6143 87207

U.V.S. BIBLIOTEEK

RDIE EKSEMPLAAR MAG ONDER
EN OMSTANDIGHEDE UIT DIE
LIOTEEK VERWYDER WORD NIE

University Free State
34300001330152
Universiteit Vrystaat

Maryke Craven

THE IMPROVEMENT OF LEAF RUST RESISTANCE IN SELECTED BREAD WHEAT LINES

by

MARYKE CRAVEN

Submitted in partial fulfillment of the requirements for the degree Magister Scientiae Agriculturae

In the Department of Plant Sciences
(Plant Pathology and Plant Breeding)
Faculty of Natural and Agricultural Sciences
University of the Free State

Supervisor: Prof. Z.A. Pretorius
Co-supervisors: Prof. M.T. Labuschagne and Dr. R. Prins

Bloemfontein
November 2002

Acknowledgements

My sincere gratitude to the following persons and institutions:

The Small Grain Institute of the Agricultural Research Council for their germplasm and to my colleagues Danie, Willem, Otilia and Fanus for their assistance.

To the Department of Genetics of the University of Stellenbosch for allowing me to perform my molecular research in their facility. Many thanks to all the wonderful M.Sc students for accepting a Kovsie in their midst, as well as to Viresh who taught me all the shortcuts.

To Annelie for her enormous support and to Sarita, who kept the Falling Number and Preharvest Sprouting programmes afloat during the stormy seasons.

To my parents, brothers and sister, as well as my in-laws for their continued interest and sympathy during the difficult times.

To my supervisors:
Prof. M.T. Labuschagne, thank you for your calm guidance.
Dr. R. Prins, you taught me what research was all about and opened the magical door of molecular markers. Thank you for everything.
Prof. Z.A. Pretorius, thank you for never giving up, it was an honour to be your student.

To my husband, Pieter: of all you asked the least and probably had to suffer the most. Thank you for always understanding.

Then lastly, but certainly not the least, to my Heavenly Father for allowing me to go on this journey and guiding me all the way.

Contents

General introduction

Various pathogens occur in the grain producing areas of South Africa each year, resulting in yield reduction as well as the downgrading of wheat (*Triticum aestivum*). Of these pathogens the three rusts (leaf, stem and stripe rust) are probably the most prevalent, with stripe rust (*Puccinia striiformis f. sp. tritici*) the most aggressive. In South Africa, leaf rust, caused by *Puccinia triticina,* occurs annually with the severity and distribution depending on the prevailing seasonal weather conditions. In the Western Cape nearly 300 000 ha of spring wheat are currently cultivated in environments that are highly conducive for leaf rust epidemics. Despite being seasonal, the impact of leaf rust on the local wheat industry should not be underestimated. To address this problem, breeders have focused on breeding for resistance. However, as it became clear that the pathogen is capable of regularly adjusting its pathogenicy, durabile resistance has become the ultimate goal. Various theories have been suggested as to how durability can be obtained, but most if not all remains to be proved.

The pyramiding of leaf rust resistance genes, as well as the incorporation of *Lr* genes obtained from wild relatives, have been suggested as durable strategies. It is further speculated that the incorporation of adult, as well as seedling resistance genes within a pyramiding strategy, will eventually result in durability. In this regard *Lr34* is considered a valuable gene, as it is known to enhance the effect of accompanying resistance genes. Although gene pyramiding is in theory a simple concept, the reality is that the incorporated genes are difficult to follow, and are quite often lost as the breeding programme progresses.

Molecular marker technology can be used to confirm more than one effective gene in a wheat line. These markers simplify the breeding process of gene pyramiding, as the presence or absence of each of the genes can be determined within the same plant, independent of their phenotypic expression. Several *Lr* genes, however, need to be mapped. The mapping of these genes is difficult, expensive

and time consuming, but molecular marker technology should be seen as a powerful and invaluable tool within current crop improvement programmes.

The objective of this study was, therefore, not only to improve leaf rust resistance in selected wheats, but to focus on durability as well as agronomic acceptability of resistant lines. This was achieved by using traditional breeding and pathology techniques, as well as a morphological marker, to combine certain *Lr* genes. Furthermore, an attempt was made to find a molecular marker for an effective, yet undesignated *Lr* gene obtained from *T. monococcum* with the use of AFLP technology.

Chapter 1
An overview of wheat leaf rust and strategies in breeding for resistance

1.1. Introduction

Cereal rusts appear to be as old as human guided evolution of cereal crops, as evidence of *Puccinia graminis* on wheat lemma fragments dated at 1400–1200 B.C. exists (Kislev, 1982). Since the beginning of agriculture mankind has been plagued by an enemy co-evolving with the cultivated plants themselves (Zadoks, 1993). Spontaneous mutation, sexual recombination, somatic hybridisation and recombination (during the parasexual cycle) provide the necessary means whereby new combinations of virulence may be generated within individual pathogen populations (Burdon, 1993). Migration and long distance dispersal of spores are also known to be important in the epidemiology of cereal rusts throughout the world (Luig, 1985; Nagarajan and Joshi, 1985; Roelfs, 1985; Park and Felsenstein, 1998). This was confirmed by Bayles *et al.* (2000) who concluded that the migration of spores plays a significant role in determining the virulence composition of populations of the stripe rust pathogen. Furthermore, the reduction of genetic variation in cultivated wheat makes it more vulnerable to diseases, with consequent limiting effects on food production (McIntosh *et al.*, 1995a).

As knowledge of cereal rusts accumulated, a new science of disease stabilization and management emerged. Improved understanding of the complexities of rust diseases is being utilized to slow the evolution of new pathotypes, to retard epidemics and consequently minimize losses (Shafer *et al.*, 1984). Crop protection is therefore necessary for the maintenance of production capacity and stability for an ever-growing population. Disease control also plays an important role in the prevention of negative effects on the quality of the product. Resistance is the preferred method of protection against diseases. It is the most cost effective, the best for the farmer, the end user of the product, and the

environment. Crucial, however, to the efficiency of breeding, is the durability of resistance. Correct management of available resistance can improve durability, which includes regional deployment of genes, multiline cultivars, mixtures of cultivars, gene stacking and polygenic resistance (Hogenboom, 1993).

The selection of genotypes containing several leaf rust (*Lr*) resistance genes using traditional infection studies is time-consuming and often not possible due to limitations in the array of pathotypes available (Roelfs *et al.,* 1992). Various molecular marker approaches have increased the ability to characterize and manipulate disease resistance genes in plants. These molecular techniques are important tools in the characterization of the interaction between plants and pathogens (Michelmore, 1995). The development of molecular markers for specific *Lr* genes allows the detection of these genes independently of the phenotype and presence of other *Lr* genes. Molecular markers can therefore be used for efficient combination of genes in the pyramiding strategy to create a more durable resistance (Roelfs *et al.,* 1992).

The objective of this chapter is to provide an overview of wheat leaf rust and its importance, as well as strategies in breeding for resistance. Emphasis is placed on the nature and application of molecular markers for disease resistance, particularly as justified by the potential of this technology to construct complex and hopefullly durable resistance to leaf rust.

1.2. Pathogen

1.2.1. Systematics and nomenclature

Based on the early distinction of leaf rust from stem rust, the name *Puccinia rubigo-vera* was assigned to the leaf rust fungus. According to Dickson (1956) this name was changed to *P. triticina* Eriks. following studies on specialization. However, Cummings and Caldwell (1956) suggested that *P. recondita* becomes the binomial applicable to leaf rust fungi. It should be noted that the name *P. recondita* was assigned to the rye leaf rust pathogen, with the wheat attacking

type as a specialized form (Anikster *et al.,* 1997). Wheat leaf rust was therefore given the name *P. recondita* f. sp. *tritici* (Knott, 1989). When it became clear that wheat leaf rust was a specialized and independent species, the name was changed to *P. triticina* (Anikster *et al.,* 1997).

Various factors lead to the conclusion that *P. triticina* was a species different from *P. recondita*. Firstly, their preferred host plants during the aecial stage differ. The alternate host for *P. triticina* is *Thalictrum speciosissimum* L. in the Ranunculaceae (d'Oliveira and Samborski, 1966) with *Isopyrum* and *Clematis* also acting as alternate hosts (Anikster *et al.,* 1997). *P. recondita* prefers *Lycopsis arvensis* L. in the Boraginaceae (Markovà and Urban, 1977) with *Anchusa* and *Echium* also being reported as alternate hosts. Secondly, the teliospores of *P. recondita* are 37% larger than those of *P. triticina*. Thirdly, the DNA content of *P. recondita* is on average 56% greater than that of *P. triticina (*Anikster *et al.,* 1997).

Throughout this dissertation *Puccinia triticina* will be used to indicate the wheat leaf rust pathogen, irrespective of whether authors used the *P. recondita* f. sp. *tritici* notation.

1.2.2. Symptoms

The wheat leaf rust pathogen primarily attacks the leaf blades, although it also, to a lesser extent, infects leaf sheaths and glumes (Knott, 1989). Following infection the fungal mycelium ramifies the leaf tissue and pustules (uredia) rupture through the epidermis (Gooding and Davies, 1997).

The orange-red uredia are round to ovoid, up to 1.5 mm in diameter, and scattered or clustered primarily on the upper surface of the leaf blades. Uredia are erumpent, without the conspicuously torn epidermal tissues at their margins, as with stem rust. Pigmented urediospores are released as the uredia rupture through the epidermis. The urediospores are 15-30 µm in diameter, subgloboid and red-brown, with three to eight germpores (Wiese, 1987).

Almost the entire surface of the leaf blade can be covered with pustules during a severe epidemic. Eventually the leaves senesce and dry out, resulting in a reduction in photosynthesis (Knott, 1989).

1.2.3. Environmental requirements

P. triticina is known to be a biotrofic, airborne pathogen that is most prevalent where wheat matures late (Wiese, 1987).

Requirements regarding temperature and light are fairly similar for leaf and stem rust, but differs for yellow rust. In general, three stages are identified for effective leaf rust infection. The first important requirement is a period of free water on plants at 15-24°C in the dark (Sharp *et al.*, 1958). This should be followed by an additional 2 to 4 h period with free water on plants but at a higher temperature of approximately 25°C. Lastly a slow drying period is required. A cool night with dew deposition followed by rising temperature and increasing light will therefore suffice for disease development (Knott, 1989).

1.2.4. Variability

Leaf rust, according to Schafer and Roelfs (1985), is more diverse for virulence than stem rust. The reason for this diversity has been attributed to population size as more inoculum survives between wheat crops, while the population size during the cropping season is also larger. Furthermore, the type of resistance being deployed against leaf rust has often been monogenic. McIntosh *et al.* (1995b) indicated significant pathogenic variation between regions of major geographical areas, which reflects the influence of geographical barriers, local inoculum survival between seasons and wheat genotype on pathogen populations.

A large array of mechanisms currently exists whereby pathogenicity may arise and new combinations of virulence are generated within individual pathogen populations (Burdon, 1993). Statler (1990) indicated that *P. triticina* has a spontaneous mutation rate for virulence of 4.7×10^{-4} which is high when compared to *Erysiphe graminis hordei* (2×10^{-8}; Torp and Jensen, 1985). Sexual recombination and somatic hybridization are also responsible for the adaptation of a pathogen to new resistances. Sexual recombination increases genetic diversity whilst the role of somatic hybridization is unclear. The process of somatic hybridization involves the exchange of whole nuclei and / or cytoplasm, and is postulated to be the origin of much of the pathogenic variation found in a range of fungal pathogens with reputations for high levels of variability but which lack sexual recombination systems (Burdon, 1993). The actual mechanisms involved with such somatic recombination as well as the frequency of such events are not known, but Watson (1981) speculated that the field survival of such new variants is rare. Evidence has, however, been presented that somatic hybridization occurs within *P. triticina* (Park *et al.,* 1999).

1.3. Economic importance

1.3.1. General economic importance

The three rusts have been considered the most important diseases of wheat world wide, despite progress made in their control in many countries (Saari and Prescott, 1985). Breeding for resistance to leaf rust has special significance because it is the most widespread rust disease of wheat (Sawhney, 1992). Leaf rust severity depends on crop development stage when initial infections occur, the relative resistance of the wheat cultivars (Kolmer, 2001), as well as the environment.

Yield losses attributed to leaf rust of 5–25 % have been reported in Canada (Kolmer, 2001). In Eastern Europe, leaf rust is considered the most damaging wheat disease (Dwurazna *et al.,* 1980) resulting in yield reductions of between 2 and 5%, but is not considered a serious problem in Western Europe (Samborski,

1985). Wheat crops in Egypt, Ethiopia and India also suffer severe yield losses due to leaf rust (Saari and Wilcoxson, 1974; Dmitriev and Gorshkov, 1980). *P. triticina* infection prematurely defoliates wheat plants, resulting in the shriveling of kernels (Knott, 1989). Yield reductions by leaf rust on susceptible varieties can be as high as 50% (Gair *et al.*, 1987). Experimentally, *P. triticina* has been demonstrated to inhibit yield potential by up to 70% (Johnston, 1967). Historically, one of the most significant leaf rust epidemics occurred in northwestern Mexico in 1976-1977. With approximately 80% of the area planted to Jupateco 73, severities of up to 50% were encountered. The effect of this epidemic could, however, have been minimized by avoiding large-scale monoculture of a single cultivar, seed multiplication programmes coordinated with breeding programmes to provide farmers with a choice of cultivars, strict observance of planting dates as well as a disease surveillance programme (Dubin and Torres, 1981).

The reason for the yield reductions can be attributed to various factors. Rusts increase transpiration and respiration and are also responsible for the reduction in photosynthesis and export of assimilates from the leaves. These pathogens can reduce plant vigour and root growth (Gooding and Davies, 1997).

It has been reported that the 1000-grain mass is a reliable indicator of yield loss due to leaf rust infection (Pretorius and Kemp, 1988). Kloppers and Pretorius (1995) indicated a 10.4% reduction in the 1000-grain mass of 'Thatcher' due to leaf rust.

1.3.2. Distribution and importance in South Africa

Even though leaf rust causes less damage than stem rust, it sometimes results in greater losses due to its frequent occurrence (Knott, 1989). In South Africa, leaf rust occurs annually with severity and distribution depending on the prevailing weather conditions (Pretorius *et al.*, 1987). The Western Cape environment is conducive for leaf rust epidemics and substantial economic losses can occur when a virulent pathotype coincides with a susceptible host under favourable

weather conditions. Approximately 300 000 ha of spring wheat are cultivated under these conditions (Boshoff *et al.*, 2002). During 1986, flag leaf severities of 100% were often recorded on leaf rust susceptible cultivars in commercial fields in the winter rainfall areas (Pretorius and Le Roux, 1988). During the 1999 wheat season, yield reductions of up to 40% due to leaf rust were experienced in the major wheat producing areas of South Africa (Boshoff, personal communication). In the Western Cape in particular, recent studies have indicated yield losses of up to 56%, with a significant reduction in hectolitre mass. A significant, but weak correlation was also obtained between protein content and area under the leaf rust progress curve (Boshoff *et al.*, 2002).

1.4. Resistance terminology and types

Vanderplank (1963) was the first to name and describe two types of resistance, namely vertical and horizontal resistance. Vertical resistance (synonym: race-specific resistance [Knott, 1989]) was defined as effective against some races, but ineffective against others, whilst horizontal resistance (synonym: race-nonspecific resistance [Knott, 1989]) refers to a type of resistance that is evenly spread against all races of the pathogen. Although the definition of vertical resistance is clear, the interpretation of horizontal resistance has been indistinct (Knott, 1989). Parlevliet (1995) similarly recognised two types of resistance according to their respective mechanisms and phenotypes, namely hypersensitive (HR) and non-hypersensitive or partial resistance.

A wide range of terms has been used for resistance in plants, i.e. seedling, adult plant, mature tissue, overall, field, complete, partial, quantitative, general, specific, horizontal, vertical, race-specific and durable resistance, all of which suggest different types of resistance, but actually describe certain aspects of resistance (Parlevliet, 1995). The different resistance terms are briefly reviewed in the following sections, despite some of them having common characteristics.

1.4.1. Pathotype-specific resistance

Pathotype-specific resistance refers to an interaction between genotypes of the host and genotypes of the pathogen and gene-for-gene relationships are therefore involved. This results in the resistance being effective against some races but ineffective against others. Pathotype-specific resistance is mostly monogenic, thus referring to a single major gene conditioning resistance. It is a qualitative trait usually expressed as a hypersensitive reaction (HR) (Roy, 2000).

The HR is an intense and rapid response characterized by premature death (necrosis) of the infected tissue surrounding an infection site, thereby inactivating and localizing the attacking agent. In rust terminology HR is characterized by a low infection type. The genes that characterize HR have large effects (major genes) and are usually dominant and non-durable (Parlevliet, 1995). This type of resistance may be complete or incomplete (Parlevliet, 1981) and is operative in the seedling stage as well as in the adult plant stage (Van Silfhout, 1993)

Immunity refers to the type of specific resistance where the plant is immune to infection by the pathogen, whilst moderate to intermediate resistance refers to the type of resistance where the pathogen penetrates the host and some rust development occurs before an incompatible reaction becomes apparent (Dyck and Kerber, 1985).

1.4.1.1. Seedling resistance

Many *Lr* genes are expressed in primary wheat leaves, e.g. *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr3bg, Lr3ka, Lr9, Lr10, Lr11, Lr14a, Lr14b, Lr15, Lr16, Lr17, Lr18, Lr19, Lr20, Lr21, Lr23, Lr24, Lr25, Lr26, Lr27, Lr28, Lr29, Lr30, Lr31, Lr32, Lr33, Lr36, Lr38, Lr39, Lr40, Lr41, Lr42, Lr43, Lr44, Lr45* and *Lr47* (Browder, 1972; Dyck and Samborski, 1974; Browder, 1980; McIntosh *et al.*, 1982; Dvorak and Knott, 1990; Pretorius *et al.*, 1990; Friebe *et al.*, 1992; Gupta and Saini, 1993; Parlevliet, 1993; Bariana and McIntosh, 1994; Cox *et al.*,1994; Dyck and Sykes, 1994; McIntosh *et al.*,1995a; Liu and Kolmer, 1997; Dubcovsky *et al.*, 1998). It is

assumed that most genes that are active in the seedling stage are also effective in adult plant stage.

All seedling resistance genes confer a hypersensitive response (Browder, 1980). Many of these produce an immune response to some rust cultures but visible fleck infection types to other (Dyck and Samborski, 1974). *Lr11, Lr16, Lr17, Lr18* and *Lr30* are examples of genes associated with an intermediate infection type (Dyck and Kerber, 1985).

1.4.1.2 Adult-plant resistance

Adult plant resistance (APR) can be defined as a type of resistance only expressed in the adult plant stage (Parlevliet, 1995) and was previously referred to as field resistance (Dyck and Kerber, 1985). *Lr12, Lr13, Lr22a, Lr22b, Lr34, Lr35, Lr37, Lr46, Lr48* as well as *Lr49* are expressed in the adult plant stage (Singh *et al.,* 1998; Kolmer, 1999; Saini *et al.,* 2002). Certain APR genes, e.g. *Lr13, Lr34* and *Lr37,* can be detected in seedlings following manipulation of the environment (Pretorius *et al.*, 1984; Drijepondt and Pretorius, 1989; Kloppers and Pretorius, 1997).

APR genes are highly influenced by background genotype, temperature, growth stage, light and in some cases, cytoplasmic factors. *Lr34* has shown variable reactions, even under controlled test conditions. For the efficient use and transfer of APR genes test conditions and pathotypes must be well defined (Gupta and Saini, 1993).

Genetic studies have indicated that inheritance of some APR forms is simple and may show race specificity (Gupta and Saini, 1993). APR is often expressed as hypersensitive resistance in flag leaves (Parlevliet, 1976).

1.4.1.3. Sources of pathotype-specific resistance

Several *Lr* genes have their origin in common wheat (McIntosh *et al.*, 1995b). Additionally, a wide range of disease resistance, including rust resistance, is contained within the wild relatives of wheat (Knott, 1989) and is essential for the creation of genetic variation within bread wheat (Kerber and Dyck, 1990). Successful interspecific and intergeneric crosses can be made among the Triticeae, due to the fact that wide crosses and polyploidy have played a major role in the evolution of the Triticeae (Knoblock, 1968).

In contradiction to hopes that resistance conferred by these wild relatives might be more durable, many forms of resistance obtained from wild relatives have been overcome by new virulent rust pathotypes. The advantage of these types of resistance is that they are initially effective against a wide range of rust pathotypes. The stem rust resistance gene *Sr*26, transferred from *Agropyron elongatum* (*Thinopyrum elongatum*), is an example of resistance obtained from wild relatives that has remained effective despite its wide cultivation in Australia (Knott, 1989).

Lr9 (*Aegilops umbellulata;* Sears, 1956), *Lr19* (*Agropyron elongatum;* Sharma and Knott, 1966), *Lr24* (*Agropyron elongatum;* Smith *et al.,* 1968), *Lr25* (*Secale cereale*; Driscoll and Jensen, 1964) *Lr26* (*Secale cereale*; Zeller, 1973), *Lr28* (*Aegilops speltoides;* McIntosh *et al.,* 1982), *Lr35* (*Triticum speltoides*; Kerber and Dyck, 1990) and *Lr37* (*Aegilops ventricosa*; Dyck and Lukow, 1988) are some of the resistance genes obtained from wild relatives.

None of the named leaf rust resistance genes obtained from wild relatives originates from *T. monococcum* (Jacobs *et al.,* 1996), despite the fact that this species has been reported highly resistant to wheat leaf rust (Niks and Dekens, 1991; Dyck and Bartos, 1994). According to Jacobs *et al.* (1996), however, the type of leaf rust resistance transferred from *T. monococcum* is not different from other existing sources conferring hypersensitive resistance in common bread wheat. Hussien *et al.* (1998) reported three undesignated *Lr* genes that originated from *T. monococcum*.

The usefulness of single genes for resistance is, however, limited since the pathogen has developed virulence to *Lr* genes in previously resistant cultivars (Kolmer, 1999).

1.4.2. Pathotype non-specific resistance

A variety is said to possess pathotype non-specific resistance if it shows resistance against all or a range of pathotypes. No gene-for-gene interaction therefore occurs (Parlevliet, 1985).

This type of resistance is quantitative in nature and the different genotypes differ in the extent of development of disease (Roy, 2000). In the case of a non-hypersensitive response (also referred to as partial resistance, Parlevliet, 1995), no cell collapse occurs. The pustules appear normal (high infection type) but epidemic development is slower. This type of resistance is often controlled by genes with small effects (oligogenic and polygenic, Parlevliet, 1995). Partial resistance is thought to be durable (Parlevliet, 1981).

1.4.2.1 Partial resistance

According to Parlevliet and Ommeren (1975), partial resistance (PR) is characterised by a reduced rate of epidemic development, despite a susceptible infection type.

PR results in a longer latent period and smaller colony size (Jacobs and Buurlage, 1990). One, two or three recessive genes condition a long latent period (Lee and Shaner, 1985). Jacobs and Broers (1989) confirmed that the inheritance of longer latent period is expressed as a recessive or partially recessive characteristic and that the gene action is to a large extent additive.

PR operates after penetration of the host (Niks, 1986). The delayed epidemic build-up observed in PR genotypes is due to the abortion of infection structures.

Reduction in mycelium growth in partial resistance genotypes compared to the growth in susceptible genotypes was also reported (Jacobs, 1990).

The identification of this type or resistance can be influenced by the growth stage, environmental sensitivity, inoculum pressure in disease nurseries, and final infection type in the case of stripe rust (Singh *et al.,* 2000). Although temperature-insensitive in barley (Parlevliet, 1975), latent period was better expressed at lower temperatures in wheat. Cultivar differences in PR also increased with a decrease in temperature in both seedling and adult plants (Broers and Wallenburg, 1989).

PR screening can be done on the basis of assessment of latent period, uredium number, uredium size and inoculum production on adult plants (Jacobs, 1990) in the greenhouse or in field nurseries (Broers, 1989). There is, however, no substitute for field evaluation for the usefulness of the resistance (Singh *et al.*, 2000).

Parlevliet (1978) indicated that small pathotype-specific effects within previously assumed non-specific resistance (partial resistance) occurred. This was confirmed by Johnson (1988) who presented examples of adult resistance genes that are race specific in nature. Habgood and Clifford (1981) however indicated that, despite the occurrence of small pathotype-specificity, no erosion of partial resistance to barley leaf rust was observed in Western Europe.

1.4.2.2. Oligogenic resistance

The boundary between oligogenic and polygenic systems is somewhat arbitrary (Roy, 2000). Parlevliet (1995) defined oligogenic resistance as a resistance where several genes have effects that are between those of *major* and *minor* genes, resulting in a *quantitative* expression (Parlevliet, 1995).

On the basis of phenotypic expression of infection types, Samborski and Dyck (1982) showed that the gene combinations *Lr13* + *Lr16, Lr30* + *Lr3ka, Lr30* +

Lr11, and *Lr33* + *Lr34* exhibited higher levels of resistance than either the respective *Lr* genes alone, especially those expressing adult plant resistance. The gene-for-gene relationship assumes that when more than one set of corresponding gene pairs are involved, the resulting level of resistance is at least that conferred by the most incompatible of the interaction gene set (Sawhney, 1992).

Kolmer *et al.* (1993) indicated that *Lr13* and *Lr34* conditioned increased resistance when paired with the seedling resistance genes *Lr3ka, Lr16, Lr17, Lr18, Lr21, Lr30* and *Lr33*. Gene combinations *Lr13* + *Lr16* and *Lr13* + *Lr34* conditioned effective resistance. Kloppers and Pretorius (1997) also compared the effects of gene combinations of lines containing *Lr13* + *Lr34, Lr13* + *Lr37* and *Lr34* + *Lr37* to that of the single gene lines. Significant reduction in both fungal growth and colony size was reported in all three lines containing the two *Lr* genes.

1.4.2.3 Polygenic resistance

Polygenic resistance refers to a form of durable resistance that is controlled by several genes, each having a small effect (Knott, 1989), but collectively providing protection to a wide spectrum of pathotypes (Roy, 2000).

In an effort to obtain near immunity to leaf rust and stripe rust in wheat by combining slow rusting genes, Singh *et al.* (2000) crossed parents that showed moderate to high levels of slow rusting. F_1s were top-crossed (three-way) with a third parent that had high yield potential and at least some form of resistance. This strategy allowed for the selection of high yield potential lines, which showed near immunity, presumably as a result of polygenic interaction.

1.5. Durable resistance

Irrespective of the many terms and descriptions used, durability is the primary objective in breeding wheat for rust resistance. According to Knott (1989) durable resistance can be defined as the capability of a variety to retain its resistance over several generations or an extended period, despite its wide cultivation and the presence of an environment that favours the development of diseases and pests. For a cultivar to be legitimately described as possessing durable resistance, it must be judged in relation to the performance of other cultivars and to what is known of the relevant pathosystem. Widespread cultivation is therefore a stronger indicator of durable resistance rather than a particular phenotype (Johnson, 1993).

Gene pyramiding (Kloppers and Pretorius, 1997), partial resistance (Parlevliet, 1995), the incorporation of resistance genes obtained from wild relatives (Knott, 1989) into the wheat genome as well as the use of multiple crosses for increasing genetic diversity (Dubin and Rajaram, 1981), are considered to be possible solutions for the creation of durable resistance.

Several leaf rust resistance genes have been identified (McIntosh *et al.*, 1995b), but due to the fact that pathogens are capable of adjusting their virulence, most of these resistance genes are now ineffective (Singh, 1992b). One such an example of non-durability is the leaf rust resistance gene *Lr16*. As many of the wheat cultivars cultivated in Canada have *Lr16*, leaf rust severity has increased due to the erosion of the *Lr16* resistance gene (Kolmer, 2001).

1.6. Breeding strategies

1.6.1. Breeding for pathotype-specific resistance

Single gene resistance can be introduced to an otherwise desirable variety through a standard backcrossing (BC) procedure. The choice of recurrent parent is important as an increase in the inherent yielding ability of the cultivar is not

expected (Dyck and Kerber, 1985). The backrossing procedure allows for the creation of BC lines which could be released as new cultivars when existing ones become susceptible (Johnson and Lupton, 1987). It also allows for the accumulation of effective genes into a single genotype (Knott, 1989).

When breeding for leaf rust resistance, the presence of the desired gene should be determined in individuals of a BC population by exposure to the pathogen. Resistance can be dominant or recessive, thus influencing the genotype of selected donor plants. In the case of a dominant trait, continuous screening and selecting of seedling resistant plants, followed by selfing, will eventually result in homozygous dominant resistant plants (Roy, 2000).

Lr genes can also be incorporated to wheat lines through a pedigree system where selections are made among and within families or lines. Record is then kept of entries throughout all selection and testing phases. This allows the tracing of any progeny plant in any generation back to the original selection and cross (Roy, 2000). The breeding strategy employed at the International Maize and Wheat Improvement Centre (CIMMYT) emphasizes pedigree breeding with multiple or double crosses that lead to a rapid increase in genetic diversity (Dubin and Rajaram, 1981). In general, selections for leaf rust resistance are made in the early stages of cultivar development. However, as resistance is frequently dominant or partially dominant, it is important that further selections be made in later generations to ensure that homozygous lines are obtained (Dyck and Kerber, 1985).

With the bulk method, seeds produced in a given generation are harvested in bulk. Only a fraction of these seeds are sown to give rise to the next generation (Roy, 2000). With breeding for leaf rust resistance, segregating generations (F_2 to F_6) are grown in bulk and exposed to a disease epidemic. This technique allows for many crosses to be handled with minimal labour (Dyck and Kerber, 1985).

1.6.2. Breeding for pathotype non-specific resistance

In principle, breeding for non-specific resistance relies on the accumulation of several genes, whether minor or major, in a single, pure-breeding genotype. In a way gene pyramiding is analogous to partial resistance breeding. According to Burdon (1993) the reason for the success achieved with pyramiding, lies in the likelihood of simultaneous mutation for virulence at any loci. The result is a reduced rate of evolution and a less diverse pathogen population.

The South American wheat 'Frontana', judged one of the best sources of durable resistance to leaf rust, carries *Lr34* in addition to *Lr13* and *LrT3*. According to Samborski and Dyck (1982), its high level of resistance has been attributed to interaction of *Lr34* and *LrT3* in a complementary manner. Improved APR was also obtained with the combination of *Lr34* and *Lr12* (Dyck, 1991).

According to Sawhney *et al.* (1989) the answer to durable resistance lies in the combination of seedling resistance and APR genes within the same wheat genome. The importance of the APR gene *Lr34* in this regard is emphasised due to its interaction with other resistance genes (Sawhney, 1992). *Lr13* enhances the resistance of other *Lr* genes in a similar manner to *Lr34* (German and Kolmer, 1992). *Lr34* is an APR gene located on chromosome 7D, although it may change to another position due to translocation (Dyck *et al.,* 1994). *Lr34* has been reported to increase the latent period, decrease infection frequency and uredial size. The gene is expressed by infection type 2^+ but without accompanying chlorosis. In wheat cultivars that lack other effective *Lr* genes, *Lr34* expresses resistance in a quantitative way (Drijepondt and Pretorius, 1989).

The complex interactions between temperature, light, host growth stage, pathogen and wheat genotype are determinative in the *Lr34* resistance phenotype (Dyck and Samborski, 1982; Singh and Gupta, 1992). The identification of *Lr34* in glasshouse-grown adult plants is however possible, providing adequate temperature control is available (Pretorius *et al.,* 1994). According to Drijepondt *et al.* (1991a), South African pathotypes do not detect

Lr34 in seedlings between 15-25°C and that such tests should be conducted at continuous 10°C.

Lr34 is associated with leaf tip necrosis. Symptoms of this condition become visible at flowering and include 2 to 3 cm of necrosis at the distal end of leaves, extending an additional 2 to 4 cm down the edges. The gene causing leaf tip necrosis is designated *Ltn*. This association between *Lr34* and *Ltn* is of practical value since it allows the detection of *Lr34* without the necessary scoring for leaf rust response (Singh, 1992a). *Lr34* is also associated with an APR gene for stem rust (Dyck, 1987), the APR gene *Yr18* to stripe rust (Singh, 1992a), and with tolerance to barley yellow dwarf virus (Singh, 1993). *Lr34* is therefore considered to be an important resistance gene, that should protect plants during growth stages when serious yield losses could be incurred (Drijepondt *et al.*, 1991b).

According to Rubiales and Niks (1995), the resistance conditioned by *Lr34* fits the definition of partial resistance and demonstrates that this phenotype can result from a single gene. Kolmer (1992) stated that *Lr13* and/or *Lr34* may in fact be present in wheat with 'partial resistance' to leaf rust. This statement was confirmed by Kolmer and Liu (2001) as one of the cultivars, 'BH1146', previously characterized as having partial resistance, was shown to have the APR genes *Lr13* and *Lr34*.

Recent studies indicated that *Lr46* resembles the type of reactions obtained with *Lr34* in adult plants. *Lr46* confers a similar non-hypersensitive type of response to wheat leaf rust as *Lr34*, as latent period is prolonged, with a higher percentage of abortion being observed. A reduced colony size together with a lower disease severity were reported (Martínez *et al.*, 2001).

Although field resistance conferred by *Lr34* remains highly effective throughout wheat growing areas in South Africa (Pretorius *et al.*, 1984), variations in expression have been reported in Mexican environments (Singh and Gupta, 1992).

When breeding for PR, the objective is to achieve an acceptable and manageable level of disease rather than total immunity. Non-specific resistance is similar to any other quantitative trait in being polygenically determined. Various methods of population breeding can be used, with the most appropriate method being recurrent selection. Recurrent selection increases the opportunity for recombination and expression of new blocks of genes which allows the breeder to maximise the progress through selection (Roy, 2000).

According to Parlevliet (1995) selections within a genetically heterogeneous population should be aimed at the removal of the most susceptible entries and those who do not show any disease. With the elimination of the latter entries, the danger of selecting major genes is diminished. Further selection among the remaining entries should be done according to agronomically desirable traits. With such a continuous selection program, resistance of the non-major gene type is accumulated. However, it is unlikely that major advances will be made through selection only and that further cycles of gene recombination followed by selection should be encouraged.

1.6.3. Molecular markers

More than 46 leaf rust resistance genes (*Lr*) have been designated and mapped in wheat (Feuillet *et al.*, 1995). In addition, DNA markers have been linked to *Lr1, Lr2, Lr9, Lr10, Lr13, Lr18-20, Lr23-25, Lr27, Lr29, Lr31, Lr32, Lr34, Lr35, Lr37 and Lr41* (Lottering *et al.*, 1999; Botha and Venter, 2000). These molecular markers allow the detection of the specific genes within genetic backgrounds of wheat that, in turn, simplify gene pyramiding within breeding programmes.

1.6.3.1. The ideal marker

According to Weising *et al.* (1995) the ideal marker should show highly polymorphic behaviour and inherit co-dominantly, which will result in homo-and heterozygotic states in diploid organisms being observed. The marker should

occur frequently as well as be evenly distributed throughout the genome. It should show selectively neutral behaviour (no pleiotropic effects) and be detectable at all plant stages. Their assays should be procedures that are easy, fast and amenable to automation. Lastly, the marker should be highly reproducible with easy exchange of data between laboratories.

Even though various marker systems have been developed, none are capable of delivering a marker that would fulfill all of the above requirements. Each marker system therefore has its advantages and disadvantages, which should be taken into consideration when choosing a molecular marker system. The main factors that need to be taken into consideration are the intent of the application, convenience and the cost involved (Gupta *et al.*, 1999).

Marker technology is, in general, expensive. Since the various techniques have different requirements, some are more expensive than others. Cost involved with a specific molecular marker system is mainly dependent of:

- Time required for DNA extraction
- Amount of DNA required
- Necessity of cloning and sequencing
- Amount and type of genetic information required
- Type of marker (dominant or co-dominant)
- Automation of a marker system
- Use of the resulting genetic map
- Proprietary status of the technique (Roy, 2000)

1.6.3.2. Molecular marker systems

In an effort to develop the ideal marker, various new DNA marker systems have been developed by modifying existing techniques. The basic principles are therefore the same in many of the systems. Up to date molecular markers can be classified into three groups:

1. Hybridization-based DNA markers such as restriction fragment length (RFLPs) and oligonucleotide fingerprinting.
2. PCR-based DNA markers
3. DNA chip and sequencing-based DNA markers such as single nucleotide polymorphisms (SNPs) (Gupta *et al.*, 1999).

Restriction Fragment Length Polymorpisms (RFLPs)

Poehlman and Sleper (1995) define RFLPs as *different fragment lengths of restriction endonuclease digested DNA detected by a defined probe between individuals*. DNA is cleaved by restriction enzymes that recognize specific DNA sequences resulting in different length fragments. Fragments so formed are identified by Southern blotting, a technique by which fragments can be separated by gel electrophoresis according to size and then transferred to a membrane (Southern, 1975). Specific sequences of DNA, cloned by a vector and called a *probe*, hybridize with complementary segments of DNA cleaved by the restriction enzymes. The probe is often labeled radioactively which will make the detection of hybridization possible with autoradiography. Each probe detects one or more genetic loci that share sequence homology whilst each allele at a locus is identified as a mobility variant of an endonuclease restriction fragment (Roy, 2000).

RFLPs are stable, universal as well as convenient. RFLP markers are also inherited co-dominantly (identifying homo- and heterozygote individuals), are detectable in all living tissues at all stages of development and are not affected by the environment (Poehlman and Sleper, 1995).

Markers based on RFLPs are however restricted to low copy sequences (William *et al.*, 1997) and due to its low frequency, not very effective in wheat (Gupta *et al.*, 1999). The RFLP technique is also laborious. Automation is difficult, resulting in the technique not being used on a routine basis within plant breeding programmes. A substantial amount of DNA (5-10 ug) in comparison to the Polymerase Chain Reaction (PCR) based techniques is also required (Weising

and Kahl, 1997). Detection of single nucleotide differences is restricted to differences that are present in restriction sites. Deletion/insertion mutations or methylation modification at the restriction sites are also detected by this technique (Appels *et al.,* 1986). In comparison to some of the PCR based marker techniques, the level of polymorphism obtained with RFLPs is quite low (Williams *et al.,* 1990).

RFLPs are used for cultivar identification, genetic mapping, germplasm evaluation and as indirect selection criteria (Poehlman and Sleper, 1995). RFLPs are better suited for QTL mapping than RAPDs due to their co-dominant nature. Since RFLPs can be scored as alleles at a locus, various statistical methods can be applied for estimating heterozygosity, genetic distances and gene flow. RFLP technology has been found to be more useful for the selection of chromosomal regions carrying useful genes derived from wild relatives (Koebner *et al.,* 1988; Jia *et al.,* 1994). RFLPs are detectable both within and among species and fragments detected in different individuals or species contain homologous sequences. RFLP linkage maps constructed with one population will be useful in other populations of the same species as well as closely related species (Roy, 2000).

PCR-based methods and markers

The analysis of nucleotide sequence variability has been revolutionized by the polymerase chain reaction (PCR, Saiki *et al.,* 1988). Before the application of PCR-based markers, the construction of wheat genetic maps was slow due to the limited level of polymorphism in wheat (Chao *et al.*, 1989).

PCR is an *in vitro* technique for the enzymatic amplification of specific DNA segments for genomic DNA or RNA (following reverse transcription). No construction of genomic or cDNA libraries (for probes) is needed, as is the case with RFLPs (Evola *et al.,* 1986; Roy, 2000).

PCR has been used to develop several marker systems. These marker systems can be divided into two groups: i) arbitrarily primed PCR and other multi-locus profiling techniques and ii) sequence targeted and single locus PCR (Karp and Ewards, 1997).

- Arbitrary primed PCR and multi-locus profiling can be subdivided into two types of markers:

  - Arbitrary primed PCR where a single arbitrarily chosen primer is used to amplify short segments of the genomic DNA that share sequence similarity to the single primer. Primers that are binding to opposite strands and which are sufficiently close together will succesfully amplify. Random amplified polymorfic DNA (RAPDs) and DNA amplification fingerprinting (DAFs) are examples of this type of marker.

  - Semi-arbitrary PCR markers imply that their primers are based upon fragments that correspond with restriction enzyme sites or sequences that are interspersed in the genome, such as repetitive elements, transposable elements and microsatellites. Amplified fragment length polymorphisms (AFLPs) and a number of versions in which microsatellites are used as primers e.g. randomly amplified microsat polymorphisms (RAMPs) and single primer amplification reaction (SPAR) are examples of this type of marker.

- Sequence targeted and single locus PCR refer to simple sequence repeats (SSR), sequence tagged microsatellite sites (STMS), sequence characterized amplified regions (SCARs), sequence tagged sites (STSs) etc. PCR is directed to specific, single-locus targets. Knowledge of the sequence of the target or flanking target regions is required.

With the use of PCR, larger populations can be screened in less time. PCR is a safe and efficient method, which requires smaller amounts of DNA due to its automated nature (Paabo *et al.,* 1988). A major disadvantage of certain PCR

methods is the requirement of sequence information. Another limiting factor is the size of the PCR products. Cohen (1994), however, reports on improvements made in amplifying longer stretches of DNA with the so-called long-PCR technique.

RAPDs are rapid, require small amounts of DNA with no radioactivity, are usually dominant (Botha and Venter, 2000) and have succesfully been used in the mapping of the leaf rust resistance genes *Lr9*, *Lr24, Lr28, Lr29* and *Lr34* (Schachermayr *et al.,* 1994; Procunier *et al.,* 1995; Schachermayr *et al.,*1995; Dedryver *et al.,* 1996; William *et al.*, 1997; Naik *et al.,* 1998). No knowledge of the targed DNA sequence is needed (Williams *et al.*, 1990). RAPDs detect the presence of only one allele at a locus (amplified allele) wherearas the absence of an amplification band represents all other alleles at that locus that failed primer amplification (Roy, 2000). Mismatched pairing between primer and template, deletions of primer sites and insertion between primers sites are considered to be responsible for polymorphisms observed (Williams *et al.*, 1990; Paran and Michelmore, 1993).

RAPD however show poor reproducibility between laboratories due to the sensitivity of the random amplified step (Devos and Gale, 1992; Schachermayr *et al.*, 1994) and as with RFLPs, has proved to be not as useful in wheat. This is also due to the low level of polymorphism within wheat (Gupta *et al.*, 1999).

Microsatellites are generally referred to as SSRs (Jacob *et al.,* 1991) or simple tandem repeats (STRs; Archibald, 1991). Loci are amplified by PCR using primers (18-25 base pairs long) which are specific for sequences flanking hypervariable regions of tandem repeats of two to four base pairs (Manifesto *et al.,* 2001). SSRs are highly abundant and evenly distributed, highly polymorphic, co-dominant, easily assayed by PCR and very accessible due to published primer sequences (Litt and Luty, 1989; Weber, 1990; Saghai-Maroof *et al.,* 1994) and have made significant contributions to plant genetic studies. Microsatellites are restricted to intraspecific and intragenomic analysis. They are therefore not suitable for comparative analysis or for introgression studies involving wild species related to wheat (Gupta *et al.*, 1999). SSRs are locus-specific in most

species (Condit and Hubbell, 1991; Senior and Heun, 1993; Wu and Tanksley, 1993; Taramingo and Tingey, 1996) and an extensive effort is needed to screen the whole genome with SSR markers in attempts to identify markers for a gene with an unknown chromosomal location. The research effort and cost involved therefore restrict their use in many laboratories (Brown *et al.,* 1996).

- ***Amplified Fragment Length Polymorphisms (AFLPs)***

Amplified fragment length polymorphisms (AFLPs) are a DNA marker analysis system based on a combination of PCR and restriction enzyme analysis (Vos *et al.,* 1995). With this technique, PCR products are resolved on denaturing polyacrylamide gels.

AFLPs are highly efficient in revealing polymorphisms compared with other DNA marker systems (Shan *et al.,* 1999). An unlimited amount of loci can be assayed with different combinations of a relative small number of oligonucleotide primers and the ability of AFLPs to distinguish among genotypes is not hindered by their bi-allelic nature, i.e. presence or absence (Mackill *et al.,* 1996). AFLPs are also more reproducible, exhibit intraspecific homology (Powell *et al.*, 1996; Law *et al.*, 1998) and require no sequence information (Ma and Lapitan, 1998).

AFLPs are time consuming and expensive (Mackill *et al.*, 1996). Single bands on a gel can sometimes comprise of several co-migrating amplification products, making analysis difficult (Botha and Venter, 2000). The technique is difficult to perform and not optimal for high through-put screenings associated with breeding programmes. This time problem is, however, being overcome by the use of fluorescence-based, semi-automated methods. Fluorescence dyes with distinguishable wavelength emissions allow for the electrophoresis of different samples simultaneously in a single lane, resulting in an three fold enhancement in throughput compared to conventional electrophoresis systems (Schwarz *et al.,* 2000).

The technical complexity and the associated high costs of AFLP result in it not being suited for high throughput marker-assisted selection situations. The aim is therefore to convert AFLP markers to systems, such as sequence-tagged (STS) markers, which are capable of handling a high throughput of material. For this purpose the conventional electrophoresis and visualisation systems are still nesessary, because access to fragments is not possible using automated DNA sequences (Schwarz *et al.,* 2000).

- ***Sequence specific PCR markers (SCARs and STSs)***

The conversion of multi-locus marker types such as AFLPs and RAPDs through cloning, sequencing and primer design to sequence specific markers successfully addresses the above mentioned problems of reproducibility, high throughput and co-migrating amplification products (Lottering *et al.,* 2002). Paran and Michelmore (1993) resolved the problem of RAPD reproducibility by deriving SCAR markers from the initial RAPD markers for the *Dm* resistance genes in lettuce. They defined SCARs as *a genomic DNA fragment at a single genetically defined locus that is identifiable by PCR amplificaton using a pair of specific oligonucleotide primers.* The two ends of the RAPD amplified product are cloned and sequenced and used as primers for the amplification of the single bands or SCARs. SCARs, however, differ from STSs as repetitive sequences might occur within the amplified fragment. They can also be dominant or co-dominant. SCARs are more reproducible than RAPDs, can be developed into plus/minus arrays where electrophoresis is not required and show less variability among different thermocyclers and when different DNA polymerases are used (Paran and Michelmore, 1993; Schachermayr *et al.*, 1994, 1995; Roy, 2000). The inability to convert RAPD markers to SCARs has however been reported (Adam-Blondon *et al.,* 1994; Borovkova *et al.,* 1997; Venter and Botha, 2000). This is probably due to the loss of uniqueness of the primer-binding site through the loss of the base subsitution on which the polymorphism is based (Masuelli *et al.,* 1995). Another hypothesis results from the fact that the annealing of a short primer (10bp) to a long template results in the middle base pairs annealing more tightly than those at the 3' and 5' ends (Chen and Wu, 1997). This results in a

specific primer that contains two bases that are not complementary to the template. The incidence of repetitive sequences might also play an important role, as it causes the specific primer to amplify a homoeologous loci located on another chromosome which also gives rise to a similar banding pattern (Penner, 1996).

Success has however been reported with the conversion of markers to SCARs within wheat. Dedryver *et al.* (1996) reported a SCAR-marker developed from a RAPD marker for *Lr24*, while a RAPD-SCAR marker was developed for the wheat aphid resistance gene *Dn2* (Myburg *et al.,* 1998). Other studies that report successes include sorghum (Boora *et al.,* 1999), rye (Gallego *et al.,* 1998), tomato (Kawchuk *et al.,* 1998), apple (Tartarini *et al.,* 1999), sugar beet (Giorio *et al.,* 1997) and rape seed (Delourme *et al.,* 1994).

The construction of SCARs is, however, not limited to the use of RAPD technology. Xu *et al.* (2001) reported on the conversion of AFLP markers linked to the *Vf* gene in apples to SCARs. As is the case with RAPDs, the internal sequences from both ends of the AFLP marker were used to design the 25 base pair SCAR primer.

A sequence-tagged site (STS) is a short unique sequence (200-500 bases long) amplified by PCR that identifies a known location on a chromosome. Its sequence does therefore not occur anywhere else in the genome. STSs can be amplified by PCR from a genomic library or gDNA using specific oligonucleotide primers (Olson *et al.,* 1989). As a result, a single band will be obtained with electrophoresis, corresponding to the size of the target region.

Olson *et al.* (1989) proposed the use of STSs as a common language for the development and synthesis of a physical map of the human genome, as STS markers that vary in length serve as both physical and genetic markers. According to Primrose (1995) such type of markers should be referred to as polymorphic STS markers. Conventionally, the term STS is used for the primers which are designed on the basis of mapped low-copy RFLP probes (Gupta *et al.*, 1999) but primers designed on the basis of RAPDs, have also been referred to

as STSs (Naik *et al.*, 1998). Once constructed, STS primer sets offer advantages of safety (no radio-isotopes), relative ease, greater throughput, convenience of sharing primer sequences over RFLPs while incorporating the advantages of PCR (Martin *et al.*, 1995; Erpelding *et al.*, 1996). STS primers developed in cereals are also potentially transferable between related species, as is the case for RFLPs (Talbert *et al.*, 1994).

Schachermayr *et al.* (1997) developed an RFLP-STS marker for *Lr10,* while Hu *et al.* (1997) were successful in developing a RAPD-STS marker for the powdery mildew resistance gene *Pm1*. The process of AFLP marker conversion to STS markers has, however, proved to be non-trivial in barley and wheat (Shan *et al.*, 1999; Seo *et al.*, 2001). Shan *et al.* (1998) managed to developed chromosome specific STS primer pairs from polymorphic AFLP fragements. Prins *et al.* (2001) also succeeded in the conversion of a fragment accociated with *Lr19* to a dominant STS marker. In these studies it became clear that not all AFLP products will be suited for conversion. One of the disadvantages of this technique is the requirement to isolate and develop the markers for each new crop (Brady *et al.*, 1996). Time is therefore spent on the cloning of fragments (Talbert *et al.*, 1994; Thomas and Scott, 1994).

1.6.3.3. Marker system selection

With the development of a functional marker, the technique chosen should fulfill certain criteria. The intended application, convenience and the cost involved are the main factors that influence the choice of marker system (Gupta *et al.*, 1999). The advantages of PCR based marker technology are the small sample requirement, high throughput and early selection (Roy, 2000).

In general, the development of molecular markers that are specific for one particular gene appears to be difficult for a gene derived from the wheat gene pool, whereas it is easier to find specific markers if the gene originates from a wild relative of wheat (Schachermayr *et al.*, 1997). This is due to the large genome size of bread wheat, the low levels of molecular polymorphisms within

the species and the overwhelming presence of repetitive sequences (William *et al.*, 1997).

A well-suited marker system for fingerprinting should reveal a high degree of polymorphisms. In a study of diversity among legumes (Azuki), 83% of the AFLP primer pairs used generated polymorphic bands, compared to the 26% of RAPDs (Yee *et al.*, 1999). Garg *et al.* (2001) found that AFLPs delivered the highest number of polymorphic bands per assay, followed by RAPDs and SSRs, but that SSRs delivered the highest polymorphic information content (PIC). In contradiction, Bohn *et al.* (1999) found that the PIC values were similar for RFLPs, AFLPs and SSRs in wheat. They also indicated that the marker index, which is a product of the number of polymorphic loci in the analyzed cultivars and the average PIC values (Powell *et al.*, 1996), was low for RFLPs and SSRs but high for AFLPs. The same type of data was generated with soybean (Powell *et al.*, 1996) and barley (Russell *et al.*, 1997). AFLPs are therefore recommended for fingerprinting, quality control as well as for the identification of essentially derived varieties (Bohn *et al.*, 1999).

Even though polymorphic microsatellite markers within wheat were only 22%, Ma *et al.* (1996) speculates that microsatellite markers can detect more polymorphic alleles per marker than RFLP. With the comparison of differentiation capability of RAPD and SSR markers in barley, Kriac *et al.* (1998) concluded that SSR has a higher differentiation efficiency than RAPDs. Ma and Lapitan (1998), however, found that the availability of SSRs is limited as well as time consuming. They concluded that AFLP markers do not require DNA sequence information, as is the case with STS-PCR and microsatellite markers. With AFLP there are no costs required for marker development. Compared to RFLP or other PCR-based marker systems, AFLP is fast, reliable and cost-effective (Ma and Lapitan, 1998). The technique contributed significantly to the development of plant genetic maps (Gupta *et al.*, 1999) and the identification of DNA tags for useful genes such as *Lr41* (Lottering *et al.*, 2002) and *Lr19* (Prins *et al.*, 2001) for leaf rust and *Pm1c* for powdery mildew resistance (Hartl *et al.*, 1999).

With their comparative studies into the different technologies, Powell *et al.* (1996) concluded that SSRs and AFLPs will become popular due to their efficiency, despite their high developmental costs, whilst RAPDs will become popular due to their low cost and simplicity, despite the fact that they are the least efficient.

1.6.3.4. Bulk segregant analysis (BSA)

In an effort to simplify the identification of markers in the absence of near-isogenic lines, bulk segregant analysis (BSA) was introduced (Melchinger, 1990). BSA was initially proposed for screening qualitative traits known to express variation at a single locus of large effect (Giovannoni *et al.,* 1991; Michelmore *et al.,* 1991). With this technique DNA from two or more individuals in a segregating population that are identical for the gene of interest is pooled. These individuals may be arbitrary for all other genes. According to Michelmore (1994), the arbitrary nature of the pooled segregants will ensure that the chance of identifying a molecular marker that differentiates between the segregating offspring is improved.

The simplicity and the low cost of this technique resulted in it being used for more complex traits and is often restricted to segregating generations which are simpler and cheaper to produce, such as backcross and F_2 generations (Mackay and Caligari, 2000).

In several studies, a BSA approach led to the identification of DNA markers linked to useful genes in wheat, apparently irrespective of the type of marker system. Hartl *et al.* (1999) were able to link an AFLP marker to powdery mildew resistance, William *et al.* (1997) used BSA for the detection of quantitative trait loci associated with leaf rust resistance in wheat, while Shi *et al.* (1998) had success with identifying a RAPD marker for powdery mildew resistance.

Kölliker *et al.* (2001) reported on the use of bulked leaf samples (opposed to DNA) from individual white clover plants for the assessment of genetic diversity using AFLP analysis. General high levels of genetic variation have previously

been detected in white clover (Gustine and Huff, 1997). The bulking of plant material rather that DNA has been successfully used in various studies (Sweeney and Danneberger, 1995; Golembiewski *et al.,* 1997), but none of which included AFLP analysis. Kölliker *et al.* (2001) concluded that the bulking of leaf material for AFLP analysis was highly reproducible, with genetic relationships being obtained that are comparable to ones obtained through analysis of individual plants. The use of this type of bulked AFLP analysis may in future be used to detect duplicate samples in large germplasm collections as well as for cultivar identification.

1.7. Influence of resistance genes on yield and quality

Lr genes derived from alien sources often show linkage with undesirable agronomic or quality attributes (Knott and Dvorak, 1976). An example of such negative quality characteristics is the yellow flour pigment in wheat with *Lr19* (Knott, 1980) and the sticky dough types that are associated with the 1BL/1RS chromosome translocation (Van Lill *et al.,* 1990).

Singh and Huerta-Espino (1997) indicated that *Lr34* in 'Jupateco 73R' was associated with slight reductions in grain yield, as well as with other traits that influence grain yield. The reduction observed varied between 2 and 5.9%. At a high rainfall, high altitude location no such significant differences in grain yield were observed between 'Jupateco 73R' and 'Jupateco 73S' (Ma and Singh, 1996). Two possible reasons for the reduction in yield were mentioned by Singh and Huerta-Espino (1997). Leaf tip necrosis might be responsible for a reduction in photosynthesis by reducing the overall photosynthetic area. The reduction in photosynthesis in turn results in a reduction in yield. Alternatively, leaf rust resistance conferred by *Lr34* may involve the production and accumulation of a toxic metabolite which induces leaf tip necrosis. It is also possible that the metabolite may be mildly toxic to plant metabolism at normal tissue concentrations, thus explaining the grain differences in *Lr34* and non-*Lr34* near isolates.

Dyck and Lukow (1988), indicated effective resistance combined with higher grain protein associated with the leaf rust resistance genes *Lr29* and *Lr37*. In a similar study, Labuschagne *et al.* (2002) concluded that the presence of *Lr29, Lr34, Lr35* as well as *Lr37* (in a 'Karee' background) increased flour protein and water absorption significantly. Both of these characteristics are advantageous for bread making quality. The study therefore provided proof that the introgression of alien genes into cultivated bread wheat is not necessarily associated with quality penalties.

1.8. Conclusion

From reviewing the literature it appears that gene pyramiding has the best potential for creating durable rust resistance. The combination of seedling and adult plant resistance genes within the same genotype allows for the evaluation of these genes at different stages within the same growth cycle, while it also results in a more complex resistance system. If one or more of these genes are associated with morphological and/or molecular markers, the screening process is even more efficient. As to the type of breeding method preferred, the pedigree system appears to be the most practical when combined with the pyramiding strategy. With this approach record can be kept of the incorporated genes within the various families and a thorough assessment can be made of selected progenies.

The financial investments in and application of molecular markers are justified by the time and costs saved in comparison to conventional breeding systems. Currently the AFLP method is favoured due to its high polymorphism revealing rate and therefore efficiency. However, the inability of AFLP technology to distinguish between co-dominant bands is undesirable. Furthermore, the identification of markers that differentiate between segregating offspring can be improved by using BSA.

In conclusion, wheat breeders should identify genes that have the best potential to remain durable, develop or improve conventional and molecular methods to

track these genes in segregating populations, and release cultivars that have complex resistance genotypes as well as acceptable agronomic and quality traits.

1.9. References

Adam-Blondon, A.F., Sévignac, M., Bannerot, H. and Dron, M., 1994. SCAR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to antracnose in common bean. *Theor. Appl. Genet.* 88: 865-870.

Anikster, Y., Bushnell, W.R., Eilam, T., Manisterski, J. and Roelfs, A.P., 1997. *Puccinia recondita* causing leaf rust on cultivated wheats, wild wheats and rye. *Can. J. Bot.* 75: 2082-2096.

Appels, R., McIntyre, C.L., Clarke, B.C. and May, C.E., 1986. Alien chromatin in wheat: ribosomal DNA spacer probes for detecting specific nucleolar organizer region loci introduced into wheat. *Can. J. Genet. Cytol.* 28: 645-657.

Archibald, A.L., 1991. Molecular biological approaches and their possible applications. Pages 100-122 in: Breeding for disease resistance in farm animals, 1991. Eds. J.B. Owen and R.F.E. Axford. CAB International. Wallingford. U.K.

Bariana, H.S. and McIntosch, R.A., 1994. Characterisation and origin of rust and powdery mildew resistance genes in VPM1 wheat. *Euphytica* 76: 53-61.

Bayles, R.A., Flath, K., Hovmoller, M.S. and de Vallavieille-Pope, C., 2000. Breakdown of the *Yr17* resistance to yellow rust of wheat in northern Europe. *Agronomie* 20: 805-811.

Bohn, M., Utz, H.F. and Melchinger, E., 1999. Genetic similarities among winter wheat cultivars eterined on the basis of RFLPs, AFLPs and SSRs and their use for predicting progeny variance. *Crop Sci.* 39: 228-237.

Boora, K.S., Frederiksen, R.A. and Magill, C.W., 1999. A molecular marker that segregates with sorghum leaf blight resistance in one cross is maternally inherited in another. *Mol. Gen. Genet.* 26: 317-322.

Borovkova, I.G., Jin, Y., Steffenson, B.J., Kilian, A., Blake, T.K. and Kleinhofs, A., 1997. Identification and mapping of a leaf rust resistance gene in barley line Q21861. *Genome* 40: 236-241.

Boshoff, W.H.P., Pretorius, Z.A. and Van Niekerk, B.D., 2002. The impact of leaf rust on spring wheat in the winter rainfall region of South Africa. *S. A. J. Plant Soil* 19: 84-88.

Boshoff, W.H.P., 1999. Unpublished data. Small Grain Institute. Bethlehem. South Africa.

Botha, A.M. and Venter, E., 2000. Molecular marker technology linked to pest and pathogen in wheat breeding. *S. A. J. Sci.* 96: 233-240.

Brady, J.L., Scott, N.S. and Thomas, M.R., 1996. DNA typing of hops (*Humulus lupulus)* through application of RAPD and microsatellite marker sequences converted to sequence tagged sites (STS). *Euphytica* 91: 277-284.

Broers, L.H.M., 1989. Partial resistance to wheat leaf rust in 18 spring wheat cultivars. *Euphytica* 44: 247-258.

Broers, L.H.M. and Wallenburg, S.C., 1989. Influence of post-infection temperature on three components of partial resistance in wheat to wheat leaf rust. *Euphytica* 44: 215-224.

Browder, L.E., 1972. Designation of two genes to *Puccinia recondita* in *Triticum aestivum*. *Crop Sci.* 12: 705-706.

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

Brown, S.M., Hopkins, M.S., Mithcell, S.E., Senior, M.L., Wang, T.Y. and Duncan, R.R., 1996. Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. *Theor. Appl. Genet.* 93: 190-198.

Burdon, J.J., 1993. Genetic variation in pathogen populations and its implication to host resistance. Pages 41-56 in: Durability of disease resistance. Current plant science and biotechnology in agriculture, Vol 18. Eds. Th. Jacobs and J.E. Parlevliet. Kluwer Academic Publishers. Dordrecht, The Netherlands.

Chao, S., Sharp, P.J., Worland, A.J., Warham, E.J., Koebner, R.M.D. and Gale, M.D., 1989. RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theor. Appl. Genet.* 78: 493-504.

Chen, X. and Wu, R., 1997. Direct amplification of unknown genes and fragments by uneven polymerase chain reaction. *Gene* 185: 195-199.

Cohen, J., 1994. 'Long PCR' leaps into large DNA sequences. *Science* 263: 1564-1565.

Condit, R. and Hubbell, S., 1991. Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. *Genome* 34: 66-67.

Cox, T.S., Raupp, W.J. and Gill, B.S., 1994. Leaf rust resistance genes *Lr41*, *Lr42* and *Lr43* transferred from *Triticum tauscii* to common wheat. *Crop Sci.* 34: 339-343.

Cummings, G.B. and Caldwell, R.M., 1956. The validity of binomials in the leaf rust fungus complex of cereals and grasses. *Phytopathology* 46: 81-82.

Dedryver, F., Jubier, M-F., Thouvenin, J. and Goyeau, H., 1996. Molecular markers linked to the leaf rust resistance gene *Lr24* in different wheat cultivars. *Genome* 39: 830-835.

Delourme, R., Bouchereau, A., Hubert, N., Renard, M. and Landry, B.S., 1994. Identification of RAPD markers linked to a fertility restorer gene for the Ogura radish cytoplasmic male sterility of rapeseed (*Brassica napus* L). *Theor. Appl. Genet.* 88: 741-748.

Devos, K.M. and Gale, M.D., 1992. The use of random amplified polymorphic DNA markers in wheat. *Theor. Appl. Genet.* 84: 567-572.

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

Dmitriev, A.P. and Gorshkov, A.K., 1980. The results of some wheat rusts investigation in Ethiopia. Pages 157-159 in: *Proc. Eur. Mediterr. Cereal Rusts Conf., 5th, 1980.*

d'Oliveira, B. and Samborski, D.J., 1966. Aecia stage of *Puccinia recondita* on Ranunculaceae and Boraginaceae in Portugal. Pages 130-150 in: Proceedings of the first European brown rust conference, Cereal rust conference, June 29-July 24, 1964, Cambridge, U.K. Plant Breeding Institute, Cambridge, U.K.

Drijepondt, S.C. and Pretorius, Z.A., 1989. Greenhouse evaluation of adult plant resistance conferred by the gene *Lr34* to leaf rust of wheat. *Plant Dis.* 73: 669-671.

Drijepondt, S.C., Pretorius, Z.A. and Rijkenberg, F.H.J., 1991a. Exspression of two wheat leaf rust resistance gene combinations involving *Lr34*. *Plant Dis.* 75: 526-528.

Drijepondt, S.C., Pretorius, Z.A. and Rijkenberg, F.H.J., 1991b. Effects of growth stage, leaf position and races of *Puccinia recondite* f.sp. *Tritici* on *Lr34* resistance in wheat. *Phytophylactica* 23: 53-58.

Driscoll, C.J. and Jensen, N.F., 1964. Characteristics of leaf rust resistance transferred from rye to wheat. *Crop Sci.* 4: 372-374.

Dubcovsky, J., Lukaszewski, J.A., Echaide, M., Antonelli, E.F. and Porter, D.R., 1998. Molecular characterization of two *Triticum speltoides* interstitial translocations carrying leaf rust and greenbug resistance genes. *Crop Sci.* 38: 1655-1660.

Dubin, H.J. and Rajaram, S., 1981. The strategy of the International Maize and Wheat Improvement Center (CIMMYT) for breeding disease resistant wheat: an international approach. Pages 27-35 in: Strategies for the control of cereal disease. Eds. J.F. Jenkyn and R.T. Plumb. Blackwell, Oxford.

Dubin, H.J. and Torres, E., 1981. Causes and consequences of the 1976-1977 wheat leaf rust epidemic in northwest Mexico. *Ann. Rev. Phytopathol.* 19: 41-49.

Dvorak, J. and Knott, D.R., 1990. Location of a *Triticum speltoides* chromosome segment conferring resistance to leaf rust in *Triticum aestivum. Genome* 33: 892-897.

Dwurazna, M., Bialota, M. and Gajda, Z., 1980. Resistance of wheat cultivars to rust in Poland. Pages 147-150 in: *Proc. Eur. Mediterr. Cereal rusts Conf. 5th, 1980.*

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

Dyck, P.L., 1991. Genetics of adult plant leaf resistance in 'Chinese Spring' and 'Sturdy' wheats. *Crop Sci.* 31: 309-311.

Dyck, P.L. and Bartos, P., 1994. Attempted transfer of leaf rust resistance from *Triticum monococcum* and durum wheat to hexaploid wheat. *Can. J. Plant Sci.* 74: 733-736.

Dyck, P.L. and Kerber, E.R., 1985. Resistance of the race-specific type. Pages 469-500 in: The cereal rusts, Vol 2, Disease, distribution, epidemiology and control. Eds. A.P. Roelfs and W.R. Bushnell. Academic Press. Inc. Orlando, Florida.

Dyck, P.L., Kerber, E.R. and Aung, T., 1994. An interchromosomal reciprocal translocation in wheat involving leaf rust resistance gene *Lr34*. *Genome* 37: 556-559.

Dyck, P.L. and Lukow, O.M., 1988. The genetic analysis of two interspecific sources of leaf rust resistance and their effect on the quality of common wheat. *Can. J. Plant Sci.* 68: 633-639.

Dyck, P.L. and Samborski, D.J., 1974. Inheritance of virulence in *Puccinia recondita* on alleles at the *Lr2* locus for resistance in wheat. *Can. J. Genet. Cytol.* 16: 323-332.

Dyck, P.L. and Samborski, D.J., 1982. The inheritance to *Puccinia recondita* in a group of common wheat cultivars. *Can. J. Genet. Cytol.* 24: 273-283.

Dyck, P.L. and Sykes, E.E., 1994. Genetics of leaf rust resistance in three speltoides wheats. *Can. J. Plant Sci.* 74 :231-233.

Erpelding, J.E., Blake, N.K., Blake, T.K. and Talbert, L.E., 1996. Transfer of sequence tagged site PCR markers between wheat and barley. *Genome* 39: 802-810.

Evola, S.V., Burr, F.A. and Burr, B., 1986. The suitability of restriction fragment length polymorphisms as genetic markers in maize. *Theor. Appl. Genet.* 71: 765-771.

Feuillet, C., Messmer, M., Schachermayr, G. and Keller, B., 1995. Genetic and physical characterization of the *Lr1* leaf rust resistance locus in wheat *(Triticum aestivum* L.). *Mol. Gen. Genet.* 248: 553-562.

Friebe, B., Zeller, F.J., Mukai, Y,, Forster, B.D., Bartos, P. and McIntosh, R.A., 1992. Characterization of rust resistant wheat *Agropyron* intermedium derivitives by C-banding, in situ hybridization and isozyme analysis. *Theor. Appl. Genet.* 83: 775-782.

Gair, R., Jenkins, J.E.E. and Lester, E., 1987. Cereal pests and diseases. Farming Press. Ipswich.

Gallego, F.J., Calles, B. and Benito, C., 1998. Molecular markers linked to the aluminium tolerance gene Alt1 in rye (*Secale cereale* L). *Theor. Appl. Genet.* 97: 1104-1109.

Garg, M., Singh, S., Singh, B., Singh, K. and Dhaliwal, H.S., 2001. Estimates of genetic similarities and fingerprinting of wheats (Tritium species) and triticale cultivars using molecular markers. *Indian J. Agric. Sci.* 71: 438-443.

German, S.E. and Kolmer, J.A., 1992. Effect of the gene *Lr34* in the enhancement of resistance to leaf rust of wheat. *Theor. Appl. Genet.* 84: 97-105.

Giorio, G., Gallitelli, M. and Carriero, F., 1997. Molecular markers linked to rhizomania resistance in sugar beet, *Beta vulgaris*, from two different sources map to the same linkage group. *Plant Breed.* 116: 401-408.

Giovannoni, J.J., Wing, R.A., Ganal, M.W. and Tanksley, S.D., 1991. Isolation of molecular markers form specific chromosomal intervals using DNA pools from existing mapping populations. *Nucl. Acids Res.* 19: 6533-6558.

Golembiewski, R.C., Danneberger, T.K. and Sweeney, P.M., 1997. Potential of RAPD markers for use in the identification of creeping bentgrass cultivars. *Crop Sci.* 37: 212-214.

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

Gupta, A.K. and Saini, R.G., 1993. Leaf rust resistance in wheat. Pages 235-237 in: Durability of disease resistance. Current plant science and biotechnology in agriculture, Vol 18. Eds. Th. Jacobs and J.E. Parlevliet. Kluwer Academic Press, The Netherlands.

Gupta, P.K., Varshney, R.K., Sharma, P.C. and Ramesh, B., 1999. Molecular markers and their applications in wheat breeding. *Plant Breed.* 118: 369-390.

Gustine, D.L. and Huff, D.R., 1997. Genetic variation within and among white clover populations from managed permanent pastures of the northeastern USA. *Crop Sci.* 37: 524-530.

Habgood, R.M. and Clifford, B.C., 1981. Breeding barley for disease resistance: The essence of compromise. Pages 15-25 in: Strategies for the control of cereal diseases. Eds. J.F. Jenkyn and R.T. Plumb. Blackwell Scientific Publications, Oxford, UK.

Hartl, L., Mohler, V., Zeller, F.J., Hsam, S.L.K. and Schweizer, G., 1999. Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat (*Triticum aestivum* L.). *Genome* 42: 322-329.

Hogenboom, N.G., 1993. Economic importance of breeding for disease resistance. Pages 11-22 in: Durability of disease resistance. Current plant science and biotechnology in agriculture, Vol 18. Eds. Th. Jacobs and J.E. Parlevliet. Kluwer Academic Publishers. Dordrecht, The Netherlands.

Hu, X.Y., Ohm, H.W. and Dweikat, I., 1997. Identification of RAPD markers linked to the gene *Pm*1 for resistance to powdery mildew in wheat. *Theor. Appl. Genet.* 94: 832-840.

Hussien, T., Bowden, R.L., Gill, B.S. and Cox, T.S., 1998. Chromosomal locations in common wheat of three new leaf rust resistance genes from *Triticum monococcum*. *Euphytica* 101:127-131.

Jacob, H.J., Lindpaintner, K., Lincoln, S.E., Kusumi, K., Bunker, R.K., Mao, Y.P., Ganten, D., Dzau, V.J. and Lander, E.S., 1991. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypersensitive rat. *Cell-Cambridge* 67: 213-224.

Jacobs, Th., 1990. Abortion of infection structures of wheat leaf rust in susceptible and partially resistant wheat genotypes. *Euphytica* 45: 81-86.

Jacobs, Th. and Broers, L.H.M., 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. I. Estimation of gene action and number of effective factors in F_1, F_2 and backcross generations. *Euphytica* 44: 197-206.

Jacobs, Th. and Buurlage, M.B., 1990. Growth of wheat leaf rust colonies in susceptible and partially resistant spring wheats. *Euphytica* 45: 71-80.

Jacobs, A.S., Pretorius, Z.A., Kloppers, F.J. and Cox, T.S., 1996. Mechanisms associated with wheat leaf rust resistance derived from *Triticum monococcum*. *Phytopathology* 86: 588-595.

Jia, J., Devos, M., Chao, S., Miller, T.E., Reader, S.M. and Gale, M.D., 1994. RFLP-based maps of the homoelogous group-6 chromosomes of wheat and their application in the tagging of *Pm12*, a powdery mildew resistance gene transferred form *Aegilops speltoides* to wheat. *Theor. Appl. Genet.* 92: 559-565.

Johnston, C.O., 1967. Leaf rust of wheat. Pages 317-325 in: Wheat and wheat improvement, 1st Edition, Agronomy 13. Eds. K.S. Quisenberry and L.P. Reitz. The American Society of Agronomy, Inc. Madison, Wisconsin, USA.

Johnson, R., 1988. Durable resistance to yellow (stripe) rust in wheat and its implications in plant breeding. Pages 63-75 in: Breeding Strategies for resistance to the rusts of wheat. Eds. N. W. Simmonds and S. Rajaram. CIMMYT, Mexico.

Johnson, R., 1993. Durability of disease resistance in crops: some closing remarks about the topic and the symposium. Pages 283-300 in: Durability of disease resistance. Current plant science and biotechnology in agriculture, Vol. 18. Eds. Th. Jacobs and J.E. Parlevliet. Kluwer Academic Publishers, The Netherlands.

Johnson, R. and Lupton, F.G.H., 1987. Breeding for disease resistance. Page 566 in: Wheat breeding – its scientific basis. Ed. F.G.H. Lupton. Chapman and Hall. London.

Karp, A. and Edwards, K.J., 1997. DNA markers: a global overview. Pages 1-14 in: DNA markers: Protocols, Applications and Overviews. Eds. G. Caetano-Anollés and P.M. Gresshoff. Wiley-Liss, Inc. New York.

Kawchuk, L.M., Hachey, J. and Lynch, D.R., 1998. Development of sequence characterized DNA markers linked to a dominant Verticillium wilt resistance gene in tomato. *Genome* 41: 91-95.

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

Kislev, M.E., 1982. Stem rust of wheat 3 300 years old found in Israel. *Science* 216: 993-994.

Kloppers, F.J. and Pretorius, Z.A., 1995. Field evaluation of leaf rust severity, yield loss and quality characteristics in near-isogenic wheat lines with *Lr29, Lr35* and *Lr37*. *S. A. J. Plant Soil* 12: 55-58.

Kloppers, F.J. and Pretorius, Z.A., 1997. Effects of combinations amongst genes *Lr13, Lr34* and *Lr37* on components of resistance in wheat to leaf rust. *Plant Pathology* 46: 737-750.

Knoblock, I.W., 1968. A check list of crosses in the Gramineae. Michigan State Univ. Press, East Lansing.

Knott, D.R., 1980. Mutation of a gene for yellow flour pigment in wheat with *Lr19* in wheat. *Can. J. Genet. Cytol.* 23: 651-654.

Knott, D.R., 1989. The wheat rusts – Breeding for resistance. Springer-Verlag, Berlin.

Knott, D.R. and Dvorak, J., 1976. Alien germ plasm as a source of resistance to disease. *Ann. Rev. Phytopathol.* 14: 211-235.

Koebner, R.M.D., Miller, T.E., Snape, J.W. and Law, C.N., 1988. Wheat endopeptidase: genetic control, polymorphism, intra-chromosomal gene location, and alien variation. *Genome* 30: 186-192.

Kölliker, R., Jones, E.S., Jahufer, M.Z.Z. and Forster, J.W., 2001. Bulked AFLP analysis for the assessment of genetic diversity in white clover (*Trifolium repens* L.). *Euphytica* 121: 305-315.

Kolmer, J.A., 1992. Enhanced leaf rust resistance in wheat conditioned by resistance gene pairs with *Lr13*. *Euphytica* 61: 123-130.

Kolmer, J.A., 1999. Physiologic specialization of *Puccinia triticina* in Canada in 1997. *Plant Dis.* 83: 194-197.

Kolmer, J.A., 2001. Physiologic specialization of *Puccinia triticina* in Canada in 1998. *Plant Dis.* 85: 155-158.

Kolmer, J.A., German, S.E., Dyck, P.L., 1993. Resistance gene combinations that condition enhanced resistance to *Puccinia recondita*, leaf rust in wheat. Page 330 in: Durability of disease resistance. Current plant science and biotechnology in agriculture, Vol 18. Eds. T. Jacobs and J.E. Parlevliet. Kluwer Academic Publishers, The Netherlands.

Kolmer, J.A. and Liu, J.Q., 2001. Simple inheritance of partial resistance to leaf rust in two wheat cultivars. *Plant Pathology* 50: 546-551.

Kriac, J., Žáková, M. and Gregová, E., 1998. Comparison of differentiation capability of RAPD and SSR markers in commercial barley (*Hordeum vulgare* L.) cultivars. *Cereal Res. Commun.* 24: 375-382.

Labuschagne, M.T., Pretorius, Z.A. and Grobbelaar, B., 2002. The influence of leaf rust resistance genes *Lr29, Lr34, Lr35* and *Lr37* on breadmaking quality in wheat. *Euphytica* 124: 65-70.

Law, J.R., Donini, P.R.M.D., Koebner, R.M.D., Reeves, J.C. and Cooke, R.J., 1998. DNA profiling and plant variety registration. III: The statistical assessment of distinctness in wheat using amplified fragment length polymorphisms. *Euphytica* 102: 335-342.

Lee, T.S. and Shaner, G., 1985. Oligogenic inheritance of length of latent period in six slow leaf rusting wheat cultivars. *Phytopathology* 75: 636-643.

Litt, M. and Luty, J.A., 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinculeotide repeat within the cardiac muscle actin gene. *Am. J. Human Genet.* 44: 397-401.

Liu, J.Q. and Kolmer, J.A., 1997. Inheritance of leaf rust resistance in wheat cultivars Grandin and CDC Teal. *Plant Dis.* 81: 505-508.

Lottering, J-M., Botha, A-M. and Kloppers, F.J., 1999. DNA markers linked to leaf rust resistance gene *Lr41*. Pages 176-177 in: Proceedings of the 9th Assembly of the Wheat Breeding Society, Australia, The University of Southern Queensland, Toowoomba, 27 Septemper-1 October 1999. Ed. P. Williamson. Toowoomba, Qld.

Lottering, J-M., Botha, A-M. and Kloppers, F.J., 2002. AFLP and RAPD markers linked to leaf rust resistance gene *Lr41* in wheat. *S. A. J. Plant Soil* 19: 17-22.

Luig, N.H., 1985. Epidemiology in Australia and New Zealand. Pages 301-328 in: The cereal rusts Vol 2. Disease, distribution, epidemiology, and control. Eds. A.P. Roelfs and W.R. Bushnell. Academic Press, Inc. London.

Ma, H. and Singh, R.P., 1996. Contribution of adult plant resistance gene *Yr18* in protecting wheat from yellow rust. *Plant Dis.* 80: 66-69.

Ma, Z-Q. and Lapitan, N.L.V., 1998. Comparison of amplified and restriction fragment length polymorphism in wheat. *Cereal Res. Commun.* 26: 7-13.

Ma, Z-Q., Röder, M. and Sorrells, M.E., 1996. Frequencies and sequence characteristics of di, tri and tetra-nucleotide microsatellites in wheat. *Genome* 39: 123-130.

Mackay, I.J. and Caligari, P.D.S., 2000. Efficiencies of F_2 and backross generations of Bulked Segregant Analysis using dominant markers. *Crop Sci.* 40: 626-630.

Mackill, D.J., Zhang, Z., Redoña, E.D. and Colowit, P.M., 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome* 39: 969-977.

Manifesto, M.M., Schlatter, A.R., Hopp, H.E., Suárez, E.Y. and Dubcovsky, J., 2001. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop Sci.* 41: 682-690.

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

Martin, J.M., Talbert, L.E., Lanning, S.P. and Blake, N.K., 1995. Hybrid performance in wheat as related to parental diversity. *Crop Sci.* 35: 104-108.

Martínez, F., Niks, R.E., Singh, R.P. and Rubilaes, D., 2001. Characterization of *Lr46*, a gene conferring partial resistance to wheat leaf rust. *Hereditas* 135: 111-114.

Masuelli, R.W., Tanimoto, E.Y., Brown, C.R. and Comai, L., 1995. Irregular meiosis in a somatic hybrid between *S. bulbocastanum* and *S. tuberosum* detected by species-specific PCR markers and cytological analysis. *Theor. Appl. Genet.* 91: 401-408.

McIntosh, R.A., Miller, T.E. and Chapman, V., 1982. Cytogenetical studies in wheat. XII. *Lr*28 for resistance to *Puccinia recondita* and *Sr*34 for resistance to *P. graminis tritici. Z. Pflanzenzücht.* 89: 295-306.

McIntosh, R.A., Friebe, B., Jiang, J., The, D. and Gill, B.S., 1995a. Cytogenetical studies in wheat XVI. Chromosome location of a new gene for resistance to leaf rust in a Japanese wheat rye translocation line. *Euphytica* 83: 141-147.

McIntosh, R.A., Wellings, C.R. and Park, R.F., 1995b. Wheat rusts: An atlas of resistance genes. CSIRO: Australia, East Melbourne.

Melchinger, A.E., 1990. Use of molecular markers in breeding for oligogenic disease resistance. *Plant Breeding* 104: 1-19.

Michelmore, R.W., 1994. Molecular approaches to manipulation of disease resistance genes. *Ann. Rev. Phytopathol.* 88: 865-870.

Michelmore, R., 1995. Molecular approaches to manipulation of disease resistance genes. *Ann. Rev. Phytopathol.* 15: 393-427.

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

Myburg, A.A., Cawood, M., Wingfield, B.D. and Botha, A-M., 1998. Development of RAPD and SCAR markers linked to the Russian wheat aphid resistance gene in wheat. *Theor. Appl. Genet.* 96: 1162-1169.

Nagarajan, S. and Joshi, L.M., 1985. Epidemiology in the Indian Subcontinent. Pages 371-402 in: The cereal rusts Vol 2. Disease, distribution, epidemiology, and control. Eds. A.P. Roelfs and W.R. Bushnell. Academic Press, Inc., London.

Naik, S., Gill, K.S., Prakasa Rao, V.S., Gupta, V.S., Tamhankar, S.A., Pujar, S., Gill, B.S. and Ranjekar, P.K., 1998. Identification of a STS marker linked to the *Aegilops speltoides*-derived leaf rust resistance gene *Lr28* in wheat. *Theor. Appl. Genet.* 97: 535-540.

Niks, R.E., 1986. Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley seedlings. *Physiological and Molecular Plant Pathology* 28: 309-322.

Niks, R.E. and Dekens, R.G., 1991. Prehaustorial and posthaustorial resistance to wheat leaf rust indiploid wheat seedlings. *Phytopathology* 81: 847-851.

Olson, M., Hood, L., Cantor, C. and Botstein, D., 1989. A common language for physical mapping of the human genome. *Science* 245: 1434-1435.

Paabo, S., Gifford, J.A. and Wilson, A.C., 1988. Mitochondrial DNA sequences from a 7000-year-old brain. *Nuc. Acids Res.* 16: 9775-9787.

Paran, I. and Michelmore, R.W., 1993. Development of reliable PCR-based markers linked to downey mildew resistance genes in lettuce. *Theor. Appl. Genet.* 85: 985-993.

Park, R.F., Burdon, J.J. and Jahoor, A., 1999. Evidence for somatic hybridization in nature in *Puccinia recondita* f. sp. *tritici,* the leaf rust pathogen of wheat. *Mycological Research* 103: 715-723.

Park, R.F. and Felsenstein, F.G., 1998. Physiological specialization and pathotype distribution of *Puccinia recondita* in western Europe, 1995. *Plant Pathology* 47: 157-164.

Parlevliet, J.E., 1975. Partial resistance to barley to leaf rust *Puccinia hordei*.I. Effect of cultivar and development stage on latent period. *Euphytica* 24: 21-27.

Parlevliet, J.E., 1976. Evaluation of the concept of horizontal resistance in the barley/*Puccinia hordei* host-pathogen relationship. *Phytopathology* 66: 494-497.

Parlevliet, J.E., 1978. Race specific aspects of polygenic resistance of barley to leaf rust, *Puccinia hordei. Neth. J. Pl. Path.* 84: 121-126.

Parlevliet, J.E., 1981. Race-non-specific disease resistance. Pages 47-54 in: Strategies for the control of cereal diseases. Eds. J.F. Jenkyn. and R.T. Plumb. Blackwell Scientific Publishers, Oxford.

Parlevliet, J.E., 1985. Resistance of the non-race-specific type. Pages 501-523 in: The cereal rusts, Vol II, Disease, distribution, epidemiology and control. Eds. A.P. Roelfs and W.R. Bushnell. Academic Press. Inc. Orlando, Florida.

Parlevliet, J.E., 1993. What is durable resistance? A general outline. Pages 23-29 in: Durability of disease resistance. Current plant science and biotechnology in agriculture, Vol 18. Eds. Th. Jacobs and J.E. Parlevliet. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Parlevliet, J.E., 1995. Durable resistance and how to breed for it. Breeding for disease resistance with emphasis on durability. Pages 1-14 in: Proceedings of a regional workshop for eastern, centeral and southern Africa, Njoro, Kenya, October 2-6, 1994. Wageningen Agricultural University, Wageningen, The Netherlands.

Parlevliet, J.E. and Ommeren, A., 1975. Partial resistance between field trials, micro plot tests and latent period. *Euphytica* 24: 293-303.

Penner, G.A., 1996. RAPD analysis of plant genomes. Pages 251-268 in: Methods of genome analysis in plants. Ed. P.P. Jauhar. CRC Press, Boca Raton.

Poehlman, J.M. and Sleper, D.A., 1995. Breeding Field Crops. 4th edition. Iowa State University Press, Ames, Iowa.

Powell, W., Morgante, M., Andrè, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A., 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplam analysis. *Mol. Breed.* 2: 225-238.

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

Pretorius, Z.A., Kloppers, F.J. and Drijepondt, S.C., 1994. Effects of inoculum density and temperature on three components of leaf rust resistance controlled by *Lr34* in wheat. *Euphytica* 74: 91-96.

Pretorius, Z.A. and Le Roux, J., 1988. Occurrence and pathogenicity of *Puccinia recondita* f. sp. *tritici* on wheat in South Africa during 1986 and 1987. *Phytophylactica* 20: 349-352.

Pretorius, Z.A., Le Roux, J. and Drijepondt, S.C., 1990. Occurrence and pathogenicity of *Puccinia recondita* f. sp. *tritici* on wheat in South Africa during 1988. *Phytophylactica* 22: 225-228.

Pretorius, Z.A., Rijkenberg, F.H.J. and Wilcoxson, R.D., 1987. Occurrence and pathogenicity of *Puccinia recondita* f.sp. *tritici* wheat in South Africa from 1983 through 1985. *Plant Dis.* 71: 1133-1137.

Pretorius, Z.A., Wilcoxson, R.D., Lang, D.L. and Shafer, J.F., 1984. Detecting wheat leaf rust resistance gene *Lr13* in seedlings. *Plant Dis.* 68:585-586.

Primrose, S.B., 1995. Principles of Genome Analysis. A guide to mapping and sequencing DNA from different organisms. Blackwell Science Ltd., Oxford.

Prins, R., Groenewald, J.Z., Marais, G.F., Snape, J.W. and Koebner, R.M.D., 2001. AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. *Theor. Appl. Genet.* 103: 618-624.

Procunier, J.D., Townley-Smith, T.F., Fox, S., Prashar, S., Gay, M., Kim, W.K., Czarnecki, E. and Dyck, P.L., 1995. PCR-based RAPD/DGGE markers linked to leaf rust resistance genes *Lr29* and *Lr25* in wheat (*Triticum aestivum* L.). *Genet. Breed.* 49: 176-179.

Roelfs, A.P., 1985. Epidemiology in Northern America. Pages 403-434 in: The cereal rusts Vol 2. Disease, distribution, epidemiology, and control. Eds. A.P. Roelfs and W.R. Bushnell. Academic Press, Inc. London.

Roelfs, A.P., Singh, R.P. and Saari, E.E., 1992. Rust diseases of wheat: Concepts and methods of disease management. International Maize and Wheat Improvement Centre (CIMMY), Mexico, D.F.

Roy, D., 2000. Plant breeding. Analysis and exploitation of variation. Alpha Science International Ltd. Pangbourne, UK.

Rubiales, D. and Niks, R.E., 1995. Characterization of *Lr34*, a major gene conferring nonhypersensitive resistance to wheat leaf rust. *Plant Dis.*79: 1208-1212.

Russell, J.R., Fuller, J.D., Macaulay, M., Hatz, B.G., Jahoor, A., Powell, N.Y. and Waugh, R., 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, SSRs and RAPDs. *Theor. Appl. Genet.* 95: 714-722.

Saari, E.E. and Prescott, J.M., 1985. World distribution in relation to economic losses. Pages 260-298 in: The cereal rusts, Vol. 2. Diseases, distribution, epidemiology and control, Eds. A.P. Roelfs and W.R. Bushnell. Academic Press. Orlando.

Saari, E.E. and Wilcoxson, R.D., 1974. Plant disease situation of high yielding dwarf wheats in Asia and Africa. *Annu. Rev. Phytopathol.* 12: 49-68.

Saghai-Maroof, M.A., Biyashev, R.M., Yang, G.P., Zhang, Q. and Allard, R.W., 1994. Extraordinary polymorphic microsatellite DNA in barley: species diversity, chromosomal locations and population dynamics. *Proc. Natl. Acad. Sci. USA* 91: 5466-5470.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higurchi, R., Horn, G.T., Mullis, K.B. and Erlich, K.A., 1988. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for the diagnostics of sickle cell anaemia. *Science* 239: 487-491.

Saini, R.G., Kaur, M., Singh, B., Sharma, S., Nanda, G.S., Nayar, S.K., Gupta, A.K. and Nagarajan, S., 2002. Genes *Lr48* and *Lr49* for hypersensitive adult plant leaf rust resistance in wheat (*Triticum aestivum* L.). *Euphytica* 124: 365-370.

Samborski, D.J., 1985. Wheat leaf rust. Pages 39-59 in: The Cereal rusts, Vol 2, Diseases, distribution, epidemiology and control. Eds. A.P. Roelfs and W.R. Bushnell. Academic Press, Inc., Orlando, Florida.

Samborski, D.J. and Dyck, P.L., 1982. Enhancement of resistance to *Puccinia recondita* by interactions of resistance genes in wheat. *Can. J. Plant Pathol.* 4: 152-156.

Sawhney, R.N., 1992. The role of *Lr34* in imparting durable resistance to wheat leaf rust through gene interaction. *Euphytica* 61: 9-12.

Sawhney, R.N., Nayar, S.K., Sharma, J.B. and Bedi, R., 1989. Mechanism of durable resistance: A new approach. *Theor. Appl. Genet.* 78: 229-232.

Schachermayr, G., Fueillet, C. and Keller, B., 1997. Molecular markers for the detection of the wheat leaf rust resistance gene *Lr10* in diverse genetic backgrounds. *Mol. Breed.* 3: 65-74.

Schachermayr, G., Siedler, H., Gale, M.D., Winzeler, H., Winzeler, M. and Keller, B., 1994. Identification and localization of molecular markers linked to the *Lr9* leaf rust resistance gene of wheat. *Theor. Appl. Genet.* 88: 110-115.

Schachermayr, G.M., Messmer, M.M., Feuillet, C., Winzeler, H., Winzeler, M. and Keller, B., 1995. Identification of molecular markers linked to the *Agropyron elongatum*-derived leaf rust resistance gene *Lr24* in wheat. *Theor. Appl. Genet.* 90: 982-990.

Schafer, J.F. and Roelfs, A.P., 1985. Estimated relation between numbers of urediniospores of *Puccinia graminis* f.sp. *tritici* and rates of occurrence of virulence. *Phytopathology* 75: 749-750.

Schafer, J.F., Roelfs, A.P. and Bushnell, W.R., 1984. Contributions of early scientist to knowledge of cereal rusts. Pages 4-38 in: The cereal rusts – Origins, specificity, structure and physiology, Vol 1. Eds. W.R. Bushnell and A.P. Roelfs. Academic Press, Inc., London.

Schwarz, G., Herz, M., Huang, X.Q., Michalek, W., Jahoor, A., Wenzel, G. and Mohler, V., 2000. Application of fluorescence-base semi-automated AFLP analysis in barley and wheat. *Theor. Appl. Genet.* 100: 545-551.

Sears, E.R., 1956. The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. *Brookhaven Symp. Biol.* 9: 1-21.

Senior, M.I. and Heun, M., 1993. Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT Primer. *Genome* 36: 884-889.

Seo, Y.W., Jang, C.S. and Johnson, J.W., 2001. Development of AFLP and STS markers for identifying wheat-rye translocations possessing 2RL. *Euphytica* 121: 279-287.

Shan, X., Blake, T.K. and Talbert, L.E., 1998. Conversion of AFLPs to sequence-tagged-site PCR markers. *Plant and Animal Genome VII Conf.,* 18-22 Jan., 1998, San Diego Abstr. 81 pp.

Shan, X., Blake, T.K. and Talbert, L.E., 1999. Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. *Theor. Appl. Genet.* 98: 1072-1078.

Sharma, D. and Knott, D.R., 1966. The transfer of leaf-rust resistance from *Agropyron* to *Triticum* by irradiation. *Can J. Genet. Cytol.* 8: 137-143.

Sharp, E.L., Schmitt, C.G., Staley, G.M. and Kingsolver, C.H., 1958. Some critical factors involved in establishiment of *Puccinia graminis* var. *tritici. Phytopathology* 48: 469-474.

Shi, A.N., Leath, S. and Murphy, J.P., 1998. A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. *Phytopathology* 88: 144-147.

Singh, R.P., 1992a. Association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. *Crop Sci.* 32: 874-878.

Singh, R.P., 1992b. Genetic association of leaf rust resistance to stripe rust in bread wheat. *Phytopathology* 82: 835-838.

Singh, R.P., 1993. Genetic association of gene *Bdv1* for tolerance to barley yellow dwarf virus with genes *Lr34* and *Yr18* for adult plant resistance to rusts in bread wheat. *Plant Dis.* 77: 1103-1106.

Singh, R.P. and Gupta, K.A., 1992. Expression of wheat leaf rust resistance gene *Lr34* in seedlings and adult plants. *Plant Dis.* 76: 489-491.

Singh, R.P. and Huerta-Espino, J., 1997. Effect of leaf rust resistance gene *Lr34* on grain yield and agronomic traits of spring wheat. *Crop Sci.* 37: 390-395.

U.V.S. BIBLIOTEEK

1163 3790 6

Singh, R.P., Huerta-Espino, J. and Rajaram, S., 2000. Achieving near-immunity to leaf and stripe rust in wheat by combining slow rusting resistance genes. *Acta Phytopath. et Entom. Hung.* 35: 133-139.

Singh, R.P., Mujeeb-Kazi, A. and Huerta-Espino, J., 1998. *Lr46:* a gene conferring slow-rusting resistance to leaf rust in wheat. *Phytopathology* 88: 980-894.

Smith, E.L., Schlehuber, A.M., Young, H.C. and Jr. Edwards, L.H., 1968. Registration of Agent wheat. *Crop Sci. 8: 511-512.*

Southern, E.M., 1975. Detection of specific sequences among DNA fragements separated by gel electrophoresis. *J. Mol. Biol.* 98: 503-517.

Statler, G.D., 1990. New mutations from a mutant culture of Puccinia recondita. *Can. J. Plant Pathol.* 12: 243-246.

Sweeney, P.M. and Danneberger, T.K., 1995. RAPD characterization of *Poa annua* L. populations in golf course greens and fairways. *Crop Sci.* 35: 1676-1680.

Talbert, L.E., Blake, N.K., Chee, P.W., Blake, T.K. and Magyar, G.M., 1994. Evaluation of 'sequence tagged site' PCR products as molecular markers in wheat. *Theor. Appl. Genet.* 87: 789-794.

Taramingo, G. and Tingey, S., 1996. Simple sequence repeats for germplasm analysis and mapping in maize. *Genome* 39: 277-287.

Tartarini, S., Gianfranceschi, L., Sansavini, S. and Gessler, C., 1999. Development of reliable PCR markers for the selection of the *Vf* gene conferring scab resistance in apple. *Plant Breed.* 118: 183-186.

Thomas, M.R. and Scott, N.S., 1994. Sequence tagged site markers for microsatellites: Simplified technique for rapid obtaining flanking sequences. *Plant Mol. Biol. Reporter* 12: 58-64.

Torp, J. and Jensen, H.P., 1985. Screening for spontaneous virulent mutants of *Erysiphe graminis* DC. f. sp. *hordei* on barley lines with resistance genes M1-a1, M1-a6, M1-a12 and M1-g. *Phytopathology* 112: 17-27.

Van Lill, D., Howard, N.L. and Van Niekerk, H.A., 1990. The dough handling properties of two South African wheats with 1B/1R chromosome translocation. *S. A. J. Plant Soil* 7: 197-200.

Van Silfhout, C.H., 1993. Durable resistance in the pathosystem: Wheat – stripe rust. Pages 135-145 in: Durability of disease resistance. Current plant science and biotechnology in agriculture, Vol 18. Eds. Th. Jacobs and J.E. Parlevliet. Kluwer Academic Publishers, The Netherlands.

Vanderplank, J.E., 1963. Plant diseases: epidemics and control. Academic Press, New York. London.

Venter, E. and Botha, A-M., 2000. Development of markers linked ot *Diuraphis noxia* resistance in wheat using a novel PCR-RFLP approach. *Theor. Appl. Genet.* 100: 965-970.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. *Nucl. Acids Res.* 23: 4407–4414.

Watson, I.A., 1981. Wheat and its rust parasites in Australia. Pages 129-147 in: Wheat Science – Today and Tomorrow. Eds. L.T. Evens and W.J. Peacock. Cambridge University Press. Cambridge.

Weber, J.L., 1990. Informativeness of human $(dC\text{-}DA)_n$) $(dG\text{-}dT)_n$ polymorphisms. *Genomics* 7: 524-530.

Weising, K. and Kahl, G., 1997. Hybridization-based microsatellite fingerprinting of plant and fungi. Pages 27-54 in: DNA markers: Protocols, Applications and Overviews. Eds. G. Caetano-Anollés and P.M. Gresshoff. Wiley-Liss, Inc., New York.

Weising, S., Nybom, H., Wolff, K. and Meyer, W., 1995. DNA fingerprinting in plants and fungi. CRC Press, London.

Wiese, M.V., 1987. Compendium of wheat diseases, 2nd ed. APS Press. Minnesota, USA.

William, H.M., Hoisington, D., Singh, R.P. and Gonzalez-de-Leon, D., 1997. Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. *Genome* 40: 253-260.

Williams, J.G.K., Kubelik, A.R., Livak, J., Rafalski, J.A. and Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* 18: 6531-6535.

Wu, K.S. and Tanksley, S.D., 1993. Abundance polymorphism and genetic mapping of microsatellites in rice. *Mol. Gen. Genet.* 241: 225-235.

Xu, M., Huaracha, E. and Korban, S.S., 2001. Development of sequence-characterized amplified regions (SCARs) from amplified fragment length polymorphism (AFLP) markers tightly linked to the *Vf* gene in apple. *Genome* 44: 63-70.

Yee, E., Kidwell, K.K., Sills, G.R. and Lumpkin, T.A., 1999. Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. *Crop Sci.* 39: 168-275.

Zadoks, J.C., 1993. The partial past. Comments on the history of thinking about resistance of plants against insects, nematodes, fungi, and other harmful agents. Pages 11-22 in: Durability of disease resistance. Current plant science and biotechnology in agriculture, Vol 18. Eds. Th. Jacobs and J.E. Parlevliet. Kluwer Academic Publishers, The Netherlands.

Zeller, F.J., 1973. 1B/1R wheat-rye substitutions and translocations. Pages 209-21 in: Proc 4th International Wheat Genetics Symposium. Alien genetic material. Eds. E.R. Sears and L.M.S. Sears. University of Missouri. Colombia, USA.

Chapter 2

Combining genes for seedling and adult plant resistance to leaf rust in wheat

2.1. Introduction

Leaf rust, caused by *Puccinia triticina* Eriks. (previously referred to as *P. recondita)*, is a major foliar pathogen of wheat (*Triticum aestivum* L.) in South Africa (Pretorius and Le Roux, 1988). Under favourable conditions, *P. triticina* has the potential to reduce yield by up to 70% (Johnston, 1967). As nearly 300 000 ha of spring wheat are grown under moderate to high-risk environmental conditions in the Western Cape (Boshoff *et al.*, 2002), the impact of leaf rust on the South African wheat industry should not be underestimated.

The use of genetic resistance is considered the most economical as well as environmentally friendly means of controlling leaf rust (Martínez *et al.*, 2001). In the past, the incorporation of resistance to *P. triticina* to wheat cultivars has focused on major, race-specific genes. These genes, however, soon succumbed to virulent strains, rendering the cultivars susceptible (Sawhney, 1992). Therefore, a major concern of plant breeders is the durability of resistance that is incorporated into crops, especially to specialized airborne fungal pathogens such as powdery mildew and rusts (Niks and Rubiales, 2002).

Parlevliet (1995) defined durability as *a quantitative term indicating a relatively long period of effectiveness of the resistance when exposed to the pathogen.* Partial resistance (Parlevliet, 1995) and the incorporation of resistance genes obtained from wild relatives (Knott, 1989) into the wheat genome, are two suggested strategies for durability. Pyramiding of genes (Pederson and Leath, 1988; Van Silfhout, 1993; Kloppers and Pretorius, 1997), will in theory

also result in durability, as several mutation events will be required to produce a new virulent pathotype. Kolmer *et al.* (1991), however, postulated that the influence of specific virulence on the fitness, and the effect of mutation to multiple virulence on fitness, might play a larger role in the occurrence of durability due to gene pyramiding, than the possibility of a low mutation rate. Until recently it was not considered practical to incorporate more than one resistance gene using traditional breeding methods, as it was not possible to discriminate between plants containing one or more effective resistance genes (Pink, 2002). Molecular markers have, however, put a new perspective on gene combination (Roelfs *et al.,* 1992).

The concept of gene pyramiding was taken a step further by Sawhney *et al.* (1989) who suggested that seedling and adult plant resistance genes should be combined within the same wheat genome. In this regard the adult plant resistance gene *Lr34* could play an important role, as it has been found to interact with other resistance genes, providing enhanced levels of resistance (Dyck and Samborski, 1982; Kolmer *et al.,* 1991).

The objective of this study was not only to improve leaf rust resistance in selected wheats, but to focus on durability as well as agronomic acceptability of resistant lines.

2.2. Material and Methods

2.2.1. Parental lines

Seven bread wheat cultivars and lines, viz. BSP97/1, BSP98/4, BSP98/16, T96/6, Elands, W98/6 and W98/22, developed at the Small Grain Institute, Bethlehem, South Africa, were obtained for the improvement of leaf rust resistance. Pedigrees of the selected cultivars/lines are indicated in Table 2.1. Five sources of leaf rust resistance (Palmiet*6/*Lr21,* Palmiet*6/*Lr32,* Palmiet*6/*Lr34,* Palmiet*6/*Lr36* and Palmiet*6/*Lr41* - which will be referred to as Palmiet/*Lr21,* Palmiet/*Lr32,* Palmiet/*Lr34,* Palmiet/*Lr36* and Palmiet/*Lr41*,

Table 2.2), obtained from the University of the Free State, were used. All the resistance genes used, except *Lr34,* are seedling genes.

2.2.2. Growing conditions and inoculation procedures

Seedlings

For detection of seedling genes, plants were grown in 10 cm diameter pots filled with steam sterilised soil (four replications). Eight to 10 days after planting, the primary leaves were inoculated with freshly collected urediospores suspended in light mineral oil. Seedlings were dried for 45 min in fan-circulated air before being placed in the dark in a dew-simulation chamber at 18-22°C. After a 16 h dew period, plants were transferred to a controlled-environment cabinet.

Adult plants

Greenhouse – F_1 Ltn screening: Plants were allowed to mature at 22°C/18°C (16 h photoperiod) in a greenhouse. Micro-element supplements were administered via an irrigation system whereas 3:2:1 N:P:K fertiliser (10 g/l: 50 ml per pot) was applied weekly. Aphicides were sprayed as needed. Adult plants were not inoculated due to the natural high powdery mildew (*Erysiphe graminis*) pressure within the greenhouses.

Field trial – F_2 plants: The rust-susceptible cultivar, Morocco, was planted as spreader to ensure inoculum throughout the trial. The trial (spreaders included) was inoculated with a fresh mixture (cultured under greenhouse conditions) of the leaf rust pathotypes UVPrt2, UVPrt9 and UVPrt13, suspended in light mineral oil. Inoculation was done by injecting the spore suspension into the leaf whorls of Morocco plants during stem elongation, as well as by spraying all plots. Both the susceptible and resistant parent cultivars/lines were included as controls. Plots were regularly irrigated to ensure humid conditions for infection and optimal moisture for plant development.

Greenhouse – Agronomic evaluations: Resistant F_3 seedlings of each line were transferred to 2-l pots. Two plants from each selection were planted per pot. Where an uneven number of F_3 seedlings were available, the corresponding parent plant was included to ensure that all the plants were subjected to the same growing conditions.

The growth medium consisted of a 3:1 ratio of potting soil and sterilised soil. Each 2-l pot contained 2080 g of the 3:1 growth medium. Pots were randomised and irrigated automatically. Fertiliser, pesticides and fungicides were administered as mentioned.

2.2.3. Crosses and selection procedure

The breeding strategy according to Sawhney *et al.* (1989) was followed. This implied that a seedling as well as an adult plant resistance gene were combined in the same line. The initial crosses were therefore made during two phases:

Phase one: Crosses were made between each of the susceptible cultivars/lines (maternal parents) and resistance donors. An F_1 progeny series for each wheat cultivar/line was produced, with each line within the series containing a different resistance gene (e.g. Elands+*Lr21,* Elands+*Lr32,* Elands+*Lr34*, Elands+*Lr36* and Elands+*Lr41.*

Phase two: During this phase each of the F_1 lines containing a seedling gene was crossed to the respective F_1 containing *Lr34.* The intercrossed (top-crossed) F_1s therefore contained both a seedling (*Lr21, Lr32, Lr36* or *Lr41*) and an adult plant resistance gene (*Lr34)* (e.g. 'Elands+*Lr21+Lr34',* 'Elands+*Lr32+Lr34',* 'Elands+*Lr36+Lr34'* and 'Elands+*Lr41+Lr34'*). The order of *Lr* genes, e.g. Elands+*Lr34+Lr21,* indicates the sequence in which the genes were incorporated within the cultivar/line. In 'Elands+*Lr34+Lr21*', the first cross was made between 'Elands' and 'Palmiet+*Lr34*'. The F_1 was therefore assigned 'Elands+*Lr34*'. In the second phase, 'Elands+*Lr34'* was

used as the female plant and crossed with 'Elands+*Lr21*'. The new F_1 was designated 'Elands+*Lr34+Lr21*'. This allowed for the tracing of possible maternal effects that might become apparent during later analyses.

Parent line evaluation for seedling infection types: The infection types (IT) (Roelfs *et al.,* 1992) of both the susceptible and resistant parent lines were determined using pathotypes UVPrt2, UVPrt3, UVPrt9 and UVPrt13. 'Morocco' was included as a susceptible control.

F_1 seedling evaluation (single Lr resistance gene): Due to the occurrence of powdery mildew at an early age, the F_1 seedlings containing the single leaf rust resistance gene could not be evaluated.

F_1 seedling evaluation (two Lr resistance genes): F_1 seed obtained from inter-crossing was pooled and evaluated. Each cross was screened using a pathotype virulent on the adapted parent, i.e. 'BSP97/1' – UVPrt2; 'T96/6' and 'Elands '– UVPrt9; and 'BSP98/4', 'BSP98/16', 'W98/6' and 'W98/22' – UVPrt13.

Adult plant screening (two Lr resistance genes): Resistant F_1 seedlings were transferred to 2 L pots containing potting soil and screened at anthesis for 'leaf tip necrosis' (*Ltn*). *Ltn* indicates the presence of *Lr34* and typical symptoms include 2 to 3 cm of necrosis at the end of the leaves, extending an additional 2 to 4 cm down the edges of the leaf (Singh, 1992). These symptoms become apparent after anthesis.

Single plant selection in field trial: F_2 seed from 10 plants per cross (e.g. seedling-resistant 'Elands+*Lr32+Lr34'* plants also exhibiting *Ltn*) was pooled and planted in rows in a field trial at the Small Grain Institute. From this progeny only plants exhibiting *Ltn* were selected. Furthermore, *Ltn* selections could be correlated to stripe rust resistance observed. Stripe rust occurred from natural infection. Resistance to leaf rust was used to a limited degree due to the low levels of infection achieved.

F_3 seedling evaluation: A total of 120 F_3 plants were selected from the field trial and evaluated, as described above, for the retention of the appropriate seedling gene. Lines were inoculated with pathotypes virulent on the background genotype. Susceptible and resistant parents were included as checks.

2.2.4. Agronomic evaluation

Resistant F_3 seedlings were transferred to 2 L pots and grown to maturity as described previously (2.2.2). Growth conditions were given in section 2.2.2. The plants were evaluated for the following agronomic traits:

- **Days to anthesis (DA):** Date of anthesis was recorded when 50% of anthers of the floret brackets were extruded.

- **Flag leaf length (FLL):** Flag leaf length of main tillers was measured at anthesis.

- **Plant height (PH):** Plant height was measured from soil level to the uppermost floral bracket of the spike of the tallest tiller. Awns were not considered.

- **Days to physiological maturity (DPM):** Plants were assumed to be physiologically mature when the whole spike as well as 10 cm of the stem (below the spike) had discoloured (i.e. <15% moisture content, Mares, 1989).

- **Number of tillers (NT):** The number of tillers produced by each line was determined.

- **Yield per plant and yield per spike (YP and YS)**: Each line was harvested and the kernel mass per plant was determined. The total kernel

mass per plant was divided by the number of tillers to obtain yield per spike.

To evaluate the response patterns of individual plants containing a seedling and adult plant resistance gene to *P. triticina*, dot plots were constructed for each *Lr* gene genotype. Data were further analysed using a two-sample t-test. Dot plots indicated variation in plant architecture and other agronomic traits among single plants within each cross, in comparison to the adapted wheat line or cultivar.

The two-sample t-test is generally used to compare the means of two populations. The t-test was developed under the assumption that the sample size of each group would be equal, and that at least 30 samples per group are available. In the present study this was not the case but the procedure was nevertheless applied to determine whether the means of selected F_3 -lines differed significantly from their control for each agronomic trait evaluated. The NCSS 2000 program was used for both the construction of the dot plots as well as the t-test.

2.3. Results and discussion

2.3.1. Parental seedling evaluation

According to the ITs observed (Table 2.3), 'BSP97/1' was most susceptible to UVPrt2, 'T96/6' and 'Elands' to UVPrt9, and 'BSP98/16', 'BSP98/4', 'W98/22'and 'W98/6' the most susceptible to UVPrt13. The results obtained for BSP98/4 and W98/6 indicated that these two lines were either segregating or impure for leaf rust reaction. The resistant parent 'Palmiet-*Lr34-Yr18*' did not give the expected 2^+ IT described by both Dyck (1977) and Drijepondt and Pretorius (1989). Singh (1992) however indicated that a range of variation of ITs (from ; to 3) could be associated with *Lr34*, even when the tests are carried out at similar temperatures. This phenomenon may be attributed to variation in light intensity and duration during different months (Singh, 1992).

ITs obtained with *Lr41* correlated well with the ITs of 0 to 0; obtained by Pretorius *et al.* (1995). According to Cox *et al.* (1992), *Lr41* mediates an exceptionally high level of resistance to a wide range of leaf rust pathotypes. *Lr36* was expressed by (;) IT, compared to the (;1NC to 2^+) indicated by Pretorius *et al.* (1995).

2.3.2. F_1 seedling infection types and adult plant *Ltn*

Table 2.4 shows results obtained in screening of F_1 seedlings derived from two-gene crosses. Seedlings expressing the appropriate IT were transferred to 2 L pots and screened at adult plant stage for *Ltn*. The number of plants selected for field evaluation based on the occurrence of the desired seedling IT and *Lr34* marker, is also shown (Table 2.4). Three of the 33 F_1 progeny lines ('BSP98/4+*Lr21*+*Lr34*', 'BSP98/4+*Lr34*+*Lr21*' and 'W98/22+*Lr34*+*Lr32*') had to be eliminated at seedling stage due to an absence of leaf rust resistance. The absence of resistant plants can probably be attributed to an insufficient amount of F_1 material being screened. 'BSP97/1+*Lr34*+*Lr36*' was eliminated at the adult plant stage due to an absence of *Ltn*. Strict selection procedures were followed, resulting in only 23.6% of the evaluated progeny being selected.

2.3.3. Field trial infection types and selections

Results obtained in the screening of F_2 adult plant populations in the field are shown in Table 2.5. Due to suitable weather conditions and abundant *P. striiformis* f. sp. *tritici* inoculum, natural inoculation with stripe rust occurred (Fig. 2.1, 2.2 and 2.3), which influenced the development of leaf rust. As *Lr34* is associated with the adult plant resistance gene *Yr18* to stripe rust (Singh, 1992), indirect selections were made for leaf rust resistance according to the stripe rust phenotype. Leaf rust development on the parent line BSP98/4 (Fig. 2.5) and F_2 lines BSP98/4+*Lr32*+*Lr34* and W98/22+*Lr32*+*Lr34* were, however, clearly visible. Leaf tip necrosis, associated with *Lr34/Yr18*, was also visible.

Host reaction types (S = susceptible, MS = moderately susceptible, R = resistant and MR = moderately resistant) for each entry in general, as well as within-entry variation, are indicated in Table 2.5. Figs. 2.6 and 2.7 are examples of progress made with the incorporation of leaf rust resistance.

Humid conditions led to the manifestation of pseudo-black-chaff (PBC), a morphological marker for the stem rust resistance gene *Sr2* (Brown, 1997). *Sr2* is a valuable adult plant gene that confers durable resistance to wheat stem rust, caused by *P. graminis* f. sp. *tritici.* According to Roelfs (1988), *Sr2* confers 'slow rusting' as well as enhanced resistance and durability to stem rust in combination with other genes. PBC is usually displayed post-anthesis with increasing intensity as the head matures. In Australia, PBC is modified environmentally with only sporadic expression at high levels. PBC is completely dominant and the level of expression can be reduced by a number of modifying genes (Bhowal and Narkhede, 1981). Single plant selections were therefore made according to leaf and stripe rust resistance, while the degree of expression of stem rust resistance gene *Sr2* was also recorded for each selection. No attempt to select lines with high expression of PBC was made as it has been suggested that it may reduce yield (Hare and McIntosh, 1979). Another implication surrounding PBC is that it can reduce farmer acceptance (Sheen *et al.,* 1968). In general, breeders select moderate levels of PBC in order to ensure selection for *Sr2*, rather than be confronted with the occasional over-expression of this character (Brown, 1997). In the present material *Sr2* was introduced from the Palmiet background, as this cultivar has been shown to contain the gene (Pretorius and Brown, 1998). Selections were therefore made according to leaf and stripe rust resistance, with the degree of PBC indicated on a scale of 1 to 3 (1 = no visible melanism; 2 = few to half of the floral brackets show melanism; 3 = nearly all floral brackets show melanism). Results are indicated in Table 2.5. Care was also taken to ensure that field selected plants showed *Ltn*.

2.3.4. F3 seedling infection types

To confirm the presence of seedling genes in the field selections, ITs were determined as previously described (Table 2.6). Most of the lines scored a fleck (;) IT. Eight of the 120 lines screened, 'W98/6+*Lr34+Lr36*' #1, 'W98/6+*Lr36+Lr34*' #6, 'W98/22+*Lr21+Lr34*' #4, 'BSP98/4+*Lr32+Lr34*' #1 and #2, 'BSP98/16+ *Lr41+Lr34*' #4, 'Elands+*Lr34+Lr41*' #1 and #2, were excluded from further tests due to an absence of resistant ITs in seedlings. These ITs could, however, also have been influenced by the presence of *Lr34*, a gene which has often been shown to interact with other *Lr* genes (German and Kolmer, 1992, Kolmer, 1992; Kloppers and Pretorius, 1997) Once again strict selections were made, resulting in 1151 seedlings being selected from the initial 3 022 evaluated. Approximately 38% of the F_3 seedlings were therefore selected for the determination of agronomic characters.

2.3.5. Agronomic evaluation

Figures 2.8 to 2.33 indicate the dot plot analyses obtained for agronomic characteristics of wheat lines advanced through the different phases of selection. Due to an unknown greenhouse effect, reduced plant growth and short flag leaves were experienced in a minority of plants.

Results obtained with the t-test, in which the mean of each resistant line was compared with the mean of the control, are indicated in Table 2.7. The test is based on the assumption that the populations are normally distributed and that their variances are equal. Whenever these assumptions are violated, the standard two-sample t-test can not be used directly, in which case the nonparametric Mann-Whitney U test or the Kolmogorov-Smirnov tests are used.

The Mann Whitney U test (or Wilcoxon rank sum test) is therefore the nonparametric substitute for the equal-variance t-test when the assumption of

normality is not valid. The Kolmogorov-Smirnov test is the nonparametric substitute for the unequal-variance t-test

Therefore, based on the normality and equal variance status of the data, different statistical procedures were followed. The four procedures therefore included were:

- Equal Variance t-test (normal data with equal variances),
- Unequal Variance t-test (normal data with unequal variances),
- Mann-Whitney U or Wilcoxon Rank-Sum Test (non-normal data with equal variances)
- Kolmogorov-Smirnov Test (non-normal data with unequal variances).

Whenever the two-tailed test, [diff <>0 with diff = (control)-(selection)] which is standard, indicated a significant difference, the left/right-tail test (diff<0 or diff>0) was indicated within the table to distinguish whether the mean of the selected line was significantly larger/smaller than that of the control.

Various factors influenced the the t-test, resulting in questionable conclusions. The generally small n, as well as the difference in sample size between the control and a specific F_3 –line, were undesirable. Furthermore, the single n plants within each selection were still segregating and could not be grouped into specific replications. The results obtained should therefore be seen as a general guideline as to the performance of the selected lines and not as a statistical fact. It is, however, interesting to note that the t-test results correlate well with the dot plots.

'BSP97/1+*Lr32*+*Lr34*' (Fig. 2.8): The dot plots indicated that the growth period of the four selected lines appeared to have been slightly increased, with the exception of selection #1. The t-test however indicated that the means of all four selections were significantly higher than that of the control. All of the selected lines produced significantly ($P<0.05$) longer FLL than the control. According to the means, all but one of the selections (#1) were significantly taller ($P<0.05$). None of the progeny produced significantly lower yields than the control.

'BSP97/1+*Lr34+Lr21'* (Fig. 2.9; Table 2.7): The t-test indicated a significant increase in growth period for #1, #3 and #7 which is reflected in the dot plots Longer FFL were measured for #1, #2, #3 and #7 ($P<0.05$). Selections #1 and #2 were generally taller ($P<0.05$) than the control. According to the dot plot, selections #4, #6 and #8 did not perform well with regards to yield per plant, which can be attributed to reduced tiller production. The t-test however indicated that the average of these lines did not differ significantly from the control. Selection #1 showed promise as a higher yielding line (YP and YS: $P<0.05$).

'BSP98/4+*Lr36+Lr34'* (Fig. 2.10; Table 2.7): An increased growth period and FLL were observed for all four selections ($P<0.05$), with #1 and #2 indicating a decrease in PH ($P<0.05$). Several progenies within the #1, #2 and #4 selections, showed promise as higher yielding lines, which was, however, not reflected by the t-test analysis of the means.

'BSP98/4+*Lr41+Lr34'* (Fig. 2.11; Table 2.7): Dot plot analysis indicated that selections #1 and #2 had similar to shorter growth periods as well as FLL. The means indicated significantly longer growth periods and FLL. Selection #1 had significantly higher YP ($P=0.004761^{**}$) which can be attributed to its increased NT ($P=0.002774^{*}$).

'BSP98/16+*Lr32+Lr34'* (Fig. 2.12; Table 2.7): Selections #1 and #2 indicated similar to shorter growth periods as well as FLL. Selection #2 produced significantly less YP and YS ($P<0.05$).

'BSP98/16+*Lr36+Lr34'* (Fig. 2.13; Table 2.7): A general increased growth period was observed for all selections ($P<0.01$) with the exception of #1. Selection #1 also performed significantly poorer with regards to FLL ($P=0.00395^{*}$), PH ($P=0.0039^{*}$), YP ($P=0.000405^{*}$) and YS ($P=0.000026^{*}$). The rest of the selected lines performed better than the control with regards to FLL and PH. According to the dot plot, both selections #2 and #3 appeared to be high yielding lines, but with the t-test #3, #5 and #6 produced significantly higher YP and YS ($P<0.01$) than the control.

'BSP98/16+*Lr41+Lr34'* (Fig. 2.14; Table 2.7): A slightly longer growth period was observed with the #1, #2 ($P<0.01$) and #3 ($P<0.01$) selections. Selection #2 showed an increased FLL ($P=0.000569$*). PH varied among the selections. Selection #2 showed increased PH ($P=0.02847$**). Dot plot analysis indicated that the #2 selection showed good tiller formation, resulting in increased yield per plant. The t-test only confirmed a significant increase in tiller production ($P=0.001727$**). YS was similar to the control for all the selected lines ($P<0.05$). Several progenies within the #2 and #3 selections showed potential for future use (dot plot analysis).

'Elands+*Lr32+Lr34'* (Fig. 2.15; Table 2.7): Selection #3 was excluded from the t-test due to insufficient n. Selections #1, #2 #4 and #5 showed a substantial shorter growth period ($P<0.01$). Selections #4 and #5 showed decreased FLL, PH and NT ($P<0.01$). None of the entries outperformed the control regarding YP, but all produced offspring that were superior to the control regarding YS (dot plot). The t-test indicated only #1 and #2 as significant better ($P<0.01$) than Elands.

'Elands+*Lr34+Lr21'* (Fig. 2.16; Table 2.7): T-test analysis could not be performed due to insufficient n. A shorter growth period was observed in some plants. FLL appeared to be longer in most of the progenies, with a general shorter plant stature. Poor YP was measured, due to reduced tiller formation as well as YS. Some exceptions within #3 and #5 progenies occurred.

'Elands+*Lr34+Lr32'* (Fig. 2.17; Table 2.7): (#2 – insufficient n for t-test) A general shorter growth period was observed ($P<0.05$). Selections #2 and #3 had FLL similar to the control (dot plot), with several progenies within #3 and #4 measuring significantly ($P<0.01$) longer lengths of up to 52.4 and 53.3 cm. Selection #1 was clearly a shorter genotype ($P<0.01$). Selections #1, #2 and several progeny of #4 produced below average YP, attributable to a reduced tiller production. This was supported by the observation that the YS appeared to be similar for all the entries, with few exceptions in #2.

'Elands+*Lr34+Lr36*' (Fig. 2.18; Table 2.7): (#3 – insufficient n for t-test) Selection #1 had a shorter growth period than the control ($P<0.05$). Selection #2 measured impressive FLL ($P<0.05$). Selection #5 showed promise as a high yielding line.

'Elands+*Lr34+Lr41*' (Fig. 2.19; Table 2.7): (#1 and #3 – insufficient n for t-test) The growth period of all the selected lines appeared severely shortened (#4: $P<0.01$). Similar FLL, PH and YS were obtained.

'Elands+*Lr41+Lr34*' (Fig. 2.20; Table 2.7): (#1 and #4 – insufficient n for t-test) A general reduced growth period was observed (#3: $P<0.01$). FLL and PH for #1 appeared to be shorter than the control. None of the selections outperformed the control with regards to YP. The dot plot analysis indicated that some progenies within selection #3 outperformed the control regarding YS. The t-test could however not confirm this general trend.

'T96/6+*Lr34+Lr21*' (Fig. 2.21; Table 2.7): (#1 – insufficient n for t-test) The single plant evaluated had a shorter growth period. A FLL of 41.4 cm was measured, with a PH similar to the control. An acceptable yield was produced, although not as high as the control. YS was comparable to that of the control.

'T96/6+*Lr34+Lr41*' (Fig. 2.22; Table 2.7): Selections #1, #2, #3, #4 and #6 had shorter growth periods than the control ($P<0.05$). All the selections had similar FLL, with the exception of #1 which produced longer FLL ($P=0.00019^{**}$). Selections #1, #4 and #6 were shorter than the control ($P<0.05$). NT was significantly smaller ($P<0.05$) for all the selections except #6. Selections #3, #4 and #6 produced significantly higher YS ($P<0.01$).

'T96/6+*Lr36+Lr34*' (Fig. 2.23; Table 2.7): (#3 – insufficient n for t-test) A general decrease in growth period was observed. (#1 and #2: $P<0.05$) Dot plot analysis indicated similar to shorter FLL for the #2 and #3 selections, with similar to longer leaves for #1. The t-test could not confirm the findings. A

reduced NT was observed (#1 and #2: P<0.01), with #1 indicating a significant increased YS (P=0.005239**).

'T96/6+*Lr41+Lr34'* (Fig. 2.24; Table 2.7): A general decrease in growth period was observed and NT was observed for all the selected lines (P<0.01). FLL and PH were similar to the control (P=0.05). YP was also smaller for #1 and #4 (P<0.01) with YS similar to the control for all four the selected lines.

'W98/6+*Lr21+Lr34'* (Fig. 2.25; Table 2.7): A general increase in growth period was observed (#1, DPM: P=0.0000**). FLL was similar to the control, with a significant increase in PH measured (#1, PH: P=0.000**). A significantly smaller NT was also produced (#1, NT: P=0.00115*). Some #1 offspring showed promise for future selection (#1, YS: P=0.02247**).

'W98/6+*Lr32+Lr34'* (Fig. 2.26; Table 2.7): (#5 – insufficient n for t-test) Selections #1, #2, #3, #4, #6 and #7 produced progenies with longer growth periods. (P<0.05) Selection #8 produced plants with significantly longer FLL (P=0.0008095*). PH of selections #4 to #8 is significantly longer than the control (P<0.05). Potential higher yielding plants were encountered within the #3 and #7 selections, although it could not be confirmed by the t-test.

'W98/6+*Lr34+Lr21'* (Fig. 2.27; Table 2.7): Significantly longer growth periods and PH were measured for all the selections, with selection #2 and #4 producing higher YP and YS (P<0.05).

'W98/6+*Lr34+Lr36'* (Fig. 2.28; Table 2.7): Selections #2 and #5 indicated a longer growth period (P<0.01). FLL varied within all selections. None of the selections outperformed the control in any of the traits measured.

'W98/6+*Lr36+Lr34'* (Fig. 2.29; Table 2.7): (#3 and #5 – insufficient n for t-test) Selections #2 and #4 indicated increased growth period (P<0.01). Selection #1 showed increased FLL (P=0.000016**) with decreased YS (P=0.002031**). Selection #2 however produced a significantly increased YS (P=0.039942**)

'W98/22+*Lr21*+*Lr34*' (Fig. 2.30; Table 2.7): Similar growth periods were observed for the control and selections. Significantly longer FLL were observed for #1 and #2 ($P<0.01$). In general all selections performed the same or slightly better than the control regarding the yield components. Some exceptions within selections #1 and #2 were, however, observed, but only #2 showed significant importance (#2, YP: P=0.001978**).

'W98/22+*Lr32*+*Lr34*' (Fig. 2.31; Table 2.7): (#2 – insufficient n for t-test) A similar growth period was observed for all the lines, except #2 and some of the progenies in #4 (#4, DPM: P=0.000201**). FLL was similar to the control for most of the lines (#5, FLL: P=0.010212** larger). PH appeared to be shorter for #1 (P=0.00155**) and #4 (P=0.001084**). In general poor YP was obtained by both the control and selections ($P<0.05$). .

'W98/22+*Lr36*+*Lr34*' (Fig. 2.32; Table 2.7): (#2 and #6 – insufficient n for t-test) Similar growth periods were obtained for all selections, with the exception of #3 (P=0.01894** larger). Selection #1 produced longer FLL (P=0.000327**). PH of all the selections was also similar to the control, excepting #3 (P=0.0000052** smaller). Similar results were also obtained for NT, YP and YS. Certain exceptions were observed where higher yields than the control were achieved (dot plots).

'W98/22+*Lr41*+*Lr34*' (Fig. 2.33; Table 2.7): Selections #1 and #2 produced significantly longer growth periods ($P<0.01$). Increased PH was measured for #1, #3 and #4 ($P<0.01$). Only #2 produced a significantly higher YP than the control.

2.4. Conclusions

Sawhney (1992) emphasised the importance of *Lr34* and its use in conjunction with other genes for the production of durable resistance to leaf rust. Sawhney (1992) further stated that *'the unique association of Lr34 with field resistance to stem and stripe rust permits achievement of combined*

resistance to all three rusts.' The present study therefore focused on the combination of the *Lr34* with the seedling resistance genes *Lr21, Lr32, Lr36 and Lr41.* No virulence has been reported in field samples of *P. triticina* for these genes in South Africa and the assumption is that these combinations will contribute to varieties with durable resistance. Widespread cultivation over space and time is considered the only true confirmation of durable resistance (Johnson, 1993). By incorporating several effective genes in advanced breeding material, it is anticipated that durability can be managed to some extent.

Future intercrossing can produce lines with three or more effective genes, or create new hybrids between lines differing from the adapted parent, but containing the same *Lr* gene combination. In the latter case selection could concentrate on agronomic performance as all progeny will have the two *Lr* genes. However, to detect more than one effective seedling gene verification by molecular markers, e.g. as described for *Lr41* (Lottering *et al.,* 1999), is needed.

The preliminary agronomic evaluation indicated that a breeding programme which focused on leaf rust resistance did not decrease the yield potential of most of the selections. In some of the cases the control parent lines were outperformed by the F_3 progenies. The progress in desirable phenotypes can to a large extent be ascribed to the use of adapted parental stocks. The background genotypes were identified as having potential by the Small Grain Institute, whereas the resistant Palmiet lines were developed not only for leaf rust resistance, but also agronomic adaptation. For a true indication of the yield and quality potential of the lines developed in this study, replicated field data need to be generated.

Table 2.1. Pedigrees of bread wheat cultivars and lines identified for improvement of leaf rust resistance

Cultivar/line	Pedigree
BSP97/1	Kariega*2/4/SSt3//Scout*5/AG/3/Kasteel/PY487(W82-1)
BSP98/4	YD"S"BON//DOVE"S"(BSP89-24)/3/V881(DICOC.DER)/V979 (BTL*TAU-1063)(BSP91-8)
BSP98/16	MARICO*2//TURTSIKUM/5*PALMIET
T96/6	SA463/5/4*T84-22BET//TMP/CI13523;STW646408/4/FLAM* 3/3/W66135//MAYO/WRR 4255-49-5
Elands	SA1684/4*MOLOPO-77
W98/6	IAS/ALDAN"S"/5/SST3//SCOUT*5/AG/3/KAST/PY487/4/80SOMERW214 -17-T1-TM
W98/22	KARIEGA*2/4/SST3//SCOUT*5/AG/3/KASTEEL/PY487(W82-1)

Table 2.2. Sources used for improving wheat leaf rust resistance

Resistance line	**Generation**	**Type of resistance gene**
Palmiet/*Lr21*	BC6F3	Seedling
Palmiet/*Lr32*	BC6F3	Seedling
Palmiet/*Lr34-Yr18*	BC6F3	Adult
Palmiet/*Lr36*	BC6F3	Seedling
Palmiet/*Lr41*	BC6F3	Seedling

Table 2.3. Infection types of parental lines and cultivars used in leaf rust resistance improvement

Cultivar/line	UVPrt 2	UVPrt 3	UVPrt 9	UVPrt 13
Susceptible parents				
BSP97/1	2	0	;1	0;
BSP98/4	;1C,2	;N	;1,3	$3,3^{+}$
BSP98/16	3	;1C	3^{-}	3^{++}
T96/6	0	;	3^{++}	$3^{-},3$
Elands	2^{-}	;,;1	3^{++}	2
W98/6	;1	;	;1	;,3
W98/22	;1	;	;1	2
Resistant parents				
Palmiet+*Lr21*	;	;	;,X	;
Palmiet+*Lr32*	;	;1	;	;1
Palmiet+*Lr34+Yr18*	0	;,;1	;	2
Palmiet+*Lr36*	;	;	;	;
Palmiet+*Lr41*	0;	0	0;	0
Control				
Morocco	3^{++}	3^{++}	3^{++}	3^{++}

Table 2.4. F_1 seedling infection type range and number of plants selected at anthesis for leaf tip necrosis

Adapted parent line	*Lr* gene combination	Pathotype	No. of seedlings with leaf rust infection type											Total F_1s evaluated	Selected F_1 seedlings	Selected *Ltn* adult plants
			0	0;	;	;1	;1N	1	;1+C	;1+CN	2	2+	3			
BSP97/1	32 X 34	UVPrt2				7					10		2	19	5	4
BSP97/1	34 X 21	UVPrt2				6	8							14	10	3
BSP97/1	34 X 36	UVPrt2				3					2			5	3	-
BSP98/4	21 X34	UVPrt13											3	3	-	-
BSP98/4	32 x 34	UVPrt13								2			15	17	2	2
BSP98/4	34 X 21	UVPrt13											3	3	-	-
BSP98/4	36 X 34	UVPrt13								3			6	9	3	3
BSP98/4	41 X 34	UVPrt13			6								6	12	6	5
BSP98/16	21 X 34	UVPrt13							10				9	19	4	3
BSP98/16	32 X 34	UVPrt13								4			7	11	4	4
BSP98/16	36 X 34	UVPrt13								7			10	17	4	1
BSP98/16	41 X 34	UVPrt13			8	2							5	15	6	6
T96/6	34 X 21	UVPrt9								1			15	16	1	1
T96/6	34 X 32	UVPrt9							2				4	6	1	1
T96/6	34 X 36	UVPrt9				1			2				8	11	3	2
T96/6	34 X 41	UVPrt9		2		5							4	11	7	6
T96/6	41 X 34	UVPrt9			3	4							13	20	7	6
Elands	32 x 34	UVPrt9				3			12				15	30	12	10
Elands	34 x 21	UVPrt9			1				3				22	26	3	3
Elands	34 x 32	UVPrt9			1	7							7	15	5	4
Elands	34 x 36	UVPrt9							8				8	16	8	7
Elands	34 x 41	UVPrt9		2	10	5							14	31	8	5
Elands	41 x 34	UVPrt9			5	4							11	20	7	4
W98/6	21 X 34	UVPrt13			1	4							20	24	5	4
W98/6	32 X 34	UVPrt13				7				1			11	19	6	5
W98/6	34 X 21	UVPrt13				2				3			3	8	2	2
W98/6	34 X 36	UVPrt13			3	2				7			4	16	6	6
W98/6	36 X 34	UVPrt13		1		1				8			3	13	5	5
W98/22	21 X 34	UVPrt13				2				4		8		14	5	4
W98/22	32 X 34	UVPrt13							1			5		6	1	1
W98/22	34 X 32	UVPrt13									13			13	-	-
W98/22	36 X 34	UVPrt13		2		5				6		10		23	7	5
W98/22	41 X 34	UVPrt13		4		3				4		8		19	7	6

Table 2.5. Adult reaction of selected F_2 plants to leaf and stripe rust in the field

Entry	Average infection within row		Reaction type of selected plants within rows Reaction types for leaf rust / stripe rust / and the presence of pseudo black chaff									
	Leaf rust	Stripe rust	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10
BSP97/1	0-TMS	TR										
BSP98/4	TR;70MS	TR										
BSP98/16	20S	TR;50R										
Elands	TS	70-80MR										
T96/6	0	70-80MR										
W98/6	0-TMS	TR										
W98/22	0-TMS	TR										
Palmiet+*Lr21*	0	60MR										
Palmiet+*Lr32*	0	90MR/MS (B)										
Palmiet+*Lr34*	0	TR										
Palmiet+*Lr36*	0	20-30R										
Palmiet+*Lr41*	0	20-30R										
BSP97/1+*Lr32+Lr34*	TS	30-60MS	0/TR/2**	0/TR/3***	0/TR/2	O/TR/1*						
BSP97/1+*Lr34+Lr21*	TS	40;80MS	0/5R/1	TMR/TR/1	TMS/TR/1	0/TR/1	TMS/TR/3	0/TR/2	0/TR/2	TS/TR/1		
BSP98/4+*Lr32+Lr34*	20;80MS	TR	40MS/TR/1	40MS/TR/1								
BSP98/4+*Lr36+Lr34*	TR	30MR	TR/TR/2	TR/10R/3	TR/5R/2	0/10R/3						
BSP98/4+*Lr41+Lr34*	TR	90MS/MR (B)	TR/20R/1	0/20R/2								
BSP98/16+*Lr21+Lr34*	-	-										
BSP98/16+*Lr32+Lr34*	0	60;80MR	TMS/10MS/2	TR/10R/2								
BSP98/16+*Lr36+Lr34*	TR	60MR	TR/TR/3	TR/TR/1	MS/20MS/3	TR/20MS/2	TR/TR/1	TR/20R/3				
BSP98/16+*Lr41+Lr34*	TMS	30MR;90MS (B)	0/TR/1	TMR/10MR/1	TMR/10MR/1	TMS/5MR/1	TMR/5R/1	TR/TR/2				
Elands+*Lr32+Lr34*	TMS	60MR	0/10MR;30MR/1	TR/TR/2	0/TR/1	TR/10R/2	0/10R/3					
Elands+*Lr34+Lr21*	0	60MR	0/20MR/3	0/10MR/3	0/TR/3	0/5R/3	0/5R/3					
Elands+*Lr34+Lr32*	TS	40-60MR	TR/10R/3	0/10R/3	0/20MS/3	TMS/10MR/1						
Elands+*Lr34+Lr36*	TMS	40-60MR	0/TR/2	0/10R/1	0/TR/3	0/10R/2	TR/TR/3					

1* = No melanism

2** = Few to half of florets show melanism

3*** = Nearly all floret brackets show melanism

Table 2.5. Adult reaction of selected F_2 plants to leaf and stripe rust in the field

Entry	Average infection within row		Reaction type of selected plants within rows Reaction types for leaf rust / stripe rust / and the presence of pseudo black chaff									
	Leaf rust	Stripe rust	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10
Elands+*Lr34+Lr41*	TS	60MR	TMS/20MS/1*	TR/10R/3***	TR/10R/3	TMS/10R/2**						
Elands+*Lr41+Lr34*	0	70-80MR	0/5R/1	TR/5R/3	TR/10R/1	TMS/5R/1						
T96/6+*Lr34+Lr21*	TMS	60MR	TMS/10R									
T96/6+*Lr34+Lr41*	0	80MR/MS	TR/30R/1	0/40MR/2	0/20R/3	0/10R/1	0/20R/1	0/20R/1				
T96/6+*Lr34+Lr32*	0	90MR/MS (B)										
T96/6+*Lr36+Lr34*	TMS	60MR	0/10R/1	0/5R/1	0/10R/2							
T96/6+*Lr41+Lr34*	TS	70-80MR	0/30MR/1	0/30MR/1	0/20MR/1	0/30MR/1						
W98/6+*Lr21+Lr34*	0	60MS	0/20R/1									
W98/6+*Lr32+Lr34*	TR;20MS	TR;60MS	0/5R/3	0/5R/3	0/10R/2	0/5R/3	TMS/TR/3	0/5R/3	0/TR/3	0/TR/3	O/TR/3	0/5R/3
W98/6+*Lr34+Lr21*	0-TMS	TR;60-80MS	0/5R/1	0/10R/3	0/TR/3	O/TR/1						
W98/6+*Lr34+Lr36*	0	TR;80MS	0/5R/2	0/20MR/2	0/5R/2	0/5R/3	0/10R/3	0/5R/2				
W98/6+*Lr36+Lr34*	0-TMS	20-30R	TR/TR/3	TMR/TR/3	0/10R/3	TMR/10MR/3	TR/TR/2	TMS/10R/3				
W98/22+*Lr21+Lr34*	TR;30MS	TR;80MS	TR/TR/3	TR/TR/1	TMS/TR/1	TR/TR/3						
W98/22+*Lr32+Lr34*	TR;60-70MS	TR;60MS	5MS/TR/3	TMS/TR/1	TR/5R/3	TMR/TR/3	TR/TR/1					
W98/22+*Lr36+Lr34*	0-TR	20-30R	TR/TR/2	TR/10R/3	TR/TR/2	TR/TR/3	TR/TR/3	TR/TR/3				
W98/22+*Lr41+Lr34*	TMS	20-30R	TR/TR/3	TMS/TR/1	TMS/TR/1	TR/TR/1						

1* = No melanism

2** = Few to half of florets show melanism

3*** = Nearly all floret brackets show melanism

Table 2.6. Number of F_3 seedlings in leaf rust infection type classes

Entry	Selection	Infection types															
		0	0;	;	;N	;1N	1	;1	;1+	;1+C	1+	1+C	1NC	2	2+	2+C	2+CN	3
BSP97/1+*Lr32+Lr34*	1			29				2										
BSP97/1+*Lr32+Lr34*	2				17	12												
BSP97/1+*Lr32+Lr34*	3				25	3												
BSP97/1+*Lr32+Lr34*	4			14		6										2		
BSP97/1+*Lr34+Lr21*	1			22				4						1				
BSP97/1+*Lr34+Lr21*	2			18		1								2				
BSP97/1+*Lr34+Lr21*	3			13						8				7				
BSP97/1+*Lr34+Lr21*	4			15					2							13		1
BSP97/1+*Lr34+Lr21*	5				1			1								26		4
BSP97/1+*Lr34+Lr21*	6			23				1						1				
BSP97/1+*Lr34+Lr21*	7			19		1										9		
BSP97/1+*Lr34+Lr21*	8				22													
BSP98/4+*Lr32+Lr34*	1																	22
BSP98/4+*Lr32+Lr34*	2													24				
BSP98/4+*Lr36+Lr34*	1			4		17										2		
BSP98/4+*Lr36+Lr34*	2			6	12			10										
BSP98/4+*Lr36+Lr34*	3			4	3			9		5						3		
BSP98/4+*Lr36+Lr34*	4			3	3			3								3		2
BSP98/4+*Lr41+Lr34*	1			13				2						2				3
BSP98/4+*Lr41+Lr34*	2		16															4
BSP98/16+*Lr32+Lr34*	1			2					4							11		3
BSP98/16+*Lr32+Lr34*	2			3						5						7		3
BSP98/16+*Lr36+Lr34*	1			16					6					3				6
BSP98/16+*Lr36+Lr34*	2		2	10				2						7				2
BSP98/16+*Lr36+Lr34*	3			12				1								6		2
BSP98/16+*Lr36+Lr34*	4			8					3					3				1
BSP98/16+*Lr36+Lr34*	5			3				4						2				2
BSP98/16+*Lr36+Lr34*	6			2		9								4				7
BSP98/16+*Lr41+Lr34*	1	1							2					23				1
BSP98/16+*Lr41+Lr34*	2		13	14										4				2

Table 2.6. Number of F_3 seedlings in leaf rust infection type classes

Entry	Selection	Infection types																
		0	0;	;	;N	;1N	1	;1	;1$^+$	;1$^+$C	1$^+$	1$^+$C	1NC	2	2$^+$	2$^+$C	2$^+$CN	3
BSP98/16+*Lr41+Lr34*	3			22										7				2
BSP98/16+*Lr41+Lr34*	4													28				
BSP98/16+*Lr41+Lr34*	5			2					1					18				7
BSP98/16+*Lr41+Lr34*	6																	
Elands+*Lr32+Lr34*	1				5	14												
Elands+*Lr32+Lr34*	2				5	3								22				
Elands+*Lr32+Lr34*	3				17			4										
Elands+*Lr32+Lr34*	4				30													
Elands+*Lr32+Lr34*	5				7	8										7		
Elands+*Lr34+Lr21*	1			1												27		
Elands+*Lr34+Lr21*	2												4				15	
Elands+*Lr34+Lr21*	3												6				30	
Elands+*Lr34+Lr21*	4							1								28		
Elands+*Lr34+Lr21*	5				10					9						14		
Elands+*Lr34-Lr32*	1				24			7						6				
Elands+*Lr34-Lr32*	2			4						4				5				
Elands+*Lr34-Lr32*	3			22				1						1		1		2
Elands+*Lr34-Lr32*	4			7	2	7								5				
Elands+*Lr34+Lr36*	1			3				14						10				
Elands+*Lr34+Lr36*	2				13	8								4				2
Elands+*Lr34+Lr36*	3			2		5											5	3
Elands+*Lr34+Lr36*	4				3	10								4				1
Elands+*Lr34+Lr36*	5			14					10									
Elands+*Lr34+Lr41*	1												6			26		
Elands+*Lr34+Lr41*	2															21		7
Elands+*Lr34+Lr41*	3							7								21		
Elands+*Lr34-Lr41*	4			20				1						10				2
Elands+*Lr41+Lr34*	1																	
Elands+*Lr41+Lr34*	2												5				20	4
Elands+*Lr41+Lr34*	3		11	5										5				2

Table 2.6. Number of F_3 seedlings in leaf rust infection type classes

Entry	Selection	Infection types																
		0	0;	;	;N	;1N	1	;1	;1+	;1+C	1+	1+C	1NC	2	2+	2+C	2+CN	3
Elands+*Lr41+Lr34*	4			1					1					18				5
T96/6+*Lr34+Lr21*	1				2									9				1
T96/6+*Lr34+Lr41*	1			10											22			
T96/6+*Lr34+Lr41*	2			19				6							6			1
T96/6+*Lr34+Lr41*	3			25														
T96/6+*Lr34+Lr41*	4		2	23														
T96/6+*Lr34+Lr41*	5													23	11			1
T96/6+*Lr34+Lr41*	6			21				2						6				4
T96/6+*Lr36+Lr34*	1			24												13		
T96/6+*Lr36+Lr34*	2				12	4						2						
T96/6+*Lr36+Lr34*	3									10		4				11		2
T96/6+*Lr41+Lr34*	1				20			8							8			1
T96/6+*Lr41+Lr34*	2			16				2						7				5
T96/6+*Lr41+Lr34*	3		6	17				1						6				
T96/6+*Lr41+Lr34*	4			23														
W98/6+*Lr21+Lr34*	1				28									6				6
W98/6+*Lr32+Lr34*	1			16				3										
W98/6+*Lr32+Lr34*	2			17					3					2				1
W98/6+*Lr32+Lr34*	3			23												9		
W98/6+*Lr32+Lr34*	4			30														
W98/6+*Lr32+Lr34*	5			4				5						10				6
W98/6+*Lr32+Lr34*	6			10							1				14			11
W98/6+*Lr32+Lr34*	7			27										9				3
W98/6+*Lr32+Lr34*	8			30														
W98/6+*Lr32+Lr34*	9			29										2				1
W98/6+*Lr34+Lr36*	1													11				5
W98/6+*Lr34+Lr36*	2			21				5						1				1
W98/6+*Lr34+Lr36*	3			32										1				
W98/6+*Lr34+Lr36*	4			30														
W98/6+*Lr34+Lr36*	5			31					2									

Table 2.6. Number of F_3 seedlings in leaf rust infection type classes

Entry	Selection	Infection types																	
		0	0;	;	;N	;1N	1	;1	;1$^+$	;1$^+$C	1$^+$	1$^+$C	1NC	2	2$^+$	2$^+$C	2$^+$CN	3	3^{++}
W98/6+*Lr34+Lr36*	6			4				8						16				2	
W98/6+*Lr34+Lr21*	1		6	19										7				5	
W98/6+*Lr34+Lr21*	2			17		1								2				1	
W98/6+*Lr34+Lr21*	3			21										4				3	
W98/6+*Lr34+Lr21*	4			21										3				9	
W98/6+*Lr36+Lr34*	1				10									10					14
W98/6+*Lr36+Lr34*	2		3	8	5		2							6				3	
W98/6+*Lr36+Lr34*	3					4											18	8	
W98/6+*Lr36+Lr34*	4			20	3														
W98/6+*Lr36+Lr34*	5				3										20			3	
W98/6+*Lr36+Lr34*	6															9		5	
W98/22+*Lr21+Lr34*	1			8												6		7	
W98/22+*Lr21+Lr34*	2			12				4								5			8
W98/22+*Lr21+Lr34*	3			15				7							2	4			6
W98/22+*Lr21+Lr34*	4								1							12			12
W98/22+*Lr32+Lr34*	1			13										4					1
W98/22+*Lr32+Lr34*	2	1		1		1													14
W98/22+*Lr32+Lr34*	3																		
W98/22+*Lr32+Lr34*	4			11				6						6					6
W98/22+*Lr32+Lr34*	5			5				4						6		1			4
W98/22+*Lr36+Lr34*	1			7		5									6				6
W98/22+*Lr36+Lr34*	2		1					3	3						9				
W98/22+*Lr36+Lr34*	3		11	8				5											
W98/22+*Lr36+Lr34*	4			10				2						4					
W98/22+*Lr36+Lr34*	5			10				4						3					8
W98/22+*Lr36+Lr34*	6			8				4	1					4					7
W98/22+*Lr41+Lr34*	1			23												8			
W98/22+*Lr41+Lr34*	2			10		3								6					
W98/22+*Lr41+Lr34*	3			18	4														
W98/22+*Lr41+Lr34*	4		5	1										1				1	

Table 2.7. Two-sample t-test analyses of means for various traits measured for each F_3 -line compared to the respective control

Entry	Selection	Flag leaf length	Plant height	Days to anthesis	Days to physiological maturity	Tillers per plant	Yield per plant	Yield per spike
BSP97/1 +*Lr32+Lr34*	1	[d] 0.0005*	[c] 0.947379	[c] 0.0001806*(>)	[c] 0.000161*(>)	[a] 0.795995	[c] 0.5525	[c] 0.82971
BSP97/1 +*Lr32+Lr34*	2	[c] 0.000001*(>)	[c] 0.000721*(>)	[d] 0.0000*(>)	[d] 0.001*(>)	[a] 0.058267	[c] 0.1037	[a] 0.316458
BSP97/1 +*Lr32+Lr34*	3	[d] 0.0003*	[c] 0.000264*(>)	[c] 0.000464*(>)	[d] 0.0004*(>)	[c] 0.466369	[c] 0.495013	[c] 0.473158
BSP97/1 +*Lr32+Lr34*	4	[c] 0.000138*(>)	[c] 0.018496*(>)	[c] 0.0037*(>)	[c] 0.000847*(>)	[a] 1.0000	[c] 0.702899	[a] 0.22594
BSP97/1 +*Lr34+Lr21*	1	[c] 0.000077*(>)	[c] 0.001534*(>)	[d] 0.000003*(>)	[d] 0.0005*(>)	[a] 0.001737**(>)	[c] 0.000049*(>)	[b] 0.0000**(>)
BSP97/1 +*Lr34+Lr21*	2	[c] 0.001381*(>)	[c] 0.000825*(>)	[c] 0.138257	[c] 0.005876*(>)	[b] 0.400497	[c] 0.441361	[c] 0.165803
BSP97/1 +*Lr34+Lr21*	3	[c] 0.000221*(>)	[c] 0.091367	[c] 0.000127*(>)	[c] 0.005732*(>)	[a] 0.87737	[c] 0.524597	[c] 0.271283
BSP97/1 +*Lr34+Lr21*	4	[c] 0.008406*(<)	[c] 0.979711	[c] 0.00365*(<)	[c] 0.09717	[b] 0.000055** (<)	[d] 0.0092*(<)	[a] 0.104257
BSP97/1 +*Lr34+Lr21*	6	[c] 0.000006*(<)	[c] 0.097833	[c] 0.00228*(<)	[c] 0.000778*(<)	[b] 0.000809**(<)	[c] 0.012605*(<)	[a] 0.085715
BSP97/1 +*Lr34+Lr21*	7	[c] 0.000385*(>)	[c] 0.811922	[d] 0.0000*(>)	[c] 0.060342	[a] 0.21955	[c] 0.096023	[a] 0.121118
BSP97/1 +*Lr34+Lr21*	8	[c] 0.436156	[c] 0.830465	[c] 0.015816*(<)	[c] 0.000294*(<)	[b] 0.289984	[c] 0.83045	[a] 0.989409
BSP98/4 +*Lr36+Lr34*	1	[a] 0.0000**(>)	[a] 0.0000**(<)	[b] 0.01336**(>)	[c] 0.0002*(>)	[a] 0.09738	[d] 0.147167	[a] 0.507766
BSP98/4 +*Lr36+Lr34*	2	[b] 0.001789**(>)	[c] 0.00177*(<)	[b] 0.000456**(>)	[a] 0.00054**(>)	[b] 0.577107	[d] 0.79527	[a] 0.352147
BSP98/4 +*Lr36+Lr34*	3	[b] 0.002888**(>)	[a] 0.89339	[b] 0.005537**(>)	[b] 0.021965**(>)	[a] 0.124484	[d] 0.98298	[a] 0.384268
BSP98/4 +*Lr36+Lr34*	4	[b] 0.006634**(>)	[d] 0.165739	[b] 0.011561**(>)	[a] 0.010779**(>)	[d] 0.15257	[d] 0.346076	[d] 0.731858
BSP98/4 +*Lr41+Lr34*	1	[a] 0.0000**(>)	[a] 0.0000**(>)	0.000593**(>)	[a] 0.001867**(>)	[c] 0.002774* (>)	[c] 0.004761*(>)	[a] 0.082461
BSP98/4 +*Lr41+Lr34*	2	[a] 0.017559**(>)	[a] 0.337061	[b] 0.001318**(>)	[a] 0.353698	[a] 0.001702**(<)	[c] 0.174372	[c] 0.951492
BSP98/16 +*Lr32+Lr34*	1	[c] 0.873546	[b] 0.014338**(<)	[a] 0.948921	[a] 0.74767	[d] 0.531401	[d] 0.51643	[a] 0.318734
BSP98/16 +*Lr32+Lr34*	2	[c] 0.06033	[a] 0.112111	[b] 0.122997	[a] 0.434804	[a] 0.00262**(<)	[a] 0.002693**(<)	[a] 0.003167**(<)
BSP98/16 +*Lr36+Lr34*	1	[c] 0.0003959*<	[b] 0.003906**(<)	[a] 0.191966	[a] 0.342778	[a] 0.097676	[a] 0.000405**(<)	[a] 0.000026** (<)
BSP98/16 +*Lr36+Lr34*	2	[d] 0.0168*	[b] 0.122786	[b] 0.004972**(>)	[a] 0.02044**(>)	[b] 0.091349	[b] 0.096067	[b] 0.389938
BSP98/16 +*Lr36+Lr34*	3	[c] 0.000099*	[b] 0.0000**(>)	[a] 0.000147**(>)	[a] 0.000008**(>)	[b] 0.000254**(<)	[b] 0.002208**(>)	[b] 0.010733**(>)
BSP98/16 +*Lr36+Lr34*	4	[c] 0.071333	[b] 0.910138	[a] 0.0000**(>)	[a] 0.017601**(>)	[a] 0.566533	[a] 0.777932	[a] 0.313029
BSP98/16 +*Lr36+Lr34*	5	[c] 0.00021*(>)	[a] 0.000004**(>)	[a] 0.0000**(>)	[a] 0.000106**(>)	[a] 0.000412**(>)	[a] 0.00069**(>)	[a] 0.013662**(>)
BSP98/16 +*Lr36+Lr34*	6	[c] 0.005596*(>)	[a] 0.000135**(>)	[a] 0.0000**(>)	[a] 0.00003**(>)	[a] 0.000279**(>)	[d] 0.000611**(>)	[a] 0.013129**(>)
BSP98/16 +*Lr41+Lr34*	1	-	-	-	-	-	-	-
BSP98/16 +*Lr41+Lr34*	2	[c] 0.000569*	[a] 0.02847**(>)	[a] 0.0000**(>)	[b] 0.000012**(>)	[b] 0.001727**(>)	[c] 0.168283	[a] 0.550344
BSP98/16 +*Lr41+Lr34*	3	[c] 0.585868	[b] 0.97117	[b] 0.013924**(>)	[a] 0.012207**(>)	[a] 0.490404	[a] 0.978072	[a] 0.282485
Elands +*Lr32+Lr34*	1	[a] 0.36478	[d] 0.719409	[a] 0.0000**(<)	[d] 0.0000*	[b] 0.00347**(<)	[a] 0.42677	[b] 0.019019**(>)
Elands +*Lr32+Lr34*	2	[c] 0.116796	[c] 0.25762	[d] 0.0000**(<)	[d] 0.0000*	[d] 0.0205*	[d] 0.382189	[d] 0.004337**(>)
Elands +*Lr32+Lr34*	3	-	-	-	-	-	-	-

* - P<0.05
** - P<0.01
(>) – Significant larger than control mean
(<) – Significant smaller than the control mean

a - Equal variance T-test
b - Unequal variance T-test
c - Mann-Whitney U or Wilcoxon Rank-Sum test
d - Kolmogorov-Smirnov test

- - insufficient n

Table 2.7. Two-sample t-test analyses of means for various traits measured for each F_3-line compared to the respective control

Entry	Selection	Flag leaf length	Plant height	Days to anthesis	Days to physiological maturity	Tillers per plant	Yield per plant	Yield per spike
Elands +*Lr32+Lr34*	4	[a] 0.011668**(<)	[a] 0.000197**(<)	[b] 0.0000**(<)	[d] 0.0000*	[b] 0.000142**(<)	[b] 0.001879**(<)	[b] 0.050169
Elands +*Lr32+Lr34*	5	[a] 0.000698**(<)	[a] 0.000058**(<)	[a] 0.0000057**(<)	[d] 0.0927	[b] 0.021628**(<)	[b] 0.065909	[a] 0.899195
Elands +*Lr34+Lr21*	1	-	-	-	-	-	-	-
Elands +*Lr34+Lr21*	2	-	-	-	-	-	-	-
Elands +*Lr34+Lr21*	3	-	-	-	-	-	-	-
Elands +*Lr34+Lr21*	5	-	-	-	-	-	-	-
Elands +*Lr34+Lr32*	1	[a] 0.997708	[a] 0.0000**(<)	[a] 0.0000**(<)	[c] 0.000001*	[a] 0.0007*	[b] 0.000149**(<)	[a] 0.540264
Elands +*Lr34+Lr32*	2	-	-	-	-	-	-	-
Elands +*Lr34+Lr32*	3	[a] 0.004963**(>)	[a] 0.229033	[c] 0.000066*	[d] 0.0000*	[a] 0.475918	[d] 0.63941	[a] 0.492422
Elands +*Lr34+Lr32*	4	[a] 0.000702**(>)	[a] 0.246259	[a] 0.014351**(<)	[c] 0.77468	[a] 0.699554	[a] 0.915653	[a] 0.895696
Elands +*Lr34+Lr36*	1	[a] 0.502437	[a] 0.0000*(<)	[a] 0.0000*(<)	[c] 0.0000035*(<)	[a] 0.141604	[a] 0.575856	[a] 0.007683**(<)
Elands +*Lr34+Lr36*	2	[a] 0.000022**(>)	[a] 0.000001**(<)	[a] 0.00155**(<)	[c] 0.750340	[a] 0.444366	[a] 0.308045	[a] 0.43581
Elands +*Lr34+Lr36*	3	-	-	-	-	-	-	-
Elands +*Lr34+Lr36*	4	[d] 0.182791	[a] 0.0000**	[a] 0.143749	[c] 0.409628	[a] 0.773441	[a] 0.094556	[d] 0.001988**(<)
Elands +*Lr34+Lr36*	5	[a] 0.023379**(>)	[b] 0.827575	[a] 0.00004**(<)	[c] 0.002022*(<)	[a] 0.970558	[a] 0.631167	[a] 0.150323
Elands +*Lr34+Lr41*	1	-	-	-	-	-	-	-
Elands +*Lr34+Lr41*	3	-	-	-	-	-	-	-
Elands +*Lr34+Lr41*	4	[a] 0.192064	[d] 0.60163	[d] 0.0000**(<)	[c] 0.0000035*(<)	[d] 0.001808**(<)	[d] 0.0647989	[d] 0.283953
Elands +*Lr41+Lr34*	1	-	-	-	-	-	-	-
Elands +*Lr41+Lr34*	3	[a] 0.993339	[a] 0.004939**(<)	[a] 0.000001*(<)	[c] 0.000001*(<)	[b] 0.00542**(<)	[a] 0.008116**(<)	[a] 0.68586
Elands +*Lr41+Lr34*	4	-	-	-	-	-	-	-
T96/6 +*Lr34+Lr21*	1	-	-	-	-	-	-	-
T96/6 +*Lr34+Lr41*	1	[a] 0.00019**(>)	[c] 0.001263*(<)	[b] 0.000048**(<)	[b] 0.000437**(<)	[a] 0.01701**(<)	[a] 0.169774	[a] 0.347285
T96/6 +*Lr34+Lr41*	2	[a] 0.890462	[c] 0.157213	[a] 0.0000**(<)	[b] 0.0000**(<)	[b] 0.00042**(<)	[a] 0.075227	[b] 0.895131
T96/6 +*Lr34+Lr41*	3	[a] 0.064826	[c] 0.100456	[d] 0.0000**(<)	[c] 0.0000105*(<)	[b] 0.000066**(<)	[c] 0.018354*(<)	[b] 0.017665**(>)
T96/6 +*Lr34+Lr41*	4	[a] 0.785131	[c] 0.000014*(<)	[c] 0.000002*(<)	[b] 0.0000**(<)	[b] 0.000137**(<)	[c] 0.076547	[a] 0.003417**(>)
T96/6 +*Lr34+Lr41*	6	[d] 0.261077	[c] 0.003256*(<)	[b] 0.0000**(<)	[b] 0.000282**(<)	[a] 0.136683	[a] 0.892706	[d] 0.018198**(>)
T96/6 +*Lr36+Lr34*	1	[a] 0.162062	[c] 0.319766	[a] 0.0000**(<)	[b] 0.0000**(<)	[d] 0.000109**(<)	[d] 0.376157	[a] 0.005239**(>)
T96/6 +*Lr36+Lr34*	2	[d] 0.05321	[c] 0.069786	[b] 0.000175**(<)	[c] 0.0000415*(<)	[d] 0.014273**(<)	[d] 0.059768	[a] 0.971415
T96/6 +*Lr36+Lr34*	3	-	-	-	-	-	-	-

* - P<0.05
** - P<0.01
(>) – Significant larger than control mean
(<) – Significant smaller than the control mean

a - Equal variance T-test
b - Unequal variance T-test
c - Mann-Whitney U or Wilcoxon Rank-Sum test
d - Kolmogorov-Smirnov test

- - insufficient n

Table 2.7. Two-sample t-test analyses of means for various traits measured for each F_3-line compared to the respective control

Entry	Selection	Flag leaf length	Plant height	Days to anthesis	Days to physiological maturity	Tillers per plant	Yield per plant	Yield per spike
T96/6 +*Lr41+Lr34*	1	[a] 0.56654	[c] 0.298601	[b] 0.000002**(<)	[b] 0.000626**(<)	[a] 0.001333**(<)	[d] 0.01064**(<)	[a] 0.919701
T96/6 +*Lr41+Lr34*	2	[a] 0.21078	[c] 0.766076	[a] 0.000046**(<)	[b] 0.006913**(<)	[b] 0.099248**(<)	[a] 0.084684	[a] 0.854807
T96/6 +*Lr41+Lr34*	3	[a] 0.161187	[c] 0.080276	[a] 0.0000**(<)	[b] 0.000076**(<)	[a] 0.008121**(<)	[c] 0.074721	[a] 0.617032
T96/6 +*Lr41+Lr34*	4	[a] 0.00042**(<)	[c] 0.783166	[a] 0.0000**(<)	[b] 0.0000**(<)	[b] 0.000166**(<)	[b] 0.011322**(<)	[d] 0.053761
W98/6+*Lr21+Lr34*	1	[b] 0.914154	[b] 0.0000**(>)	[a] 0.000009**(<)	[a] 0.0000**(>)	[c] 0.0115045*(<)	[c] 0.735268	[a] 0.022472**(>)
W98/6+*Lr32+Lr34*	1	[b] 0.81577	[b] 0.008823**(>)	[b] 0.00065**(>)	[a] 0.006839**(>)	[a] 0.002428**(<)	[c] 0.352876	[a] 0.329108
W98/6+*Lr32+Lr34*	2	[b] 0.174417	[a] 0.005270**(>)	[b] 0.109374	[a] 0.008049*(>)	[a] 0.01359**(<)	[c] 0.150495	[a] 0.569296
W98/6+*Lr32+Lr34*	3	[a] 0.6052399	[c] 0.0005505*(>)	[a] 0.01172**(>)	[a] 0.00009**(>)	[a] 0.210292	[c] 0.115685	[a] 0.395947
W98/6+*Lr32+Lr34*	4	[a] 0.001081**(>)	[c] 0.0000155*(>)	[a] 0.000002**(>)	[a] 0.0000**(>)	[a] 0.904667	[c] 0.47348	[a] 0.653486
W98/6+*Lr32+Lr34*	5	-	-	-	-	-	-	-
W98/6+*Lr32+Lr34*	6	[a] 0.328505	[c] 0.0002025*(>)	[d] 0.111158	[a] 0.01612**(>)	[a] 0.071048	[c] 0.401411	[a] 0.935667
W98/6+*Lr32+Lr34*	7	[a] 0.958729	[c] 0.172802	[a] 0.001466**(>)	[a] 0.0000**(>)	[a] 0.516823	[c] 0.746603	[a] 0.944
W98/6+*Lr32+Lr34*	8	[a] 0.0008095*(>)	[c] 0.0021515*(<)	[a] 0.76278	[a] 0.196886	[d] 0.002142**(<)	[c] 0.004893*(<)	[c] 0.0025575*(<)
W98/6+*Lr32+Lr34*	9	[a] 0.018973**(<)	[c] 0.008011*(<)	[a] 0.00871**(>)	[a] 0.000015**(<)	[a] 0.000342**(<)	[c] 0.000423*(<)	[b] 0.00267**(<)
W98/6+*Lr34+Lr21*	1	[a] 0.262534	[d] 0.0000*	[b] 0.001036**(>)	[b] 0.000002**(>)	[a] 0.000852**(<)	[c] 0.352619	[a] 0.281581
W98/6+*Lr34+Lr21*	2	[a] 0.000007**(>)	[c] 0.000004*(>)	[d] 0.0000**(>)	[a] 0.0000**(>)	[d] 0.0000**(>)	[c] 0.000017*(>)	[c] 0.000101*(>)
W98/6+*Lr34+Lr21*	3	[a] 0.004873**(<)	[c] 0.000183*(>)	[a] 0.639329	[a] 0.000345**(>)	[a] 0.97075	[c] 0.711541	[a] 0.82886
W98/6+*Lr34+Lr21*	4	[a] 0.000002**(>)	[c] 0.0000005*(>)	[a] 0.0000**(>)	[a] 0.0000**(>)	[c] 0.32726	[c] 0.000168*(>)	[a] 0.000013**(>)
W98/6+*Lr34+Lr36*	2	[a] 0.002678**(>)	[c] 0.000056*(>)	[b] 0.002746**(>)	[a] 0.000165**(>)	[c] 0.272089	[c] 0.725639	[d] 0.294832
W98/6+*Lr34+Lr36*	3	[a] 0.001946**(<)	[c] 0.286052	[a] 0.024112**(<)	[a] 0.811879	[a] 0.00344**(<)	[c] 0.07748	[b] 0.562931
W98/6+*Lr34+Lr36*	4	[a] 0.058465	[c] 0.000039*(>)	[a] 0.01587**(>)	[a] 0.789363	[a] 0.01277**(<)	[c] 0.260353	[a] 0.837516
W98/6+*Lr34+Lr36*	5	[a] 0.505416	[c] 0.018608*(>)	[c] 0.011285*(>)	[a] 0.000452**(>)	[b] 0.842905	[c] 0.402656	[a] 0.361551
W98/6+*Lr34+Lr36*	6	[b] 0.002039**(<)	[c] 0.97287	[a] 0.012893**(<)	[a] 0.303365	[a] 1.0000	[c] 0.324580	[a] 0.917363
W98/6+*Lr36+Lr34*	1	[a] 0.000016*(<)	[c] 0.52318	[a] 0.195311	[b] 0.739514	[c] 0.022604*(<)	[c] 0.010453*(<)	[b] 0.002031**(<)
W98/6+*Lr36+Lr34*	2	[a] 0.148078	[c] 0.000129*(<)	[d] 0.0124*(>)	[a] 0.000003**(>)	[b] 0.702507	[c] 0.165803	[a] 0.039942**(>)
W98/6+*Lr36+Lr34*	3	-	-	-	-	-	-	-
W98/6+*Lr36+Lr34*	4	[a] 0.003856**(>)	[c] 0.000389*(>)	[a] 0.0244**(>)	[a] 0.001177**(>)	[c] 0.908284	[c] 0.138777	[d] 0.170001
W98/6+*Lr36+Lr34*	5	-	-	-	-	-	-	-
W98/22+*Lr21+Lr34*	1	[a] 0.000936**(>)	[a] 0.265315	[a] 0.003989**(>)	[d] 0.116703	[d] 0.147295	[c] 0.170904	[a] 0.284464
W98/22+*Lr21+Lr34*	2	[a] 0.006095**(>)	[c] 0.84300	[a] 0.985405	[c] 0.658377	[a] 0.00013**	[c] 0.001978*(>)	[a] 0.072729
W98/22+*Lr21+Lr34*	3	[a] 0.679256	[b] 0.007247**(<)	[a] 0.239332	[a] 0.15688	[c] 0.981817	[c] 0.467898	[b] 0.62214

* - P<0.05
** - P<0.01
(>) – Significant larger than control mean
(<) – Significant smaller than the control mean

a - Equal variance T-test
b - Unequal variance T-test
c - Mann-Whitney U or Wilcoxon Rank-Sum test
d - Kolmogorov-Smirnov test

- - insufficient n

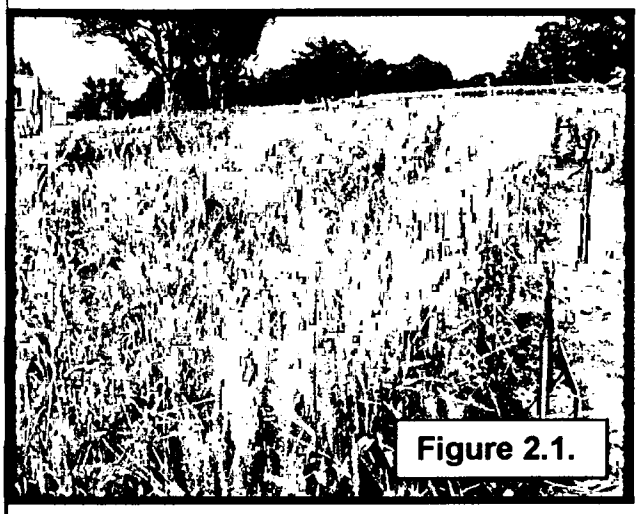
Table 2.7. Two-sample t-test analyses of means for various traits measured for each F_3-line compared to the respective control

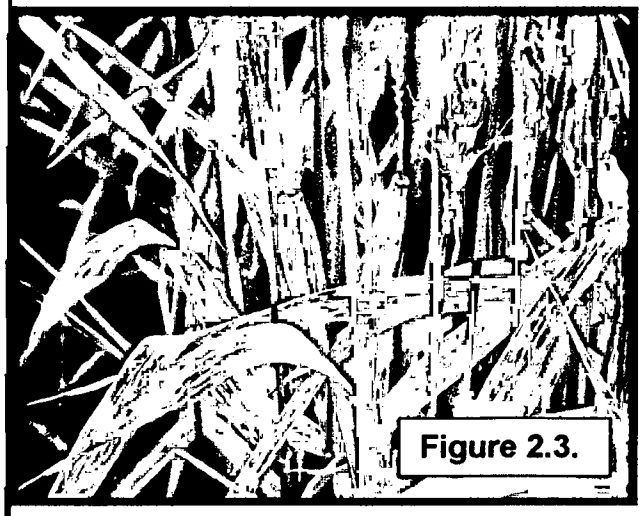
Entry	Selection	Flag leaf length	Plant height	Days to anthesis	Days to physiological maturity	Tillers per plant	Yield per plant	Yield per spike
W98/22+*Lr32+Lr34*	1	[a] 0.282373	[a] 0.00155**(<)	[a] 0.322146	[b] 0.250806	[a] 0.024426**(<)	[c] 0.011817*(<)	[a] 0.252762
W98/22+*Lr32+Lr34*	2	-	-	-	-	-	-	-
W98/22+*Lr32+Lr34*	4	[a] 0.77096	[a] 0.001084**(<)	[b] 0.480721	[b] 0.488766	[a] 0.00155**(<)	[c] 0.004057*	[a] 0.017712**(<)
W98/22+*Lr32+Lr34*	5	[a] 0.010212**(>)	[b] 0.053564	[a] 0.235704	[a] 0.000201**(>)	[b] 0.006751**(>)	[c] 0.001727*(>)	[a] 0.122834
W98/22+*Lr36+Lr34*	1	[a] 0.000327**(>)	[a] 0.288733	[b] 0.119334	[a] 0.153956	[a] 0.169993	[c] 0.446821	[a] 0.757903
W98/22+*Lr36+Lr34*	2	-	-	-	-	-	-	-
W98/22+*Lr36+Lr34*	3	[a] 0.013574**(<)	[b] 0.0000052**(<)	[a] 0.308319	[b] 0.015894**(>)	[a] 0.756113	[c] 0.808805	[a] 0.448215
W98/22+*Lr36+Lr34*	4	[a] 0.569461	[a] 0.870656	[a] 0.085673	[a] 0.28733	[a] 0.178367	[c] 0.933858	[a] 0.198732
W98/22+*Lr36+Lr34*	5	[a] 0.00013**(<)	[a] 0.193859	[b] 0.004021**(<)	[a] 0.865935	[b] 0.649959	[c] 1.0000	[a] 0.575925
W98/22+*Lr36+Lr34*	6	-	-	-	-	-	-	-
W98/22+*Lr41+Lr34*	1	[a] 0.4444303	[a] 0.003967**(<)	[b] 0.66172	[a] 0.011547**(>)	[a] 0.06247	[c] 0.001283*(<)	[b] 0.000048**(<)
W98/22+*Lr41+Lr34*	2	[a] 0.000047**(>)	[a] 0.42058	[a] 0.0005**(>)	[a] 0.003917**(>)	[c] 0.732465	[c] 0.133559	[a] 0.01567**(>)
W98/22+*Lr41+Lr34*	3	[a] 0.762395	[a] 0.000001**(<)	[d] 0.0137*	[a] 0.086923	[c] 0.023528*(>)	[c] 0.922478	[a] 0.336341
W98/22+*Lr41+Lr34*	4	[b] 0.000936**(<)	[a] 0.017307**(<)	[b] 0.000078**(<)	[a] 0.0000**(<)	[a] 0.442176	[c] 0.120691	[a] 0.124304

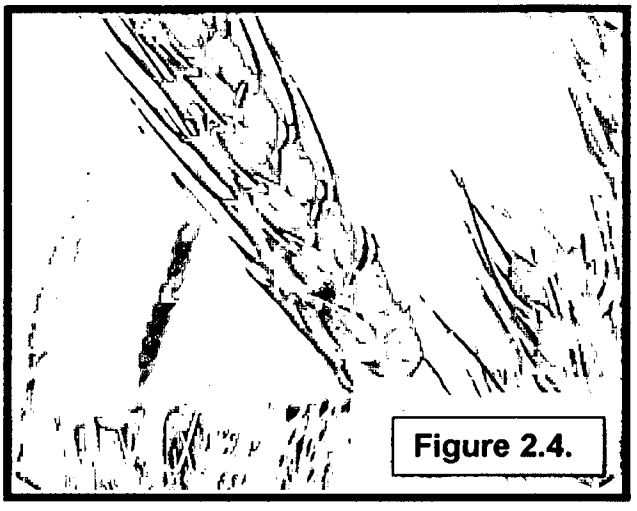
* - P<0.05
** - P<0.01
(>) – Significant larger than control mean
(<) – Significant smaller than the control mean

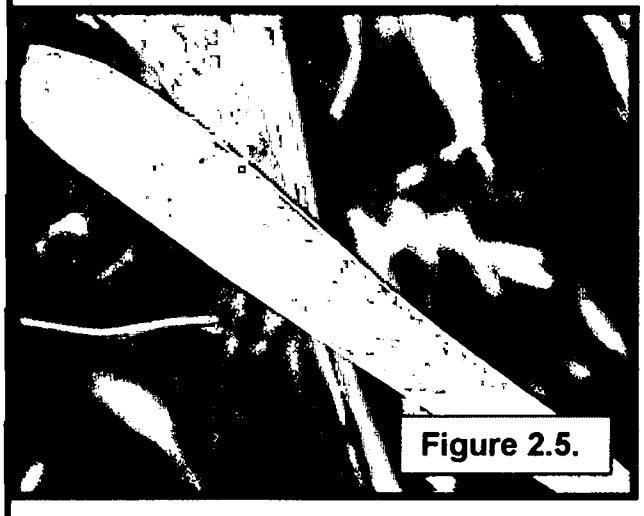
a - Equal variance T-test
b - Unequal variance T-test
c - Mann-Whitney U or Wilcoxon Rank-Sum test
d - Kolmogorov-Smirnov test

- - insufficient n









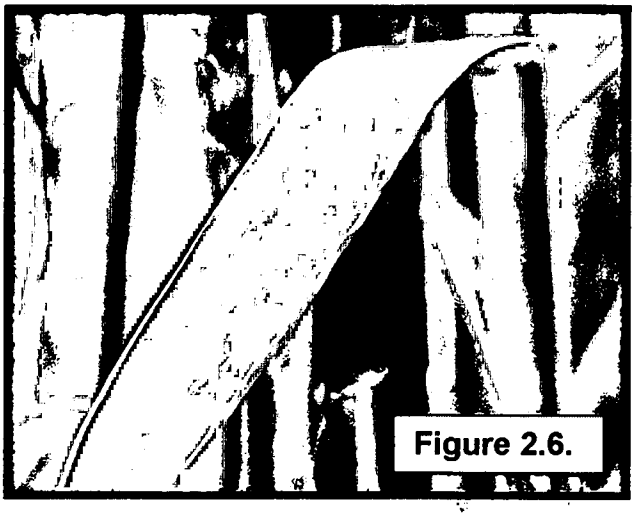


Figure 2.1. Infection pressure with field trial. Marocco (left) completely dead.

Figure 2.2. Stripe rust infection on Elands

Figure 2.3. Stripe rust infection on T96/6

Figure 2.4. 'Pseudo black chaff' on Elands+*Lr32+Lr34* (score: 3)

Figure 2.5. Leaf rust infection on BSP98/4

Figure 2.6. Hypersensitive reaction to leaf rust infection on BSP98/4+*Lr32+Lr34*

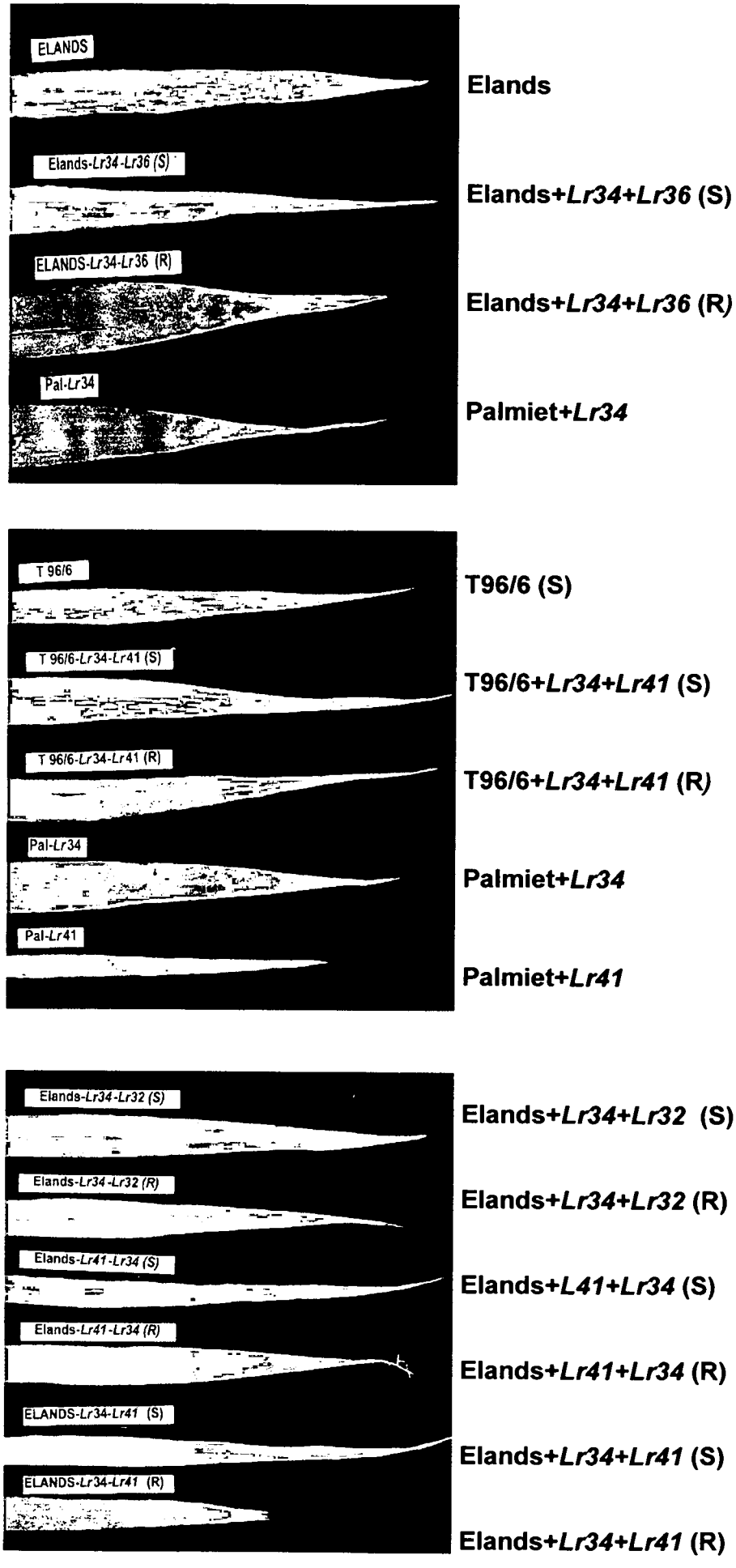


Figure 2.7. Resistant and susceptible infection types of parental cultivars and their respective F_2 progenies recorded in the field. Leaf tip necrosis is clearly visible in resistant entries.

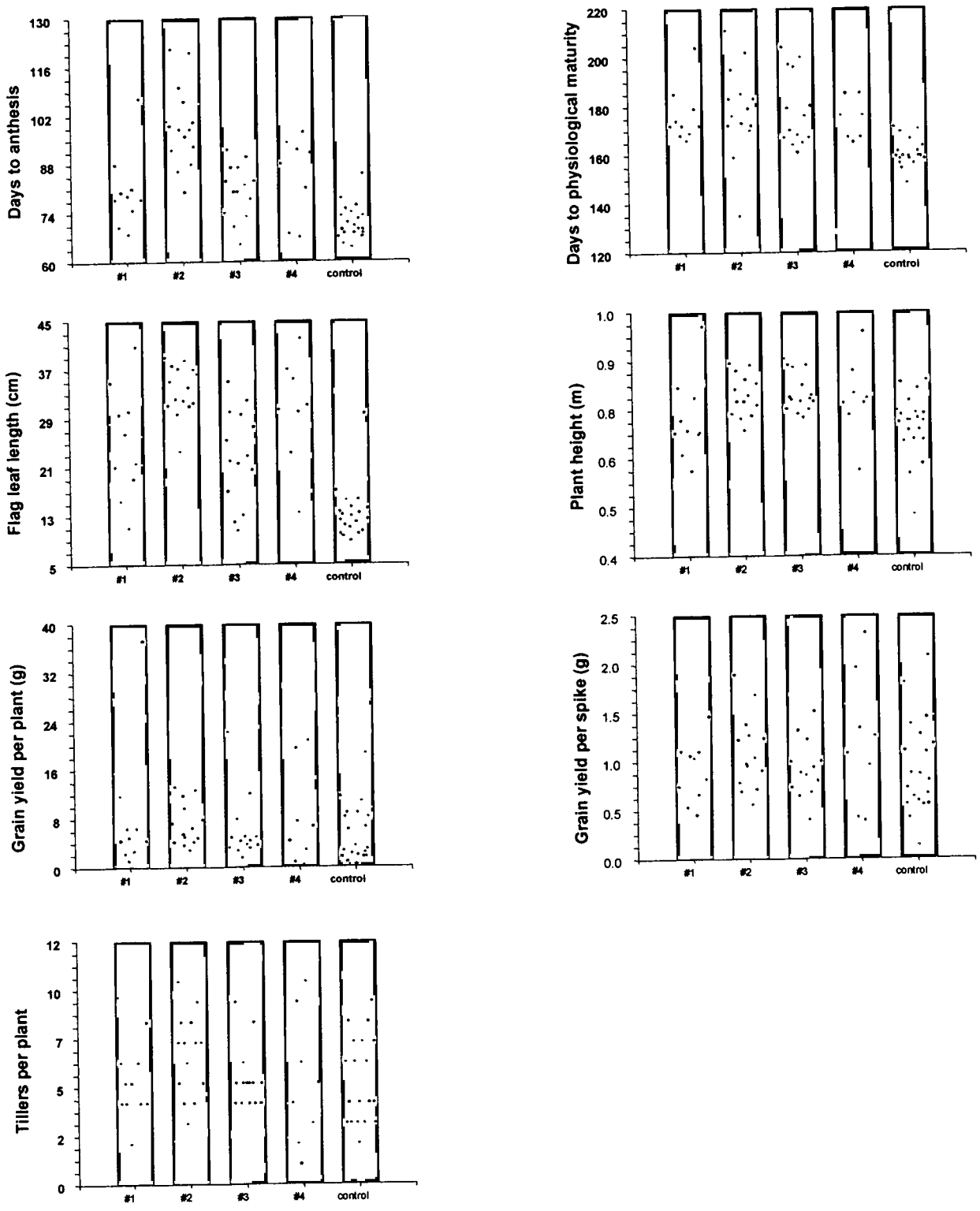


Figure 2.8. Dot plot of agronomic traits of selections #1, #2, #3 and #4 of wheat line BSP97/1+*Lr32*+*Lr34* compared to BSP97/1 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

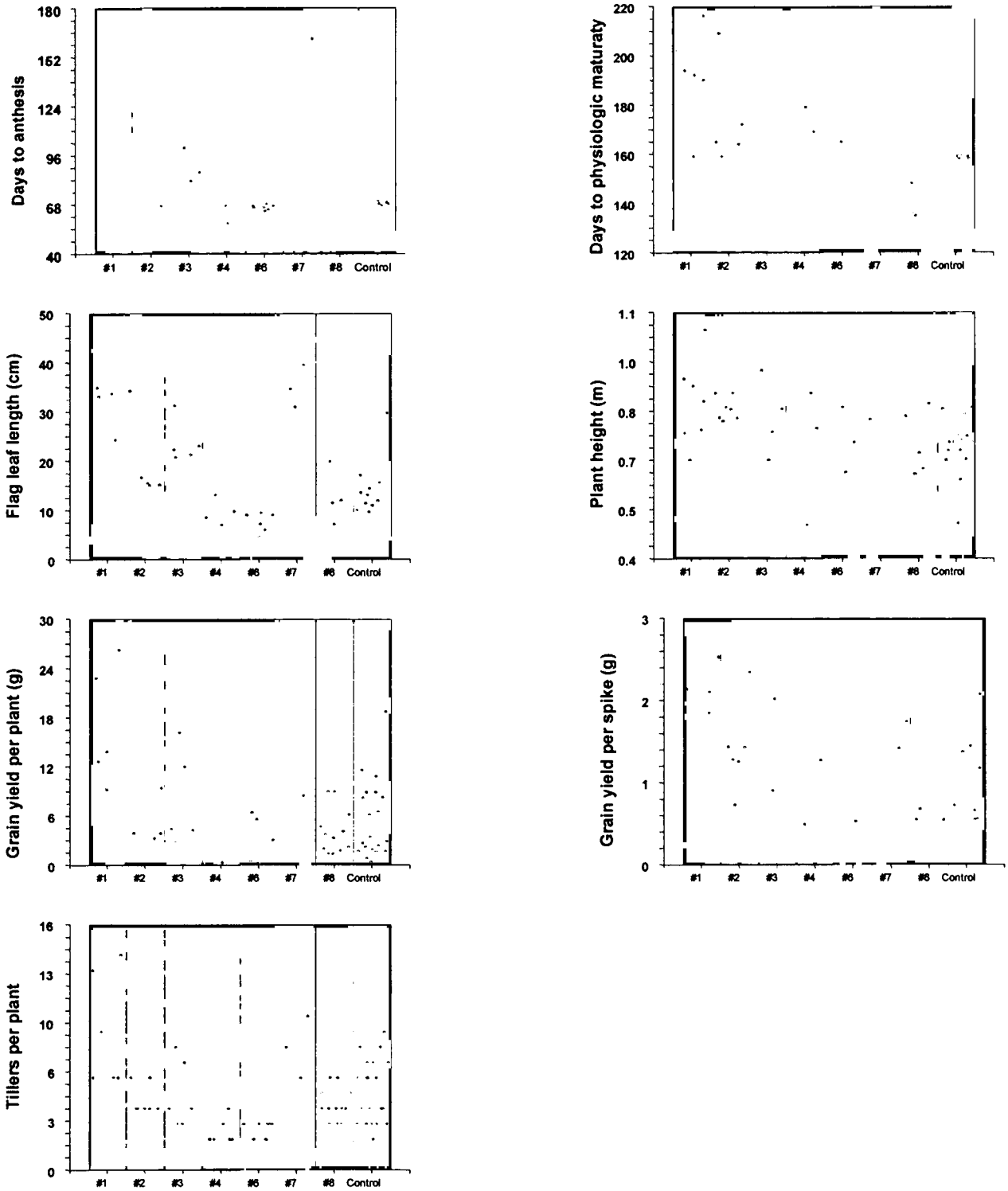


Figure 2.9. Dot plot of agronomic traits of selections #1, #2, #3, #4, #6, #7 and #8 of wheat line BSP97/1+*Lr34+Lr21* compared to BSP97/1 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

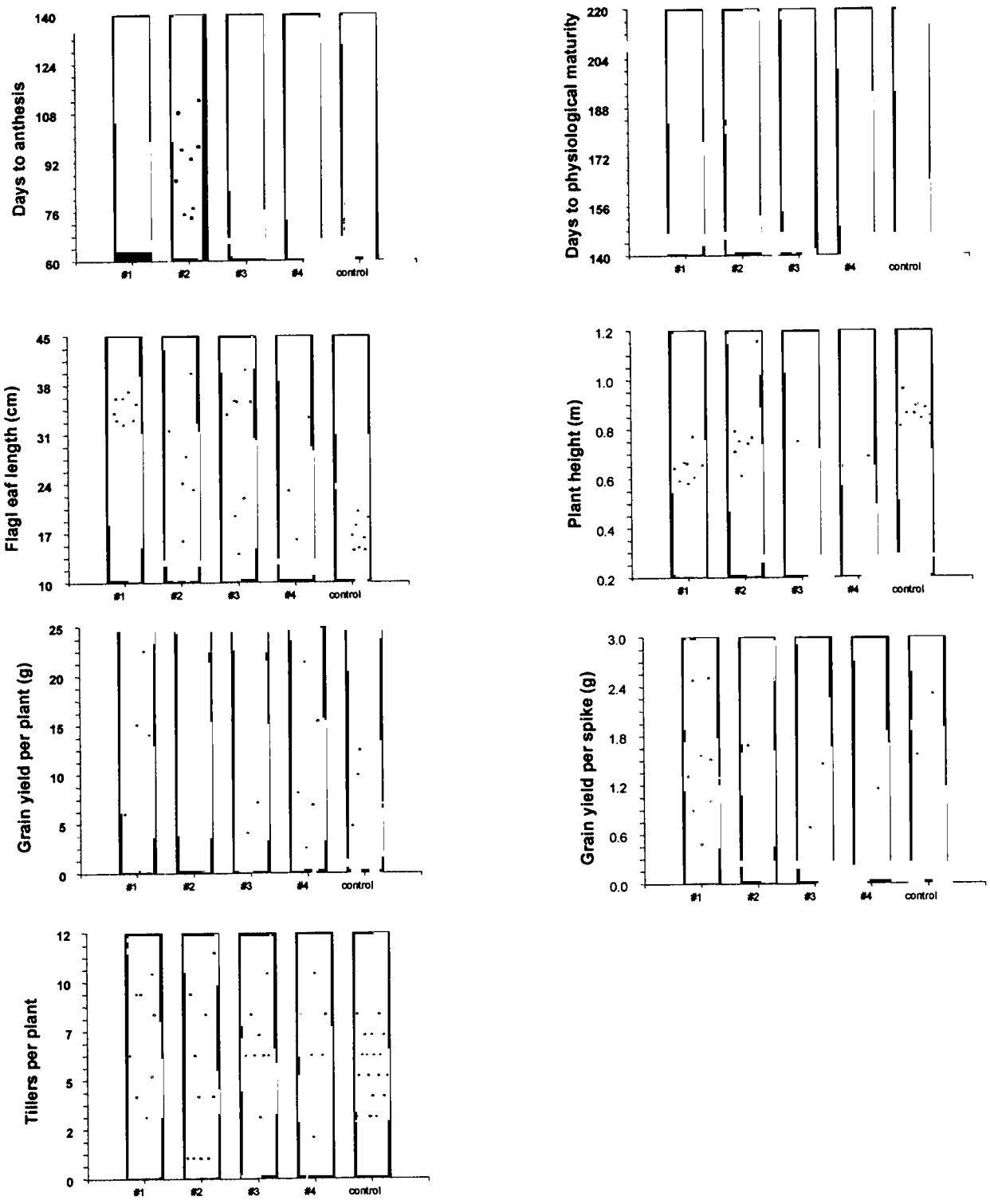


Figure 2.10. Dot plot of agronomic traits of selections #1, #2, #3 and #4 of wheat line BSP98/4+*Lr36*+*Lr34* compared to BSP98/4 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection or within the adapted line

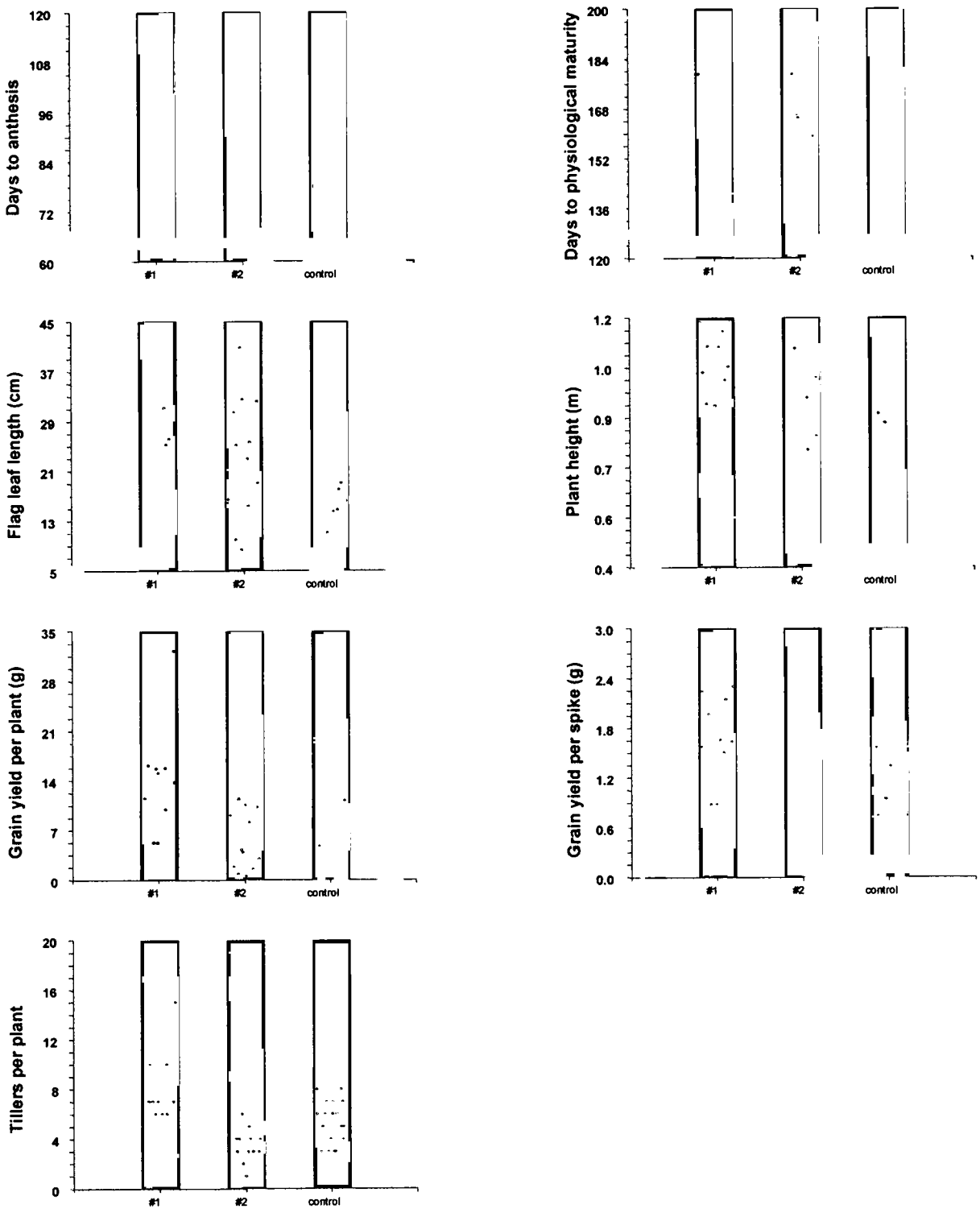


Figure 2.11. Dot plot of agronomic traits of selections #1 and #2 of wheat line BSP98/4+*Lr41*+*Lr34* compared to BSP98/4 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection or within the adapted line

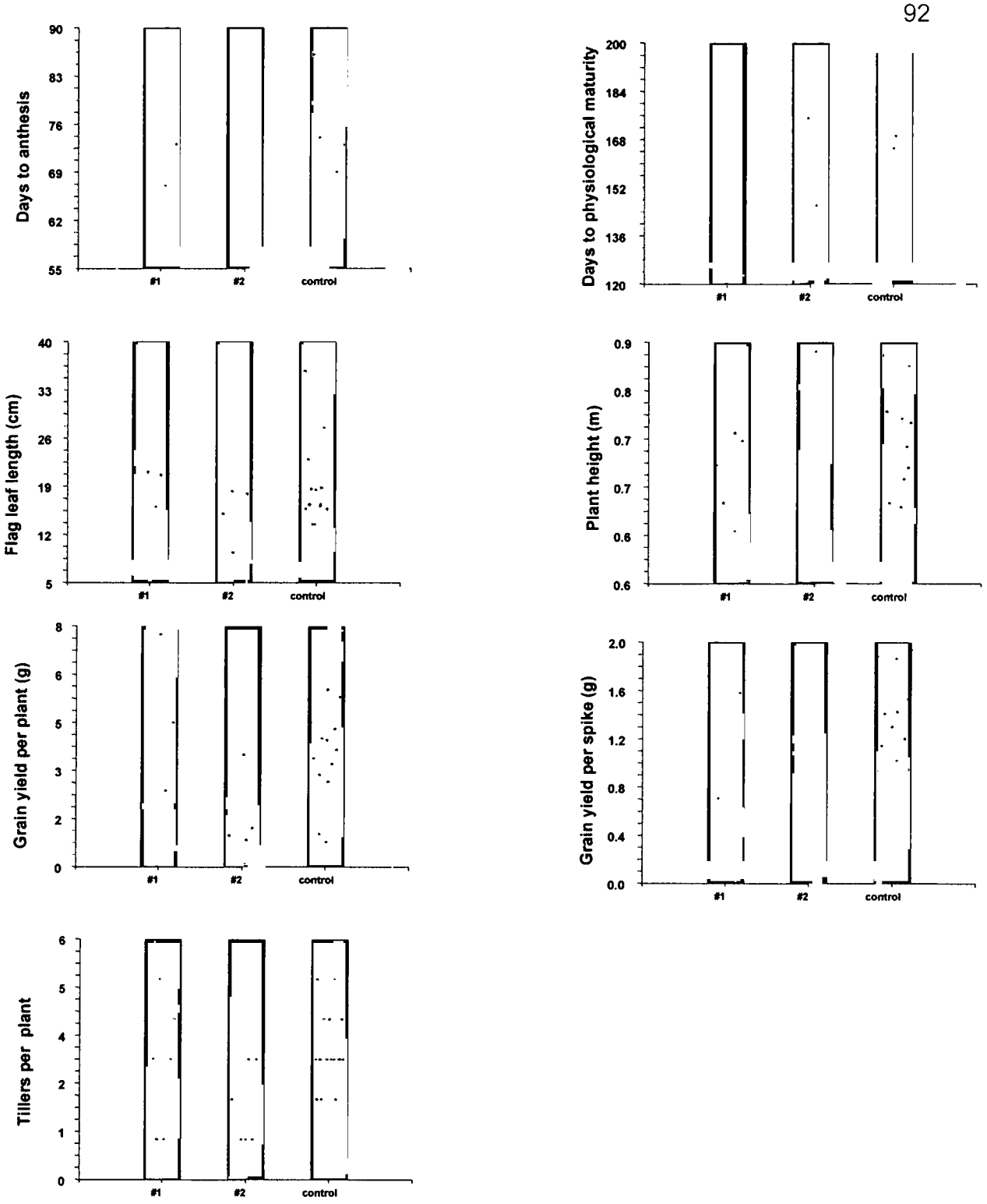


Figure 2.12. Dot plot of agronomic traits of selections #1 and #2 of wheat line BSP98/16+*Lr32*+*Lr34* compared to BSP98/16 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

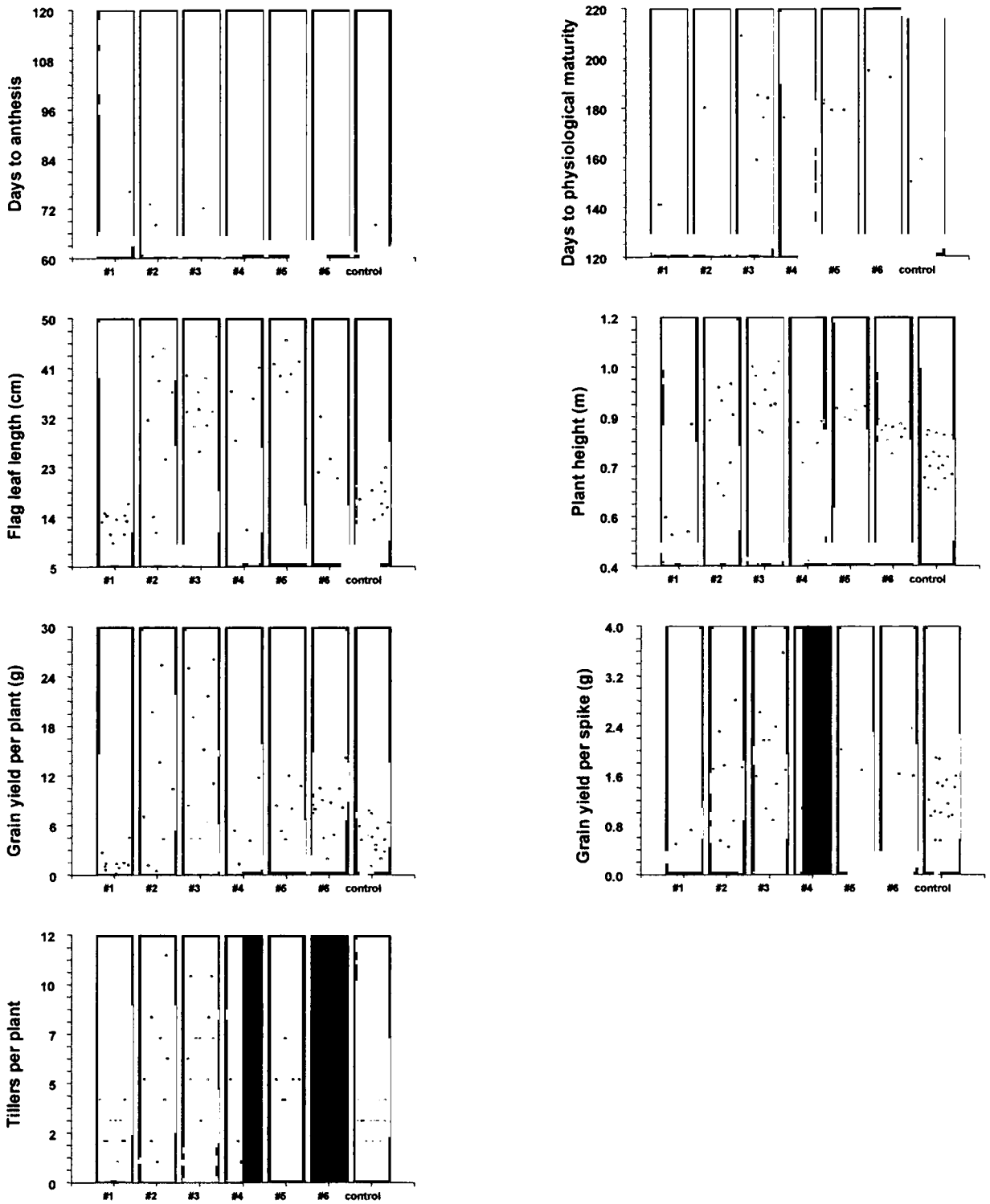


Figure 2.13. Dot plot of agronomic traits of selections #1, #2, #3, #4, #5 and #6 of wheat line BSP98/16+*Lr36*+*Lr34* compared to BSP98/16 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

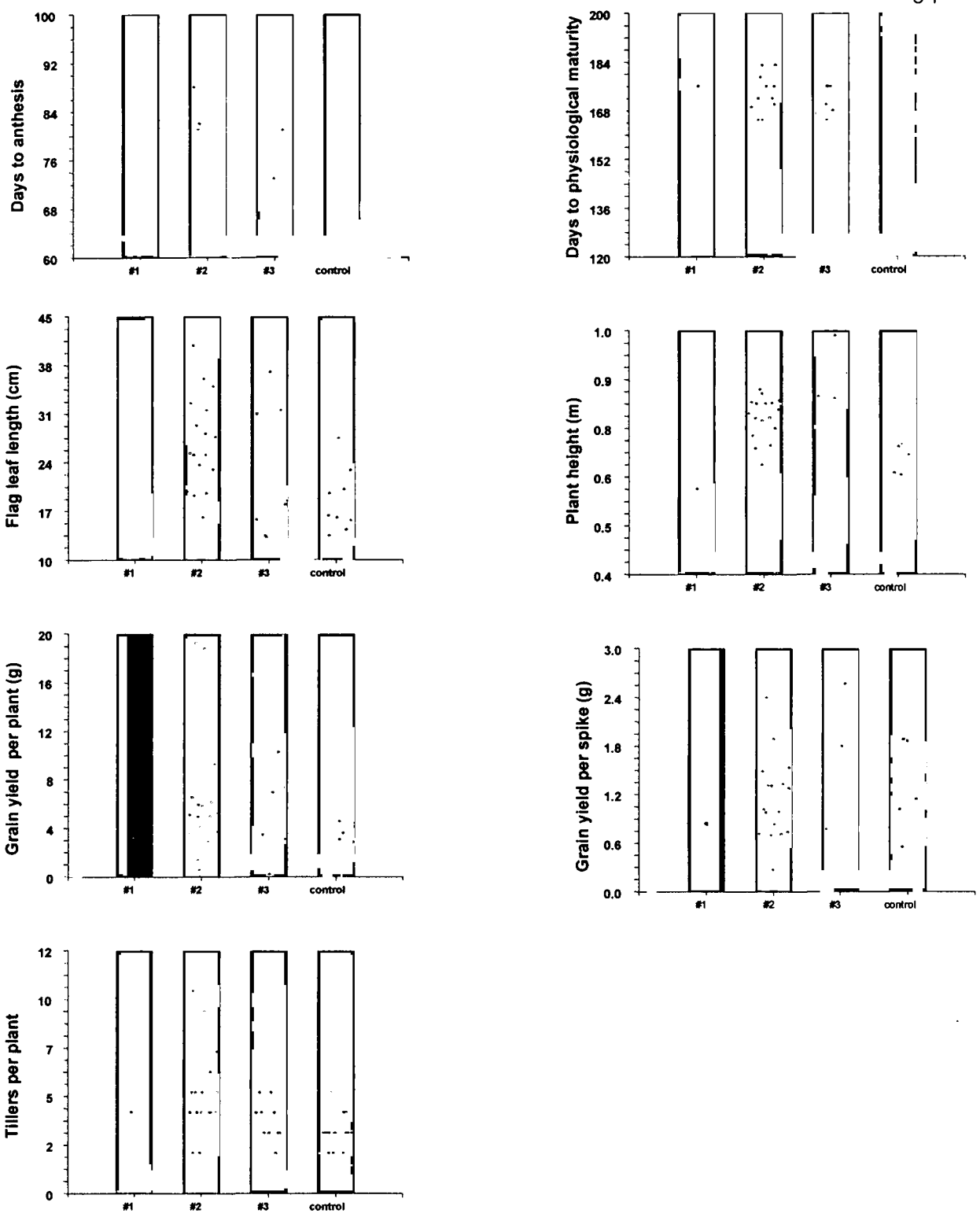


Figure 2.14. Dot plot of agronomic traits of selections #1, #2 and #3 of wheat line BSP98/16+*Lr41*+*Lr34* compared to BSP98/16 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

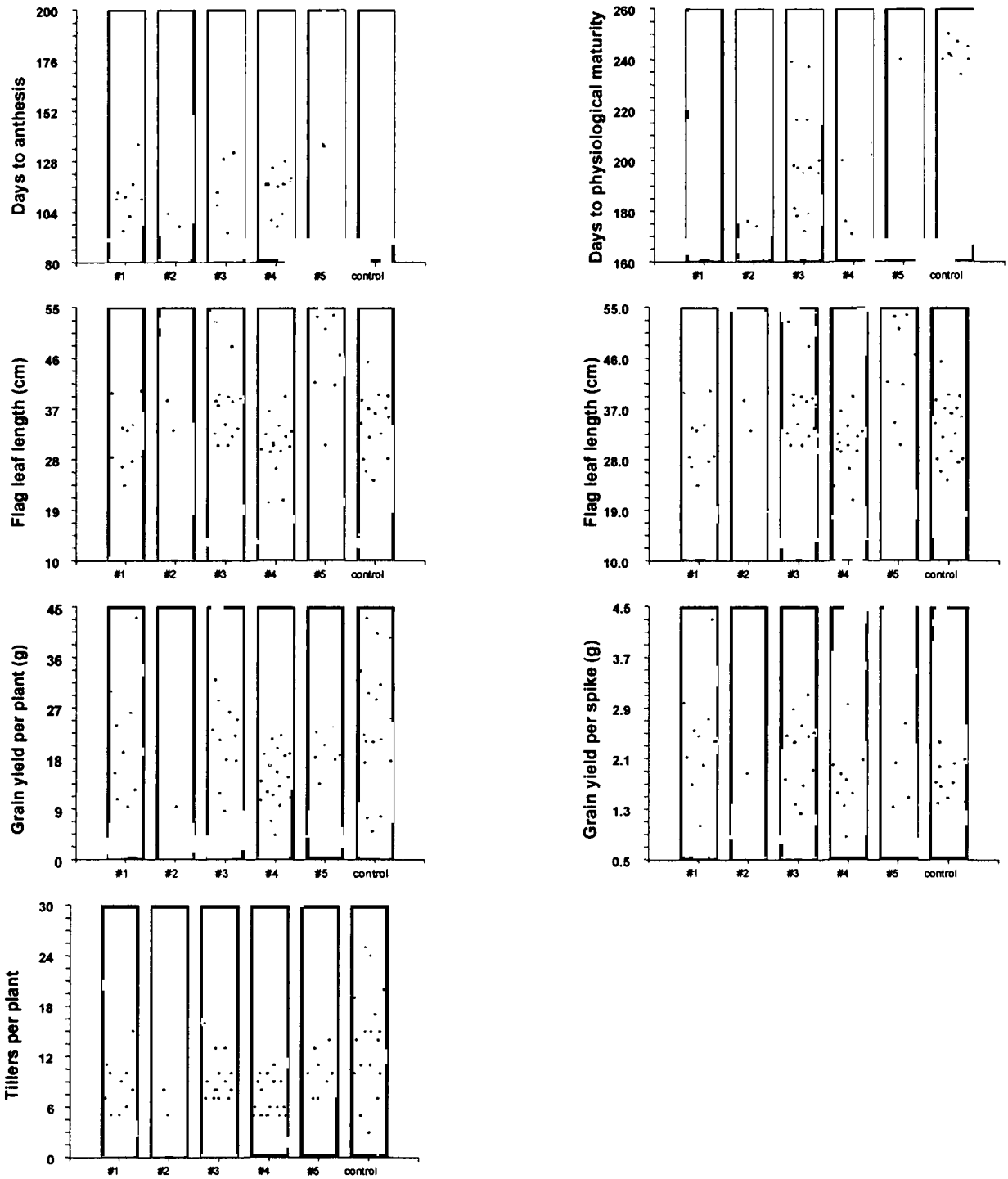


Figure 2.15. Dot plot of agronomic traits of selections #1, #2, #3, #4 and #5 of wheat line Elands+*Lr32+Lr34* compared to Elands (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

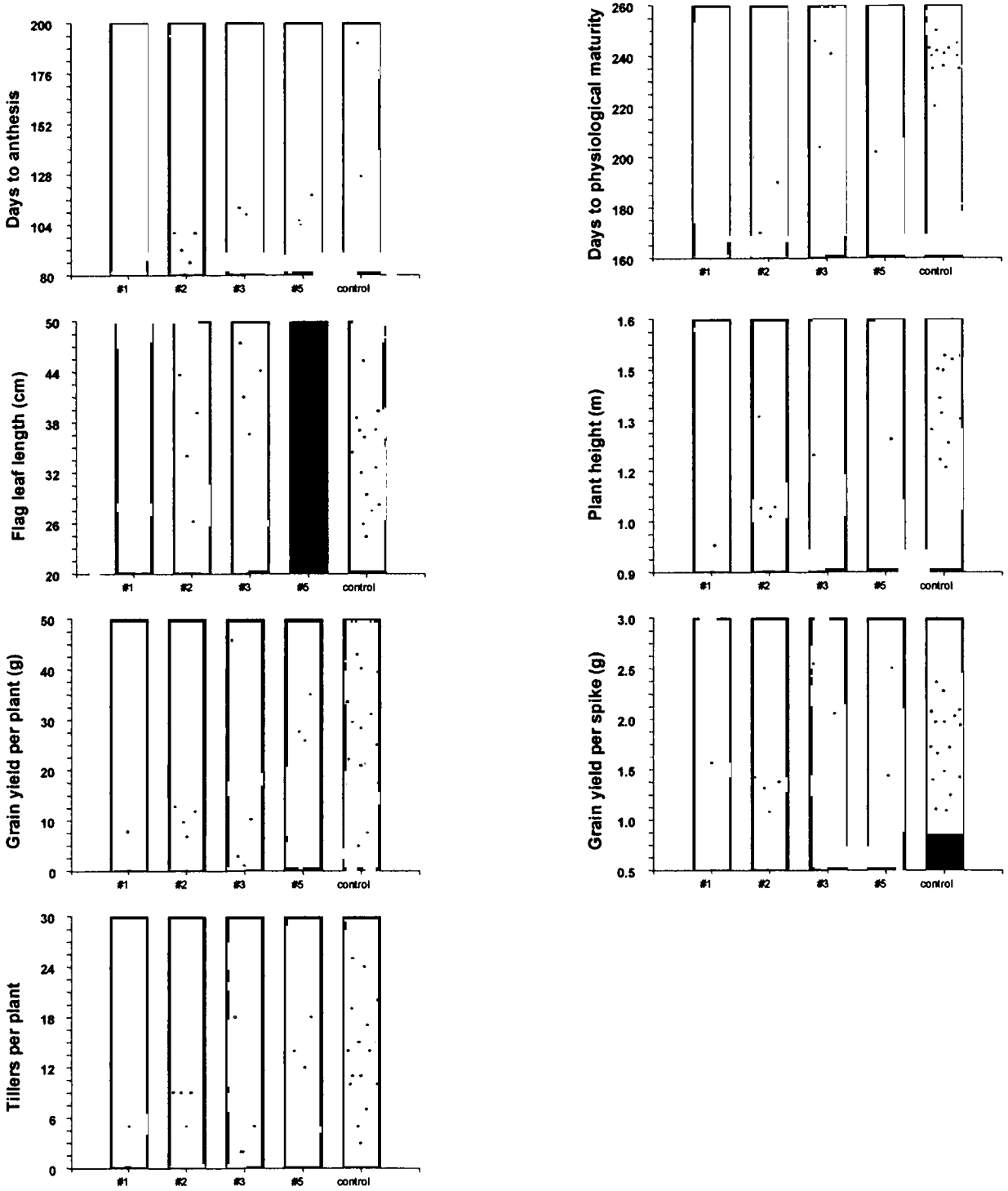


Figure 2.16. Dot plot of agronomic traits of selections #1, #2, #3 and #5 of wheat line Elands+*Lr34+Lr21* compared to Elands (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

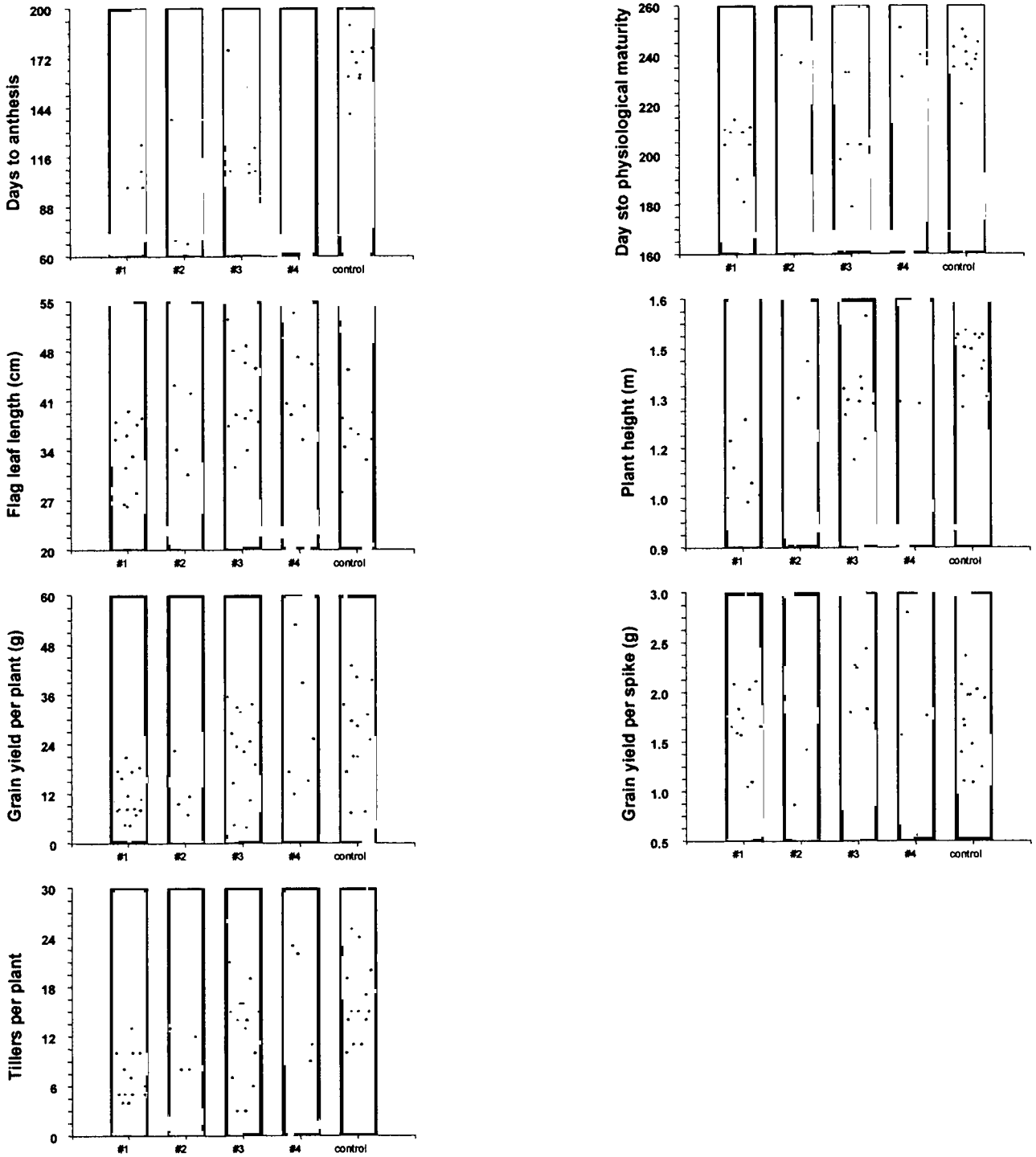


Figure 2.17. Dot plot of agronomic traits of selections #1, #2, #3 and #4 of wheat line Elands+*Lr34+Lr32* compared to Elands (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

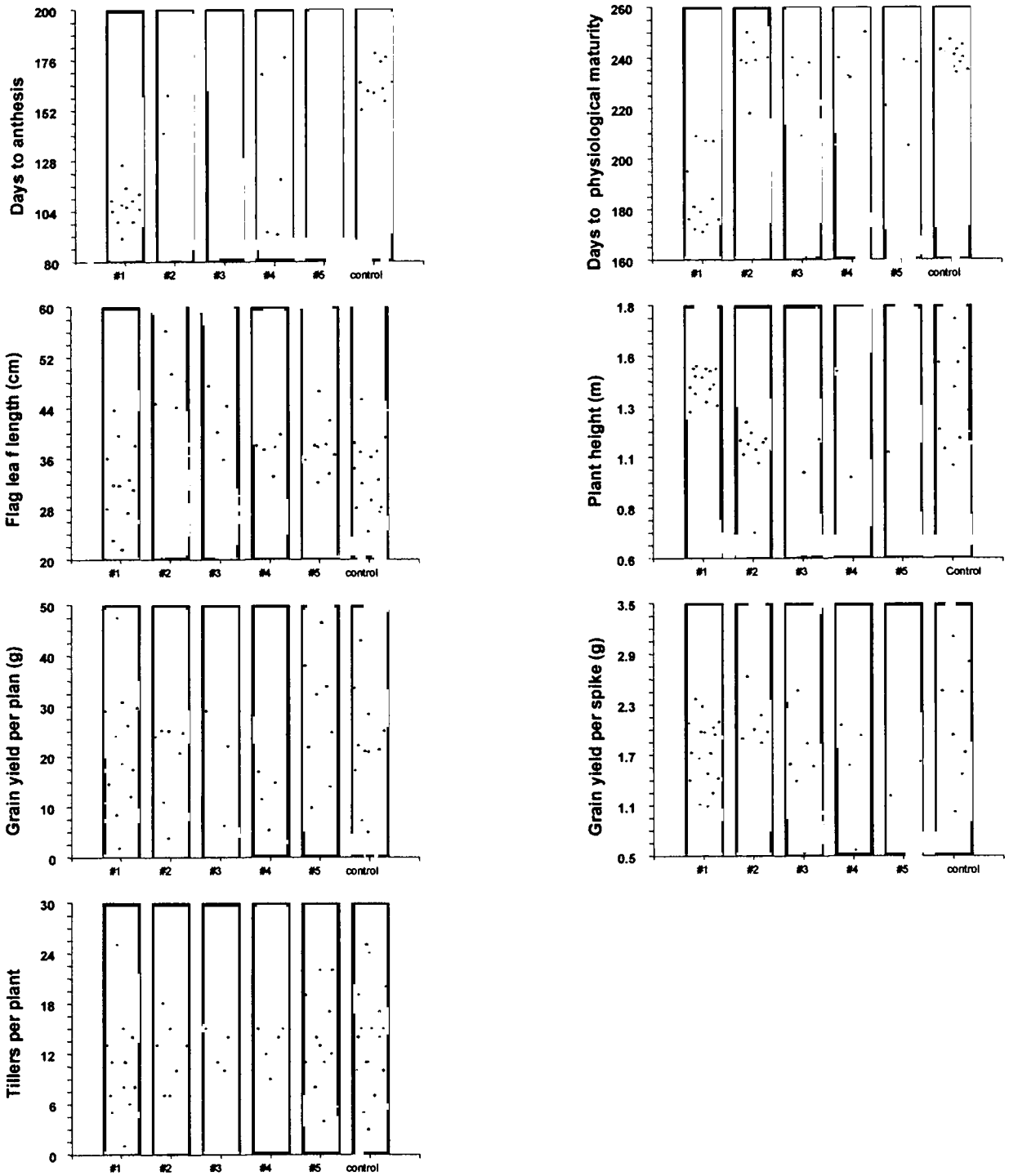


Figure 2.18. Dot plot of agronomic traits of selections #1, #2, #3, #4 and #5 of wheat line Elands+*Lr34+Lr36* compared to Elands (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

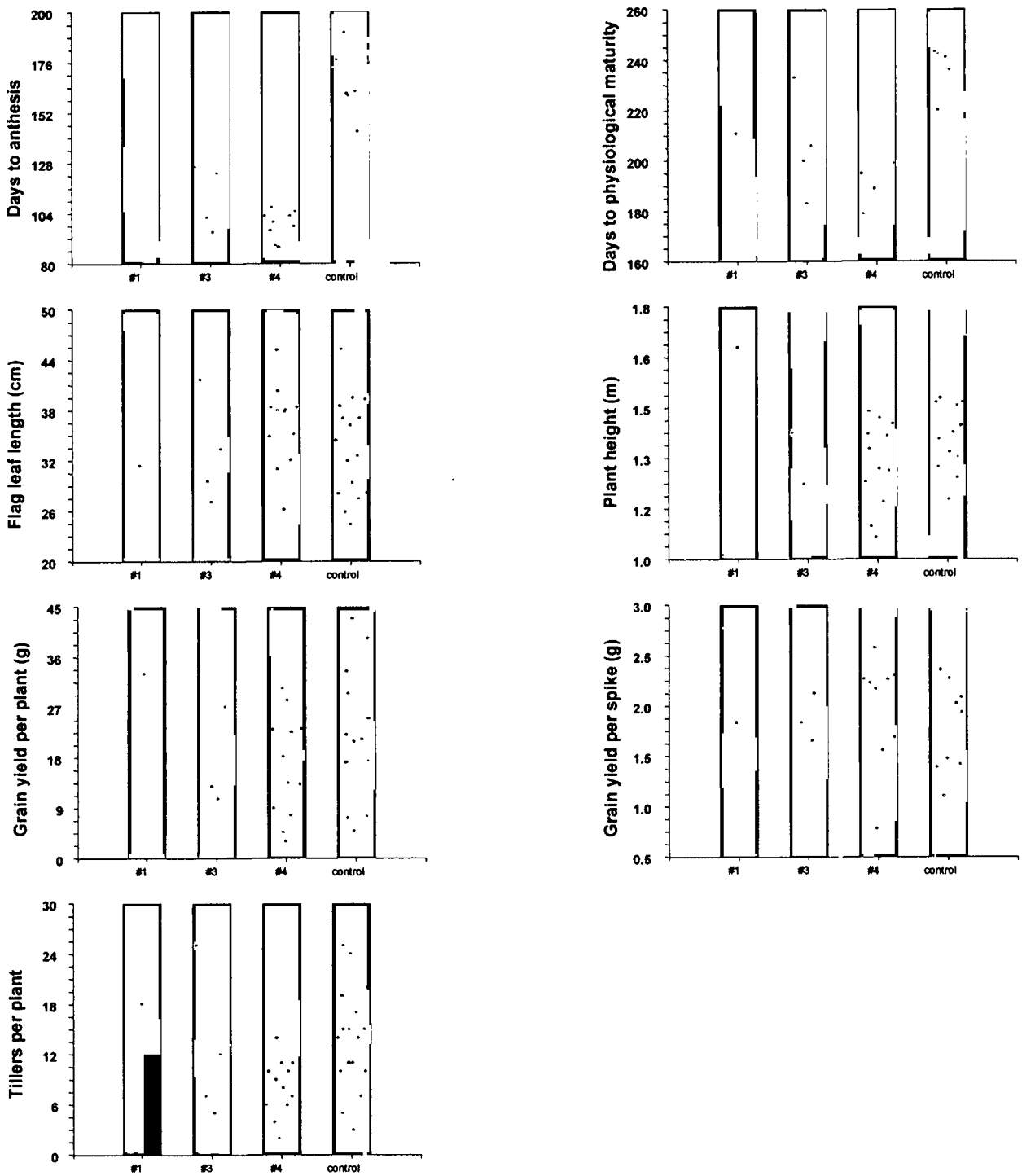


Figure 2.19. Dot plot of agronomic traits of selections #1, #3 and #4 of wheat line Elands+*Lr34+Lr41* compared to Elands (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

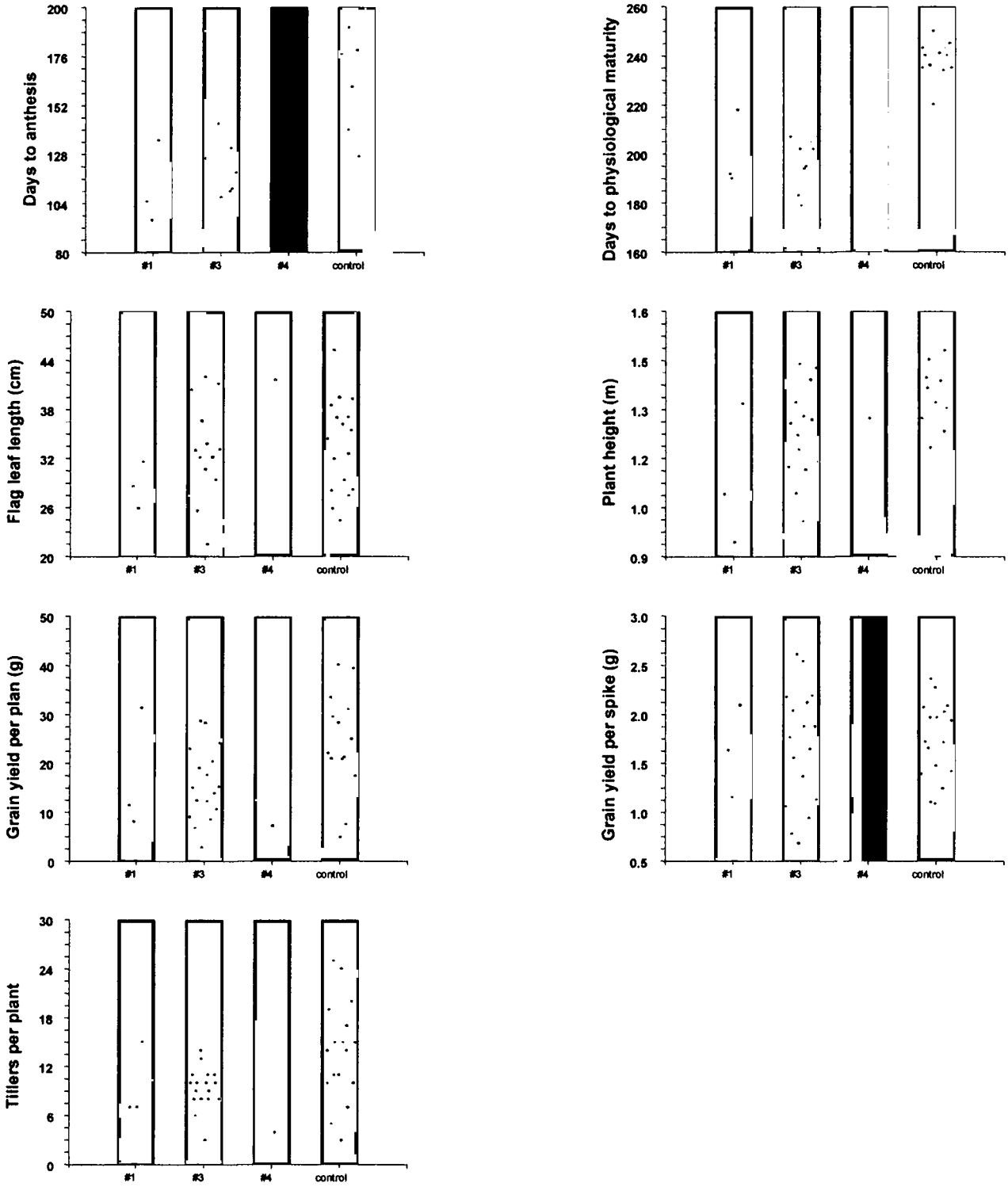


Figure 2.20. Dot plot of agronomic traits of selections #1, #3 and #4 of wheat line Elands+*Lr41+Lr34* compared to Elands (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

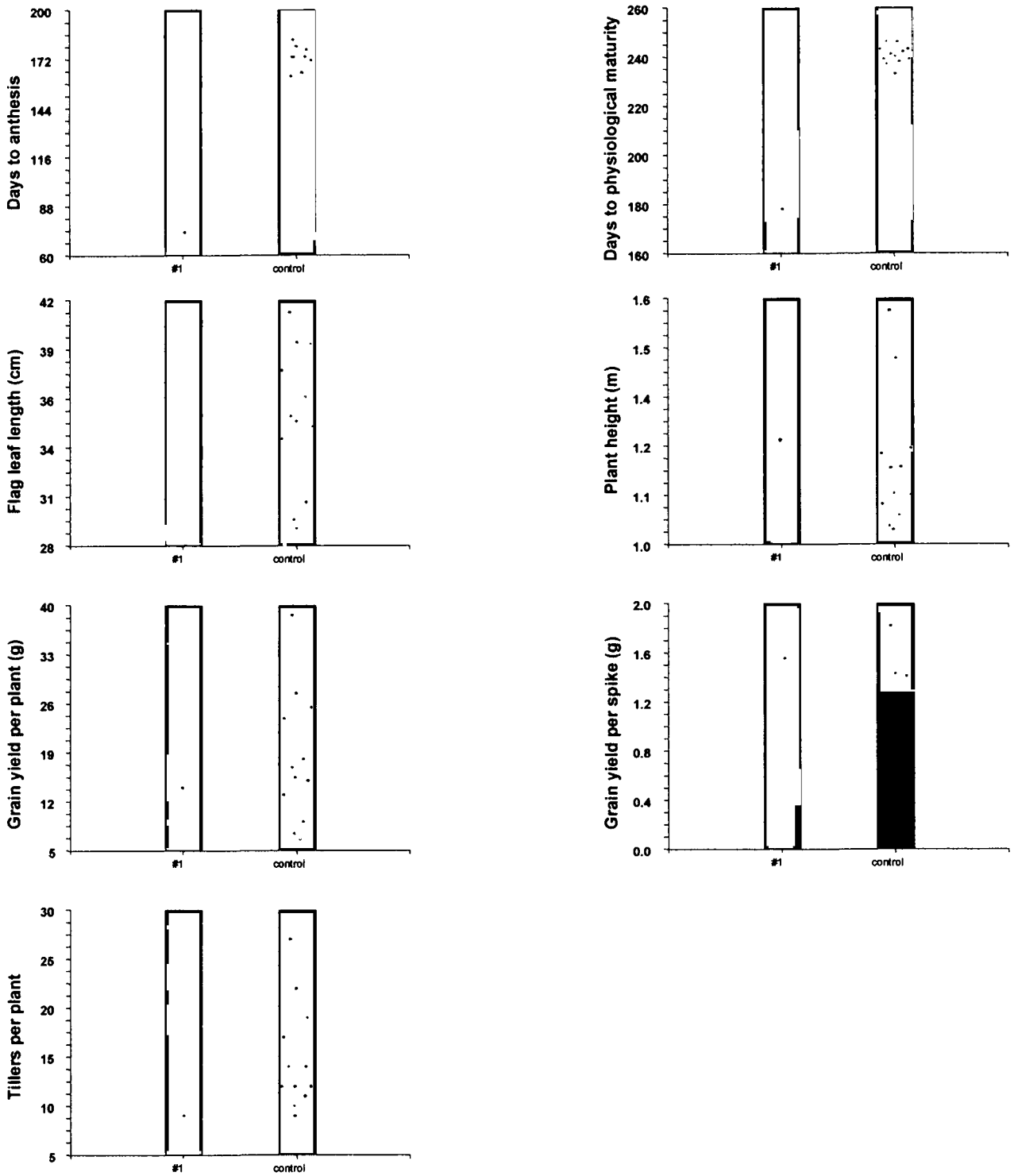


Figure 2.21. Dot plot of agronomic traits of selection #1 of wheat line T96/6+*Lr34*+*Lr21* compared to T96/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

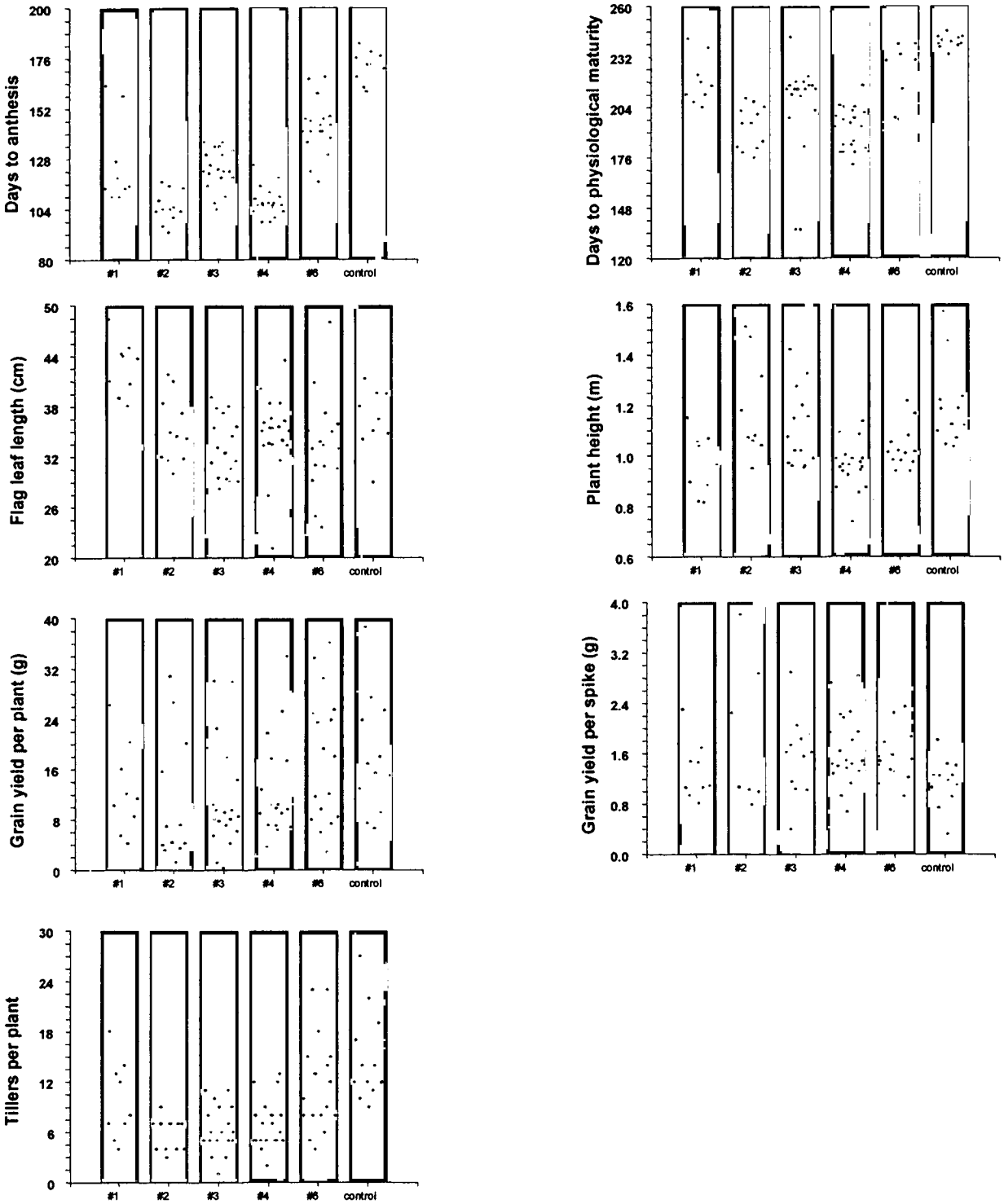


Figure 2.22. Dot plot of agronomic traits of selections #1, #2, #3, #4 and #6 of wheat line T96/6+*Lr34+Lr41* compared to T96/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

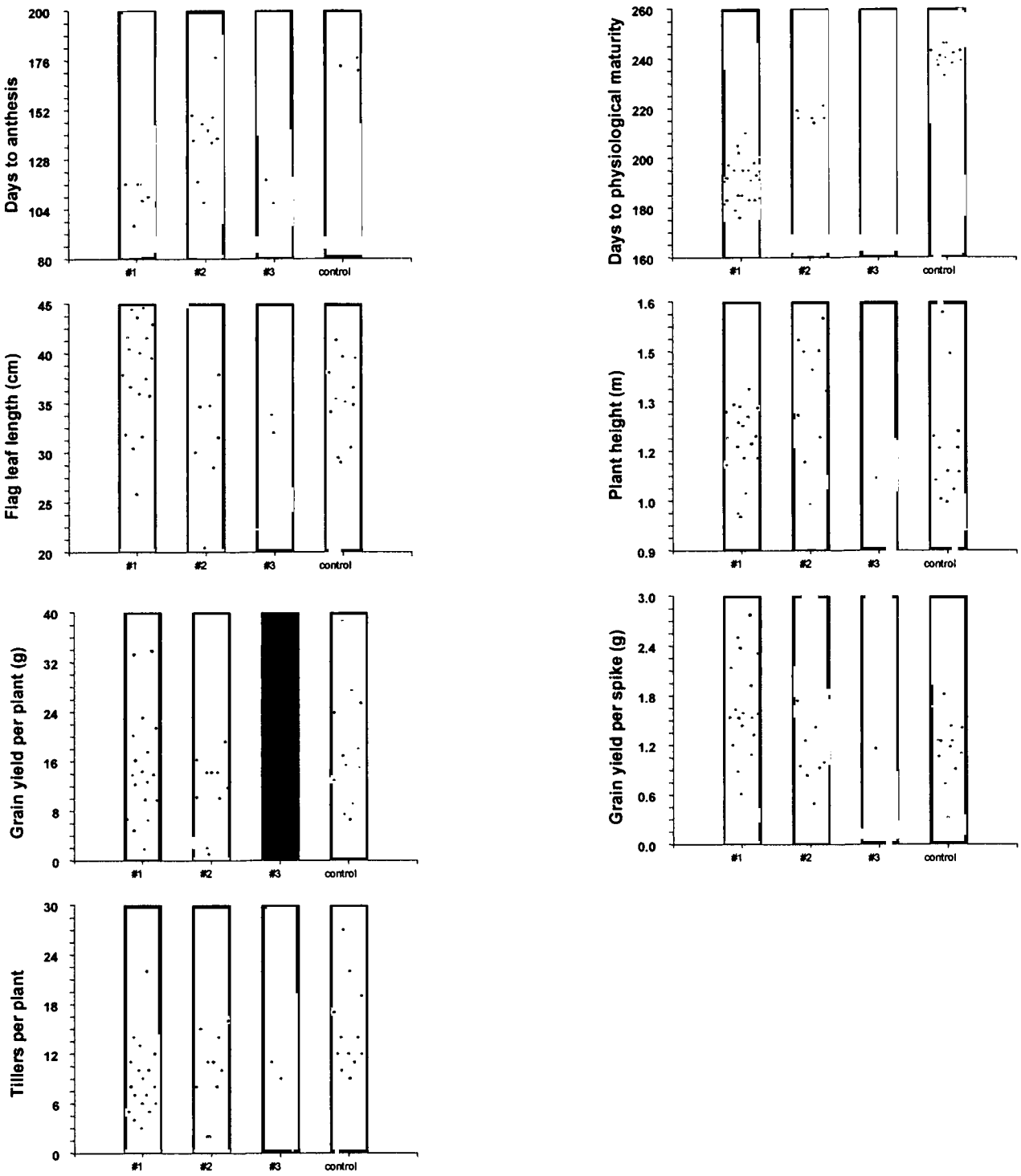


Figure 2.23. Dot plot of agronomic traits of selections #1, #2 and #3 of wheat line T96/6+*Lr36*+*Lr34* compared to T96/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

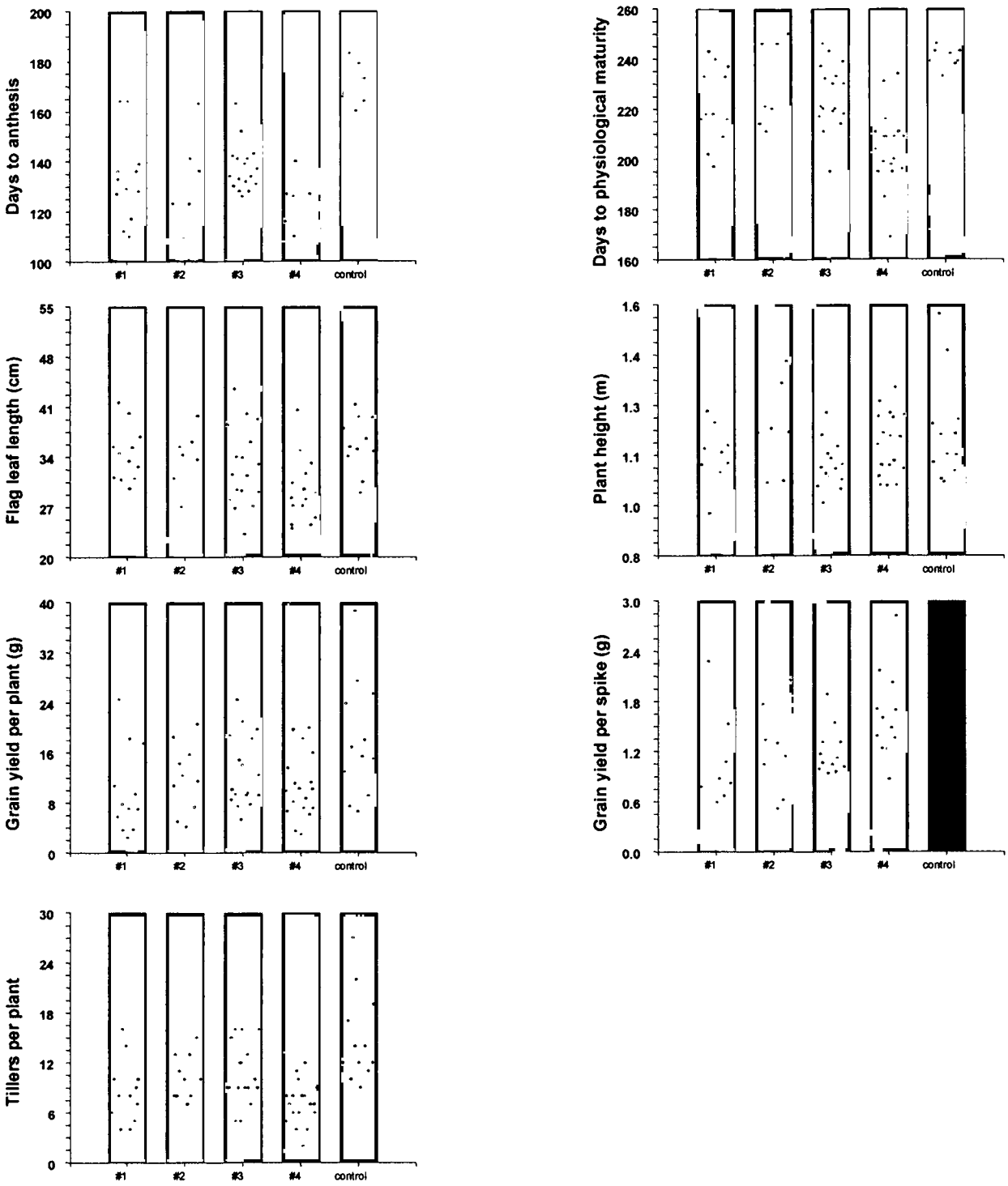


Figure 2.24. Dot plot of agronomic traits of selections #1, #2, #3 and #4 of wheat line T96/6+*Lr41*+*Lr34* compared to T96/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

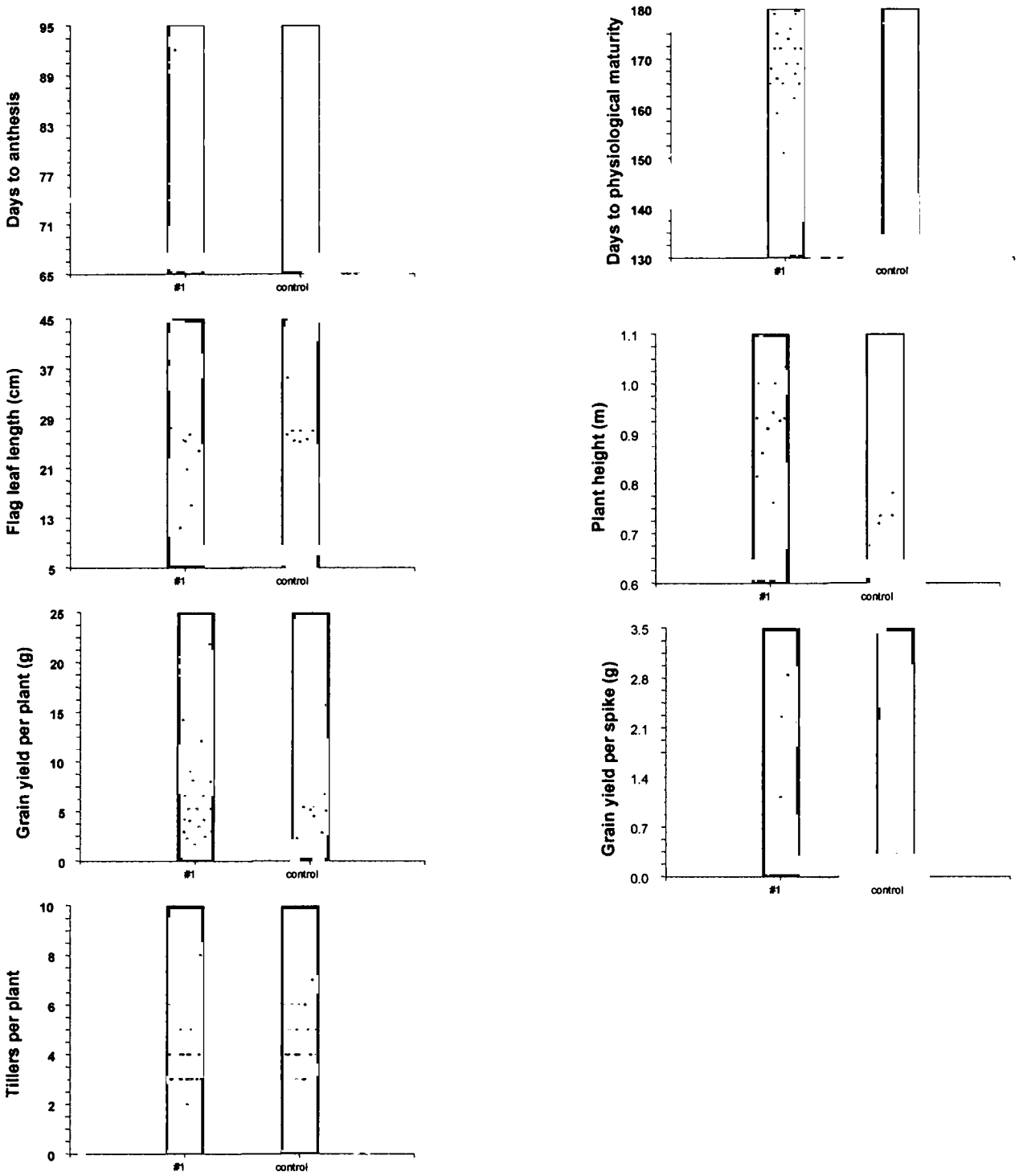


Figure 2.25. Dot plot of agronomic traits of selection #1 of wheat line W98/6+*Lr21*+*Lr34* compared to W98/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

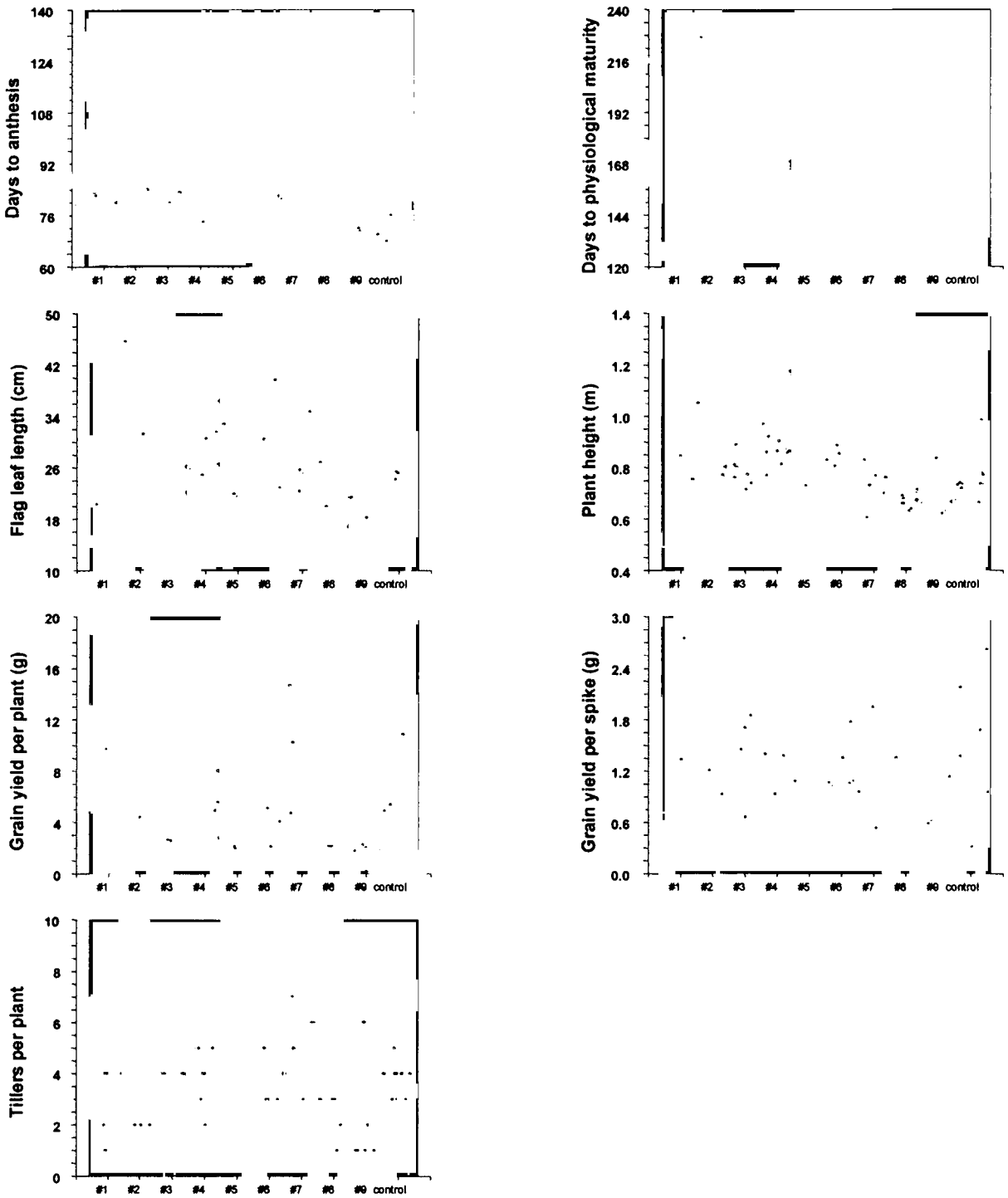


Figure 2.26. Dot plot of agronomic traits of selection #1, #2, #3, #4, #5, #6, #7, #8 and #9 of wheat line W98/6+*Lr32*+*Lr34* compared to W98/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

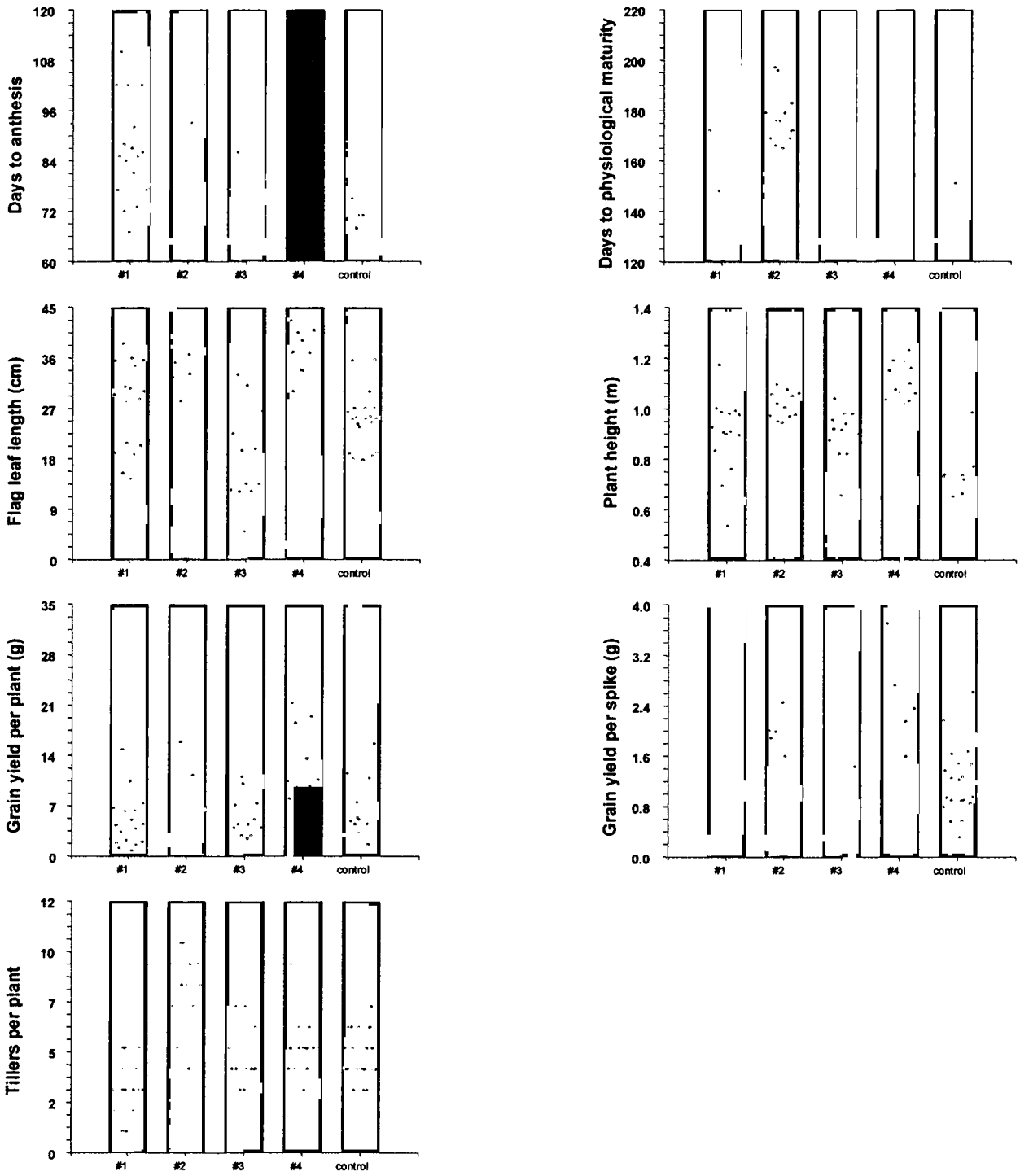


Figure 2.27. Dot plot of agronomic traits of selection #1, #2, #3 and #4 of wheat line W98/6+*Lr34+Lr21* compared to W98/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

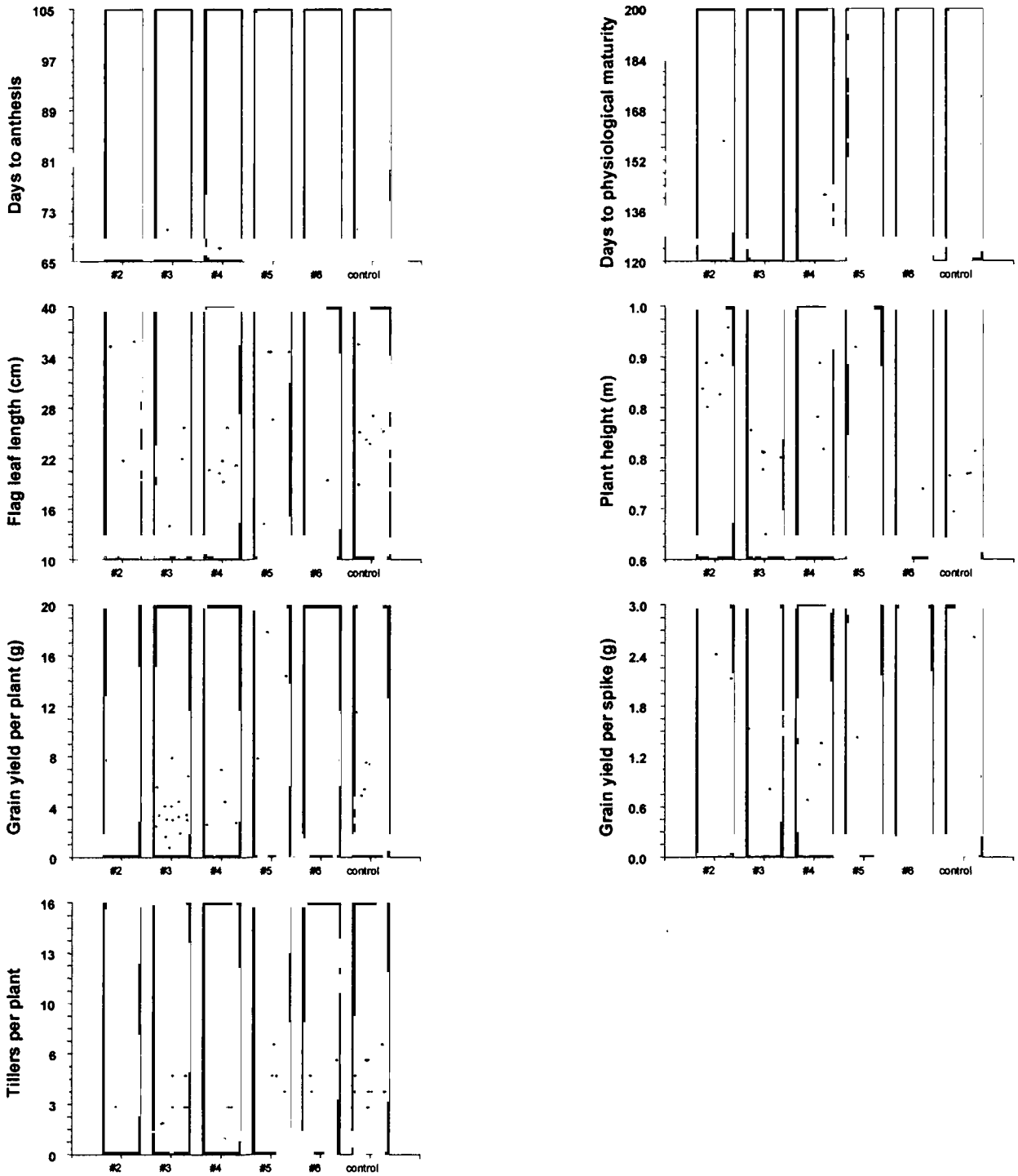


Figure 2.28 Dot plot of agronomic traits of selections #2, #3, #4, #5 and #6 of wheat line W98/6+*Lr34+Lr36* compared to W98/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

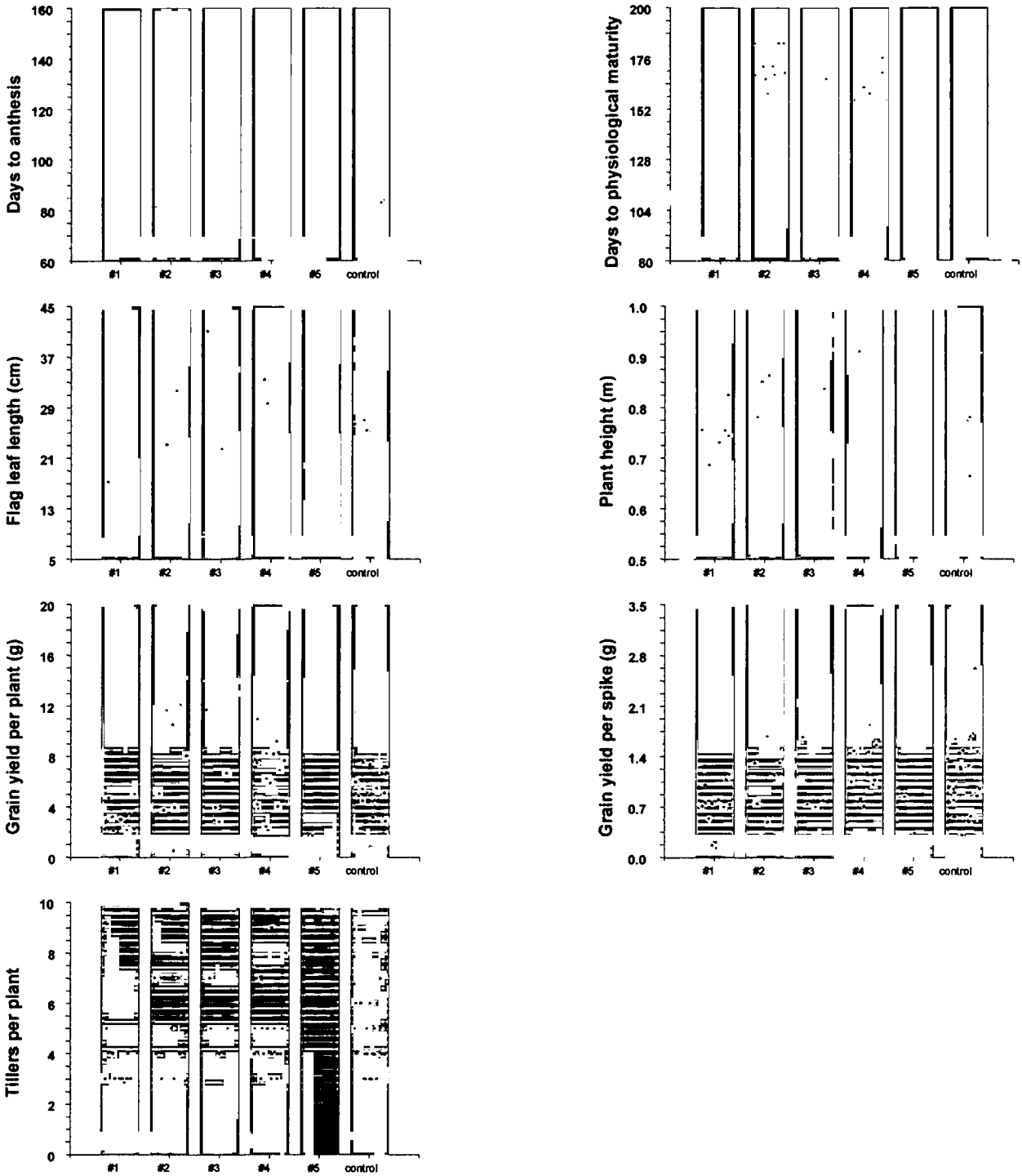


Figure 2.29 Dot plot of agronomic traits of selections #1, #2, #3, #4 and #5 of wheat line W98/6+*Lr36*+*Lr34* compared to W98/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

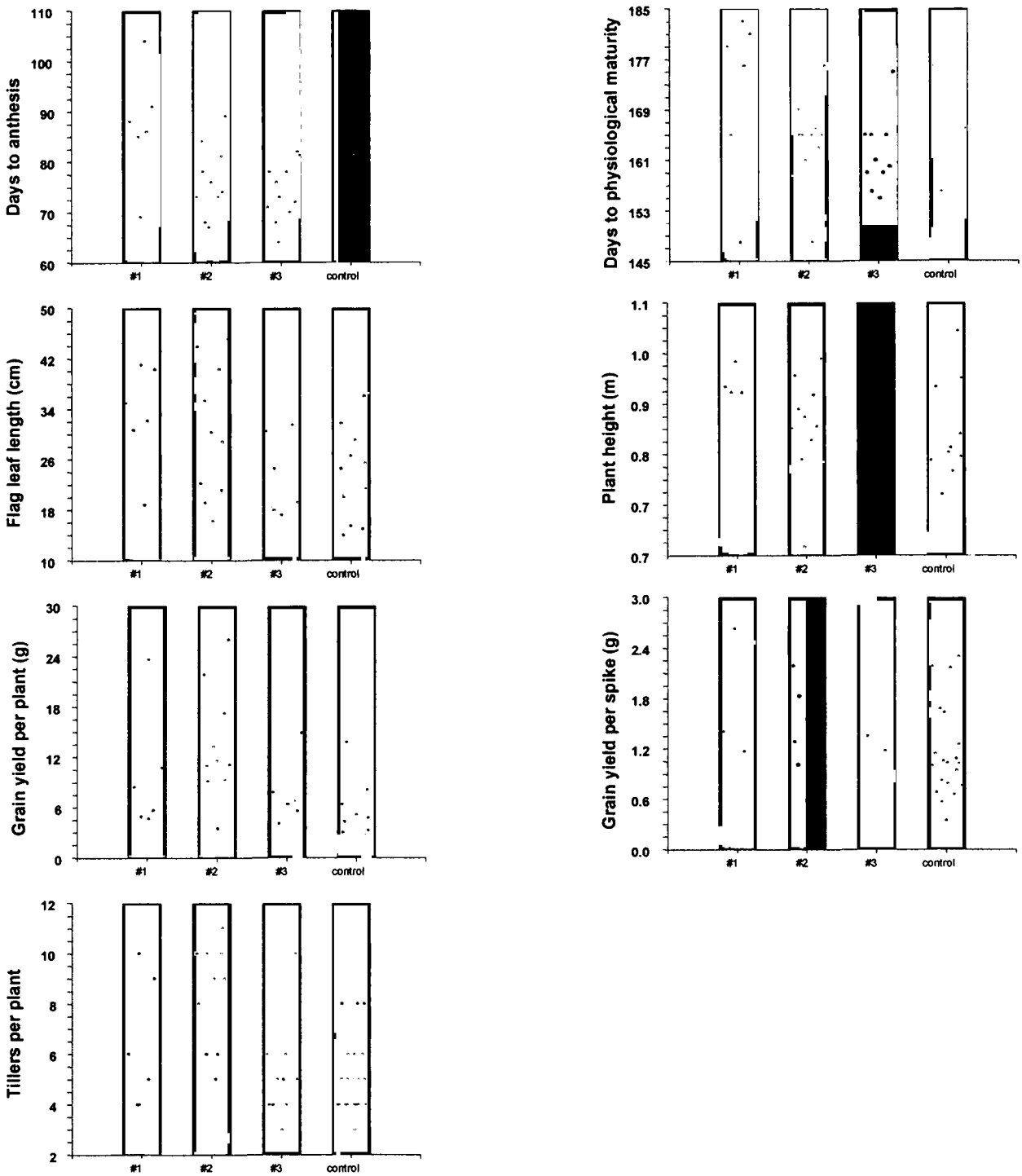


Figure 2.30. Dot plot of agronomic traits of selections #1, #2 and #3 of the wheat line W98/22+*Lr21*+*Lr34* compared to W98/22 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

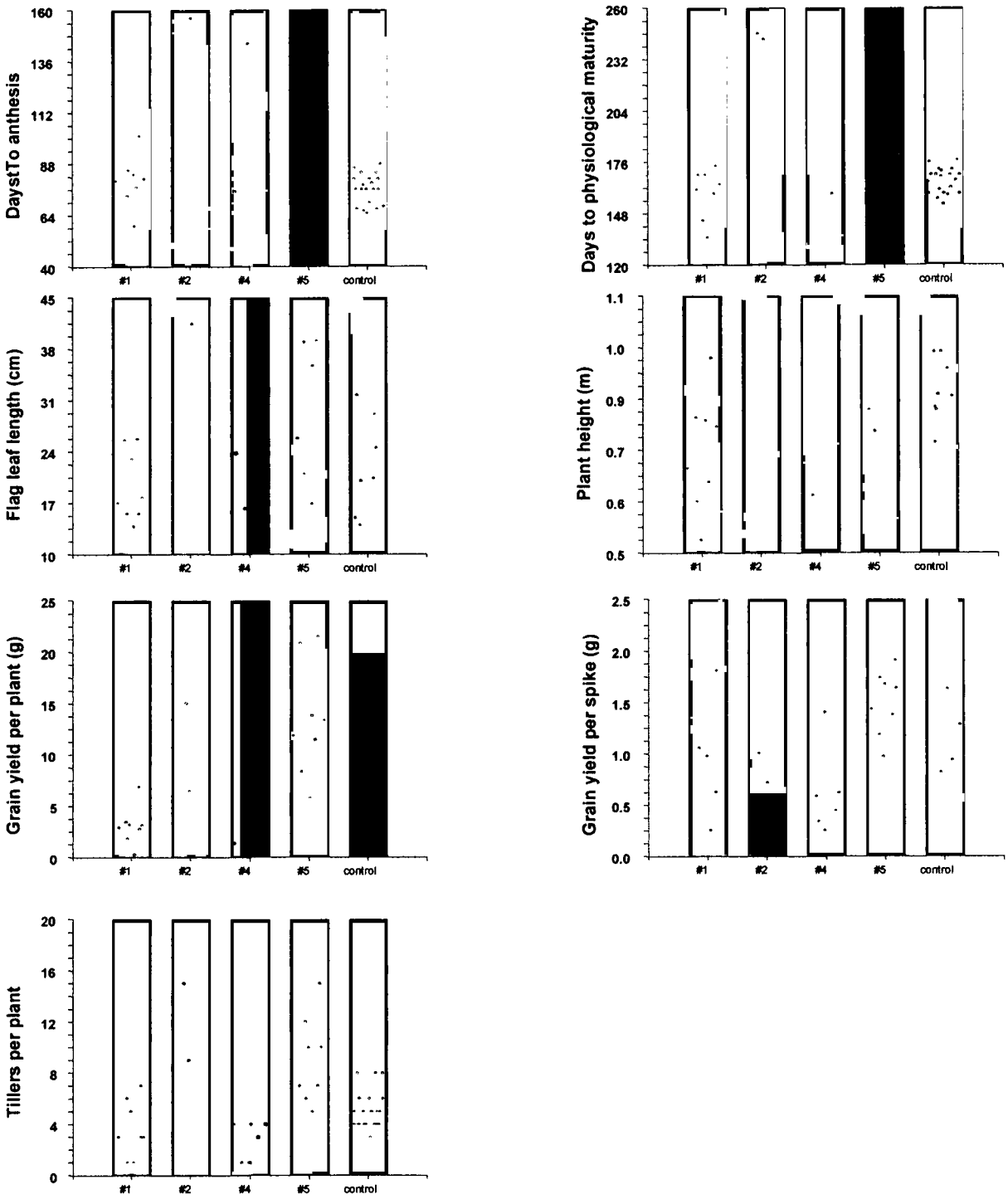


Figure 2.31. Dot plot of agronomic traits of selections #1, #2, #4 and #5 of wheat line W98/22+*Lr32*+*Lr34* compared to W98/22 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

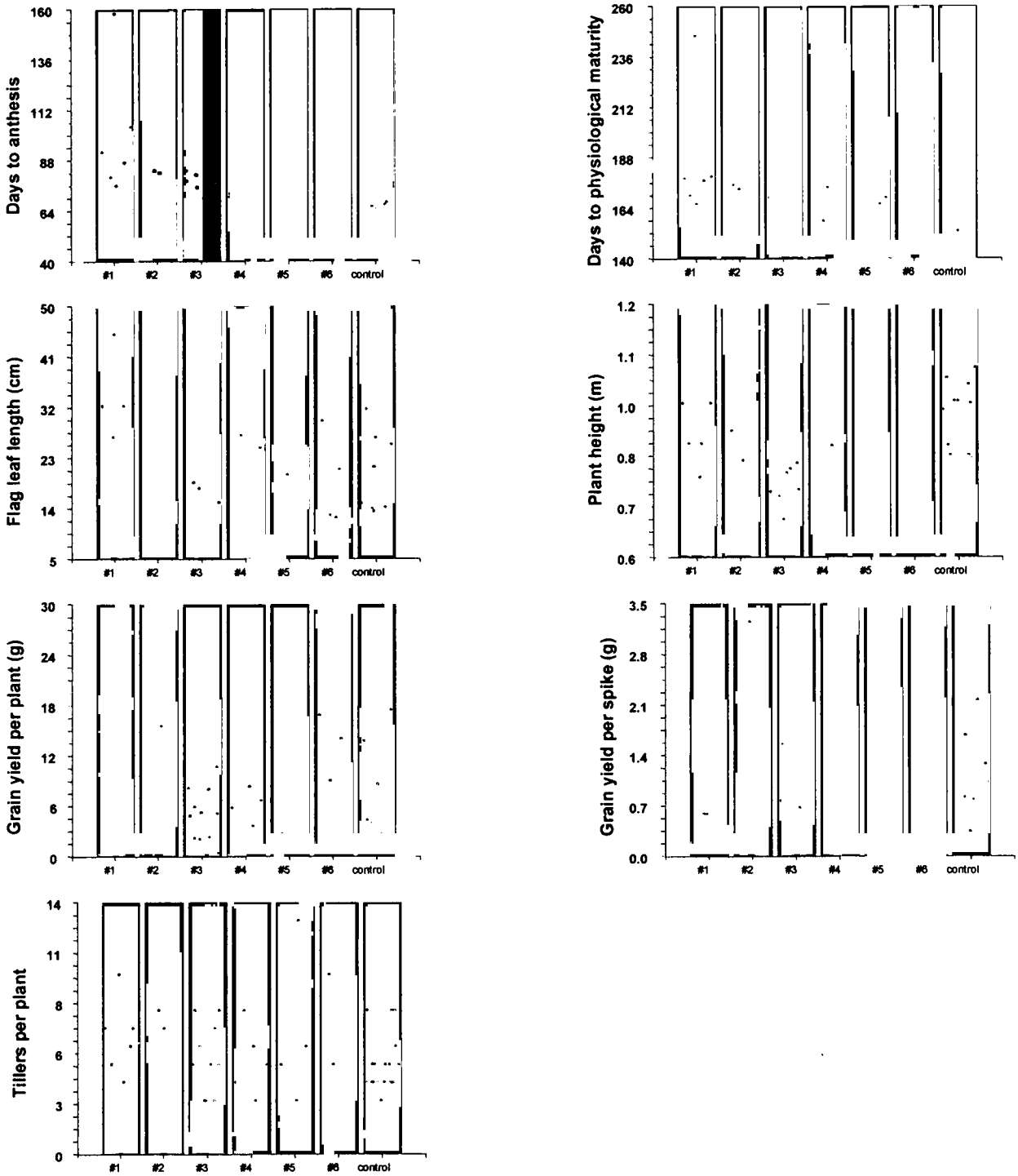


Figure 2.32. Dot plot of agronomic traits of selections #1, #2, #3, #4, #5 and #6 of wheat line W98/22+*Lr36*+*Lr34* compared to W98/22 (control) Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

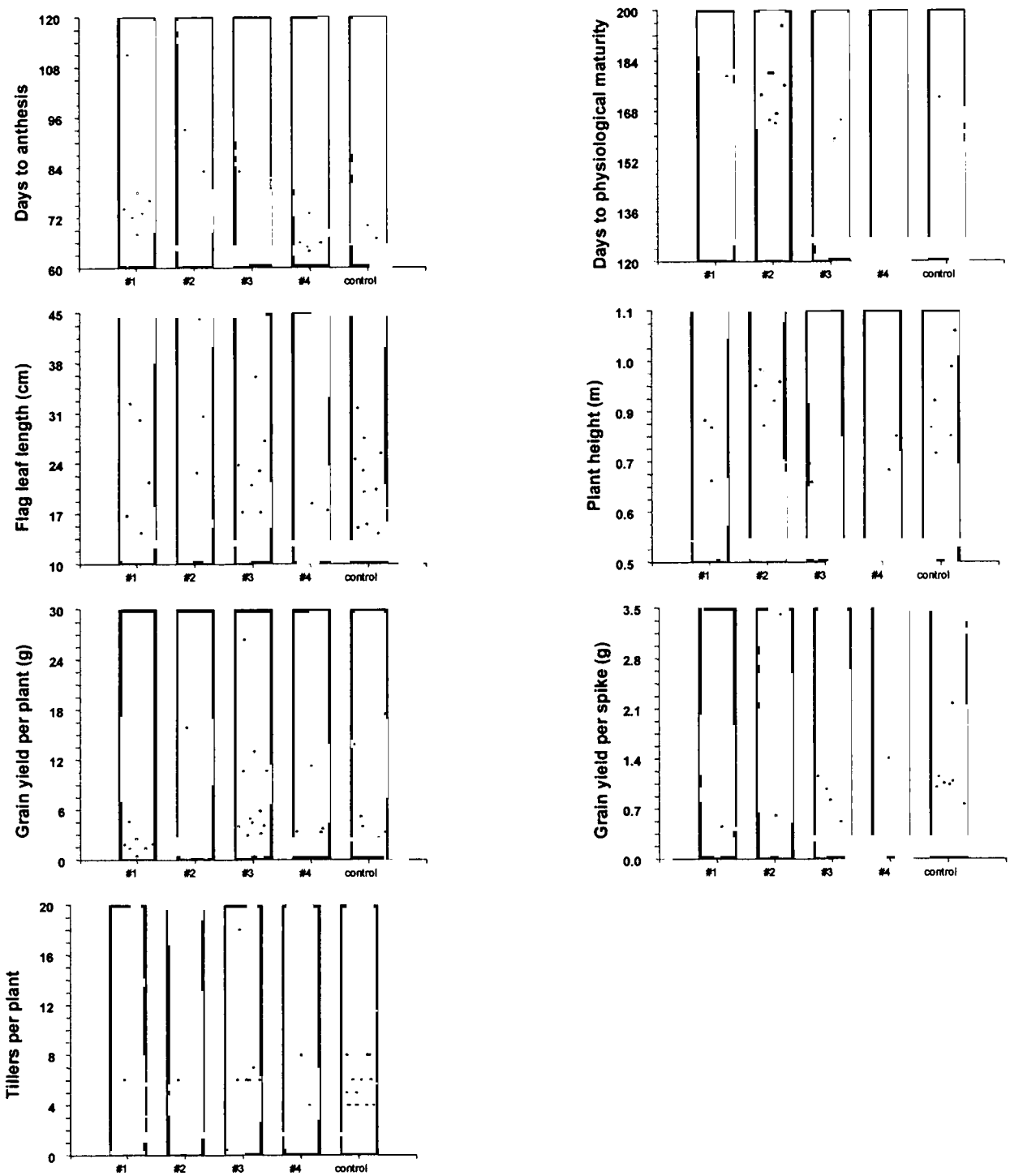


Figure 2.33. Dot plot of agronomic traits of selections #1, #2, #3 and #4 of wheat line W98/22+*Lr41*+*Lr34* compared to W98/6 (control). Within each bar, dots represent the variation observed among single plants derived from a specific selection, or within the adapted line.

2.5. References

Bhowal, J.G. and Narkhede, M.N., 1981. Genetics of pseudo-black chaff wheat. *Z. Pflanzenzucht.* 86: 298-304.

Boshoff, W.H.P., Pretorius, Z.A. and Van Niekerk, B.D., 2002. The impact of leaf rust on spring wheat in the winter rainfall region in South Africa. *S. A. J. Plant Soil* 19: 84-88.

Brown, G.N., 1997. The inheritance and expression of leaf chlorosis associated with gene *Sr2* for adult plant resistance to wheat stem rust. *Euphytica* 95: 67-71.

Cox, T.S., Sears, R.G. and Gill, B.S., 1992. Registration of KS90WGRC10 leaf rust resistant hard red winter wheat germplasm. *Crop Sci.* 32: 339-343.

Drijepondt, S.C. and Pretorius, Z.A., 1989. Greenhouse evaluation of adult plant resistance conferred by the gene *Lr34* to leaf rust of wheat. *Plant Dis.* 73: 669-671.

Dyck, P.L., 1977. Genetics of leaf rust reaction in three introductions of common wheat. *Can. J. Genet. Cytol.* 19: 711-716.

Dyck, P.L. and Samborski, D.J., 1982. The inheritance of resistance to *Puccinia recondita* in a group of common wheat cultivars. *Can. J. Genet. Cytol.* 24: 273-283.

German, S.E. and Kolmer, J.A., 1992. Effect of the gene *Lr34* in the enhancement of resistance to leaf rust of wheat. *Theor. Appl. Genet.* 84: 97-105.

Hare, R.A. and McIntosh, R.A., 1979. Genetic and cytogenetic studies of durable adult-plant resistance in 'Hope' and related cultivars to wheat rusts. *Z. Pflanzenzucht.* 83: 350-367.

Johnson, R., 1993. Durability of disease resistance in crops: some closing remarks about the topic and the symposium. Pages 283-300 in: Durability of disease resistance. Current plant science and biotechnology in agriculture, Vol. 18. Eds. Th. Jacobs and J.E. Parlevliet. Kluwer Academic Publishers, The Netherlands.

Johnston, C.O., 1967. Leaf rust of wheat. Pages 317-325 in: Wheat and wheat improvement, 1st Edition, Agronomy 13. Eds. K.S. Quisenberry and L.P. Reitz. American Society of Agronomy, Wisconsin.

Kloppers, F.J. and Pretorius, Z.A., 1997. Effects of combinations amongst genes *Lr13, Lr34* and *Lr37* on components of resistance in wheat to leaf rust. *Plant Pathology* 46: 737-750.

Knott, D.R., 1989. The wheat rusts – Breeding for resistance. Springer-Verlag Berlin. Heideberg.

Kolmer, J.A., 1992. Enhanced leaf rust resistance in wheat conditioned by resistance gene pairs with *Lr13*. *Euphytica* 61: 123-130.

Kolmer, J.A., Dyck, P.L. and Roelfs, A.P., 1991. An appraisal of stem and leaf rust resistance in North American hard red spring wheats and the probability of multiple mutations to virulence in populations of cereal rust fungi. *Phytopathology* 81: 237-239.

Lottering, J.M., Botha, A-M. and Kloppers, F.J., 1999. DNA markers linked to leaf rust resistance gene *Lr41*. Pages 176-177 in: Proceedings of the 9th Assembly of the Wheat Breeding Society, Australia, The University of Southern Queensland, Toowoomba, 27 Septermper – 1 October 1999. Ed. P. Williamson. Toowoomba, QLd.

Mares, D.J., 1989. Preharvest sprouting damage and sprouting tolerance: Assay methods and instrumentation. Pages 129-170 in: Preharvest field sprouting in cereals. Ed. N.F. Derera. CRC Press. Inc., Florida.

Martínez, F., Niks, R.E., Singh, R.P. and Rubilaes, D., 2001. Characterization of *Lr46*, a gene conferring partial resistance to wheat leaf rust. *Hereditas* 135: 111-114.

Niks, R.E. and Rubiales, D., 2002. Potentially durable resistance mechanisms in plants to specialised fungal pathogens. *Euphytica* 124: 210-216.

Parlevliet, J.E., 1995. Durable resistance and how to breed for it. Breeding for disease resistance with emphasis on durability. Pages 1-14 in: Proceedings of a regional workshop for eastern, centeral and southern Africa, held at Njoro, Kenya, October 2-6, 1994. Wageningen Agricultural University, Wageningen, The Netherlands.

Pederson, W.L. and Leath, S., 1988. Pyramiding major genes for resistance to maintain residual effects. *Annu. Rev. Phytopathology* 26: 369-378.

Pink, D.A.C., 2002. Strategies using genes for non-durable disease resistance. *Euphytica* 124: 227-236.

Pretorius, Z.A. and Brown, G.N., 1998. Detection of the *Sr2*-linked gene for seedling chlorosis in South African wheat cultivars. Pages 376-380 in: Proceedings of the tenth regional wheat workshop for eastern, central and southern Africa, University of Stellenbosch, 14-18 September 1998.

Pretorius, Z.A. and Le Roux, J., 1988. Occurrence and pathogenicity of *Puccinia recondita* f. sp. *Tritici* on wheat in South Africa during 1986 and 1987. *Phytophylactica* 20: 349-352.

Pretorius, Z.A., Van Niekerk, B.D., Kloppers, F.J. and Vorster, A.L., 1995. Managing certain recently named *Lr* genes in breeding wheat for resistance to *Puccinia recondita* f. sp. *tritici* in South Africa. *S. A. J. Plant Soil* 12: 32-37.

Roelfs, A.P., 1988. Resistance to leaf and stem rust of wheat. Pages 10-22 in Breeding strategies for resistance to the rusts of wheat. Eds. N.W. Simmonds and S. Rajaram. CYMMIT, Mexico D.F.

Roelfs, A.P., Singh, R.P. and Saari, E.E., 1992. Rust diseases of wheat: Concepts and methods of disease management. International Maize and Wheat Improvement Centre (CIMMY), Mexico, D.F.

Sawhney, R.N., 1992. The role of *Lr*34 in imparting durable resistance to wheat leaf rust through gene interaction. *Euphytica* 61: 9-12.

Sawhney, R.N., Nayar, S.K., Sharma, J.B. and Bedi, R., 1989. Mechanism of durable resistance: A new approach. *Theor. Appl. Genet.* 78: 229-232.

Sheen, S.J., Ebeltoft, D.C. and Smith, G.S., 1968. Association and inheritance of 'black chaff' and stem rust reactions in Conley wheat crosses. *Crop Sci.* 8: 477-480.

Singh, R.P., 1992. Expression of wheat leaf rust resistance gene *Lr34* in seedling and adult plants. *Plant Dis.* 76: 489-491.

Van Silfhout, C.H., 1993. Durable resistance in the pathosystem: wheat – stripe rust. Pages 135-145 in: Durability of disease resistance. Eds. TH. Jacobs and J.E. Parlevliet. Kluwer Academic Publishers, London.

Chapter 3

Identification of AFLP markers linked to a leaf rust resistance gene in wheat line KS93U9

3.1. Introduction

A continuous search for new genes and their utilisation are necessary to guarantee progress in disease resistance breeding (Hartl *et al.*, 1999). For this purpose, various methods, including pedigree (Cox *et al.*, 1985) and DNA marker (Karp *et al.*, 1996) analysis, have been used to quantify genetic diversity among genotypes. The development of molecular markers linked to genes of agronomic importance accelerates the incorporation of the gene of interest into a new variety and makes the concept of gene pyramiding possible (Feuillet *et al.*, 1995). Using traditional infection studies, the selection of genotypes containing several leaf rust (*Puccinia triticina*) resistance (*Lr*) genes is time-consuming and often not possible, due to limitations in the array of pathotypes available. The development of molecular markers for specific *Lr* genes allows the detection of these genes independently of the phenotype and presence of other *Lr* genes (Roelfs *et al.*, 1992). Furthermore, Penner *et al.* (1998) found that marker assisted selection (MAS) significantly increased the efficiency of backcrossing.

In general, the development of molecular markers for genes derived from the common wheat gene pool has been difficult, whereas markers for genes originating from wild relatives have been more readily found (Schachermayr *et al.*, 1997). This is due to the large genome size of bread wheat, the low levels of molecular polymorphisms within the species, and the overwhelming presence of repetitive sequences (William *et al.*, 1997).

Hussien *et al.* (1998) conducted monosomic analysis to determine the chromosomal locations of three new leaf rust resistance genes transferred from *T. monococcum* to common wheat. One of the undesignated genes, originally transferred from *T. monococcum* subsp. *monococcum* to wheat line KS92WGRC23, is located on chromosome 6A (Hussien *et al.,* 1998). No useful linked DNA marker has been reported for this gene.

In wheat line 'KS93U9' (pedigree Karl*3//PI 266844/PI 355520), which is a selection from 'KS92WGRC23' (Jacobs *et al.,* 1996), this *Lr* gene conditions an immune response to the prevailing South African pathotypes of *P. triticina*. The objective of this study was to find an AFLP marker linked to the *T. monococcum*-derived leaf rust resistance gene in 'KS93U9'.

3.2. Materials and methods

3.2.1. Plant material

3.2.1.1. Initial screening and identification

Leaf rust-resistant plants from a 'Karee*2/KS93U9' line (seed provided by Prof Z.A. Pretorius, University of the Free State, South Africa) were used in crosses with the leaf rust-susceptible winter wheats T96/6 and Elands, respectively. For molecular marker analysis, it was important to evaluate all available parental lines. Thus, 'KS93U9' and 'Karee*2/KS93U9' were included as resistant controls whereas 'Karl', 'Karee', 'T96/6' and 'Elands' served as the rust-susceptible parents. The two *T. monococcum* sources, 'PI 266844' and 'PI 355520', could not be included since both stocks had lost viability. The 'Karee*2/KS93U9//T96/6' and 'Karee*2/KS93U9//Elands' F_1 were selfed and the F_2 used in the analysis.

3.2.1.2. Validation of marker

For marker validation, F_2 progeny of crosses between 'Karee*2/KS93U9' and, respectively, 'BSP97/1', 'BSP98/4' and 'BSP98/16' were evaluated for their leaf rust resistance and presence of the putative AFLP marker

3.2.2. Expression of resistance to *P. triticina*

Leaf rust ratings on 'T96/6' and 'Elands' progeny were done on greenhouse-grown seedlings inoculated at the primary leaf stage with fresh urediospores of pathotype UVPrt 9. Similar procedures were followed for the other backgrounds, except that 'BSP97/1' progeny were inoculated with pathotype UVPrt2, and 'BSP98/4' and 'BSP98/16' derivatives with UVPrt13. In all cases the pathotype used was virulent on the locally adapted wheat but avirulent on 'KS93U9'. The inoculated plants were incubated at 18-20°C in a dew chamber for 16 h, after which the plants were transferred to a greenhouse cubicle set at a 23°C/18°C diurnal cycle. A 0-4 rating scale (Roelfs *et al.,* 1992) was used to score the disease reactions.

Following the leaf rust phenotyping, two plants from each parent line (Table 3.1) were transplanted to 2-l pots. Ten resistant and 10 susceptible F_2 plants from each of the 'Karee*2/KS93U9//T96/6' and 'Karee*2/KS93U9//Elands' crosses were similarly transferred. For marker validation, two susceptible and five resistant F_2 plants of each of the 'BSP97/1', 'BSP98/4' and 'BSP98/16' backgrounds (Table 3.2) were transferred to larger pots. Leaf tissue from new, uninoculated growth was subsequently sampled for DNA extraction.

3.2.3. Solutions used with DNA extractions

2% CTAB isolation buffer

- 20 g CTAB (2%)
- 280 ml 5 M NaCl (1.4 M)
- 40 ml O.5 M EDTA pH8 (20 mM)
- 100 ml 1 M Tris-Cl pH8 (100 mM)
- Dissolve on hot plate.
- Adjust to pH 8
- Autoclave

0.5 M EDTA

- 186.12 g $Na_2EDTA.2H_20$
- Add approximately 750 ml distilled water and adjust pH to 8 with NaOH pellets until the solution becomes clear. Make up volume to 1000 ml with distilled water
- Autoclave

Wash buffer

- 76 ml absolute ethanol
- 0.1 ml 10 M NH_4OAc
- Make up volume of 1000 ml with distilled water

24.1 Chloroform:isoamylalcohol [24:1(v/v)]

- 20 ml isoamylalcohol
- 480 ml chloroform
- Store covered in foil at 4°C

5 M NaCl

- 292.2 g NaCl
- Make up volume to 1000 ml with distilled water
- Autoclave

10 M NH_4OAc

- 38.5 g NH_4OAc
- Make up volume to 1000 ml with ddH_20

3.2.4. Methodology for DNA extractions

Phase One

- Add one gram of fresh leaf material to 10 ml of 2% CTAB extraction buffer and 20 µl ß-mercaptoethanol (BME) and incubate for 1 h at 60°C in 50 ml polypropylene tubes.
- Add 15 ml of chloroform:isomylalcohol [24:1(v/v)] and centrifuge for 10 min at 7000 rpm.
- Transfer upper-phase to new polypropylene tubes and add isopropanol equal to two thirds of the volume transferred.
- Precipitate DNA for 1 h at -20°C.
- Centrifuge at 10 000 rpm (4°C) for 10 min.
- Add 15 ml of wash buffer to tubes and leave on the bench for 20 min or more.
- Centrifuge at 10 000 rpm (4°C) for 5 min.
- Discard supernatant.
- Dissolve DNA in 1 ml of ddH_2O and store at 4°C overnight.

Phase Two

- Digest pellets with 5 µl RNase (1 mg/ml) for 30 min at 37°C.
- Precipitate DNA with 2 ml ddH_2O, 1 ml 7.5 M NH_4OAc (pH 7.7) and 10 ml cold 100% EtOH.
- Store samples at -20°C for 1 h or more.

Phase Three

- Centrifuge at 10 000 for 10 min at room temperature (21°C).
- Extract DNA with 500 µl phenol and 500 µl chloroform:isomylalcohol [24:1(v/v)].
- Centrifuge at 10 000 for 10 min (21°C).
- Precipitated DNA by adding NH_4OAc to a final concentration of 2.5 M and 100% EtOH (double the volume of DNA solution and salt).

- Incubate at -20°C for 1 h.
- Centrifuge at 14 000 rpm for 45 min at 4°C.
- Wash pellets with 1 ml of 70% EtOH.
- Centrifuge at 14 000 rpm for 30 min.
- Wash pellets again with 1 ml of 70% EtOH.
- Centrifuge at 14 000 rpm for 15 min.
- Speed-vac dry the tubes for approximately 15 min.
- Dissolve DNA in 40 µl of ddH_2O (depends on size of pellet).

3.2.5. DNA quantification

Extracted DNA was quantified on a 0.8% agarose gel (1.04 g Seakem LE agarose dissolved in 130 ml 0.5xTBE), which was run in 0.5 x TBE at 60 V for 1.5 h in a 20 cm x 20 cm gel tray. Six µl of ethidium bromide (10 µg/ml) were added to the 130 ml gel mix. A Pharmacia LKB GNA 200 gel apparatus with Pharmacia LKB GPS 200/400 power pack was used. The DNA bands were compared against lambda concentration standards of 0.1 µg, 0.2 µg, 0.3 µg, 0.4 µg and 0.5 µg. Dilutions (250 ng/µl for AFLPs) were made accordingly.

3.2.6. Solutions used with AFLP-protocol

6% sequencing gel mix (7M urea)

- 15 ml 40% Acrylamide (Promega)
- 42.04 g Urea (Riedel-de-Hahn)
- 10 ml 10 x TBE
- Dilute to 100 ml with ddH_20

10xTBE

- 108 g Tris
- 55 g Boric Acid
- 20 ml 0.5 M EDTA pH 8
- Dilute to 1000 ml with ddH_20
- Autoclave

10% Ammonium persulphate

- 1 g Ammonium persulphate
- Add ddH_20 to 10 ml
- Aliquot
- Store at -20°C

Preparation for gel

- 0.4 mm spacers (Gibco BRL)
- Model S2 gel casting clamps (Gibco BRL)
- Add 250 µl of 10% Ammonium persulphate
- Add 50 µl Temed (Sigma)

1x$TE_{0.1}$ buffer

- 1 ml 50xTE buffer
- 0.545 g Tris
- pH with concentrated HCl
- Dilute to 500 ml with ddH_2O
- Autoclave

5xRL Buffer

- 50 mM Tris Hac pH 7.5
- 50 mm MgAc
- 250 mM Kac
- 250 ng/µl BSA
- 25 mM DTT

AFLP Loading dye

- 39.2 ml Formamide
- 0.8 ml EDTA (from 0.5 M stock)
- 0.02 g Bromophenol blue
- 0.02 g Xylene cyanol FF
- dissolve and aliquot
- store at –20°C

3.2.7. AFLP: Initial screening and testing

The AFLP-protocol for wheat, as described by Donini *et al.* (1997), was used with minor modifications. A bulk segregant analyses (BSA, Michelmore *et al.*, 1991) strategy was followed, which implied that susceptible and resistant bulks were constructed of F_2 plants derived from the 'T96/6' and 'Elands' backgrounds. A total of four bulks were therefore used during the initial screenings. Each of the bulks contained DNA of 10 F_2 plants. The parental wheat lines were included as controls.

3.2.7.1. Restriction digestion and ligation of genomic DNA

Five hundred nanogram of genomic DNA were digested with 5U *Sse*I8387I (Amersham) and 5U *Mse*I (New England Biolabs) in a 40 µl mixture containing 1x One-Phor-All buffer (USB), 0.1 µg/µl BSA (New England Biolabs) and AFLP grade water. The mixture was digested for 2 to 3 h at 37°C. After restriction, 5 pmol *Sse*I adaptor and 50 pmol *Mse*I adaptor were ligated to the restriction fragments with 1U T4 DNA ligase (Amersham). The final ligation volume of 10 µl consisted of 1 mM of ATP, 1xOne-Phor-All buffer, 0.1 µg/µl BSA (New England Biolabs) and AFLP grade water. The restriction-ligation mixture (50 µl) was incubated overnight at 37°C. A 1:9 dilution with $TE_{0.1}$ buffer was performed on 45 µl of the restriction-ligation reaction. The remaining 5 µl of each reaction was tested on a 1% agarose gel to determine whether the DNA was restricted during the restriction-ligation step.

3.2.7.2. Pre-amplification reactions (Cold AMP)

After adaptor ligation, non-selective amplification (pre-amplification) of DNA fragments was performed using 75 ng of each of the non-selective (zero base pair extension) primers M00 and S00. The pre-amplification mix of 50 µl also consisted of 200 µM dNTPs, 1U *Taq* polymerase, 1xPCR buffer (Bioline), 1.5 mM $MgCl_2$ (Bioline) and AFLP grade water. The PCR reaction was performed on a DNA Engine MJR PTC 200 thermal cycler. The amplification protocol was: 72°C for 5 min, followed by a 30 cycle sequence of 94°C for 30 sec, 56°C for 60 sec and 72°C for 60 sec. The PCR protocol was concluded with 72°C for 5 min. The soak temperature was 4°C. A 1:9 dilution with $TE_{0.1}$ buffer was performed on 45 µl of the Cold Amp reaction. The remaining 5 µl of each reaction was tested on a 1% agarose gel to determine whether the DNA was restricted during the Cold Amp step.

3.2.7.3. Selective amplification (Hot Amp)

Seven *Sse*I primers (5 ng/reaction) (Table 3.3) were each labeled radioactive with 0.05 μCi [γ-^{33}ATP] (New Life Sciences) for 2 h at 37°C in a labeling mix containing 0.049U Kinase (Bioline), 1xOne-Phor-All buffer (USB) and AFLP grade water. The reaction was stopped by incubating the radioactive primer mix at 65°C for 10 min.

3.2.7.4. PCR

The PCR mix of 10 μl contained 2.5 μl of diluted preamplification template DNA, 200 μM dNTPs, 1.5 mM $MgCl_2$ (Bioline), 1 x PCR buffer (Bioline), 15 ng of unlabeled *Mse*I primer (New England Biolabs), AFLP grade water, 0.025U *Taq* Polymerase (Bioline) and 0.5 μl labeled primer (*Sse*I, Amersham). The PCR reaction was performed on a DNA engine MJR PTC 200 thermal cycler. The amplification protocol was: 94°C for 30 sec, 65°C for 30 sec and 72°C for 1 min. This was repeated for 13 cycles with the temperature dropping 0.7°C with each cycle. The second phase of the protocol consisted of 94°C for 30 sec, 56°C for 30 sec and 72°C for 1 min (for 26 cycles). The soak temperature was 4°C.

3.2.7.5. Electrophoresis

A model S2 sequencing gel apparatus (Life Technologies) was used during the assays. Gels were 'pre-run' at 80 W for approximately 30 min with 1XTBE buffer. DNA was denatured before electrophoresis at 94°C for 4 min. Electrophoresis was carried out for 2 h at 80 W.

3.2.7.6. Fixation of gels

After electrophoresis, the gel was transferred to a 3 mm Whatman filter paper (or chromatography paper). The gel was then clingwrapped before it was dried on a Savant SGD 4050 slab gel drier at 80°C for approximately 1.5 h. When completely

dry, an X-ray film (Kodax Biomax MR) was placed on top of the gel and allowed to develop for 3 to 5 days.

3.2.7.7. Reproducibility of the AFLP technique

The reproducibility of the AFLP protocol was verified by including control checks at all the various steps involved, i.e. DNA extraction, restriction and ligation (R-L), Cold Amp as well as Hot Amp. DNA from the same T96/6 plant was used in the verification process. DNA was extracted twice from the selected T96/6 plant in separate experiments. These two samples served as a DNA extraction control and were both included in the restriction-ligation step. The restriction-ligation reaction of the second sample was repeated as well. One of the restricted-ligated templates derived from the second T96/6 sample was then used in two different Cold Amp reactions. One of these Cold Amp reactions was then used twice in the Hot Amp reactions. Fig. 3.1 gives a graphic description of the procedure followed in determining the reproducibility of the AFLP protocol.

The profile of all five AFLP samples derived from the same T96/6 plant was expected to be the same. This would indicate that the AFLP technique as used in this study is reproducible. Any difference detected should serve as a warning that there is a problem and the specific step at which it is detected will give an indication as to what the problem may be.

3.2.7.8. Increased primer specificity

Initial screenings indicated that the S12/M14 primer combination amplified an AFLP fragment that is unique to all resistant plants. In order to obtain a simpler AFLP profile with a more discrete band for the putative marker, the S12 (*Sse*I+2) primer in combination with each of the four +3 extensions to the relevant *Mse*I+2 (M14:-AT) primer was tested. These primers were M43 (-AT**A**), M44 (-AT**C**), M45 (-AT**G**) and M46 (-AT**T**). All the parental lines and bulks that were used in the initial screening

were included. The S12/M14 primer combination was also repeated as a control on some of these lines. The wheat cultivars 'Kariega' and 'Avocet S' were included to establish whether the 'Kariega' x 'Avocet S' doubled haploid (DH) mapping population (R. Prins, personal communication, 2002) would be useful to map the relevant AFLP marker.

3.2.8. Marker validation

The S12/M44 marker was further tested on three different groups of F_2 plants derived from a cross between 'Karee*2/KS39U9' and the leaf rust-susceptible lines 'BSP97/1', 'BSP98/4' and 'BSP98/16'.

3.3. Results and discussion

3.3.1. Reproducibility of the AFLP technique

The identical AFLP-profile that was obtained with the primer combination S26 (*Sse*-TT) and M42 (*Mse*-GT) confirmed the reproducibility of the AFLP technique (Fig. 3.2).

3.3.2. AFLP analysis on bulks

One of the 130 primer combinations (Table 3.5) that were tested on the bulks and parental lines generated a putative marker for the target *Lr* gene. Primer combination S12 (*Sse*-AC) and M14 (*Mse*-AT) amplifies an AFLP fragment that is unique to all the resistant parental lines and bulks (Fig. 3.3, arrow 1). A smaller but unique allele is visible within the 'T96/6' bulk (Fig. 3.3, arrow 2), that might be an indication of co-dominance. A third allele, characteristic to 'Elands' and its progeny, is also visible (Fig. 3.3, arrow 3). This might be useful in cultivar identification

studies. In order to verify this linkage of the putative marker in 'Elands', its occurrence within other backgrounds needs to be determined.

3.3.3. Increased primer specificity

The combination of S12 with each of the four +3 extensions of M14 [M43 (-AT**A**), M44 (-AT**C**), M45 (-AT**G**) and M46 (-AT**T**)] identified S12/M44 as the critical primer combination (Fig. 3.4). Once again the *Lr* carrying parental lines and bulks amplified the band of interest. This marker is, however, more discrete than the one obtained with S12/M14. There are also fewer background bands in its vicinity, which will also simplify cloning attempts. Similar to the S12/M14 marker (Fig 3.3 and 3.4), the S12/M44 marker appears to be co-dominant in the 'T96/6' background. However, as the AFLP bands are more discrete in Fig. 3.4, a faint band of similar size as the strong 'co-dominant' band in the 'T96/6' S bulk is visible, not only in the parental 'T96/6', but also in the 'Elands' S bulk and all the other susceptible parental lines. The amplification of the band in the susceptible lines and bulks in this experiment is probably due to the increased primer specificity, resulting in reduced primer competition in the PCR reaction. The S12/M14 band characteristic of the 'T96/6' S bulk or the marker band of the resistant bulks was not visible or polymorphic in the 'Kariega' and 'Avocet S' genotypes and can thus not be mapped in the 'Kariega' x 'Avocet S' DH mapping population. In the 'T96/6' S bulk and 'T96/6' parental line a faint background band on approximately the same level as the band associated with the resistance gene, was confounding (Fig. 3.4 and 3.6).

3.3.4. Screening of individual F_2 plants

F_2 plants, initially used to construct the resistant and susceptible bulks of 'Karee*2/KS93U9//Elands' and 'Karee*2/KS93U9//T96/6', were screened individually with S12/M44. All 10 of the resistant 'Karee*2/KS93U9//Elands' F_2 plants revealed the band associated with the leaf rust resistance gene (Fig. 3.5), while it was absent in the corresponding susceptible plants. With the screening of the

'Karee*2/KS93U9//T96/6' F_2 plants, the band was absent in one of the samples (F_2 #4; Fig. 3.6 and 3.7). A 30-330bp AFLP™ DNA ladder (Gibco-BR6, Life Technologies) included with the screening, indicated that the marker is approximately 240 base pairs in size.

However, in the susceptible plants derived from 'T96/6' the less faint background band on the same level as the band associated with the resistance gene, was complicating analysis of the gel (Fig. 3.6). When the gel was run for longer the profile was much clearer and more discrete (Fig. 3.7). This background band was separated from the band of interest and it became clear that the smaller 'co-dominant' band, which was first noticed in 'T96/6' and its susceptible progeny, is also a background band. This marker is thus indeed a dominant marker in both backgrounds and not co-dominant in the 'T96/6' background as initially suspected (Fig. 3.7).

3.3.5. Validation of marker

The S12/M44 marker also proved to be useful in the three additional backgrounds tested as it is absent in the three susceptible parental lines (Fig. 3.8). This marker is thus useful in all five different susceptible wheat backgrounds tested in this study, which is promising as wheat markers' usefulness are often limited to a small number of genetic backgrounds.

However, these initial validation tests revealed that recombination has taken place in several instances between the marker S12/M44 band and the resistance gene (resistant 'Karee*2/KS93U9//BSP97/1' #1, #3, #4 and #5 and 'Karee*2/KS93U9//BSP98/16' #6, susceptible 'Karee*2/KS93U9//BSP98/16' #7). This explains the absence of the fragment in F_2:'Karee*2/KS93U9//T96/6' plant #4 (Fig 3.7). The number of F_2's tested was too small to determine the recombination/linkage distance and emphasizes the need to expand the F_2 population to accurately determine the linkage distance between the S12/M44 marker and the resistance gene. The absence of the S12/M44 band of interest in

'Kariega' and 'Avocet' was also demonstrated (Fig. 3.8), thereby confirming the results of S12/M14 (Fig. 3.4), that the 'Kariga' and 'Avocet' population cannot be used to determine its chromosome location.

3.4. Conclusions

The application of AFLP marker technology has provided a powerful tool for the detection of a larger number of DNA polymorphism within plants, especially those species with low polymorphism (Thomas *et al.,* 1995; Vos *et al.,* 1995). In the present study one (S12:-AC/M14:-AT) of 130 (0.8%) AFLP primer combinations tested generated a polymorphic fragment in the resistant bulks and parental lines carrying the undesignated *Lr* resistance gene. By increasing primer specificity using *Mse*I+3 primers, S12:-AC/M44:-ATC was identified as the critical primer combination. Using 96 primer combinations, Hartl *et al.* (1999) applied a BSA strategy to identify several AFLP markers closely linked to powdery mildew resistance genes in common wheat. There is little doubt as to the superiority of AFLP over other marker techniques such as RFLPs and RAPDs. With their investigation into AFLP markers linked to a major quantitative trait locus controlling scab resistance in wheat, Bai *et al.* (1999) found that in comparison to the 1% of RAPDs, 20 of 300 (7%) AFLP primer combinations tested, resulted in polymorphic fragments distinguishing the same two bulks. Lottering *et al.* (2002) also found that the number of polymorphisms (30%) determined by AFLP was significantly higher than that obtained by RAPDs (Botha and Venter, 2000). Ma and Lapitan (1998) demonstrated the increased resolution power of AFLPs to RFLPs with 100% of the AFLP primer pairs tested on 11 wheat genotypes detecting polymorphisms compared to the 61% of the RFLP markers.

The BSA strategy followed proved to be successful. The identification of an AFLP marker can, however, also be attributed to the fact that the *Lr* gene originates from a wild relative (*T. monococcum*). As wheat has low levels of polymorphism, it simplifies the search for markers, if the gene originates from a wild relative of wheat (Schachermayr *et al.*, 1997). Lottering *et al.* (2002), using only 64 primer

combinations, managed to develop an AFLP marker for the leaf rust resistance gene *Lr41* originated from *Triticum taucshii*, a diploid ancestor of common bread wheat.

Once the putative marker for leaf rust resistance generated by S12/M44 was developed with the pooled DNA, the marker was tested on the 'Elands' and 'T96/6' F_2 populations in an attempt to verify linkage to the *Lr* resistance gene. The analysis confirmed the dominant status of the marker but indicated that it might not be closely linked to the *Lr* gene, as one of the 10 F_2 'Karee*2/KS93U9//T96/6' resistant plants screened, did not have the band associated with resistance. Validation on three additional backgrounds revealed several instances of recombination between the marker and the resistance/susceptible gene emphasizing the need to do a proper linkage study. A true indication of linkage can, however, only be given with the screening of a larger F_2 population (i.e. 100 plants). Should the linkage distance be acceptable, it might still be a very useful marker as it proved to be polymorphic in five wheat backgrounds. The linkage distance is also needed before the intensive effort to clone and convert the AFLP band to a user-friendlier STS marker will be considered. The success rate of converting AFLP markers to STS markers in wheat is limited (Shan *et al.,* 1999), except in cases such as in the present study where AFLP markers linked to genes that have been introgressed from the wild species of wheat (Prins *et al.* 2001) are used. Preliminary results, however, suggests that further attempts are justified to identify more markers, preferably closer linked to the *Lr* gene, which can be used in combination with this marker to ensure accurate molecular tagging of the *Lr* genes. No confirmation on the chromosomal location of the *Lr* gene and marker could be obtained.

Table 3.1. Plant material used in the initial AFLP analysis

Wheat line or cross	Susceptible		Resistant	
	Infection type*	Number of plants selected	Infection type*	Number of plants selected
Elands	3	2	-	-
T96/6	3	2	-	-
KS93U9	-	-	0	2
Karee*2/KS93U9	-	-	0	2
Karl	3	2	-	-
Karee	3	2	-	-
Karee*2/KS93U9//Elands (F_2)	3	10	0;	10
Karee*2/KS93U9//T96/6 (F_2)	3	10	0;	10

* Infection types according to Roelfs *et al.* (1992).

Table 3.2. Plant material used in marker validation

Wheat line	Susceptible		Resistant	
	Infection type*	Number of plants selected	Infection type*	Number of plants selected
BSP97/1	2,3	2	-	-
BSP98/4	3^+	2	-	-
BSP98/16	3^+	2	-	-
Karee*2/KS93U9XBSP97/1 (F_2)	2^+,3	2	;,;1N	5
Karee*2/KS93U9XBSP98/4 (F_2)	3	2	;,;N	5
Karee*2/KS93U9XBSP98/16 (F_2)	2^+,3	2	;	5

* Infection types according to Roelfs *et al.* (1992).

Table 3.3. *Sse* primers labeled radioactive for AFLP screenings

***Sse* 3871 adaptor**	**Name**	
*Sse*I + 2	S12	5-GAC TGC GTA CAT GCA GG AC-3
	S15	5-GAC TGC GTA CAT GCA GG CA-3
	S16	5-GAC TGC GTA CAT GCA GG CC-3
	S18	5-GAC TGC GTA CAT GCA GG CT-3
	S19	5-GAC TGC GTA CAT GCA GG GA-3
	S25	5-GAC TGC GTA CAT GCA GG TG-3
	S26	5- GAC TGC GTA CAT GCA GG TT-3

Table 3.4. *Mse* primers used in AFLP screenings

***Mse*I – adaptor**	**Name**	
Primers +2	M11	5-GAT GAG TCC TGA GTA A AA-3
	M12	5-GAT GAG TCC TGA GTA A AC-3
	M13	5-GAT GAG TCC TGA GTA A AG-3
	M14	5-GAT GAG TCC TGA GTA A AT-3
	M15	5-GAT GAG TCC TGA GTA A CA-3
	M16	5-GAT GAG TCC TGA GTA A CC-3
	M17	5-GAT GAG TCC TGA GTA A CG-3
	M18	5-GAT GAG TCC TGA GTA A CT-3
	M19	5-GAT GAG TCC TGA GTA A GA-3
	M20	5-GAT GAG TCC TGA GTA A GC-3
Primers +3	M31	5-GAT GAG TCC TGA GTA A AA A-3
	M32	5-GAT GAG TCC TGA GTA A AA C-3
	M33	5-GAT GAG TCC TGA GTA A AA G-3
	M34	5-GAT GAG TCC TGA GTA A AA T-3
	M35	5-GAT GAG TCC TGA GTA A AC A-3
	M36	5-GAT GAG TCC TGA GTA A AC C-3
	M37	5-GAT GAG TCC TGA GTA A AC G-3
	M38	5-GAT GAG TCC TGA GTA A AC T-3
	M39	5-GAT GAG TCC TGA GTA A AG A-3
	M40	5-GAT GAG TCC TGA GTA A AG C-3
	M41	5-GAT GAG TCC TGA GTA A AG G-3
	M42	5-GAT GAG TCC TGA GTA A AG T-3
	M44	5-GAT GAG TCC TGA GTA A AT C-3
	M47	5-GAT GAG TCC TGA GTA A CA A-3
	M48	5-GAT GAG TCC TGA GTA A CA C-3
	M49	5-GAT GAG TCC TGA GTA A CA G-3
	M50	5-GAT GAG TCC TGA GTA A CA T-3
	M51	5-GAT GAG TCC TGA GTA A CC A-3
	M52	5-GAT GAG TCC TGA GTA A CC C-3
	M53	5-GAT GAG TCC TGA GTA A CC G-3
	M54	5-GAT GAG TCC TGA GTA A CC T-3
	M55	5-GAT GAG TCC TGA GTA A CG A-3

Table 3.4. *Mse* primers used in AFLP screenings

***Msel* - adaptor**	**Name**	
	M57	5-GAT GAG TCC TGA GTA A CG G-3
	M58	5-GAT GAG TCC TGA GTA A CG T-3
	M59	5-GAT GAG TCC TGA GTA A CT A-3
	M62	5-GAT GAG TCC TGA GTA A CT T-3
	M63	5-GAT GAG TCC TGA GTA A GA A-3
	M64	5-GAT GAG TCC TGA GTA A GA C-3
	M65	5-GAT GAG TCC TGA GTA A GA G-3
	M66	5-GAT GAG TCC TGA GTA A GA T-3
	M67	5-GAT GAG TCC TGA GTA A GC A-3
	M68	5-GAT GAG TCC TGA GTA A GC C-3
	M69	5-GAT GAG TCC TGA GTA A GC G-3
	M70	5-GAT GAG TCC TGA GTA A GC T-3
	M71	5-GAT GAG TCC TGA GTA A GG A-3
	M72	5-GAT GAG TCC TGA GTA A GG C-3
	M73	5-GAT GAG TCC TGA GTA A GG G-3
	M74	5-GAT GAG TCC TGA GTA A GG T-3
	M75	5-GAT GAG TCC TGA GTA A GT A-3
	M76	5-GAT GAG TCC TGA GTA A GT C-3
	M77	5-GAT GAG TCC TGA GTA A GT G-3
	M78	5-GAT GAG TCC TGA GTA A GT T-3
	M80	5-GAT GAG TCC TGA GTA A TA C-3
	M81	5-GAT GAG TCC TGA GTA A TA G-3
	M83	5-GAT GAG TCC TGA GTA A TC A-3

Table 3.5. *Sse* and *Mse* primer combinations used in the AFLP assay

***Sse* primer**	***Mse* +2 Primer**	***Mse* +3 Primer**
S12	M11, M12, M14, M15, M16, M17, M18, M19, M20	
S15	M11, M12, M14, M15, M16, M17, M18, M19, M21	M35, M39, M40, M41, M65, M76, M80, M81, M83
S16	M11, M12, M14, M15, M16, M17, M18, M19, M21	M35, M39, M40, M41, M65, M76, M80, M81, M83
S18	M11, M12, M14, M15, M16, M17, M18, M19, M21	M35, M39, M40, M41, M65, M76, M80, M81, M83
S19	M12, M13, M14, M19, M20, M21	M35, M39, M40, M41, M65, M75, M76, M77, M78, M80, M81, M83
S25		M35, M39, M40, M41, M65, M76, M80, M81, M83
S26		M12, M31, M32, M33, M34, M35, M36, M37, M38, M39, M40, M41, M42, M43, M44, M47, M48, M49, M50, M51, M52, M53, M54, M55, M57, M58, M59, M62, M63, M64, M65, M66, M67, M68, M69, M70, M71, M72, M73, M74

Table 3.6. AFLP results obtained with verification of the marker in F_2 plants derived from crosses made between the resistant parent line Karee*2/KS93U9 and BSP97/1, BSP98/4 and BSP98/16

	Number of plants with S12/M44 band present	Number of plants with S12/M44 band absent	Number of plants tested
F2:K/KS x BSP97/1 Resistant	1	4	5
F2:K/KS x BSP97/1 Susceptible		2	2
F2:K/KS x BSP98/4 Resistant	5		5
F2:K/KS x BSP98/4 Susceptible		2	2
F2:K/KS x BSP98/16 Resistant	4	1	5
F2:K/KS x BSP98/16 Susceptible	1	1	2

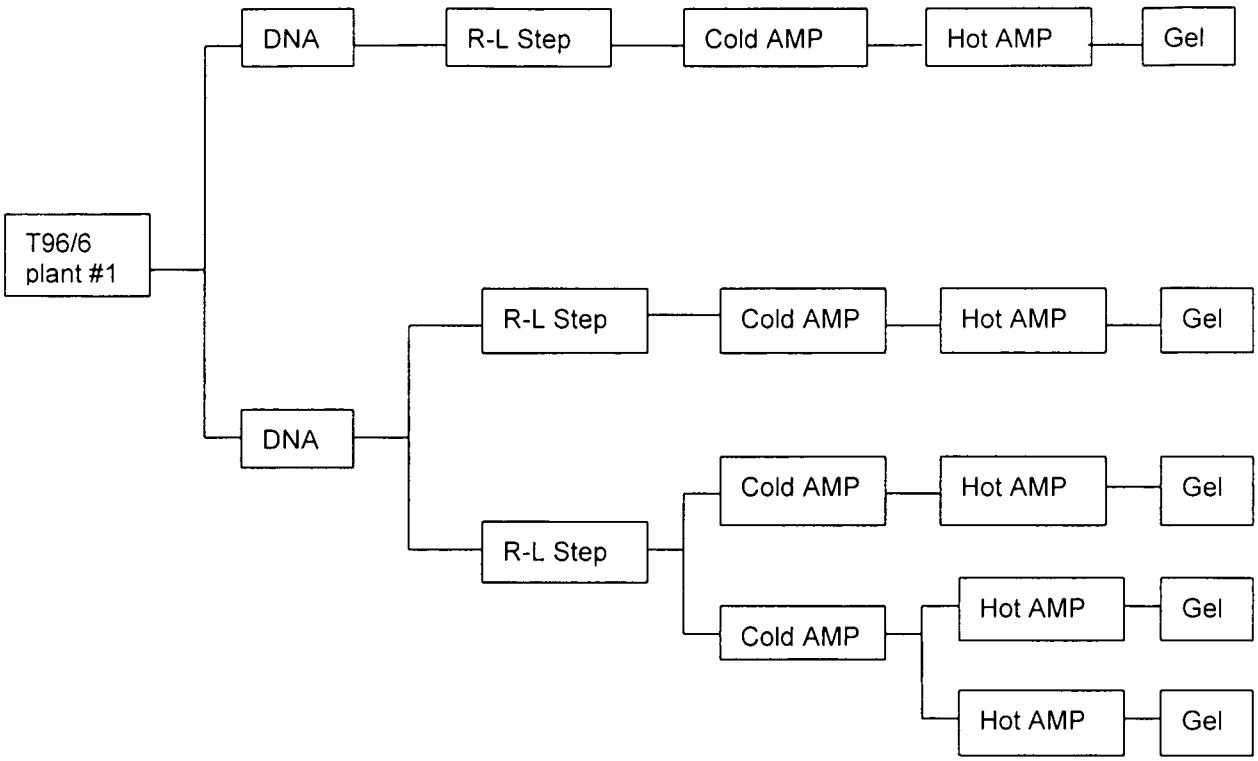


Figure 3.1. Protocol followed to determine reproducibility of the AFLP technique.

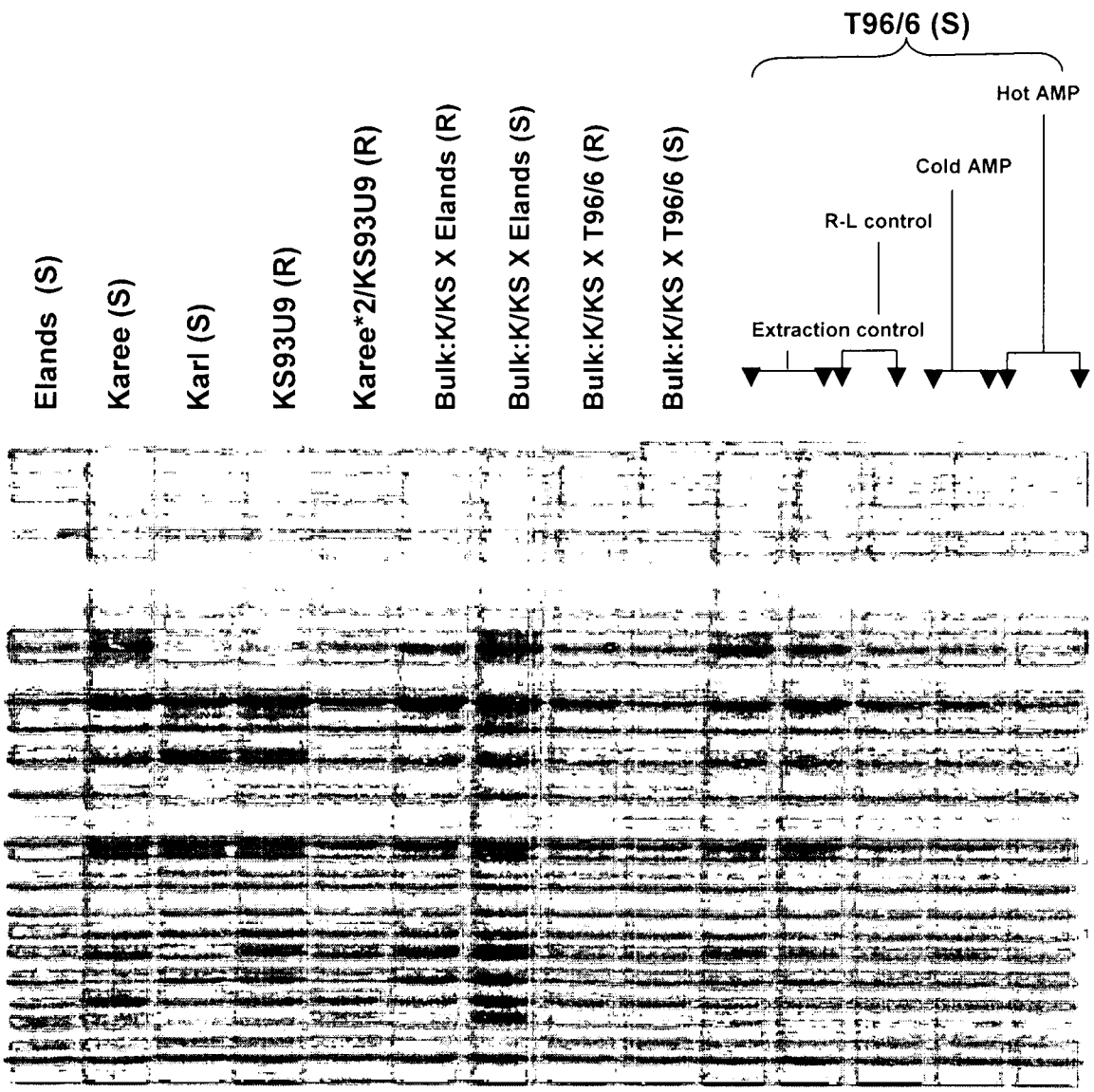


Figure 3.2. Electrophoresis pattern obtained with S26 (*Sse*-TT) and M42 (*Mse*-GT) primer combination. Extraction DNA, restriction and ligation DNA, Cold AMP as well as Hot AMP DNA were included as controls to demonstrate the repeatability of the AFLP protocol. (S = leaf rust susceptible, R = leaf rust resistant).

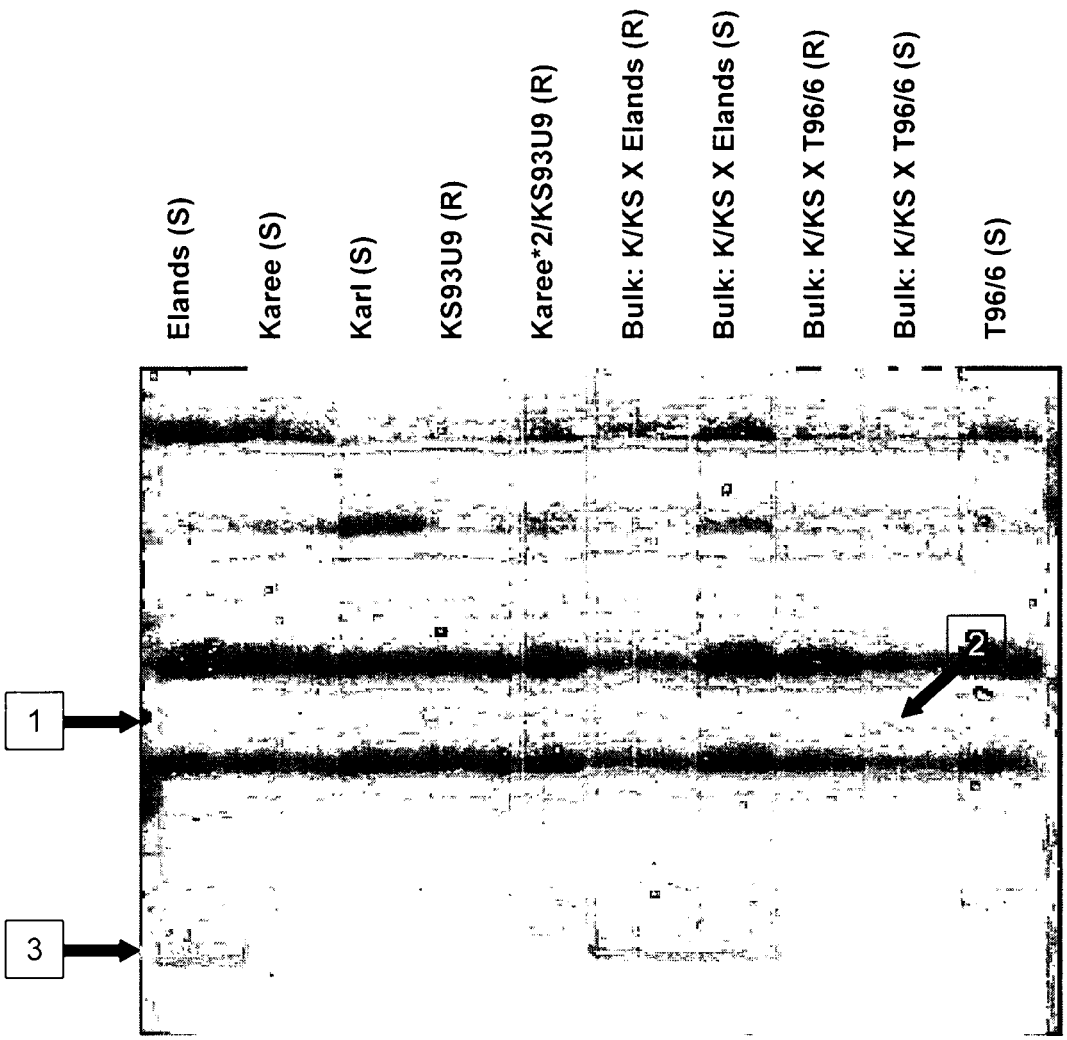


Figure 3.3. AFLP marker obtained from S12/M14 primer combination (arrow 1). A faint and smaller band is observed in the Karee*2/KS93U9 x T96/6 S bulk (arrow 2). A marker that can be used to distinguish Elands from T96/6 is shown by arrow 3. (S = leaf rust susceptible and R = leaf rust resistant)

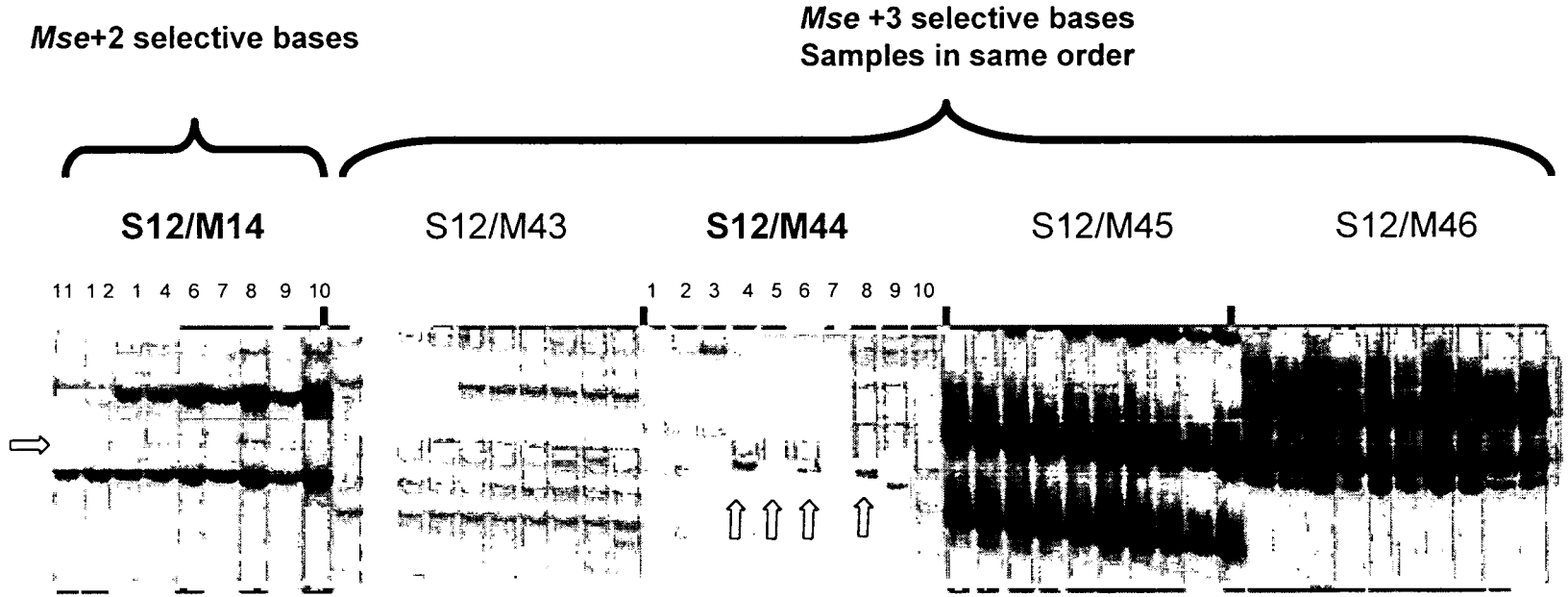


Figure 3.4. AFLP results obtained with S12/*Mse* +2 primer and S12/*Mse* +3 primer combinations. Putative molecular marker for rust resistance is indicated with arrows. (1) Elands (S), (2) Karee (S), (3) Karl (S), (4) KS93U9 (R), (5) Karee*2/KS93U9 (R), (6) Bulk Karee*2/KS93U9 x Elands (R), (7) Bulk Karee*2/KS93U9 x Elands (S), (8) Bulk Karee*2/KS93U9 x T96/6 (R), (9) Bulk Karee*2/KS93U9 x T96/6 (S), (10) T96/6 (S), (11) Kariega, (12) Avocet S.

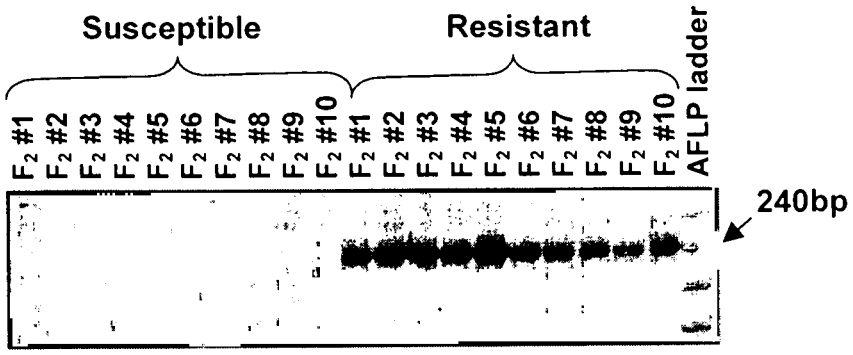


Figure 3.5. AFLP profiles of the F_2 susceptible and resistant Karee*2/KS93U9 x Elands individual plants.

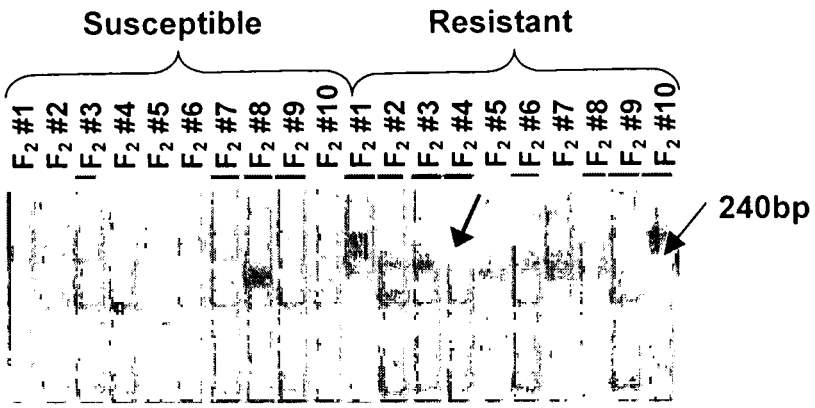


Figure 3.6. AFLP profiles of the F_2 susceptible and resistant Karee*2/KS93U9 x T96/6 individual plants. Band absent in resistant F_2 #4 plant.

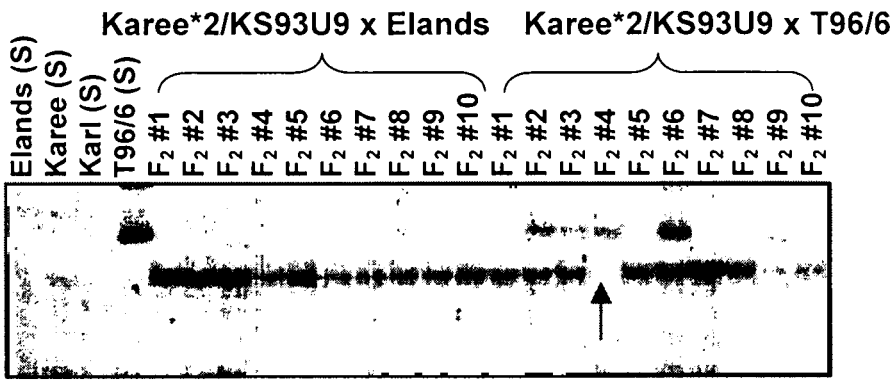


Figure 3.7. AFLP profiles of the resistant F_2 individuals of both Karee*2/KS93U9 x Elands and Karee*2/KS93U9 x T96/6. Elands, Karee, Karl and T96/6 are included as susceptible controls.

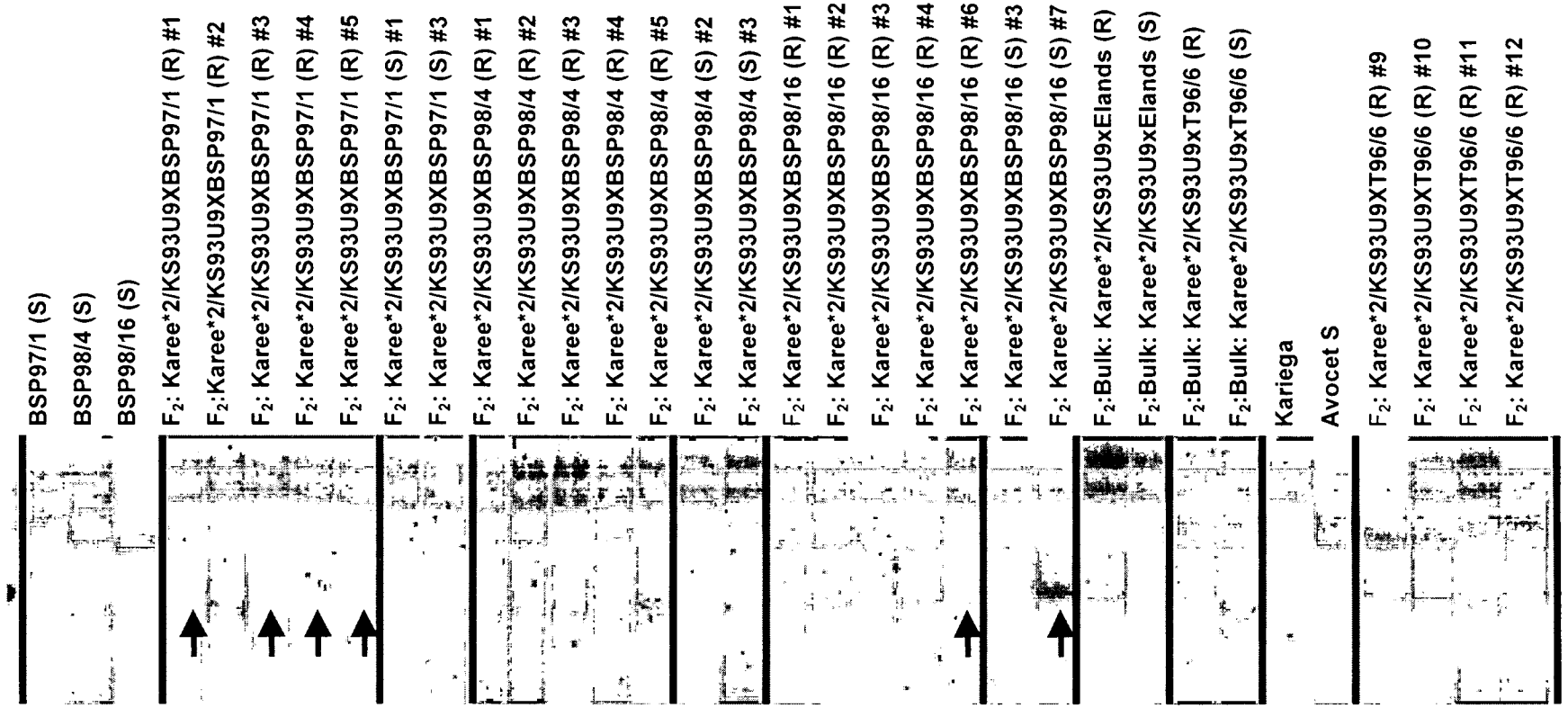


Figure 3.8. Validation of AFLP marker for an undesignated leaf rust resistance gene in the BSP97/1, BSP98/4 and BSP98/16 backgrounds. Recombination is evident (arrows) as bands are absent in resistant Karee*2/KS93U9xBSP97/1 #1, #3, #4, and #5 as well as Karee*2/KS93U9xBSP98/16 #6 lanes, whilst the band is present in the susceptible Karee*2/KS93U9xBSP98/16 #7 lane.

3.5. References

Bai, G., Kolb, F.L., Shaner, G. and Domier, L.L., 1999. Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology* 89: 343-348.

Botha, A-M. and Venter, E., 2000. Molecular marker technology linked to pest and pathogen resistance in wheat breeding. *S. A. J. Plant Soil* 96: 233-240.

Cox, T.S., Kiang, Y.T., Gorman, M.B. and Rodgers, D.M., 1985. Relationship between coefficient of parentage and genetic similarity indices in soybean. *Crop Sci.* 25: 529-532.

Donini, P., Elias, M.L., Bougourd, S.M. and Koebner, R.M.D., 1997. AFLP fingerprinting reveals pattern differences between template DNA extracted from different plant organs. *Genome* 40: 521-526.

Feuillet, C., Messmer, M., Schachermayr, G. and Keller, B., 1995. Genetic and physical characterization of the *Lr1* leaf rust resistance locus in wheat (*Triticum aestivum* L.). *Mol. Gen. Genet.* 248: 553-562.

Hartl, L., Mohler, V., Zeller, F.J., Hsam, S.L.K. and Schweizer, G., 1999. Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat (*Triticum aestivum* L.). *Genome* 42: 322-329.

Hussien, T., Bowden, R.L., Gill, B.S. and Cox, T.S., 1998. Chromosomal locations in common wheat of three new leaf rust resistance genes from *Triticum monococcum*. *Euphytica* 101: 127-131.

Jacobs, A.S., Pretorius, Z.A., Kloppers, F.J. and Cox, T.S., 1996. Mechanisms assosiated with wheat leaf rust resistance derived from *Triticum monococcum*. *Phytopathology* 86: 588-595.

Karp, A., Seberg, O. and Buiatti, M., 1996. Molecular techniques in the assessment of botanical diversity. *Ann. Bot.* 78: 143-149.

Lottering, J-M., Botha, A-M. and Kloppers, F.J., 2002. AFLP and RAPD markers linked to leaf rust resistance gene *Lr41* in wheat. *S. A. J. Plant Soil* 19: 17-22.

Ma, Z-Q. and Lapitan, N.L.V., 1998. Comparison of amplified and restriction fragment length polymorphism in wheat. *Cereal Res. Commun.* 26: 7-13.

Michelmore, R.W., Paran, I. and Kesseli, R.V., 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. *Proc. Natl. Acad. Sci. USA* 88: 9828-9832.

Penner, G.A., Zirino, M., Kruger, S. and Townley-Smith, F., 1998. Accelerated recurrent parent selection in wheat with microsatellite markers. Pages 131-134 in: Proceedings of the 9th International Wheat Genetics Symposium. Saskatoon, Saskatchewan, Canada, 2-7 August 1998. Ed. A.E. Slinkard. University Extension Press, Canada.

Prins, R., Groenewald, J.Z., Marais, G.F., Snape, J.W. and Koebner, R.M.D., 2001. AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. *Theor. App. Genet.* 103: 618-624.

Roelfs, A.P., Singh, R.P. and Saari, E.E., 1992. Rust diseases of wheat: concepts and methods of disease management. International Maize and wheat improvement centre (CIMMYT), Mexico, D.F.

Schachermayr, G., Fueillet, C. and Keller, B., 1997. Molecular markers for the detection of the wheat leaf rust resistance gene *Lr10* in diverse genetic backgrounds. *Mol. Breed.* 3: 65-74.

Shan, X., Blake, T.K. and Talbert, L.E., 1999. Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. *Theor. Appl. Genet.* 98: 1072-1078.

Thomas, C.M., Vos, P., Zabeau, M., Jones, D.A., Norcott, K.A., Chadwick, B.P. and Jones, J.D.G., 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato Cf-9 gene for resistance to *Cladosporium fulvum*. *Plant J.* 8: 785-794.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. *Nucl. Acids Res.* 23: 4407-4414.

William, H.M., Hoisington, D., Singh, R.P. and González-de-León, D., 1997. Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. *Genome* 40: 253-260.

Summary

The objective of this study was not only to improve leaf rust (caused by *Puccinia triticina*) resistance in selected wheats (*Triticum aestivum* L.), but to focus on durability as well as agronomic acceptability of resistant lines. This was achieved by traditional breeding techniques as well as with the use of AFLP analysis.

Seven bread wheat lines were obtained from ARC-Small Grain Institute (SGI) where they were developed. Six leaf rust resistance sources were obtained from the University of the Free State. The breeding strategy focused on creating lines that contained both seedling and adult plant resistance genes. *Lr34* was chosen as the adult plant resistance source as it is an important gene due to its durability and interaction with other leaf rust resistance genes. It is also an easy gene to follow as it is associated with leaf tip necrosis. In an attempt to create genotypes that will remain durable, four seedling leaf rust resistance genes (*Lr21, Lr32, Lr36 and Lr41)* were each combined with *Lr34* in the seven SGI backgrounds. By combining seedling and adult plant genes, and selecting only the lowest infection types throughout all phases of testing, a significant shift in the leaf rust resistance of the population occurred.

Greenhouse evaluations of plant architecture and agronomic performance of lines containing both a seedling gene and *Lr34,* indicated that individual plants, similar or better than the original SGI parent, had been selected. Several of these selections can therefore be incorporated in larger, mainstream breeding programmes. It is, however, imperative that the agronomic and quality value of selections be determined under field conditions.

More efficient manipulation of an undesignated *Lr* gene was also attempted. This gene, which originates from *T. monococcum*, was incorporated in certain bread wheat lines and cultivars. Since the gene is characterized by an

immune response to South African pathotypes of leaf rust, it is considered valuable in terms of rust resistance. However, its single gene nature implies it will not remain durable and that it has to be protected in complex resistance gene combinations. To achieve this, linked molecular markers are needed. In this study a putative AFLP marker for leaf rust resistance was generated by S12/M14 and S12/M44 using pooled DNA. Analysis of individual plants from which the DNA bulks were constructed indicated that the marker might not be closely linked to the *Lr* gene of interest. Validation in three additional backgrounds revealed several instances of recombination between the marker and the gene, emphasising the need to do a proper linkage study. Should the linkage distance be acceptable, the marker might still be useful as it proved to be polymorphic in five different wheat backgrounds. The linkage distance is also needed before the intensive effort to clone and convert the AFLP band to a more user-friendly STS marker will be considered.

Key words: Leaf rust resistance, gene pyramiding, wild relatives, seedling resistance genes, adult plant resistance genes, *Lr34*, durability, molecular marker technology, AFLP

Opsomming

Die studie het nie net gefokus op die verbetering van blaarroesweerstand van geselekteerde broodkoringlyne nie, maar op die skepping van volhoubare sowel as agronomies aanvaarbare weerstandstandslyne. Dit is moontlik gemaak deur van tradisionele veredelingstegnieke asook AFLP analises gebruik te maak.

Sewe broodkoringlyne is verkry vanaf die LNR-Kleingraaninstituut, waar hulle ontwikkel is. Ses blaarroesweerstandslyne (*Triticum aestivum*) is van die Universiteit van die Vrystaat verkry. Die veredelingstrategie het gefokus op die inkorporering van beide saailing- en volwasseplantweerstandsgene binne dieselfde lyn. *Lr34* is gekies as die geenbron van volwasseplantweerstand aangesien dit, vanweë sy volhoubaarheid en sy interaksie met ander weerstandsgene, 'n baie waardevolle geen is. Verder is *Lr34* ook 'n maklike geen om te volg aangesien dit met blaarpuntnekrose geassosiëer word. In 'n poging om lyne te skep wat volhoubare weerstand teen blaarroes sal hê, is vier saailingweerstandsgene (*Lr21, Lr32, Lr34* en *Lr41*) elk afsonderlik met die volwasseplantweerstandsgeen *Lr34* gekombineer binne die sewe KGI agtergronde. Deur die kombinering van saailing- en volwasseplantweerstandsgene, asook deur deurlopende selektering vir die laagste infeksietipe gedurende alle evalueringsfases, het daar 'n betekenisvolle verskuiwing in die blaarroesweerstand binne die populasie plaasgevind.

Glashuisevaluasies van agronomiese prestasie sowel as planttipe van die onderskeie lyne wat beide 'n saaling- en die *Lr34* geen bevat, het aangedui dat individuele plante wat dieselfde, of beter, gevaar het as die oorspronklike KGI ouerlyn, geselekteer is. Verskeie van hierdie seleksies kan dus in die hoofstroom-veredelingsprogramme geïnkorporeer word. Dit is egter noodsaaklik dat beide die agronomiese en kwaliteitwaarde van die seleksies onder veldtoestande geëvalueer word.

'n Poging is ook aangewend om 'n onbekende *Lr* geen te manipuleer. Hierdie geen wat afkomstig is vanaf *T. monococcum*, is geïnkorporeer in sekere broodkoringlyne. Die geen word as waardevol beskou in terme van roesweerstand, aangesien dit gekarakteriseer word deur 'n immuunreaksie teenoor Suid-Afrikaanse

blaarroespatotipes. Weens sy enkelgeenstatus sal die geen egter gou sy volhoubaarheid prysgee. Dit is dus noodsaaklik dat hierdie geen beskerm word binne komplekse weerstandsgeenkombinasies. Om dit te kan bereik, is molekulêre merkers noodsaaklik. In hierdie studie is 'n voorlopige AFLP merker vir die blaarroesweerstandgeen gegenereer deur van gebulkte DNA monsters gebruik te maak. Die AFLP-merker is deur die S12/M14 en S12/M44 priemstukkombinasies gegenereer. Bevestigingsstudies in drie verskillende agtergronde as dié waaruit die merker ontwikkel is, het aangedui dat verskeie gevalle van rekombinasie tussen die merker en die geen voorgekom het, wat die noodsaaklikheid van 'n koppelingstudie benadruk. Indien dit sou blyk dat die koppelingsafstand aanvaarbaar is, kan die merker bruikbaar wees, aangesien dit in vyf verskillende koringagtergronde polimorfies was. Die koppelingsafstand word ook benodig voordat 'n intensiewe poging aangewend kan word om die merker te kloon. Dit sal die omskakeling van die AFLP-band na 'n meer gebruikersvriendelike STS merker moontlik maak.

Sleutelwoorde: Blaarroesweerstand, geenstapeling, wilde verwantes, saailingweerstandgene, volwasseplantweerstandgene, *Lr34*, volhoubaarheid, molekulêre merkertegnologie, AFLP

U.O.V.S. BIBLIOTEEK