr*37,	*T. ventricosa*	;C - ;1	MR	VPM1		test at 10 °C	Bariana and McIntosh (1993)
18	7D	*Lr*34	Frontana	-	3	Jupateco 73R		adult-plant resistance	Singh (1992)
19	5B		Compair				8	see *Yr*Com	Chen and Line (1992)
20	6D		Fielder					see *Yr*Fie	Chen and Line (1992)
21	1B		Lemhi					see *Yr*Lem	Chen and Line (1992)
22	4D		Lee			Lee	7, 23	see *Yr*Le1	Chen and Line (1992)
23	6D		Lee			Lee	7, 22	see *Yr*Le2	Chen and Line (1992)
24	1B		K733	;1N					McIntosh, RA (www.crl.umn)
25	1D		TP1295			Strubes Dickkopf			Calonnec, A and Johnson, R (www.crl.umn)
26	6AS		*Haynaldia villosa*			Yangmai-5			Yildirim, A. *et al*. (www.crl.umn)
27	2BS		Selkirk						McIntosh, RA (www.crl.umn)
28	4DS		*T. tauschii* W-219						Singh, RP (www.crl.umn)
29	1BL	*Lr*46	Lalbahadur		;	Lalbahadur		adult-plant resistance	Singh, RP (www.crl.umn)
30	3BS				;	Opata 85		adult-plant resistance	Singh, RP (www.crl.umn)

Temporary designations of *Yr* genes

Yr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Other genes in Tester	Remarks	Reference
A			Anza	;CN1 to 2+					Wellings, CR *et al*. (www.crl.umn)
A			Avocet	4	4	Avocet			Unpublished (www.crl.umn)
A1			Gaines					adult-plant resistance	Milus, E (www.crl.umn)
A2			NuGaines				A1	adult-plant resistance	Milus, E (www.crl.umn)
A3			Luke				A4	adult-plant resistance	Milus, E (www.crl.umn)
A4			Luke				A3	adult-plant resistance	Milus, E (www.crl.umn)
A5			Durch				A6	adult-plant resistance	Chen, XM and Line, RF (www.crl.umn)
A6			Durch				A5	adult-plant resistance	Chen, XM and Line, RF (www.crl.umn)
A7			Stephens				A8	adult-plant resistance	Chen, XM and Line, RF (www.crl.umn)
A8			Stephens				A7	adult-plant resistance	Chen, XM and Line, RF (www.crl.umn)
Ab			Alba			Alba	Alb	adult-plant resistance	Stubbs, RW (www.crl.umn)
Alb			Alba			Alba	Ab		Stubbs, RW (www.crl.umn)
B-a	R		Bersee			Bersee	14, B-b, B-c	adult-plant resistance	Bariana and McIntosh (1993)
B-b			Bersee			Bersee	14, B-a, B-c	adult-plant resistance	Bariana and McIntosh (1993)
B-c			Bersee			Bersee	14, B-a, B-b	adult-plant resistance	Bariana and McIntosh (1993)
C			Yecora Rojo					adult-plant resistance	Zwer, PK and Qualset, CO (www.crl.umn)
CaV			Zdar			Zdar	4b, 5		Bartos, P (www.crl.umn)
Ck			Cook, Oxley			Cook			Bariana and McIntosh (1993)
Cle	4B		Clement			Clement	9		Chen, XM and Line, RF (www.crl.umn)
Com	5B		Compair			Compair	8	see *Yr* 19	Chen, XM and Line, RF (www.crl.umn)
CV1			Carstens V			Carstens V	12, CV2 ,		Chen, XM and Line, RF (www.crl.umn)
CV2			Carstens V			Carstens V	12, CV1 ,		Chen, XM and Line, RF (www.crl.umn)
CV3			Carstens V			Carstens V	12, CV1 ,		Chen, XM and Line, RF (www.crl.umn)
D	6A		Druch			Druch	3a, Dru		Chen, XM and Line, RF (www.crl.umn)
D			Yecora Rojo						Zwer, PK and Qualset, CO (www.crl.umn)
Da1	1A		Daws			Daws	Da2		Zwer, PK and Qualset, CO (www.crl.umn)
Da2	5D		Daws			Daws	Da1		Zwer, PK and Qualset, CO (www.crl.umn)
Dru	5B		Druch			Druch	3a, D		Zwer, PK and Qualset, CO (www.crl.umn)
DT			Dippes Triumph			Dippes Triumph		adult-plant resistance	Stubbs, RW (www.crl.umn)
E	3E		Elytrigia (Lophoprum)	0		Cns sub line	Yr18		Ma, JX (www.crl.umn)
EDC			Etoile de Choisy			Etoile de Choisy		adult-plant resistance	Ma, JX (www.crl.umn)
Falco			Falco			Falco		adult-plant resistance	Ma, JX (www.crl.umn)
Fie	6D		Fielder			Fielder	6	See *Yr* 20	Chen, XM and Line, RF (www.crl.umn)
Fmg			Flamingo			Flamingo		adult-plant resistance	Stubbs, RW (www.crl.umn)
G			Gaby			Gaby			Stubbs, RW (www.crl.umn)
H			Anza					adult-plant resistance,	Zwer, PK and Qualset, CO (www.crl.umn)

Yr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Other genes in Tester	Remarks	Reference
Hr			Harrier		MRMS	Harrier		adult-plant resistance	Bariana and McIntosh (1993)
H Peko			Heines Peko			Heines Peko		adult-plant resistance	Stubbs, RW (www.crl.umn)
H4			Heines IV			Heines IV			Stubbs, RW (www.crl.umn)
H46	6A		Hybrid 46			Hybrid 46	3b, 4b		Chen, XM and Line, RF (www.crl.umn)
H52	1BL		*T. turgidum*						Peng, J.H. *et al* (www.crl.umn)
HVII	4A		Heines VII			Heines VII	2		Peng, J.H. *et al* (www.crl.umn)
J			Glennson 81						Zwer, PK and Qualset, CO
K733	1B		K733 durum			K733			McIntosh, RA (www.crl.umn)
KK-1			Kenya Kubangu						McIntosh, RA (www.crl.umn)
KK-2			Kenya Kubangu						McIntosh, RA (www.crl.umn)
Kg1			King		MRMS	Flinders		adult-plant resistance	Bariana and McIntosh (1993)
Kg2			King		MR	King	Kg1	adult-plant resistance	Bariana and McIntosh (1993)
L			Ollanta						Zwer, PK and Qualest, CO
LD			Langs Dfoerfler 5111			Langs Dfoerfler 5111			Stubbs, RW (www.crl.umn)
Le1	4D		Lee			Lee	7, Le2	See *Yr*22	Chen, XM and Line, RF (www.crl.umn)
Le2	6D		Lee			Lee	7, Le1	See *Yr*23	Chen, XM and Line, RF (www.crl.umn)
Lely			Lely			Lely		adult-plant resistance	Stubbs, RW (www.crl.umn)
Lem	1B		Lemhi			Lemhi			Chen, XM and Line, RF (www.crl.umn)
Luq	2B		Luqiyu			Luqiyu			Chen, XM and Line, RF (www.crl.umn)
Min	4A		Minster			Minster	3c		Chen, XM and Line, RF (www.crl.umn)
Mor	4B		Moro			Moro	10		Chen, XM and Line, RF (www.crl.umn)
ND	4A		Nord Desprez			Nord Desprez	3a		Chen, XM and Line, RF (www.crl.umn)
Opal			Opal			Opal		adult-plant resistance	Stubbs, RW (www.crl.umn)
P1			Pavon 76		MRMS	Pavon 76	P2	partial additive APR	Singh and Rajaram (www.crl.umn)
P2			Pavon 76		MRMS	Pavon 76	P1	partial additive APR	Singh and Rajaram (www.crl.umn)
Pa1			Paha			Paha	Pa2, Pa3		Chen, XM and Line, RF (www.crl.umn)
Pa1			Paha			Paha	Pa1, Pa3		Chen, XM and Line, RF (www.crl.umn)
Pa1			Paha			Paha	Pa2, Pa3		Chen, XM and Line, RF (www.crl.umn)
Pr1			Produra			Produra	Pr1, Pr2		Chen, XM and Line, RF (www.crl.umn)
Pr2			Produra			Produra	Pr1, Pr3		Chen, XM and Line, RF (www.crl.umn)
Pr3			Produra			Produra	Pr1, Pr2		Chen, XM and Line, RF (www.crl.umn)
S	3B		Stephens			Stephens	3a, Ste		Chen, XM and Line, RF (www.crl.umn)
SD			Strubes Dickkopf			Strubes Dickkopf			Stubbs, RW (www.crl.umn)
Slk	2B		Selkirk			Selkirk		see *Yr*27	McIntosh, RA (www.crl.umn)
SP			Spaldings Proflic			Spaldings Proflic			Chen, XM and Line, RF (www.crl.umn)
Ste	2B		Stephens			Stephens	3a, S		Chen, XM and Line, RF (www.crl.umn)
Su92	2B		Suwon 92/Omar			Suwon 92/Omar			Chen, XM and Line, RF (www.crl.umn)
T1			Tonichi 81		MRMS	Tonichi 81	18, T2	additive APR	Singh and Rajaram (www.crl.umn)

Yr gene	Chromosome Location	Linkage	Original source	Seedling reaction	Adult reaction	Tester	Other genes in Tester	Remarks	Reference
T2			Tonichi 81		MRMS	Tonichi 81	18, T1	additive APR	Singh and Rajaram (www.crl.umn)
Tr1	6D		Tres			Tres	Tr2		Chen, XM and Line, RF (www.crl.umn)
Tr2	3A		Tres			Tres	Tr1		Chen, XM and Line, RF (www.crl.umn)
Tye	6D		Tyee			Tyee			Chen, XM and Line, RF (www.crl.umn)
V23	6A		Vilmorin 23			Vilmorin 23	3a		Chen, XM and Line, RF (www.crl.umn)
Yam	4B		Yamhill			Yamhill	2, 4a		Chen, XM and Line, RF (www.crl.umn)
Z			Zaragoza 75		MS	Zaragoza 75		adult-plant resistance	Singh and Rajaram (www.crl.umn)

(McIntosh *et al*., 1995; http://www.crl.umn.edu/res_gene/wyr.html).

CHAPTER 2

THE TRANSFER OF LEAF RUST RESISTANCE FROM *TRITICUM TURGIDUM* TO *TRITICUM AESTIVUM*

2.1 INTRODUCTION

The aim of wheat breeding is the development of a widely adapted cultivar that displays all the traits and qualities desired by the breeder and farmer (Peterson, 1965). Leaf rust, caused by *Puccinia triticina* Eriks., is a disease affecting quality and yield of wheat and therefore has an economical impact. The existence of rusts on wheat has been recognized since Biblical times (Cooke, 1977; Harlan, 1981) but breeding for resistance against leaf rust was only initiated in the early 1900s. The objective of breeding wheat for resistance to leaf rust is to obtain a cultivar that will remain resistant for at least its commercial life span (Bender *et al.*, 2000). Continued research on the genetics of leaf rust resistance and concurrent breeding for resistance is therefore necessary for sustained production and minimal losses.

As a cause of inbreeding, cultivated wheat has a higher sensitivity to pathogens (Jiang *et al.*, 1994). The wild *Triticum* species have not been extensively used for wheat production and can therefore be a valuable source of resistance genes (Knott and Dvorak, 1976; Knott, 1989; Jones *et al.*, 1995). It was hoped that resistance genes derived from wild species would be more durable than those from domesticated cultivars, but unfortunately most single resistance genes from alien species have become ineffective (Knott, 1989; Antonov and Marais, 1996).

However, the search for new or unused resistance genes should not be discouraged as these genes could be used in combination with sources of known durability.

Breeding techniques allow wide crosses not generally expected in nature and species sharing a primary gene pool are usually sexually compatible (McIntosh, 1991). Despite the successful production of F_1 hybrid seed, especially when resistance genes are transferred from a lower ploidy level to a higher one, negative effects e.g. infertility, chromosome breakage, interactions between resistance genes and suppressor genes, or aneuploidy, may result from such crosses (Lin and Kuo, 1995).

The aim of this study was to transfer adult-plant resistance genes conferring resistance to *Puccinia triticina* from *T. turgidum* to *T. aestivum* and to determine if these genes were inherited according to Mendelian ratios.

2.2 MATERIAL AND METHODS

2.2.1 Wheat cultivars and lines

Seed of the bread wheat cultivar SST55 and wild species of *Triticum turgidum* were planted in 1-litre-capacity pots. The five tetraploid lines were *T. turgidum* ssp. *dicoccoides* (Körn. ex Aschers. and Graebn.) Thell. var. *kotchianum* (also known as *Triticum dicoccoides* [(Körn. ex Aschers. and Graebn.) Schweinf.] (UFS accession 91), *T. turgidum* ssp. *pyramidales* (UFS accession 318), *T. turgidum*

ssp. *durum* (Desf.) Husn var. *libycum* (also known as *Triticum durum* (Desf.) (UFS accession 129), *T. turgidum* ssp. *persicum* var. *rubiginosum* (UFS accession 353) and *T. turgidum* ssp. *abyssinicum* (UFS accession 116).

The flowering period of the wild species was obtained from preliminary tests done by Barnard (1999b). To ensure corresponding flowering dates a series of SST55 plantings was made at two-week intervals. To grow vigorous plants, 50 ml of a 3 g/l hydroponic nutrient solution (6.5:2.7:13 N:P:K plus micro elements) were administered per pot and continued for three days per week for the remainder of the experiment.

When the full spike was visible, prior to anthesis, SST55 was emasculated and used as the female parent in the crosses. F_1 seeds were obtained by inserting pollen shedding spikes of wild wheat parents into glassine bags (25 x 6.2 cm) along with the female spike of SST55. To ensure pollen dissemination to the stigmas of SST55 bags were regularly tapped.

2.2.2 F_1 progeny

The F_1 progeny was planted four months after seed harvesting. The F_1 seeds were washed in 30 % ethanol for 1 min. followed by soaking in a 1:6 sodium hyperchlorite and water solution for 1 min. before washing twice in distilled water (modified from Baxter and Van der Linde, 1999). The seeds were placed on filter paper drenched in 1 % H_2O_2 solution in glass Petri dishes. Filter paper was kept

moist using the above mentioned solution until germinated seeds were ready to transfer to the glasshouse. Twenty F_1 seeds of UFS accession 116, 19 seeds of UFS accession 91, 20 seeds of UFS accession 129 and 10 seeds of UFS accession 318 germinated. Plants were grown in soil in 1-litre-capacity pots at 15 to 20 °C in rust-free cubicles in a glasshouse. Daylight was supplemented by 14 h of light emitted by fluorescent tubes (120 $\mu Em^{-2}s^{-1}$).

2.2.3 F_2 progeny

F_2 seeds were germinated on plates containing filter paper moistened with a 1 % hydrogen peroxide solution. F_2 seedlings were planted in 1-litre-capacity pots (10 plants per pot) and fertilized three times per week. In total, 162 plants of SST55/*T. turgidum* ssp. *abyssinicum* (116), 159 of SST55/*T. turgidum* ssp. *pyramidales* (318), 223 seedling plants of SST55/*T. turgidum* ssp. *durum* v. *libycum* (129) and 135 of SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* (91) were planted. All plants were grown in a glasshouse with conditions as described for the parental lines. Infection studies were performed on F_2 plants when they reached the flag leaf stage. Inoculum of pathotype UVPrt9 of *P. triticina* was used for infection.

2.2.4 F_3 progeny

For progeny testing, 780 F_2 seeds of the cross SST55/*T. turgidum* ssp. *pyramidales* (318), 600 of SST55/*T. turgidum* ssp. *durum* v. *libycum* (129), 870 of SST55/*T. turgidum* ssp. *abyssinicum* (116) and 530 of SST55/*T. turgidum* ssp.

dicoccoides v. *kotchianum* (91) were planted, grouped per F_2 reaction class, in 1-litre-capacity pots and placed in a glasshouse. A nutrient supplement, as described previously, was administered once a week. To test the adult-plant reaction, inoculations with leaf rust pathotype UVPrt9 of *P. triticina* were done on the flag leaves of adult plants.

2.2.5 Backcrosses

Crosses were also attempted between the F_1's and *T. aestivum* plants. Both the F_1's and *T. aestivum* (SST55, SST825, Palmiet and Nantes) were used alternately as male and female parents. Backcrosses of resistant F_2 and F_3 plants to SST55 were also made.

2.2.6 Inoculation with *Puccinia* species

Rust infections with *P. triticina* were done on seedlings and the adult *T. turgidum* parental lines, the SST55 bread wheat parent, and F_1, F_2 and F_3 progeny. *T. turgidum* seedlings were sprayed with a suspension of fresh spores of *P. triticina* pathotypes UVPrt2, UVPrt3, UVPrt9 and UVPrt13 in light mineral oil. Seedlings were also infected with *P. graminis* f. sp. *tritici* pathotypes UVPgt50 and UVPgt51 and pathotypes 6E16 and 6E22 of *P. striiformis* f. sp. *tritici*. The leaf and stem rust pathotypes have virulence to several *Lr* and *Sr* genes whereas the stripe rust pathotypes are representative of the variations occurring in South Africa. The flag leaves of adult plants were spray-infected by a suspension of UVPrt9 of *P. triticina*. UVPrt9 was used because it has been the dominant leaf rust pathotype

in South Africa (Van Niekerk, 2001). Plant growth stage at inoculation was determined according to the Zadoks scale (Zadoks *et al.*, 1974). Infection types (0 to 4 scale; McIntosh *et al.*, 1995) were recorded two weeks after inoculation.

2.2.7 Pollen viability

In order to determine the fertility and viability of pollen, both parents, their F_1 and other bread wheat controls were tested according to two methods. For the first method, mature pollen was coloured with 0.75 % toluidin blue on a glass microscope slide smeared with Mayers albumen. Viable pollen coloured light blue while dead pollen were dark blue when observed at 400x with a Nikon Optiphot microscope (Figure 2.1). The second method made use of the fluorescent colourant fluorescein diacetate (FDA) (Huang and Johnson, 1996). Pollen was suspended in 100 µl distilled water. Five µl of 0.5 % FDA was placed on an object slide and allowed to dry. Twenty five µl of the pollen suspension was then placed on the slide, covered with a glass cover slip after 10 min. and observed using a Nikon Labophot epifluorescence microscope. With filter combination UV-1A (excitation filter 330 – 380 nm and barrier filter 420 nm) dead pollen was blue and living pollen was blue and yellow, but a better distinction between viable and non-viable pollen was made with filter set B-2A (excitation filter 450 – 490 nm and barrier filter 520 nm) where living pollen fluoresced bright yellow (Figure 2.2).

2.2.8 Statistical analysis

To test the observed segregation ratios for adherence to Mendelian inheritance standard chi-square analysis was conducted (Steel and Torrie, 1980). For the purpose of statistical Mendelian analysis intermediate reactions were considered as resistance and grouped in the resistant category.

2.3 RESULTS AND DISCUSSION

2.3.1 P_o

Results of P_0, F_1 and F_2 are tabulated in Table 2.1. Seedling infection types produced by the *T. turgidum* parents are tabulated in Table 2.2. Of the tested seedling plants all but *T. turgidum* ssp. *persicum* v. *rubiginosum* (353) and *T. turgidum* ssp. *pyramidales* (318) were susceptible to leaf rust. No resistance against stem rust was recorded, but *T. turgidum* ssp. *persicum* v. *rubiginosum* (353) and *T. turgidum* ssp. *durum* v. *libycum* (129) were resistant to pathotypes 6E16 and 6E22 of *P. striiformis* f. sp. *tritici* (Figure 2.3).

All *T. turgidum* parental lines were resistant to UVPrt9 in the adult stage with reactions ranging between ; and ;1. *T. turgidum* ssp. *dicoccoides* v. *kotchianum* (91), *T. turgidum* ssp. *pyramidales* (318) and *T. turgidum* ssp. *abyssinicum* (116) had hypersensitive flecks whereas *T. turgidum* ssp. *durum* v. *libycum* (129) showed a similar response (;1CN) except for severe associated chlorosis and necrosis. The *T. aestivum* parent SST55 had a 3++ susceptible reaction when infected with UVPrt9.

Preliminary studies by Barnard (1999b) on adult *T. turgidum* species inoculated with a mixture of pathotypes 2, 3, 9 and 13 of *P. triticina* showed the following reactions: *T. turgidum* ssp. *dicoccoides* v. *kotchianum* (UFS accession 91), *T. turgidum* ssp. *persicum* var. *rubiginosum* (UFS accession 353) and *T. turgidum* ssp. *durum* v. *libycum* (UFS accession 129) had ;1 adult-plant resistance, while *T. turgidum* ssp. *pyramidales* (UFS accession 318) and *T. turgidum* ssp. *abyssinicum* (UFS accession 116) had hypersensitive flecks. This data correlate with the results found in the present study. The only difference between data from Barnard (1999b) and this experiment was accession 91 which showed necrotic flecks and not a ;1 reaction as reported by Barnard (1999b). Although a few small, sporulating pustules formed, *T. turgidum* ssp. *durum* v. *libycum* (129) showed a very strong necrotic reaction. From these results it was clear that all resistance sources conditioned an HR resistance type.

Cultivars carrying genes conferring HR-associated resistance have been an effective and economical way of controlling wheat leaf rust (Nelson, 1978). Almost all major genes belong in this phenotypic category (Parlevliet, 1988). Although this type of resistance is considered to be non-durable (Nelson, 1978) it can be used in combination with other genes in gene stacking to produce effective durable resistance. Many breeders avoid the HR type of resistance due to historical examples of pathogen adaptation to these resistance barriers. However, vertical resistance can prove extremely useful due to the high levels of

resistance it conditions. If used wisely in resistance complexes, where the vulnerability of single genes is protected, vertical resistance will continue to play a role in the genetic control of leaf rust.

2.3.2 F_1

Thirty one F_1 seeds were obtained from the SST55/*T. turgidum* ssp. *abyssinicum* (UFS accession 116) cross. From the SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* (UFS accession 91) cross, 35 seeds, from SST55/*T. turgidum* ssp. *durum* v. *libycum* (UFS accession 129), 24 seeds, from SST55/*T. turgidum* ssp. *pyramidales* (UFS accession 318), 20 seeds and from *T. turgidum* ssp. *persicum* var. *rubiginosum* (UFS accession 353), five seeds were obtained. Viable F_1 progeny was obtained from the crosses SST55 with UFS accessions 91, 318, 129 and 116. A high percentage of germination was recorded for all F_1 crosses, but not all germinating seeds produced viable plants. In some cases no roots developed, while in extreme cases no radicles were produced.

F_1 SST55/*T. turgidum* ssp. *durum* v. *libycum* (129) was the only accession that showed adult-plant resistance (Figure 2.4). Resistance is therefore the effect of a dominant gene or genes. A high frequency of necrosis was observed in this cross.

Pollen viability of the F_1's was considerably lower than that of either parent (Figure 2.5). Viability of *T. aestivum* plants ranged between 61.3 % and 71.2 %.

The pollen viability of the *T. turgidum* parents was lower and ranged between 37.6 % and 52.1 %. Incompatibilities often occur in a cross between wild tetraploid and hexaploid bread wheat. The non-viability of F_1 seeds, low pollen viability and sterility of backcrosses were therefore expected. Future crosses between such genotypes should thus take into account the low seed set in hybrids.

2.3.3 F_2

Adult F_2 plants showed a wide spectrum of reactions to *P. triticina*. It differed from fully susceptible (infection type 4) and intermediate (infection type ;1-2) to very resistant (infection type ; or 1N) (Figure 2.6). In most cases the spikes of resistant plants resembled the phenotype of their wild wheat parent, indicating that several backcrosses may be necessary to regain the bread wheat agrotype. In all crosses some of the plants were sterile and produced no seeds. SST55/*T. turgidum* ssp. *durum* v. *libycum* (129) showed a clear hypersensitive response that can be used as a phenotypical marker for the gene. Although this resistance appears effective, the considerable amount of leaf necrosis will contribute to yield losses in epidemic situations. Samborski and Peturson (1960) showed that yield losses in leaf rust-resistant wheats due to HR amounted to 28 %.

Despite a clear distinction between resistance and susceptibility the genes did not necessarily segregate according to Mendelian ratios. Chi-square (X^2) tests (Table 2.1) supported a 9:7 ratio for F_2 populations derived from SST55/*T.*

turgidum ssp. *durum* v. *libycum* (129) and SST55/*T. turgidum* ssp. *abyssinicum* (116). This ratio suggests complementary dominant gene action. However, all F_1 plants of the cross with accession 116, which theoretically contained both dominant alleles, were susceptible and thus did not support the complementary model. It is possible that the resistance genes of this cross were suppressed in the F_1 where only one D-genome was present as described in other work of similar nature (Bai and Knott, 1992). In SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* (91) and SST55/*T. turgidum* ssp. *pyramidales* (318) the F_1 and F_2 data suggested a recessive gene, or genes, for adult-plant resistance. The chi-square value indicated that the resistance of accession 91 could be the result of two recessive genes, but the segregation ratio deviated significantly from the expected 1:3 or 7:9 ratios for accession 318.

Major genes contributing to leaf rust resistance, as observed particularly in UFS accession 129, are common in wheat breeding (Lupton, 1987; McIntosh *et al.*, 1995). On the other hand recessive resistance genes as observed in the rest are not so abundant and are more difficult to use in wheat breeding programs. Inheritance of resistance genes differs between cultivars and what might be a dominant gene in one cultivar might be expressed as a recessive gene in another (Pretorius *et al.*, 1995). It would thus be interesting to observe behaviour of these genes once they have been reconstituted through backcrossing in hexaploid backgrounds.

2.3.4 F_3

Results from progeny testing F_2 plants with a particular leaf rust phenotype are presented in Figure 2.7. More than 90 % of the F_2 seeds germinated. Plants from all crosses that were rated as intermediate in the F_2 segregated into resistant, intermediate and susceptible plants in the F_3. In the SST55/129 population of the 291 plants rated as resistant in the F_2 all except 17, reacted similarly in the F_3. Of the 17 plants that responded differently, 15 were rated as intermediate and two as susceptible. All susceptible F_2 plants reacted accordingly in the F_3.

In SST55/116 plants rated as resistant in the F_2, 241 reacted similarly in the F_3. Of the 130 plants that responded differently, 70 were rated as intermediate and 60 as susceptible. The susceptible F_2 plants responded differently in the F_3 and the 71 plants segregated into 13 % intermediate and 87 % susceptible.

Resistant F_2 plants of the SST55/91 cross segregated into resistant, intermediate and susceptible F_3's. Of the 277 resistant F_2 plants, 179 were resistant, 81 had an intermediate response and 17 were susceptible. All susceptible F_2 plants reacted accordingly in the F_3.

In the SST55/318 population of the 449 plants rated as resistant in the F_2, 301 reacted similarly in the F_3, while 57 were rated as intermediate and 91 as

susceptible. The susceptible F_2 plants responded differently in the F_3 and segregated into two resistant, two intermediate and 38 susceptible plants.

From these results it is clear that resistant F_2 plants from all crosses gave rise to resistant F_3 plants, showing that the initial rating was accurate. The fact that resistant or intermediate F_2 plants produced susceptible F_3's was expected as those individuals heterozygous for the resistance gene would have segregated for the susceptible allele. This, however, indicates towards dominance of resistance because in recessivity, only the *rr* genotype would have been rated as resistant. In crosses involving accessions 318 and 116, and assuming dominance of resistance, a limited number of susceptible F_2 plants were incorrectly classified as they produced resistant or intermediate offspring. If resistance was indeed recessive, then susceptible *Rr* F_2 plants would have produced resistant and susceptible F_3's. Due to these small numbers it is unlikely that aberrant F_2 ratios resulted from misclassifications during leaf rust assessment. If the F_3 progeny tests are taken as indicative of dominance of resistance, then other genetic factors influenced gene behaviour in the tetraploid x hexaploid hybrids. Recently Bower (2002) warned that early generation segregation in these types of crosses should be interpreted with caution. For example, the single D genome will influence rust resistance in the F_1, many gametes probably abort due to variation in chromosome numbers, and other unknown segregation distortion mechanisms are also operative. Cross

pollination could also have contributed to error, especially as a high degree of male sterility was indicated in the pollen viability tests.

2.3.5 Backcrosses

Successful backcrossing was performed with bread wheat parents and F_1's as male or female parents. SST55/*T. turgidum* ssp. *pyramidales* (318) backcrosses rendered the most seeds. The BC_1-seeds were collected and replanted. Four successful crosses were made with F_2 plants. These consisted of two SST55/*T. turgidum* ssp. *durum* v. *libycum* (129), one SST55/*T. turgidum* ssp. *pyramidales* (318) and one SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* (91) accession. No BC_1 could be crossed successfully with a bread wheat parent either. Some of the resistant F_2-plants (of all crosses) and most of the BC_1- plants were sterile. Of the backcrosses made with F_3 plants to SST55, four were from SST55/*T. turgidum* ssp. *pyramidales* (318) and two from SST55/*T. turgidum* ssp. *durum* v. *libycum* (129).

Seed germination does not guarantee viable adult plants (Brown-Guedira *et al.*, 1997) and the degree of sterility of the observed adult F_2's therefore is not unusual. SST55 was chosen as the only backcross parent for Nantes, Palmiet and SST825 did not render viable BC_2's. When backcrosses to the other bread wheat parents were successful, the progeny was non-viable. SST825 could not be used because it was resistant to UVPrt9, thus masking the introduced genes.

In this study resistance genes were successfully transferred from *T. turgidum* species to *T. aestivum* and resistance expression was followed up to the F_3. *T. turgidum* ssp. *durum* v. *libycum* (129), *T. turgidum* ssp. *pyramidales* (318) and *T. turgidum* ssp. *dicoccoides* v. *kotchianum* (91) should be valuable sources of resistance genes in wheat breeding programmes if appropriate source stocks are developed and the genes wisely used.

Figure 2.1. Pollen coloured with toluidin blue. Living pollen (400x) colours light blue (left) and non-viable pollen (200x) dark blue (right).

Figure 2.2. Differentiation between viable and non-viable pollen grains stained with FDA. Two filter sets were used with combination UV-1A (excitation filter 330 – 380 nm and barrier filter 420 nm) (400x) (left) and B-2A (excitation filter 450 – 490 nm and barrier filter 520 nm) (400x) (right). The two blue pollen grains on the right of the pictures were considered viable according to their fluorescence in the corresponding micrograph.

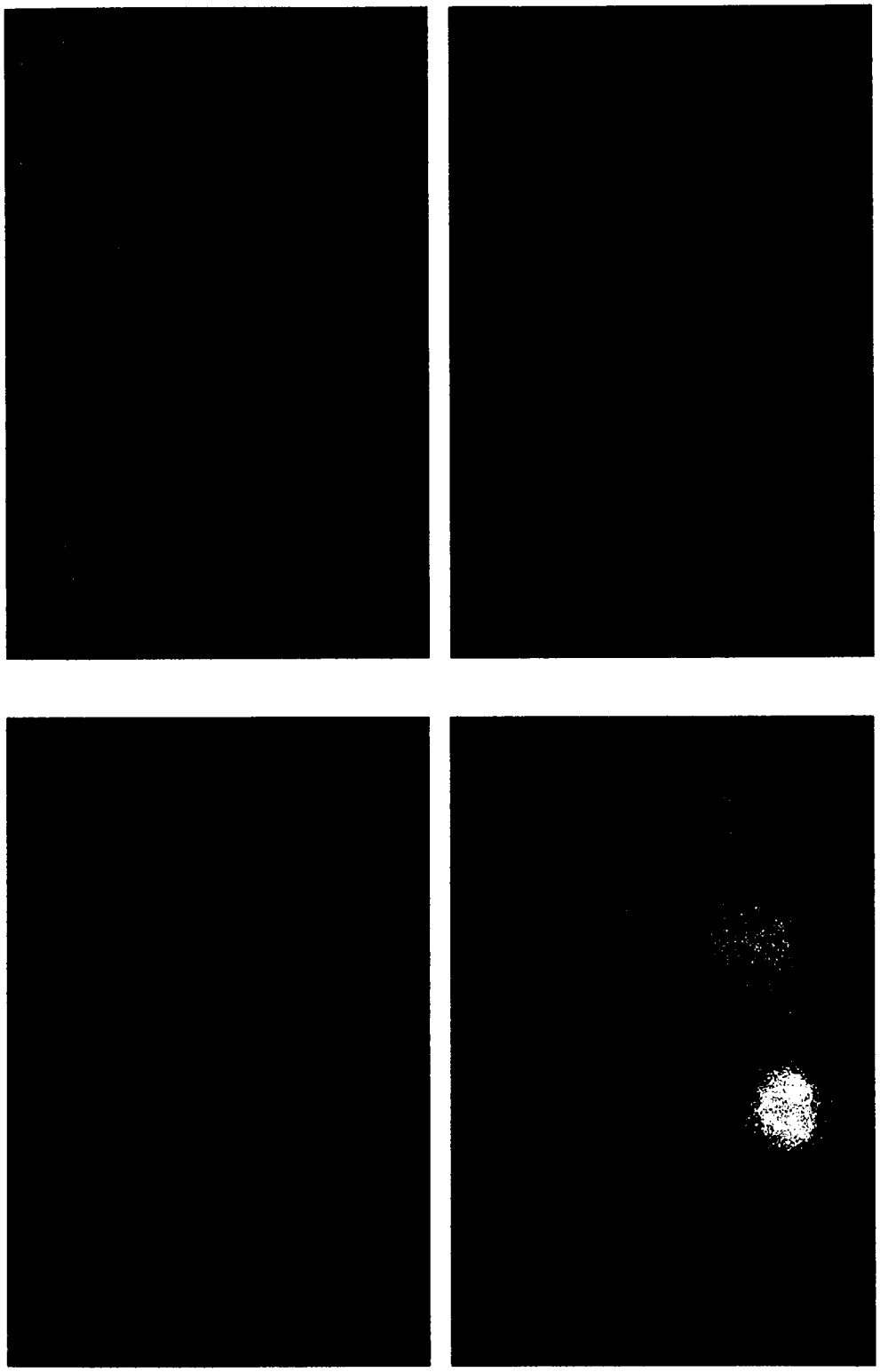
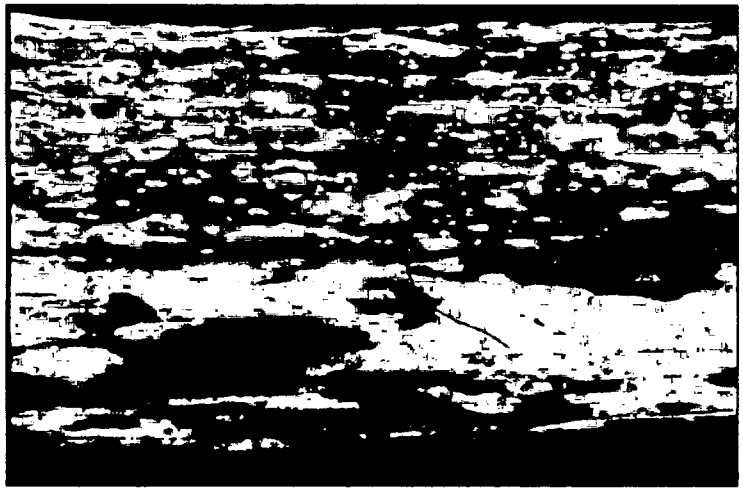


Figure 2.3. Seedling reactions of *T. turgidum* ssp. *durum* var. *libycum* (left) and *T. aestivum* (Morocco) (right) when infected by pathotype 6E16A- of *Puccinia striiformis* f. sp. *tritici*.

Figure 2.4. The flag leaf reaction of *T. turgidum* ssp. *durum* v. *libycum* (129) (left) and its F_1 from a cross with *T. aestivum* cv SST55 (right) to pathotype UVPrt9 of *Puccinia triticina*.



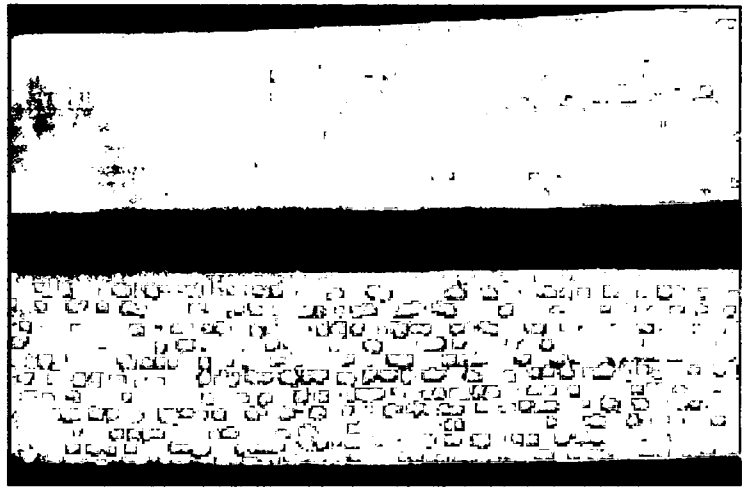


Figure 2.5. Pollen viability of *T. turgidum* and *T. aestivum* parents and their F_1's; (129) represents *T. turgidum* ssp. *durum* v. *libycum*, (318) represents *T. turgidum* ssp. *pyramidales*, (116) represents *T. turgidum* ssp. *abyssinicum* and *T. turgidum* ssp. *dicoccoides* v. *kotchianum* is represented by (91).

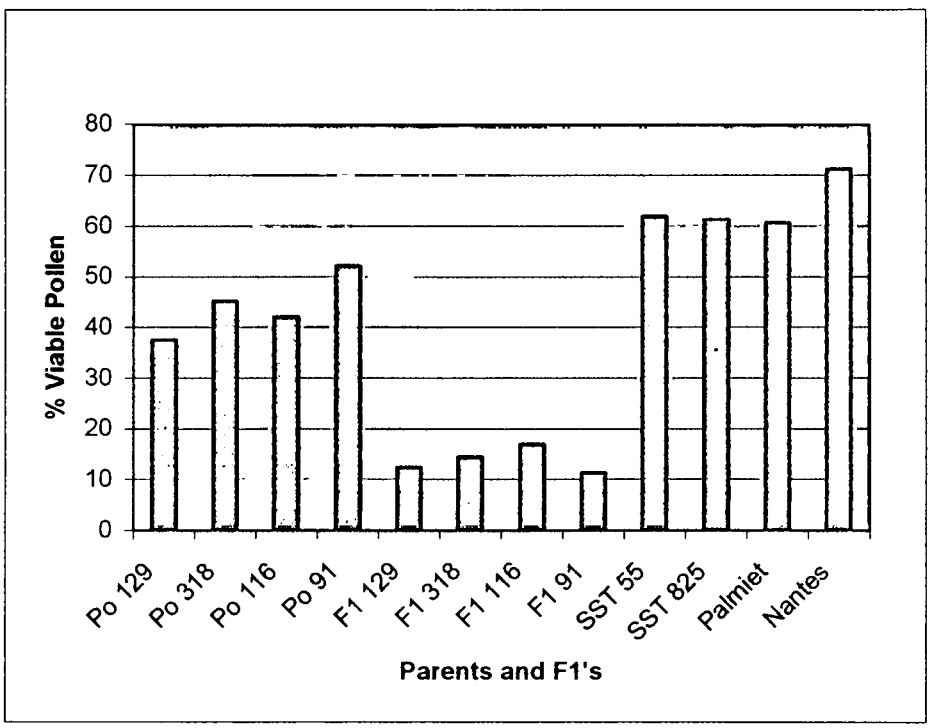

80
70
60
50
40
30
20
10
0
% Viable Pollen
Po 129
Po 318
Po 116
Po 91
F1 129
F1 318
F1 116
F1 91
SST 55
SST 825
Palmiet
Nantes
Parents and F1's

Figure 2.6. Flag leaf reactions of F_2 plants of crosses between SST55 and *T. turgidum* to *Puccinia triticina* ranging from fully resistant (top) to fully susceptible (bottom).

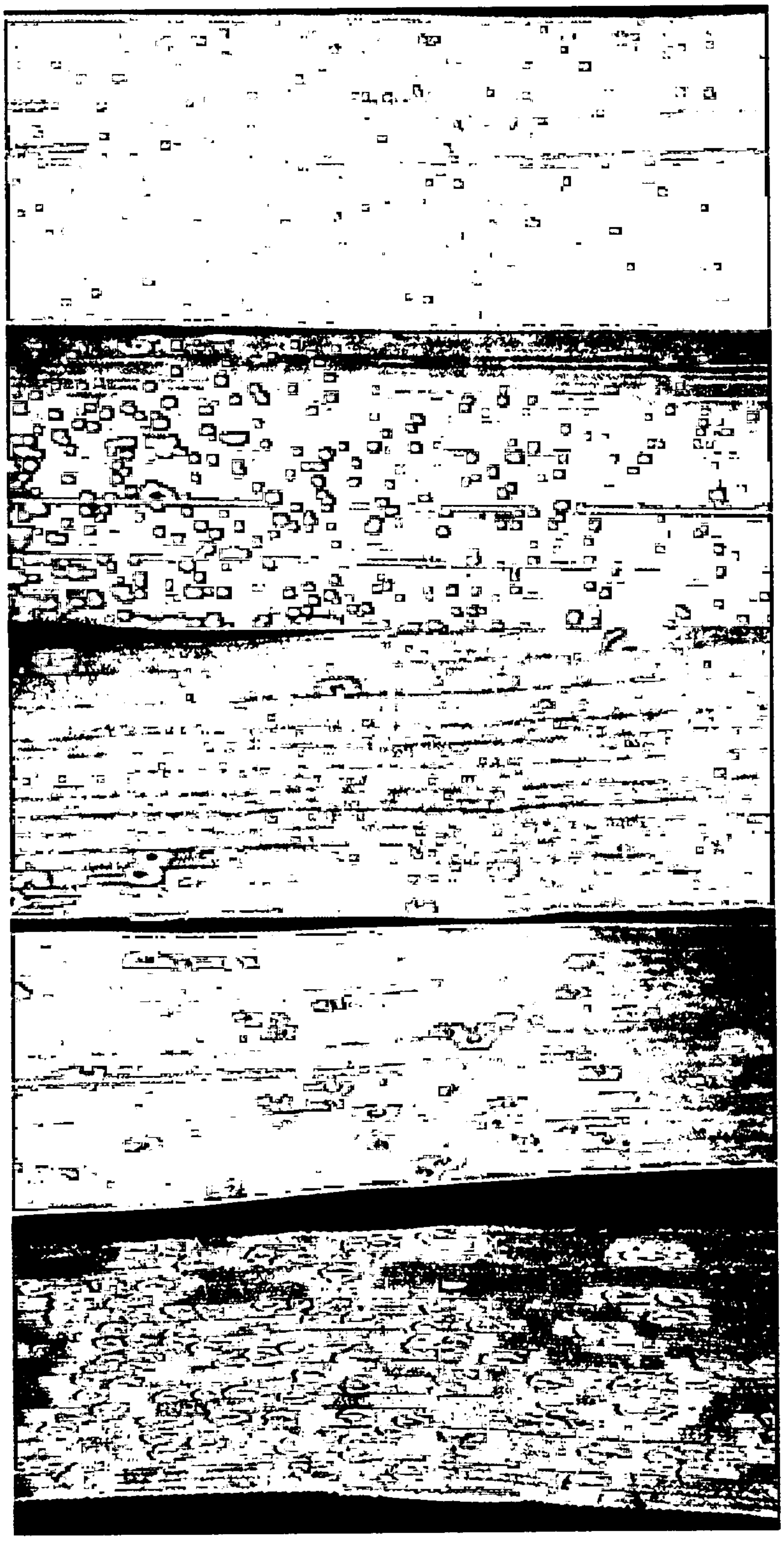


Figure 2.7. The adult plant reaction of the F_3 progeny grouped per F_2 reaction class. *T. turgidum* ssp. *durum* v. *libycum* is represented by 129, 318 represents *T. turgidum* ssp. *pyramidales*, 91 represents *T. turgidum* ssp. *dicoccoides* v. *kotchianum* and *T. turgidum* ssp. *abyssinicum* is represented by 116. Plants were infected by pathotype UVPrt9 of *Puccinia triticina*.

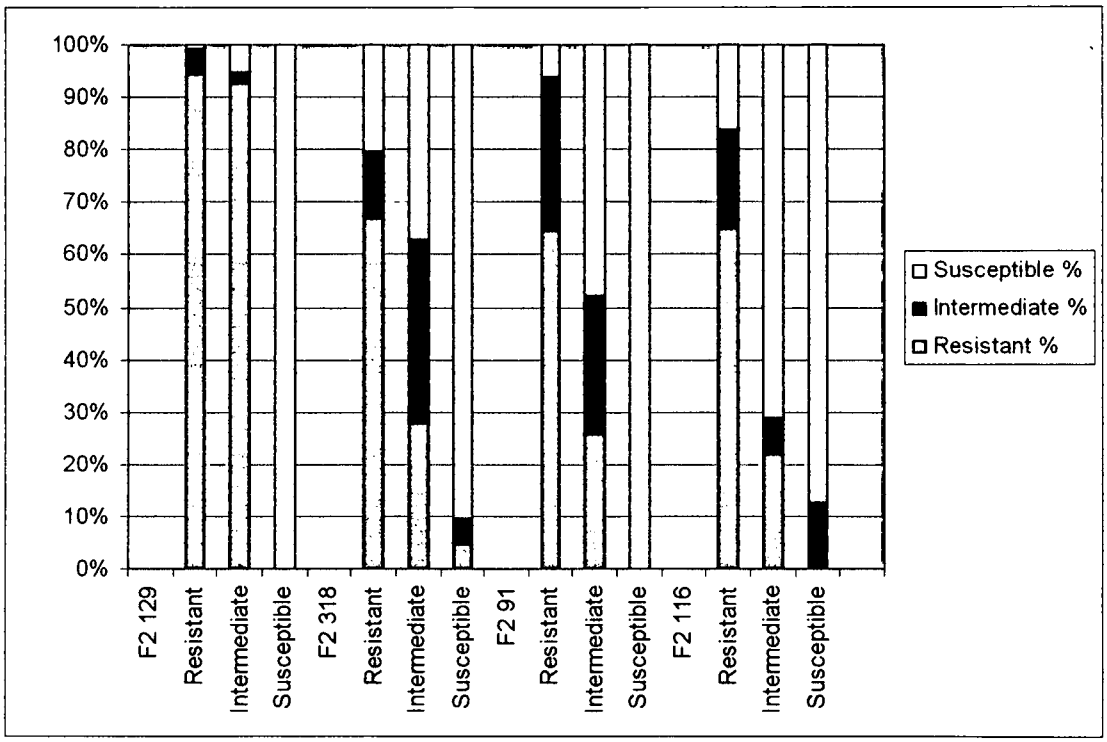

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
F2 129
Resistant
Intermediate
Susceptible
F2 318
Resistant
Intermediate
Susceptible
F2 91
Resistant
Intermediate
Susceptible
F2 116
Resistant
Intermediate
Susceptible
Susceptible %
Intermediate %
Resistant %

Table 2.1. Reaction of progeny derived from crosses between leaf rust-resistant *Triticum turgidum* accessions and the susceptible bread wheat cultivar SST55

Parental lines and crosses	Generation	Infection type UVPrt9 Seedling	Infection type UVPrt9 Adult	Number of Plants Resistant IT range	Number of Plants Susceptible IT range	Chi-square 1:3	Chi-square 3:1	Chi-square 7:9	Chi-square 9:7
T. turgidum ssp. *dicoccoides* v. *kotchianum* (UFS 91)	P0	4	;						
T. turgidum ssp. *abyssinicum* (UFS 116)	P0	3++	;						
T. turgidum ssp. *pyramidales* (UFS 318)	P0	;1-	;						
T. turgidum ssp. *durum* v. *libycum* (UFS 129)	P0	3+	;;1 CN						
T. turgidum ssp. *persicum* v. *rubiginosum* (UFS 353)	P0	1Z	;1						
SST55	P0	4	3++						
SST55 X 91	F1	4	3++		35				
SST55 X 116	F1	3++	2+ 3		31				
SST55 X 318	F1	1	2+ 3		20				
SST55 X 129	F1	3	;;1 CN	24					
SST55 X 353	F1	1Z			5				
SST55 X 91	F2		;1 - 3++	50	85	10.43*		2.54	
SST55 X 116	F2		; - 3	89	73	8.24*			0.11
SST55 X 318	F2		; - 3++	53	106	5.89*		6.96*	
SST55 X 129	F2		;;CN - 3	128	95		36.84*		0.12

* Deviated significantly (P<0.05) from expected ratio.
N: Necrosis.
C: Chlorosis.
Z: pustules closer to leaf base.

Table 2.2. Seedling infection types produced by *Triticum turgidum* spp. to various wheat rust cultures

	Pathotypes*								
***Triticum turgidum* accession**	**UVPrt2**	**UVPrt3**	**UVPrt9**	**UVPrt13**	**UVPgt50**	**UVPgt52**	**UVPgt53**	**6E16A-**	**6E22A-**
T. turgidum ssp. *abyssinicum*	3-	3++/4	3++	3++	3	3-	2	2+/3	2 C
T. turgidum ssp. *pyramidales*	2+	1-	;1-	1;	3++	3	4	3++	4
T. turgidum ssp. *dicoccoides* v. *kotchianum*	4	3++/4	4	3++/4	3++	3	3++	3++	3
T. turgidum ssp. *persicum* v. *rubiginosum*	4	2+/4	1Z	4	3-	3	4	; C	; C
T. turgidum ssp. *durum* var. *libycum*	3+	3 N	3+	4	3++	4	4	;	;

* UVPrt refers to *Puccinia triticina*, UVPgt to *Puccinia graminis* f. sp. *tritici* and 6E to *Puccinia striiformis* f. sp. *tritici*.

CHAPTER 3

THE USE OF AFLP TECHNOLOGY TO DETERMINE INTROGRESSION OF WHEAT LEAF RUST RESISTANCE FROM *TRITICUM TURGIDUM* TO *TRITICUM AESTIVUM*

3.1 INTRODUCTION

Wheat leaf rust can, under favourable conditions, cause crop losses of up to 78 % (Singh, 1999; Boshoff *et al.*, 2002a). Leaf rust resistant cultivars have become an economic barrier against the disease, but the rapidity by which the pathogen overcomes leaf rust genes, makes it necessary to introduce new resistance genes to widen this gene pool and to maintain rust-free cultivars. Is has been suggested that wild relatives of wheat, such as *T. turgidum*, are possible sources of new resistance against pathogens (Knott and Dvorak, 1976; Knott, 1989).

Traditionally, breeders have had to rely on time consuming breeding procedures to introduce new genes into crops and make selections of new variants on the basis of phenotype. The availability of molecular markers provides the breeder with the option of selecting for the presence or absence of genes in the laboratory rather than the field. Molecular markers can also provide insight in terms of the evolution of a genome, the phyletic origins of cultivated species and the current levels of diversity in modern agricultural crops (Hill *et al.*, 1996). The identification of molecular markers for disease resistance is important in assisting the breeding process by enabling breeders to select plants with desirable traits according to genotype (Tanksley *et al.*,

1989; Cervera *et al.*, 1996). Genotypic markers are used to tag the desired genes by distinguishing between variations in DNA sequences (Mohan *et al.*, 1997). These markers can also be used in the study of quantitative traits and are used as "labels" to determine the presence (or absence) of alleles in a segregating population (Smith *et al.*, 1990; Young, 1999). The introduction of DNA-markers has thus accelerated efforts to develop disease resistance (Mohan *et al.*, 1997).

Different techniques are available for the production of DNA markers. These include RFLPs (Random Amplified Length Polymorphisms) (Rognli *et al.*, 1992; Powell *et al.*, 1996), RAPDs (Random Amplified Polymorphic DNA) (Welsh and McClelland, 1990), micro-satellites or simple sequence repeats (SSRs) (Tautz and Rentz, 1984) and AFLPs (Amplified Fragment Length Polymorphism) (Zabeau and Vos, 1993). RFLPs use a more classical approach of DNA extraction, digestion with endonucleases, Southern blotting and probe hybridization and detection (Prins *et al.*, 1996). The RFLP technique is laborious, time consuming and expensive, but it is highly reproducible and has proved effective in identifying, marking and isolating genes (Powell *et al.*, 1996). Due to its intensive nature, RFLPs are not considered to be a breeder friendly method for generating markers.

The development of the Polymerase Chain Reaction (PCR) has resulted in a new generation of fingerprinting techniques such as RAPDs, AFLPs and SSRs, and has made marker technology more accessible to breeders. These techniques use the PCR method to generate fragments that can be resolved

according to size and identified in one step through gel staining or fluorescence detection (Saiki *et al.*, 1985; Mullis and Faloona, 1987). In comparison to RFLPs, RAPDs require small amounts of DNA, is relatively inexpensive and produces numerous dominant markers, but has been shown to have problems with reproducibility between laboratories, due to the sensitivity of PCR reaction conditions (Penner *et al.*, 1993; Dedryver *et al.*, 1996; Hill *et al.*, 1996). Micro-satellites, also known as SSRs, allow the identification of co-dominant markers (Mackill *et al.*, 1996). This technique can be used as inexpensively as RAPDs to detect polymorphisms, but requires the development of primers to amplify repeat sequences (Mackill *et al.*, 1996).

In comparison to these, the AFLP-technique, although not as cost effective as RAPDs, is reliable, easy to perform and highly repeatable and does not require any prior development work (Cho *et al.*, 1996; Hill *et al.*, 1996; Mackill *et al.*, 1996; Jones *et al.*, 1997). For AFLPs, genomic DNA is digested by restriction endonucleases, the resulting fragments are ligated to adapters and these fragments are PCR amplified by using primers that are complementary to the adapter sequence (Zabeau and Vos, 1993) AFLP primers often contain additional arbitrary nucleotides that selectively amplify restriction fragments (Lin and Kuo, 1995). The resulting amplification is resolved and visualized using sequencing gel electrophoresis or capillary gel electrophoresis with the aid of radioactive or fluorescent labelling, respectively (Lockhart and McLenachan, 1997). Finally, AFLPs generate more data points per assay

than any other molecular technique currently available making it ideal to study the introgression of alien genes into domesticated wheat.

Different molecular techniques have been used to follow the introgression of alien genes into existing wheat cultivars and have been used to detect polymorphisms between cultivated and wild wheat (Ishii *et al.*, 1993; Joshi and Nguyen, 1993; Law *et al.*, 1998; Bohn *et al.*, 1999). RFLP mapping was used by Ishii *et al.* (1993) to follow the introgression of flowering earliness and brown plant hopper resistance from *Oryza australiensis* into *O. sativa*. RAPDs was used by Joshi and Nguyen (1993) to determine the genetic relationship between wild and cultivated wheat, while Bohn *et al.* (1999) used RFLPs, AFLPs and SSRs to investigate the genetic similarity of winter wheat cultivars. In the latter study, it was concluded that although the number of average polymorphic bands generated was similar for RFLPs, AFLPs and SSRs, the marker index was highest for AFLPs (Bohn *et al.*, 1999). Furthermore, 10 to 100 times more markers are produced with the AFLP technique than other methods, allowing greater coverage of the genome and making AFLPs ideal to determine the extent of introgression of DNA into a cultivated crop from a wild variety (Lin and Kuo, 1995; Law *et al.*, 1998).

Thus, the aim of this study was to determine the extent of introgression of DNA from two wild *Triticum turgidum* species, *T. turgidum* ssp. *durum* v. *libycum* and *T. turgidum* ssp *dicoccoides* v. *kotchianum*, into a cultivated wheat variety SST55, in order to identify new possible sources of leaf rust resistance.

3.2 MATERIAL AND METHODS

3.2.1 Wheat material

A rust-susceptible wheat cultivar SST55 was crossed with two subspecies of *Triticum turgidum*, namely *T. turgidum* ssp. *dicoccoides* v. *kotchianum* (UFS accession 91) (Table 3.1) and *T. turgidum* ssp. *durum* v. *libycum* (UFS accession 129) (Table 3.2). The F_1 and F_2 populations derived from these crosses were self-fertilized. Plants were grown in 1-liter capacity pots under controlled glasshouse conditions. A nutrient solution (50 ml of a 3 g/l hydroponic solution) (6.5:2.7:13 N:P:K plus micro elements) was administered three days a week per pot and continued for the remainder of the experiment. Leaf material for DNA extraction was taken from plants prior to pathogen inoculation and rating. Phenotypic IT rating was done according to the 1-4 scale (McIntosh, 1995).

3.2.2 DNA preparation

DNA was extracted from four *T. turgidum* ssp. *durum* v. libycum, three *T. turgidum* ssp. *dicoccoides* v. *kotchianum* and four SST55 parental plants. For the SST55/*T. turgidum* ssp. *durum* v. *libycum* cross, DNA was extracted from 17 F_2 individuals, while for the SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* cross DNA was extracted from 13 F_2 plants. Fresh leaf material (0.5 g) was collected and DNA extracted according to the method of Edwards *et al.* (1991). Leaf material was grounded in liquid nitrogen until a fine powder, re-suspended in 10 ml extraction buffer (0.25 M EDTA (pH 8), 20 % SDS, 0.1 M Tris-HCl (pH 8), 0.5 M NaCl and 1 % (w/v) CTAB) and incubated

at 65 °C for one hour. Chloroform extractions were performed by the addition of 10 ml chloroform:isoamylalcohol (24:1) followed by centrifugation at 8 krpm for 10 minutes. The supernatant was extracted twice with chlofororm:isoamylalcohol followed by the addition of two volumes of absolute ethanol to precipitate the nucleic acids (on ice for 2 hours). DNA was spooled using a sterile Pasteur pipette and washed twice by immersion in 70 % ethanol. The spooled DNA was re-dissolved in 100 µl sterile double distilled water (Sabax™). Where necessary, the chloroform extraction was augmented by additional phenol:chloroform extractions prior to precipitation. The DNA concentration was determined and the DNA stored at – 20 °C.

The DNA concentration was determined with the use of a spectrophotometer at 260 nm. The formula [DNA] = Optic density x dilution x constant (50 µg/ml) was used to determine the DNA concentration and purity using the 260/280 OD ratio (Sambrook *et al.*, 1989). The genomic DNA was resolved according to size and visualized on a 0.8 % agarose gel using gel electrophoresis at 60 V in 0.5x TAE (0.438 g/l Tris, 0.09 ml/l acetic acid and 0.022 g/l acid EDTA) and visualized under UV-light using ethidium bromide (Sambrook *et al.*, 1989).

3.2.3 AFLP-protocol

The AFLP™ Analysis System I and AFLP Starter Primer Kit (GibcoBRL) was used to generate AFLP profiles. Genomic DNA (250 ng) was digested with 2 µl of *Eco*R1/*Mse*1 (1.25 U/µl) in 25 µl reactions containing 5 x reaction buffer (50 mM Tris-HCl, 50 mM Mg-acetate and 250 mM K-acetate) and AFLP-grade

water for 2 h at 37 °C. Adapters were ligated to the digested DNA in 50 µl reactions containing 250 ng of digested DNA, 24 µl adapter ligation solution (*Eco*R1/*Mse*1 adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 mM Mg-acetate and 50 mM K-acetate), 1 µl T4 DNA ligase (1 U/µl in 10 mM Tris-HCl (pH 7.5)), 1 mM DTT, 50 mM KCl and 50 % glycerol (v/v) at 20 °C for 2 h. The ligation product was diluted 1:10 in TE-buffer (10 mM Tris-HCl and 0.1 mM EDTA) and stored at 4 °C.

Pre-selective amplification was performed as described by Maughan *et al.* (1996) using 5 µl of diluted ligation product, 40 µl pre-amplification primer mix (27,8 ng/µl *Eco*R1, 6.78 ng/µl *Mse*1 with dNTPs) (Table 3.3), 5 µl of 10 x PCR buffer (GibcoBRL) (200 mM Tris-HCl, 15 mM MgCl2, 500 mM KCl and 1 U Ampli *Taq* DNA polymerase) under the following conditions: 20 cycles at 94 °C for 30 s, 56 °C for 60 s and 72 °C for 60 s. Pre-selective amplification was confirmed by gel electrophoresis at 60 V in 0.5x TAE (0.438 g/l Tris, 0.09 ml/l acetic acid and 0.022 g/l acid EDTA) and visualized under UV-light using ethidium bromide.

The pre-selective amplification product was diluted 1:50 in TE-buffer (10 mM Tris-HCl and 0.1 mM EDTA). Selective amplification was performed in 20 µl reactions containing 5 µl pre-selective diluted template, 4.5 µl *Mse*-primer (*Mse*+CAA, *Mse*+CTC or *Mse*+CTA) (6.7 ng/µl) (Table 3.3), 1 µl *Eco*-primer (*Eco*+ACA or *Eco*+AAC) (1 µM/µl) (Table 3.3) and 2 µl of 10x PCR buffer (200 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCL and 5 U of Ampli *Taq* polymerase). Eco-primers were labelled fluorescently with NED and FAM, respectively.

The PCR cycle consisted of 35 cycles of 30 s at 94 °C, 30 s at 65 °C and 1 min at 72 °C with a temperature reduction of 0.7 °C per cycle for 12 cycles. AFLP fragments were prepared for separation by the addition of 5 μl of the selective amplification product to 1 μl Rox size standard marker (35, 50, 75, 100, 139, 150, 160, 200. 300, 340, 350, 400, 450, 490, 500 bp) (Applied Biosystems) and 24 μl formamide with denaturation at 94 °C for 5 min and quick cooling on ice. Fragments were resolved and visualized using an ABi Prism 310 automated capillary sequencer (Applied Biosystems).

3.2.4 Data analysis

AFLP fragments were scored manually using Genescan® 3.1 on an Apple McIntosh computer. Profiles were coded into a binary matrix using Microsoft Excel on the basis of presence (1) and absence (0) of fragments. The minimum size of fragment coded was 45 bp with a minimum peak height of 40. Comparisons were made between resistant and susceptible parents, between parents and resistant, susceptible and intermediate F_2's as well as between the different F_2's. Distance analysis and dendrograms were determined using the UPGMA clustering method (NCSS, 2000).

3.3 RESULTS

A total of six AFLP primers were tested on each of the two crosses between SST55 and UFS accessions 91 and 129 as well as on the resulting F_2 individuals from these crosses (Figure 3.1) (Table 3.4). This resulted in a total of 545 fragments for the parents and F_2 progeny of the SST55/*T. turgidum*

ssp. *durum* v. *libycum* cross and 486 fragments for the parents and F_2 progeny of the SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* cross (Table 3.4). An average of 129 fragments was identified per primer combination for both crosses. Amplification products were obtained for four AFLP primers with the exception of *Mse*+CAA/*Eco*+ACA and *Mse*+CAA/*Eco*+AAC (Table 3.3).

In the SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* cross primer combination *Mse*+CTA/*Eco*+ACA rendered a total of 118 fragments. These fragments ranged in size from 45 to 500 bp. Of these fragments two fragments (103 and 110 bp) were present in resistant plants only while three (172, 277 and 306 bp) were detected in susceptible plants only. The 110 bp fragment was detected in all resistant plants, but the 103 bp fragment was absent in one resistant plant. The fragments detected in susceptible plants were detected in all but two plants (Table 3.5).

Primer combination *Mse*+CTA/*Eco*+AAC detected 73 fragments in the parents and progeny of the SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* cross. Of these, four fragments (81, 197, 220 and 311 bp) were present in resistant plants only, while one fragment (196 bp) was present in susceptible plants only. However, the 220 and 311 bp fragment was absent in one of the resistant plants. The 81 and 197 bp fragments were present in all resistant plants, while the 196 bp fragment was present in all susceptible plants (Table 3.5).

Primer combination *Mse*+CTA/*Eco*+AAC detected 120 fragments ranging in size from 47 to 416 bp in the parents and progeny of the SST55/*T. turgidum* ssp. *durum* v. *libycum* cross. Of these, three fragments (168, 238 and 366 bp) were present in resistant plants only. However, two plants did not have the 168 bp fragment, while 3 did not posses the 366 bp fragment. Three fragments (87, 218 and 308 bp) were only detected in the susceptible plants (Table 3.6).

Primer combination *Mse*+CTA/*Eco*+ACA detected 139 fragments ranging in size from 45 to 499 bp in parents and progeny of the SST55/*T. turgidum* ssp. *durum* v. *libycum* cross. Of these, four fragments (174, 237, 268 and 391 bp) were present in resistant plants only. However, two plants did not have the 174, 268 and 391 bp fragments, while three resistant plants did not posses the 237 bp fragment. Two fragments (157 and 239 bp) were detected in all the susceptible plants (Table 3.6).

Primer combination *Mse*+CTC/*Eco*+ACA rendered a total of 161 fragments ranging in size between 48 and 319 bp for the parents and progeny of SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* cross. Three fragments (267, 276 and 292 bp) were detected in resistant plants only, while ten fragments (62, 91, 119, 129, 147, 153, 159, 175, 179 and 211 bp) were present in susceptible plants only (Table 3.5).

Primer combination *Mse*+CTC/*Eco*+AAC rendered a total of 133 fragments ranging in size from 53 to 298 bp for the progeny and parents of the SST55/*T.*

turgidum ssp. *dicoccoides* v. *kotchianum* cross. Five fragments (117, 128, 200, 216 and 233 bp) were detected in resistant plants only. Of these, the 216 bp was absent in only one resistant plant. Fourteen fragments (71, 83, 92, 95, 116, 139, 149, 178, 183, 190, 194, 198, 203 and 231 bp) were only present in the susceptible plants. Fragments 92, 95, 116, 198, 203 and 231 bp were detected in all susceptible plants, while fragments 83, 139 and 190 bp were absent in one susceptible plant. Fragments 71, 149, 178, 183 and 194 bp were absent in two susceptible plants (Table 3.5).

Primer combination *Mse*+CTC/*Eco*+AAC detected 136 fragments ranging in size from 54 to 424 bp in parents and progeny of SST55/*T. turgidum* ssp. *durum* v. *libycum*. Of these, six fragments (185, 188, 200, 221, 233 and 285 bp) were present in resistant plants only. The 185 and 188 bp fragment was absent in one resistant plant, while 2 resistant plants did not posses the 221 or 233 bp fragment. The 200 and 285 bp fragments were found in all resistant plants. Only one fragment (183 bp) was detected in all susceptible plants (Table 3.6).

Primer combination *Mse*+CTC/*Eco*+ACA produced a total of 204 fragments ranging in size between 48 and 267 bp for the parents and progeny of the cross SST55/*T. turgidum* ssp. *durum* v. *libycum*. Three of these fragments (82, 162 and 212 bp) were present in resistant plants only. The 212 bp fragment was present in all resistant plants while the other fragments, 82 and 162 bp, were absent in one resistant plant, respectively. One fragment (77 bp) was detected in all susceptible plants (Table 3.6).

Of the fragments present in the F_2 plants of the SST55/*T. turgidum* ssp. *durum* v. *libycum* cross, 230 out of 545 fragments over all the primer combinations tested were not present in any of the parent individuals (Table 3.7). Primer combination *Mse*+CTC/*Eco*+ACA identified 102 fragments in the F_2 progeny, not present in the parents, while primer combination *Mse*+CTC/*Eco*+AAC identified 41, *Mse*+CTA/*Eco*+ACA identified 32 and primer combination *Mse*+CTA/*Eco*+AAC identified 55 fragments present in the F_2 progeny, but not in any of the parents.

A total of 155 out of 486 fragments were present the F_2 individuals of the SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* cross, but not in any of the parents for all the primer combinations tested (Table 3.8). Primer combination *Mse*+CTC/*Eco*+ACA identified 68 fragments in the F_2 progeny, not present in the parents, primer combination *Mse*+CTC/*Eco*+AAC identified 48, *Mse*+CTA/*Eco*+ACA identified 29 and primer combination *Mse*+CTA/*Eco*+AAC identified 10 fragments present in the F_2 progeny, but not in any of the parents.

For the cross SST55/*T. turgidum* ssp. *durum* var. *libycum*, a total of 61 fragments were present in the *T. turgidum* parent as well as F_2 progeny, but not in the *T. aestivum* parent SST55 (Table 3.9). Similarly, 91 fragments were present in the *T. aestivum* parent as well as F_2's, but not in *T. turgidum* ssp. *durum* var. *libycum* (Table 3.10).

For the cross SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*, a total of 67 fragments were present in *T. turgidum* ssp. *dicoccoides* v. *kotchianum* and F_2 plants, but not in any of the SST55 plants (Table 3.11). Similarly, 110 fragments were present in SST55 and F_2 progeny, but not in any of the individual *T. turgidum* ssp. *dicoccoides* v. *kotchianum* plants (Table 3.12). The AFLP fragment data of SST55/*T. turgidum* ssp. *durum* v. *libycum* and SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* parents as well as F_2 progeny is summarized in Table 3.13.

The pair wise distance matrix for SST55/*T. turgidum* ssp. *durum* v. *libycum* and SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* parents as well as F_2 progeny is based on the total number of AFLP fragments for all the primer combinations used (Table 3.14 and 3.15). The genetic distance between the SST55 parent individual plants 296 and 297 was 0.44 and the average between SST55 and the individual resistant *T. turgidum* ssp. *dicoccoides* v. *kotchianum* parent was 0.71. The average distance between SST55 and the resistant and susceptible F_2 progeny of the same cross was 0.69 and 0.62, respectively. The genetic distance between the *T. turgidum* parent and the resistant and susceptible F_2 progeny was 0.60 and 0.70, respectively. Only a single *T. turgidum* ssp d*icoccoides* v. *kotchianum* was used to represent the donor parent.

In the resulting dendrograms from the distance matrix for the parents and resistant, intermediate and susceptible F_2 progeny of the SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* cross, two main clusters were identified

(Figure 3.2). Resistant and susceptible plants grouped in separate clusters, respectively. In the resistant cluster, *T. turgidum* formed a sub-cluster on its own, while the resistant progeny grouped into two separate sub-clusters with an intermediate plant clustering in the resistant group. In the susceptible cluster, the two SST55 plants grouped together in a sub-cluster while the three susceptible plants comprised the second sub-cluster.

In the data matrix based on the parents and F_2 progeny of the SST55/*T. turgidum* ssp. *durum* v. *libycum* cross, the genetic distance between the SST55 parent was 0.44, the distance between the *T. turgidum* parent was 0.32 and the genetic distance between SST55 and *T. turgidum* 0.67. The average genetic distance between SST55 and the resistant and susceptible F_2 progeny was 0.66 and 0.63, respectively. The genetic distance between the *T. turgidum* parent and resistant and susceptible F_2 progeny was 0.51 and 0.61, respectively.

The resulting dendrogram (Figure 3.3) for the parents and F_2 progeny of the SST55/*T. turgidum* ssp. *durum* v. *libycum* cross grouped the *T. aestivum* parent together with the susceptible F_2 plants, while the resistant F_2 progeny grouped with the *T. turgidum* parent. Of the three plants in the intermediate group, two grouped with the resistant cluster, while one grouped in the susceptible cluster.

3.4. DISCUSSION

AFLP fingerprinting was successfully used in this study to analyse the introgression of new resistance from two tetraploid wild wheat relatives into a hexaploid domestic cultivar. In total, only four fragments (200 bp and 285 bp [*Mse*+CTC/*Eco*+AAC], 212 bp [*Mse*+CTC/*Eco*+ACA], as well as 238 bp, [*Mse*+CTA/*Eco*+AAC]) were shown to be solely introgressed from the *T. turgidum* ssp. *durum* v. *libycum* parent into the resistant progeny (Table 3.16). The progeny of *T. turgidum* ssp. *dicoccoides* v. *kotchianum* displayed greater introgression from the wild wheat parent. In total, nine fragments (110 bp [*Mse*+CTA/*Eco*+ACA], 197 bp [*Mse*+CTA/*Eco*+AAC], 267, 276 and 292 bp [*Mse*+CTC/*Eco*+ACA], 117, 128, 200, and 233 bp [*Mse*+CTC/*Eco*+AAC]) were shown to be solely introgressed from the *T. turgidum* ssp. *dicoccoides* v. *kotchianum* parent into the resistant progeny (Table 3.17). The amount of introgression from wild wheat into domesticated varieties, in terms of number of AFLP fragments correlates with results from Feuntes *et al.* (1999), in a study of rice varieties and Hongtrakul *et al.* (1997) on the genetic diversity of sunflowers, who found that between 4 and 19 AFLP fragments were unique to the varieties studied, respectively. Similar findings were reported by Bower (2002) in following the introgression of AFLP fragments from *T. turgidum* to *T. aestivum,* who determined that 12 fragments were solely introgressed from the wild parent.

Several introgressed fragments from both *T. turgidum* parents were also present in most, but not all resistant progeny. These fragments were,

however, absent in the SST55 parent and all the susceptible progeny (Tables 3.16 and 3.17). According to Gold *et al.* (1999) there is a possibility that these markers are linked to the flanking regions of the introgressed segment or in the case of polygenic resistance, to only one of the genes responsible for resistance. From the pathogenic screening of the F_2 progeny (Chapter 2) it is known that two dominant genes have been introgressed from the *T. turgidum* parent in the SST55/*T. turgidum* ssp. *durum* v. *libycum* cross and two recessive genes in the SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* cross. Furthermore, due to the wide cross, it is possible for markers associated with a certain chromosome or chromosome region to be absent in some of the progeny (Gold *et al.*, 1999). Therefore, it is probable that markers only partially linked to the resistance phenotype are single gene tags or are situated within the area of the chromosome undergoing recombination.

Many fragments were present in the resistant F_2, as well as intermediate or susceptible progeny. A possible reason for this could be the presence of a suppressor gene. In wheat, a suppressor gene is located on chromosome 7D (Kerber and Green, 1980). This suppressor inhibits the expression of stem rust resistance genes and has been proposed to also suppress leaf rust resistance genes (Dyck, 1987). The progeny of the cross between *T. aestivum* and *T. turgidum* have one D genome only and if the specific suppressor gene was present, it could inhibit the expression of the resistance genes, resulting in a false negative or intermediate phenotype. This could account for the presence of certain markers in the resistant progeny as well as the intermediate or susceptible progeny.

Several fragments were present in the F_2 progeny but could not be accounted for in the parent lines. This would suggest that recombination is producing novel sequences in the progeny. An average of 37 % recombination was detected in the progeny of SST55/*T. turgidum* ssp. *durum* v. *libycum* and 29 % in the progeny of SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum.* Therefore, the functioning of other genes may be affected during the introgression of genes from wild wheat into domestic varieties. Furthermore, the introgression is likely to introduce instability into the genome which would require additional backcrossing to stabilize.

In the progeny of SST55/*T. turgidum* ssp. *durum* v. *libycum,* 13 % of fragments was solely inherited from the *T. turgidum* parent, 16 % from SST55 and 33 % from either parent. In the progeny of SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* 21 % of polymorphisms was inherited from SST55 14 % from the *T. turgidum* parent and 36 % from either parent. This correlates with data from Peil *et al.* (1997) who found that polymorphisms increase with polyploidy level. Although the data suggest that the parents may contribute similar amounts of genetic material to the F_2 progeny, the hexaploid parent contributed slightly more. This is not surprising, considering the findings of Peil *et al.* (1997) and the fact that SST55 was the maternal parent in the original cross and would have contributed the cytoplasm which has more genetic material than the pollen grain of *T. turgidum.*

As expected, the genetic distance between the two wild wheat parents, *T. turgidum* ssp. *dicoccoides* v. *kotchianum* and *T. turgidum* ssp. *durum* v. *libycum,* and SST55 was greater than the genetic distance between individual plants of the same group. The genetic distance data based on AFLP fragments indicated significant differences between resistant and susceptible progeny for both crosses. The genetic distance between SST55 and *T. turgidum* ssp. *durum* v. *libycum* and SST55 and *T. turgidum* ssp. *dicoccoides* v. *kotchianum* was 0.67 and 0.71, respectively. The genetic distance between susceptible progeny and resistant plants for the cross SST55/*T. turgidum* ssp. *durum* v. *libycum* and SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* was an average of 0.6 and 0.51, respectively. The genetic distance between susceptible parents and progeny for the cross SST55/*T. turgidum* ssp. *durum* v. *libycum* and SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* was an average of 0.69 and 0.66, respectively. The closer relationship between the resistant plants and resistant parent indicates that resistance in the progeny is based on the introgression of a larger donor segment. It is interesting to note that F_2 progeny with an intermediate pathotype grouped between the resistant and susceptible F_2 progeny.

The dendrogram based on the AFLP data of the SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum* cross had two main clusters, containing the resistant and susceptible plants, respectively. The plant that was scored as intermediate (IT ;2) clustered in the resistant group indicating that grouping the intermediate with resistant plants in the Mendelian tests, was correct (Chapter 2). The dendrogram based on the AFLP data of the SST55/*T.*

turgidum ssp. *durum* v. *libycum* cross grouped resistant and susceptible plants in different clusters, respectively. The three intermediate plants did not form a separate sub-cluster. Two intermediate plants clustered with the resistant group, while the other clustered with the susceptible F_2 progeny. This indicates that this intermediate group is truly intermediate, showing characteristics of both susceptible and resistant plants.

In this study the AFLP technique has been successfully used to follow the introgression of resistance from two tetraploids, *T. turgidum* ssp. *dicoccoides* v. *kotchianum* and *T. turgidum* ssp. *durum* v. *libycum* into hexaploid *T. aestivum*. Due to the limitation of the number of individual plants used in this study, further research is needed to comprehend the full significance of the statistical data.

Between four and nine AFLP fragments out of a total of 486 and 545 fragments, respectively, were shown to be solely introgressed from the wild parents into the resistant domestic variety. Several AFLP fragments were found to be partially introgressed into the resistant progeny indicating partial linkage to one or the other resistance gene. Certain markers were identified in resistant as well as intermediate progeny and suggest the possible action of a suppressor gene to silence the resistant phenotype in suppressed intermediate plants. The introduction of novel sequences during introgression from wild varieties into domestic lines is a potential source of variation but may serve to destabilize the genome requiring several backcrosses to

restabilize it. Genetic distance data confirm a greater degree of introgression form the resistant parent to the resistant progeny has occurred.

Figure 3.1. A typical AFLP profile obtained from primer combination *Mse*+CTA/*Eco*+AAC on resistant SST55/*T. turgidum* ssp. *durum* v. *libycum* F_2 plant 211.

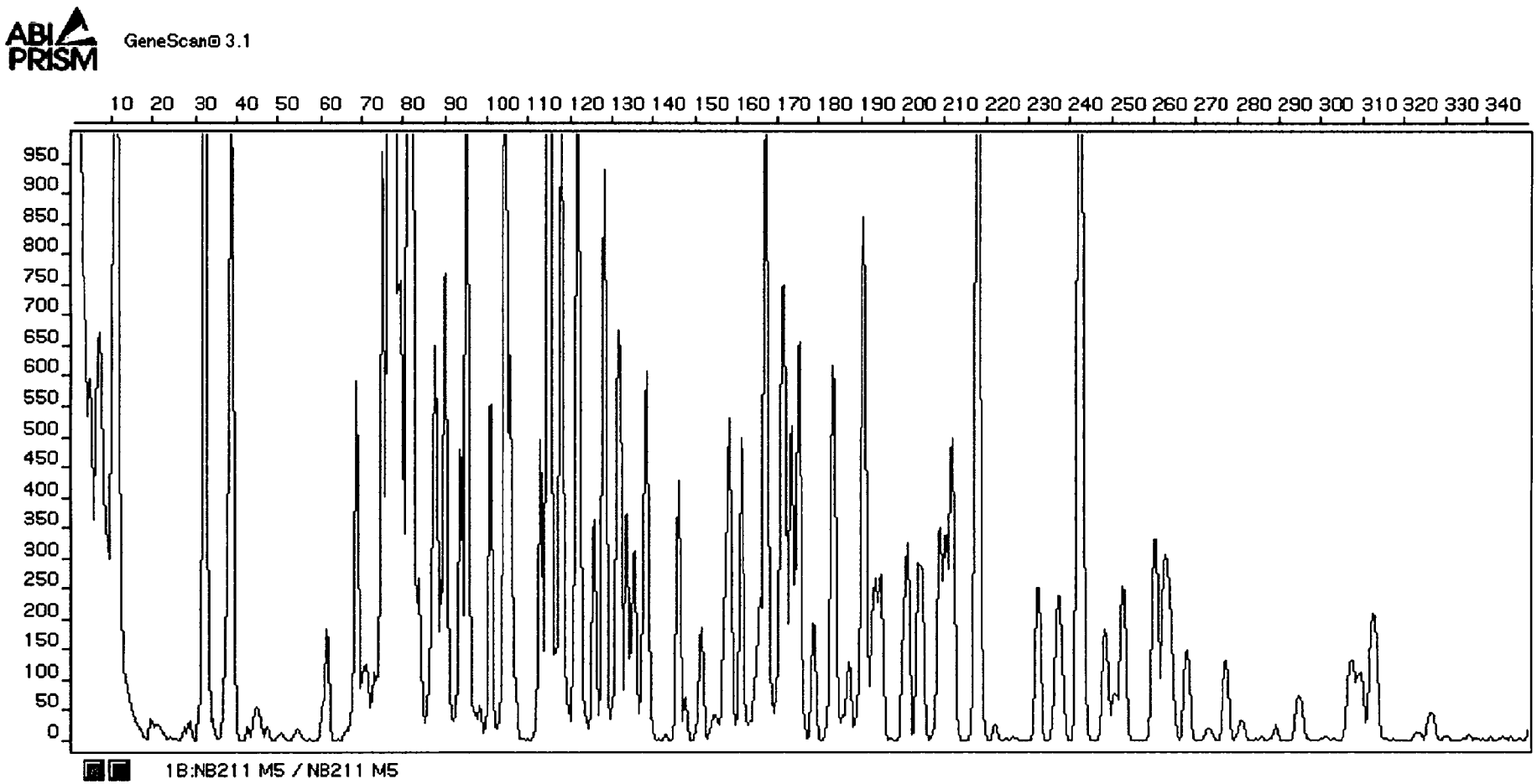

ABI PRISM
GeneScan® 3.1
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340
950 900 850 800 750 700 650 600 550 500 450 400 350 300 250 200 150 100 50 0
1B:NB211 M5 / NB211 M5

Figure 3.2. A dendrogram of the parents, resistant, intermediate and susceptible F_2 plants of the cross *T. turgidum* ssp. *dicoccoides* v. *kotchianum* based on the AFLP fragments obtained by primer pairs *Mse*+CTC/*Eco*+AAC, *Mse*+CTC/*Eco*+ACA, *Mse*+CTA/*Eco*+AAC and *Mse*+CTA/*Eco*+ACA showing the genetic distances between the respective plants.

Dendrogram

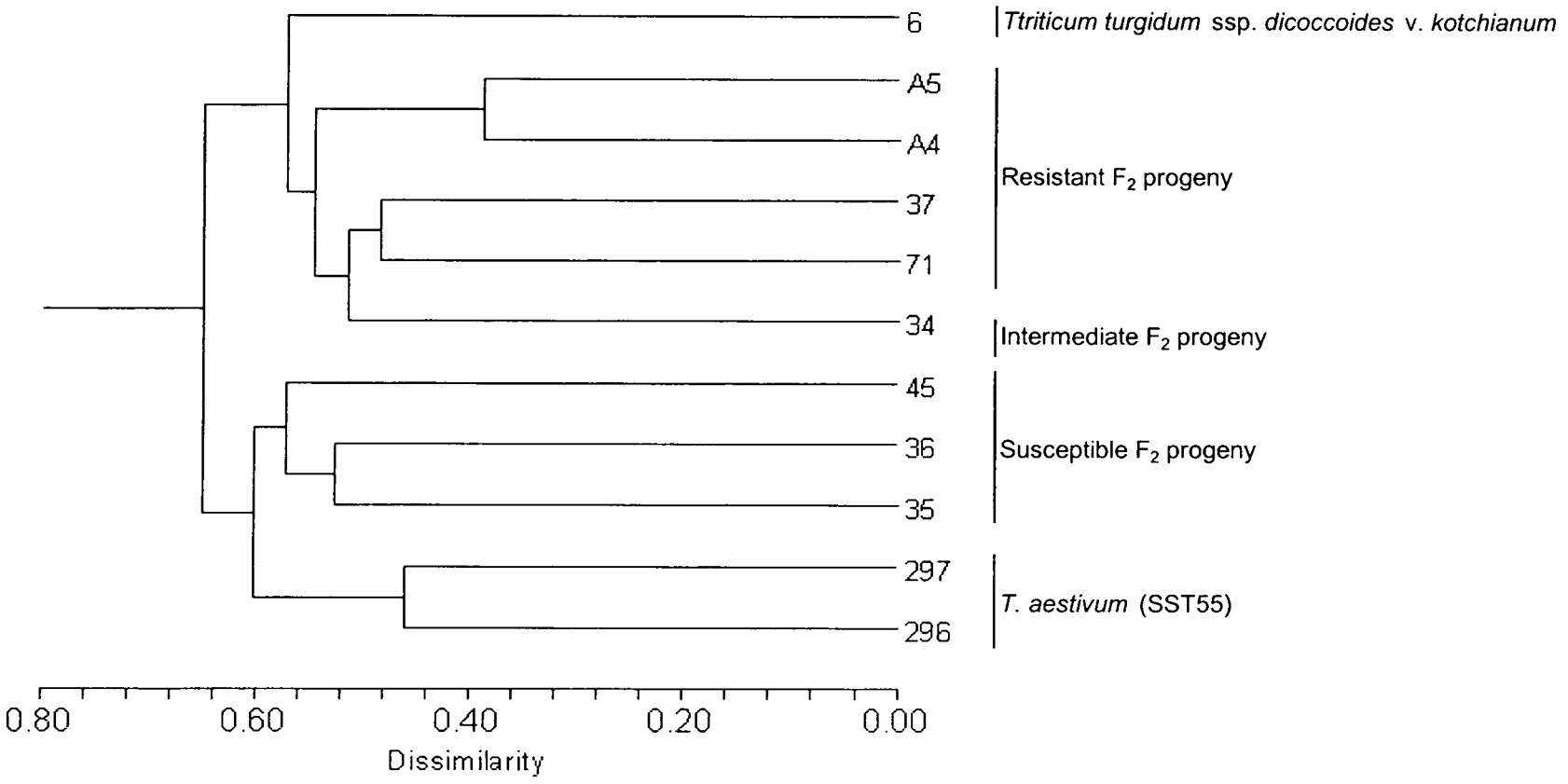

6
A5
A4
37
71
34
45
36
35
297
296
Ttriticum turgidum ssp. dicoccoides v. kotchianum
Resistant F2 progeny
Intermediate F2 progeny
Susceptible F2 progeny
T. aestivum (SST55)
0.80
0.60
0.40
0.20
0.00
Dissimilarity

Figure 3.3. A dendrogram of the parents, resistant, intermediate and susceptible F_2 plants of the cross *T. turgidum* ssp. *durum* v. *libycum* based on the AFLP fragments obtained by primer pairs *Mse*+CTC/*Eco*+AAC, *Mse*+CTC/*Eco*+ACA, *Mse*+CTA/*Eco*+AAC and *Mse*+CTA/*Eco*+ACA showing the genetic distances.

Dendrogram

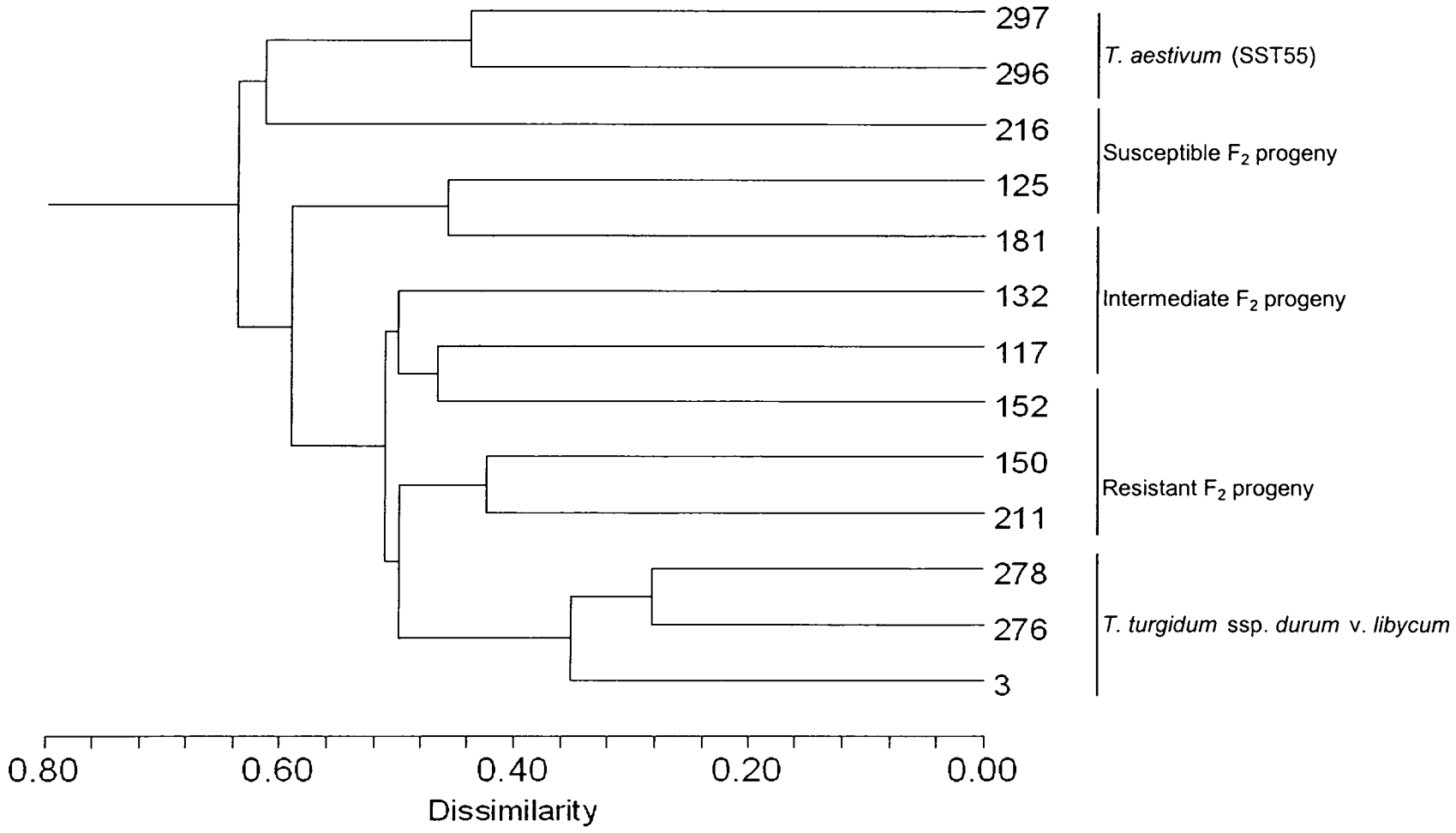

297
296
216
125
181
132
117
152
150
211
278
276
3
T. aestivum (SST55)
Susceptible F2 progeny
Intermediate F2 progeny
Resistant F2 progeny
T. turgidum ssp. durum v. libycum
0.80
0.60
0.40
0.20
0.00
Dissimilarity

Table 3.1. Phenotypic reaction of SST55 (leaf rust-susceptible parent), *T. turgidum* ssp *dicoccoides* v. *kotchianum* (leaf rust-resistant parents) and F_2 plants resulting from this cross when inoculated with UVPrt9 of *Puccinia triticina*

Generations	Sample number	Phenotypic reaction*
Po SST55	296	3++
Po SST55	297	3++
Po SST55	301	3++
Po SST55	303	3++
Po *T. turgidum* ssp *dicoccoides* v. *kotchianum*	4	;
Po *T. turgidum* ssp *dicoccoides* v. *kotchianum*	6	;
Po *T. turgidum* ssp *dicoccoides* v. *kotchianum*	9	;
F_2 Resistant plant	71	;
F_2 Resistant plant	75	;
F_2 Resistant plant	A4	;
F_2 Resistant plant	A5	;
F_2 Resistant plant	37	;1
F_2 Intermediate plant	34	;2
F_2 Intermediate plant	45	;2+
F_2 Intermediate plant	41	;2
F_2 Susceptible plant	35	3
F_2 Susceptible plant	36	3++
F_2 Susceptible plant	38	3
F_2 Susceptible plant	43	3
F_2 Susceptible plant	A3	3++

* IT based on 1-4 scale (Roelfs, 1988)

Table 3.2. Phenotypic reaction of SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *durum* v. *libycum* (leaf rust-resistant parents) and F_2 plants resulting from this cross when inoculated with UVPrt9 of *Puccinia triticina*

Generations	Sample number	Phenotypic reaction*
Po SST55	296	3++
Po SST55	297	3++
Po SST55	301	3++
Po SST55	303	3++
Po *T. turgidum* ssp *durum* v. *libycum*	3	;;1CN
Po *T. turgidum* ssp *durum* v. *libycum*	5	;;1CN
Po *T. turgidum* ssp *durum* v. *libycum*	278	;;1CN
Po *T. turgidum* ssp *durum* v. *libycum*	276	;;1CN
F_2 Resistant plant	211	;;1CN
F_2 Resistant plant	152	;;1CN
F_2 Resistant plant	217	;;1CN
F_2 Resistant plant	206	;;1CN
F_2 Resistant plant	209	;;1CN
F_2 Resistant plant	150	;;1CN
F_2 Intermediate plant	131	;;2+
F_2 Intermediate plant	132	;;2
F_2 Intermediate plant	181	;;2
F_2 Intermediate plant	153	;2+
F_2 Intermediate plant	603	;;3
F_2 Intermediate plant	117	;;2
F_2 Intermediate plant	145	;2++
F_2 Susceptible plant	219	3
F_2 Susceptible plant	125	3++
F_2 Susceptible plant	216	3
F_2 Susceptible plant	129	3++

* IT based on 1-4 scale (Roelfs, 1988)

Table 3.3. *Mse* 1 and *Eco* R1 adapters and primers used to generate AFLP profiles

Adapters		
Mse		5'-GACGAGTCCTGAG-3' 3'-TACTCAGGACTCAT-5'
Eco		5'-CTCGTAGACTGCGTACC-3' 3'CATCTGACGCATGGTTAA-5'
Primers		
Mse		5'-GATGAGTCCTGAGTAA-3'
	Mse-CTA Mse-CTC Mse-CAA	
Eco		5'-GATGCGTACCAATTC-3'
	EcoR-ACA (FAM) EcoR-AAC (NED)	

Table 3.4. Polymorphic fragments (bp) and number of recombination fragments in the parents and progeny of the crosses SST55 and *T. turgidum* ssp. *dicoccoides* v. *kotchianum* and *T. turgidum* ssp. *durum* v. *libycum*

Primer combination	Number of fragments (bp)	Number of polymorphic fragments (bp)
SST/*T. turgidum* ssp. *durum* v. *libycum*		
Mse + CTC/Eco + ACA	154	66
Mse + CTC/Eco + AAC	132	31
Mse + CTA/Eco + ACA	139	23
Mse + CTA/Eco + AAC	120	46
Total	**545**	**166**
Average	**136**	**42**
SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*		
Mse + CTC/Eco + ACA	166	41
Mse + CTC/Eco + AAC	132	36
Mse + CTA/Eco + ACA	115	25
Mse + CTA/Eco + AAC	73	14
Total	**486**	**116**
Average	**122**	**29**

Table 3.5. AFLP fragments (bp) only present in resistant or susceptible parents and progeny only for SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*

	Susceptible					I/med	Resistant				
	T. aestivum		F_2								
Primer combination	296	297	35	36	45	34	71	37	A4	A5	6
AFLP fragments in resistant plants only											
Mse + CTC/Eco + ACA						119	119	119	119	119	119
						267	267		267	267	267
						276	276		276	276	276
							292		292	292	292
Mse + CTC/Eco + AAC						117	117	117	117	117	117
						128	128	128	128	128	128
						200	200	200	200	200	200
						216		216	216	216	216
						233	233	233	233	233	233
Mse + CTA/Eco + ACA						103		103	103	103	103
						110	110	110	110	110	110
Mse + CTA/Eco + AAC							81	81	81	81	81
							197	197	197	197	197
							220		220	220	220
								311	311		311
AFLP fragments in susceptible plants only											
Mse + CTC/Eco + ACA	91	91	91	91	91						
	129	129	129	129	129						
	147	147	147	147							
	153	153	153								
	159	159	159	159							
		175	175	175							
	179	179	179								
	211		211	211	211						
Mse + CTC/Eco + AAC	71			71	71						
	83	83	83	83	83						
	92	92	92	92	92						
	95	95	95	95	95						
	116	116	116	116	116						
	139		139	139	139						
	149	149	149	149							
	178	178	178	178							
	183	183	183	183							
	190	190	190	190	190						
	194		194	194	194						
	198	198	198	198	198						
	203	203	203	203	203						
	231	231	231	231	231						
Mse + CTA/Eco + ACA	172	172	172	172	172	172					
	277	277	277		277	277					
	306	306	306		306						
Mse + CTA/Eco + AAC	196	196	196	196	196	196					

Table 3.6. AFLP fragments (bp) only present in resistant or susceptible parents and offspring only for SST55/*T. turgidum* ssp. *durum* v. *libycum*

	Resistant						Intermediate			Susceptible			
	T. turgidum			F_2								*T. aestivum*	
Primer combination	3	276	278	211	152	150	132	181	117	216	125	296	297
Fragments in resistant plants only													
Mse + CTC/Eco + ACA	82	82	82	82	82			82					
	162	162	162		162	162	162	162					
	212	212	212	212	212	212							
		217	217	217	217	217	217						
Mse + CTC/Eco + AAC	185	185	185	185	185	185	185	185					
	188	188	188		188	188	188	188					
	200	200	200	200	200	200	200	200					
	221	221	221										
	233	233	233	233	233	233	233	233					
	285	285	285	285	285	285	285	285					
Mse + CTA/Eco + ACA	174	174	174	174	174			174					
	237	237	237	237		237	237	237					
	268	268	268	268	268	268			268				
	391		391	391	391	391			391				
Mse + CTA/Eco + AAC	168	168	168	168	168	168			168				
	238	238	238	238	238	238							
	366	366		366	366								
Fragments in susceptible plants only													
Mse + CTC/Eco + ACA								77	77	77	77	77	77
							211	211	211		211		211
Mse + CTC/Eco + AAC								183		183	183	183	183
Mse + CTA/Eco + ACA							157	157	157	157	157	157	157
								239		239	239	239	239
Mse + CTA/Eco + AAC									218	218	218	218	218
							308		308	308		308	308

Table 3.7. AFLP fragments (bp) present in F_2 plants of SST55/*T. turgidum* ssp. *durum* v. *libycum*, but not in either *T. turgidum* ssp. *durum* v. *libycum* or SST55

Primers	Fragment length in base pairs
Mse+CTA/*Eco*+ACA	47, 80, 85, 110, 117, 127, 137, 141, 148, 150, 154, 165, 169, 185, 197, 208, 214, 235, 255, 284, 300, 317, 318, 321, 331, 356, 374, 382, 385, 401, 447
Mse+CTA/*Eco*+AAC	47, 51, 53, 61, 72, 79, 81, 82, 91, 98, 125, 129, 134, 137, 138, 153, 158, 164, 179, 180, 184, 187, 192, 196, 198, 204, 207, 209, 211, 213, 222, 227, 245, 249, 260, 265, 268, 274, 285, 295, 301, 314, 324, 329, 333, 358, 367, 378, 381, 387, 391, 403, 411, 406
Mse+CTC/*Eco*+ACA	67, 68, 72, 75, 79, 88, 89, 107, 112, 114, 119, 121, 124, 125, 126, 131, 133, 135, 138, 141, 145, 144, 150, 151, 154, 155, 157, 161, 163, 169, 171, 172, 177, 181, 195, 196, 201, 203, 204, 208, 211, 216, 217, 218, 221, 222, 225, 232, 235, 234, 238, 239, 243, 245, 247, 251, 253, 256, 258, 259, 260, 263, 266, 267, 268, 269, 271, 279, 280, 282, 284, 285, 355, 361, 367, 374, 375, 378, 384, 387, 394, 395, 403
Mse+CTC/*Eco*+AAC	57, 66, 78, 97, 99, 101, 105, 117, 118, 121, 123, 127, 128, 129, 144, 151, 152, 153, 160, 161, 162, 174, 175, 187, 195, 204, 215, 228, 239, 241, 242, 254, 266, 271, 283, 285, 303, 321, 351, 360

Table 3.8. AFLP fragments (bp) present in F_2 plants of the cross SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*, but not in either SST55 or *T. turgidum* ssp. *dicoccoides* v. *kotchianum*

Primers	Fragment length in base pairs
***Mse*+CTA/*Eco*+ACA**	45, 49, 54, 60, 75, 78, 85, 135, 137, 150, 175, 187, 193, 221, 236, 340, 250, 255, 259, 307, 331, 366, 377, 385, 397, 418, 452, 488, 500
***Mse*+CTA/*Eco*+AAC**	73, 79, 84, 105, 106, 157, 185, 193, 279, 311
***Mse*+CTC/*Eco*+ACA**	40, 44, 64, 68, 75, 79, 81, 93, 107, 112, 116, 119, 120, 124, 126, 131, 132, 133, 135, 138, 140, 142, 144, 149, 150, 161, 163, 165, 171, 181, 188, 191, 194, 195, 196, 198, 200, 203, 205, 208, 209, 211, 212, 217, 218, 222, 223, 224, 225, 232, 238, 239, 240, 243, 245, 250, 251, 253, 254, 256, 258, 259, 266, 268, 276, 280, 282, 283
***Mse*+CTC/*Eco*+AAC**	63, 68, 78, 88, 91, 94, 97, 99, 102, 123, 127, 129, 132, 143, 144, 145, 146, 147, 148, 151, 161, 162, 164, 165, 166, 169, 171, 179, 180 ,181, 187, 191, 204, 210, 215, 216, 218, 224, 233, 235, 254, 255, 256, 257, 258, 266, 279, 287

Table 3.9. AFLP fragments (bp) present in *T. turgidum* ssp. *durum* v. *libycum* and F_2 plants of SST55/T. *turgidum* ssp. *durum* v. *libycum*, but not in SST55

Primers	Fragment length in base pairs
***Mse*+CTA/*Eco*+ACA**	45, 56, 69, 75, 88, 136, 158, 174, 175, 187, 222, 237, 268, 312, 315, 359, 374, 391, 407, 419, 448, 470, 488
***Mse*+CTA/*Eco*+AAC**	58, 76, 84, 93, 100, 109, 122, 128, 149, 157, 165, 168, 171, 178, 190, 195, 220, 235, 238, 262, 267, 278, 233
***Mse*+CTC/*Eco*+ACA**	82, 85, 90, 93, 116, 120, 132, 140, 149, 162, 165, 191, 212, 217
***Mse*+CTC/*Eco*+AAC**	82, 96, 102, 146, 148, 185, 188, 196, 200, 221, 233, 285

Table 3.10. AFLP fragments (bp) present in *T. aestivum* (SST55) and F_2 plants of SST55/*T. turgidum* ssp. *durum* v. *libycum*, but not in *T. turgidum* ssp. *durum* v. *libycum*

Primers	Fragment length in base pairs
Mse+CTA/*Eco*+ACA	40, 43, 60, 106, 131, 155, 157, 194, 217, 239, 260, 309
Mse+CTA/*Eco*+AAC	66, 71, 87, 112, 120, 218, 243, 308, 310, 364
Mse+CTC/*Eco*+ACA	69, 71, 76, 77, 80, 91, 92, 94, 113, 117, 118, 122, 129, 139, 143, 145, 147, 156, 159, 170, 175, 184, 187, 193, 211, 215, 241, 261
Mse+CTC/*Eco*+AAC	64, 71, 79, 83, 92, 98, 104, 106, 116, 120, 131, 135, 139, 150, 156, 159, 168, 176, 183, 190, 194, 198, 207, 225, 231, 235, 245, 247, 252, 280, 284, 292, 298, 308, 315, 320, 342, 369, 382, 392, 415

Table 3.11. AFLP fragments (bp) present in *T. turgidum* ssp. *dicoccoides* v. *kotchianum and the* F_2 plants of the cross SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*, but not in SST55

Primers	Fragment length in base pairs
***Mse*+CTA/*Eco*+ACA**	57, 69, 71, 87, 103, 108, 110, 173, 313, 335, 401, 432, 448, 469
***Mse*+CTA/*Eco*+AAC**	81, 98, 99, 102, 128, 165, 167, 171, 180, 197, 220, 226, 262, 264, 273, 286, 311, 366
***Mse*+CTC/*Eco*+ACA**	38, 61, 67, 88, 90, 95, 110, 121, 157, 169, 177, 201, 212, 242, 267, 276, 292
***Mse*+CTC/*Eco*+AAC**	57, 77, 82, 101, 105, 117, 121, 128, 153, 185, 188, 195, 200, 216, 221, 228, 233, 242

Table 3.12. AFLP fragments (bp) present in *T. aestivum* (SST55) and F_2 plants of SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*, but not in *T. turgidum* ssp. *dicoccoides* v. *kotchianum*

Primers	Fragment length in base pairs
***Mse*+CTA/*Eco*+ACA**	73, 98, 111, 132, 144, 155, 167, 172, 215, 239, 245, 277, 281, 306, 324, 327, 352, 355, 362, 390, 420, 435, 460
***Mse*+CTA/*Eco*+AAC**	101, 116, 174, 196, 217, 288, 351
***Mse*+CTC/*Eco*+ACA**	49, 62, 66, 71, 78, 91, 111, 113, 117, 118, 119, 122, 129, 130, 134, 143, 145, 146, 147, 153, 156, 159, 168, 170, 175, 179, 183, 184, 187, 193, 211, 219, 241, 267
***Mse*+CTC/*Eco*+AAC**	54, 58, 64, 71, 83, 89, 92, 95, 104, 106, 112, 116, 120, 124, 135, 137, 139, 149, 151, 155, 156, 159, 168, 172, 176, 178, 183, 190, 194, 198, 203, 207, 212, 219, 231, 236, 238, 245, 260, 262, 270, 280, 284, 292, 296

Table 3.13. A summary of the number of AFLP fragments (bp) per primer combination for the parents and progeny of the crosses SST55/*T. turgidum* ssp. *durum* v. *libycum* and SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*

	Mse+CTC		*Mse*+CTA	
	Eco+ACA	*Eco*+AAC	*Eco*+ACA	*Eco*+AAC
SST/*T. turgidum* ssp. *durum* v. *libycum*				
Fragments present in the progeny, but not in either of the parents	102	41	32	55
Fragments present in *T. turgidum* and progeny, but not in *T. aestivum*	15	11	12	23
Fragments present in *T. aestivum* and progeny, but not in *T. turgidum*	28	41	12	10
Fragments present in both parents and progeny	9	39	83	32
Total fragments	**154**	**132**	**139**	**120**
SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*				
Fragments present in the progeny, but not in either of the parents	68	48	29	10
Fragments present in *T. turgidum* and progeny, but not in *T. aestivum*	14	18	17	18
Fragments present in *T. aestivum* and progeny, but not in *T. turgidum*	35	45	23	7
Fragments present in both parents and progeny	49	21	46	38
Total fragments	**166**	**132**	**115**	**73**

Table 3.14. Genetic distances calculated using a total of 486 AFLP fragments (bp) for primer combinations *Mse*+CTC/*Eco*+ACA, *Mse*+CTC/*Eco*+AAC, *Mse*+CTA/*Eco*+ACA and *Mse*+CTA/*Eco*+AAC for *T. turgidum* ssp. *dicoccoides* (6), Resistant F_2 plants (A3, A5, 37 and 71), Intermediate F_2 plant (34), Susceptible F_2 plants (45, 36 and 35) and *T. aestivum* (297 and 296)

0.439261									
0.596795	0.563340								
0.617478	0.604398	0.525600							
0.674046	0.658627	0.649903	0.557227						
0.613769	0.611906	0.610038	0.536350	0.615626					
0.684132	0.668946	0.649903	0.581291	0.505676	0.641061				
0.682461	0.674046	0.668946	0.598705	0.521238	0.642839	0.484933			
0.734039	0.710328	0.658627	0.628472	0.555175	0.621164	0.551047	0.592957		
0.708719	0.680787	0.648145	0.610038	0.534217	0.613769	0.503413	0.544797	0.388182	
0.702247	0.660358	0.600609	0.632094	0.598705	0.660358	0.563340	0.581291	0.573382	0.544797

Table 3.15. Genetic distance calculated using a total of 545 AFLP fragments for primer combintions *Mse*+CTC/*Eco*+ACA, *Mse*+CTC/*Eco*+AAC, *Mse*+CTA/*Eco*+ACA and *Mse*+CTA/*Eco*+AAC for *T. aestivum* (297 and 296), Suscpetible F_2 plants (216 and 125), Intermediate F_2 plants (191, 132 and 117), Resistant F_2 plants (150, 152 and 211) and *T. turgidum* ssp. *durum* v. *libycum* (278, 276 and 3)

0.360375											
0.341882	0.282449										
0.607620	0.607620	0.593721									
0.505994	0.483494	0.473804	0.585857								
0.537982	0.502314	0.525774	0.663101	0.483494							
0.525774	0.507824	0.494872	0.609145	0.424220	0.529290						
0.525774	0.500464	0.505994	0.653235	0.504157	0.473804	0.494872					
0.629214	0.584814	0.592707	0.742997	0.581627	0.570332	0.586401	0.513751				
0.627153	0.603023	0.607620	0.752238	0.587438	0.603023	0.595281	0.585857	0.458298			
0.529290	0.525774	0.513275	0.752238	0.496743	0.465906	0.491108	0.527535	0.591137	0.598390		
0.634506	0.604559	0.606092	0.612183	0.628630	0.634506	0.656069	0.644658	0.680313	0.709072	0.624188	
0.628630	0.592157	0.596838	0.615206	0.622700	0.610666	0.635966	0.609145	0.660911	0.682405	0.593721	0.439261

Table 3.16. Summary of AFLP fragments (bp) present in the resistant parent and progeny of SST55/*T. turgidum* ssp. *durum* v. *libycum* and SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*

Primer combinations	Fragment size in base pairs			
SST55/*T. turgidum* ssp. *durum* v. *libycum*				
Mse+CTA/*Eco*+AAC	238			
Mse+CTC/*Eco*+AAC	200	285		
Mse+CTC/*Eco*+ACA	212			
SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*				
Mse+CTA/*Eco*+ACA	110			
Mse+CTA/*Eco*+AAC	197			
Mse+CTC/*Eco*+ACA	267	276	292	
Mse+CTC/*Eco*+AAC	117	128	200	233

Table 3.17. Summary of AFLP fragments (bp) partially present in the resistant parents and progeny of SST55/*T. turgidum* ssp. *durum* v. *libycum* and SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*

Primer combinations	Fragment size in base pairs			
SST55/*T. turgidum* ssp. *durum* v. *libycum*				
Mse +CTA/*Eco* +AAC	168	366		
Mse +CTA/*Eco* +ACA	174	237	268	391
Mse +CTC/*Eco* +AAC	185	188	221	233
Mse +CTC/*Eco* +ACA	82	162		
SST55/*T. turgidum* ssp. *dicoccoides* v. *kotchianum*				
Mse +CTA/*Eco* +ACA	103			
Mse +CTA/*Eco* +AAC	220	311		
Mse +CTC/*Eco* +AAC	216			

CHAPTER 4

HISTOPATHOLOGY OF RESISTANCE TO WHEAT LEAF RUST IN *TRITICUM TURGIDUM* SSP. *DURUM* VAR. *LIBYCUM*

4.1 INTRODUCTION

The genus *Triticum* consists of diploid, tetraploid and hexaploid species. The species most commonly used in cultivation are hexaploid *T. aestivum* L. and tetraploid *T. turgidum* (Knott, 1989). Because changes and mutations in virulence of the leaf rust pathogen *Puccinia triticina* are frequent (Statler *et al.*, 1982), it is necessary to widen the current resistance gene pool in order to maintain rust-free cultivars. One of the most valuable resources for resistance genes is uncultivated species of wheat (Knott and Dvorak, 1976; Knott, 1989).

Two general mechanisms of resistance to obligate parasites have been recognized, namely pre-haustorial and post-haustorial resistance. Pre-haustorial resistance is resistance expressed before the first haustorium forms and post-haustorial is resistance manifested after the first haustorium is produced (Heath, 1981). Pre-haustorial resistance is considered the best type of resistance because there is a clear incompatibility between host and pathogen and infection is terminated well in advance of any established parasitic relationship. This defense mechanism is typical of non-host infections (Heath, 1974; Heath, 1977; Heath, 1981) and suggests that breeders should search for sources with similar effect. However, most leaf rust resistance genes described at present condition a post-haustorial resistance type in common wheat (Jacobs, 1989).

In most developed plants post-haustorial resistance is expressed as a hypersensitive response to infection by pathogens, i.e. a rapid death of cells surrounding the infection site (Keen, 1990). Race-specific, hypersensitive resistance to rust fungi, similar to most specific resistances, is often ephemeral, since the pathogen is able to develop races that will render the resistance ineffective (Niks and Dekens, 1991; Smale *et al.*, 1998). It should be noted, however, that hypersensitive resistance is a characteristic of both race-specific and race-nonspecific resistance (Gilchrist, 1998; Parlevliet, 1988). If the leaf rust response of wild relatives of bread wheat is race-nonspecific, and characterised by pre-haustorial resistance, such germplasm would be excellent sources of diversity for breeding purposes.

The aim of this study was to characterize the infection pathway of *P. triticina* in a leaf rust-resistant accession of *T. turgidum* and to determine if infection structure development is altered when the resistance is transferred to common hexaploid wheat.

4.2 MATERIAL AND METHODS

4.2.1 Host material

Histological investigations were conducted on *T. turgidum* ssp. *durum* var *libycum*, SST55 (leaf rust-susceptible *T. aestivum* cultivar), and the F_1 of a cross between these two genotypes. Since studies were done on flag leaves, plants were grown in sterile soil in 1-L-capacity plastic pots. Three plants of the parent lines were grown per pot whereas F_1's were planted individually. Plants were raised at 15°C (night) to 25°C (day) in a rust-free glasshouse cubicle where daylight was supplemented with

120 $\mu Em^{-2}s^{-1}$ photosynthetic active radiation per day. Standard practices for watering and fertilization (see Chapter 2) were applied.

4.2.2 Inoculum production, inoculation and incubation

Prior to inoculation of the parents and F_1 plants, pathotype UVPrt9 of *P. triticina* was produced on seedlings of the susceptible bread wheat cultivar Karee. Karee is a selective host for this pathotype and thus minimizes contamination among cultures. The upper surface of flag leaves of adult plants (Zadoks growth stage 99) were inoculated with a suspension of sterile, distilled water, rust spores (82.6 x 10^4 spores per ml) and the surfactant Tween 20. After inoculation, plants were allowed to air dry for 1 h before they were put in a dark dew cabinet for 16 h. Upon removal, plants were allowed to air dry for 2 h before they were returned to the glasshouse. Infection types were determined 14 days after inoculation according to the 1-4 scale (Roelfs, 1988) (Table 4.1).

4.2.3 Fluorescence microscopy

The protocol described by Bender *et al.* (2000) was followed. One leaf per adult plant was sampled 14 days after inoculation. It was cut into 1 cm^2 pieces and kept in an ethanol:dichloromethane (3:1 v/v) and 0.15 % tricholoroacetic acid solution for 24 h. Thereafter, leaf segments were washed twice with 100 % ethanol and 0.05 M NaOH for 15 min per wash. The segments were rinsed three times with distilled water. It was soaked in Tris/HCl (pH 5.8), stained for 5 min with 0.1 % Uvitex (Novartis, now Syngenta, Basel, Switzerland), washed with water, followed by a 25 %

aqueous glycerol wash. Thereafter, leaf segments were stored in 50 % glycerol with a trace of lacto-phenol.

4.2.3.1 Microscopic examination

Leaf segments were observed as whole mounts. Observations were made at 100x, 200x and 400x magnification on a Nikon Optiphot epifluorescence microscope. Two different filter set combinations were used: UV1A (excitation filter 330 – 380 nm and barrier filter 420 nm) for the observation of fungal structures and B-2A (excitation filter 450 – 490 nm and barrier filter 520 nm) for the observation of plant cell necrosis. With the first filter set, fungal structures were bright blue. Using the second filter set for observation of hypersensitivity, normal cells were a light brown-yellow while all necrotic cells fluoresced a bright yellow.

At least 300 infection sites per parent and accession were observed. Fungal structures, at different stages of development or infection interruption, were counted for SST55 (susceptible control), *T. turgidum* parent (resistant control) and their F_1 progeny.

The histological components prestomatal exclusion, abortive penetration, early abortion and colony formation (Bender *et al.*, 2000), were quantified. Prestomatal exclusions (PE) were defined as germ tubes failing to produce appressoria or forming non-stomatal appressoria (NSA). Abortive penetration (AP) consisted of non-penetrating appressoria (NPA) and aborted substomatal vesicles (ASSV) (Parlevliet and Kievit, 1986). Early abortions (EA) are defined as sites where six or less haustorium mother cells (HMC's) were formed whereas those infection sites with

more than six HMC's were considered successful colonies (Niks, 1983). Early abortions and colonies with necrosis were also distinguished and a distinction was made between sporulating and non-sporulating colonies.

Measurements of uredia and necrotic areas were made with the use of a calibrated eyepiece micrometer. The elliptical dimensions were calculated in mm^2 using the formula: (π x lenth x width)/4. The hypersensitivity index (HI) (Kloppers and Pretorius, 1995b) was calculated by dividing the necrotic area by the colony area to demonstrate the size of the necrotic area in relation to the colony area.

4.3 RESULTS AND DISCUSSION

4.3.1 Infection types

SST55 was susceptible to UVPrt9 showing infection type 3++ (moderate to large, sporulating pustules) on flag leaves (Figure 4.1). *T. turgidum* ssp. *durum* var *libycum* and the adult F_1 both produced ;;1 CN infection types (mostly flecks with the occasional small, sporulating pustules, accompanied by chlorosis and necrosis) to this pathotype (Figure 4.1).

4.3.2 Fluorescence microscopy

Examples of the histological components enumerated are given in Figure 4.2 and results are summarized in Figure 4.3. From the relative contributions of each component it did not appear that *T. turgidum* ssp. *durum* var *libycum* posesses a novel or pre-haustorial resistance mechanism.

4.3.2.1 Prestomatal exclusion

Prestomatal exclusions were marginally more frequent in SST55, (13.3 %) the receptive parent, than in either *T. turgidum* ssp. *durum* var *libycum* (8.8 %) or the F_1 (11.4 %). In all three lines NSA appeared to be the most important cause of prestomatal exclusion, but it was noted that in SST55 45 % of this component was attributed to the failure of germ tubes to form appressoria, whereas the corresponding values for the F_1 and resistant parent were 28 % and 29 %, respectively. There was no significant difference ($P<0.05$) between the prestomatal analysis of SST55, the F_1 and the *T. turgidum* parent. This proved that resistance expressed and inherited from *T. turgidum* ssp. *durum* var *libycum* is post-haustorial.

4.3.2.2 Abortive penetration

Abortive penetration is defined as NPA (non-penetrating appressoria), ASSV (aborted substomatal vesicles) and ASSVN (those aborted vesicles associated with necrosis). There was a significant difference ($P< 0.05$) in the frequency of abortive penetration between both the different parents and the F_1 (Figure 4.4). In SST55 abortive penetration was constituted by about 50% each of NPA and ASSV, with negligible necrosis. In contrast, approximately 40% of AP's in the F_1 and *T. turgidum* parent showed necrosis, indicating an early onset of the HR. Aborted substomatal vesicles were more conspicuous in the F_1 than the resistant parent.

4.3.2.3 Early abortion

In SST55 17 % of all observed sites fell in this category while 14.6 % of sites in the *T. turgidum* parent and 6.1 % in the F_1 aborted early (Figure 4.5). There was a significant difference between accessions for the number of EA's with and without

necrosis. In SST55 almost no EA sites were associated with necrosis, as opposed to *T. turgidum* ssp. *durum* var *libycum* and the F_1 where in excess of 94 % of EA's showed necrosis. This correlates with results of Barnard (1999). ASSVN is associated with early hypersensitive resistance. This type of resistance is characterized by many early abortions, with small to medium sized necrotic colonies and a low infection type (Niks and Dekens, 1987). The infection type of UFS accession 129 was ;; 1 CN which correlates with the above description, but the amount of early abortions in the resistant parent was low (only 14.6 %) which concludes that the resistance of *T. turgidum* ssp. *durum* var *libycum* is probably the result of late hypersensitive response.

4.3.2.4 Formation of colonies

More than 40% of infection sites in all three lines resulted in successful colonies according to the definition used (Figure 4.6). Furthermore, lines did not differ statistically for this parameter. When dissecting the nature of colonies, those in SST55 colonies were almost all necrosis free, while the opposite was true for colonies in *T. turgidum* ssp. *durum* var *libycum* and the F_1 (Figure 4.6). Necrosis was highly conspicuous in the latter and colonies were enveloped in necrotic tissue. The HI of the *T. turgidum* parent was 2.50 ± 0.28 and that of the F_1 1.22 ± 0.12. With the necrotic area surrounding colonies in both parents and F_1 (Figure 4.1) it is not surprising that the HI values exceeded 1. In many cases it seemed as if the necrotic reaction was systemic and carried along the veins (Figure. 4.7). This phenomenon should be investigated further in terms of biochemical resistance mechanisms to determine which signals result in this extensive expression of host cell death.

In studies by Jacobs *et al.* (1996) it was concluded that necrosis resulted from haustorium-induced hypersensitive cell death, which inhibited fungal growth. A lack of correlation between colony growth and hypersensitive response suggested that necrosis is not the only or most important resistance factor in plants showing this resistance (Brown *et al.*, 1966). This was obvious from the present results showing a high frequency of colonies in the resistant entries, despite severe host necrosis. It is also possible that the extensive death of leaf tissue occurred later during the infection process by which time many infections have proceeded beyond the six HMC stage. A late hypersensitive response has previously been associated with similar mechanisms as an early hypersensitive response, with the exception of few early abortions (Niks and Dekens, 1987).

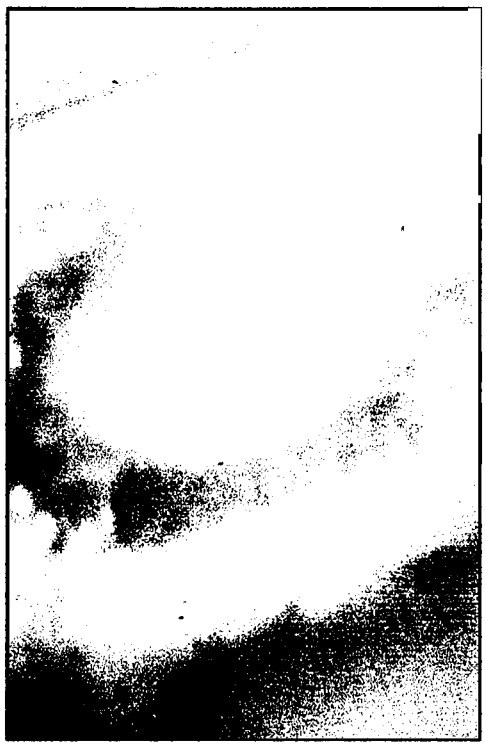
It can be concluded that *T. turgidum* ssp. *durum* var *libycum* conditions posthaustorial resistance. Some evidence of arrestation of fungal growth during early stages was found, but colonies mostly continued to form. This phenomenon is not uncommon since other researchers have noted that posthaustorial resistance often inhibits the pathogen rather than killing it (Chakravarti, 1966; Heath, 1981). The genes in *T. turgidum* ssp. *durum* var *libycum* condition a very strong necrotic reaction, this resistance therefore can not be considered potentially durable. An important observation was that resistance expression was not altered when crossed with bread wheat.

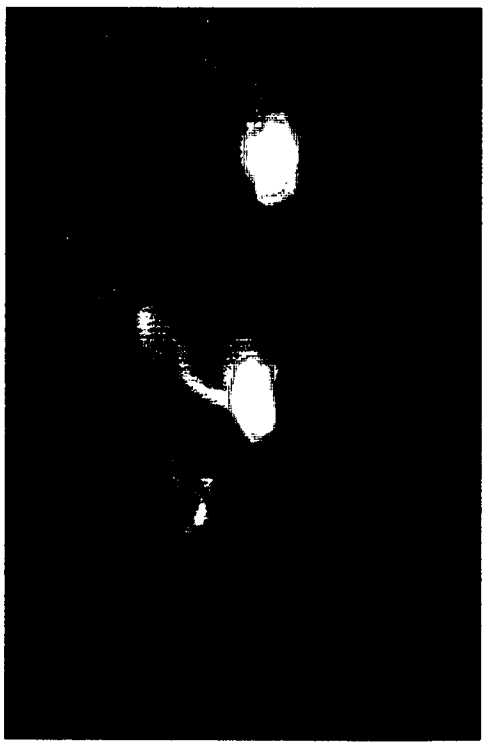
In the search for new resistance sources, genes that contribute to HR should not be overlooked. Such genes often are effective against a range of pathotypes and can be

used in combination with other *Lr* genes for gene stacking. It should be more a question of sensible deployment within durable genotypes rather than ruling out single genes as a resistance resource in principle.

Figure 4.1. Leaf rust reaction of the F_1 of the cross SST55/*T. turgidum* ssp. *durum* v. *libycum* (left) and the *T. aestivum* parent SST55 (right).

Figure 4.2. Histology of leaf rust infection structures in *T. turgidum* ssp. *durum* v. *libycum* and SST55. Colonies of *T. turgidum* ssp. *durum* v. *libycum* were associated with necrosis and under filter set UV1A (excitation filter 330 – 380 nm and barrier filter 420 nm) (400x) the colony is blue (top left), while necrosis of the same colony (right) fluoresces bright yellow using filter set B-2A (excitation filter 450 – 490 nm and barrier filter 520 nm) (top right). Early abortions (bottom left) as well as non-penetrating appressoria (bottom right) were frequently observed.





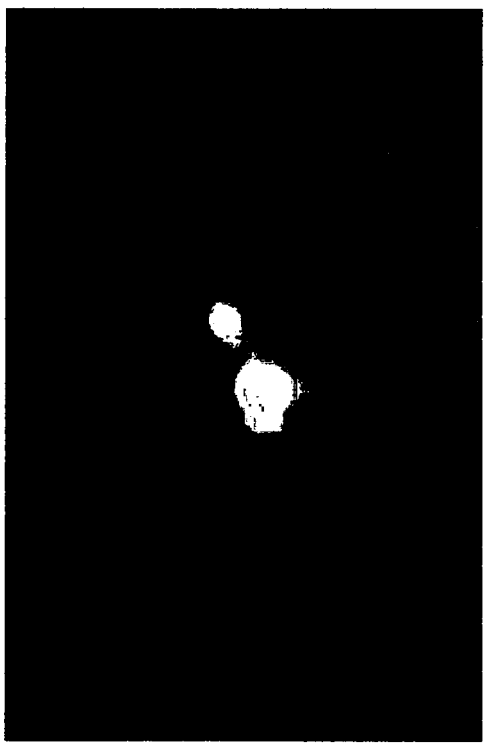
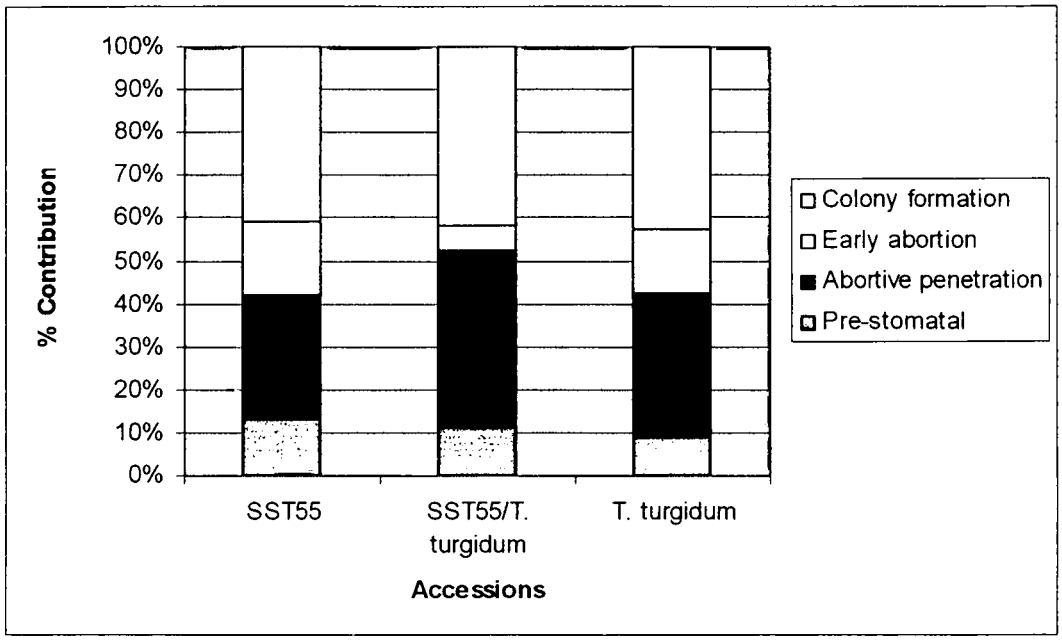


Figure 4.3. Relative proportions of histological components of resistance to *Puccinia triticina* in *Triticum aestivum* cv. SST55, *T. turgidum* ssp. *durum* v. *libycum* and their F_1 hybrid.

Figure 4.4. Abortive penetration, presented as aborted substomatal vesicles with (ASSVN) or without (ASSV) necrosis, and non-penetrating appressoria (NPA), in *Triticum aestivum* cv. SST55, *T. turgidum* ssp. *durum* v. *libycum* and their F_1 hybrid.


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Contribution
SST55
SST55/T. turgidum
T. turgidum
Accessions
Colony formation
Early abortion
Abortive penetration
Pre-stomatal

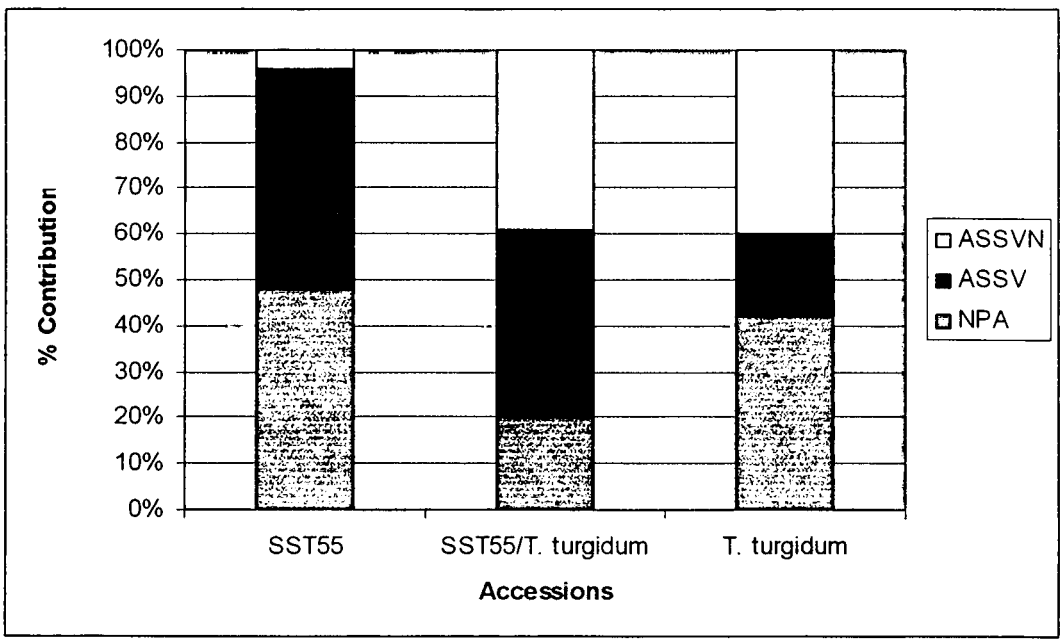
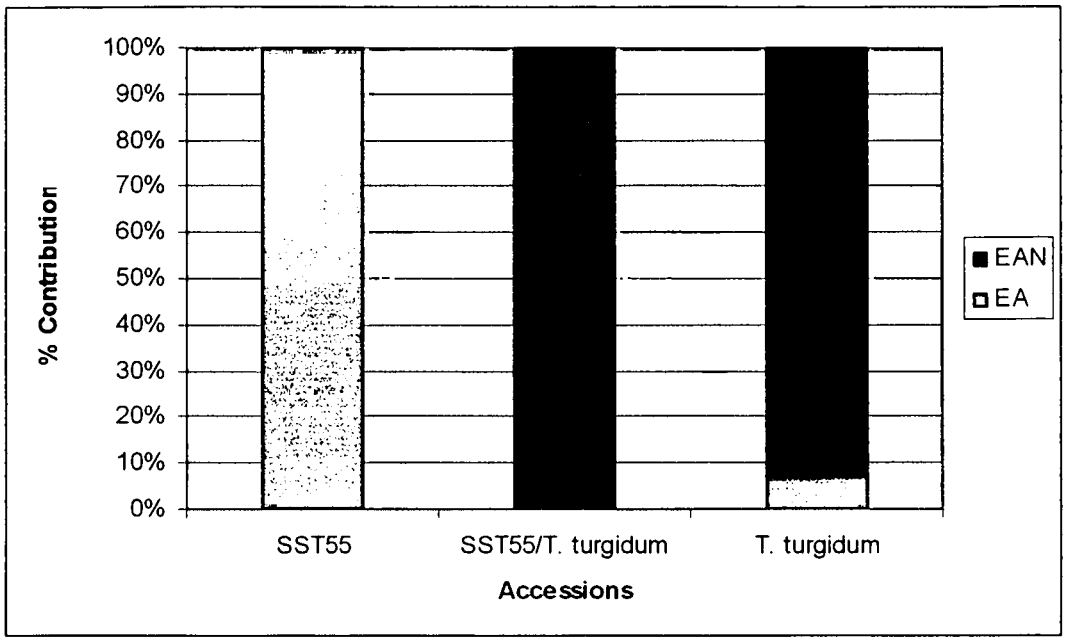

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Contribution
SST55
SST55/T. turgidum
T. turgidum
Accessions
ASSVN
ASSV
NPA

Figure 4.5. Early abortion, presented as early abortions (EA) and early abortions with necrosis (EAN) in *Triticum aestivum* cv. SST55, *T. turgidum* ssp. *durum* v. *libycum* and their F_1 hybrid.

Figure 4.6. Colony formation, presented as sporulating colonies (CS) and sporulating colonies with necrosis (CSN) in *Triticum aestivum* cv. SST55, *T. turgidum* ssp. *durum* v. *libycum* and their F_1 hybrid.


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Contribution
SST55
SST55/T. turgidum
T. turgidum
Accessions
EAN
EA

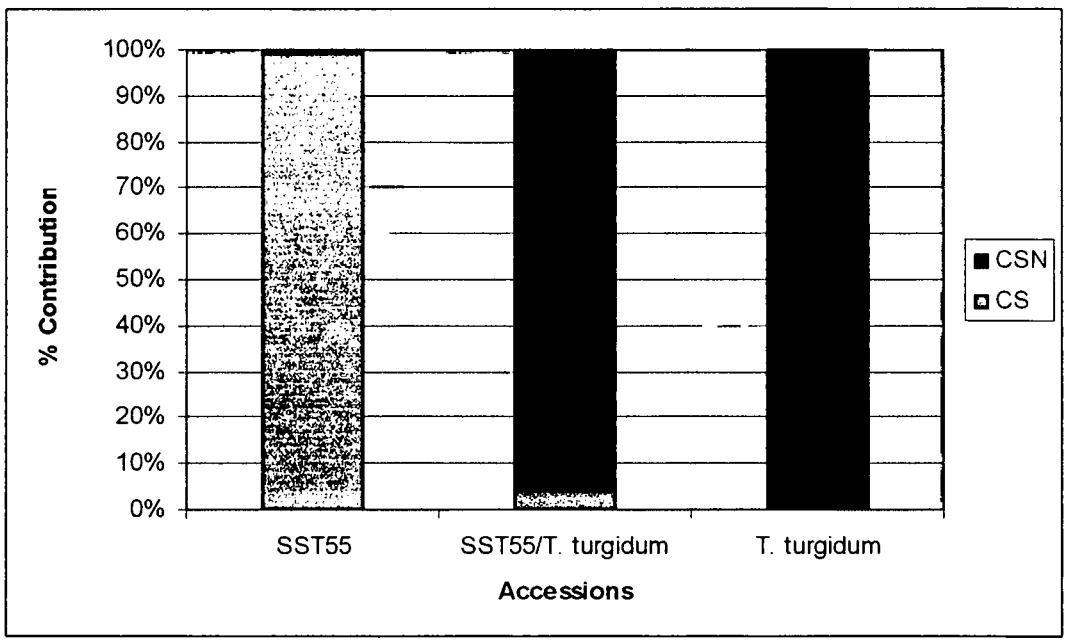
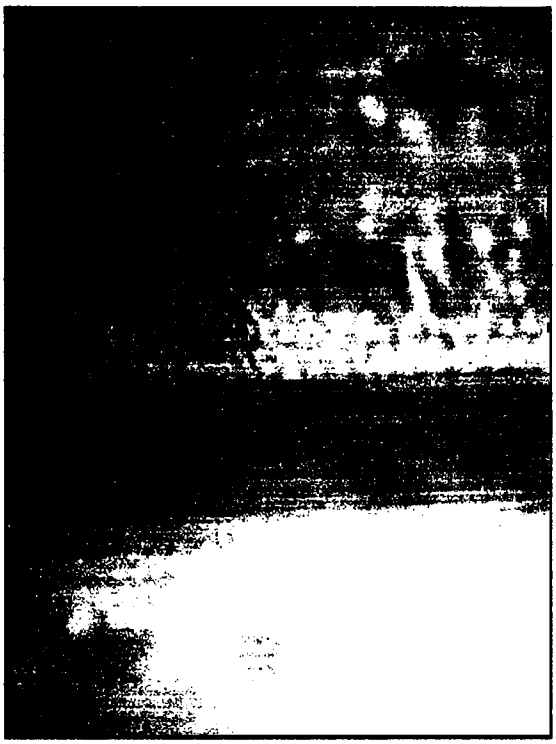
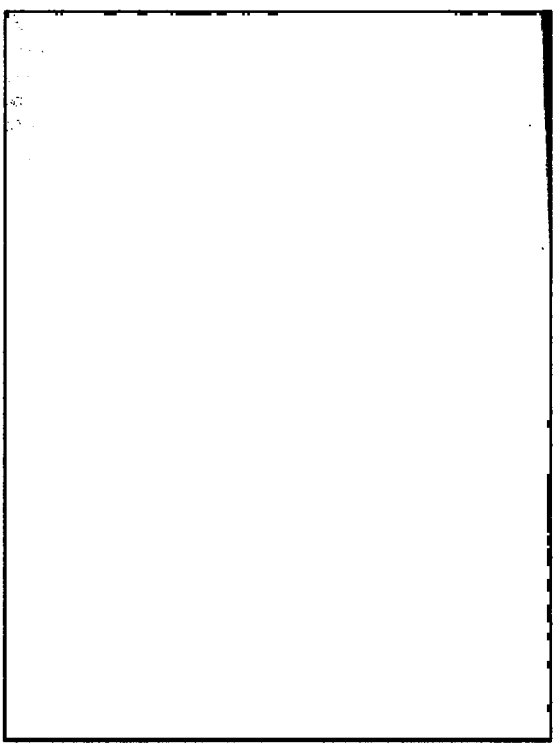

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Contribution
SST55
SST55/T. turgidum
T. turgidum
Accessions
CSN
CS

Figure 4.7. Systemic necrosis in *T. turgidum* ssp. *durum* v. *libycum*. Filter set UV1A (excitation filter 330 – 380 nm and barrier filter 420 nm) (top) shows the blue colony and the bright yellow stripe on filter set B-2A (excitation filter 450 – 490 nm and barrier filter 520 nm) (bottom) indicates dead tissue.





Tabel 4.1. Host response and infection type descriptions used in wheat leaf rust evaluation (Roelfs, 1988)

Host response (class)	IT	Disease symptoms
Immune	0 low	No uredia or macroscopic signs of infection
Nearly immune	; low	No uredia, but necrotic or chlorotic flecks
Very resistant	1 low	Small uredia with necrotic border
Moderately resistant	2 low	Small to medium uredia with chlorosis or necrosis
Moderately susceptible	3 high	Medium-sized uredia
Susceptible	4 high	Large uredia without chlorosis or necrosis
Heterogenous	X low	Random distribution of variable-sized uredia
Heterogenous	Y low	Variable sized uredia, decreasing in size with distance from the leaf tip
Heterogenous	Z low	Variable sized uredia, decreasing in size with distance from the leaf base

SUMMARY

Wheat and the wheat leaf rust pathogen *Puccinia triticina* co-evolved for several millennia. The frequency by which host resistance genes are overcome by the pathogen has lead to a constant search for new genes, in particular in wild species related to wheat, to enlarge the resistance gene pool. For this reason four subspecies of *T. turgidum* that was known to have adult-plant resistance to leaf rust were crossed with the bread wheat cultivar SST55 and studied in terms of expression, inheritance and molecular markers.

Seedling plants of *T. turgidum* ssp. *dicoccoides* (Körn. ex Aschers. and Graebn.) Thell. var. *kotchianum*, *T. turgidum* ssp. *pyramidales*, *T. turgidum* ssp. *durum* (Desf.) Husn var. *libycum* and *T. turgidum* ssp. *abyssinicum* were inoculated with different races of *P. triticina*, *P. graminis* f. sp. *tritici* and *P. striiformis* f. sp. *tritici*. A differential interaction was observed between accessions and pathotypes, indicating that the plants had race-specific resistance. All showed adult-plant resistance with leaf rust infection types ranging between ";" (flecking) and ";1CN" (flecks and small pustules associated with chlorosis and/or necrosis).

Despite of low pollen viability, sterility and recessiveness of resistance genes in three of the accessions, F_1, F_2 and F_3's were produced. *T. turgidum* ssp. *durum* v. *libycum* was the only accession that produced resistant F_1's, indicating a dominant gene or genes. Mendelian ratios in the F_2 progeny suggested that

resistance in this accession resulted from two major genes. The F_2 of the *T. turgidum* ssp. *abyssinicum* and *T. turgidum* ssp. *pyramidales* crosses did not segregate according to Mendelian ratios and two minor genes conferred resistance of *T. turgidum* ssp. *dicoccoides* v. *kotchianum*.

The molecular AFLP technique was used to to follow the introgression of resistance from tetraploid *T. turgidum* ssp. *dicoccoides* v. *kotchianum* and *T. turgidum* ssp. *durum* v. *libycum* to the hexaploid bread wheat cultivar SST55. In total nine fragments were solely introgressed from *T. turgidum* ssp. *dicoccoides* v. *kotchianum* and four from *T. turgidum* ssp. *durum* v. *libycum* and were present in all resistant accessions. These fragments have potential to be developed into molecular markers.

Histology tests done on accession *T. turgidum* ssp. *durum* v. *libycum* confirmed a post-haustorial expression of resistance. Resistance in this accession was strongly associated with a hypersensitive response. Necrosis started at the aborted sub-stomatal vesicle stage and continued through to colony formation. All colonies were enveloped in necrotic leaf tissue with the parent having a higher hypersensitivity index than the F_1. In some cases it seemed as if necrosis was systemic and carried along the veins.

Although hypersensitive resistance as observed in this study is not considered durable it should not be overlooked in the search for new resistance genes. The

challenge to the breeder is to use these genes in genetic backgrounds where it will be protected against pathogenic adaptation in the leaf rust fungus.

OPSOMMING

Koring en die koringblaarroes patogeen *Puccinia triticina* het gekoëvoleer vir etlike millennia. Die frekwensie waarteen weerstandsgene deur die patogeen oorkom word, het gelei tot 'n konstante soektog na nuwe gene, veral van die wilde families van koring, om die weerstandsgeenpoel aan te vul. Om hierdie rede is vier subspesies van *T. turgidum* wat oor volwasseplantsweerstand beskik, gekruis met die broodkoring kultivar SST55 en bestudeer op grond van geenuitdrukking, oorerwing en molekulêre merkers.

Saailingplante van *T. turgidum* ssp. *dicoccoides* (Körn. ex Aschers. en Graebn.) Thell. var. *kotchianum*, *T. turgidum* ssp. *pyramidales*, *T. turgidum* ssp. *durum* (Desf.) Husn var. *libycum* en *T. turgidum* ssp. *abyssinicum* is geïnokuleer met verskillende rasse van *P. triticina*, *P. striiformis* f. sp. *tritici* asook *P. graminis* f. sp. *tritici*. Die verskeie interaksies wat waargeneem is tussen koringlyne en roesrasse het daarop gedui dat die plante oor ras-spesifieke weerstand beskik. Alle plante het volwasseplantsweerstand getoon met blaarroes infeksietipes wat gewissel het tussen ";" (vlek) en ";CN" (vlekke en klein roespuisies geassosieer met chlorose en nekrose).

Ten spyte van die lae stuifmeel lewensvatbaarheid, steriliteit en resessiwiteit van weerstandsgene in drie van die koringlyne, is F_1, F_2 en F_3 plante geproduseer. *T. turgidum* ssp. *durum* v. *libycum* was die enigste koringlyn wat weerstandbiedende F_1's geproduseer het. Hierdie aanduiding dat weerstand in dié lyn die resultaat van 'n dominante geen of gene is, is bevestig deur Mendeliese toetse op die F_2 nageslag wat

aangedui het dat twee dominante gene verantwoordelik was vir weerstand. Alhoewel die F_2 plante van die kruisings *T. turgidum* ssp. *pyramidales* en *T. turgidum* ssp. *abyssinicum* nie volgens Mendeliese verhoudings gesegregeer het nie, het dieselfde toetse aangedui dat weerstand deur twee resessiewe gene veroorsaak word in *T. turgidum* ssp. *dicoccoides* v. *kotchianum*.

Die molekulêre AFLP tegniek is gebruik om die introgressie van weerstand vanaf die tetraploïede *T. turgidum* ssp. *durum* v. *libycum* en *T. turgidum* ssp. *dicoccoides* v. *kotchianum* na heksaploïede broodkoring kultivar SST55 te volg. In totaal is nege fragmente uitsluitlik verkry vanaf *T. turgidum* ssp. *dicoccoides* v. *kotchianum* en vier vanaf *T. turgidum* ssp. *durum* v. *libycum*. Hierdie fragmente was teenwoordig in alle weerstandbiedende plante en besit die potensiaal om as molekulêre merkers ontwikkel te word.

Die histologie toetse gedoen op *T. turgidum* ssp. *durum* v. *libycum* het post-haustoriale weerstand bevestig. Weerstand in hierdie koringlyn is geassosieer met die hipersensitiewe reaksie. Nekrose het begin by die geaborteerde substomatale fase en was deurlopend tot by die kolonies. Alle kolonies was omring met nekrotiese blaarweefsel en die ouer het 'n hoër hipersensitiewe indeks as die F_1 gehad. In sommige gevalle het dit voorgekom asof die nekrose sistemies was en met are vervoer is.

Ten spyte daarvan dat hipersensitiewe weerstand, soos waargeneem in hierdie studie, nie as lanklewend geag word nie, moet dit nie geïgnoreer word in die soektog na nuwe

weerstandsgene nie. Die uitdaging vir die koringteler is om hierdie gene te gebruik in 'n genetiese agtergrond waar hulle beskermd sal wees teen patogeniese mutasies van die blaarroespatogeen.

REFERENCES

ACOSTA, A.C. 1962. The transfer of stem rust resistance from rye to wheat. Dissertation Abstracts 23: 34 – 35.

ANDERSON, R.G., WILLIAMS, N.D. and MAAN, S.S. 1971. Monosomic analyses of genes for resistance derived from Marquis and Reliance wheat. Crop Science 11: 556 – 558.

ANIKSTER, Y., BUSHNELL, W.R., EILAM, T., MANISTERSKI, J. and ROELFS, A.P. 1997. *Puccinia recondita* causing leaf rust on cultivated wheats, wild wheats and rye. Canadian Journal of Botany 75: 2082 – 2096.

ANTONOV, A.I. and MARAIS, G.F. 1996. Identification of leaf rust resistance genes in *Triticum* species for transfer to common wheat. South African Journal of Plant and Soil 13: 55 – 60.

AUSEMUS, E.R., HARRINGTON, J.B., REITZ, L.P. and WORZELLA, W.W. 1946. A summary of genetic studies in hexaploid and tetraploid wheats. Journal of the American Society of Agronomy 38: 1083 -1099.

BADAEVA, D., FRIEBE, B. and GILL, B.S. 1996. Genome differentiation in *Aegilops*. 1. Distribution of highly repetitive DNA sequences on chromosomes of diploid species. Genome 39: 293 – 306.

BAI, D. and KNOTT, D.R. 1992. Suppression of rust resistance in bread wheat (*Triticum aestivum* L.) by D-genome chromosomes. Genome 35: 276 – 282.

BAKER, E.P., SANGHI, A.K., McINTOSH, T.A. and LUIG, N.H. 1970. Cytogenetical studies in wheat III. Studies of a gene conditioning resistance to stem rust strains with unusual genes for avirulence. Australian Journal of Biological Sciences 23: 369 – 375.

BARIANA, H.S. 1991. Genetic studies on stripe rust resistance in wheat. PhD Thesis, University of Sydney.

BARIANA, H.S. and McINTOSH, 1993. Cytogenetical studies in wheat XIV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. Genome 36: 476 – 482.

BARNARD, A.D. 1999a. Identification of genetic variation in bread wheat quality characteristics in the Western Cape. Magister Scientiae Thesis. Department of Plant Breeding, Faculty of Agriculture. UOFS.

BARNARD, J.E. 1999b. Adult-plant resistance to *Puccinia recondita* f. sp. *tritici* in a collection of wild *Triticum* species. Magister Scientiae Thesis. Department of Plant Pathology, Faculty of Agriculture. UOFS.

BAXTER, A.P. and VAN DER LINDE, E. 1999. Collecting and preserving fungi – A manual for mycology. SAFRINET, The South African (SADC) LOOP of Bio NET- INTERNATIONAL. Ultra Litho (Pty) Ltd. Hereotdale, Johannesburg.

BENDER, C.M., PRETORIUS, Z.A. and SPIES, J.J. 1997. Assessment of macroscopic components of leaf rust resistance in wheat genotypes containing *Lr*12 and *Lr*13. South African Journal of Plant Soil. 14: 2.

BENDER, C.M., PRETORIUS, Z.A., KLOPPERS, F.J. and SPIES, J.J. 2000. Histopathology of leaf rust infection and development in wheat genotypes containing *Lr*12 and *Lr*13. Journal of Phytopathology 146: 65 – 76.

BOHN, M., UTZ, H.F. and MELCHINGER, A.E. 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs and SSRs and their use for predicting progeny variance. Crop Science 39: 228 – 237.

BOSHOFF, W.H.P. 2000. Control of foliar rusts of wheat in South Africa with special emphasis on *Puccinia striiformis* f. sp. *tritici*. Doctor of Philosophy

Thesis. Faculty of Natural and Agricultural Sciences, Department of Plant Pathology, UOFS.

BOSHOFF, W.H.P., PRETORIUS, Z.A. and VAN NIEKERK, B.D. 2002a. The impact of leaf rust on spring wheat in the winter rainfall region of South Africa. South African Journal of Plant and Soil 19: 84 – 88.

BOSHOFF, W.H.P., PRETORIUS, Z.A. and VAN NIEKERK, B.D. 2002b. Resistance in South African and foreign wheat cultivars to pathotypes 6E16A – and 6E22A – of *Puccinia striiformis* f. sp. *tritici*. South African Journal of Plant Soil 19: 27 – 36.

BOWER, J-M. 2002. Wheat leaf rust resistance in selected *Triticum turgidum* accessions. Magister Scientiae Thesis. Department of Plant Sciences (Genetics). Faculty of Natural and Agricultural Sciences, UOFS.

BROWDER, L.E. 1972. Designation of two genes for resistance to *Puccinia recondita* in *Triticum aestivum*. Crop Science 12: 705 – 706.

BROWDER, L.E. 1973. Probable genotypes of some *Triticum aestivum* 'Agent' derivatives for reaction to *Puccinia recondita* f. sp. *tritici*. Crop Science. 13: 203 – 206.

BROWDER, L.E. 1980. A compendium of information about named genes for low reaction to *Puccinia recondita* in wheat. Crop Science 20: 775 – 779.

BROWN, J.F., SHIPTON, W.A. and WHITE, N.H. 1966. The relationship between hypersensitive tissue and resistance in wheat seedlings infected with *Puccinia graminis tritici*. Annual Applied Biology 53: 279 – 290.

BROWN-GUEDIRA, G.L., GILL, B.S., COX, T.S. and LEATH, S. 1997. Transfer of disease resistance genes from *Triticum araraticum* to common wheat. Plant Breeding 116: 105 – 112.

BURGER, F.H. 1995. The value of certain cytogenetic and molecular techniques in plant systematics. Magister Scientiae Thesis. Department of Botany and Genetics, University of the Orange Free State.

CERVERA, M.T., GUSMAO, J., STEENACKERS, M., VAN GYSEL, A, VAN MONTAGU, M. and BOERJAN, W. 1996. Application of AFLP^{TM}-based molecular markers to breeding of *Populus* spp. Plant Growth Regulation 20: 47 – 52.

CHAKRAVARTI, B.P. 1966. Attempts to alter infection processes and aggressiveness of *Puccinia graminis* var. *tritici*. Phytopathology 56: 223 – 229.

CHEN, X. and LINE, R.F. 1992. Inheritance of stripe rust resistance in wheat cultivars used to differentiate races of *Puccinia striiformis* in North America. Phytopathology 82: 633 – 637.

CHO, Y.G., BLAIR, M.W., PANAUD, O. and McCOUCH, S.R. 1996. Cloning and mapping of variety-specific rice genomic DNA sequences: amplified fragment length polymorphisms (AFLP) from silver-stained polyacrylamide gels. Genome 39: 373 – 378.

CHOUDHURI, H.C. 1958. The inheritance of stem and leaf rust resistance in common wheat. Indian Journal of Genetics 18: 90 – 115.

COOKE, R.C. 1977. Fungi, Man and his Environment. Longman group Limited. London.

COX, T.S., RAUPP, W.J. and GILL, B.S. 1994. Leaf rust-resistance genes *Lr*41, *Lr*42 and *Lr*43 transferred from *Triticum tauschii* to common wheat. Crop Science 34: 339 – 343.

CRAIGIE, J.H. 1927. Discovery of the function of the pycnia of the rust fungi. Nature 120: 765 – 767.

CRAVEN, M. 2002. The improvement of leaf rust resistance in selected bread wheat lines. Magister Scientiae Thesis. Department of Plant Sciences, University of the Orange Free State.

d'OLIVEIRA, B. and SAMBORSKI, D.J. 1966. Aecia stage of *Puccinia recondita* on Ranunculaceae and Boraginaceae in Portugal. Proceedings of the first European brown rust conference. Cereal Rust conference. June 29 – July 24, 1964, Plant Breeding Institute, Cambridge, U.K.

DEDRYVER, F., JUBIER, M-F., THOUVENIN, J. and GOYEAU, H. 1996. Molecular markers linked to the leaf rust resistance gene *Lr*24 in different wheat cultivars. Genome 39: 830-835.

DICKSON, J.G. 1956. Disease of field crops, Second edition. McGraw-Hill book company, Inc., New York.

DRISCOLL, C.J. and ANDERSON, L.M. 1967. Cytogenetic studies of Transec – a wheat-rye translocation line. Canadian Journal of Genetics and Cytology 9: 375 – 380.

DUBIN, H.J. and TORRES, E. 1981. Causes and consequences of the 1976-1977 wheat leaf rust epidemic in northwest Mexico. Ann. Rev. Phytopathology 19: 41-49.

DVORAK, J. 1977. Transfer of leaf rust resistance from *Aegilops speltoides* to *Triticum aestivum*. Canadian Journal of Genetics and Cytology 19: 133 – 141.

DYCK, P.L. 1979. Identification of the gene for adult plant leaf rust resistance in Thatcher. Canadian Journal of Plant Science 59: 499 -501.

DYCK, P.L. 1987. The association of a gene for leaf rust with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29: 467-469.

DYCK, P.L. 1992. Transfer of a gene for stem rust resistance from *Triticum araraticum* to hexaploid wheat. Genome 35: 788 – 792.

DYCK, P.L. and JEDEL, P.E. 1989. Genetics of resistance to leaf rust in two accessions of common wheat. Canadian Journal of Plant Science 69: 531 – 534.

DYCK, P.L. and KERBER, E.R. 1977. Chromosome location of gene *Sr*29 for reaction to stem rust. Canadian Journal of Genetics and Cytology 19: 371 – 373.

DYCK, P.L. and KERBER, E.R. 1981. Aneuploid analysis of a gene for leaf rust resistance derived from the common wheat cultivar Terenzio. Canadian Journal of Genetics and Cytology 23: 405 – 409.

DYCK, P.L. and SAMBORSKI, D.J. 1968a. Host-parasite interactions involving two genes for leaf rust resistance in wheat. Pages 245 – 250 in: K.W. Findlay and K.W. Shepherd, eds. Proceedings of the Third International Wheat Genetics Symposium. Australian Academy of Science, Canberra, Australia.

DYCK, P.L. and SAMBORSKI, D.J. 1968b. Genetics of resistance to leaf rust in the common wheat varieties Webster, Loros, Brevit, Carina, Malakof and Centenario. Canadian Journal of Genetics and Cytology 10: 7 – 17.

DYCK, P.L., KERBER, E.R. and LUKOW, OM. 1987. Chromosome location and linkage of a new gene (*Lr*33) for reaction to *Puccinia recondita* in common wheat. Genome 29: 463 – 466.

DYCK, P.L., SAMBORSKI, D.J. 1970. The genetics of two alleles for leaf rust resistance at the *Lr*14 locus in wheat. Canadian Journal of Genetics and Cytology 12: 689 – 694.

DYCK, P.L., SAMBORSKI, D.J. 1974. Inheritance of virulence in *Puccinia recondita* of alleles at the *Lr*2 locus for resistance in wheat. Canadian Journal of Genetics and Cytology 16: 323 – 332.

DYCK, P.L., SAMBORSKI, D.J. 1982. The inheritance of resistance to *Puccinia recondita* in a group of common wheat cultivars. Canadian Journal of Genetics and Cytology 24: 273 – 283.

DYCK, P.L., SAMBORSKI, D.J. and ANDERSON, R.G. 1966. Inheritance of adult-plant resistance derived from the common wheat varieties Exchange and Frontana. Canadian Journal of Genetics and Cytology 8: 665 – 671.

EDWARDS, K., JOHNSTONE, C and THOMPSON, C. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCT analysis. Nucleic Acids Research 19: 1349.

FRIEBE, F., JIANG, J., GILL, B.S. and DYCK, P.L. 1993. Radiation-induced nonhomoeologous wheat – *Agropyron intermedium* chromosomal translocatons conferring resistance to leaf rust. Theoretical and Applied Genetics 86: 141 – 149.

FRIEBE, F., ZELLER, F.J., MUKAI, Y., FORSTER, B. P., BARTOS, P. and McINTOSH, R.A. 1992. Characterization of rust resistant wheat – *Agropyron intermedium* derivatives by C-banding *in situ* hybridization and isozyme analysis. Theoretical and Applied Genetics 83: 775 – 749.

FUENTES, J.L., ESCOBAR, F., ALVAREZ, A., GALLEGO, G., DUQUE, M.C., FERRER, M., DEUS, J.E. and THOME, J.M. 1999. Analyses of diversity in Cuban rice varieties using isozyme, RAPD and AFLP markers. Euphytica 109: 107 – 115.

GAINES, C.S., FINNEY, P.L. and RAUBENTHALER, G. 1996. Milling and baking qualities of some wheats developed for Eastern or North-Western regions of the United States and grown at both locations. Cereal Chemistry 73: 521 – 252.

GERECHTER-AMITAI, Z.K., VAN SILFHOUT, C.H., GRAMA, A. and KLEITMAN, F. 1989. *Yr*15 – a new gene for resistance to *Puccinia striiformis* in *Triticum dicoccoides* sel. G-25. Euphytica 43: 187 – 190.

GIBBS RUSSELL, G.E., WATSON, L., KOEKEMOER, M., SMOOK, L., BARKER, N.P., ANDERSON, H.M. and DALLWITZ, M.J. 1990. Grasses of Southern Africa. National Botanic Garden/Botanical Research Institute. South Africa.

GILCHRIST, D.G. 1998. Programmed cell death in plant disease: The purpose and promise of cellular suicide. Annual Review of Phytopathology 36: 393 – 414.

GOLD, J., HARDER, D., TOWNLEY-SMITH, F., AUNG, T. and PROCUNIER, J. 1999. Development of a molecular marker for rust resistant genes *Sr*39 and *Lr*35 in wheat breeding lines. Electronic Journal of Biotechnology 2: 1 – 6.

GOODING, M.J. and DAVIES, W.P. 1997. Wheat production and utilization. Systems, quality and the environment. University Press, Cambridge.

GOODMAN, R.N. and NOVACKY, A.J. 1994. The hypersensitive reaction in plant to pathogens: A resistance phenomenon. The American Phytopathological Society, Minnesota. USA.

GRAHAM, T.L. and GRAHAM, M.Y. 1999. Role of hypersensitive cell death in conditioning elicitation competency and defence potentiation. Physiological and Molecular Plant Pathology 55: 13 – 20.

GREEN, G.J., KNOTT, D.R., WATSON, I.A and PUGSLEY, A.T. 1960. Seedling reaction to stem rust of lines of Marquis wheat with substituted genes for rust resistance. Canadian Journal of Plant Science 40: 524 – 538.

HAGGAG, M.E.A. and DYCK, P.L. 1973. The inheritance of leaf rust resistance in four common wheat varieties possessing genes at or near the *Lr*3 locus. Canadian Journal of Genetics and Cytology 15: 127 – 134.

HARLAN, J.R. 1981. Wheat Science – Today and Tomorrow. University Press, Cambridge.

HEATH, M.C. 1974. Light and electron microscope studies of the interaction of host and non-host plants with cowpea rust – *Uromyces phaseoli* var. *vignae*. Physiology and Plant Pathology 4: 403 – 414.

HEATH, M.C. 1976. Hypersensitivity, the cause or the consequence of rust resistance? Phytopathology 66: 935 – 936.

HEATH, M.C. 1977. A comparative study of non-host interactions with rust fungi. Physiology and Plant Pathology 10: 73 – 88.

HEATH, M.C. 1981. Resistance of plants to rust infection. Phytopathology 71: 971 - 974.

HEATH, M.C. 1982. Host defence mechanisms against infection by rust fungi. The rust fungi. K.J. Scott & Chakravorty, eds. Academic Press, Inc.

HEATH, M.C. 1998. Involvement of reactive oxygen species in the response of resistant (hypersensitive) or susceptible cowpeas to the cowpea rust fungus. New Phytologist 138: 251 – 263.

HILL, M., WITSENBOER, H., ZABEAU, M., VOS, P., KESSELI, R. and MICHELMORE, R. 1996. PCR-based fingerprinting using AFLPs as a tool for studying genetic relationships in *Lactuca* spp. Theoretical and Applied Genetics 93: 1202-1210.

HONGTRAKUL, V., HUESTIS, G.M. and KNAPP, S.J. 1997. Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm:

genetic diversity among oilseed inbred lines. Theoretical and Applied Genetics 95: 400 – 407.

HUANG, Y.H. and JOHNSON, C.E. 1996. A convenient and reliable method to evaluate blueberry pollen viability. Hort. Science 31: 1235.

ISHII, T., BRAR, D.S., MULTANI, D.S. and KHUSH, G.S. 1993. Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O. sativa*. Genome 37: 217 – 221.

JACOBS, A.S., PRETORIUS, Z.A., KLOPPERS, F.J. and COX, T.S. 1996. Mechanisms associated with wheat leaf rust resistance derived from *Triticum monococcum*. Phytopathology 86: 588 – 595.

JACOBS, T.H. 1989. The occurrence of cell wall appositions in flag leafs of spring wheat, susceptible and partially resistant to what leaf rust. Journal of Phytopathhology 127: 239 – 249.

JACOBS, A.J., PRETORIUS, Z.A. and COUTINO, T.A. 2002. Quantification of early infection structures of *Puccinia recondita* f. sp. *tritici* in wheat with leaf rust resistance derived from *Triticum monococcum*. South African Journal of Science 96: 86 – 90.

JIANG, J., FRIEBE, B. and GILL, B.S. 1994. Recent advances in alien gene transfer in wheat. Euphytica 73: 199 – 212.

JOHNSON, R. 1979. The concept of durable resistance. Phytopathology 69: 198 – 199.

JOHNSON, R. 1981. Durable resistance: definition of, genetic control and attainment in plant breeding. Phytopathology 71: 567-568.

JONES, C.J., EDWARDS, K.J., CASTAGLIONE, S., WINFIELD, MO., SALA, F., VAN DE WIEL, C., BREDEMEIJER, G., VOSMAN, B., MATTHES, M., DALY, A., BRETTSCHNEIDER, R., BETTINI, P., BUIATTI, M., MAESTRI, E., MALCEVSCHI, A., MARMIROLI, N., AERT, R., VOLCKAERT, G., RUEDA, J., LINACERO, R., VAZQUIZ, A. and KARP, A. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Molecular Breeding 3: 381 – 390.

JONES, S.S., MURRAY, T.D. and ALLAN, R.E. 1995. Use of alien genes for the development of disease resistance in wheat. Annual Review of Phytopathology 33: 429 – 443.

JOSHI, C.P. and NGUYEN, H.T. 1993. Application of the random amplified polymorphic DNA technique for the detection fo polymorphism among wild and cultivated tetraploid wheats. Genome 32: 724 – 732.

KEEN, N.T. 1990. Gene-for-gene complementarity in plant-pathogen interactions. Annual Review of Genetics 24: 447-463.

KENDRICK, B. 1992. The Fifth Kingdom. Second Edition. Mycologue Publications.

KERBER, E.R. 1987. Resistance to leaf rust in hexaploid wheat, *Lr*32, a third gene derived from *Triticum tauschii*. Crop Science 27: 204 – 206.

KERBER, E.R. and DYCK, P.L. 1973. Inheritance of stem rust resistance transferred from diploid wheat (*Triticum monococcum*) to tetraploid and hexaploid wheat and chromosome location of the gene involved. Canadian Journal of Genetics and Cytology 15: 397 – 409.

KERBER, E.R. and DYCK, P.L. 1979. Resistance to stem rust and leaf rust of wheat in *Aegilops squarrosa* and transfer of a gene for stem rust resistance to

hexaploid wheat. Pages 358 – 364 in: S. Ramanujam, eds. Proceedings of the Fifth International Wheat Genetics Symposium.

KERBER, E.R. and DYCK, P.L. 1990. Transfer to hexaploid wheat of linked genes for adult-plant leaf rust and seedling stem rust resistance from an amphiploid of *Aegilops speltoides* x *Triticum monococcum*. Genome 33: 530 – 537.

KERBER, E.R. and GREEN, G.J. 1980. Suppression of stem rust resistance in the hexaploid wheat cultivar Canthatch by chromosome 7DL. Canadian Journal of Botany 58: 1347 – 1350.

KIBIRIGE-SEBUNYA, I. and KNOTT, D.R. 1983. Transfer of stem rust resistance to wheat from an *Agropyron* chromosome having a gametocidal effect. Canadian Journal of Genetics and Cytology 25: 215 – 221.

KLOPPERS, F.J. 1994. Characterization of resistance conferred by selected *Lr* genes with emphasis on histopathology, leaf rust development and associated quality attributes in wheat. Doctor of Philosophy Thesis. Department of Plant Pathology, University of the Orange Free State.

KLOPPERS, F.J., and PRETORIUS, Z.A. 1995a. Field evaluation of leaf rust severity, yield loss and quality characteristics in near-isogenic wheat lines with *Lr*13, *Lr*35 and *Lr*37. South African Journal of Plant Soil 12: 55 – 58.

KLOPPERS, F.J., and PRETORIUS, Z.A. 1995b. Histology of the infection and development of *Puccinia recondita* f. sp. *tritici* in a wheat line with *Lr*37. Journal of Phytopathology 143: 261 – 267.

KLOPPERS, F.J., and PRETORIUS, Z.A. 1997. Effects of combinations amongst genes *Lr*13, *Lr*34 and *Lr*37 on components of resistance in wheat to leaf rust. Plant Pathology 46: 737 – 750.

KLUG, W.S. and CUMMINGS, M.R. 1994. Concepts of Genetics. Fourth Edition. Prentice Hall.

KNOTT, D.R. 1961. The inheritance of rust resistance. VI. The transfer of stem rust resistance from *Agropyron elongatum* to common wheat. Canadian Journal of Plant Science 41: 109 – 123.

KNOTT, D.R. 1962. The inheritance of rust resistance, IX. The inheritance of resistance to races 15B and 56 of stem rust in the wheat variety Khapstein. Canadian Journal of Plant Science 42: 415 – 419.

KNOTT, D.R. 1966. The inheritance of stem rust resistance in wheat. Pages 156 – 166 in: J. MacKey, eds. Proceedings of the Second International Wheat Genetics Symposium. Lund, Sweden.

KNOTT, D.R. 1968. The inheritance of resistance to stem rust races 56 and 15B-1L (Can) in the wheat varieties Hope and H-44. Canadian Journal of Genetics and Cytology 10: 311 – 320.

KNOTT, D.R. 1989. The Wheat Rusts – Breeding for Resistance. Springer-Verlag.

KNOTT, D.R. and ANDERSON, R.G. 1956. The inheritance of rust resistance. I. The inheritance of stem rust resistance in ten varieties of common wheat. Canadian Journal of Agricultural Science 36: 174 – 195.

KNOTT, D.R. and DVORAK, J. 1976. Alien germplasm as a source or resistance to disease. Annual Review of Phytopathology 14: 211 – 235.

KNOTT, D.R. and YADAV, B. 1993. The mechanism and inheritance of adult plant leaf rust resistance in 12 wheat lines. Genome 36: 877 – 883.

KOLMER, J.A. 1996. Genetics of resistance to wheat leaf rust. Annual Review of Phytopathology 34: 435 – 455.

KOLMER, J.A., LIU, J.Q. and SIES, M. 1995. Virulence and Molecular Polymorphisms in *Puccinia recondita* f. sp. *tritici* in Canada. Genetics 85: 276 – 285.

KUHN, R.C., OHM, H.W. and SHANER, G.E. 1978. Slow leaf rusting resistance in wheat against twenty-two isolates of *Puccinia recondita*. Phytopathology 68: 651 – 656.

KULKARNI, R.N. and CHOPRA, V.L. 1980. Slow-rusting resistance: Its components, nature and inheritance. Journal of Plant diseases and Protection 87: 562 – 573.

LAW, C.N. and WOLFE, M.S. 1966. Location of genetic factors for mildew resistance and ear emergence time on chromosome 7B of wheat. Canadian Journal of Genetics and Cytology 8: 462 – 470.

LAW, J.R., DONINI, P., KOEBNER, R.M.D., REEVES, J.C. and COOKE, R.J. 1998. DNA profiling and plant variety registration. III : The statistical assessment of distinctness in wheat using amplified fragment length polymorphisms. Euphytica 102: 335 – 342.

LIN, J. and KUO, J. 1995. AFLP^{TM}: A novel PCR-based assay for plant and bacterial DNA fingerprinting. Focus 17: 2.

LITTLEFIELD, L.J. and HEATH, M.C. 1979. The Ultrastructure of Rust Fungi. Academic Press. London.

LOCKHART, P.J. and McLENACHAN, P.A. 1997. Isolating polymorphic plant DNA fragments identified using AFLP^{TM} technology without acrylamide gels: Markers for evolutionary studies. Focus 19: 70 – 71.

LOEGERING, W.Q. 1975. An allele for low reaction to *Puccinia graminis tritici* in Chinese Spring wheat. Phytopathology 65: 925.

LOEGERING, W.Q. and SEARS, E.R. 1966. Relationships among stem-rust genes on wheat chromosomes 2B, 4B and 6B. Crop Science 6: 157 – 160.

LUIG, N.H. and McINTOSH, R.A. 1968. Location and linkage of genes on wheat chromosome 2D. Canadian Journal of Genetics and Cytology 10: 99 – 105.

LUIG, N.H. and RAJARAM, S. 1972. The effect of temperature and genetic background on host gene expression and interaction to *Puccinia graminis tritici*. Phytopathology 66: 1435 – 1438.

LUPTON, F.G.H. 1987. Wheat Breeding – its scientific basis. Chapman and Hall Ltd. University Press. Cambridge.

LUPTON, F.G.H. and MACER, R.C.F. 1962. Inheritance or resistance to yellow rust *(Puccinia glumarum* Erikss. & Henn) in seven varieties of wheat. Transactions of the British Mycological Society 45: 21 – 45.

MACER, R.C.F. 1966. The formal and monosomic genetic analysis of stripe rust (*Puccinia striiformis*) resistance in wheat. Pages 127 – 142 in: J. MacKey, eds. Proceedings of the Second International Wheat Genetics Symposium.

MACER, R.C.F. 1975. Plant pathology in a changing world. Transactions of the British mycological Society 65: 351 – 374.

MACKILL, D.J., ZHANG, Z., REDONA, E.D. and COLOWIT, P.M. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome 39: 969 – 977.

MARKOVÀ, J.R. and URBAN, Z. 1977. To the knowledge of the brown rust of coach grass in Bohemia and Moravia 2. Ĉeskā Mykol 31: 72 – 80. (In Czech with English summary).

MARAIS, G.F. 1992. The modification of a common wheat – *Thinopyrum disticum* translocated chromosome with a locus homoeoallelic to *Lr*19. Theoretical and Applied Genetics 85: 73 – 78.

MAUGHAN, P.J., SAGHAI MAROOF, M.A., BUSS, G.R. and HUESTIS, G.M. 1996. Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance and near-isogenic line analysis. Theoretical and Applied Genetics 93: 392 – 401.

MAUSETH, J. D. 1991. Botany; an introduction to plant biology. Saunders College Publishing, Philadelphia.

McINTOSH, R.A. 1978. Cytogenetical studies in wheat X. Monosomic analysis and linkage studies involving genes for resistance to *Puccinia graminis* f. sp. *tritici* in cultivar Kota. Heredity 41: 71 – 82.

McINTOSH, R.A. 1988. Catalogue of gene symbols for wheat. Pages 1225 – 1323 in: T.E. Miller and R.M.D. Koebner, eds. Proceedings of the Seventh International Wheat Genetics Symposium. Institute of Plant Science Research, Cambridge, UK.

McINTOSH, R.A. 1991. Alien sources of disease resistance in bread wheat. Pages 320 – 332 in: T. Sasakuma and T. Kinoshita eds. Proceedings of Dr H. Kihara Memorial International Symposium. On Cytoplasmic Engineering in Wheat: Nuclear and Organellar Genomes of Wheat Species. Yokohoma, Japan.

McINTOSH, R.A. 1992. Close genetic linkage of genes conferring adult plant resistance to leaf rust and stripe rust in wheat. Plant Pathology 41: 523 – 527.

McINTOSH, R.A. and DYCK, P.L. 1975. Cytogenetical studies in wheat VII. Gene *Lr*23 for reaction to *Puccinia recondita* in Gabo and related cultivars. Australian Journal of Biological Sciences 28: 201 – 211.

McINTOSH, R.A., DYCK, P.L. and GREEN, G.J. 1976. Inheritance of leaf rust and stem rust resistance in wheat cultivars Agent and Agatha. Australian Journal of Agricultural Research 28: 37 – 45.

McINTOSH, R.A., DYCK, P.L., THE, T.T., CUSICK, J.E. and MILNE, D.L. 1984. Cytogenetical studies in wheat XIII. *Sr*35 – a third gene from *Triticum monococcum* for resistance to *Puccinia graminis tritici*. Zeitschrift fűr Pflanzenzűchtung 92: 1 – 14.

McINTOSH, T.A. and LUIG, N.H. 1973a. Recombination between genes for reaction to *P. graminis* at or near the *Sr*9 locus. Pages 425 – 432 in: E.R. Sears and L.M.S. Sears, eds. Proceedings of the Fourth International Wheat Genetics Symposium. Agricultural Experiment Station, University of Missouri, Columbia, Missouri, USA)

McINTOSH, T.A. and LUIG, N.H. 1973b. Linkage of genes for reaction to *Puccinia graminis* f. sp. *tritici* and *P. recondita* in Selkirk wheat and related cultivars. Australian Journal of Biological Sciences 26: 1145 – 1152.

McINTOSH, T.A., LUIG, N.H. and BAKER, E.P. 1967. Genetic and cytogenetic studies of stem rust, leaf rust and powdery mildew resistance in Hope and related wheat cultivars. Australian Journal of Biological Sciences 20: 1181 – 1192.

McINTOSH, T.A., MILNE, D.L. and CHAPMAN, V. 1982. Cytogenetic studies in wheat XIII. *Lr*28 for resistance to *Puccinia recondita* and *Sr*37 for resistance to *P. graminis tritici*. Zeitschrift fűr Pflanzenzűchtung 92: 1 – 14.

McINTOSH, T.A., WELLINGS, C.R. and PARK, R.F. 1995. Wheat rusts: An atlas of resistance genes. CSIRO, Kluwer Academic Publishers, East Melbourne, Australia.

METTIN, D., BLŰTHNER, W.D. and SCHLEGEL, G. 1973. Additional evidence on spontaneous 1B/1R wheat-rye substitutions and translocations. Pages 179 – 184 in: E.R. Sears and L.M.S. Sears, eds. Proceedings of the Fourth International Wheat Genetics Symposium. Agricultural Experiment Station, University of Missouri, Columbia, Missouri, USA.

MOHAN, M., NAIR, S., BHAGWAI, A., KRISHMA, T.G., YANO, J., BHAITA, C.R. and SASAKI, T. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular Breeding 2: 87 – 103.

MORRISON, L.A. 1993. Taxonomy of the wheats: a commentary. Proceedings of the eighth International wheat genetics symposium.

MOULD, M.J.R. and HEATH, M.C. 1999. Ultrastructural evidence of differential changes in transcription, translation, and cortical microtubules during in planta penetration of cells resistant or susceptible to rust infection. Physiological and Molecular Plant Pathology 55: 225 – 236.

MULLIS, K.B. and FALOONA, F.A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods in Enzymology 155: 335 – 350.

MURRAY, G.M., ELLISON, P.F., WATSON, A. and CULLIS, B.R. 1994. The relationship between wheat yield and stripe rust as affected by length of epidemic and temperature at the grain development stage of crop growth. Plant Pathology 43: 397 – 405.

NEL, A., KRAUSE, M., RAMAUTER, N and VAN ZYL, K. 1999. A guide for the control of Plant Diseases. National Department of Agriculture, Pretoria.

NELSON, R. R. 1978. Genetics of horizontal resistance to plant diseases. Annual Review of Phytopathology 16: 359 – 378.

NEWTON, M., JOHNSON, T. and BROWN, A.M. 1930. A preliminary study on the hybridization of physiologic forms of *Puccinia graminis tritici*. Science and Agriculture 10: 721 – 731.

NIKS, R.E. 1982. Early abortion of colonies of leaf rust *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60: 714 – 723.

NIKS, R.E. 1983. Comparative histology of partial resistance and the non-host reaction to leaf rust pathogens in barley and wheat seedling. Phytopathology 73: 60 – 64.

NIKS, R.E. and DEKENS, R.G. 1987. Histological studies on the infection of *Triticale*, wheat and rye by *Puccinia recondita* f. sp. *tritici* and *Puccinia recondita* f. sp. *recondita*. Euphytica 36: 725 – 731.

NIKS, R.E. and DEKENS, R.G. 1991. Prehaustorial and posthaustorial resistance to wheat leaf rust in diploid seedlings. Phytopathology 81: 847 - 851.

PARLEVLIET, J.E. 1988. Strategies for the utilization of partial resistance for the control of cereal rusts. P 48 – 62. Breeding strategies for resistance to the rusts of wheat. CIMMYT. Simonds, N.W. and Rajaram, S. Mexico.

PARLEVLIET, J.E. and KIEVIT, C. 1986. Development of barley leaf rust, *Puccinia hordei*, infections in barley 1. Effect of partial resistance and plant stage. Euphytica 35: 953 – 959.

PEIL, A., SCHUBERT, E., SCHUMANN, E. and WEBER, W.E. 1997. RAPDs as molecular markers for the detection of *Aegilops markgrafi* chromatin in addition and euploid introgression lines of hexaploid wheat. Theoretical and Applied Genetics 94: 934 – 940.

PENNER, B.A., BUSH, A., WISE, R., KIM, W., DOMIER, L., KASHA, L., LAROCHE, A., SCOLES, G., MOLNAR, S.J. and FEDACK, G. 1993.

Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods Applied 2: 341 – 345.

PETERSON, R.F. 1965. Wheat. Botany, cultivation and utilization. Interscience Publishers Inc. New York.

POWELL, W., MORGANATNTE, M., ANDRE, C. HANAFEY, M., VOGEL, J., TINGEY, S. and RAGALSKI, A. 1996. The comparison of FRLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2: 225 – 238.

PRETORIUS, Z.A. and KEMP, G.H.J. 1988. Effect of adult-plant resistance on leaf rust development and grain yield in wheat. Phytophylactica 20: 341 – 343.

PRETORIUS, Z.A., KLOPPERS, F.J. and VAN NIEKERK, B.D. 1995. Wheat leaf rust resistance in South Africa: Present status and future prospects. The Ninth Regional Wheat Workshop for Eastern, Central and Southern Africa. CIMMYT/CIDA Eastern Africa Cereals Program and the CIMMYT/EU Eastern Africa and SADC Wheat Programs. Ethiopia.

PRIESTLEY, R.H. 1978. Detection of increased virulence in populations of what yellow rust. In 'Plant Disease Epidemiology'. (Eds. P.R. Scott and A. Bainbridge.) pp. 63 – 70. Blackwell Scientific Publications, Oxford.

PRINS, R., MARAIS, G.F., JANSE, B.J.H., PRETORIUS, Z.A., and MARAIS, A.S. 1996. A physical map of the *Thinopyrum*-derived *Lr*19 translocation. Genome 39: 1013 – 1019.

PRUSKY, D., DINOOR, A. and JACOBY, B. 1980. The sequence of death of haustoria and host cells during the hypersensitive reaction of oat to crown rust. Physiological Plant Pathology 17: 33 – 40.

RICHAEL, C. and GILCHRIST, D. 1999. The hypersensitive response: A case of hold or fold? Physiological and Molecular Plant Pathology 55: 5 – 12.

RILEY, R., CHAPMAN, V. and JOHNSON, R. 1968. The incorporation of alien disease resistance in wheat by genetic interference with the regulation of meiotic chromosome synapsis. Genetical Research, Cambridge 12: 199 – 219.

ROELFS, A.P. 1985. Wheat and rye stem rust. The Cereal Rusts. Vol II. Diseases, Distribution, Epidemiology and Control. Academic Press, Orlando.

ROELFS, A.P. 1988. Genetic control of phenotypes in wheat stem rust. Annual Review of Phytopathology 26: 351 – 361.

ROELFS, A.P. and GROTH, J.V. 1980. A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. Phytopathology 70: 855 – 862.

ROELFS, A.P., SINGH, R.P. and SAARI, E.E. 1992. Rust diseases of wheat – concepts and methods of disease management. CIMMYT. Mexico D.F. 81pp.

ROGNLI, O.A., DEVOS, K.M., CHINOY, C.N., HARCOURT, T.L., ATKINSON, M.D. and GALE, M.D. 1992. RFLP mapping of rye chromosome 7R reveals a highly translocated chromosome relative to wheat. Genome 35: 1026 – 1031.

ROWLAND, G.G. and KERBER, E.R. 1974. Telocentric mapping in hexaploid wheat of genes for leaf rust resistance and other character derived from *Aegilops squarrosa*. Canadian Journal of Genetics and Cytology 16: 137 – 144.

SAIKI, R.K., SCHARF, SL, FALOONA, F., MULLIS, K.B., HORN, G.T., ERLICH, H.A. and ARNHEIM, N. 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230: 1350 – 1354.

SALISBURY, F.B. and ROSS, C.W. 1992. Plant physiology. Fourth edition. Wadsworth Publishing Company.

SAMBORSKI, D.J. and DYCK, P.L. 1976. Inheritance of virulence in *Puccinia recondita* on six backcross lines of wheat with single genes for resistance to leaf rust. Canadian Journal of Botany 54: 1666 – 1671.

SAMBORSKI, D.J. and DYCK, P.L. 1982. Enhancement of resistance to *Puccinia recondita* by interactions of resistance genes in wheat. Canadian Journal of Plant Pathology 4: 152 – 156.

SAMBORSKI, D.J. and PETURSON, B. 1960. Effect of leaf rust on the yield of resistant wheats. Canadian Journal of Plant Science 40: 620 – 622.

SAMBROOK, J., FRITSCH, E.F. and MANIATIS, T. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor.

SCHAFER, J. F. 1971. Tolerance to plant disease. Annual Review of Phytopathology 9: 235 – 252.

SCHAFER, J.F. and ROELFS, A.P. 1985. Estimated relation between numbers of urediniospores of *Puccinia recondita* f. sp. *tritici* and rates of occurrence of virulence. Phytopathology 75: 749 – 750.

SEARS, E.R. 1954. The aneuploids in common wheat. Missouri Agric Exp. Stn. Res. Bull. Missouri.

SEARS, E.R. 1977. Analysis of wheat – *Agropyron* recombinant chromosomes. In 'Proceedings in The Eighth European Association of Research in Plant Breeding Congress'. (Eds E. Samchez Monge and F. Garcia-Olmedo.) pp. 663 – 72. Madrid, Spain.

SEARS, E.R., LOEGERING, W.Q., and RODENHISER, H.A. 1957. Identification of chromosomes carrying genes for stem rust resistance in four varieties of wheat. Agronomy Journal 49: 206 – 212.

SHARMA, D. and KNOTT, D.R. 1966. The transfer of leaf-rust resistance from *Agropyron* to *Triticum* by irradiation. Canadian Journal of Genetics and Cytology 8: 137 – 143.

SHARP, E.L., SALLY, B.K. and TAYLOR, G.A. 1976. Incorporation of additive genes for stripe rust resistance in winter wheat. Phytopathology 66: 794 – 797.

SHEEN, S.J. and SNYDER, L.A. 1964. Studies on the inheritance of resistance to six stem rust cultures using chromosome substitution lines of a Marquis wheat selection. Canadian Journal of Genetics and Cytology 6: 74 – 82.

SILVERMAN, W. 1959. The effect of variations in temperature on the necrosis associated with infection type 2 uredia of the wheat stem rust fungus. Phytopathology 49: 827 – 830.

SINGH, R.P. 1992. Genetic association of leaf rust resistance gene *Lr*34 with adult plant resistance to stripe rust in bread wheat. Phytopathology 82: 835 – 838.

SINGH, R.P. 1999. Yield losses due to brown rust in two popular cultivars of wheat. Plant Disease Resistance 14: 60 – 62.

SINGH, R.P. and McINTOSH, R.A. 1984. Complementary genes for resistance to *Puccinia recondita* in *Triticum aestivum*. II Cytogenetic studies. Canadian Journal of Genetics and Cytology 26: 736 – 742.

SINGH, R.P. and McINTOSH, R.A. 1986. Cytogenetical studies in wheat XIV. *Sr*8b for resistance to *Puccinia graminis tritici*. Canadian Journal of Genetics and Cytology 28: 189 – 197.

SINGH, R.P. and RAJARAM, S. 1995. Strategies to achieve durable resistance to rust disease of wheat. The Ninth Regional Wheat Workshop for Eastern, Central and Southern Africa. CIMMYT/CIDA Eastern Africa Cereals Program and the CIMMYT/EU Eastern Africa and SADC Wheat Programs. Ethiopia.

SKIPP, R.A., HARDER, D.E. and SAMBORSKI, D.J. 1974. Electron microscopy studies on infection of resistant (*Sr*6 gene) and susceptible near-isogenic wheat lines by *Puccinia graminis* f. sp. *tritici*. Canadian Journal of Botany 52: 2615 – 2620.

SMALE, M., SINGH, R.P., SAYRE, K., PINGALI, P., RAJARAM, S. and DUBIN, H.J. 1998. Estimating the economic impact of breeding non-specific resistance to leaf rust in modern bread wheats. Plant Disease 82: 1055 – 1061.

SMITH, O.S., BOWEN, S.L., TENBORG, R.A. and WALL, S.J. 1990. Similarities among a group of elite maize inbreds as measured by pedigree F_1 grain yield, heterosis and RFLPs. Theoretical and Applied Genetics 80: 833 – 840.

SOLIMAN, A.S., HEYNE, E.G. and JOHNSON, C.O. 1963. Resistance to leaf rust in wheat derived from *Aegilops umbellulata* translocation lines. Crop Sciences 3: 254 – 256.

SOLIMAN, A.S., HEYNE, E.G. and JOHNSON, C.O. 1964. Genetic analysis of leaf rust resistance in eight differential varieties of wheat. Crop Science 4: 246 – 248.

STAKMAN, E.C. 1915. Relation between *Puccinia graminis* and plants highly resistant to its attack. Journal of Agricultural Research 4: 193 – 199.

STARR, C. and TAGGART, R. 1992. Biology – the unity and diversity of life. Sixth edition. Wadsworth Publishing Company.

STATLER, G.D., MILLER, J.D. and LEBENS, S. 1982. Wheat leaf rust in North Dakota during 1979 – 1981. Plant Disease 66: 1174 – 1176.

STEEL, R.G.D. and TORRIE, J.H. 1980. Principals and procedures of statistics. Second Edition. McGraw-Hill. New York.

STEVENS, R.B. 1974. Plant Disease. Ronald Press Company, New York.

TANKSLEY, S.D., YOUNG, N.D., PATERSON, A.H. and BONIERBALE, M.W. 1989. RFLP mapping in plant breeding: New tools for an old science. Biotechnology 7: 257 – 264.

TAUTZ, D. and RENTZ, M. 1984. Simple sequences are ambiquitous repetitive components of eukaryotic genomes. Nucleic Acid Research 12: 4127 – 4138.

TENG, P.S. and BOWEN, K.L. 1985. Disease modelling and simulation. Pp 435 – 466 in A.P. Roelfs and W.R. Bushnell, eds., The Cereal Rusts, Vol. II. Diseases, Distribution, Epidiomology and Control. Academic Press, Orlando.

THE, T.T. 1973. Chromosome location of genes conditioning stem rust resistance transferred from diploid to hexaploid wheat. Nature New Biology 241: 256.

TRENCH, T. N., WILKINSON, D. J., and ESTERHUYSEN, S. P. 1992. South African plant disease control handbook. University of Natal.

VAN DER PLANK, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York.

VAN NIEKERK, B.D. 2001. Genetic control of leaf rust in wheat. Pages 52 – 53 in Small Grain Institute Technology Report 2001. Agricultural Research Council, Bethlehem.

VOS, P., BLEEKER, M, REIJANS, M., VAN DE LEE, T., HORNES, M., FRIJTERS, A., POT, J., PELEMAN, J., KUIPER, M. and ZABEAU, M. 1995.

AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407 – 4414.

WAHL, I., ANIKSTER, Y., MANISTERSKI, J. and SEGAL, A. 1984. Evolution at the centre of origin. The cereal rusts: Volume I, Origins, specificity, structure and physiology. W.R. Bushnell & A.P. Roelfs, eds. Academic Press, Inc., Orlando.

WATSON, I.A. and LUIG, N.H. 1966. *Sr*15 – a new gene for use in the classification of *Puccinia graminis* var *tritici*. Euphytica 15: 239 – 250.

WELSH, J. and McCLELLAND, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Research 18: 7213 – 7218.

WIESE, M.V. 1987. Compendium of wheat diseases, Second edition. APS Press. Minnesota, USA.

WILSON, J. and SHANER, G. 1987. Slow leaf-rusting resistance in triticale. Phytopathology 77: 458 – 462.

WORLAND, A.J. and LAW, C.N. 1986. Genetic analysis of chromosome 2D in wheat I. The location of genes affecting height, day-length insensitivity, hybrid dwarfism and yellow-rust resistance. Zeitschrift fűr Pflanzenzűchtung. 96: 331 – 345.

WORLAND, A.J., LAW, C.N., HOLLINS, T.W., KOEBNER, R.M.D. and GIURRA, A. 1988. Location of a gene for resistance to eyespot (*Pseudocercosporella herpotrichoides*) on chromosome 7D of wheat. Plant Breeding 101: 43 – 51.

YOUNG, N.D. 1996. QTL mapping and quantitative disease resistance in plants. Annual Review Phytopathol 34: 479 – 501.

YOUNG, N.D. 1999. A cautiously optimistic vision for marker-assisted breeding. Molecular Breeding 5: 505 – 510.

ZABEAU, M. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application No. 0-534-858-A1.

ZABEAU, M. and VOS, P. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application No. 92402697.

ZADOKS, J.C. 1961. Yellow rust of wheat. Studies in epidemiology and physiologic specialization. Tijdschrift Over Plantenziekten 67: 69 – 256.

ZADOKS, J.E., CHANG, T.T. and KONZAK, C.F. 1974. A decimal code for the growth stages of cereal. Weed Research 14: 415 – 421.

ZELLER, F.J. 1973. 1B/1R wheat-rye chromosome substitutions and translocations. Pages 209 – 211 in: E.R. Sears and L.M.S. Sears, eds. Proceedings of the Fourth International Wheat Genetics Symposium. University of Missouri, Columbia, Missouri, USA.

ZIMMER, D.E. and SCHAFER, J.F. 1961. Relation of temperature to reaction type of *Puccinia coronata* on certain oat varieties. Phytopathology 51: 202 – 203.

APPENDIX

Table 1. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *dicoccoides* v. *kotchianum* (91) (leaf rust resistant parent) and the subsequent segregating F_2 population using the primer combination *Mse*+CTA/*Eco*+ACA

Fragment	Susceptible											Intermediate			Resistant							
	T. aestivum (SST55)				F_1		F_2													*T. turgidum*		
Size	296	297	301	303	7	10	35	36	38	45	A3	34	43	41	71	37	75	A4	A5	4	9	6
45								X						X	X							
49														X								
54																X						
57					X											X					X	
60																	X					
62	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
66	X								X					X	X	X	X				X	
69					X				X												X	
71											X					X	X	X	X			X
73			X		X	X					X	X	X			X	X	X	X			
75													X									
77				X	X			X	X		X		X	X		X		X	X	X	X	X
78					X		X						X				X					
82	X	X	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X
75											X											
87					X	X	X	X	X		X	X	X		X	X	X	X	X	X		X
90	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X
94			X	X		X	X	X	X	X			X		X	X	X	X	X	X	X	X
95	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
98			X	X		X	X				X	X			X	X	X					
101	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
103																X	X	X	X	X	X	X
105	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
108																		X		X	X	
110													X		X	X	X	X	X	X	X	X
111			X	X		X	X	X	X	X	X		X	X	X	X	X					
113				X	X		X	X	X				X			X	X	X	X	X	X	X
115	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
118			X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X

Fragment	Susceptible											Intermediate			Resistant							
	T. aestivum (SST55)				F_1		F_2													*T. turgidum*		
Size	296	297	301	303	7	10	35	36	38	45	A3	34	43	41	71	37	75	A4	A5	4	9	6
122			X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
126	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
128			X	X	X	X	X		X		X	X	X			X	X	X	X			X
129			X	X																		
132			X	X					X		X		X		X	X	X					
134		X	X	X		X	X	X	X	X	X			X	X	X	X	X	X			X
135											X						X					
137							X															
139	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X			X
143		X	X	X		X	X	X	X	X	X		X	X	X		X	X	X			X
144	X	X	X	X		X				X		X	X				X					
147		X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X			X
150											X							X				
152	X	X	X	X	X	X	X	X	X	X	X		X	X	X		X	X	X			X
155		X	X	X							X											
157	X	X	X	X		X	X	X	X	X	X	X	X		X		X	X	X			X
161	X	X	X	X		X	X	X	X	X	X		X	X	X		X	X	X			X
167		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X			X
172	X	X	X	X	X		X	X	X		X	X	X									
173						X		X	X	X	X	X	X	X	X		X	X	X			X
175					X		X						X									
179	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X			X
184	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X			X
186							X			X			X				X	X	X			X
187										X	X		X				X	X				
191	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X			X
193													X				X					
194	X		X	X			X	X	X	X	X		X		X		X	X	X			X
200		X	X	X		X	X	X	X	X	X		X		X		X	X	X			X
204	X	X	X	X		X	X	X	X	X	X		X	X	X		X	X	X			X
209	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X			X
211			X	X			X	X	X				X					X	X			X
215			X	X							X				X		X					

Fragment Size	Susceptible											Intermediate			Resistant							
	T. aestivum (SST55)				F_1		F_2													*T. turgidum*		
	296	297	301	303	7	10	35	36	38	45	A3	34	43	41	71	37	75	A4	A5	4	9	6
218	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X			X
222							X										X					
232	X	X	X	X		X	X			X	X		X		X		X	X	X			X
236													X									
239	X	X	X	X		X	X			X	X		X				X	X	X			
240														X	X							
242	X	X	X	X		X	X	X	X	X	X	X	X		X		X	X	X			X
245				X							X							X	X			
249	X	X	X	X		X	X			X	X		X	X	X		X					X
250																		X	X			
253	X	X	X	X		X	X			X	X		X		X		X	X	X			X
255														X								
259							X				X		X				X	X	X			
260	X		X	X		X				X		X			X							X
263		X	X	X		X	X			X			X				X	X	X			X
268										X	X		X		X		X	X	X			X
273		X																				
277	X	X	X	X	X	X	X			X	X	X	X									
281			X	X											X		X	X	X			
295	X		X	X			X			X	X				X	X	X	X	X			X
306	X	X	X	X	X	X	X			X	X											
307					X													X				
309		X		X						X			X	X	X		X		X			X
313							X				X				X		X	X	X			X
324				X							X											
327		X	X	X							X							X	X			
331							X				X							X	X			
335							X				X							X	X			X
349		X	X																			
352				X			X				X							X	X			
355			X																			
362	X	X	X	X							X							X	X			
366											X							X	X			

Fragment Size	Susceptible											Intermediate			Resistant							
	T. aestivum (SST55)				F_1		F_2													*T. turgidum*		
	296	297	301	303	7	10	35	36	38	45	A3	34	43	41	71	37	75	A4	A5	4	9	6
377											X							X				
385							X															
390				X							X							X				
397																		X				
398	X																					
401							X				X			X				X	X			X
408	X		X	X							X							X	X			X
418																		X				
420	X		X	X							X											
430	X		X	X																		
432							X											X	X			X
435			X	X							X											
438				X			X				X			X				X	X			X
448							X				X			X				X	X		X	X
452											X							X	X			
460	X		X	X			X				X							X	X			
464	X	X	X	X			X				X			X				X	X			X
469											X							X				X
488											X							X	X			
500					X						X							X				

Table 2. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *dicoccoides* v. *kotchianum* (91) (leaf rust resistant parent) and the subsequent segregating F_2 population using the primer combination *Mse*+CTA/*Eco*+AAC

Fragment	Susceptible								Intermediate			Resistant				
	T. aestivum (SST55)				F_1	F_2										*T. turgidum*
Size	296	297	301	303	10	35	36	45	34	43	41	71	75	A4	A5	6
59		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
64	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
66			X					X		X	X					X
69		X	X	X	X	X	X	X								X
70		X		X				X								X
72		X	X	X	X			X			X	X	X			X
73										X						
77		X	X	X	X	X	X	X	X	X				X		X
79															X	
81												X	X	X	X	X
84								X								
86	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
88		X		X	X	X	X	X	X	X		X		X	X	X
91	X	X	X	X	X	X		X	X	X	X	X		X		X
93	X				X	X	X	X	X					X	X	X
96	X		X		X	X	X	X	X	X	X			X	X	X
98							X					X		X	X	X
99					X	X	X	X	X			X				X
101	X	X	X	X	X		X	X						X	X	
102						X	X	X	X			X				X
104			X	X	X	X	X	X	X	X	X	X		X	X	X
105										X						
106										X		X				
108		X	X	X	X	X	X	X	X	X		X	X	X	X	X
110	X				X	X	X	X	X	X		X	X	X	X	X
112		X		X			X	X	X							X
114		X		X	X	X	X	X	X	X	X	X	X	X	X	X
116	X	X	X	X	X	X	X	X	X			X				
118		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
122	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X

Fragment Size	Susceptible								Intermediate			Resistant				
	T. aestivum (SST55)				F_1	F_2										*T. turgidum*
	296	297	301	303	10	35	36	45	34	43	41	71	75	A4	A5	6
124			X	X	X	X	X	X	X	X	X	X	X		X	X
128					X	X	X	X	X							X
131		X	X	X	X	X	X	X	X	X	X	X		X	X	X
132	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
140				X	X	X	X	X		X	X	X	X	X	X	X
142		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
146	X			X	X	X	X	X	X	X		X	X	X		X
149	X					X	X	X		X		X			X	X
155	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
157							X	X								
160	X	X	X	X	X		X		X							X
165						X		X				X				X
167							X	X		X				X		X
171					X	X	X	X	X		X	X		X	X	X
174	X	X	X		X	X	X	X	X			X				
178	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
180					X	X		X				X				X
185								X								
190			X	X	X		X	X		X	X	X	X	X	X	X
193										X						
196	X		X	X	X	X	X	X	X	X						
197											X	X	X	X	X	X
217	X	X	X	X				X				X				
220											X	X		X	X	X
224	X															
226						X										X
238	X	X	X	X	X	X		X	X			X		X	X	X
246	X		X	X	X	X		X	X	X		X		X		X
255	X															
262																X
262														X		X
264												X				X
273						X								X		X
279														X		

Fragment Size	Susceptible								Intermediate			Resistant				
	T. aestivum (SST55)				F_1	F_2										*T. turgidum*
	296	297	301	303	10	35	36	45	34	43	41	71	75	A4	A5	6
286														X		X
288	X		X	X		X						X			X	
298	X		X	X		X		X	X		X	X	X	X	X	X
311									X		X	X	X	X		
248			X	X		X					X			X		X
351			X	X										X		
366						X								X		X
403																X
420																X

Table 3. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *durum* v. *libycum* (129) (leaf rust resistant parent) and the subsequent segregating F_2 population using the primer combination *Mse*+CTA/*Eco*+ACA

Fragment	Resistant										Intermeditiate						Susceptible						
	T. turgidum				F_2															*T. aestivum*			
Size	5	3	276	278	217	211	152	206	209	150	145	131	132	181	153	117	216	125	129	296	297	301	303
45	X		X	X	X	X	X			X	X				X		X	X	X				
46			X					X										X				X	
47			X		X				X							X			X	X			
49	X	X		X		X	X	X		X	X	X	X	X			X				X		
51	X														X			X				X	X
52				X	X						X							X		X	X	X	
53	X		X	X		X			X	X					X		X	X	X				
55							X	X			X	X		X									
56		X														X			X				
60																		X					X
62	X		X	X	X	X	X	X		X	X	X		X	X		X	X	X	X	X		
67																				X			
69	X	X		X			X	X		X	X	X	X	X	X	X	X	X	X				
72					X	X	X		X	X	X	X		X	X	X							
73	X	X						X								X						X	
75		X					X		X		X	X				X		X					
78		X					X		X			X	X	X				X					X
80									X														
82	X	X	X	X	X	X			X		X				X	X	X	X	X	X	X	X	X
65																		X					
88	X	X	X				X	X	X	X	X	X	X	X		X	X	X					
69		X																					
90	X	X		X	X	X			X				X		X	X	X			X	X	X	X
93		X																				X	X
95	X	X	X	X	X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
99		X					X	X				X	X	X				X		X	X	X	X
101	X	X	X	X	X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
105	X	X	X	X	X	X			X	X	X		X		X	X	X	X	X	X	X		X
106																		X				X	
110							X	X				X		X									
111		X											X				X					X	X
113	X	X																X					X
115	X	X	X	X	X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
117								X															
118	X						X	X	X				X	X		X	X	X				X	X
122	X	X	X		X	X	X		X	X	X		X	X	X	X	X	X	X			X	X
126	X	X	X		X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
127							X	X				X		X									
128	X	X	X		X	X			X	X	X		X		X	X		X	X			X	X

Frag-	Resistant										Intermeditiate						Susceptible						
ment	*T. turgidum*				F_2															*T. aestivum*			
Size	5	3	276	278	217	211	152	206	209	150	145	131	132	181	153	117	216	125	129	296	297	301	303
131								X						X								X	X
132	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X
134	X	X			X				X	X					X	X	X	X			X	X	X
136		X						X						X									
137							X	X			X	X		X									
139	X	X	X		X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
141							X	X				X		X									
143		X	X										X			X	X				X	X	X
145		X											X							X	X	X	X
146	X	X	X	X	X	X			X	X	X		X		X	X	X	X	X		X	X	X
148								X						X									
150							X					X											
152	X	X	X	X	X	X			X	X	X	X	X		X	X	X	X	X	X	X	X	X
154																		X	X				
155								X					X								X	X	X
157												X		X		X	X	X	X	X	X	X	X
158	X	X	X	X	X	X	X		X	X	X		X		X		X	X	X				
161	X	X	X	X	X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
165										X													
167	X	X	X	X	X	X			X		X		X		X	X	X	X	X		X	X	X
169							X	X				X		X									
171		X			X	X			X				X			X	X	X	X	X	X	X	X
174	X	X	X	X		X	X	X	X		X	X		X									
175	X		X	X						X	X		X		X	X	X	X					
179	X	X		X	X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
183	X	X	X	X	X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
185								X				X											
187	X	X				X	X		X		X			X				X					
191	X	X	X	X	X	X			X	X	X		X			X	X	X	X	X	X	X	X
194													X	X				X		X			X
195	X	X			X	X									X		X					X	
197												X											
201	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X	X		X	X	X
205	X	X	X		X	X			X	X	X		X		X	X	X	X	X	X	X		X
207																						X	
208												X											
210		X					X	X				X		X						X	X	X	X
212	X		X	X	X	X			X	X	X		X		X	X	X	X	X			X	X
214							X	X				X		X									
217													X			X						X	X
218	X	X	X	X	X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
222	X	X			X				X		X		X		X	X	X						

Fragment	Resistant										Intermeditiate						Susceptible						
	T. turgidum				F_2															*T. aestivum*			
Size	5	3	276	278	217	211	152	206	209	150	145	131	132	181	153	117	216	125	129	296	297	301	303
232	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
235											X						X	X					
237	X	X	X	X	X	X			X	X			X		X	X							
239														X			X	X	X	X	X	X	X
242	X	X	X	X	X	X			X	X	X		X		X	X	X	X	X	X	X	X	X
245																							X
248	X	X	X	X	X				X	X	X		X		X	X	X			X	X	X	X
253	X	X	X	X	X				X	X	X				X	X	X			X	X	X	X
255																X	X						
258			X																				
260					X	X			X	X	X	X			X			X		X		X	X
263	X	X		X												X	X				X	X	X
268	X	X	X	X	X	X	X		X	X	X				X	X							
273																					X		
277	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X			X		X	X
280		X		X			X		X		X	X			X	X	X					X	X
284							X	X				X		X									
294	X	X		X	X	X			X	X	X				X	X	X			X		X	X
300											X												
306	X		X			X	X		X	X	X				X	X			X	X		X	X
309					X												X				X		X
312			X	X	X	X			X	X					X		X						
315	X										X												
317							X					X											
318																	X						
321																	X						
323	X																						X
327		X		X	X	X			X	X	X					X					X	X	X
331																X							
345	X																			X			
349																					X	X	
351	X		X	X	X	X			X	X	X				X	X	X						X
353																						X	
356							X																
359	X						X		X		X			X	X								
364					X					X					X	X				X	X	X	X
374	X								X														
382																X							
385					X		X		X														
390									X		X												
391	X	X		X	X	X	X	X	X	X	X				X	X							
397	X								X											X			X

Fragment Size	Resistant										Intermeditiate						Susceptible						
	T. turgidum				F_2															*T. aestivum*			
	5	3	276	278	217	211	152	206	209	150	145	131	132	181	153	117	216	125	129	296	297	301	303
401					X					X	X				X	X	X						
407	X				X					X					X	X	X						
414	X			X																X		X	X
419	X				X				X		X				X	X							
426																				X		X	X
429	X																			X		X	X
433	X		X		X	X			X	X	X				X	X	X					X	X
439	X				X				X	X	X				X	X	X						X
447																X							
448	X			X	X	X			X	X	X				X	X	X						
452											X					X							
460	X				X	X			X	X	X				X	X				X		X	X
464	X		X	X	X	X			X	X	X				X	X	X			X	X	X	X
470	X				X				X		X					X							
488	X				X				X							X							
497	X																						
499					X				X												X		

Table 4. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *durum* v. *libycum* (129) (leaf rust resistant parent) and the subsequent segregating F_2 population using the primer combination *Mse*+CTA/*Eco*+AAC

Frag-	Resistant								Intermeditiate					Susceptible				
ment	*T. turgidum*			F_2													SST 55	
Size	3	276	278	217	211	152	209	150	145	132	181	153	117	219	125	216	296	297
47					X									X				
51						X					X							
53						X					X							
58	X									X			X			X		
59				X	X		X		X			X			X		X	X
61						X					X							
63	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
66					X					X	X						X	X
69	X					X			X	X		X						X
70	X																	
71				X		X	X	X			X	X					X	X
72						X												
76	X					X	X		X									
78		X	X	X	X			X		X	X	X		X	X	X	X	X
79													X					
81						X	X								X	X		
82						X				X	X							
84	X						X								X	X		
85	X	X	X	X	X		X	X	X	X		X	X		X	X	X	X
87				X	X		X	X	X	X				X	X	X	X	X
89	X									X		X		X		X	X	X
91						X					X							
93	X	X	X	X	X		X		X	X			X		X	X		
96	X	X	X	X	X	X	X	X	X	X		X		X	X	X	X	
98																		
100	X				X	X	X			X	X	X	X		X	X		
102	X	X	X	X	X		X	X	X	X		X	X	X	X	X	X	X
103	X			X	X	X					X	X	X		X	X	X	X
108		X	X	X	X		X	X		X		X	X		X	X	X	X
109	X			X	X					X			X	X	X			
112				X		X		X		X						X		X

Fragment Size	Resistant								Intermeditiate					Susceptible				
	T. turgidum			F_2													SST 55	
	3	276	278	217	211	152	209	150	145	132	181	153	117	219	125	216	296	297
114	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X
116	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	X
118	X			X			X	X	X	X		X	X	X	X	X	X	X
120						X				X	X						X	X
122	X	X	X		X		X	X	X			X	X	X	X	X		
124	X			X	X		X	X		X		X	X	X	X	X	X	X
125						X												
128	X		X	X			X	X	X	X								
129				X			X	X		X				X	X	X		
131	X	X	X	X	X	X	X		X	X		X	X	X	X	X	X	X
134								X										
136	X	X	X	X	X		X	X	X	X		X	X	X	X	X	X	X
138						X					X							
140	X			X	X	X	X	X	X	X		X	X	X	X	X		X
142	X			X	X		X	X	X	X		X	X	X	X	X	X	X
126	X	X	X	X			X	X	X	X			X	X	X	X		X
137											X							
149		X	X	X	X		X	X	X	X		X		X	X	X		
153						X												
155			X	X	X		X	X	X	X		X	X	X		X	X	X
157		X													X			
158						X		X		X	X							
160	X		X	X			X	X	X	X						X	X	X
164						X					X							
165	X												X					
168	X	X	X		X		X	X					X					
171	X		X	X	X		X	X	X	X			X	X	X	X		
172						X												
174			X	X	X			X	X	X	X	X	X	X	X	X	X	
177	X	X	X	X	X		X	X		X	X	X	X		X	X	X	
178			X						X	X						X		
179						X						X						
180							X		X						X			
184					X		X									X		

Fragment	Resistant								Intermeditiate					Susceptible				
	T. turgidum			F_2													SST 55	
Size	3	276	278	217	211	152	209	150	145	132	181	153	117	219	125	216	296	297
187						X					X							
190	X	X	X	X	X		X	X	X	X		X	X	X	X	X		
192						X					X							
195	X	X	X	X			X	X	X	X		X	X	X		X		
196					X	X					X				X			
198									X						X			
204						X					X							
207						X					X							
209							X	X								X		
211																X		
213							X		X									
316	X																	
218									X				X	X	X	X	X	X
220			X	X	X		X	X	X				X		X	X		
222													X			X		
224		X																
226				X			X		X					X				
227						X												
235			X	X			X		X									
238	X	X	X	X	X	X	X	X	X									
243									X					X			X	X
245						X												
246	X	X	X	X	X		X	X	X			X	X	X			X	X
249						X												
257									X									
260						X			X		X							
262	X		X		X								X	X				
265				X			X		X									
267	X	X	X	X			X	X										
268													X	X				
274						X												
278	X		X	X	X		X	X	X				X	X				
285				X		X					X							
289	X		X	X	X	X	X	X	X			X	X	X			X	X

Fragment	Resistant								Intermeditiate					Susceptible				
	T. turgidum			F_2													SST 55	
Size	3	276	278	217	211	152	209	150	145	132	181	153	117	219	125	216	296	297
295						X					X							
298	X	X	X	X	X		X	X	X		X	X	X	X			X	X
301						X												
308											X		X	X		X	X	X
310									X		X			X			X	X
314						X	X		X				X					
324											X							
329													X					
333						X												
348	X	X	X	X	X	X	X	X	X			X	X	X			X	X
358				X			X		X				X	X				
366	X	X		X	X	X	X		X			X	X					
367						X												
378									X									
381						X												
387					X		X	X	X					X				
391						X												
402						X												
403					X		X		X				X					
411									X									
416						X												

Table 5. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *dicoccoides* v. *kotchianum* (91) (leaf rust resistant parent) and the subsequent segregating F_2 population using the primer combination *Mse*+CTC/*Eco*+AAC

Frag-	Susceptible					Intermediate	Resistant				
ment	*T. aestivum*		F_2								*T. turgidum*
Size	296	297	35	36	45	34	71	37	A4	A5	6
53											X
54	X	X	X	X	X		X				
57						X				X	X
58	X		X	X			X	X	X		
62	X	X	X	X	X		X				X
63						X		X	X	X	
64	X		X	X	X						
67	X	X									
68			X	X							
71	X			X	X						
73	X	X	X	X	X	X	X	X	X	X	X
77							X	X	X	X	X
78			X	X	X	X					
79	X	X									
82										X	X
83	X	X	X	X	X						
88						X	X				
89	X	X			X					X	
91						X					
92	X	X	X	X	X						
94						X					
95	X	X	X	X	X						
97						X					
98	X	X			X		X	X	X	X	X
99			X	X							
101							X				X
102				X	X						
104	X	X		X	X						
105						X	X	X	X	X	X
106		X	X	X	X						

Frag-	Susceptible					Intermediate	Resistant				
ment	*T. aestivum*		F_2								*T. turgidum*
Size	296	297	35	36	45	34	71	37	A4	A5	6
112	X	X	X	X	X				X		
114	X	X				X	X			X	X
116	X	X	X	X	X						
117						X	X	X	X	X	X
120	X	X	X	X	X	X	X			X	
121				X	X						X
122	X	X									
123				X							
124		X			X						
127			X		X						
128						X	X	X	X	X	X
129											
130	X	X									
131	X	X	X	X	X						
132							X	X	X	X	
135	X		X	X	X						
136		X				X	X	X	X	X	X
137		X			X						
139	X		X	X	X						
143											
144					X						
145			X	X							
146							X	X	X	X	
147				X							
148					X	X	X				
149	X	X	X	X							
150	X	X			X						
151				X			X				
153				X		X	X		X	X	X
155	X				X						
156		X	X	X							
159		X			X						
160											X
161						X	X	X	X	X	

Frag-	Susceptible					Intermediate	Resistant				
ment	*T. aestivum*		F_2								*T. turgidum*
Size	296	297	35	36	45	34	71	37	A4	A5	6
162			X	X							
163	X	X									
164					X						
165						X					
166										X	
168	X	X			X						
169			X	X				X		X	
171							X			X	
172	X				X						
173	X	X	X	X	X						X
176		X			X	X	X	X	X	X	
177											X
178	X	X	X	X							
179					X						
180						X			X		
181							X			X	
183	X	X	X	X							
185						X			X	X	X
187					X	X			X	X	
188				X			X				X
190	X	X	X	X	X						
191										X	
194	X		X	X	X						
195						X	X				X
198	X	X	X	X	X						
200						X	X		X	X	X
203	X	X	X	X	X						
204							X				
206	X										
207	X				X						
208		X									
210					X						
212	X	X			X						
215											

Frag-	Susceptible					Intermediate	Resistant				
ment	*T. aestivum*		F_2								*T. turgidum*
Size	296	297	35	36	45	34	71	37	A4	A5	6
216						X			X	X	X
218			X	X							
219	X	X			X						
221						X	X		X	X	X
224				X	X						
225	X	X									
228						X	X		X	X	X
231	X	X	X	X	X						
233						X	X		X	X	X
235				X							
236	X	X			X						
238	X				X						
242				X		X	X				X
244		X									
245	X				X						
247	X										X
252	X	X									
254					X						
255			X								
256									X		
257					X						
258						X	X			X	
260	X	X			X						
262	X				X						
266					X						
270	X				X						
274	X										
279						X			X		
280	X				X						
284	X	X			X						
285						X	X			X	X
287			X								
292	X	X		X	X						
298	X	X			X						

Table 6. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *dicoccoides* v. *kotchianum* (91) (leaf rust resistant parent), and the subsequent segregating F_2 population using the primer combination *Mse*+CTC/*Eco*+ACA

Fragment	Susceptible						I/med	Resistant					
	T. aestivum		F_2									*T. turgidum*	
Size	296	297	35	36	45	A3	34	71	37	A4	A5	4	6
48	X	X											X
49		X	X										
61				X			X	X	X			X	
62	X	X											
63	X		X	X	X		X	X	X	X	X	X	
64								X	X				
66	X	X	X	X	X	X	X	X	X	X	X		
67			X		X							X	X
68				X	X		X			X			
69		X	X			X			X			X	X
71	X			X	X		X	X	X	X			
72													
73	X	X	X	X		X	X	X	X	X	X	X	X
75				X	X		X	X	X	X	X		
76		X	X										
77	X	X										X	
78	X	X	X	X	X	X	X	X	X	X	X		
79					X				X				
80	X	X		X		X	X	X		X	X	X	
81			X			X							
82	X												
83		X		X	X		X	X	X	X	X	X	
84	X	X	X			X							X
85													
88					X		X				X	X	X
90							X	X	X	X	X	X	
91	X	X	X	X	X	X							
92		X	X			X							X
93				X					X				
94	X	X	X		X	X	X	X		X	X	X	

Fragment Size	Susceptible						I/med	Resistant					
	T. aestivum		F_2									*T. turgidum*	
	296	297	35	36	45	A3	34	71	37	A4	A5	4	6
95						X							X
101	X			X	X	X	X	X	X	X	X	X	
102		X	X										X
106	X	X		X	X	X	X	X	X	X	X	X	
107			X										
110						X							X
111	X	X	X	X	X				X				
112										X			
113	X	X		X		X	X	X	X		X		
114													
115	X	X	X	X		X		X					X
116					X		X		X	X	X		
117	X	X				X							
118	X	X	X	X		X							
119								X	X	X	X	X	X
120				X	X		X	X		X	X		
121									X				X
122	X	X	X	X	X	X	X			X	X		
124			X	X	X	X	X	X	X	X			
125													
126				X			X	X	X	X	X		
129	X	X	X	X	X	X							
130	X							X					
131				X	X		X		X	X			
132													
133										X			
134	X	X	X			X							
135								X		X			
136	X												
137	X											X	X
138			X			X							
140				X	X		X	X	X	X			
141													X
142									X				

Fragment Size	Susceptible						I/med	Resistant					
	T. aestivum		F_2									*T. turgidum*	
	296	297	35	36	45	A3	34	71	37	A4	A5	4	6
143	X	X	X	X	X	X	X	X		X			
144				X	X		X			X	X		
145		X				X							
146	X		X										
147	X	X	X	X		X							
149								X					
150							X						
152	X	X											
153	X	X	X			X	X						X
156	X	X		X		X	X	X					
157					X					X	X		X
159	X	X	X	X		X							
161				X			X	X		X	X		
163			X			X							
165				X	X			X		X	X		
167	X			X	X	X	X	X		X	X		X
168		X	X										
169						X		X					X
170	X	X	X										
171						X							
172													
173		X		X	X		X	X		X	X		X
174	X	X											
175		X	X	X		X	X						
177				X			X	X		X	X		X
179	X	X	X			X							
181				X									
183	X					X							
184		X	X	X	X		X	X		X	X		
187	X	X	X			X		X					
188				X	X		X			X	X		
191			X	X	X	X	X	X		X	X		
193	X	X				X							
194			X										

Frag-ment Size	Susceptible						I/med	Resistant					
	T. aestivum		F_2									*T. turgidum*	
	296	297	35	36	45	A3	34	71	37	A4	A5	4	6
195							X						
196								X					
197													X
198			X							X			
200				X									
201							X	X					X
202				X									
203								X					
204													
205						X							
208				X	X			X		X	X		
209											X		
211			X	X		X	X						
212					X			X		X			X
214			X			X							
215	X	X											
216													X
217					X		X			X			
218								X			X		
219	X		X			X							
220		X											
222							X	X					
223				X	X					X	X		
224			X										
225						X							
232					X		X	X		X	X		
238							X						
239				X	X			X		X	X		
240			X										
241	X	X				X							
242					X		X						X
243								X		X	X		
245						X							
250													

Fragment Size	Susceptible						I/med	Resistant					
	T. aestivum		F_2									*T. turgidum*	
	296	297	35	36	45	A3	34	71	37	A4	A5	4	6
251					X		X	X		X	X		
253						X							
254					X		X	X		X	X		
256						X				X			
258								X					
259					X					X	X		
267							X	X		X	X		
268								X					
276							X	X		X	X		
280							X						
282							X						
283										X	X		
285													
287						X	X	X					
288										X			
291							X						
292								X		X	X		
302							X						
308													
309											X		
310							X	X		X			
315							X	X		X	X		
316								X					
319						X		X					

Table 7. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *durum* v. *libycum* (129) (leaf rust resistant parent) and the subsequent segregating F_2 population using the primer combination *Mse*+CTC/*Eco*+ACA

Fragment size	Resistant								Intermediate			Susceptible			
	T. turgidum				F_2									*T. aestivum*	
	278	276	3	5	152	211	209	150	132	117	181	216	125	296	297
54		X	X	X	X	X	X	X	X	X	X	X	X	X	X
57													X		
58	X	X	X	X		X	X	X	X	X	X	X		X	
62	X	X		X	X	X		X	X		X	X	X	X	X
64					X									X	
65					X										
67														X	X
71					X									X	
73	X	X		X	X	X		X	X		X	X	X	X	X
78	X		X				X		X	X	X	X	X		
79					X									X	X
82	X			X					X		X	X			
83					X									X	X
89				X										X	X
92	X				X									X	X
94				X											
95														X	X
96				X	X										
97		X				X		X	X		X	X	X		
98					X									X	X
99					X										
101	X		X				X	X		X			X		
102				X					X		X	X			
104	X				X									X	X
105		X				X			X		X	X	X		
106			X				X	X		X					X
112				X	X									X	X
114	X	X		X		X		X	X		X	X	X	X	X
116					X									X	X
117	X	X				X		X					X		

Fragment size	Resistant								Intermediate			Susceptible			
	T. turgidum				F_2									*T. aestivum*	
	278	276	3	5	152	211	209	150	132	117	181	216	125	296	297
118							X				X				
120					X					X				X	X
121					X										
122														X	X
123					X										
124															X
127									X	X			X		
128								X				X			
129									X		X				
130														X	X
131					X									X	X
135					X									X	
136		X	X	X											X
137															X
139					X									X	
144					X										
146		X		X					X	X	X		X		
148	X	X		X	X										
149														X	X
150					X									X	X
151							X								
152															
153					X	X		X	X		X	X	X		
155														X	
156					X										X
159															X
160						X	X	X	X			X	X		
161										X	X				
162					X										
163														X	X
165	X	X	X	X											
168					X					X				X	X
172														X	
173		X		X	X									X	X

Fragment size	Resistant								Intermediate			Susceptible			
	T. turgidum				F_2									*T. aestivum*	
	278	276	3	5	152	211	209	150	132	117	181	216	125	296	297
174							X		X	X					
175								X				X	X		
176											X				X
178			X	X	X									X	X
183											X	X	X	X	X
185	X	X	X	X	X	X	X	X	X	X	X				
187					X										
188	X	X		X	X		X	X	X	X	X				
190					X									X	X
194					X									X	
195								X		X		X	X		
196		X	X	X							X				
198					X					X		X	X	X	X
200	X	X	X	X	X	X	X	X	X	X	X				
203		X		X	X									X	X
204								X		X	X				
206														X	
207					X					X		X		X	
208															X
212		X	X	X	X									X	X
215					X										
219		X		X	X							X	X	X	X
221				X	X	X		X	X		X				
225					X									X	X
228						X		X			X				
231					X									X	X
233				X		X	X	X	X		X				
236					X									X	X
238														X	
239					X										
241								X							
242						X	X		X	X	X				
244															X
245					X									X	

Fragment size	Resistant								Intermediate			Susceptible			
	T. turgidum				F_2									*T. aestivum*	
	278	276	3	5	152	211	209	150	132	117	181	216	125	296	297
247					X					X				X	
252					X						X			X	X
254					X										
257								X	X			X			
260				X	X									X	X
262														X	
266					X	X									
270														X	
271					X										
274														X	
280					X									X	
283					X										
284											X			X	X
285				X	X	X	X	X	X		X				
292					X									X	X
298						X								X	X
303					X										
308					X									X	
315					X									X	X
320							X								X
321					X										
342									X		X	X		X	
345															X
351						X									
360					X										
366														X	
369					X										X
382					X									X	
384														X	
385														X	X
392					X									X	X
397															X
415								X	X			X		X	
424														X	

Table 8. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *durum* v. *libycum* (129) (leaf rust resistant parent) and the subsequent segregating F_2 population using the primer combination *Mse*+CTC/*Eco*+ACA

Fragment size	Resistant									Intermediate					Susceptible			
	T. turgidum				F_2												*T. aestivum*	
	3	276	278	5	217	211	152	209	150	132	181	153	117	603	216	125	296	297
48		X	X	X													X	X
49																		X
62	X	X	X	X						X							X	X
63		X															X	
66		X	X	X	X					X				X	X		X	X
67						X		X	X	X				X		X		
68													X	X		X		
69							X	X	X		X	X				X		X
71						X											X	
72							X	X		X		X	X	X		X		
73	X	X	X	X	X				X		X				X		X	X
75									X	X				X				
76					X	X									X			X
77											X	X	X		X	X	X	X
78	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
79										X	X							
80						X	X	X	X		X	X		X		X	X	X
82	X	X	X	X	X	X	X	X			X			X				
83	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X
84	X	X	X	X	X	X	X		X	X	X				X		X	X
85	X	X	X	X	X										X			
88						X			X	X				X				
89												X						
90	X	X	X	X		X	X	X	X	X	X	X	X	X		X		
91					X										X		X	X
92					X						X		X		X			X
93	X		X	X	X		X	X		X	X	X	X	X	X	X		
94					X	X			X						X		X	X
101		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

Frag-	Resistant									Intermediate					Susceptible			
ment	*T. turgidum*				F_2												*T. aestivum*	
size	3	276	278	5	217	211	152	209	150	132	181	153	117	603	216	125	296	297
106	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X
107					X										X			
111	X	X		X	X	X							X		X	X	X	X
112							X		X		X	X		X		X		
113								X	X	X	X	X	X	X			X	X
114					X										X			
115	X			X	X	X		X	X			X	X	X	X	X	X	X
116	X		X	X			X	X	X		X	X	X	X		X		
117					X									X	X		X	X
118					X						X	X			X	X	X	X
119		X	X			X	X	X			X		X					
120	X			X					X									
121					X									X	X	X		
122								X	X		X			X			X	X
124					X				X						X			
125											X							
126					X	X	X	X	X			X	X	X	X	X		
129					X										X		X	X
130																	X	
131								X			X		X					
132	X	X	X	X		X	X	X	X		X	X		X		X		
133										X								
134																	X	X
135					X			X	X	X	X			X	X	X		
136	X			X													X	
137																	X	
138					X										X			
139					X										X			X
140	X			X									X					
141											X							
142			X				X		X		X	X		X		X		
143								X		X			X				X	X
144						X	X	X	X	X	X			X		X		
145					X										X			X

Fragment size	Resistant									Intermediate					Susceptible			
	T. turgidum				F_2												*T. aestivum*	
	3	276	278	5	217	211	152	209	150	132	181	153	117	603	216	125	296	297
146																	X	
147					X										X		X	X
149	X			X														
150										X				X				
151																X		
152																	X	X
153																		X
154										X				X				
155											X							
156							X		X	X	X	X	X			X	X	X
157						X		X						X				
159					X										X		X	X
161								X		X				X				
162				X	X		X	X	X	X	X	X						
163					X										X			
165				X	X	X	X	X	X	X	X	X	X	X	X	X		
167		X		X	X	X		X	X	X			X	X	X	X	X	
169								X	X	X				X				
170					X										X		X	X
171					X					X					X			
172						X							X					
173				X			X	X	X	X	X	X		X		X		X
174																	X	X
175					X										X			X
177													X	X				
179																	X	X
181										X			X	X				
184							X	X	X	X	X		X	X				X
187					X										X		X	X
191				X						X			X	X				
193							X	X	X	X	X			X		X	X	X
195								X										
196					X										X			
201										X								

Fragment size	Resistant									Intermediate					Susceptible			
	T. turgidum				F_2												*T. aestivum*	
	3	276	278	5	217	211	152	209	150	132	181	153	117	603	216	125	296	297
202				X														
203														X				
204											X							
205																		
208								X	X	X				X				
211										X	X	X	X	X		X		
212				X	X	X	X	X	X									
215					X										X		X	X
216											X		X					
217						X	X	X	X	X				X				
218																X		
219				X	X										X		X	
221							X			X	X							
222						X		X	X				X	X				
225					X										X			
232						X		X	X					X				
235					X					X					X			
237							X											
238										X	X	X	X					
239						X		X	X					X				
241					X										X		X	X
243						X			X					X				
245								X										
247					X										X			
251						X		X	X		X			X				
253					X										X			
256					X										X			
258					X									X	X			
259								X	X									
260						X												
261								X	X		X			X				X
263					X										X			
266						X												
267								X	X					X				