WHEAT LEAF RUST RESISTANCE IN SELECTED *Triticum turgidum* ACCESSIONS

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Ek verklaar dat die verhandeling wat hierby vir die graad MSc. aan die Universitieit 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.

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List of abbreviations

AFLP	amplified fragment length polymorphism
AP	appressorium / abortive penetration
APR	adult-plant resistance
ASSV	aborted substomatal vesicle
ASSVI	aborted substomatal vesicle initial
bp	base pairs
C	chlorosis
CIMMYT	International Maize and Wheat Improvement Centre
cm	centimetre(s)
CTAB	cetyltrimethylammonium bromide
DNA	deoxyribonucleic acid
d.p.i.	days post-inoculation
EA	early abortion
EDTA	ethylenediamin tetraacetic acid
e.g.	*exempli gratia* (for example)
et al.	et alii (and others)
etc.	etcetera
EtOH	ethanol
Fig(s).	Figure(s)
f. sp.	*forma specialis*
G	germtube
g	gram(s)
H	haustorium

h	hour(s)
HCN	host cell necrosis
HI	hypersensitivity index
HMC	haustorium mother cell
HR	hypersensitive reaction
Hy	hyphae
i.e.	*id est* (that is)
IH	infection hyphae
IT	infection type
Lr	leaf rust resistant gene
ml	millilitre
NaCl	sodium chloride
PE	prestomatal exclusion
NPA	nonpenetrating appressorium
NSA	nonstomatal appressorium
PCR	polymerase chain reaction
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
SSR	simple sequence repeat
ssp.	subspecies
SSV	substomatal vesicle
SSVI	substomatal vesicle initial

TE	Tris EDTA
Tris-HCl	(Tris[hydroxymethyl]aminomethane) hydrochloric acid
U	urediospores
var.	variety
V	vesicle
%	percentage
°C	degree Celsius
μ	micro
π	pi

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He that will apply new remedies must expect new evils: for time is the greatest innovator.

Francis Bacon

GENERAL INTRODUCTION

Rust diseases of plants, in particular cereal crops, remain a problem in modern agriculture. Although rust has occurred on wheat throughout its evolutionary development, it is presently more damaging because of large areas sown to genetically homogeneous, or closely related cultivars (Samborski, 1985). The rust pathogens that cause the greatest losses in wheat belong to the genus *Puccinia* (Knott, 1989; Kendrick, 1992). Leaf rust (*P. triticina*) of common wheat (*Triticum aestivum* L.) does not result in total crop loss, but yield reductions of up to 40 % have been reported (Knott, 1989; Das *et al.*, 1992). The extent of crop damage can often be correlated to the time of disease onset, with more severe epidemics resulting from infections during early growth stages (Peterson, 1965; Western, 1971).

In South Africa leaf rust epidemics have been reported on spring wheat in the winter rainfall regions of the Western Cape and in the central and western Free State, under favourable conditions of moisture and temperature (Pretorius *et al.*, 1987). The disease has also been reported on irrigated wheat in KwaZulu-Natal, Mpumalanga, North West and the Northern Cape (Pretorius and Le Roux, 1988).

World-wide, control measures have been implemented over time, e.g. breeding for resistance, the application of fungicides, cultural practices and the eradication of alternate or accessory hosts. Since chemical control is usually too costly, or cultural management not effective, avenues such as breeding for resistance have been actively pursued. Ever since it has first been known that a single gene could confer resistance, breeding programs were initiated with the hope that the resistance would be durable (Crute and Pink, 1996). Durability of resistance

is defined by the ability of a variety to remain resistant during its widespread cultivation for a long period of time, in an environment favourable to a disease or pest (Johnson, 1979; Johnson, 1981). For leaf rust, most durable resistance is associated with gene combinations, specifically the adult-plant resistance genes *Lr13* and *Lr34* (Roelfs, 1988a).

Resistance has been defined by Vanderplank (1963) as either vertical or horizontal. These terms have no inherent biological meaning, but vertical resistance is defined as being effective to only some races of a pathogen. It can therefore be classified as being race-specific and a differential interaction occurs between isolates of the pathogen and varieties of the host (Parlevliet, 1988). Horizontal resistance, by definition, is effective to all races of a pathogen. Resistance can also be characterized according to its onset in terms of plant growth stage, i.e. seedling or adult plant resistance (Dyck and Kerber, 1985). At microscopic level rust resistance has been contrasted in terms of haustorium formation. Prehaustorial resistance is often associated with non-host interactions (Heath, 1977; Heath, 1981b; Heath, 1982; Elmhirst and Heath, 1987) and is the first defence mechanism (Anker and Niks, 2001) that is activated preventing the sporeling from developing a haustorium and successfully completing the parasitic relationship (Heath, 1981b; Niks and Dekens, 1991). In posthaustorial resistance the defence mechanism is activated when a fungus succeeds in penetrating the plant cell and fungus growth is halted after the formation of at least one haustorium (Niks and Dekens, 1991; Anker and Niks, 2001).

The search for more durable resistance has lead to the possible exploitation of wild relatives as the domesticated wheat gene pool appears to have been depleted

of resistance genes. The introgression of disease resistance genes from wild wheat offers a wider diversity of resistance sources (McIntosh *et al.*, 1995). Alien germplasm will thus assist in the expansion of the existing genetic variation by introducing novel variation into the crops (Knott and Dvořàk, 1976; Jones *et al.*, 1995).

To help identify resistance genes in wheat plants, breeders are increasingly using molecular marker techniques (Moore *et al.*, 1993; Keim *et al.*, 1997; Mohan *et al.*, 1997; Law *et al.*, 1998). Several techniques have been used, e.g. restriction fragment length polymorphism (RFLP) (Powell *et al.*, 1996), random amplified polymorphic DNA (RAPD) (Welsh and McClelland, 1990), simple sequence repeats (SSR) (Tautz and Renz, 1984; Tautz *et al.*, 1986) and amplified fragment length polymorphism (AFLP) (Zabeau and Vos, 1993). AFLPs represent a combination of the RFLP and PCR technique (Vos *et al.*, 1995) and usually give more dense and informative maps when compared to other techniques (Lin and Kuo, 1995; Keim *et al.*, 1997).

The objective of this study was to characterize adult-plant leaf rust resistance reported by Barnard (1999) in some *T. turgidum* accessions. Characterization studies included microscopy, pathotype effects, expression of resistance in crosses with hexaploid wheat, and preliminary work on following the resistance by means of molecular techniques.

CHAPTER 1

AN OVERVIEW OF WHEAT AND RUST DISEASES

WHEAT

Introduction Archaeological studies have shown that man has had a long and intimate association with wheat (Harlan, 1981). Evidence exists that wheat originated in western Iran, southern Turkey, northern Iraq and the areas extending along the Mediterranean basin to Israel before it was exported to the western world (Cook and Veseth, 1991).

While wild forms of diploid and tetraploid wheat occur, no wild species of hexaploid wheats exists in nature (McIntosh, 1976). The domestication of wheat began with wild einkorn, emmer and early hexaploid types, but has presently shifted almost exclusively to durum, club and bread wheats (Cook and Veseth, 1991).

Economic importance As the foundation of human nutrition, the *Triticeae* crops (barley, rye, triticale and wheat) have worldwide economic importance (Jones *et al.*, 1995). Wheat, especially bread wheat (*Triticum aestivum* L. em. Thell.), is one of the most important cultivated plants with respect to the human diet (Kam-Morgan *et al.*, 1989; http://www.cc.ndsu.nodak.edu/instruct). Cultivated tetraploid durum wheats constitute over 10 % of the wheats produced all over the world (Joshi and Nguyen, 1993).

The latest worldwide production estimate for wheat was expected to be 579 million metric tons in comparison to the 573, 558, and 132 million metric tons for maize, rice, and soybeans, respectively (http://hordeum.msu.montana.edu/genome). Wheat is important in the baking industry since it is the only cereal containing the proteins

glutenin and gliadin, which combine in dough to form gluten. Gluten is characterized by its elasticity and extensible properties that allow the dough to rise (Sim, 1965).

Taxonomy and distribution The tribe *Triticeae* Dumort *(Hordea* Benth) belongs to the family Poaceae (*Gramineae*) and includes the genus *Triticum*. This genus contains three ploidy levels and approximately thirty species (Dvořàk and McGuire, 1991). Studies by Sakamura showed that the ploidy levels of wheat consist of chromosome numbers of 14, 28 and 42, with 7 as the basic number (Knott, 1989). Diploid wheat (2n=14) usually consists of an AA genome type, whereas tetraploids (2n=28) originated from a cross between *Turgidum monococcum* (2n=14, AA) and probably *Aegilops speltoides* (2n=14, BB) to give a genome type of 2n=28, AABB (Fig. 1) (Knott, 1989; Zhang *et al.*, 1998). Hexaploid wheat originated from a cross between *T. turgidum* (2n=28, AABB) and *T. taushii* (2n=14, DD) resulting in the hexaploid *T. aestivum* (2n=42, AABBDD) (McIntosh, 1991). This makes hexaploid bread wheat (2n=42) a relative latecomer among the cereals (Harlan, 1981).

Wheat is cultivated worldwide and can be found in temperature regions in both hemispheres (Miller, 1987). In sub-Saharan Africa the largest areas of production include Ethiopia and South Africa (Knott, 1989).

WHEAT RUSTS

Introduction and taxonomy Diseases of crop plants are as old as agriculture and numerous references to fungal diseases occur in ancient writings (Cooke, 1977). Rust diseases have been described in the Bible as well as in the ancient Greek writings of

Aristotle and his students (http://www.clay.agr.okstate.edu/wheat/Feb99.html). Furthermore, it is believed that rust fungi were present on grasses ancestral to cereals long before the latter came into existence as agriculturally important crops (Johnson and Browder, 1966). The three rust diseases of wheat are caused by *Puccinia graminis* Pers. f. sp. *tritici* (stem rust), *P. triticina* Eriks. (leaf rust, previously known as *P. recondita* Rob.ex Desm. f. sp. *tritici*) and *P. striiformis* Westend. f. sp. *tritici* (yellow or stripe rust). The genus *Puccinia* belongs to the order *Uredinales*, which literally means rust fungi (Kendrick, 1992). This order constitutes one of the largest groups in the *Basidiomycetes*. The rust fungi are all obligatory biotrophic parasites of vascular plants (Schafer, 1987). They usually have a narrow host range, being restricted to a single family, a single genus, or even a single species of that genus (Kendrick, 1992).

Leaf rust

Introduction Leaf rust (*P. triticina*) is one of the most important diseases of wheat (Samborski, 1985; Pretorius and Le Roux, 1988). It is also the most common and widely distributed of the wheat rusts (Peterson, 1965; Hiratsuka and Sato, 1982; Schafer, 1987; Knott, 1989; Das *et al.*, 1993). Damage caused by leaf rust is usually not severe, however, on a worldwide basis the disease causes more damage in wheat than stem or stripe rust (Samborski, 1985; Dyck, 1987; Knott, 1989; Roelfs *et al.*, 1992). Leaf rust does not result in total crop loss, but yield reductions of up to 40 % have been reported (Knott, 1989; Das *et al.*, 1992; http://www.ksu.edu/plantpath/extension/facts/wheat11.html). The extent of yield losses can be correlated to the time of disease onset, with more severe epidemics resulting from infections during early growth stages (Peterson, 1965; Western, 1971). Leaf rust increases exponentially over time,

explaining why rust epidemics suddenly explode during favourable weather conditions (http://hordeum.msu.montana.edu/genome). Wheat varieties have different rates and mechanisms of grain filling and may differ in the actual amount of yield reduction sustained (Knott, 1989).

Leaf rust typically occurs uniformly across a field. In over-wintering locations in the northern hemisphere the disease will be more severe on the bottom leaves (Roelfs *et al.*, 1992), whereas when the spores are blown in, it will be more severe on upper leaves. The leaf rust pathogen can only survive in living leaf tissue and not in seed, soil or crop residues.

Distribution Rust diseases are found wherever wheat is cultivated (Yoneyama and Anzai, 1983; Pretorius *et al.*, 1990; Roelfs *et al.*, 1992; Kolmer, 1996). In South Africa rust was first discovered on barley in 1708, and on wheat in 1725 (Verwoerd, 1931).

Leaf rust is particularly severe on spring wheat in the winter rainfall regions of the Western Cape. Recently Boshoff *et al.* (in press) reported yield losses as high as 78 % due to leaf rust in unprotected plots of the cultivar SST75 in this region. Likewise, the disease can reach epidemic proportions in other parts of the country, in particular the central and western Free State, if moisture and temperature conditions are favourable (Pretorius *et al.*, 1987). The disease also occurs on irrigated wheat in KwaZulu-Natal, Mpumalanga, North West and the Northern Cape (Pretorius and Le Roux, 1988).

While the geographic distribution of the most important rust species is generally similar, their impact depends on the degree of resistance of the predominant cultivars

in that region and favourable environmental conditions. Leaf rust, for example, is predominant in humid regions or the rainy seasons in drier areas (Knott, 1989). One area of concern regarding plant pathogenic fungi is the rapidity with which they can spread between different areas. Rust spores can be airborne over long distances, often spanning several countries (McIntosh *et al.*, 1995). They have the potential to extend their geographic range or to suddenly appear in countries or continents where they were previously not recorded (Cooke, 1977).

Infection process To colonise plants, parasitic fungal organisms have evolved strategies to invade plant tissue, optimise their growth in the plant, and to propagate (Knogge, 1996).

Germination Germination is defined as the transformation of a mature rust spore from a resting into an active state (Teng and Bowen, 1985). Urediospores of *P. triticina* that come into contact with wheat plants will germinate if the environmental conditions are favourable. Favourable conditions include free water and temperatures ranging between 15 to 25 °C (Roelfs *et al.*, 1992). Urediospores absorb water and swell before germination takes place (Stevens, 1974). At 20 °C *P. triticina* infects with dew periods of approximately 3 h. Lower temperatures (approximately 10 °C) will require longer dew periods of about 12 h. Few, if any, urediospores will germinate if the temperature fluctuates either below 2 °C or above 32 °C (Roelfs *et al.*, 1992).

Germtube growth and appressorium formation After germination of the urediospores, the fungus must locate a stomatal opening. Several rust fungi appear to use the topography of the leaf to orientate germ tubes and to locate stoma (Littlefield and Heath, 1979). This orientation increases the chance of the germtube to have a

successful encounter with a stoma. Jacobs (1989a) found that while many germtubes grew at right angles to veins, some grew directly to the nearest stoma not following any lines parallel to either the long or short axis of the leaf.

After the location of a stoma an appressorium forms over the stomatal pore (Littlefield and Heath, 1979). Following the appropriate stimuli, an infection peg forms out of the appressorium and penetrates the stomatal aperture (Mendgen *et al.,* 1988; Kloppers, 1994). The appressorium is an adherent body providing the essential inertia against which the developing infection peg pushes as it moves downward through the stomatal opening. Without this anchoring device, the invading structure would push the germtube away from the surface of the leaf (Stevens, 1974).

Vesicle and haustorium formation Inside the leaf a substomatal vesicle is formed from which a primary infection hypha will grow towards a host cell (Knott, 1989; Roelfs *et al.*, 1992). Contact stimuli, with an appropriate host cell, control the formation of haustorium mother cells (Harder, 1989; Roelfs *et al.,* 1992; Kloppers, 1994). Direct penetration occurs when the haustorium mother cell comes into contact with a mesophyll cell. In a compatible host-pathogen interaction a haustorium will form inside the living host cell. Bushnell (1972) defined a haustorium as, *"...a specialized organ which is formed inside a living host cell as a branch of an extracellular (or intracellular) hyphae or thallus, which terminates in the host cell, and which probably has a role in the interchange of substances between host and fungus".* From a parasitic point of view it is important that the haustorium is not recognized as foreign by the plant host so that the resistance mechanisms of the host are not activated. Secondary hyphae develop, form additional haustorium mother cells and haustoria, eventually culminating in a colony (Roelfs *et al.*, 1992).

Life-cycle After successful colonisation orange-brown uredia bearing urediospores are formed. These can be distinguished from those of stem rust in that they occur primarily on the leaves rather than the stems, and the orange-red spores are almost spherical (Schafer, 1987; Knott, 1989). Sporulation occurs mainly on the upper leaf surface. Towards the end of the season when environmental conditions become unfavourable, black telia will replace the uredia (Roelfs *et al.*, 1992). The telia are elongated and covered by the epidermis (Western, 1971). The fungus over-seasons in this state. When environmental conditions become favourable again each cell in the teliospore germinates and forms basidia. Basidiospores form on the basidia and are released under humid conditions. Basidiospores are not adapted to long range transport, can not reinfect wheat and need to find another host plant for perpetuation of the life cycle (Roelfs *et al.*, 1992). A species of rust that requires the infection of another host, the alternate host, in order to complete its life-cycle is said to be heteroecious (Jackson and Mains, 1921). The basidiospores will germinate on an alternate host, and from its haploid mycelium, pycnia, containing pycniospores, are formed. Through spermatization and plasmogamy, opposite mating types in pycniospores and receptive hyphae fuse to give rise to aecium formation. Dicaryotic aeciospores represent the fifth stage in the life-cycle and are able to infect wheat plants (Nilsson, 1983) (Fig. 2). In South Africa *P. triticina* does not complete its macrocyclic life-cycle, but rather is perpetuated in the uredial state on wheat crops or volunteer plants in different agro-ecological regions.

Hosts Even though *P. triticina* can infect a wide range of hosts, the various *formae specialis* have a strict host specialisation (Kendrick, 1992). Wheat, its close relatives and the man-made crop triticale are the primary hosts for the leaf rust fungi.

Primary hosts The primary host for leaf rust is *Triticum aestivum* L. em. Thell. Other species, on which leaf rust has been of lesser importance, include *T. turgidum* L., *T. monococcum* L., *T. dicoccum*, and *T. speltoides* (Tausch) Gren. *ex* K. Richt. (Roelfs *et al.*, 1992).

Alternate hosts These hosts are necessary for completion of the sexual phase and thus recombination of avirulence and virulence genes (Samborski, 1985; Roelfs *et al.*, 1992). Alternate hosts also serve as a source of inoculum for the wheat crop before exogenous urediospores arrive. Alternate hosts for *P. triticina* include species in the *Ranunculaceae* and *Boraginaceae* families. *Thalictrum* spp.; *Anchusa* spp., *Clematis* spp. and *Isopyrum fumarioides* have been reported as alternate hosts for the wheat leaf rust pathogen (Roelfs *et al.*, 1992). No aecial infections of *P. triticina* have been observed or recorded in South Africa.

Accessory hosts Many grass species can be infected artificially by *P. triticina* including the wild and weedy species of *Triticum* and species of genera related to wheat, like *Secale* and *Agropyron* (Roelfs *et al.*, 1992). Volunteer or self-sown wheat seem to be the most common accessory crop for *P. triticina*. These plants are found in close proximity to wheat, for example along the edges of fields where wheat is grown (Roelfs *et al.*, 1992).

DISEASE CONTROL

History Disease control started when early farmers began to make certain assessments about plant diseases. For example, in the case of *P. graminis* f.sp. *tritici*

it was often noticed that the disease was worst in the presence of barberry bushes (*Berberis vulgaris*). Although there was no evidence connecting stem rust and barberry, the French passed a law to eradicate barberry in the 1600s. While eradicating barberry did not rid the country of wheat rust, an important source of infection was removed and any potential wheat rust epidemics were delayed for several weeks since there was no alternate host (http://www.ksu.edu/plantpath/extension/facts/ wheat11.html).

Although extensive research on control measures has been conducted, wheat rusts continue to cause significant crop losses. The reason for this is the plasticity and adaptation of these pathogens (Johnson and Browder, 1966). The genetic plasticity is evident from host-pathogen interactions, in particular adaptations in the pathogen to overcome resistance genes.

Cultural control This method of rust control is aimed at breaking the life-cycle of the fungus at a critical stage such as over-wintering or over-summering (Roelfs, 1985). In the USA winter wheat in some areas is infected soon after emergence by spores from nearby infected spring cultivars and volunteer plants. Delaying the planting of winter wheat may thus prevent infection. In areas where the rust inoculum arrives late in the growing season, early planting may allow a crop to mature before rust becomes serious. In areas where wheat is not grown in the summer, over-summering is a critical stage for rust. Eradication of any susceptible hosts such as volunteer wheat or other susceptible species can help to control rust (Knott, 1989). Using cultural control it must be realized that combined methods of every aspect of the cropping practise, like the inclusion of chemical control, should be used to promote the growth of plants and to kill the pathogens (Stevens, 1974).

Chemical control The last forty years have seen the investigation of rust control by chemical means. Susceptible varieties can be protected from rust with foliar fungicides. One spraying may sometimes be sufficient, but this will depend on the length of the growing season and the type of chemical used (Knott, 1989).

A major drawback of the use of chemicals as a means of control is the cost. Often the value of the crop targeted for spraying does not justify the control costs (Stevens, 1974). Another problem with existing systemic fungicides is that they are too expensive for most developing countries where yield of wheat per unit area is low, such as in regions with low rainfall (Knott, 1989). In addition to the cost of the compound, chemical control also requires application equipment. This implies capital investments, as well as training in handling and operation of chemicals and spraying procedures. In general fungicides are more effective on varieties that poses a measure of genetic resistance (Bingham and Lupton, 1981). According to Nel *et al.* (1999) seven fungicides have been registered for leaf rust control on wheat in South Africa. These compounds all belong to the triazole group, some of which are combined with carbendazim (Nel *et al.*, 1999).

Genetic resistance

Race-specific resistance and race-non-specific resistance Vanderplank (1963) differentiated between two types of disease resistance in plants, namely vertical and horizontal resistance. Vertical resistance is defined as being effective to only some races of a pathogen. It can therefore be classified as being race-specific and a differential interaction occurs between isolates of the pathogen and varieties of the host (Parlevliet, 1988). Race-specific resistance depends on the recognition of a pathogen

(Heath, 1991), and the interaction between resistance genes in a plant and the avirulence genes in the parasite (Flor, 1942). Genes conferring race-specific resistance produce highly resistant phenotypes, but their effects are short lived due to relatively easy adaptations in the pathogen (Smale *et al.*, 1998).

Horizontal resistance is defined as resistance effective against all genotypes of a pathogen (Vanderplank, 1963). This means there is no differential interaction between the host and pathogen genotypes. This type of resistance is known as race-non-specific resistance (Parleviet, 1988). It is unlikely that for race-non-specific resistance there exists a specific recognition for each potential pathotype. It can be assumed that this type of resistance is part of the plant's general defence against plant pathogens (Heath, 1991).

Seedling resistance In addition to the classification of resistance types based on race effects, resistance has also been qualified according to the growth stage of expression.

Throughout history numerous varieties, resistant to leaf rust, have been reported to host a type of seedling resistance. This resistance, which provides a very high level of protection (hypersensitive reaction), is expressed in primary leaves (Knott, 1989). However, seedling resistance is mostly monogenic, race-specific and short-lived in the presence of pathogen adaptation (http://www.botany.hawaii.edu/faculty/wong/bot135/Lect08.htm).

Adult plant resistance Adult plant resistance (APR) can be defined as a resistance which is not expressed in the seedling stage and which develops in mature plants (Zadoks *et al.*, 1974). Although examples of non-durable, single-gene APR exist

(e.g. *Lr12, Lr22b*, McIntosh *et al.*, 1995), APR is generally considered to be more long lasting than seedling resistance. Varieties with APR may exhibit low levels of leaf rust, but not enough to cause significant yield loss. Where APR is based on several genes, the leaf rust pathogen has difficulty in overcoming resistance. APR is thus an important trait, protecting the plant against pathogenic changes, ensuing leaf rust epidemics and yield losses (http://clay.agr.okstate.edu/wheat/Feb99.html). Selection for APR should preferably be in the field as APR may be underestimated in the greenhouse (Knott, 1989).

Some examples of APR genes for leaf rust in wheat are *Lr12, Lr13, Lr22a, Lr22b, Lr34, Lr35, Lr37* and *Lr46* (Dyck, 1977; Dyck and Kerber, 1985; Roelfs, 1985; McIntosh *et al.*, 1995). Considerable emphasis is also being placed at CIMMYT, and progress made, on breeding for non-specific APR to leaf rust (Singh and Rajaram, 1992; Braun *et al.*, 1996; Sayre *et al.*, 1998).

Prehaustorial resistance The time of resistance onset has also been proclaimed as important within the concept of durability of leaf rust resistance. Prehaustorial resistance is often associated with non-host interactions (Heath, 1977; Heath, 1981a; Heath, 1982; Elmhirst and Heath, 1987) and is the first defence mechanism (Anker and Niks, 2001) that is activated preventing the sporeling from developing a haustorium and successfully completing the parasitic relationship (Niks and Dekens, 1991; Heath, 1981a). Normal haustorium mother cells are formed, but often a papilla is induced at the cell wall penetration site, preventing haustorium formation (Heath, 1981a; Heath, 1982; Jacobs, 1989c; Niks and Dekens, 1991). In cases where this resistance type is expressed prior to penetration, the germination rate is not reduced (Heath, 1981a), but the germtube has difficulty in locating and

recognising stomata (Heath, 1974; Heath, 1977). When the prehaustorial defence mechanism is breached a posthaustorial defence may be elicited (Anker and Niks, 2001).

Posthaustorial resistance This defence mechanism is activated when a fungus succeeds to penetrate the plant cell (Niks and Dekens, 1991; Anker and Niks, 2001). The fungus growth is halted after the formation of at least one haustorium (Niks and Dekens, 1991). Posthaustorial resistance can be expressed morphologically in different ways. For example fibrillar material gets deposited in the extrahaustorial matrix (Littlefield and Heath, 1979) or callose collars can also develop around the necks of haustoria (Heath and Heath, 1971). Plant cells containing haustoria usually die in incompatible interactions (Littlefield and Aronson, 1969; Heath, 1981b; Niks, 1983b). This type of plant cell necrosis is called a hypersensitive response (Stakman, 1915; Kiraly, 1980; Prusky, 1988; Graham and Graham, 1999). The hypersensitive reaction (HR) was described by Stakman in 1915 as, "*The essential fact is that the fungus gains entrance in the same manner in susceptible and resistant forms, but acts differently thereafter. In susceptible it grows vigorously without seriously affecting host cells for some time. In resistant forms, on the other hand, a very rapid action results in the almost immediate death of the host cell. The degree of susceptibility is indicated to a certain extent by the rapidity of this action*".

Hypersensitive cell death is only activated once the passive defence mechanism of a plant has been passed (Kiraly, 1980; Prusky, 1988). The following reaction can be either specific or non-specific (Prusky, 1988). Responses associated with the non-specific reaction include enhanced metabolism, death of the host cell followed by the deposition of antifungal compounds in the infected tissue to block the further spread of

the fungus (Stakman, 1915; Kiraly, 1980; Prusky, 1988; Richael and Gilchrist, 1999). Usually a visual necrotic reaction can be seen (Prusky, 1988; Hammond-Kosack and Jones, 1996; Van Loon and Van Strien, 1999).

Hypersensitive resistance is usually governed by major genes, in which case it is race-specific and non-durable (Denissen, 1993). It is difficult to determine whether the hypersensitive response is the cause or consequence of resistance against rust (Heath, 1999).

Partial resistance In view of the non-durability of resistance characterised by the HR, alternative forms of resistance, e.g. partial resistance (PR), should be investigated (Parlevliet and van Ommeren, 1975). This type of resistance is associated with a reduced rate of epidemic development in spite of a susceptible infection type (Parleviet, 1978). Characteristics of PR include low receptivity, a long latent period and reduced spore production. The last two components of PR are based on obstructing haustorial formation (Niks, 1982; Niks, 1983b). Studies done on barley (Niks, 1982; Niks, 1986) suggest that partial resistance operates especially during the early phases of infection, directly after penetration. Confirmation of this was seen when aborted leaf rust (*P. triticina*) structures were seen on partially resistant bread wheat genotypes (Jacobs, 1989b). Another effect of partial resistance is smaller colony sizes compared to highly susceptible cultivars (Broers, 1989). Assuming a gene-for-gene relationship with respect to PR, differential interactions between cultivars and races will be less easily detected due to the quantitative expression of PR (Broers, 1989). PR in wheat to wheat leaf rust is a complex system with an expression that is highly dependent on the cultivar, race and environment (Broers, 1989).

Partial resistance has been divided into two phases (Jacobs, 1989a). In the first phase infection structures were aborted and associated with the presence of cell wall appositions. The aborted infection structures did not form haustoria (Jacobs, 1998b). In the second phase a continuous retardation of the growth rate was observed in partially resistant genotypes (Jacobs and Buurlage, 1990) and a post-haustorial inhibition was postulated (Jacobs, 1989b). Based on its quantitative genetic nature it is thought that partial resistance will be more durable than HR (Broers, 1989; Parlevliet, 1988).

Breeding for resistance

Gene-for-gene concept Flor (1942) was the first to formulate the gene-for-gene concept, defined as, "... *for each gene conditioning rust reaction in the host there is a specific gene conditioning pathogenicity in the parasite*". In other words this hypothesis states that resistance genes in the host have corresponding pathogenicity genes in the pathogen (Knott, 1989). Incompatibility occurs when a pathogen avirulence gene and a host resistance gene participate in the same interaction (Keen, 1990). Once a fungus successfully penetrates a host, the plant is compelled to develop some form of resistance to minimise the harmful effects of the pathogen. To do this a 'recognisable' feature of the fungus must act as a trigger of defence reaction(s). The fact that cultivar resistance is usually expressed after the first haustorium is formed suggests that it is based on various forms of interference with the metabolic relationship established at this stage between the plant and fungus (Roelfs *et al.*, 1992).

Resistance genes Breeding for resistance is based on the successful exploitation of resistance genes in crops (Kolmer, 1996; Smale *et al.*, 1998). Plant

genomes contain many genes that are involved in the detection and discrimination of potential pathogens. These genes are commonly clustered in gene families, which makes the genetics of specific pathogen recognition complex. Ever since it has been known that a single gene could confer resistance, breeding programs were initiated with the hope that the resistance would be durable (Crute and Pink, 1996). Durable resistance is defined as resistance that has remained effective in a cultivar during its widespread cultivation for several generations or a long period of time, in an environment favourable to a disease or pest (Johnson, 1979; Johnson, 1981). Two possible reasons could be found for durable resistance. Firstly, the pathogen may not be able to develop a virulent race(s), and secondly, the resistant cultivar may not come into contact with virulent races (Knott, 1989). Durability can also be influenced by the life-cycle of the rust. In leaf rust the sexual cycle is not necessary for the pathogen to survive, which means there is little chance for recombination leading to a new virulent race. In the absence of sexual recombination the pathogen thus has to rely on either mutation or possibly somatic recombination to generate variants. Since durable resistance is apparent only in retrospect, breeding for this type of resistance is difficult. To achieve durability, breeders usually incorporate polygenic resistance (Knott, 1989; Samborski and Dyck, 1982). There appears to be consensus that complex, polygenic resistance does not encourage frequent mutations in the pathogen (Roelfs, 1988a).

Resistance genes in wheat to leaf rust are called *Lr* genes (http://www.ksu.edu/plantpath/extension/facts/wheat11.html). Identification of new and existing *Lr* genes allows for incorporation of different genes into wheat germ plasm, thus helping to diversify resistance sources. There are currently more than forty different *Lr* genes available (http://wheat.pw.usda.gov/ggpages/wgc/2001upd.html). Not all these genes

are useful as different races of leaf rust can defeat different combinations of *Lr* genes in wheat (Schachermayr *et al.,* 1997).

In the case of stem rust there are several known sources of durable resistance ascribable to a single gene while, for wheat leaf rust, most durable resistance is associated with gene combinations (Roelfs, 1989). Durable resistance to leaf rust is thought to be more difficult to obtain than with stem rust since leaf rust is more diverse for virulence. This diversity may be the result of one or more factors. Firstly, more inoculum survives between wheat crops; secondly the pathogen population size is currently larger during the crop season, and thirdly, resistance deployed against leaf rust has often been a single gene at a time. Thus, population sizes are large which results in a greater probability of mutants and a greater probability that these variants can survive the non-wheat growing period (Roelfs, 1988a).

In order to maintain progress in this area, new resistance genes should be isolated, genetically characterised relative to previously designated *Lr* genes, and incorporated into breeding programs (Lin and Kuo, 1995).

Breeding systems The three main systems used in breeding for disease resistance are the pedigree, bulk and backcrossing systems. The standard pedigree system allows for the maintenance of pedigrees of each line during the breeding process (Moreno-Gonzàlez and Cubero, 1993). The pedigree system can be modified in various ways for specific objectives and to suit available sources (Knott, 1989).

In the bulk system, early generations are planted in bulk at normal seedling rates and the material is allowed to evolve through natural selection. For rust resistance, the system can be modified by growing bulks in a nursery (Knott, 1989).

Backcrossing is a particularly useful technique to transfer genes into a desirable cultivar lacking rust resistance (Knott, 1989). These backcross lines containing new resistance genes could be released as new cultivars providing they are superior to the existing ones (Johnson and Lupton, 1987). Dominant resistance genes can be backcrossed into the recurrent parent and tested with the appropriate rust race in each generation. Plants showing resistance are selected for another cycle of backcrossing (Bingham and Lupton, 1981). In the case of recessive genes, a cross and a backcross are made and the progeny selfed to recover the resistance. Another backcross can be made and the process repeated (Knott, 1989). Dominant resistance is clearly identifiable and simply inherited, its inheritance can be determined directly from segregating F_1 plants in a backcross. Normally, the F_2 families from a backcross are also tested. For one gene, the segregation ratio will be 1 segregating: 1 susceptible, for two genes 3:1 and for three genes 7:1. For two linked genes, three types of segregating families will be observed depending on whether segregation of the families occurs for the first gene, the second gene or both genes. With three genes there are seven types of segregating backcross F_2 families, three segregating for one gene, three segregating for two genes and one segregating for all three genes. It is difficult to identify all seven types, still, the ratio of segregating to susceptible families and the observed segregation within families should indicate the number of genes involved (Knott, 1989).

Quantitative resistance controlled by several genes is considered to be more difficult to transfer by backcrossing (Bingham and Lupton, 1981). Backcrossing is a conservative procedure and the basic objective is to change one character in an otherwise acceptable cultivar (Knott, 1989).

Other breeding systems that have been used with success in breeding for

resistance against wheat diseases include the doubled haploid, single seed descent (SSD) and recurrent selection systems, as well as hybrid varieties. When resistant and susceptible parents are crossed, a population of homozygous doubled haploid lines can be obtained from F_1 plants using the wheat x maize pollination and embryo rescue procedure (Laurie and Bennett, 1988).

SSD is used to produce homozygous lines with as little labour as possible. SSD usually commences from the F_2 population when single seeds are obtained and grown in pots. This process is repeated for a number of generations until the desired level of homozygocity is reached. As only one seed per plant is required, generations are often grown in an accelerated way; i.e. in small pots, at long daylengths, and without fertilizer. Individual plant progenies are then grown and tested for various desirable characters (Knott, 1989, Van Oeveren, 1993).

Recurrent selection involves the selection of several desirable parents, making all possible crosses among them, obtaining the progeny, selecting for the desired trait, and intercrossing the selected material to start another cycle. This system works on the concept of repeated recombination cycles followed by selection, thus concentrating the desired genes in individual genotypes (Knott, 1989). Recurrent selection has been used with success in breeding for stripe rust resistance (Sharp, 1979).

Many of the major genes for specific resistance are dominant making it easy to combine a number of genes in a hybrid cultivar. If each parent used in the cross carries several dominant genes for resistance, all the genes should be expressed. To have expression of recessive genes in the F_1 hybrid the genes will have to be present in both parents (Knott, 1989). An advantage of hybrid varieties is the relatively quick release of commercial F_1 hybrids to the industry (Edwards, 1987).

Selection strategies Successful breeding for disease resistance depends on an appropriate method of selection (Johnson, 1992). Screening in the glasshouse where the environmental conditions are highly controlled is less representative of actual growing conditions (Niks *et al.*, 1993). In this controlled environment certain factors such as inoculum type and distribution can be manipulated. An advantage is that it is also possible to screen plants independent of the growing season (Niks *et al.*, 1993). When plants are screened for rust resistance under controlled environment conditions, an infection type scale described by Roelfs (1988b) is often used. Infection types 0 to 2 are usually indicative of resistance and 3 to 4 of susceptibility.

Marker assisted breeding The use of molecular techniques in wheat breeding is becoming increasingly important (Moore *et al.*, 1993; Keim *et al.*, 1997; Mohan *et al.*, 1997; Law *et al.*, 1998). Molecular markers are especially advantageous for traits that are otherwise difficult to identify and follow in segregating populations. The development of a high-density genetic linkage map of cultivated wheats using conventional molecular markers has lagged behind the other major food crops such as rice and tomatoes because of the limited levels of genetic polymorphisms and the large genome size (Joshi and Nguyen, 1993).

Molecular markers are used to identify and tag desirable genes by detecting variation at the DNA sequence level (Mohan *et al.*, 1997). These variations, unlike morphologic markers, are not revealed in the phenotype of the plants. Varying in size, each of these sites might be nothing more than a single nucleotide difference in a gene or a piece of repetitive DNA (Jones *et al.*, 1997). Screening for resistance genes involves the use of closely linked markers to aid selection of resistant lines. Screening

is based on identifying tight linkages between the marker and the gene of interest so that the presence of a desirable gene can be inferred by assaying for the marker. Linkage must be as close as possible to minimise the possibility of recombination. The development of molecular markers has the advantage over phenotypic markers in that the expression of many resistance genes is strongly influenced by environmental effects such as temperature, thus requiring special conditions for screening (Johnson and Lupton, 1987).

Advantages of molecular techniques include its applicability to the transfer of any trait, qualitative and quantitative (http://clay.agr.okstate.edu/wheat/Feb99.html). Molecular techniques also hasten the transfer of selected genes among varieties (Mohan *et al.*, 1997). These markers are not environmentally regulated, they are unaffected by the plant growth conditions, and are detectable at all stages of plant growth (Mohan *et al.*, 1997). Molecular markers are also more numerous than morphological markers and allow the detection of genes independently of the phenotype (Schachermayr *et al.*, 1997).

Developments in DNA marker technology together with the concept of marker-assisted selection provide new solutions for selecting and maintaining desirable genotypes. Once molecular markers closely linked to desirable traits are identified marker assisted selection can be performed in early segregating populations and early stages of plant development. Marker-assisted selection or identification can be used to pyramid major genes including resistance genes with the ultimate goal of producing varieties with more desirable characters. Molecular marker technology is now integrated into existing plant breeding programs all over the world in order to allow researchers to access, transfer and combine genes at a rate and with a precision not previously

possible (Mohan *et al.*, 1997).

Techniques used in marker-assisted programs include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), single sequence repeat (SSR) and amplified fragment length polymorphism (AFLP).

Restriction fragment length polymorphism RFLPs were the first DNA markers to be identified. They reveal differences in the DNA that alter the length of fragments obtained by digestion with restriction enzymes (Powell *et al.*, 1996). The RFLP approach relies on the cleavage of genomic DNA by restriction enzymes (Jones *et al.*, 1997). The resulting length polymorphism between a given pair of sites is then detected by hybridisation to a labelled DNA probe. The use of the RFLP technique in certain crops, like wheat, has revealed a lack of polymorphisms, which has hampered the construction of linkage maps (Joshi and Nguyen, 1993; Powell *et al.*, 1996). Another problem is that RFLPs are labour intensive (Mohan *et al.*, 1997) and the preparations of gene libraries for the isolation of RFLP probes are time consuming.

Random amplified polymorphic DNA RAPD analysis (Welsh and McClelland, 1990), which is a PCR-based technique (Mohan *et al.*, 1997) solved some of the problems encountered by RFLPs (Powell *et al.*, 1996). It permits the erection of a saturated genetic linkage map in a relatively short time (Williams *et al.*, 1990). This technique is based on the amplification of genomic DNA directed by a single short primer of randomly chosen sequence (Strange, 1993). Several DNA fragments are amplified and separated on standard agarose gels. A disadvantage is that the PCR technique will only allow amplification of a relatively small size range of DNA so that priming sites need to be fairly close together for amplification to occur. RAPDs are used

for mapping, but because of the random nature of their generation, and short primer length, they cannot easily be transferred between species (Jones *et al.*, 1997).

Simple sequence repeats Plant genomes have a large number of simple sequence repeats of less than six base pairs. These SSRs are tandemly repeated and scattered throughout the chromosome. Typically SSRs are either dinucleotides (AC)*n*, (AG)*n*, (AT)*n*; trinucleotides (TCT)*n*, (TTG)*n* or tetranucloetides like (TATG)*n* where *n* equals the number of repeating units within the microsatellite locus (Tautz and Renz, 1984; Tautz *et al.*, 1986). To isolate a SSR at a particular locus, a small-insert genomic library is constructed. The library is subsequently screened with a number of microsatellite probes to identify inserts containing SSRs. The inserts are then sequenced and primers are chosen that match unique flanking sequences for particular loci. PCR amplifications are used to generate DNA banding patterns on a gel and to reveal polymorphisms. The use of SSRs has an economical impact, as it is expensive to establish, needs specific primers and has a long development time (Jones *et al.*, 1997).

Amplified fragment length polymorphism In the light of some of the drawbacks of the above mentioned techniques the AFLP technique is ideal for the detection of polymorphisms between resistant and susceptible cultivars in wheat. This technique is based on the amplification of genomic restriction fragment subsets using the PCR technique (Zabeau and Vos, 1993). Choosing the different base numbers and composition of nucleotides in the adapters (Lin and Kuo, 1995; Mohan *et al.*, 1997) can control the number of DNA fragments obtained. In contrast to the RFLP technique AFLPs will display the presence or absence of restriction fragments rather than the

length polymorphisms (Vos *et al.*, 1995). AFLPs can be used to distinguish between closely related organisms, including near-isogenic lines. This method generates a large number of restriction fragment bands facilitating the detection of polymorphisms. This technique is especially useful since it requires no prior sequence characterization of the target genome, and can be used for DNA of any origin or complexity (Vos *et al.*, 1995). Additionally it is easily standardised and readily automated for high throughput applications. A high reproducibility, rapid generation, and high frequency of identifiable polymorphisms make AFLP DNA analysis an attractive technique for identifying polymorphisms and determining linkages by analysing individuals from a segregating population (Vos *et al.*, 1995; Jones *et al.*, 1997).

Current criticism is that AFLPs are expensive to generate using fluorescent dye or radioactivity (Jones *et al.*, 1997) to detect the bands. Another problem with the use of unmapped AFLPs for diversity analysis in cereals is that they tend to be clustered in areas of low recombination, such as the pericentromeric regions, which have a high content of repetitive DNA (Moore *et al.*, 1993). AFLPs are a powerful means of profiling plant varieties and hence have potential applications in a range of other areas. Current investigations concentrate on use of AFLPs and other molecular markers to measure genetic diversity in wheat and other major agricultural crops (Law *et al.*, 1998).

Sources of resistance

Gene transfer from wild species Genetic variation of cultivated wheat has decreased considerably due to modern agricultural systems. This makes cultivated wheat more sensitive to new diseases (Joshi and Nguyen, 1993; Jiang *et al.*, 1994).

The introgression of disease resistance genes from wild wheat offers a wider diversity of resistance sources (McIntosh *et al.*, 1995). Alien germplasm pools will thus

assist in the expansion of the existing genetic variation by introducing novel variation into the crops (Knott and Dvořàk, 1976; Jones *et al.*, 1995).

It was noted that tetraploid *Triticum* species carried more resistance to leaf and stem rust than did the hexaploid bread wheat (Knott and Dvořàk, 1976). Major disease epidemics are rare in wheat in the wild compared with wheat under agriculture. Selection pressure in the wild favours the more resistant plants, wild wheat is genetically more diverse, the canopy of wheat in the wild is usually more open and provides a less favourable environment for epidemics, and natural enemies of pathogens are usually more abundant and diverse in the wild (Cook and Veseth, 1991).

At least six genera from the tribe *Triticeae* have been used as successful donors of disease resistance genes for domestic wheat (Jiang *et al.*, 1994; Jones *et al.*, 1995). Transferring genes from related species to wheat largely depends on the evolutionary distance between the species involved (McIntosh, 1991). Species belonging to the primary gene pool of common wheat share homologous (functionally related) genomes. The transfer of resistance genes from related species of lower ploidy into hexaploid bread wheat can be complicated by interactions between resistance genes and suppressor genes in the different genomes (Lin and Kuo, 1995). The transfer of genes can be achieved by direct hybridisation, homologous recombination, backcrossing and selection (McIntosh, 1991).

Experience with introduced alien resistance has not always supported the expectation of durability (Johnson and Lupton, 1987). In many cases high levels of resistance have been obtained, but undesirable linked traits were simultaneously transferred (Jones *et al.*, 1995). The expression of resistance is often reduced when genes are transferred to a new species, but this can vary depending on the genetic

background (Knott and Dvořàk, 1976).

Examples of wild relatives of wheat used for leaf rust resistance include, *T. umbellulatum* (Zhuk.) Bowden (*Lr9*), *T. dicoccoides* (*Lr14a*), *T. timopheevii* (*Lr18*), *Thinopyrum ponticum* (*Lr19*), *T. tauschii* (*Lr21, 22a, 32, 39, 40, 41, 42, 43*), *T. turgidum* var. *durum* (*Lr23*), *Th. ponticum* (*Lr24, 29*), *Secale cereale* (*Lr25, 26, 45*), *T. speltoides* (*Lr28, 35, 36*), *T. ventricosum* (*Lr37*), *Th. intermedium* (*Lr38*) and *T. spelta* (*Lr44*) (McIntosh, 1988; McIntosh *et al.*, 1995).

Wild species have often been screened and show potential as sources of resistance. Antonov and Marais (1996) screened 877 *Triticum* accessions for leaf rust resistance. A total of 206 of these showed low to medium infection types to the pathotypes used. In a study by Negassa (1987) 58 % of an Ethiopian wheat collection showed resistance to leaf rust. In a study by Barnard (1999) a wide variety of diploid and tetraploid wild wheat species showed high levels of seedling and adult plant resistance. Wild wheat is therefore clearly a valuable source of disease resistance that can be exploited to improve existing cultivars.

CONCLUSIONS

To minimize the harmful effects of leaf rust on wheat, research should continue to focus on the identification of new sources of resistance and ways to incorporate these in commercial cultivars. Few effective leaf rust resistance sources remain in bread wheat, implying that other sources, e.g. wild species, should be screened for possible resistance genes (McIntosh *et al.*, 1995).

Improvement of crossing techniques has widened the field for the search of

resistance genes. Breeders can now use species related to the domesticated crop to search for specific resistance genes and incorporate them successfully into existing cultivars. Posthybridisation barriers such as preferential transmission of certain alien chromosomes, and adverse genetic interactions leading to hybrid dysgenesis, chromosome elimination, chromosome breakage, and sterility impede further progress in alien transfer. The use of diverse host and donor genotypes in the initial hybridisation can often overcome some of these barriers (Jiang *et al.*, 1994).

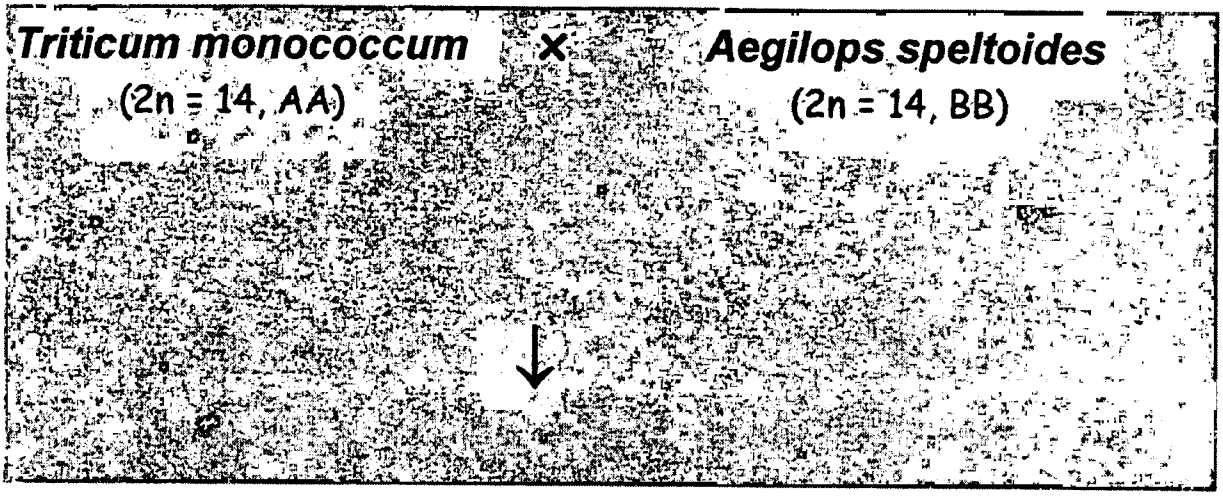
It is important to characterize the type of resistance found in the wild species. In this regard it should be determined if resistance is effective in the seedling or adult plant stage and whether it is horizontal or vertical. Furthermore, it is important to determine the number of genes responsible for resistance in order to predict its durability. Single gene resistance in the wild species may prove not to be durable (Bender and Pretorius, 1997; Nelson *et al.*, 1997), but with the use of the gene pyramiding technique (Pederson, 1988; Van Ginkel and Rajaram, 1993) may prove to be a valuable asset to breeders. Resistance being conveyed by more than one resistance gene are thought to be more durable, since two or more simultaneous mutations have to occur in the rust population to produce virulence (Roelfs *et al.*, 1992). The probability of such a mutant arising and becoming established has been considered small (Johnson, 1983; Schafer *et al.*, 1984).

Using molecular techniques such as AFLPs contributes to the characterization of resistance genes at a molecular level in segregating populations. If an informative polymorphism can be found and transformed into a breeder-friendly marker, it should help to accelerate the transfer of the desired genes into a cultivar of choice.

Thus, wild relatives of wheat are a valuable source of new disease resistance

genes. It must be remembered that not all resistance found in wild species will be durable, but it remains important to screen such species and characterize the type of resistance found.

Figure 1. Proposed origin of *Triticum turgidum* (durum wheat) and *Triticum aestivum* (bread wheat) (Knott, 1989).



T. turgidum × *T. tauschii*

(2n = 28, AABB) (2n = 14, DD)

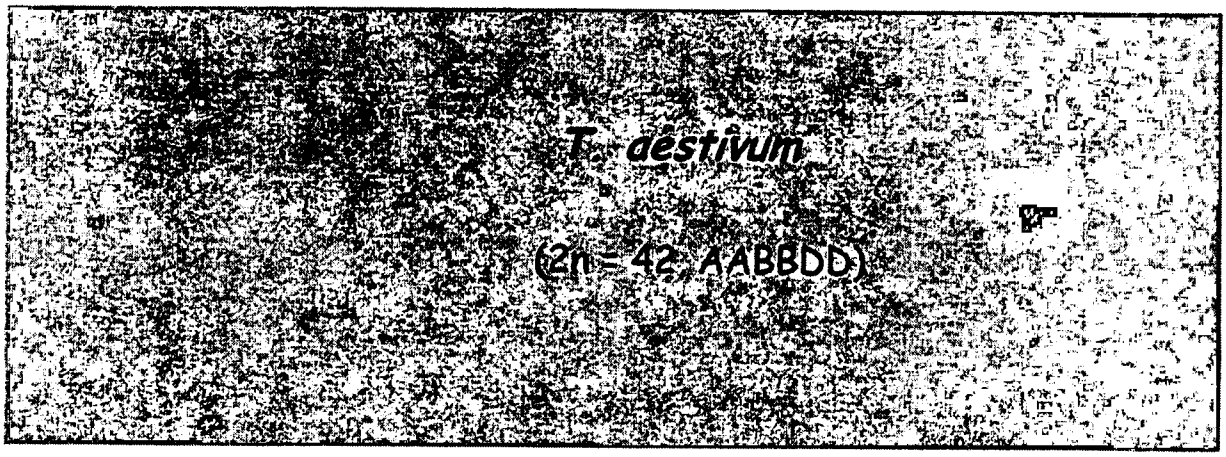
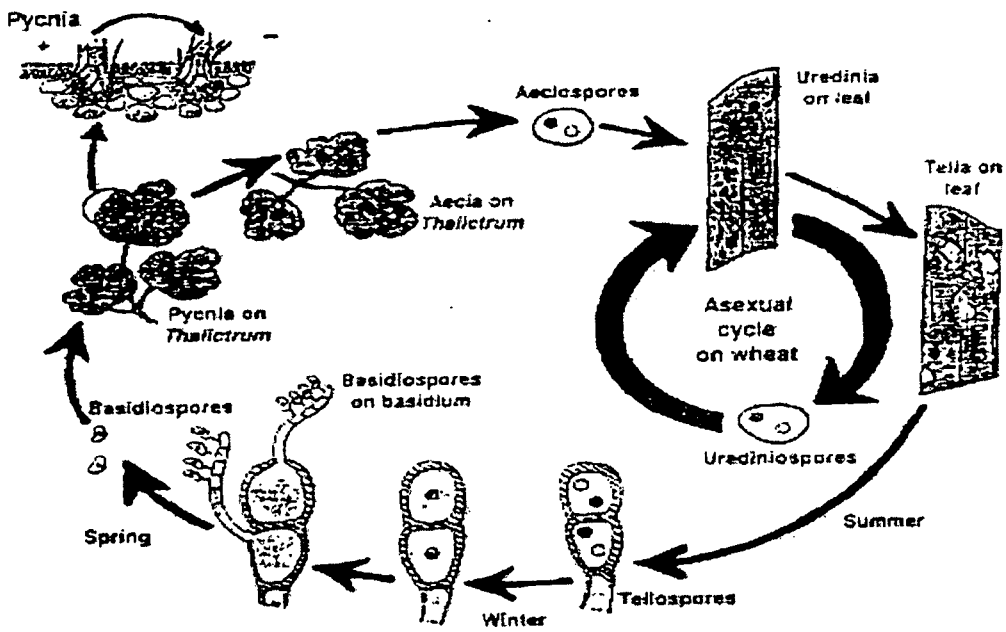


Figure 2. Life-cycle of *P. triticina* (wheat leaf rust) (Roelfs *et al.*, 1992). It should be noted that the cropping season refers to the USA and that the cycle is different in South Africa.


Pycnia
+
−
Aeciospores
Uredinia on leaf
Telia on leaf
Aecia on Thalictrum
Pycnia on Thalictrum
Asexual cycle on wheat
Basidiospores on basidium
Basidiospores
Urediniospores
Summer
Spring
Teliospores
Winter

CHAPTER 2

HISTOPATHOLOGY OF ADULT-PLANT RESISTANCE TO *PUCCINIA TRITICINA* IN *TRITICUM TURGIDUM*

ABSTRACT

Triticum turgidum ssp. *dicoccum* var. *arras*, *T. turgidum* ssp. *durum* var. *murciense*, *T. turgidum* ssp. *durum* var. a*estivum* and *T. turgidum* ssp. *polonicum* were tested for adult-plant resistance to *Puccinia triticina.* The four species showed resistance to all pathotypes tested except UVPrt5, which was virulent. This differential interaction between hosts and pathotypes indicated the presence of vertical resistance. To further classify resistance expression through histopathology, several stages of the infection process were studied on and in flag leaves. All the *Triticum* species and Morocco had low levels of prestomatal exclusion, showing that fungal behaviour prior to penetration did not influence resistance expression. Resistance was associated with the early abortion of structures in *T. turgidum* ssp. *dicoccum* var. *arras* and the two *T. turgidum* ssp. *durum* subspecies. Host cell necrosis was common at infection sites in *T. turgidum* ssp. *dicoccum* var. *arras,* indicating a typical hypersensitive response. Leaf rust pathotypes influenced microscopic components of resistance differently, emphasizing the importance of working with several isolates when apparently novel resistance mechanisms are studied. Based on infection types and hypersensitivity, resistance in the lines studied is not considered durable.

INTRODUCTION

Plant breeders are continuously searching for novel resistance genes that may be

durable and transferable to existing wheat varieties. Although it has been assumed that rust resistance found in wild wheat species would be more durable than the resistance of common wheat varieties (McIntosh *et al.*, 1995), several examples of virulence to species-derived genes exist. However, wild species remain an important source of new diversity for disease resistance. The first step in exploiting wild wheat species in breeding for disease resistance is to determine whether prospective sources show resistance against a particular pathogen. The next step would be to determine whether the donor species is resistant to other races of the same pathogen, followed by characterization of the resistance phenotype. Finally, in an attempt to attain durable disease resistance, the gene(s) needs to be transferred to an agronomically accepted *Triticum aestivum* background.

Resistance is considered durable when a cultivar remains resistant over time, despite being challenged by diverse pathogen races in environments favourable for disease development. According to Vanderplank (1963) resistance could be classified as either vertical or horizontal. Vertical (race-specific) resistance depends on the recognition of a pathogen (Heath, 1991), and functions according to the interaction between resistance genes in a plant and avirulence genes in the parasite (Flor, 1942). Genes conferring race-specific resistance produce highly resistant phenotypes, but their protection is short-lived due to relatively easy adaptations in the pathogen (Smale *et al.*, 1998). Race-specific resistance is activated once the passive defence mechanism has been passed and the ensuing interaction recognized as incompatible (Kiraly, 1980; Prusky, 1988). In incompatible interactions plant cells containing fungal haustoria usually die (Littlefield and Aronson, 1969; Heath, 1981b; Niks, 1983b), leading to host cell necrosis called a hypersensitive response; (Kiraly,

1980; Prusky, 1988; Knott, 1989; Graham and Graham, 1999). Horizontal (race-non-specific) resistance is defined as resistance effective against all genotypes of a pathogen (Vanderplank, 1963; Parlevliet, 1988). This means there is no differential interaction between the host and pathogen genotype and is demonstrated by similar disease responses in host varieties to different pathotypes. It has been assumed that this type of resistance is part of the general resistance in plants to pathogens (Heath, 1991).

The objective of this study was to characterize the expression of adult-plant resistance to *Puccinia triticina* in selected accessions of *Triticum turgidum* in terms of race-specificity and hypersensitivity.

MATERIALS AND METHODS

Wheat genotypes Four subspecies of *Triticum turgidum* were selected on the basis of their adult-plant resistance described by Barnard (1999). The species chosen were *T. turgidum* ssp. *dicoccum* var. *arras* (UFS accession 104), *T. turgidum* ssp. *durum* var. *murciense* (UFS accession 125), *T. turgidum* ssp. *durum* var. *aestivum* (UFS accession 127) and *T. turgidum* ssp. *polonicum* (UFS accession 370). Morocco was included as the leaf rust-susceptible control.

Inoculum preparation Prior to inoculation of entries, the wheat leaf rust pathotypes UVPrt2, 3, 5, 9, 13 and 17 were multiplied on seedlings of selected susceptible wheat cultivars. Pathotypes 2, 3, 9, and 13 were originally collected from bread wheat, whereas UVPrt5 was sampled from durum wheat (Pretorius *et al.*, 1987). Pathotype UVPrt17 was selected for this study as it represents a mutation for

virulence towards the *Lr41* gene transferred from *T. tauschii* (Pretorius, 1997). Emerging seedlings were drenched with 50 ml of a 0.3 g/l maleic hydrazide solution per 10 cm plastic pot to retard plant development and enhance sporulation (Knott, 1989). When seedlings were seven days old they were inoculated with urediospores from the different rust pathotypes suspended in a light mineral oil. The seedlings were subsequently kept in a dark dew-simulation chamber at 18 to 21 °C for 16 h. Upon removal from the chamber, seedlings were allowed to dry before being placed in isolation cubicles (40 x 30 x 30 cm) in a glasshouse where they were kept at 18 to 25 °C. Urediospores were harvested 14 days after inoculation.

Plant growth Seeds of the test and control entries were planted in 1-litre-capacity pots and plants (three plants per pot and four replications per treatment) were raised in a leaf rust-free, air-conditioned glasshouse cubicle at 18 to 25 °C. Seven days after seedling emergence 50 ml of a 3 g/l hydroponic nutrient solution (6.5:2.7:13 N:P:K plus microelements) was administered to each pot. This treatment continued three times a week for the remainder of the experiment.

Infection of adult plants Flag leaves of the selected *T. turgidum* species were inoculated separately with freshly harvested urediospores of the above mentioned pathotypes. Each of the pathotypes of *P. triticina* was suspended separately in sterile, distilled water containing Tween 20® and sprayed unto the upper surface of leaves. The plants were subsequently incubated for 16 h at 19 to 22 °C. Infected plant surfaces were allowed to dry before plants were transferred to a glasshouse

where assessments were made 14 days later. The plants were maintained in conditions similar to those described for the pre-inoculation period.

Fluorescence microscopy

Staining One flag leaf per replicate of each host infected with pathotypes UVPrt2, 3, 9 and 13 was sampled 14 days post-inoculation (d.p.i.). A 4 cm segment was collected from the central part of each leaf and cut into 1 to 2 cm long portions. Material was then prepared according the modified Uvitex staining method for fluorescence microscopy (Rohringer *et al.*, 1977). The leaf segments were cleared and fixed in ethanol: dichloromethane (3:1 v/v) containing 0.15 % trichloroacetic acid for 18 to 24 h. Leaf segments were washed for 15 min. (2x) with 50 % ethanol. The subsequent washing step (2x for 15 min.) with 0.05 M sodium hydroxide turned the leaves yellow. Samples were rinsed with distilled water (3x) before submerging in a Tris(hydroxymethyl)aminomethane/hydrochloric acid buffer (pH 5.8) for 30 min. The samples were stained for 5 min. in 0.1 % diethanol (Uvitex 2B, Ciba-Geigy AG) in buffer (Niks and Dekens, 1991). Rinsing with water (4x) preceded a final wash step with 25 % aqueous glycerol (30 min.). The stained leaf segments were stored in 50% glycerol containing a trace lactophenol to prevent deterioration of the preparations.

Microscopic examination The leaf segments were mounted and used for fluorescence microscopy (Rohringer *et al.*, 1977; Kuck *et al.*, 1981). Observations were made at magnifications 100x and 400x with a Nikon Optiphot epifluorescence microscope on 50 randomly selected infection structures on each of three replicates per treatment. The filter combinations UV-1A (excitation filter 330 to 380 nm and

barrier filter 420 nm) were used for fungal structures and B-2A (excitation filter 450 to 490 nm and barrier filter 520 nm) for autofluorescence measurements. The fungal structures observed fluoresced in a bright light blue colour. Orange-yellow fluorescing host cells were considered necrotic whereas unaffected cells did not fluoresce (Rohringer *et al.*, 1977).

Fungal structures were counted to determine the different proportions of prestomatal exclusion, abortive penetration, early abortion and colony formation. Prestomatal exclusion (PE) consisted of germ tubes failing to produce appressoria, or forming nonstomatal appressoria (NSA) on the leaf surface. Abortive penetration (AP) is defined as non-penetrating appressoria (NPA) and aborted substomatal vesicles (ASSV) (Parlevliet and Kievit, 1986). Early abortions (EA) included sites where six or less haustorium mother cells (HMC's) were formed, whereas those with more than six were considered colonies (Niks, 1983a). Early abortions displaying host cell necrosis (HCN) and colonies with sporulation and necrosis were also recorded. Colonies, uredium size and necrotic area were measured using a calibrated eyepiece micrometer and their dimensions (mm^2) calculated according to the formula: π x length x width / 4. Where possible, a hypersensitivity index (HI) (Kloppers and Pretorius, 1995) was calculated to reflect the size of the necrotic area as a proportion of the colony area. Here, coalescing colonies were not measured.

Scanning electron microscopy Portions of flag leaves from *T. turgidum* ssp. *dicoccum* var. *arras* were collected at 14 d.p.i. and cut into segments of approximately 5 mm^2. The leaf samples were fixed in 3 % gluteraldehyde and subsequently washed in 0.05 M phosphate buffer (2x) (pH 6.8-7.2) and post-fixed in

2 % osmium tetroxide. The samples were washed with 0.05 M phosphate buffer (pH 6.8-7.2) to dissolve the osmium tetroxide fixatives before it was dehydrated through several ethanol steps. Leaf samples were critical-point-dried in a Polaron dryer. Furthermore, segments were stripped according to a technique described by Hughes and Rijkenberg (1985) to reveal fungal development inside leaf tissues. The strips were sputter coated with gold in a Bio-Rad SEM coating system. The samples were examined using a JEOL WINSEM JSM-6400 scanning microscope operating at 5 kV. At least 50 infection sites were observed for each of the four pathotypes of *P. triticina.*

Fungal structures on the surface of, and inside leaf segments were observed but not counted for quantitative analysis. The objective was to determine if scanning electron microscopy could be used to more accurately describe the development of fungal structures in *T. turgidum* ssp. *dicoccum* var. *arras* than fluorescence microscopy.

Infection types Phenotypic assessments of infection types (ITs) that developed on the four *T. turgidum* accessions and Morocco were made 14 d.p.i. Descriptions of the scale used (Roelfs, 1988b; McIntosh *et al.*, 1995) are given in Table 1.

Statistical analysis Analysis of variance was done with SOLO (BMDP Statistical Software Inc., Los Angeles, CA), using the procedure for a general linear model. Standard deviations were calculated to compare means.

RESULTS

Infection types Morocco was susceptible to all pathotypes tested (IT 4) indicating that procedures followed were sufficient for evaluating leaf rust resistance (Table 2). With the exception of UVPrt5, which was virulent to all accessions tested (Figs. 1, 2, 3 and 4), the highest levels of adult-plant resistance (ITs 0; to ;1C) were expressed by *T. turgidum* ssp. *dicoccum* var. *arras*. *Triticum turgidum* ssp. *polonicum* showed the lowest levels of resistance with ITs ranging between 2 and 3-. Low infection types produced on *T. turgidum* ssp. *durum* var. *murciense* and *T. turgidum* ssp. *durum* var. *aestivum* were mostly within the "1" range, indicating the occurrence of small, sporulating pustules.

Fluorescence microscopy

Prestomatal exclusion Germ tubes failing to produce appressoria, or those forming nonstomatal appressoria (NSA), are illustrated in Fig. 5. Although a high degree of variation between replicates was encountered, a significant interaction ($p < 0.05$) between pathotypes and accessions occurred. Prestomatal exclusion was negligible in Morocco whereas the highest proportion sporelings failing to infect their host were detected on *T. turgidum ssp. durum* var. *aestivum* inoculated with UVPrt13 (11. 81 %) (Fig. 6). In the latter host-pathotype combination prestomatal exclusion resulted almost exclusively from the inability of germ tubes to form an appressorium. Very little distinction between NSA and the absence of appressoria was evident in any of the other treatments (Tables 3 to 6).

Abortive penetration Examples of NPA and ASSV are shown in Figs. 7 and 8. AP was influenced ($P < 0.05$) by different pathotypes, host genotype and their

interaction (Fig. 6). In *T. turgidum* ssp. *dicoccum* var. *arras,* AP was an important component of resistance and, depending on the pathotype, 30.2 % to 51 % of infection sites failed to penetrate properly (Fig. 6). It was not possible to ascribe AP in this accession to one mechanism as approximately similar proportions of NSA and ASSV occurred (Tables 3 to 6). Similar to *T. turgidum* ssp. *dicoccum* var. *arras,* AP was an important component of resistance in *T. turgidum* ssp. *durum* var. *aestivum* with 21 % (pathotype UVPrt3) to 40 % (UVPrt9) of sites aborting during the penetration phase (Fig. 6). Considering UVPrt 9 and 13, significantly more NPAs than ASSVs occurred on *T. turgidum* ssp. *durum* var. *aestivum* (Tables 5 and 6). It was clear that AP did not play a significant role in the response of *T. turgidum* ssp. *polonicum,* as less than 13.2 % of infection sites were classified as such (Fig. 6). Very few aborted vesicles in any accession were accompanied by necrosis.

Early abortion An infection site considered an EA is shown in Fig. 9. EA was common in *T. turgidum* ssp. *durum* var. *murciense* (33.0 % [UVPrt3] to 56.3 % [UVPrt9] infection sites) and in *T. turgidum* ssp. *dicoccum* var. *arras* (21.9 % [UVPrt2] to 53.9 % [UVPrt13]), but infrequent in *T. turgidum* ssp. *polonicum* (less than 14.3 %) (Fig. 10). More early abortions of UVPrt3 (18.7 %) were associated with necrosis of *T. turgidum* ssp. *dicoccum* var. *arras* than in any other pathotype-host interaction (Tables 3 to 6).

Colony formation The formation of colonies was significantly influenced ($p < 0.05$) by accessions and pathotypes. UVPrt13 produced the least number of colonies in *T. turgidum* ssp. *dicoccum* var. *arras* (1.3 % of all infection sites) whereas 85.6 % of UVPrt2 sites developed into colonies on *T. turgidum* ssp. *polonicum* (Fig. 10). Over pathotypes and species, UVPrt2 produced the most colonies. Colonies in

Morocco and *T. turgidum* ssp. *polonicum* were characterized by more than 30 HMCs at an infection site. Further differentiation was needed to distinguish between colonies with sporulation and/or necrosis formation (Fig. 11) (Tables 3 to 6). Of those sites classified as colonies on Morocco, more than 96 % culminated in sporulation. It was also apparent that pathotype significantly influenced the occurrence of necrotic colonies. In *T. turgidum* ssp. *dicoccum* var. *arras* only 3.9 % and 1.3 % of all UVPrt9 and 13 colonies, respectively, showed HCN whereas 17.5 % and 43.6 % of UVPrt3 and UVPrt2 colonies were accompanied by dead leaf tissue (Tables 3 to 6). Likewise, in *T. turgidum* ssp. *durum* var. *aestivum,* 52.8 % of UVPrt3 colonies resulted in necrotic host cells.

No sporulation was recorded in *T. turgidum* ssp. *dicoccum* var. *arras* for UVPrt2, 3, 9 and 13. In *T. turgidum* ssp. *polonicum* infected with UVPrt13 or UVPrt9, the proportion of colonies producing spores varied between 17.5 % and 82.9 % (Tables 3 to 6).

Colony area The mean colony area was influenced by the different hosts and pathotypes used ($p < 0.05$). As anticipated Morocco had the largest colonies followed by *T. turgidum* ssp. *polonicum* (Tables 7 to 10). *T. turgidum* ssp. *dicoccum* var. *arras* supported the smallest colonies.

Uredium size The mean size of uredia was significantly influenced ($p < 0.05$) by pathotype, host genotype and their interaction. Morocco produced the largest uredia and, as suggested by colony area, was followed by *T. turgidum* ssp. *polonicum* (Tables 7 to 10). This was in accordance with the macroscopic IT values recorded (Table 2).

Necrotic area Measurable necrosis was only obtained for *T. turgidum* ssp.

dicoccum var. *arras* and supported the fleck phenotype observed visually (Table 2).

Hypersensitivity index Due to the infrequency of necrosis the hypersensitivity index could only be determined for *T. turgidum* ssp. *dicoccum* var. *arras*. The index for UVPrt9 was 0.94, followed by 0.87 for UVPrt2, 0.67 for UVPrt3 and 0.23 for UVPrt13.

Scanning electron microscopy Scanning electron microscopy was done only on *T. turgidum* ssp. *dicoccum* var. *arras*. Using this approach substomatal vesicle initials (Fig. 12A), mature (Fig. 12B) or collapsed (Fig. 12C) substomatal vesicles, and hyphae (Fig. 12D) were detected.

DISCUSSION

The differential interaction between hosts and pathotypes, as reflected by the occurrence of low and high infection types, indicated that these accessions possess genes for vertical resistance to *P. triticina*. This was disappointing in view of the preliminary results reported by Barnard (1999), who found that some of these accessions exhibited a typical non-host reaction. Non-host reactions are usually not associated with necrosis and abortion of infection structures occurs early in the infection process (Heath, 1981b; 1982; Elmhirst and Heath, 1987; Jacobs, 1989a). Furthermore, as basic recognition does not occur between a non-host and pathogen this type of resistance has been assumed to be more durable. Barnard (1999) used UVPrt13 for histological studies and concluded that *T. turgidum* ssp. *dicoccum* var. *arras* exhibited a typical non-host phenotype to the particular isolate used. The present study thus emphasized the importance of working with diverse pathotypes

when phenotyping resistance expression. It was also clear that the genes occurring in these accessions will themselves not confer long-lasting resistance. It could be argued that the genes may be used in appropriate combinations to which UVPrt5, for example, does not have virulence.

Growth of fungal structures can be interrupted at several phases during infection of a host (Niks, 1982). To illustrate this, the relative percentages of PE, AP, EA and colony formation are summarised in Figs. 6 and 10. To show differences between species and the pathotypes of *P. triticina* used, a Tukey-Kramer multiple-comparison table was composed (Table 11). In general the accessions responded similarly for PE, but differences in AP, EA and colony formation were observed. All the species had significantly higher proportions of EA than Morocco whereas for PE, AP and colony formation differences were not always significant.

In all *T. turgidum* accessions PE did not seem to play a noteworthy role in resistance since the fraction of this component was low in relation to others. This is in accordance with studies done by Niks (1981; 1982) who found no evidence to suggest that spore germination, appressorium formation, and/or stoma penetration are affected by resistance of partially resistant barley (*Hordeum vulgare*) genotypes. In the wheat leaf rust interaction, Jacobs (1989a) reported that urediospore germination and appressorium formation were not related to the presence of resistance genes. In the present study there was also no significant difference between PE observed on resistant *T. turgidum* species and leaf rust-susceptible Morocco.

The percentage infection sites classified as aborted were higher on *T. turgidum* ssp. *dicoccum* var. *arras* and *T. turgidum* ssp. *durum* var. *aestivum*, varying

with pathotype, than on the susceptible control. Most aborted penetration attempts were accounted for by nonpenetrating appressoria rather than collapsed substomatal vesicles. The low incidence of AP in *T. turgidum* ssp. *polonicum* indicated a different host response than in the other accessions. Prepenetration exclusion was also reported for wheat leaf rust on leaf sheaths and penduncles by Romig and Caldwell (1964). However, these results are in contrast with those of Gavinlertvatana and Wilcoxson (1978), Lee and Shaner (1984), Poyntz and Hyde (1987) and Jacobs (1989c) who reported no genotypic effects on wheat leaf rust prior to the formation of the first haustorial mother cells.

EA is characterized by arrested fungal growth just after the formation of the first infection hyphae and haustorium mother cells (Niks, 1982), and is usually associated with a hypersensitive reaction. In Morocco only limited early abortion of infection structures was observed. *T. turgidum* ssp. *durum* var. *murciense, T. turgidum* ssp. *dicoccum* var. *arras* and *T. turgidum* ssp. *durum* var. *aestivum* expressed EA, whereas few infection sites displayed EA in *T. turgidum* ssp. *polonicum.* Host cell necrosis was frequently associated with infection sites, specifically of UVPrt2, in *T. turgidum* ssp. *dicoccum* var. *arras.* Host cell necrosis has also been found in other studies of alien resistance expression. In the bread wheat line KS93U9, which carries a *T. monococcum* gene for leaf rust resistance, the frequent occurrence of HCN at infection sites in both seedling and adult leaves suggested that the resistance transferred from wild species is not different from other existing sources conferring hypersensitive resistance to *P. triticina* (Jacobs *et al.*, 1996).

Abortion of infection structures at such an early stage clearly reduces the number of wheat leaf rust colonies. Resistance, therefore, not only leads to abortion of infection structures but it also seems to be responsible for a reduction of mycelium growth compared to the susceptible control. This results in a delayed formation of sporogenic tissue and postponed sporulation.

Scanning electron microscopy showed that resistance in *T. turgidum* ssp. *dicoccum* var. *arras* was not prehaustorial. Although the technique was suitable for observing fungal structures inside leaf tissue, quantification of components was difficult. More work in controlled infections on specified leaf areas, with a resultant increase in infection sites, is needed before scanning electron microscopy can be used for quantitative studies.

Large, sporulating colonies were frequently encountered in *T. turgidum* ssp. *polonicum* and Morocco. In *T. turgidum* ssp. *dicoccum* var. *arras* colonies were associated with necrosis, which agrees with the commonly observed fleck (;) IT. These colonies were the smallest of all host-pathotype interactions studied and no urediospores were produced. Successful colonies of all pathotypes were established in *T. turgidum* ssp. *durum* var. *murciense* and *T. turgidum* ssp. *durum* var. *aestivum*. These colonies were, however, significantly smaller and fewer than those observed in Morocco and small necrotic areas were infrequently observed. Necrosis could, however, not be quantified in these lines as it was interrupted over areas of colonized host cells. This indicated that necrosis did not play a significant role in leaf rust resistance of these two subspecies of *T. turgidum*. Working with *P. graminis* f.sp. *tritici* on wheat seedlings Brown *et al.* (1966) suggested that the lack of relationship between the growth of the rust colony and the occurrence of

hypersensitive cell death suggested that cell collapse is neither the only nor necessarily the most important factor in resistance in resistant plants. This study clearly showed that different subspecies of *T. turgidum* display adult-plant resistance to certain pathotypes of *P. triticina*. Notwithstanding this resistant phenotype, all species were susceptible to pathotype UVPrt5, indicating that they contain vertical rather than horizontal resistance. Earlier, Johnson and Lupton (1987) had warned that despite the apparent potential of *T. turgidum* as a source of resistance to wheat leaf rust, an alien origin of genes does not imply durability. Assumptions about race-specificity and durability are, however, not possible without research on the interactions between potential resistance donors and different variants of the pathogen. Considering the resistance potential of species related to bread wheat, it is suggested that these studies are continued.

Table 1. Host response and *Puccinia triticina* infection type descriptions

Host response	Infection type[a]		Symptoms
Resistant	0	Low	No uredia or macroscopic sign of infection
Nearly resistant	;	Low	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
Heterogeneous	X	Low	Random distribution of variable-sized uredia
Heterogeneous	Y	Low	Variable-sized uredia, decreasing in size with distance from the leaf tip
Heterogeneous	Z	Low	Variable-sized uredia, decreasing in size with distance from the leaf base
Moderately susceptible	3	High	Medium-sized uredia
Susceptible	4	High	Large uredia without chlorosis or necrosis

[a] According to Roelfs (1988b) and McIntosh *et al.* (1995). Infection types were refined as follows: --, uredia at lower size limit; -, uredia smaller than normal; +, uredia larger than normal; ++, uredia at the upper size limit; C, more chlorosis than normal; and N, more necrosis than expected for the infection type.

Table 2. Infection types determined on flag leaves of *Triticum turgidum* accessions after inoculation with different pathotypes of *Puccinia triticina*

	Infection type[a] to pathotype					
Accessions	**UVPrt2**	**UVPrt3**	**UVPrt5**	**UVPrt9**	**UVPrt13**	**UVPrt17**
Morocco[b]	4	4	4	4	4	4
T. turgidum ssp. dicoccum var. arras	;1C	0;	3+	0;	0;	;
T. turgidum ssp. durum var. murciense	1++	1++	3++	1++	1++	2+
T. turgidum ssp. durum var. aestivum	1++	;1--	3+	1++	;1--C	;
T. turgidum ssp. polonicum	3--	2	3-	2	2	22+

[a]Infection types were scored according to the 0 (resistant) to 4 (susceptible) scale (Roelfs, 1988b; McIntosh *et al.*, 1995). Plus and minus signs indicate variation above or below established pustule sizes.
[b]Leaf rust-susceptible control.

Table 3. Differentiation of resistance components to pathotype UVPrt2 of *Puccinia triticina* in *Triticum turgidum* species as determined by fluorescence microscopy 14 days after inoculation

Resistance component[a]	Accessions[c,d]				
	Morocco[b]	*T. turgidum* ssp. *dicoccum* var. *arras* (104)	*T. turgidum* ssp. *durum* var. *murciense* (125)	*T. turgidum* ssp. *durum* var. *aestivum* (127)	*T. turgidum* ssp. *polonicum* (370)
Prestomatal exclusion (%)					
No appressorium formed	0.17±0.24	1.94±0.01	1.67±2.36	2.25±2.32	0.33±0.46
Nonstomatal appressorium	0	1.94±0.88	1.67±2.36	3.52±2.32	0.32±0.45
Abortive penetration (%)					
Non-penetrating appressorium	1.01±0.48	15.91±3.93	9.681±0.46	15.72±5.43	3.92±0.06
Aborted substomatal vesicle	0	13.99±3.09	16.67±0	19.04±0.38	4.27±1.46
Aborted substomatal vesicle with necrosis	0	0.17±0.24	0	0	0
Early abortion (%)					
Early abortion	0	18.05±10.84	36.31±8.99	32.13±0.13	5.22±1.76
Early abortion with necrosis	0	3.89±1.76	1.66±2.36	1.62±2.28	0.32±0.45
Colony formation (%)					
Colonies	0.33±0.47	0.32±0.45	19.34±.01	15.69±2.6	11.4±3.91
Colonies with necrosis	0	43.63±0.36	0.58±0.82	0.31±0.44	0
Sporulating colonies	97.99±1.88	0	11.04±9.96	9.71±1.28	74.22±5.55
Colonies with sporulation and necrosis	0	0	0	0	0

[a]Relative proportions of microscopic components within each phase of fungal development.
[b]Leaf rust-susceptible control.
[c]*Triticum turgidum* accessions resistant to leaf rust. Numbers refer to the original UFS accession designations.
[d]Means ± standard deviation.

Table 4. Differentiation of resistance components to pathotype UVPrt3 of *Puccinia triticina* in *Triticum turgidum* species as determined by fluorescence microscopy 14 days after inoculation

Resistance component[a]	Accessions[c,d]				
	Morocco[b]	*T. turgidum* ssp. *dicoccum* var. *arras* (104)	*T. turgidum* ssp. *durum* var. *murciense* (125)	*T. turgidum* ssp. *durum* var. *aestivum* (127)	*T. turgidum* ssp. *polonicum* (370)
Prestomatal exclusion (%)					
No appressorium formed	1.33±0.94	1.28±1.81	0.64±0.91	2.26±0.31	0
Nonstomatal appressorium	0	1.27±0.03	0.471±0.33	0.65±0.04	0
Abortive penetration (%)					
Non-penetrating appressorium	0.67±0.94	13.03±0.39	18.36±17.6	11.74±1.67	3.52±0.15
Aborted substomatal vesicle	0	20.72±6.56	8.63±2.51	9.23±4.26	7.78±5.01
Aborted substomatal vesicle with necrosis	0	0.33±0.47	0	0.31±0.43	0
Early abortion (%)					
Early abortion	0	26.69±2.05	31.71±20.2	19.39±2.42	7.695±3.93
Early abortion with necrosis	0	18.72±0.01	1.33±1.89	2.29±0.61	0
Colony formation (%)					
Colonies	0.67±0.94	0	15.29±4	0.65±0.04	43.36±1.85
Colonies with necrosis	0	17.52±4.43	0	52.81±3.48	0
Sporulating colonies	96.33±1.41	0	20.72±6.82	0.34±0.48	38.46±1.78
Colonies with sporulation and necrosis	0	0	0	0.31±0.43	0

[a]Relative proportions of microscopic components within each phase of fungal development.
[b]Leaf rust-susceptible control.
[c]*Triticum turgidum* accessions resistant to leaf rust. Numbers refer to the original UFS accession designations.
[d]Means ± standard deviation.

Table 5. Differentiation of resistance components to pathotype UVPrt9 of *Puccinia triticina* in *Triticum turgidum* species as determined by fluorescence microscopy 14 days after inoculation

	Accessions[c,d]				
Resistant component[a]	**Morocco[b]**	***T. turgidum* ssp. *dicoccum* var. *arras* (104)**	***T. turgidum* ssp. *durum* var. *murciense* (125)**	***T. turgidum* ssp. *durum* var. *aestivum* (127)**	***T. turgidum* ssp. *polonicum* (370)**
Prestomatal exclusion (%)					
No appressorium formed	0.67±0.94	0.97±0.45	0	4.91±4.21	4.64±2.84
Nonstomatal appressorium	0	1.98±0.91	1.76±2.49	2.61±0.95	0.98±1.39
Abortive penetration (%)					
Non-penetrating appressorium	0	29.15±2.34	14.31±5.15	27.67±3.76	2.28±0.43
Aborted substomatal vesicle	0	20.18±2.78	13.38±0.08	12.65±6.73	0.64±0.91
Aborted substomatal vesicle with necrosis	0	1.95±2.75	0	0	0
Early abortion (%)					
Early abortion	0.34±0.47	34.99±1.36	55.3±3.73	23.47±3.08	1.64±2.32
Early abortion with necrosis	0	3.9±2.76	1±1.41	1.93±1.81	2.61±1.4
Colony formation (%)					
Colonies	0	3.25±3.68	12.23±7.21	6.22±5.14	4.61±1.82
Colonies with necrosis	0	3.93±3.71	0	13.67±0.73	0
Sporulating colonies	99±0.47	0	2±2.82	3.28±3.73	82.88±1.69
Colonies with sporulation and necrosis	0	0	0	3.55±4.1	0

[a]Relative proportions of microscopic components within each phase of fungal development.
[b]Leaf rust-susceptible control.
[c]*Triticum turgidum* accessions resistant to leaf rust. Numbers refer to the original UFS accession designations.
[d]Means ± standard deviation.

Table 6. Differentiation of resistance components to pathotype UVPrt13 of *Puccinia triticina* in *Triticum turgidum* species as determined by fluorescence microscopy 14 days after inoculation

Resistance component[a]	Accessions[c,d]				
	Morocco[b]	*T. turgidum* ssp. *dicoccum* var. *arras* (104)	*T. turgidum* ssp. *durum* var. *murciense* (125)	*T. turgidum* ssp. *durum* var. *aestivum* (127)	*T. turgidum* ssp. *polonicum* (370)
Prestomatal exclusion (%)					
No appressorium formed	1.67±0.47	0	0.33±0.47	9.84±7.41	1±1.41
Nonstomatal appressorium	0	2.9±1.47	0.34±0.47	1.98±0	0
Abortive penetration (%)					
Non-penetrating appressorium	0.67±0	19.18±6.4	16.29±5.24	20.28±1.48	11.26±1.05
Aborted substomatal vesicle	0	22.95±8.26	6.31±0.49	6.92±0.47	1.98±0.02
Aborted substomatal vesicle with necrosis	0	0	0	1.28±0.89	0
Early abortion (%)					
Early abortion	0	37.45±1.88	41.129±13.85	18.88±16.52	9.97±8.52
Early abortion with necrosis	0	2.62±0.94	0	1.65±2.33	4.29±0.41
Colony formation (%)					
Colonies	0	0	21.26±0.1	16.08±6.81	53.94±12.183
Colonies with necrosis	0	1.31±0.95	0.33±0.47	14.8±7.09	0
Sporulating colonies	97.66±0.47	0	11.66±2.35	1.32±0	17.53±1.59
Colonies with sporulation and necrosis	0	0	0	0	0

[a]Relative proportions of microscopic components within each phase of fungal development.
[b]Leaf rust-susceptible control.
[c]*Triticum turgidum* accessions resistant to leaf rust. Numbers refer to the original UFS accession designations.
[d]Means ± standard deviation.

Table 7. Dimensions of colonies and necrotic and sporulating areas produced by pathotype UVPrt2 of *Puccinia triticina* observed 14 d.p.i. on flag leaves of *Triticum turgidum* accessions

	Accessions[b]				
Colony description	**Morocco[a]**	***Triticum turgidum* ssp. *dicoccum* var. *arras* (104)**	***Triticum turgidum* ssp. *durum* var. *murciense* (125)**	***Triticum turgidum* ssp. *durum* var. *aestivum* (127)**	***Triticum turgidum* ssp. *polonicum* (370)**
Colony size (mm^2)	0.82±0.25	0.08±0.05	0.26±0.18	0.38±0.22	0.56±0.21
Necrotic area (mm^2)	0	0.05±0.03	0	0	0
Sporulation area (mm^2)	0.25±0.162	0	0	0	0.09±0.05

[a]Leaf rust-susceptible control.
[b]*Triticum turgidum* accessions resistant to leaf rust. Numbers refer to the original UFS accession designations.

Table 8. Dimensions of colonies and necrotic and sporulating areas produced by pathotype UVPrt3 of *Puccinia triticina* observed 14 d.p.i. on flag leaves of *Triticum turgidum* accessions

	Accessions[b]				
Colony description	**Morocco[a]**	***Triticum turgidum* ssp. *dicoccum* var. *arras* (104)**	***Triticum turgidum* ssp. *durum* var. *murciense* (125)**	***Triticum turgidum* ssp. *durum* var. *aestivum* (127)**	***Triticum turgidum* ssp. *polonicum* (370)**
Colony size (mm^2)	0.85±0.24	0.04±0.06	0.22±0.14	0.263±0.146	0.40±0.19
Necrotic area (mm^2)	0	0.03±0.04	0	0	0
Sporulation area (mm^2)	0.30±0.11	0	0	0	0.08±0.04

[a]Leaf rust-susceptible control.
[b]*Triticum turgidum* accessions resistant to leaf rust. Numbers refer to the original UFS accession designations.

Table 9. Dimensions of colonies and necrotic and sporulating areas produced by pathotype UVPrt9 of *Puccinia triticina* observed 14 d.p.i. on flag leaves of *Triticum turgidum* accessions

	Accessions[b]				
Colony description	**Morocco[a]**	***Triticum turgidum* ssp. *dicoccum* var. *arras* (104)**	***Triticum turgidum* ssp. *durum* var. *murciense* (125)**	***Triticum turgidum* ssp. *durum* var. *aestivum* (127)**	***Triticum turgidum* ssp. *polonicum* (370)**
Colony size (mm^2)	0.83±0.16	0.02±0.01	0.14±0.11	0.21±0.12	0.56±0.29
Necrotic area (mm^2)	0	0.015±0.007	0	0	0
Sporulation area (mm^2)	0.30±0.10	0	0	0.06±0.04	0.09±0.05

[a]Leaf rust-susceptible control.
[b]*Triticum turgidum* accessions resistant to leaf rust. Numbers refer to the original UFS accession designations.

Table 10. Dimensions of colonies and necrotic and sporulating areas produced by pathotype UVPrt13 of *Puccinia triticina* observed 14 d.p.i. on flag leaves of *Triticum turgidum* accessions

	Accessions[b]				
Colony description	**Morocco[a]**	***Triticum turgidum* ssp. *dicoccum* var. *arras* (104)**	***Triticum turgidum* ssp. *durum* var. *murciense* (125)**	***Triticum turgidum* ssp. *durum* var. *aestivum* (127)**	***Triticum turgidum* ssp. *polonicum* (370)**
Colony size (mm^2)	0.71±0.21	0.01±0.006	0.19±0.05	0.18±0.10	0.58±0.24
Necrotic area (mm^2)	0	0.009±0.005	0	0	0
Sporulation area (mm^2)	0.21±0.13	0	0	0	0.15±0.09

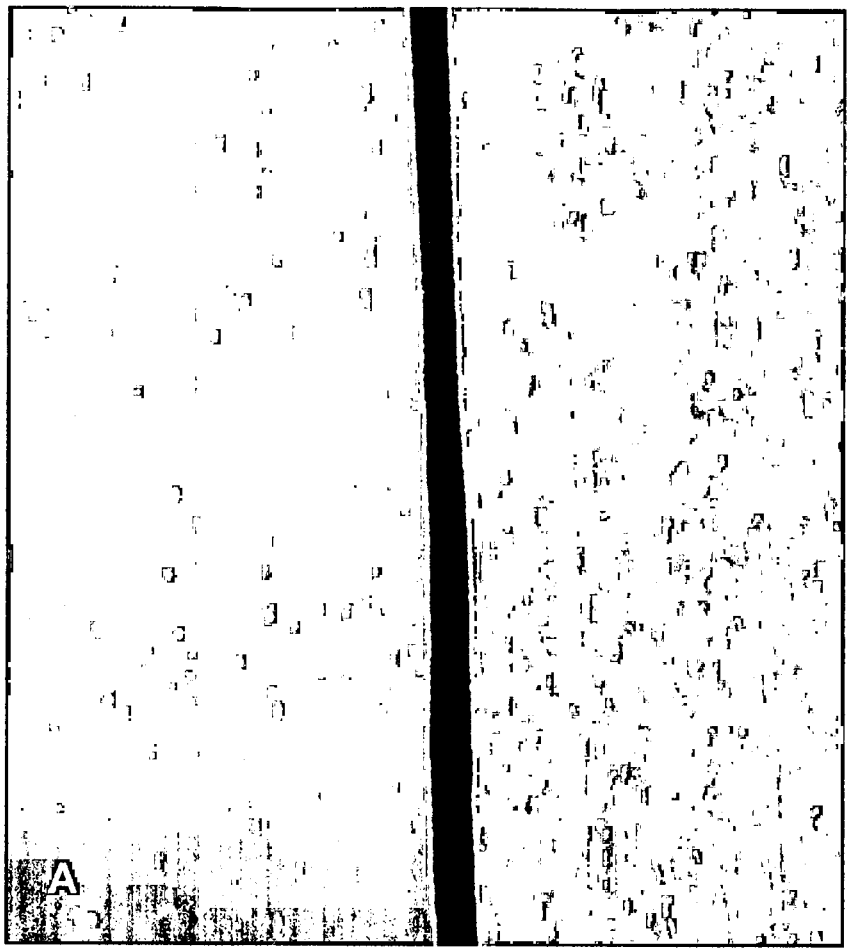
[a]Leaf rust-susceptible control.
[b]*Triticum turgidum* accessions resistant to leaf rust. Numbers refer to the original UFS accession designations.

Table 11. Tukey-Kramer test values for comparison of *T. turgidum* accessions and Morocco within each stage of fungal development. Values followed by different letters are significantly different at $p < 0.05$

Prestomatal exclusion			
	Morocco	1.25	a
	T. turgidum ssp. *dicoccum* var. *arras* (104)	3.00	ab
	T. turgidum ssp. *durum* var. *murciense* (125)	1.68	a
	T. turgidum ssp. *durum* var. *aestivum* (127)	6.98	b
	T. turgidum ssp. *polonicum* (370)	2.23	a
Abortive Penetration			
	Morocco	0.83	a
	T. turgidum ssp. *dicoccum* var. *arras* (104)	39.56	b
	T. turgidum ssp. *durum* var. *murciense* (125)	26.97	b
	T. turgidum ssp. *durum* var. *aestivum* (127)	32.92	b
	T. turgidum ssp. *polonicum* (370)	8.06	a
Early abortion			
	Morocco	0.0083	a
	T. turgidum ssp. *dicoccum* var. *arras* (104)	39.96	b
	T. turgidum ssp. *durum* var. *murciense* (125)	42.11	b
	T. turgidum ssp. *durum* var. *aestivum* (127)	25.33	bc
	T. turgidum ssp. *polonicum* (370)	7.58	c
Colony formation			
	Morocco	97.83	a
	T. turgidum ssp. *dicoccum* var. *arras* (104)	17.47	b
	T. turgidum ssp. *durum* var. *murciense* (125)	29.22	b
	T. turgidum ssp. *durum* var. *aestivum* (127)	34.77	b
	T. turgidum ssp. *polonicum* (370)	82.13	a

Figure 1. Reactions observed on *Triticum turgidum* ssp. *dicoccum* var. *arras* (A) flag leaves to pathotypes UVPrt17 (left) and UVPrt5 (right) of *Puccinia triticina*.

Figure 2. Reactions observed on *Triticum turgidum* ssp. *durum* var. *murciense* (B) flag leaves to UVPrt17 (left) and UVPrt5 (right) of *Puccinia triticina*.


A

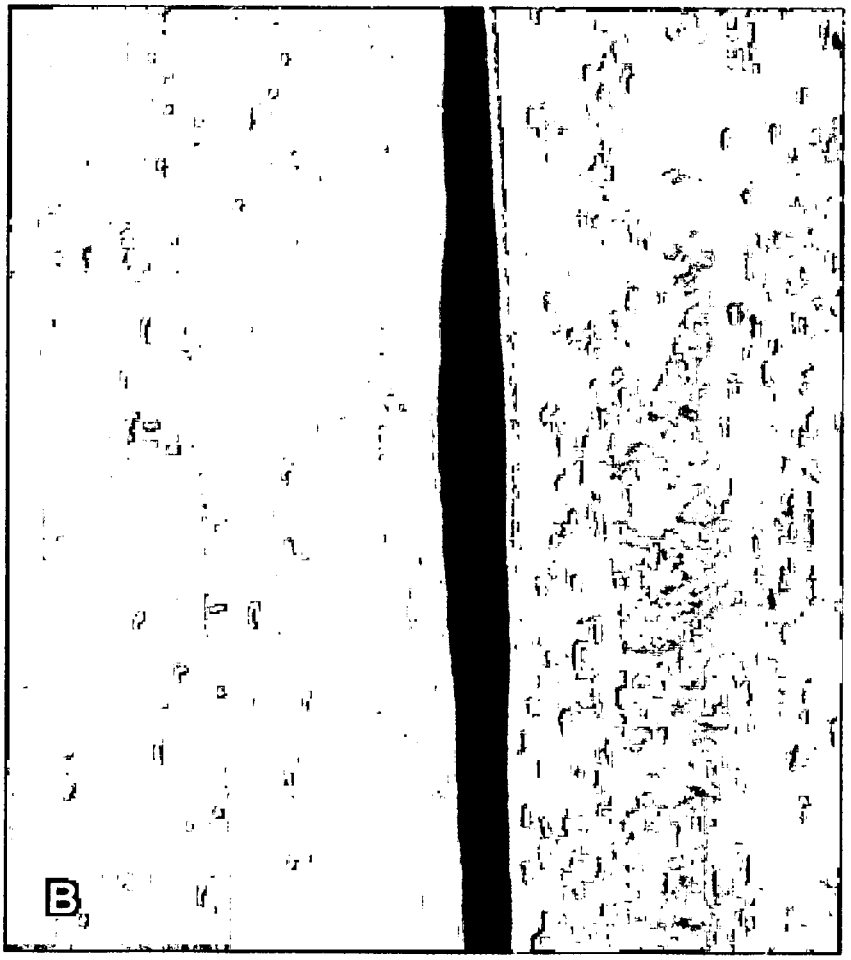

B

Figure 3. Reactions observed on *Triticum turgidum* ssp. *durum* var. *aestivum* (A) flag leaves to UVPrt17 (left) and UVPrt5 (right) of *Puccinia triticina*.

Figure 4. Reactions observed on *Triticum turgidum* ssp. *polonicum* (B) flag leaves to UVPrt17 (left) and UVPrt5 (right) of *Puccinia triticina*.

B

A

Figure 5. Non-stomatal appressorium of pathotype UVPrt2 of *Puccinia triticina* observed with fluorescence microscopy on a flag leaf of *Triticum turgidum* ssp. *durum* var. *aestivum*, 14 d.p.i.

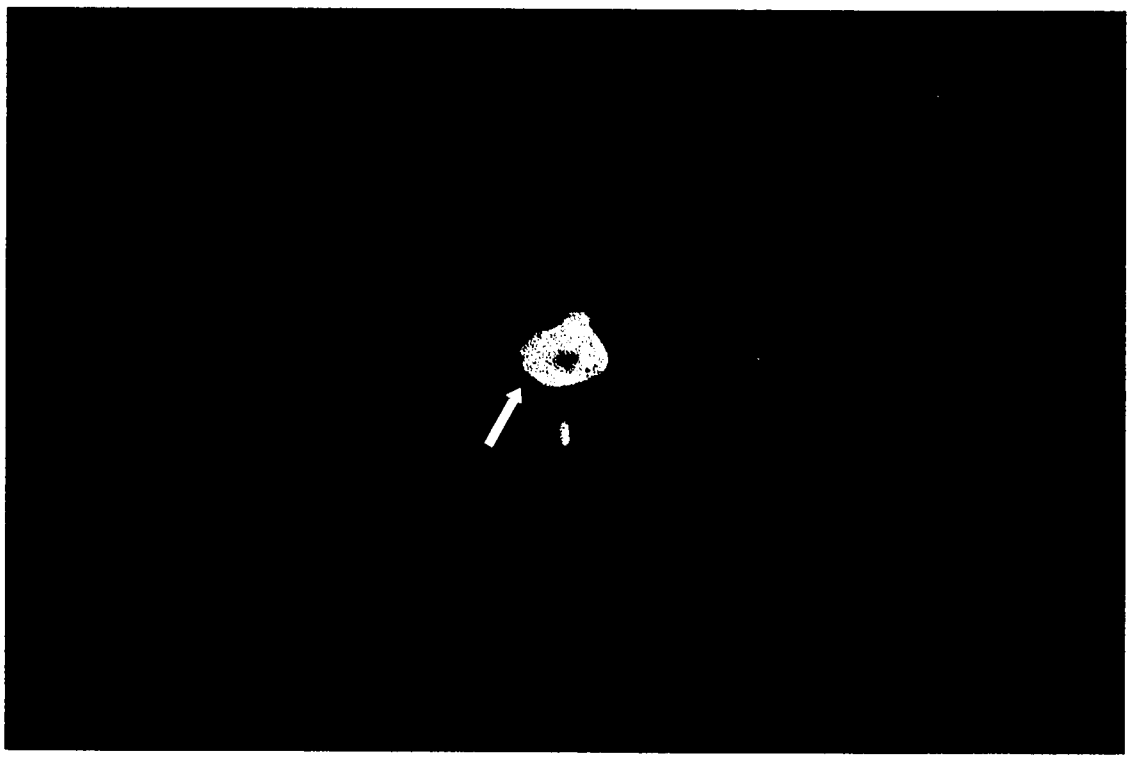


Figure 6. The percentage of all infection sites studied showing prestomatal exclusion and abortive penetration of *Puccinia triticina* in Morocco (control) and *Triticum turgidum* ssp. *dicoccum* var. *arras* (104), *T. turgidum* ssp. *durum* var. *murciense* (125), *T. turgidum* ssp. *dicoccum* var. *aestivum* (127) and *T. turgidum* ssp. *polonicum* (370) when inoculated with pathotypes UVPrt2, 3, 9 and 13.

Error bars represent standard deviations.

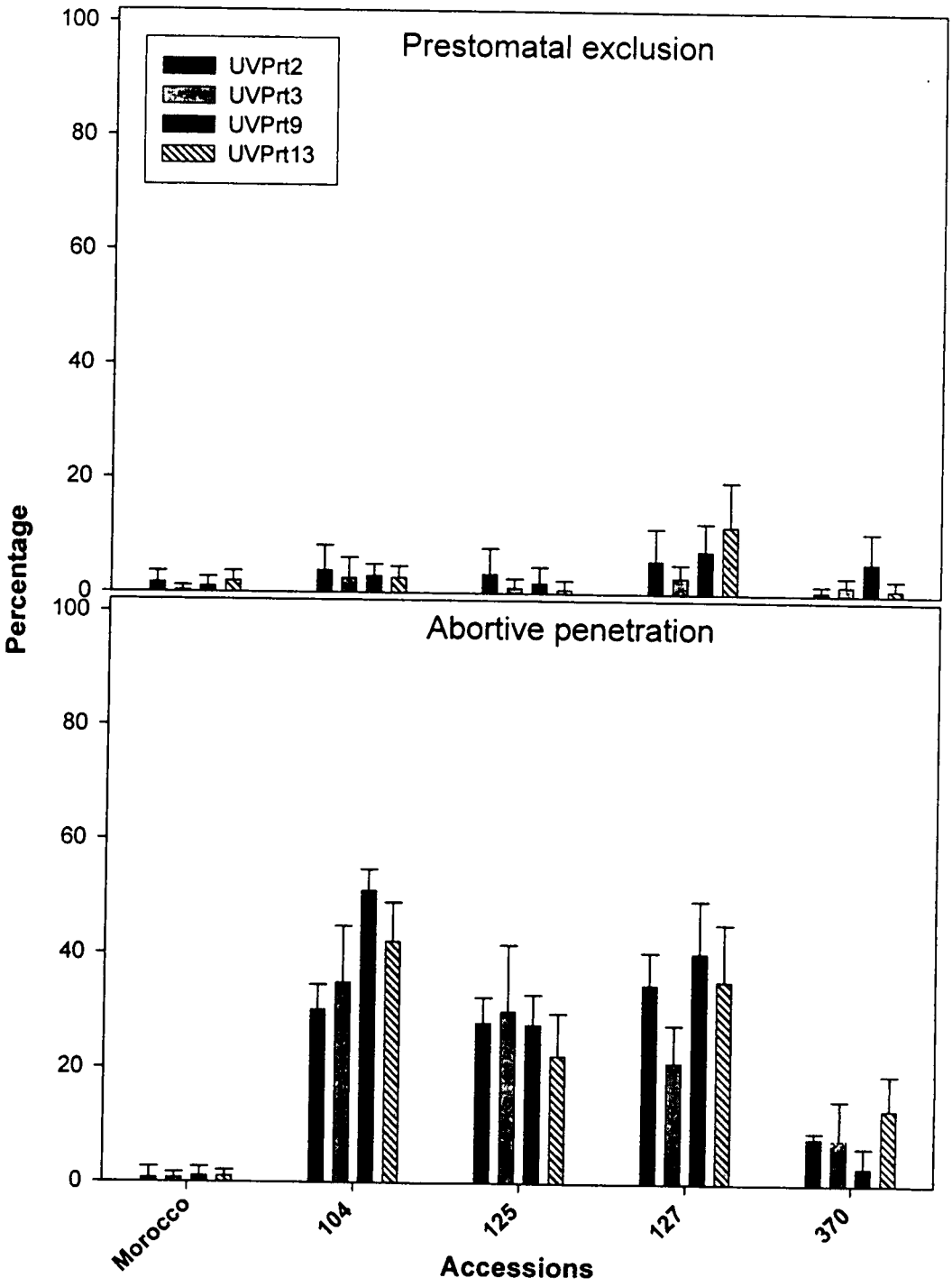
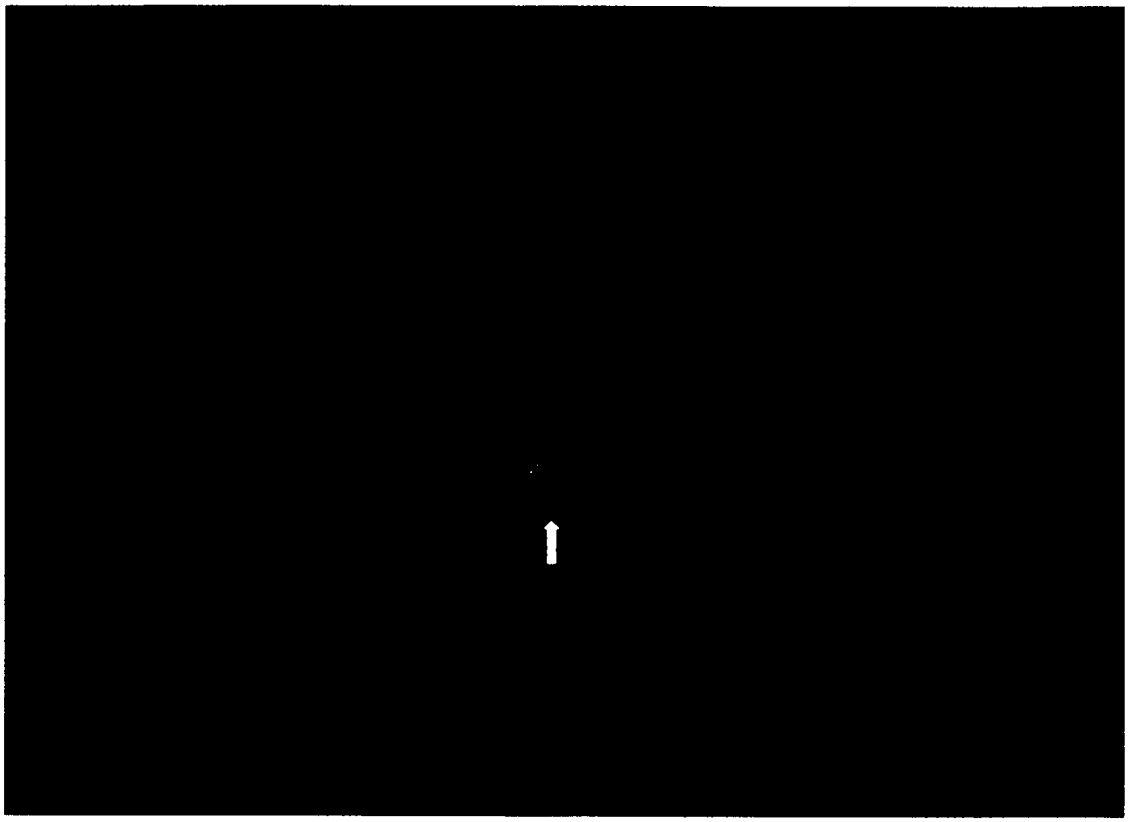

Prestomatal exclusion
UVPrt2
UVPrt3
UVPrt9
UVPrt13
Abortive penetration
Percentage
100
80
60
40
20
0
Morocco
104
125
127
370
Accessions

Figure 7. Non-penetrating appressorium of pathotype UVPrt9 of *Puccinia triticina* observed with fluorescence microscopy on a flag leaf of *Triticum turgidum* ssp. *dicoccum* var. *arras*, 14 d.p.i.

Figure 8. Aborted sub-stomatal vesicle of pathotype UVPrt9 of *Puccinia triticina* observed with fluorescence microscopy in a flag leaf of *Triticum turgidum* ssp. *dicoccum* var. *arras*, 14 d.p.i.



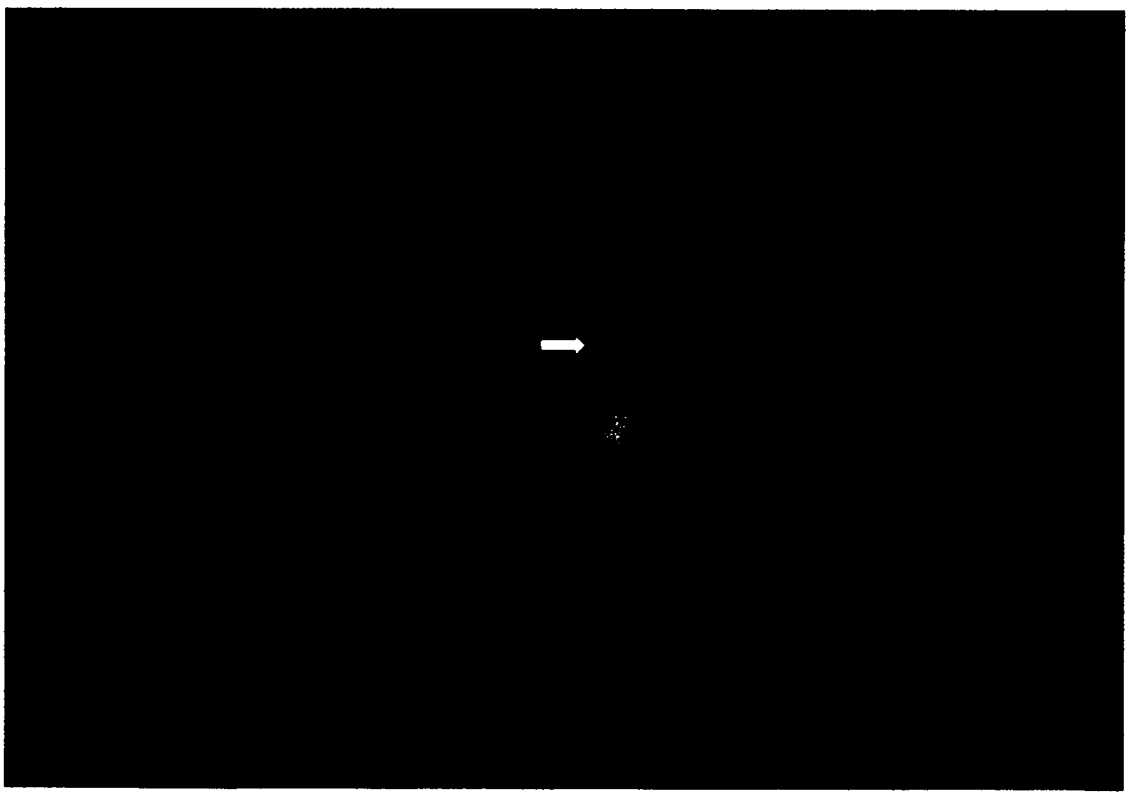


Figure 9. Early abortion of wheat leaf rust infection structures (pathotype UVPrt3) observed with fluorescence microscopy in a flag leaf of *Triticum turgidum* ssp. *dicoccum* var. *arras*.

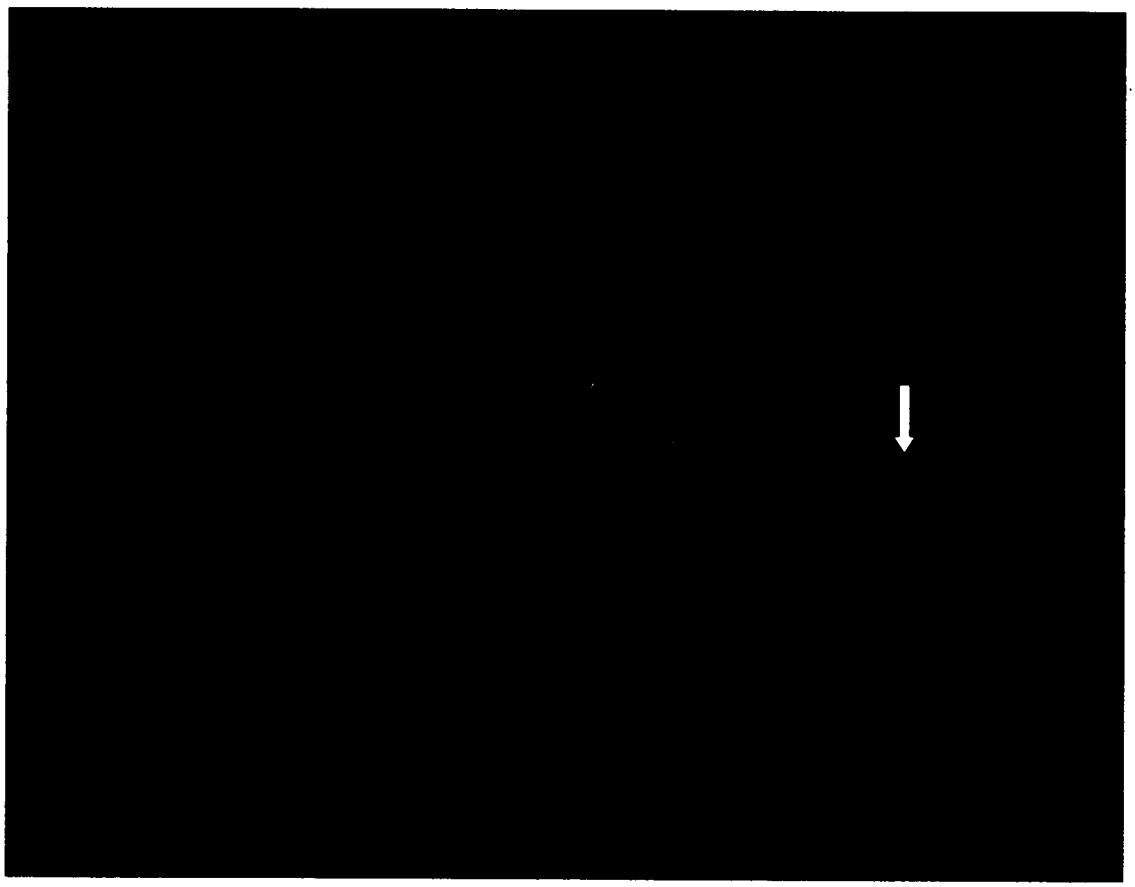


Figure 10. The percentage of all infection sites studied showing early abortion and colony formation of *Puccinia triticina* in Morocco (control) and *Triticum turgidum* ssp. *dicoccum* var. *arras* (104), *T. turgidum* ssp. *durum* var. *murciense* (125), *T. turgidum* ssp. *dicoccum* var. *aestivum* (127) and *T. turgidum* ssp. *polonicum* (370) when inoculated with pathotypes UVPrt2, 3, 9 and 13.

Error bars represent standard deviations.

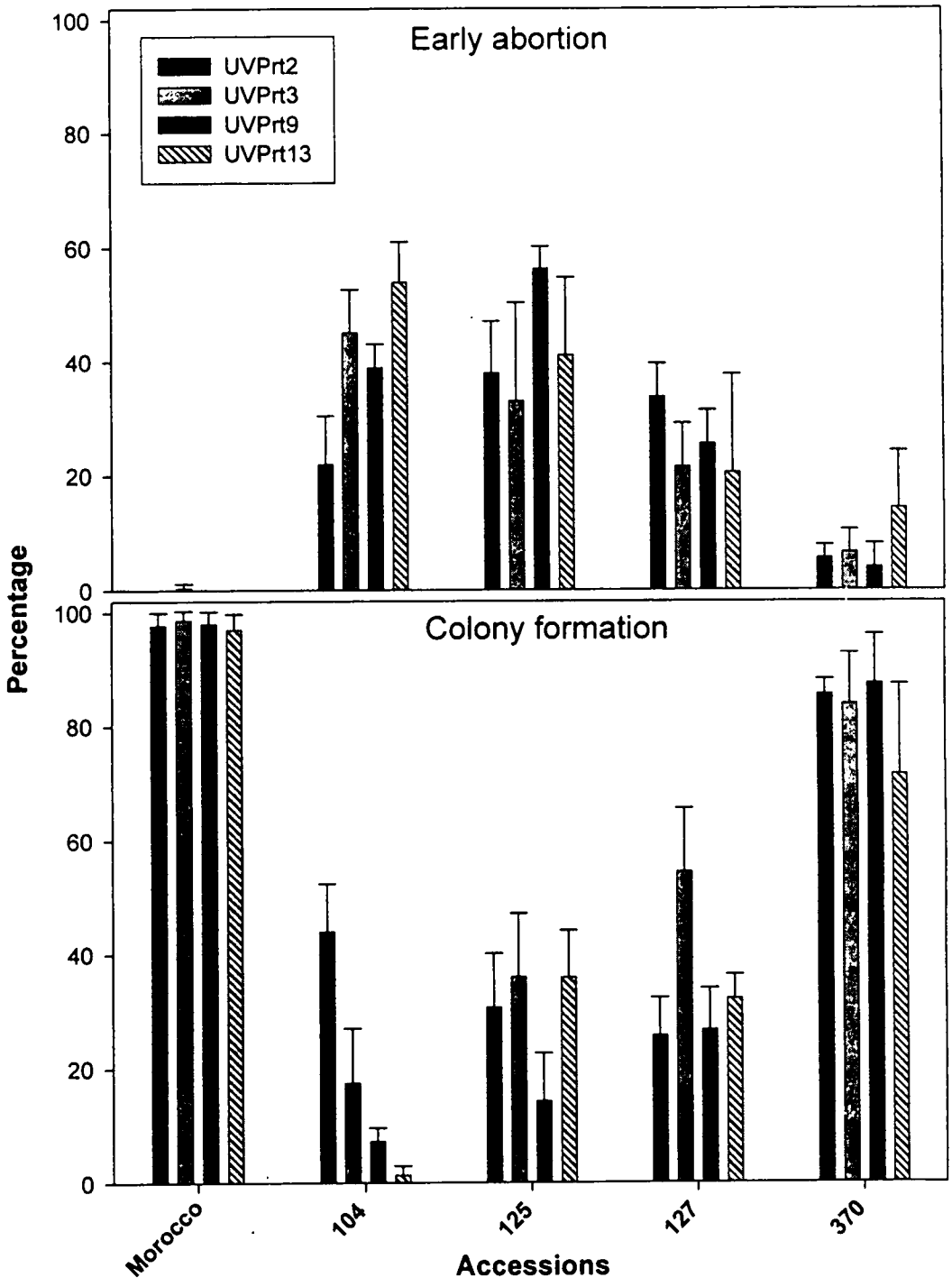
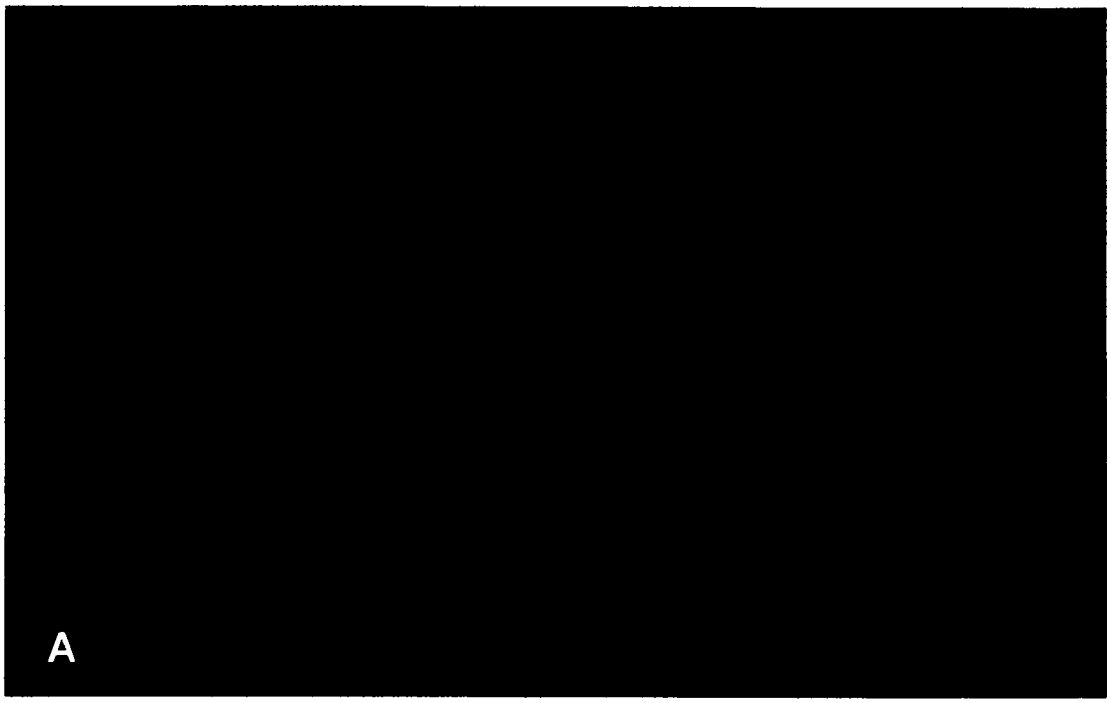

Early abortion
UVPrt2
UVPrt3
UVPrt9
UVPrt13
100
80
60
40
20
0
Colony formation
100
80
60
40
20
0
Percentage
Morocco
104
125
127
370
Accessions

Figure 11. Colony (A) of pathotype UVPrt9 of *Puccinia triticina* and associated host cell necrosis (B) in a flag leaf of *Triticum turgidum* ssp. *dicoccum* var. *arras,* 14 d.p.i.


A

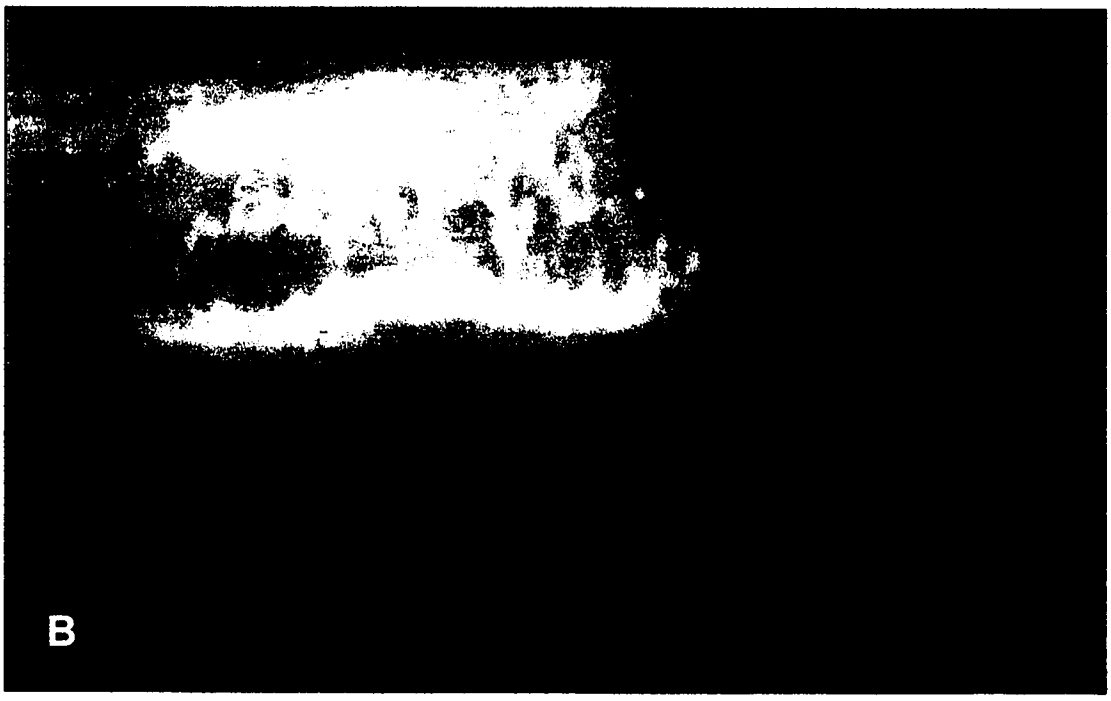
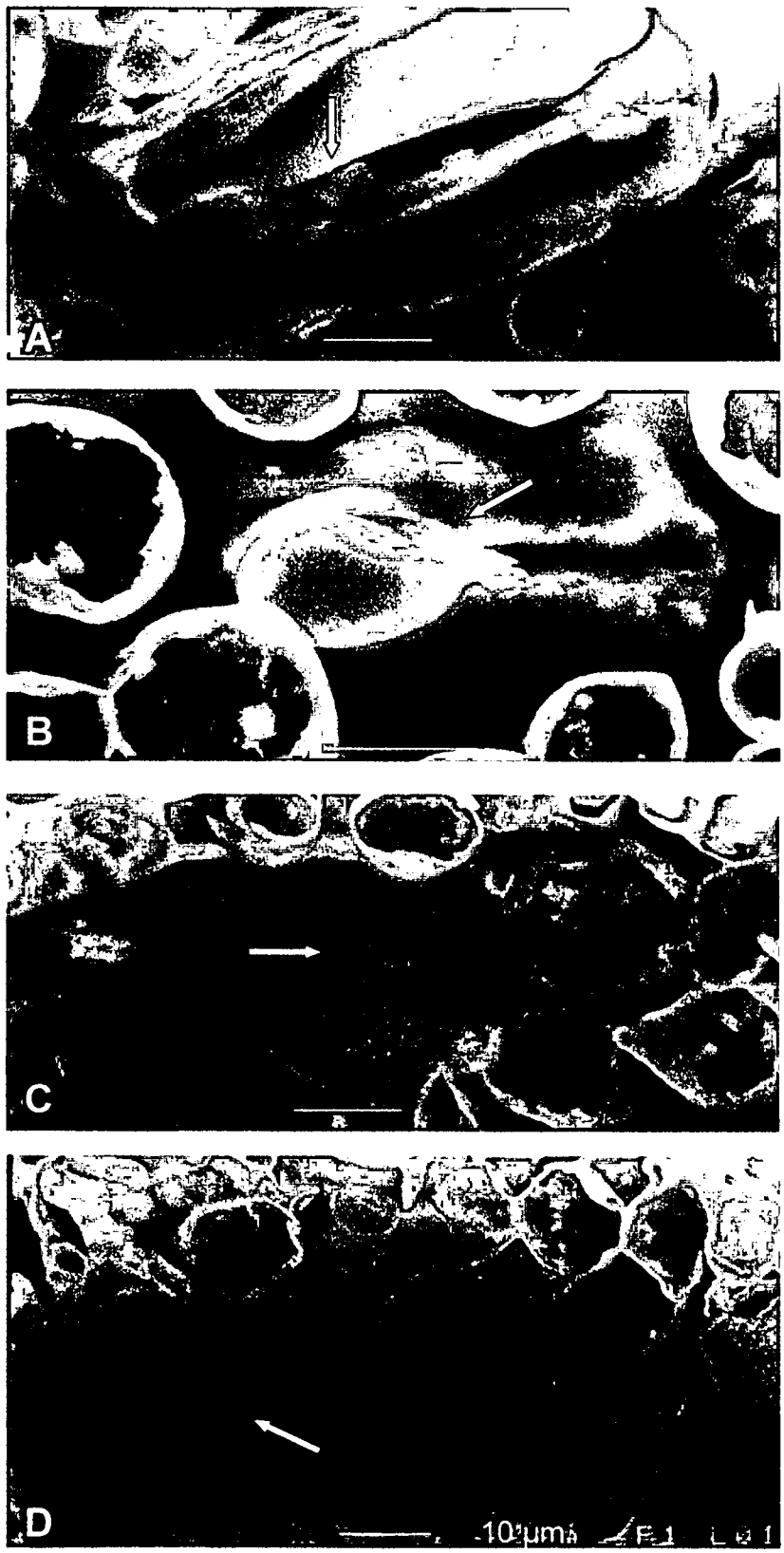

B

Figure 12. Fungal infection structures observed inside flag leaves of *Triticum turgidum* ssp. *dicoccum* var. *arras* using scanning electron microscopy. (A) Substomatal vesicle initial (SSVI), (B) substomatal vesicle (SSV), (C) collapsed substomatal vesicle (CSSV) and (D) hyphae. Scale bar represents 10 µm.


A
B
C
D
10µm

CHAPTER 3

INTROGRESSION OF LEAF RUST RESISTANCE GENES FROM TETRAPLOID *TRITICUM TURGIDUM* SPECIES INTO HEXAPLOID WHEAT

ABSTRACT

Four accessions of *Triticum turgidum* that previously displayed high levels of adult-plant resistance to certain pathotypes of *Puccinia triticina* were crossed with a leaf rust-susceptible bread wheat cultivar, SST55. Following successful hybridisation, the F_1 of all crosses was susceptible as adult plants to pathotype UVPrt9 of *P. triticina*. A decrease in pollen viability of F_1 plants was also observed. The F_2 population segregated into resistant and susceptible plants. Progeny of the *T. turgidum* ssp. *dicoccum* var. *arras* and *T. turgidum* var. *aestivum* crosses with SST55 segregated according to a 7:9 Mendelian ratio, suggesting two recessive genes for adult plant resistance to *P. triticina*. The among F_3 family segregation for *T. turgidum* ssp. *dicoccum* var. *arras* supported the F_2 data obtained and reinforced the hypothesis of two genes for resistance. *T. turgidum* ssp. *polonicum* progeny deviated significantly from the proposed 7:9 and 1:3 segregation ratios, but the population size was too small to form any valid assumption of inheritance. *T. turgidum* ssp. *durum* var. *murciense* showed a 1:3 Mendelian segregation pattern which was indicative of a single recessive resistance gene.

INTRODUCTION

Cultivated wheat is more sensitive to pathogens due to its inbred nature (Joshi and Nguyen, 1993; Jiang *et al.*, 1994) and requires continued improvement of disease

resistance. Most single genes for resistance have been overcome by pathotypes with new virulence characteristics (Knott and Dvořàk, 1976; Antonov and Marais, 1996). This ability of pathogens to continuously overcome existing resistance in cultivars necessitates the search for novel resistance genes to broaden the genetic base of resistance in wheat to rust diseases. Several species related to common wheat are known to have resistance to leaf rust (Knott and Dvořàk, 1976; Gill *et al.*, 1986; Valkoun *et al.*, 1986; Cox *et al.*, 1992; Valkoun and Mamluk, 1993; Dyck and Bartoš, 1994). It is hoped that resistance genes from wild species might add to the genetic diversity available in efforts to stay ahead of changing rust pathogens (McIntosh *et al.*, 1995).

Durability is usually obtained by combining several effective single resistance genes in a cultivar, or by using polygenic resistance. Success with introgression of resistance genes from wild species into cultivated wheat depends on the genetic distance between them, as well as the development of plant transformation technology, and gene cloning (McIntosh *et al.*, 1995). Species from the primary gene pool are usually sexually compatible (McIntosh, 1991). It was, however, noted that the transfer of resistance genes from plants with a lower ploidy level to a plant with a higher ploidy level might be complicated by interactions between resistance genes and suppressor genes in the different genomes (Lin and Kuo, 1995). It was also noted that expression of resistance could often be reduced when genes are transferred to a new species (Knott and Dvořàk, 1976).

The objective of this study was to determine if adult plant resistance in certain *Triticum turgidum* accessions is expressed when incorporated in a susceptible

hexaploid wheat species, and if resistance was inherited according to Mendelian ratios.

MATERIALS AND METHODS

Genotypes When using alien germplasm in breeding for disease resistance, species related to the crop are screened for possible resistance genes (Knott and Dvořàk, 1976). Accessions containing presumed new resistance genes are crossed with a susceptible genotype to determine the number of genes conferring resistance to a particular pathotype (Roelfs *et al.*, 1992).

In the present study four subspecies of *Triticum turgidum* (2n=4x=AABB), viz. *T. turgidum* ssp. *dicoccum* var. *arras*, *T. turgidum* ssp. *durum* var. *murciense*, *T. turgidum* ssp. *durum* var. *aestivum* and *T. turgidum* ssp. *polonicum* were selected on the basis of their adult plant resistance expressed in a study by Barnard (1999).

Inoculation Several pathotypes of *P. triticina* were used throughout this investigation. Prior to inoculation, the pathotypes were multiplied on seedlings of selected susceptible hosts (Table 1). Emerging seedlings were drenched with 50 ml per 10 cm plastic pot of a 0.3 g/L maleic hydrazide solution to retard plant growth and enhance sporulation (Knott, 1989). When primary leaves were fully unfolded, seedlings were inoculated with urediospores from the different rust pathotypes suspended in a light mineral oil (McSherry and Harris, Wedmore, Somerset, UK). The seedlings were subsequently kept in a dew-simulation chamber at 18 to 21 °C for 16 h. Seedlings were then placed at 18 to 25 °C in a glasshouse where pathotypes were kept apart in 40 x 30 x 30 cm isolation compartments.

Seedling tests To determine the expression of seedling resistance in these accessions, primary leaf infection types to the predominant South African pathotypes of *P. triticina* were determined. SST55 (2n=6x=AABBDD) was included as a hexaploid bread wheat control. Seedlings were grown in 300 ml pots containing sterilised soil-peat moss mixture (1:1 v/v). The seedlings were spray-inoculated with freshly harvested urediospores of UVPrt2, UVPrt3, UVPrt9 and UVPrt13 of *P. triticina* suspended in a light mineral oil and subsequently incubated in a dew-simulation chamber at 18 to 21 °C for 16 h. After this period the infected seedlings were allowed to dry before they were transferred to the glasshouse where they were kept at 18 to 25 °C. An assessment of leaf rust reactions was made after 10 days using the 0 to 4 infection type scale of Roelfs (1988b).

Adult plant tests Flag leaves of adult plants, grown in 1 liter-capacity pots in a glasshouse at 18 to 25 °C, were inoculated with freshly harvested urediospores of the above named pathotypes. Each pathotype was suspended separately in distilled water containing Tween 20® and sprayed onto the upper surface of leaves. The plants were subsequently incubated at 19 to 22 °C for 16 h in a dew chamber. Upon removal from the dew chamber, the plants were allowed to dry for 2 h before being returned to the glasshouse. The plants were maintained in conditions similar to those described for the pre-inoculation period.

Wheat x *T. turgidum* crosses Information on the growth period of the *T. turgidum* lines was obtained from Barnard (1999). To synchronize flowering dates of species and SST55, the bread wheat parent, different plantings were made at regular intervals over an 8 week period. Seeds were planted in 1 litre pots. Approximately seven days after seedling emergence a solution of 50 ml per pot of a 3 g/L hydroponic nutrient solution (6.5:2.7:13 N:P:K plus micro-elements) was administered to plants. Fertilization continued for three days a week for the remainder of the experiment.

When spikes were fully emerged from the leaf sheath, but prior to anthesis, SST55 plants were emasculated and used as the female parent in crosses with each of the species. Hybridisation was facilitated by inserting pollen shedding spikes of growing plants of the respective male parents alongside those of SST55 in glassine bags (25 x 6.2 cm), three to four days after emasculation. These bags were regularly tapped to increase pollen dissemination to receptive stigmas. Upon maturity the F_1 hybrid seeds were harvested.

F_1 plants were grown to maturity to determine the adult plant leaf rust reaction as well as to obtain F_2 seed. To ensure maximal germination, F_1 seeds were surface-sterilised in 30 % EtOH for 1 min., followed by soaking in a 1:6 sodium hypochlorite and water solution for 1 min., before being washed twice in dH_2O (modified from Baxter and Van der Linde (1999)). Seeds were subsequently germinated on filter paper, drenched with a 1 % H_2O_2 solution in glass petridishes (Fig. 1). These filter paper disks were kept moist with 1 % H_2O_2 solution until germination and transplanting of germlings to 1 litre-capacity pots in the glasshouse. Growing conditions for and fertilization of F_1 plants were as described before.

Flag leaves of F_1 hybrids, including the parents, were inoculated as described above in the adult plants tests with pathotype UVPrt9 of *P. triticina*. This pathotype has been the dominant leaf rust variant in South Africa for several years (Van Niekerk, 2001). Upon completion of the F_1 leaf rust evaluation, plants were grown to maturity and F_2 seeds harvested. To determine the number of genes conferring adult plant resistance, F_2 plants were grown and flag leaves inoculated with UVPrt9 in a similar way. To confirm F_2 reactions, limited progeny tests were also conducted. Resistant F_2 homozygotes, derived from the cross between SST55 and *T. turgidum* ssp. *dicoccum* var. *arras,* were identified and retained for further investigations.

Pollen viability To determine the male fertility of F_1 plants obtained from crosses between tetraploid and hexaploid wheats, pollen were collected from plants where the spike had just emerged. The pollen was placed on a microscope slide covered with Mayers albumin, stained with 0.75 % toluidine blue and viewed (400x) with a Nikon Optiphot epifluorescence microscope. The filter combinations B-2A (excitation filter 450 to 490 nm and barrier filter 520 nm) for autofluorescence observations were used. Viable pollen appeared blue whereas dead pollen fluoresced bright yellow (Fig. 2). Observations were made on approximately 1000 randomly selected pollen grains per accession. SST55 was included as control.

Statistical analysis Standard chi-square analysis was conducted to test the observed segregation ratios for adherence to Mendelian inheritance (Steel and Torrie, 1980). Plants with intermediate reactions were grouped with plants showing a resistant reaction.

RESULTS AND DISCUSSION

T. turgidum ssp. *dicoccum* var. *arras* showed seedling resistance (Fig. 3) to all pathotypes tested. Infection types ranged from a ";" to UVPrt13, to "X-" to UVPrt2. In the seedling stage *T. turgidum* ssp. *durum* var. *aestivum* was resistant to only UVPrt3 and 9 (Fig. 4; Table 2) whereas *T. turgidum* ssp. *durum* var. *murciense* was susceptible to all pathotypes tested. *T. turgidum* ssp. *polonicum* was susceptible to all pathotypes except UVPrt13 to which a mesothetic (mixed) reaction was produced. The *T. turgidum* lines all showed some degree of adult plant resistance when inoculated with pathotypes UVPrt2, 3, 9 and 13 of *P. triticina* (Table 2). The highest level of resistance (infection type range 0; to ;1C) was observed in *T. turgidum* ssp. *dicoccum* var. *arras*. Most other low infection types were in the intermediate category.

To determine if the adult-plant resistance to *P. triticina* is expressed in a hexaploid background crosses were made between the *T. turgidum* parents and a leaf rust susceptible cultivar SST55. Since both *T. turgidum* and SST55 are from the same primary gene pool, hybridisation occurred readily and a sufficient number of F_1 seeds were obtained. The F_1 progeny displayed reduced levels of resistance to UVPrt9 (Figs. 5, 6, 7 and 8) (Table 3). *T. turgidum* ssp. *dicoccum* var. *arras* and *T. turgidum* ssp. *durum* var. *aestivum* displayed IT values of "3++", while T. *turgidum* ssp. *durum* var. *murciense* and *T. turgidum* ssp. *polonicum* had infection type values of "23C". Since all the F_1 plants had a susceptible IT range, it could be assumed that the resistance is either recessive or that the genes were suppressed when the tetraploid parents were crossed with hexaploid wheat. Likewise, Bai and Knott

(1992) observed a similar suppression of resistance in F_1 progeny from crosses between leaf rust resistant accessions of *T. turgidum* var. *dicoccoides* (AABB) and susceptible bread wheat.

The F_1 plants showed signs of a decrease in male fertility when compared to SST55 (Table 4) but this did not influence the ability to give rise to F_2 progeny. Low viability of pollen is expected in wide crosses where euploid or near-euploid pollen (close to n=14 or n=21) will tend to be viable, and the intermediate types tending to be non-viable. Thompson *et al.* (1994) in a study on *Erythronium grandiflorum* also showied that pollen viability tests did not always convey the true competence of the pollen grain.

The F_2 progeny from crosses between SST55 with *T. turgidum* ssp. *dicoccum* var. *arras, T. turgidum* ssp. *durum* var. *murciense, T. turgidum* ssp. *durum* var. *aestivum* and *T. turgidum* ssp. *polonicum* segregated into resistant and susceptible plants (Fig. 9) with IT values ranging from ";" to "3". With respect to Mendelian ratios expected for single gene resistance, a reduced number of leaf rust resistant seedlings occurred in the F_2 progeny. For example, of the 122 seedlings derived from *T. turgidum* ssp. *durum* var. *murciense* x SST55, only seven were resistant, whereas the *T. turgidum* ssp. *dicoccum* var. *arras* and *T. turgidum* ssp. *durum* var. *aestivum* crosses produced five and two resistant plants out of 89 and 53 F_2's, respectively (Table 3). Since *T. turgidum* ssp. *polonicum* did not express clear primary leaf resistance, this cross was not evaluated as seedlings. Chi-square values obtained for *T. turgidum* ssp. *dicoccum* var. *arras* seedlings differed significantly from the proposed 7:9 (based on adult-plant segregation) and 1:3 ratios (Table 3).

In adult plants inoculated with UVPrt9 the resistant progeny of *T. turgidum* ssp. *dicoccum* var. *arras* exhibited ITs of ";" to ";1" whereas the susceptible F_2 plants showed ITs of "3+" to "3++". In the adult F_2 a 7:9 ratio (chi-square$_{7:9}$=0.029) was obtained for *T. turgidum* ssp. *dicoccum* var. *arras,* suggesting that the segregation pattern did not differ significantly from that expected for two recessive resistance genes. In the F_3 population of *T. turgidum* ssp. *dicoccum* var. *arras* three different populations could be identified, namely homozygous resistant, homozygous susceptible and heterozygous families. The among family segregation observed in the F_3 population supported the idea of an digenic resistance (chi-square$_{7:8:1}$=3.066).

T. turgidum ssp. *durum* var. *aestivum* x SST55 plants also segregated into a 7:9 ratio (chi-square$_{7:9}$=0.663), suggesting the occurrence of two recessive genes for adult-plant resistance. Adult *T. turgidum* ssp. *durum* var. *murciense* F_2 plants segregated according to a 1:3 Mendelian ratio (chi-square$_{1:3}$=0.481) indicating the presence of a single recessive resistance gene effective against *P. triticina*. Only 42 *T. turgidum* ssp. *polonicum* F_2 plants were tested which precluded meaningful inheritance data.

Single gene resistance, as expressed in *T. turgidum* ssp. *durum* var. *murciense*, is not considered durable when used alone in a cultivar (Bender and Pretorius, 1997; Nelson *et al.*, 1997), since the pathogen can produce a virulence gene to overcome the resistance in a relatively short period of time (Mohan *et al.*, 1997). This situation has lead to the typical boom and bust cycles where rust-resistant cultivars were produced, with a subsequent increase in popularity and cultivation, only to be discarded as virulent pathotypes appeared. Despite the non-durable nature of single genes, progress in breeding for durable resistance is possible when genes are

combined in cultivars (Pederson, 1988; Van Ginkel and Rajaram, 1993). In certain cases gene combinations also mediated a higher level of resistance than individual genes (Schafer *et al.*, 1963; Sharp *et al.*, 1976; Dyck, 1977; Samborski and Dyck, 1982).

Although F_1 and F_2 data suggested Mendelian inheritance of resistance, these ratios should be interpreted with caution. It is wise to test further generations to confirm segregation data, since several factors can influence segregation in early generations. For example, in F_1 plants the single D-genome (coming from the SST55 hexaploid) may get involved in the formation of trivalents resulting in irregular segregation. Additionally, many gametes will be aborted due to the variation in chromosome numbers, thus the effect will be particularly strong in the pollen. It is difficult to foresee if and how this may impact on the transmission of resistance. Another factor to consider is that in the F_1, gametes carrying the species-derived chromosomes may not occur at random due to reduced fitness or the presence of segregation distortion mechanisms. Finally, F_1 spikes were not bagged suggesting that some cross pollination may have occurred in the presence of male-sterility.

From the wider array of pathotypes used in Chapter 2, it was clear that pathotypes virulent to these genes already exist. However, by isolating the genes and using them in complex resistance backgrounds, for example containing the durable *Lr34* gene, these sources may still be useful. Further work is required to develop stable hexaploid lines resistant to leaf rust, to intercross them in allelism tests, and to determine possible linkages to other rust resistances. In the present South African situation linkage to *P. striiformis* resistance is especially sought after. One season of field testing showed that all four species have resistance to stripe

rust. Flag leaf severity varied between "trace resistant" for *T. turgidum* ssp. *dicoccum* var. *arras* and 30MR (moderately resistant) for *T. turgidum* ssp. *durum* var. *aestivum* (Pretorius, unpublished data). Whether this stripe rust resistance was retained in the lines with adult-plant leaf rust resistance needs to be verified.

From working with a limited number of accessions high levels of adult-plant resistance, although not necessarily durable, were detected in *Triticum turgidum*. It could be worthwhile to exploit other species expressing resistance, in particular to the UVPrt5 pathotype.

Table 1. *Puccinia triticina* pathotypes used for inoculation and selective hosts on which they were increased

Pathotype	Leaf rust resistance (*Lr*) genes[a]	Selective hosts[b]
UVPrt2	*Lr1,2a,2b,3ka,11,15,17,20,24,26,30/2c,3a,3bg,10,14a,16*	Zaragosa
UVPrt3	*Lr3a,3bg,3ka,10,11,14a,16,17,20,26,30/1,2a,2b,2c,15,24*	Agent
UVPrt9	*Lr2a,2b,3bg,15,16,17,26,30/1,2a,2b,2c,10,14a,15,17,24*	Karee
UVPrt13	*Lr3a,3bg,3ka,11,16,20,30/1,2a,2b,2c,10,14a,15,17,24,26*	RL6078

[a]South African leaf rust differentiating genes.
[b]Selective hosts chosen for the increase of respective pathotypes.

Table 2. Adult and seedling infection types[a] of *Triticum turgidum* accessions and SST55 to selected pathotypes of *Puccinia triticina*

Accessions[b]	Leaf rust pathotypes			
	UVPrt2	UVPrt3	UVPrt9	UVPrt13
Seedlings				
T. turgidum ssp. *dicoccum* var. *arras*	X-	;1	;1--n	;
T. turgidum ssp. *durum* var. *murciense*	3+	3	3++	4
T. turgidum ssp. *durum* var. *aestivum*	3-	1C	2	3-
T. turgidum ssp. *polonicum*	3	3	3++	X
SST55[c]	4	2+3	4	4
Adult plants				
T. turgidum ssp. *dicoccum* var. *arras*	;1C	0;	0;	0;
T. turgidum ssp. *durum* var. *murciense*	1++	1++	1++	1++
T. turgidum ssp. *durum* var. *aestivum*	1++	;1--	1++	;1--C
T. turgidum ssp. *polonicum*	3--	2	2	2
SST55[c]	4	2+	4	4

[a]Infection types according to Roelfs (1988b) and McIntosh *et al.* (1995).
[b]*Triticum turgidum* accessions showing adult-plant resistance to *Puccinia triticina* (Barnard,1999).
[c]Bread wheat control.

Table 3. Reaction of progeny derived from crosses between leaf rust-resistant *Triticum turgidum* accessions and the susceptible bread wheat cultivar SST55

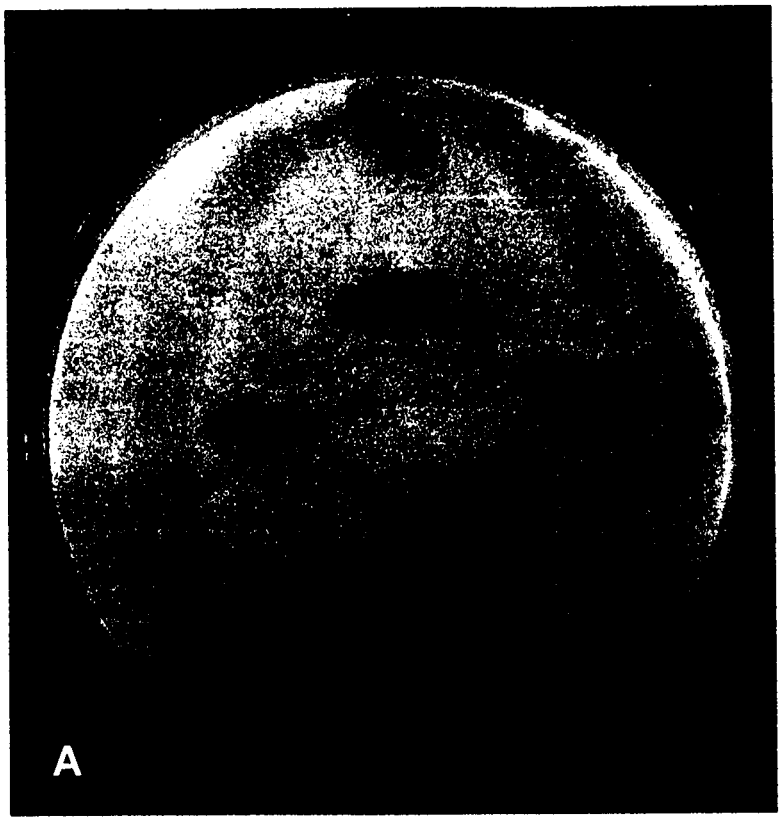
Parental lines and crosses	Generation	Infection type[a]		Number of plants					
		Seedling	Adult	Resistant IT range	Segregating	Susceptible IT range	Chi-square (1:3)	Chi-square (7:9)	Chi-square (7:8:1)
T. turgidum ssp. *dicoccum* var. *arras* (104)	P0	;1 to N	;0 to ;1C						
T. turgidum ssp. *durum* var. *murciense* (125)	P0	3++	1++						
T. turgidum ssp. *durum* var. *aestivum* (127)	P0	3++	;1- to 1++						
T. turgidum ssp. *polonicum* (370)	P0	2	2 to 3--						
SST55	P0	3++	3++						
104 X SST55	F1		3++			54			
125 X SST55	F1		23C			29			
127 X SST55	F1		3++			13			
370 X SST55	F1		23C			12			
104 X SST55	F2	; to 3		5		84	17.831*	52.57*	
125 X SST55	F2	; to 3		7		115	21.93*	47.28*	
127 X SST55	F2	; to 3		2		51	12.73*	34.42*	
104 X SST55	F2		; to 3++	95		125	38.788*	0.029	
125 X SST55	F2		; to 3++	26		91	0.481	22.031*	
127 X SST55	F2		; to 3++	34		36	20.743*	0.663	
370 X SST55	F2		; to 3++	2		42	9.818*	27.481*	
104 X SST55[b]	F3			14	12	4			3.066

[a]Host response and infection type descriptions used in the evaluation of *Puccinia triticina* (Roelfs, 1988b, McIntosh *et al.*, 1995)
[b]F_3 segregating population (among family segregation).
*Significantly different from the proposed 1:3 or 7:9 ratios.

Table 4. Comparison of pollen viability of the F_1 progeny derived from crosses between tetraploid *Triticum turgidum* species and hexaploid SST55

Accessions	Pollen viability (%)
T. turgidum ssp. *dicoccum* var. *arras*	13.3
T. turgidum ssp. *durum* var. *murciense*	19.9
T. turgidum ssp. *durum* var. *aestivum*	12.8
T. turgidum ssp. *polonicum*	9.6
SST55	63.4

Figure 1. F_1 seeds of the four *Triticum turgidum* species were surfaced sterilized and germinated on filter paper (A and B) drenched with H_2O_2 in glass petridishes.


A

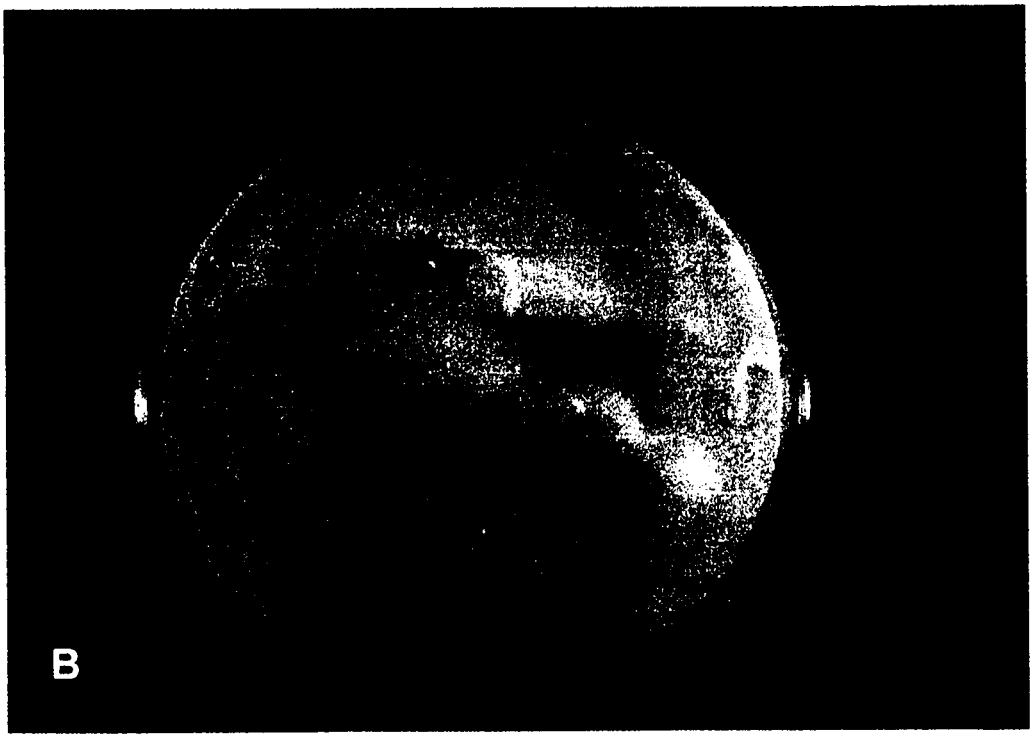

B

Figure 2. Differentiation between viable and non-viable pollen grains using fluorescence microscopy filter combinations UV-1A (excitation filter 330 to 380 nm and barrier filter 420 nm) (A) and B-2A (excitation filter 450 to 490 nm and barrier filter 520 nm) (B). The two light-blue pollen grains in A were considered non-viable according to their bright-yellow fluorescence in the corresponding micrograph (B).


A

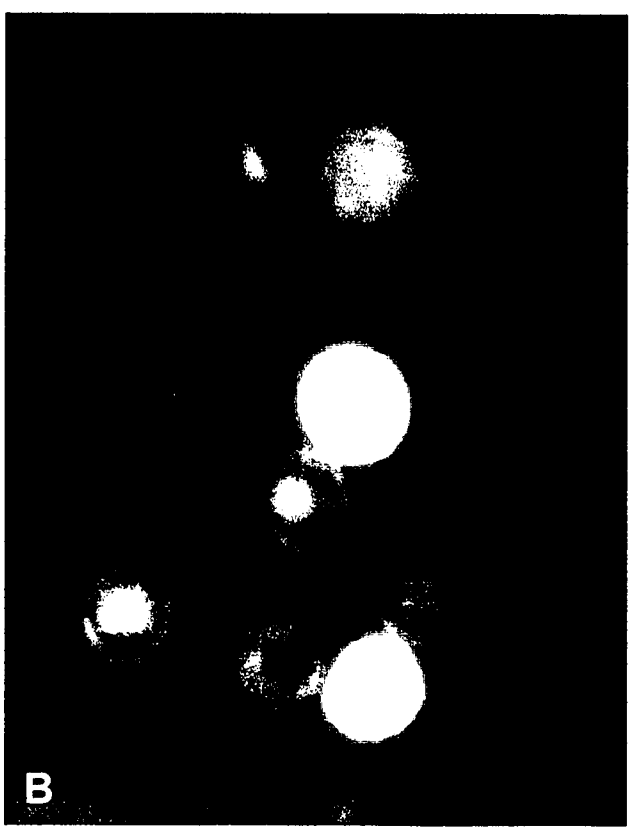

B

Figure 3. *Triticum turgidum* ssp. *dicoccum* var. *arras* seedling resistance to UVPrt2 (A), UVPrt3 (B) and UVPrt9 (C) of *Puccinia triticina*.

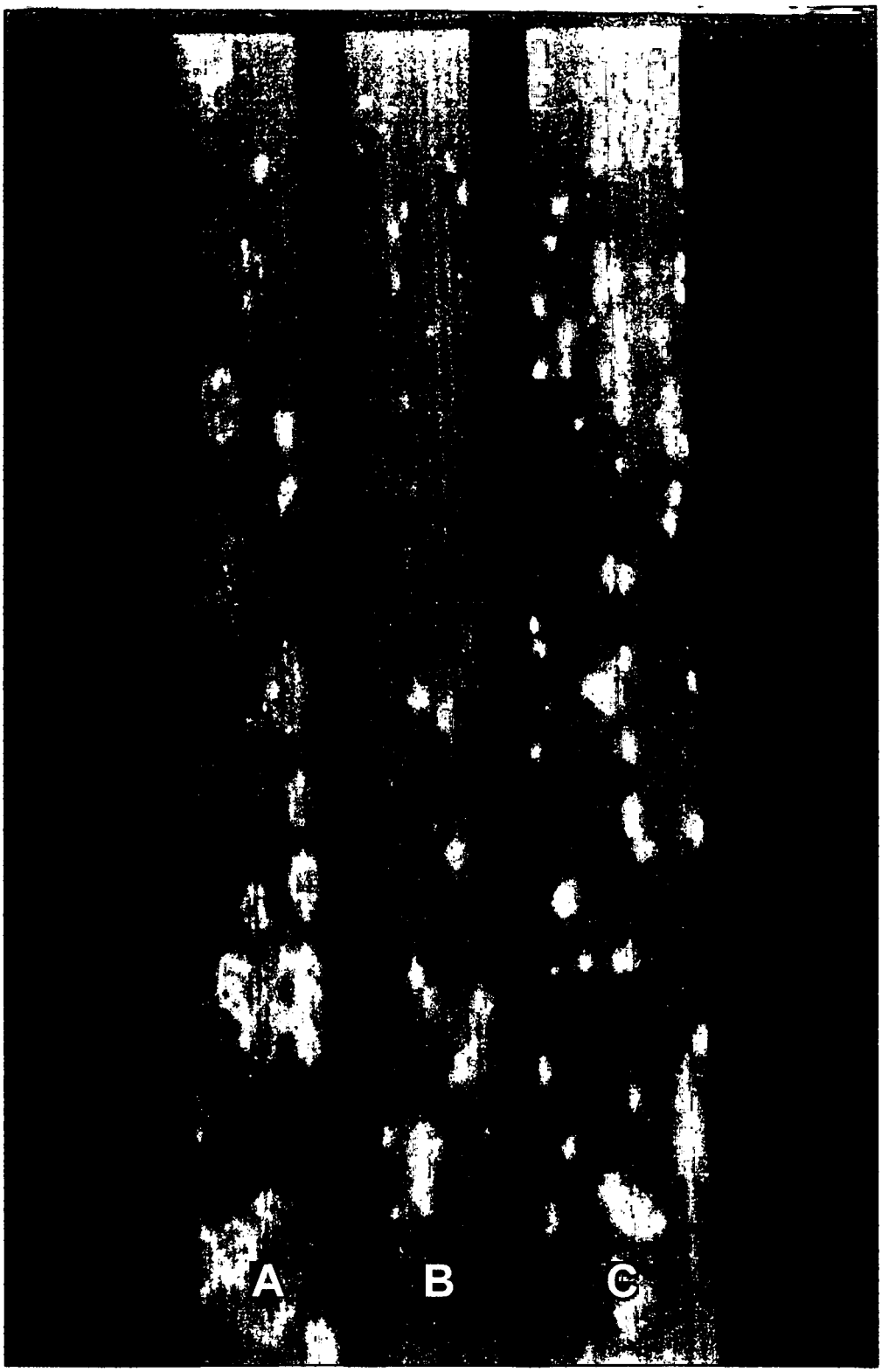

A
B
C

Figure 4. *Triticum turgidum* ssp. *durum* var. *aestivum* seedling resistance to UVPrt3 (A), and susceptibility to UVPrt2 (B) of *Puccinia triticina*.

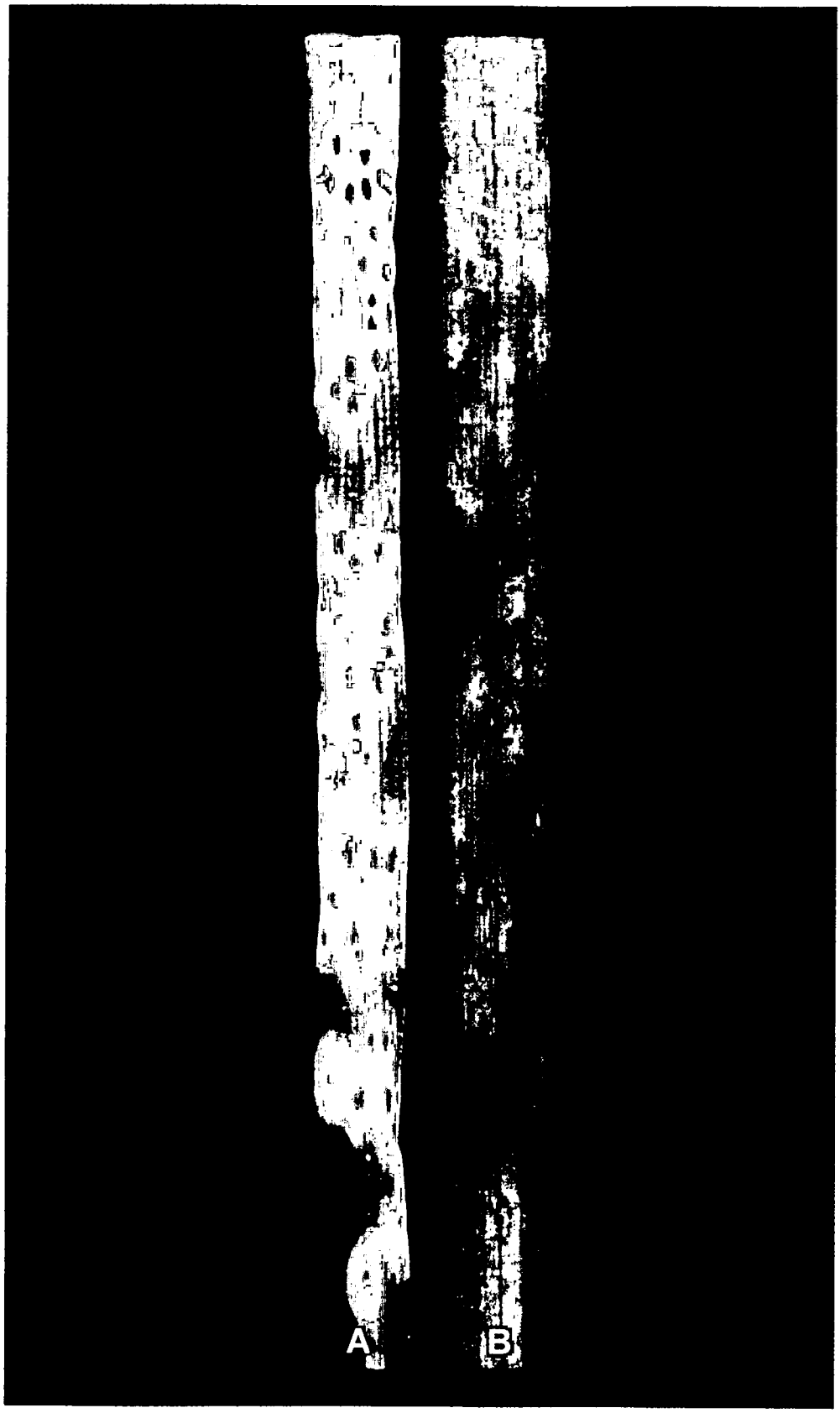

A
B

Figure 5. Parental (*Triticum turgidum* ssp. *dicoccum* var. *arras*) (A) and F_1 (SST55 x *T. turgidum* ssp. *dicoccum* var. *arras*) (B) flag leaf reaction to UVPrt9 of *Puccinia triticina*.

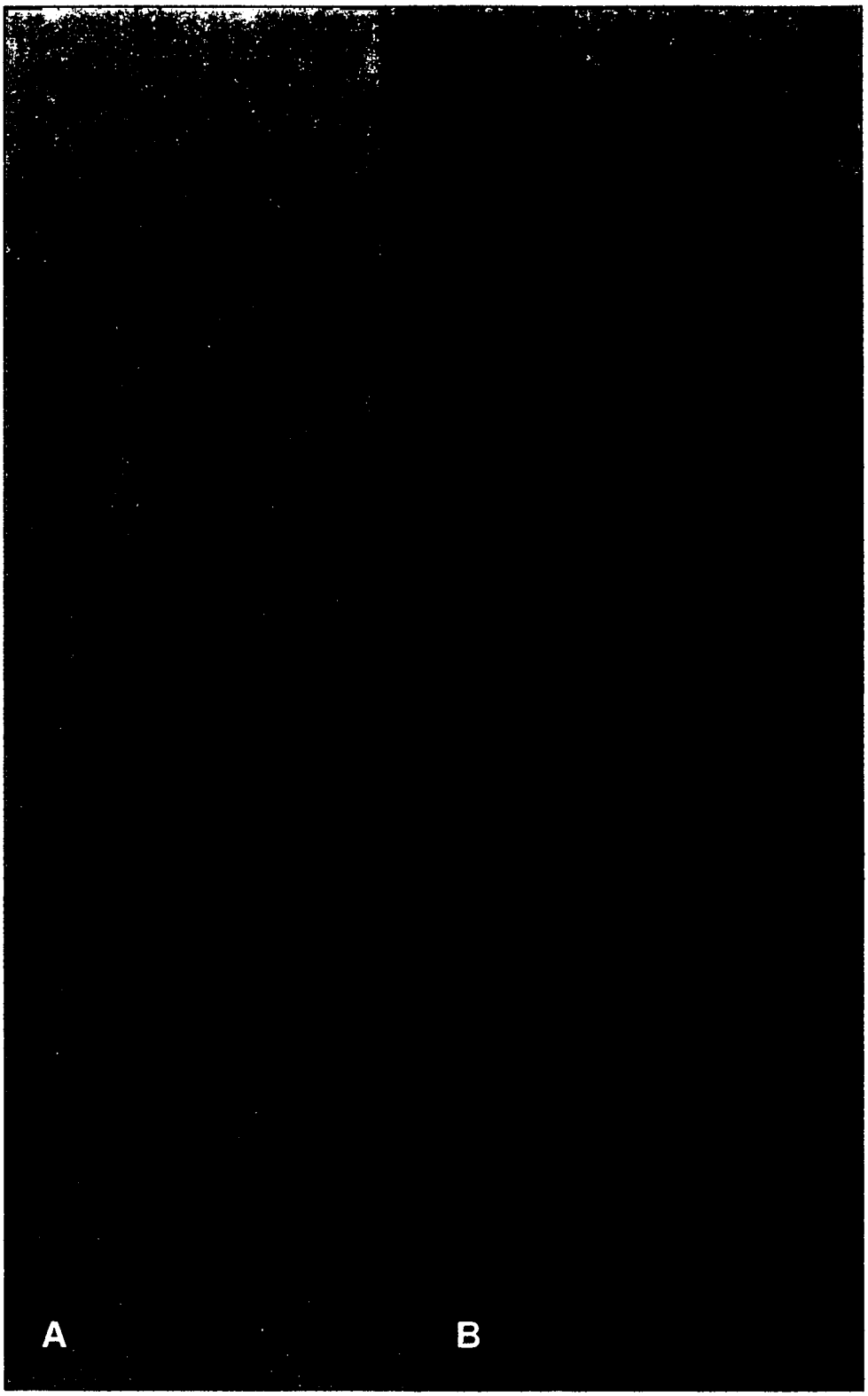

A
B

Figure 6. Parental (*Triticum turgidum* ssp. *durum* var. *murciense*) (A) and F_1 (SST55 x *T. turgidum* ssp. *durum* var. *murciense*) (B) flag leaf reaction to UVPrt9 of *Puccinia triticina.*

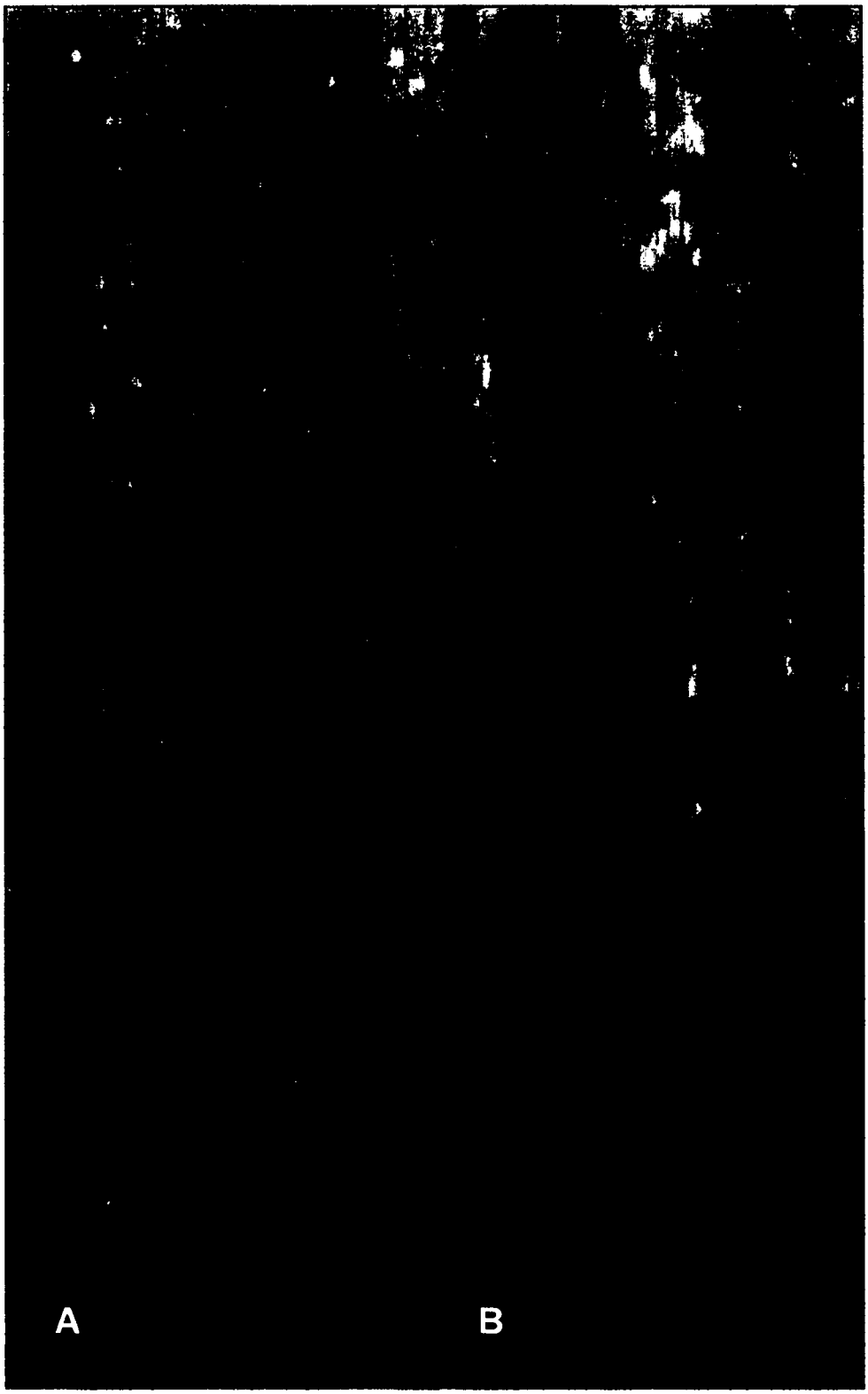

A
B

Figure 7. Parental (*Triticum turgidum* ssp. *durum* var. *aestivum*) (A) and F_1 (SST55 x *T. turgidum* ssp. *durum* var. *aestivum*) (B) flag leaf reaction to UVPrt9 of *Puccinia triticina*.

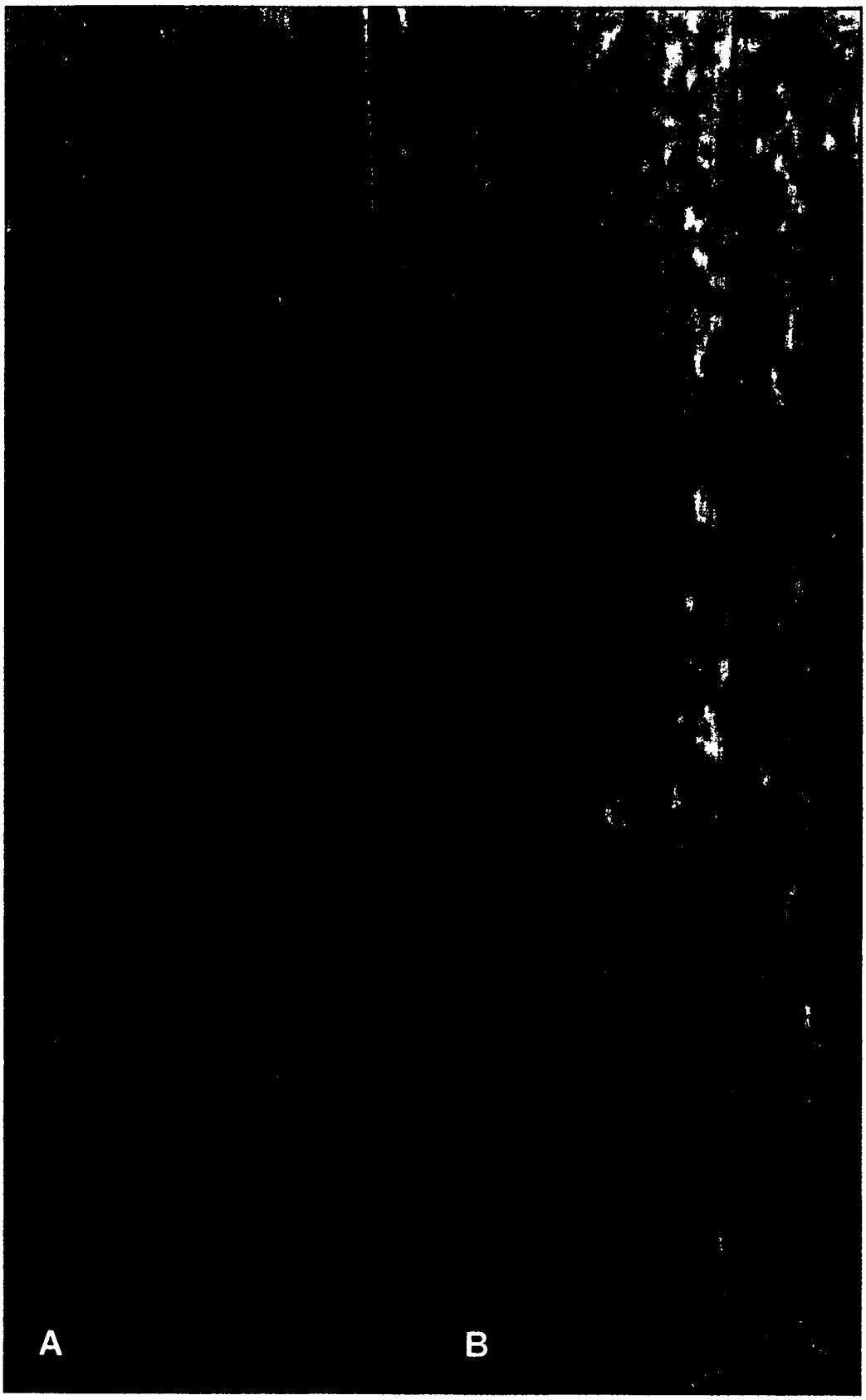

A
B

Figure 8. Parental (*Triticum turgidum* ssp. *polonicum*) (A) and F_1 (SST55 x *T. turgidum* ssp. *polonicum*) (B) flag leaf reaction to UVPrt9 of *Puccinia triticina.*

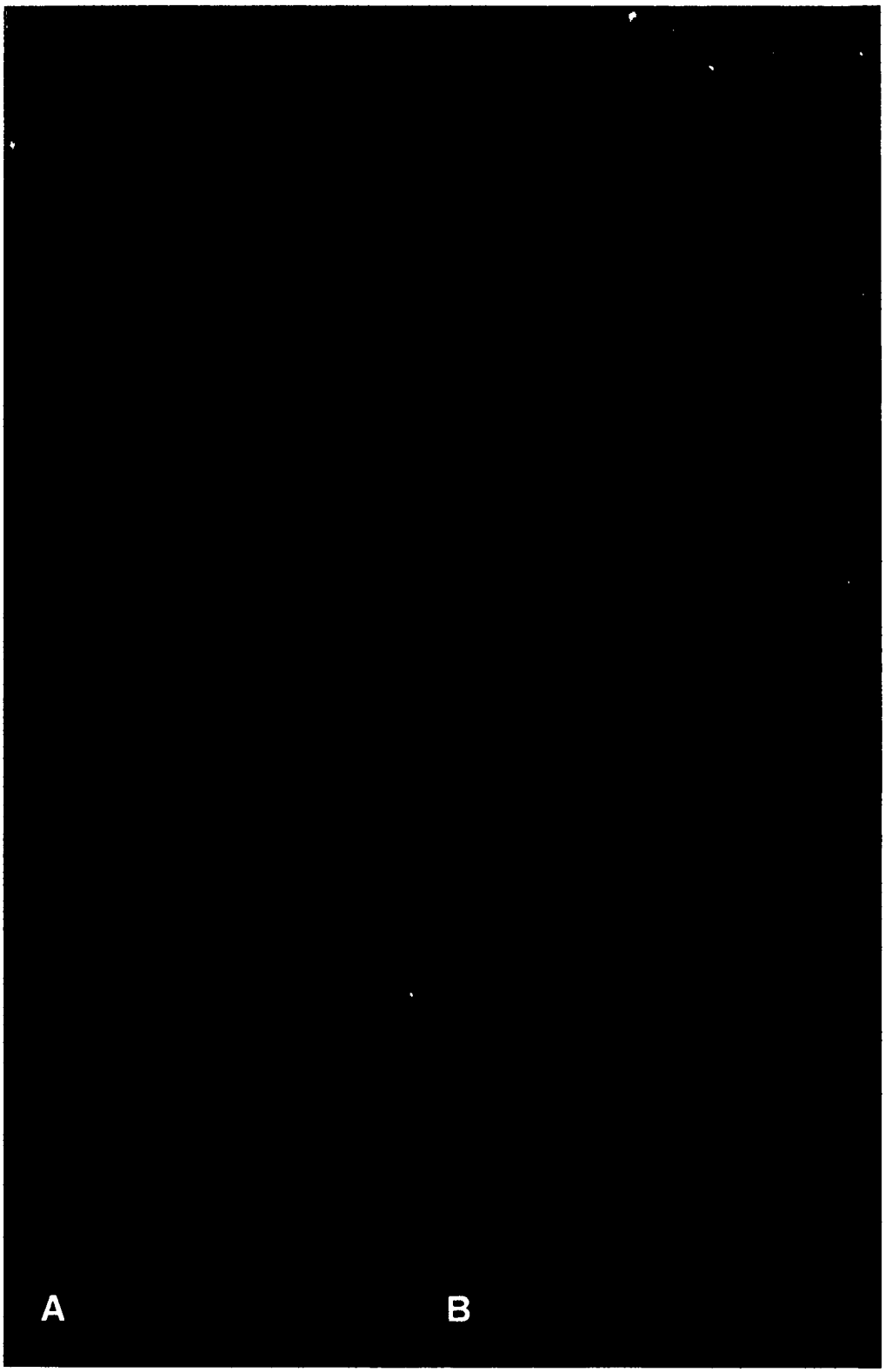

A
B

Figure 9. Resistant (A) and susceptible (B) adult F_2 progeny derived from the cross SST55 x *T. turgidum* ssp. *dicoccum* var. *arras*.


A
B

Figure 10. Resistant (A) and susceptible (B) adult F_3 progeny derived from the cross SST55 x *T. turgidum* ssp. *dicoccum* var. *arras*.


A
B

CHAPTER 4

IDENTIFICATION OF POSSIBLE POLYMORPHISMS ASSOCIATED WITH WHEAT LEAF RUST RESISTANCE IN *TRITICUM TURGIDUM* SSP. *DICOCCUM* VAR. *ARRAS*

ABSTRACT

The introgression of resistance genes from wild wheat offers a source of resistance lacking in cultivated wheat. Molecular markers can be used to follow introgression of new resistance genes into existing cultivars and to determine the genetic relationship between parents. AFLPs (amplified fragment length polymorphism) was used in this study to determine the extent of introgression between *Triticum turgidum* ssp. *dicoccum* var. *arras* and SST55. A total of 82 polymorphic fragments were observed between SST55 and *T. turgidum* ssp. *dicoccum* var. *arras*. Of these, 54 fragments were exclusive to the wild *T. turgidum* ssp. *dicoccum* var. *arras* parent. The frequency of fragment introgression into leaf rust-resistant and susceptible F_2 progeny was 0.66 from SST55 and 0.68 from *T. turgidum* ssp. *dicoccum* var. *arras*. Distance analysis of the data confirmed that a greater extent of introgression occurred from the wild parent into the resistant F_2 plants than into the susceptible F_2 plants. Furthermore, 12 fragments, unique to *T. turgidum* ssp. *dicoccum* var. *arras* were only present in resistant F_2 progeny and may be linked to the introgressed gene or segment conferring resistance.

INTRODUCTION

Several techniques are currently available to identify molecular markers including restriction fragment length polymorphism (RFLP) (Powell *et al.,* 1996), random amplified polymorphic DNA (RAPD) (Welsh and McClelland, 1990), simple sequence repeats (SSR) (Tautz and Renz, 1984; Tautz *et al.*, 1986), inter simple sequence repeats (ISSR) and amplified fragment length polymorphism (AFLP) (Zabeau and Vos, 1993). The new generation of PCR-based DNA fingerprinting techniques including RAPDs, SSRs, ISSRs and AFLPs has the advantage over other non-PCR methods that no prior development work is required (Zabeau and Vos, 1993; Vos *et al.*, 1995). Furthermore, these methods lend themselves to high throughput systems.

Molecular markers are used to identify and tag desirable genes by detecting variation at the DNA sequence level (Moore *et al.*, 1993; Jones *et al.*, 1997; Keim *et al.*, 1997; Mohan *et al.,* 1997). In the past, breeders have relied on the selection of morphological markers that are readily observable and are co-inherited with the desired trait. However, the scarcity of such markers (Jones *et al.*, 1997) usually results in an inability to discriminate between closely related lines that differ in only a single trait of interest (Powell *et al.*, 1996; Jones *et al.*, 1997). Furthermore, molecular markers have proven useful to identify agronomic traits that are difficult to tag, such as resistance to pathogens (Mohan *et al.*, 1997), especially at the seedling stage.

Molecular techniques can be used to follow the introgression of alien genes into existing cultivars (Ishii *et al.*, 1993; Chen *et al.*, 1994; Autrique *et al.*, 1995). Ishii *et al.* (1993) used RFLP mapping to determine the introgression of BPH (brown plant hopper) resistance and flowering earliness from *O. australiensis* into chromosome 12

and 10 of *O. sativa* (cultivated rice). Molecular markers have also been used to successfully detect polymorphisms between cultivated wheat and wild species (Law *et al.*, 1998). Joshi and Nguyen (1993) used RAPDs to determine the genetic relationship between wild and cultivated tetraploid wheat. In a study done by Bohn *et al.* (1999) the genetic similarity between 11 winter wheat cultivars was determined on the basis of RFLP, AFLP and SSR analysis. In this study it was found that the PIC (average polymorphic information content for polymorphic bands) was similar for RFLPs, AFLPs and SSRs. However, the MI (marker index) was highest for AFLPs indicating that this is the technique of choice for determining the introgression of DNA into a cultivated crop from a wild varietiy (Law *et al.*, 1998).

The AFLP technique is a combination of RFLPs and PCR with adapters being ligated to digested DNA fragments followed by PCR using adapter based primers (Fig. 1) (Zabeau and Vos, 1993; Vos *et al.*, 1995). The complexity of the PCR product is further reduced by using primers with 3' selective bases at the 5' end of the digested fragments (Vos *et al.*, 1995). Thus, the AFLP technique provides simultaneous coverage of many loci in a single assay and can be tuned to generate DNA fingerprints of the complexity required by altering the number of selective bases employed (Lin and Kuo, 1995; Mohan *et al.*, 1997). For example, the AFLP technique provides 10 to 100 times more markers on average than do other approaches which translates to 10 to 100 times denser maps (Lin and Kuo, 1995; Keim *et al.*, 1997).

The aim of this study was to correlate the introgression of DNA from a wild relative of wheat (*Triticum turgidum* ssp. *dicoccum* var. *arras*) into a domestic variety (SST55) with observable leaf rust resistance to pathotype UVPrt9 of *Puccinia triticina*.

Parental, F_1 and F_2 plants were fingerprinted using AFLPs to determine the respective parental contribution of genetic material considering that SST55 is a hexaploid and *T. turgidum* ssp. *dicoccum* var. *arras* a tetraploid wheat variety.

MATERIALS AND METHODS

DNA-isolation and purification DNA was extracted from two SST55 plants (leaf rust-susceptible), two *T. turgidum* ssp. *dicoccum* var. *arras* (leaf rust-resistant) plants, one F_1 hybrid and 12 plants from the subsequent F_2 segregating population (4 susceptible and 8 resistant).

Young leaf material was collected on ice and ground in liquid nitrogen using a mortar and pestle. The ground plant material was incubated at 65 °C, with periodic shaking for 1 h in 10 ml extraction buffer (0.25 M EDTA [pH 8.0], 20 % SDS, 0.1 M Tris-HCl [ph 7.0], 0.5 M NaCl) (Edwards *et al.*, 1991), with the addition of 1 % (w/v) CTAB. After incubation, the cellular debris and proteins were extracted by the addition of chloroform:iso-amylalcohol (24:1) in a 1:1 ratio by centrifugation for 10 min at 8000 rpm. Chloroform extractions were repeated until the interface was visually clear of debris. This was followed by the addition of 2 volumes 100 % cold EtOH to the supernatant to precipitate the DNA which was stored at 4 °C overnight. Precipitated DNA was spooled using a sterile pasteur pipette and washed twice by emersion in 70 % EtOH. The DNA was resuspended in 500 µl sterile distilled water (Sabex pour water ^{TM}) and stored at -20 °C.

Purity and concentration determination DNA concentration was determined spectrophotometrically using the formula, [DNA] = Optic density x dilution x constant (50 µg/ml). Purity was determined using the 260/280 OD ratio (Sambrook *et al.*, 1989).

Gel electrophoresis DNA was visualized under UV-light on a 0.8 % agarose gel stained with ethidium bromide after electrophoresis at 80 V in 0.5x TAE (0.438 g/L Tris, 0.09 ml/L acidic acid, 0.022 g/L acid EDTA) (Sambrook *et al.*, 1989).

AFLP-Protocol

Restriction endonuclease digestion and ligation of adapters Genomic DNA (500 ng) was digested in 5x reaction buffer ([50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, 250 mM K-acetate], 2 µl *Eco*R1/*Mse*1 and 25 µl AFLP-grade water) for 2 h at 37 °C according to manufacturer's recommendations (GibcoBRL). Adapters were ligated to the digested DNA (25 µl reaction volume: 24 µl adapter ligation solution and 1 µl T4 DNA ligase (1 unit/µl in 10 mM Tris-HCl [pH 7.5]), 1 mM DTT, 50 mM KCl, 50 % glycerol (v/v)) by incubation at 20 °C for 2 h. The ligation product was diluted 1:10 in TE buffer (10mM Tris-HCl [pH 8.0], 0.1 nM EDTA).

Pre-selective amplification PCR amplification was performed using 5 µl diluted ligation product in 25 µl reaction, containing 10 x PCR buffer (200 mM Tris-HCl [pH 8.4], 15 mM $MgCl_2$, 500 mM KCl and 1 U Ampli Taq DNA polymerase (GibcoBRL)).

Pre-selective PCR amplification was confirmed by gel electrophoresis and the resulting amplicons were diluted 1:50 in TE buffer.

Selective amplification Selective PCR reactions were performed in a 20 µl PCR reaction containing 5 µl template, 4.5 µl Mse primer (Mse-CTA or Mse-CAG [6.7 ng/µl]), 1µl Eco primer (Eco-ACA or Eco- AAC [1 µM/µl] (Table 3), 2µl of 10x PCR buffer (200 mM Tris-HCl [pH 8.4], 15 mM $MgCl_2$, 500 mM KCl and 5 U of Ampli Taq DNA polymerase). The PCR cycle consisted of 30 s at 94 °C, 30 s at 65 °C and 60 s at 72 °C for a total of 35 cycles with a temperature reduction of 0.7 °C per cycle for 12 cycles. *Eco*R1 primers (Eco-ACA and Eco-AAC) (PE Biosystems) were fluorescently labelled with FAM and NED, respectively.

AFLP visualisation and data analysis After amplification, 5 µl of the selective amplification reaction was added to 24 µl formamide and 1 µl Rox size standard marker (35, 50, 75, 100, 139, 150, 160, 200, 300, 340, 350, 400, 450, 490, 500 bp). The mixture was denatured at 94 °C for 5 min and fragments resolved using a Perkin Elmer ABI Prism 310 Automated capillary sequencer (PE Biosystems). Fragment data was tabulated in Microsoft Excel and distance analysis and dendrograms were determined using the UPGMA clustering method (NCSS 2000).

RESULTS

DNA was extracted from 17 plants (Fig. 2). The successful restriction digestion and ligation of adaptors was determined by gel electrophoresis of the pre-selective amplification product (Fig. 3). Both primer pairs tested, yielded polymorphic results between the susceptible and resistant F_2 plants derived from the cross between SST55 (leaf rust susceptible parent) and *T. turgidum* ssp. *dicoccum* var. *arras* (leaf

rust resistant parent). A total of 276 fragments were obtained ranging in size from 43 to 499 bp. All fragments in the F_1 *Triticum turgidum* ssp. *dicoccum* var. *arras* x SST55 hybrid were present in either or both parents (Tables 4 to 7).

A total of 82 fragments were polymorphic between SST55 and *T. turgidum* ssp. *dicoccum* var. *arras*. Of these, 28 fragments were unique to the hexaploid SST55 parent and present in F_2 plants at a frequency of 0.66. Similarly, 54 fragments unique to the wild tetraploid *T. turgidum* ssp. *dicoccum* var. *arras* parent were present in the F_2 plants at a frequency of 0.68.

Primer combination Mse-CTA and Eco-ACA detected a total of 82 fragments (Table 4). Fragment sizes ranged from 46 to 499 bp. Of these, four fragments (69, 83, 200 and 295 bp) were present in *T. turgidum* ssp. *dicoccum* var. *arras* and the resistant F_2 plants but not SST55 or the susceptible F_2 plants. However, the 69 bp fragment was absent in one of the eight resistant plants, while the 83 bp fragment was absent in four of the eight resistant plants. The 200 bp and 295 bp fragments were present in all the resistant F_2 plants (Table 8).

Primer combination Mse-CTA and Eco-AAC detected a total of 64 fragments (Table 5). Fragment sizes ranged from 43 to 499 bp. Of these, four fragments (88, 93, 127 and 180 bp) were present in *T. turgidum* ssp. *dicoccum* var. *arras* and the resistant F_2 plants but not SST55 or the F_2 plants. The 127 bp fragment was absent in four of the eight resistant plants (Table 8).

Primer combination Mse-CAG and Eco-ACA detected a total of 69 fragments (Table 6). Fragment sizes ranged from 46 to 483 bp. Of these, three fragments (82, 130 and 192 bp) were present in *T. turgidum* ssp. *dicoccum* var. *arras* and the

resistant F_2 plants but not SST55 or the susceptible F_2 plants. The 130 bp fragment was absent in two of the eight resistant plants (Table 8).

Primer combination Mse-CAG and Eco-AAC detected a total of 61 fragments (Table 7). Fragment sizes ranged from 45 to 404 bp. Of these, six fragments (58, 128, 190, 198, 226 and 231 bp) were present in *T. turgidum* ssp. *dicoccum* var. *arras* and the resistant F_2 plants but not SST55 or the susceptible F_2 plants. The 128 bp fragment was absent in two of the eight resistant plants (Table 8).

The pair wise distance matrix based on the total AFLP fragment data for both primer combinations revealed genetic distances ranging from 0.21 between A35 (SST55 parent) and A36 (SST55 parent) and 0.85 between A36 (SST55 parent) and A31 (*T. turgidum* ssp *dicoccum* var. *arras* parent) (Table 9). The average genetic distance between the parental plants was 0.84 and between the resistant and susceptible F_2 groups was 0.66. The average genetic distance between the SST55 and F_2 susceptible and resistant plants was 0.61 and 0.72, respectively, while the corresponding distance for *T. turgidum* ssp. *dicoccum* var. *arras* was 0.71 for the susceptible plants and 0.57 for the resistant plants. The resulting dendrogram based on distance data identified two main clusters (Fig. 4). Each of these consisted of two sub clusters corresponding to the resistant parent with resistant F_2 plants and the susceptible parent with susceptible F_2 plants. The F_1 plant grouped within the resistant cluster.

DISCUSSION

This study has successfully used AFLP fingerprinting to analyse the introgression of new resistance from a tetraploid wild relative into a hexaploid domestic cultivar. In

total, 12 fragments were shown to be solely introgressed from the *T. turgidum* ssp. *dicoccum* var. *arras* parent into all the resistant F_2 plants (Table 8). Although the population tested was very small, the presence of markers in the resistant F_2 plants and *T. turgidum* ssp. *dicoccum* var. *arras,* but not in susceptible F_2 plants and SST55, indicates that they are linked to the introgressed chromosomal segment from *T. turgidum* ssp. *dicoccum* var. *arras* conferring resistance (Table 8) (Peil *et al.*, 1997; Gold *et al.*, 1999).

Certain introgressed fragments from *T. turgidum* ssp. *dicoccum* var. *arras* were not present in all the resistant F_2 plants but absent in SST55 and susceptible F_2 plants (Table 8). Segregation data has already shown (Chapter 3) that two genes are responsible for resistance. It is, therefore, possible that these markers are linked to either flanking regions of the introgressed segment or to only one of the two genes responsible for resistance (Gold *et al.*, 1999). This would account for the absence of these fragments in some resistant plants.

It is interesting to note that 29 % more polymorphisms were detected in the tetraploid *T. turgidum* ssp. *dicoccum* var. *arras* than the hexaploid SST55. These findings are contrary to those of Peil *et al.* (1997) who found 20 % more polymorphisms in a hexaploid Alcedo than a diploid *Ae. markgraffi.* It is possible that the AFLP primers used in this study have a bias in terms of detecting sequences in the A and B genomes and not D. However, the high degree of dissimilarity between the parental lines suggests that the level of polymorphism in the tetraploid *T. turgidum* ssp. *dicoccum* var. *arras* is greater than the polymorphisms contributed by the D genome in SST55, of which the fate in the F_2 progeny is unknown.

The frequency of polymorphic fragments between the respective parent lines, SST55 and *T. turgidum* ssp. *dicoccum* var. *arras* and the F_2 progeny (0.66 and 0.68, respectively), indicates that a greater extent of introgression has occurred from *T. turgidum* ssp. *dicoccum* var. *arras* than SST55. Furthermore, distance analysis of all the AFLP data indicate a greater chromatin introgression from *T. turgidum* ssp. *dicoccum* var. *arras* to F_2 resistant plants (0.57) than from SST55 (0.72) (Fig. 4) (Table 9). This demonstrates the importance of determining the genetic extent of introgression when crossing distantly related wheats (Manjarrez-Sandoval *et al.*, 1997; Burkhamer *et al.*, 1998).

Finally, distance data indicate that the introgressed chromatin differs significantly between resistant and susceptible F_2 plants (0.66). Also the markers identifying the translocated segment associated with disease resistance may not prove equally effective in further backcross populations due to further recombination of the introgressed chromatin conferring resistance. Unfortunately, the small size of the population used in this study limits statistical analyses and further research will be necessary to quantify linkage between the markers and genes.

Table1. Phenotypic reaction[a] of SST55 (leaf rust-susceptible parent), *Triticum turgidum* ssp. *dicoccum* var. *arras* (leaf rust-resistant parent), F_1 (SST55 x *T. turgidum* ssp. *dicoccum* var. *arras*) and the resultant F_2 susceptible and resistant progeny when inoculated with UVPrt9 of *Puccinia triticina*. All phenotypic assessments were made 14 d.p.i.

Generations	Sample number	Phenotypic reaction
P_0 SST55	A35	3++
P_0 SST55	A36	3++
P_0 *T. turgidum* ssp. *dicoccum* var. *arras*	A31	0;
P_0 *T. turgidum* ssp. *dicoccum* var. *arras*	B	0;
F_1 (SST55 x *T. t.* ssp. *dicoccum* var. *arras*)	A29	3++
F_2 Resistant plant	N2	;
F_2 Resistant plant	N15	;
F_2 Resistant plant	B45	;
F_2 Resistant plant	B13	;
F_2 Resistant plant	D	;
F_2 Resistant plant	C26	;
F_2 Resistant plant	C33	;
F_2 Resistant plant	N11	;1C
F_2 Susceptible plant	N30	3++
F_2 Susceptible plant	N31	3++
F_2 Susceptible plant	N9	3++
F_2 Susceptible plant	N4	3++

[a]Host response and infection type descriptions used in the evaluation of *Puccinia triticina* (Roelfs, 1988b; McIntosh *et al.*, 1995).

Table 2. *Mse*1 and *Eco*R1 adapters and primers used to generate AFLP profiles

***Mse* adapter**	***Eco* adapter**
5'-GACGATGAGTCCTGAG-3'	5'-CTCGTAGACTGCGTACC
3'-TACTCAGGACTCAT-5'	3'-CATCTGACGCATGGTTAA-5'
***Mse* primers**	***Eco* primers**
5'-GATGAGTCCTGAGTAA-3'	5'-GATGCGTACCAATTC-3'
Mse-CTA	*Eco*R-ACA (FAM)
Mse-CAG	*Eco*R-AAC (NED)

Table 3. The amount of parent polymorphism between SST55 and *Triticum turgidum* ssp. *dicoccum* var. *arras*

	Mse-CTA and Eco-ACA	Mse-CTA and Eco-AAC	Mse-CAG and Eco-ACA	Mse-CAG and Eco-AAC
Total amount of polymorphisms	82	64	69	61
Parent polymorphisms	26	15	23	19
SST55	8	5	13	4
T. turgidum ssp. *dicoccum* var. *arras*	18	10	10	15

Table 4. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *dicoccum* var. *arras* (leaf rust-resistant parent) (104), F_1 (SST55 x 104) and the subsequent segregating F_2 population using the primer combination Mse-CTA and Eco-ACA

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
46	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
49	X	X	X	X	X	X	X		X	X	X	X	X		X	X	X
51	X	X	X	X	X	X	X		X	X	X	X	X		X	X	X
53	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X
54	X	X	X	X	X	X	X		X	X		X	X		X	X	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	X	X	X	X	X
66	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
69			X	X	X					X	X	X	X	X	X	X	
72	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
75	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
77	X	X	X	X	X	X	X	X	X		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
80	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
82	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
83			X	X	X							X	X	X			
87	X	X	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
93	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
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
102			X	X	X				X	X							

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
104	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
107			X	X	X			X	X	X		X	X	X	X	X	X
111	X	X			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	X	X	X	X
115	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
117	X	X	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	X	X
126	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	X	X	X
131	X	X			X			X	X				X		X	X	X
129	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
133	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
137	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X
138	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
146	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
151	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
156	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
166	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
171	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
173			X	X	X	X	X	X	X	X				X	X	X	X
175			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
190	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
194	X	X	X	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
204	X	X	X	X	X					X	X	X	X	X			

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
208	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	X	X	X	X	X	X	X	X
218	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
232	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
238	X	X	X	X	X	X	X	X	X	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
248	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
252	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
258			X	X	X	X	X	X	X	X		X		X	X	X	X
260	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
262	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
277	X	X			X	X			X			X	X		X	X	X
295			X	X	X					X	X	X	X	X	X	X	X
305	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
308	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
312	X	X	X	X	X					X	X						
348	X	X			X							X	X		X	X	X
351			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
361			X	X	X	X	X	X	X	X	X			X	X	X	X
367			X	X	X	X	X	X	X					X	X	X	X
398			X	X	X			X	X	X	X			X	X	X	X
408			X	X	X	X			X	X	X			X	X	X	X
420	X	X			X	X	X	X		X	X				X	X	X
429			X	X	X				X	X					X	X	X
432			X	X	X	X		X	X	X	X	X	X	X	X	X	X
434			X	X	X	X	X	X	X	X				X	X	X	X
438	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
448	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
459	X	X	X	X	X	X	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	X	X
499			X	X	X			X		X			X	X	X	X	X

Table 5. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *dicoccum* var. *arras* (leaf rust-resistant parent) (104), F_1 (SST55 x 104) and the subsequent segregating F_2 population using the primer combination Mse-CTA and Eco-AAC

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
43	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
45	X	X	X	X	X	X	X		X	X	X	X	X		X	X	
48	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
51	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
53	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
57	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
59	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
63	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
65	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
68			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
71	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
76	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
77	X	X	X	X	X	X	X	X	X	X	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
79	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
81	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
86	X	X	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
91	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	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
99			X	X	X	X	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
103	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
108	X	X	X	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	X
114	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	X
118	X	X	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	X	X
124	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X
127			X	X	X					X	X	X	X				
131	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
136	X	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	X	X	X	X	X
142	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
145	X	X	X	X	X					X	X	X	X		X	X	X
149	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
155	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
159	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
165	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	X	X	X	X
174			X	X	X									X	X	X	X
177	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
180			X	X	X					X	X	X	X	X	X	X	X
190	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
194	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
196	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
216	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
238	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
242	X	X	X	X	X							X	X	X	X	X	X
245	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
263	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
279	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
288	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
279	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
288	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
297	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
299	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
311			X	X	X	X	X		X	X	X	X	X	X	X	X	X
349	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	
365	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
492			X	X	X	X	X	X	X								
499	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Table 6. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *dicoccum* var. *arras* (leaf rust-resistant parent) (104), F_1 (SST55 x 104) and the subsequent segregating F_2 population using the primer combination Mse-CAG and Eco-ACA

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
46	X	X	X	X	X	X	X			X	X	X		X	X	X	X
49			X	X	X	X	X				X	X			X	X	X
52	X	X	X	X	X	X					X	X			X	X	X
55	X	X	X	X	X	X	X	X			X	X	X		X	X	X
61	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
64	X	X	X		X				X						X	X	X
66	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
68	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
70	X	X	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			X		X		X
74	X	X	X	X	X	X	X			X	X	X		X			X
77	X	X		X	X	X	X	X	X	X	X	X	X	X			X
82			X	X	X					X	X	X	X	X	X	X	X
87	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
92	X	X			X	X			X	X							
103	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
109	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
112	X	X	X	X	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
117	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
121	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
128	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
130			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	X
134	X	X			X				X	X		X		X		X	X
137	X	X			X		X	X	X	X							
140	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
148	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
153	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
156	X	X	X	X	X	X	X	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	X	X
160	X	X			X			X			X	X					
166			X	X	X	X	X	X	X	X	X	X	X	X		X	X
168	X	X	X	X	X	X	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	X	X	X	X
175	X	X			X	X	X	X	X	X		X			X	X	X
180	X	X			X			X				X					X
183	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
192			X	X	X					X	X	X	X	X	X	X	X
198	X	X			X	X	X	X	X	X	X	X					X
202	X	X		X	X	X	X	X	X	X	X	X	X	X			
206	X	X			X	X	X	X	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	X
215	X	X			X	X	X	X	X	X					X	X	X
217	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
223	X	X			X			X	X	X	X	X			X		X
231	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
235	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
242			X	X	X			X			X			X			
249	X	X			X			X	X	X							
256	X	X	X		X	X	X		X	X	X			X			

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
266			X	X	X	X	X	X	X		X	X		X			
276	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
282	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
303	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
308			X	X	X	X	X	X	X	X	X	X	X	X	X		
316	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
322	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
336	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
356	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
377	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
397	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
401	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	
425	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
437	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
448			X	X	X	X	X									X	X
483	X	X			X	X		X		X	X	X	X	X			

Table 7. AFLP fragments obtained for SST55 (leaf rust-susceptible parent), *T. turgidum* ssp. *dicoccum* var. *arras* (leaf rust-resistant parent) (104), F_1 (SST55 x 104) and the subsequent segregating F_2 population using the primer combination Mse-CAG and Eco-AAC

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
45	X	X			X	X	X	X	X	X	X	X		X	X	X	X
46			X		X	X	X	X	X			X					X
48				X	X	X	X				X	X			X		
51	X	X	X	X	X	X	X	X	X		X	X				X	
56			X	X	X	X	X	X	X	X	X		X		X	X	X
58			X	X	X					X	X	X	X	X	X	X	X
61	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
63	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
65			X	X	X	X	X	X	X		X	X	X	X	X	X	X
66	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X
68	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
70	X	X	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	X	X	X	X	X	X	X
74	X	X		X	X					X		X					
76	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
81			X		X	X	X	X	X		X	X					X
85			X	X	X		X		X	X	X	X		X			X
87	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
92	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	X	X	X	X	X	X
104	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
111	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
114	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
120	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
128			X	X	X					X	X	X	X	X		X	
137	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
141	X	X	X	X	X	X	X	X	X	X	X	X		X		X	
146	X	X	X	X	X								X	X	X	X	
148	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
152	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
156	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
162	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	X	X	X	X	X	X
175	X	X	X	X	X	X	X	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
186	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
190			X	X	X					X	X	X	X	X	X	X	X
195			X	X	X	X	X	X			X	X	X	X	X	X	X
198			X	X	X					X	X	X	X	X	X	X	X
203	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
206	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
210			X	X	X	X	X	X	X	X	X	X		X		X	X
226			X	X	X					X	X	X	X	X	X	X	X
231			X	X	X					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
246	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
248	X	X			X	X	X	X	X				X	X	X	X	X
259	X	X	X	X	X	X	X	X	X		X	X		X			X
276	X	X			X	X	X	X	X	X	X	X		X			

Fragment sizes	A35	A36	A31	B	A29	N30	N31	N9	N4	N2	N15	C26	C33	N11	B45	B13	D
	P_0 SST55	P_0 SST55	P_0 104	P_0 104	F_1 (104 x SST55)	Susceptible F_2 plants				Resistant F_2 plants							
288	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
293	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
300	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
309	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
347	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
360					X												
383	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
401	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
404			X	X	X		X				X	X			X		X

Table 8. Fragments observed in only the resistant *Triticum turgidum* ssp. *dicoccum* var. *arras* parent, the F_1 (SST55 x *T. turgidum* ssp. *dicoccum* var. *arras*), and the subsequent resistant F_2 progeny using two different primers

Mse-CTA and Eco-ACA	Mse-CTA and Eco-AAC	Mse-CAG and Eco-ACA	Mse-CAG and Eco-AAC
69*	88	82	58
83*	93	130*	128*
200	127*	192	190
295	180		198
			226
			231

* Fragments were observed in some of the resistant F_2 (SST55 x *T. turgidum* ssp. *dicoccum* var. *arras*) progeny and in none of the susceptible F_2 progeny.

Table 9. A pair-wise distance matrix based on total AFLP fragment data for all four primers for SST55 (A35 and A36), *Triticum turgidum* ssp. *dicoccum* var. *arras* (A31 and B), F_1 (SST55 x *T. turgidum* ssp. *dicoccum* var. *arras*) (A29), F_2 susceptible plants (N30, N31, N9 and N4) and F_2 resistant plants (N2, N15, C26, C33, N11, B45, B13 and D)

0.129641	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.845154	0.855039	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.835152	0.835152	0.342997	—	—	—	—	—	—	—	—	—	—	—	—	—
0.648204	0.661041	0.557606	0.557606	—	—	—	—	—	—	—	—	—	—	—	—
0.594089	0.608069	0.704129	0.715964	0.579771	—	—	—	—	—	—	—	—	—	—	—
0.601119	0.61494	0.698137	0.710072	0.61494	0.33052	—	—	—	—	—	—	—	—	—	—
0.635107	0.648204	0.761467	0.761467	0.621735	0.485071	0.476331	—	—	—	—	—	—	—	—	—
0.628457	0.641689	0.721809	0.733359	0.61494	0.439633	0.42997	0.458349	—	—	—	—	—	—	—	—
0.710072	0.721809	0.601119	0.628457	0.565091	0.673633	0.679842	0.710072	0.628457	—	—	—	—	—	—	—
0.727607	0.739066	0.565091	0.579771	0.557606	0.586973	0.594089	0.679842	0.685994	0.557606	—	—	—	—	—	—
0.704129	0.715964	0.550019	0.534522	0.493657	0.641689	0.621735	0.692092	0.685994	0.557606	0.40996	—	—	—	—	—
0.727607	0.739066	0.608069	0.621735	0.667367	0.692092	0.685994	0.692092	0.733359	0.601119	0.579771	0.550019	—	—	—	—
0.761467	0.772424	0.579771	0.579771	0.586973	0.667367	0.673633	0.679842	0.661041	0.586973	0.550019	0.550019	0.550019	—	—	—
0.721809	0.733359	0.61494	0.601119	0.579771	0.635107	0.692092	0.685994	0.679842	0.608069	0.61494	0.628457	0.542326	0.628457	—	—
0.733359	0.744729	0.572478	0.572478	0.565091	0.635107	0.692092	0.685994	0.654654	0.565091	0.61494	0.601119	0.557606	0.586973	0.388922	—
0.715964	0.727607	0.594089	0.594089	0.526603	0.61494	0.661041	0.641689	0.635107	0.586973	0.608069	0.565091	0.635107	0.621735	0.439633	0.439633

Figure 1. AFLP procedure as described by Zabeau and Vos (1993).

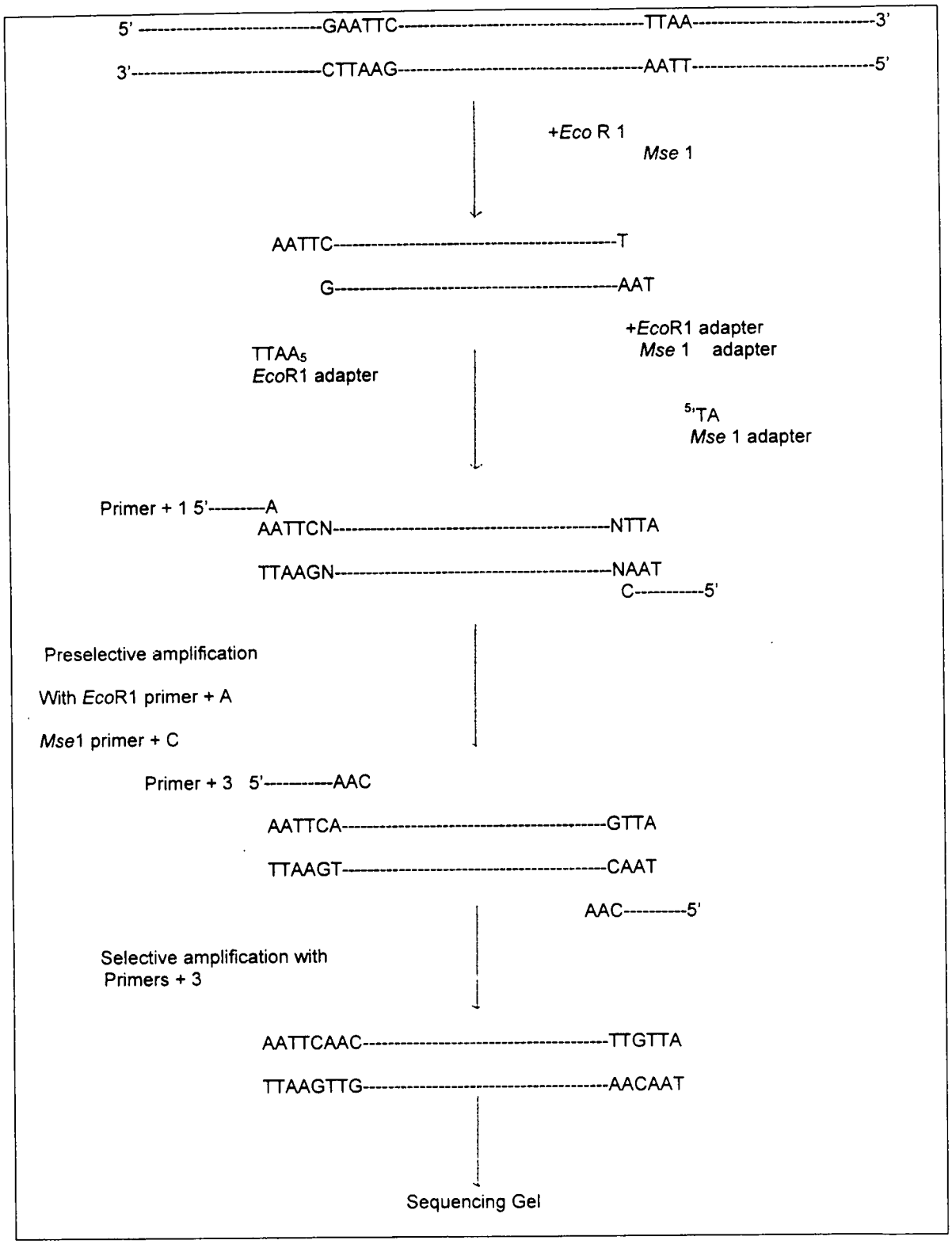
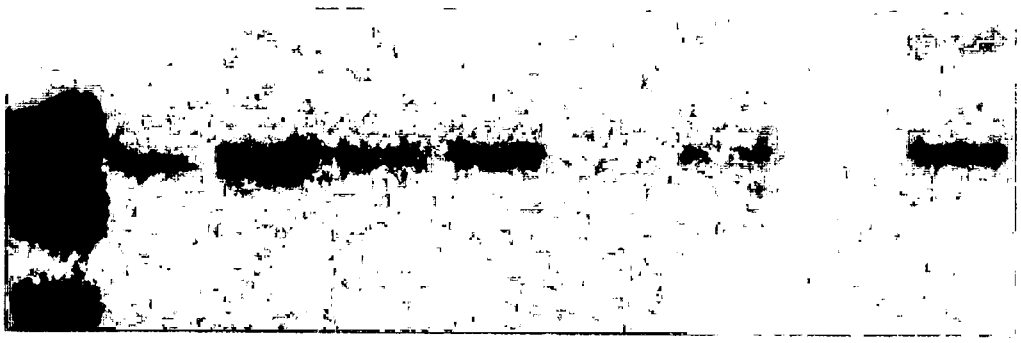

5' ----------GAATTC----------TTAA----------3'
3'----------CTTAAG----------AATT----------5'
+Eco R 1
Mse 1
AATTC----------T
G----------AAT
+EcoR1 adapter
Mse 1 adapter
TTAA5
EcoR1 adapter
5'TA
Mse 1 adapter
Primer + 1 5'--------A
AATTCN----------NTTA
TTAAGN----------NAAT
C----------5'
Preselective amplification
With EcoR1 primer + A
Mse1 primer + C
Primer + 3 5'----------AAC
AATTCA----------GTTA
TTAAGT----------CAAT
AAC----------5'
Selective amplification with
Primers + 3
AATTCAAC----------TTGTTA
TTAAGTTG----------AACAAT
Sequencing Gel

Figure 2. Extracted genomic DNA (200ng) visualized on a 0.8 % agarose gel under UV-light. Lane 1, DNA marker 3 (564, 831, 947, 1375, 1584, 1904, 2027, 3530, 4268, 4973, 5148 and 21226 bp); lane 2, A35; lane 3, A36; lane 4, A31; lane 5, B; lane 6, A28; lane 7, A29; lane 8, N14 and lane 9, N15.

Figure 3. A 0.8 % agarose gel visualizing pre-selective amplification under UV-light. Lane 1, DNA marker 3 (564, 831, 947, 1375, 1584, 1904, 2027, 3530, 4268, 4973, 5148 and 21226 bp), lane 2, N2; lane 3, N15; lane 4, C26; lane 5, B45; lane 6, B13; lane 7, D; lane 8, N30 and lane 9, N31.

1 2 3 4 5 6 7 8 9



1 2 3 4 5 6 7 8 9

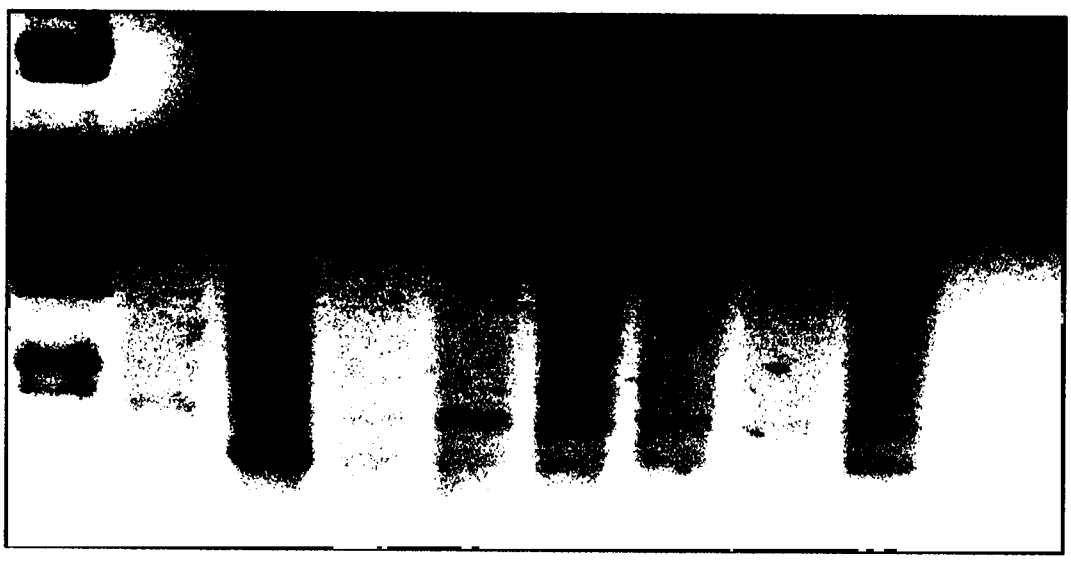
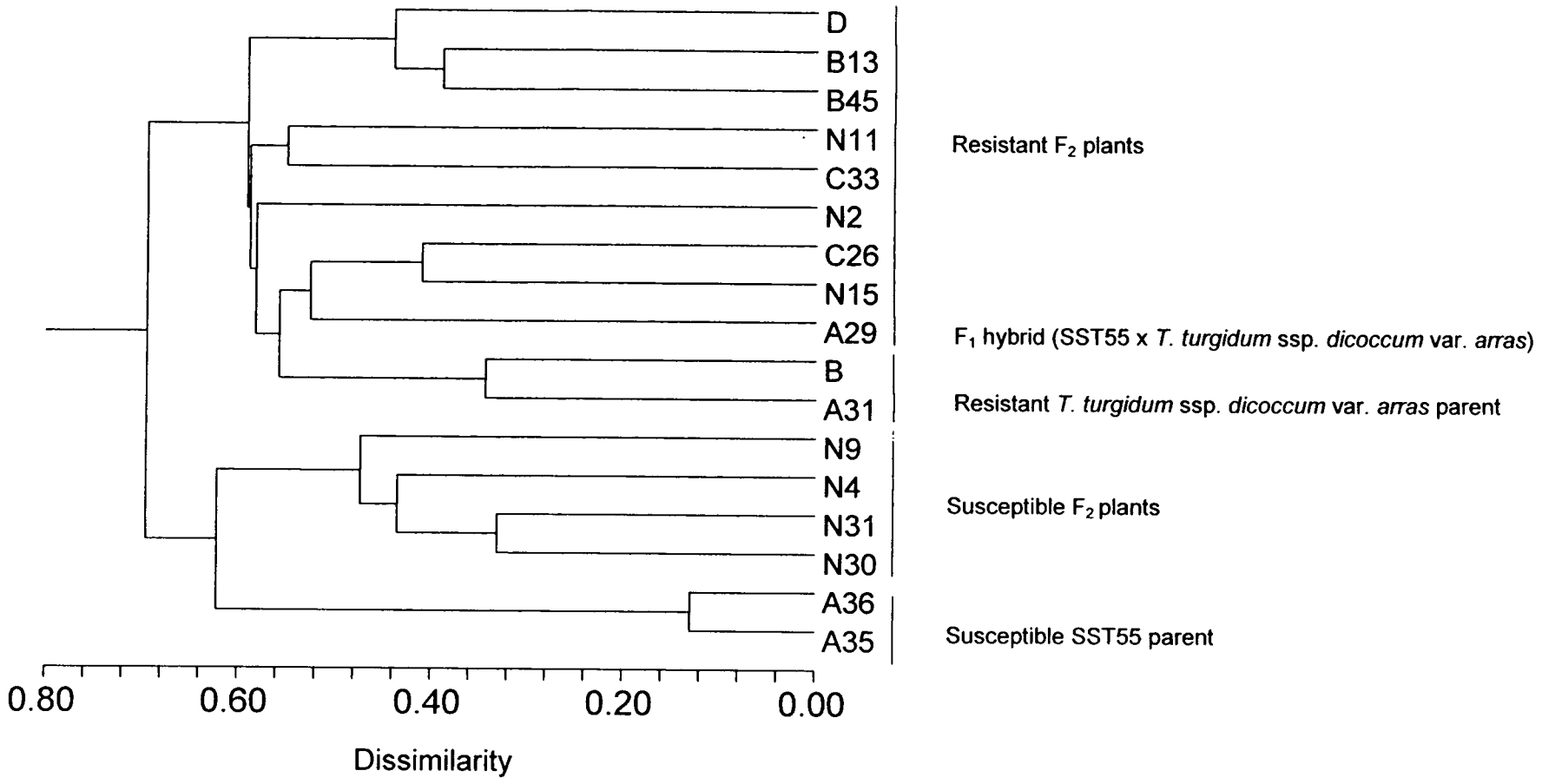


Figure 4. A dendrogram based on the polymorphic fragments obtained by AFLPs showing the genetic distances between the respective plants.


D
B13
B45
N11
C33
N2
C26
N15
A29
B
A31
N9
N4
N31
N30
A36
A35
0.80
0.60
0.40
0.20
0.00
Dissimilarity
Resistant F_2 plants
F_1 hybrid (SST55 x *T. turgidum* ssp. *dicoccum* var. *arras*)
Resistant *T. turgidum* ssp. *dicoccum* var. *arras* parent
Susceptible F_2 plants
Susceptible SST55 parent

SUMMARY

In wheat, the depletion of genes for rust resistance necessitates the search for alternative sources of diversity. Species related to wheat, e.g. *Triticum turgidum*, provide such a source of new and hopefully durable resistance genes to *Puccinia triticina*. In an effort to exploit this source, *T. turgidum* ssp. *dicoccum* var. *arras*, *T. turgidum* ssp. *durum* var. *aestivum*, *T. turgidum* ssp. *durum* var. *murciense* and *T. turgidum* ssp. *polonicum*, were crossed with the leaf rust-susceptible cultivar SST55. The F_1 progeny from all crosses were susceptible in the adult-plant stage, suggesting recessivity of resistance. F_2 tests indicated that two recessive genes may be responsible for resistance in *T. turgidum* ssp. *dicoccum* var. *arras* and *T. turgidum* ssp. *durum* var. *aestivum*, respectively. Segregation ratios suggested a single recessive gene in *T. turgidum* ssp. *durum* var. *murciense*. *T. polonicum* progeny deviated from both single and two-gene Mendelian models.

To further characterize the resistance in *T. turgidum*, accessions were inoculated with six pathotypes (UVPrt2, 3, 5, 9, 13 and 17) of *P. triticina*. Since all were susceptible to UVPrt5 but resistant to the other isolates, it appeared that these accessions have race-specific (vertical) resistance to *P. triticina*. From fluorescence microscopy of resistance components it was found that prestomatal exclusion did not play a significant role. In *T. turgidum* ssp. *dicoccum* var. *arras, T. turgidum* ssp. *durum* var. *aestivum* and *T. turgidum* ssp. *durum* var. *murciense* early abortion of structures seemed important. *T. turgidum* ssp. *polonicum* and Morocco (control) had few, if any, early aborted structures and subsequent colonies were large in comparison to the other lines. *T. turgidum* ssp. *dicoccum* var. *arras, T. turgidum* ssp. *durum* var. *aestivum* and *T. turgidum* ssp. *durum* var. *murciense* showed varying

degrees of host cell necrosis which is typical of a non-durable, hypersensitive response. No necrosis was detected in leaf rust colonies in *T. turgidum* ssp. *polonicum.*

AFLP analysis was used to follow the introgression of resistance into domestic hexaploid wheat from *T. turgidum* ssp. *dicoccum* var. *arras*, the F_1 hybrid and susceptible and resistant F_2 plants. It was found that 12 AFLP markers were solely introgressed from *T. turgidum* ssp. *dicoccum* var. *arras* into the resistant F_2 plants. Certain markers, however, introgressed from *T. turgidum* ssp. *dicoccum* var. *arras* were not present in all the resistant F_2 plants (although not present in the SST55 and the susceptible F_2 progeny). This suggests that these markers are possible linked to either flanking regions of the introgressed segment or to only one of the two genes responsible for resistance. Contrary to what was expected, more polymorphisms were detected in tetraploid *T. turgidum* ssp. *dicoccum* var. *arras* than hexaploid SST55, suggesting an unequal contribution of genetic material from the respective parents in susceptible and resistant F_2 progeny. Furthermore, the frequency of introgression into F_2 plants, especially into the resistant F_2 progeny, was higher from *T. turgidum* ssp. *dicoccum* var. *arras* than from SST55. Further research is, however, necessary to fully evaluate the statistical significance of this data.

OPSOMMING

Na aanleiding van die afname in bruikbare roesweerstandsgene in koring is dit noodsaaklik om nuwe bronne van variasie te soek. Spesies verwant aan broodkoring, soos bv. *Triticum turgidum,* kan 'n waardevolle bron van nuwe en moontlik volhoubare weerstand teen *Puccinia triticina* wees. In 'n poging om hierdie bron te ontgin is *T. turgidum* ssp. *dicoccum* var. *arras*, *T. turgidum* ssp. *durum* var. *aestivum*, *T. turgidum* ssp. *durum* var. *murciense* en *T. turgidum* ssp. *polonicum* gekruis met SST55, 'n blaarroesvatbare koringkultivar. Die F_1 nageslag van alle kruisings was vatbaar in die volwasseplantstadium, wat 'n aanduiding was van resessiewe weerstand. Die F_2 populasies het gesegregeer in weerstandbiedende en vatbare plante. Segregasiedata het getoon dat twee resessiewe gene moontlik verantwoordelik was vir weerstand in onderskeidelik *T. turgidum* ssp. *dicoccum* var. *arras* en *T. turgidum* ssp. *durum* var. *aestivum*. 'n Enkel resessiewe geen het moontlik weerstand in *T. turgidum* ssp. *durum* var. *murciense* bemiddel. *T. turgidum* ssp. *polonicum*-nageslag se segregasie data het afgewyk van beide die 7:9 en 1:3 Mendeliese verhoudings.

Ten einde weerstand in *T. turgidum* te karakteriseer is aanwinste geïnokuleer met ses patotipes (UVPrt2, 3, 5, 9, 13 en 17) van *P. triticina*. Al die spesies was vatbaar vir UVPrt5, maar bestand teen die ander isolate. Hierdie interaksie tussen gashere en isolate is bewys dat die aanwinste slegs oor rasspesifieke (vertikale) weerstand beskik. Met fluoressensie-mikroskopie van weerstandskomponente is gevind dat prestomatale uitsluiting nie 'n rol in weerstand speel nie. By *T. turgidum* ssp. *dicoccum* var. *arras*, *T. turgidum* ssp. *durum* var. *aestivum* en *T. turgidum* ssp. *dicoccum* var. *murciense* was die vroeë abortering van swamstrukture belangrik. In

T. turgidum ssp. *polonicum* en Morocco (kontrole) is enkele, indien enige, vroeë aborsies opgemerk en swamkolonies was groot in vergelyking met die ander drie spesies. *T. turgidum* ssp. *dicoccum* var. *arras*, *T. turgidum* ssp. *durum* var. *aestivum* en *T. turgidum* ssp. *dicoccum* var. *murciense* het wisselende grade van gasheerselnekrose, tipies van hipersensitiewe en nie-volhoubare bestandheid, getoon. Geen nekrose kon by blaarroeskolonies in *T. turgidum* ssp. *polonicum* waargeneem word nie.

AFLP analise is gebruik om die introgressie van weerstand vanaf *T. turgidum* ssp. *dicoccum* var. *arras* na kommersiële heksaploïede koring, hul F_1, en weerstandbiedende en vatbare F_2 nageslag te volg. In die studie is gevind dat 12 AFLP merkers eksklusief van *T. turgidum* ssp. *dicoccum* var. *arras* oorgedra is na bestande F_2 plante. Sekere van die merkers afkomstig vanaf *T. turgidum* ssp. *dicoccum* var. *arras* was nie teenwoordig in al die weerstandbiedende F_2 nageslag nie (alhoewel hulle nie teenwoordig was in SST55 of die vatbare F_2 nageslag nie). Dit kan wees dat die merkers gekoppel is aan een van die aangrensende gebiede van die oorgedraagde segment, of slegs aan een van die twee gene verantwoordelik vir weerstand. In teenstelling met wat verwag is, is meer polimorfismes opgemerk in die tetraploïde *T. turgidum* ssp. *dicoccum* var. *arras* as in heksaploïede SST55, moontlik as gevolg van 'n hoë graad van verskil tussen hulle. Verder is die introgressie-frekwensie in die F_2 plante hoër vanaf *T. turgidum* ssp. *dicoccum* var. *arras* as vanaf SST55, wat aandui dat 'n onewe bydrae van genetiese materiaal van die respektiewe ouers oorgedra is na die vatbare en weerstandbiedende F_2 nageslag. Verdere navorsing is egter nodig om die statistiese betekenisvolheid van die data te evalueer.

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