Molecular and biochemical characterisation of rust and Fusarium head blight resistant wheat lines

by

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Declaration

“I, Ansori du Plessis, do hereby declare that the dissertation hereby submitted by me for the degree Magister Scientiae Agriculturae in Plant Breeding at the University of the Free State represents my own original, independent work and that I have not previously submitted the same work for a qualification at another university.

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Dedication

This masters dissertation is dedicated to my parents,

Ryno and Annelie du Plessis,

to whom I will always be grateful for this life opportunity.

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List of abbreviations

α	Alpha
ABC	ATP-binding cassette
ACN	Acetonitrile
AFLP	Amplified fragment length polymorphism
AP-PCR	Arbitrarily primed polymerase chain reaction
APR	Adult-plant resistance
APS	Ammonium persulfate
ARC-SGI	Agricultural Research Council-Small Grain Institute
β	Beta
BC_1	Backcross one
BC_2	Backcross two
bp	Base pair(s)
BSA	Bovine serum albumin
CAPS	Cleaved amplified polymorphism sequences
cM	CentiMorgan
CTAB	Hexadecyltrimethylammonium bromide
DAF	DNA amplification fingerprinting
DArT	Diversity arrays technology
DH	Double haploid
dH_2O	Deionised water
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

dNTPs	2’-deoxynucleotide 5’-triphosphate
DON	Deoxynivalenol
DTT	Dithiothreitol
EDTA	Ethylene-diaminetetraacetate
EST’s	Expressed sequence tags
EtBr	Ethidium Bromide
F_1	First generation
FHB	Fusarium head blight
f. sp.	formae specialis
gDNA	Genomic deoxyribonucleic acid
HMW-GS	High molecular weight glutenin subunits
Indels	Insertions and deletions
ISSR	Inter-simple sequence repeats
kDa	Kilodalton
LMP	Large monomeric proteins
LMW-GS	Low molecular weight glutenin subunits
LPP	Large polymeric proteins
Lr	Leaf rust
Ltn	Leaf tip necrosis
LUMP	Large unextractable monomeric proteins
LUPP	Large unextractable polymeric proteins
MAR	Matrix-attachment region
MAS	Marker assisted selection
Mb	Million base pairs

$MgCl_2$	Magnesium chloride
mRNA	Messenger ribonucleic acid
N	Nitrogen
NaCl	Sodium chloride
NIV	Nivalenol
PBC	Pseudo black chaff
PCR	Polymerase chain reaction
Pgt	*Puccinia graminis* f.sp. *tritici*
QTL	Qualitative trait loci
RAPD	Random amplified polymorphic DNA
®	Registered
RFLP	Restriction fragment length polymorphism
RP-HPLC	Reverse phase-high pressure liquid chromatography
rpm	revolutions per minute
RSA	Republic of South Africa
SA	South Africa
SCAR	Sequence characterised amplified region
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SE-HPLC	Size exclusion-high pressure liquid chromatography
SMP	Small monomeric proteins
SNP	Single nucleotide polymorphism
SPP	Small polymeric proteins
Sr	Stem rust

SSR	Simple sequence repeats
STM	Sequenced tagged microsatellite
STR	Short tandem repeat
STS	Sequence-tagged-sites
SUMP	Small unextractable monomeric proteins
SUPP	Small unextractable polymeric proteins
TBE	Tris-HCl/borate/EDTA
TE	Tris-HCl/EDTA
TEMED	Tetramethylethylenediamine
TFA	Trifluoroacetic acid
™	Trade mark
Tris	Tris (hydroxymethyl) aminomethane
Tris-HCl	Tris (hydroxymethyl) aminomethane-hydrochloride
UFS	University of the Free State
UPP	Unextractable polymeric proteins
USA	United States of America
UV	Ultraviolet
ω	Omega
γ	Gamma
Yr	Yellow rust

List of SI Units

%	Percentage
°C	Degrees Celsius
cm	Centimetre(s)
g	Gram(s)
h	Hour(s)
ha	Hectares
M	Molar(s)
mg	Milligram
min	Minute(s)
ml	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar(s)
ng	Nanogram(s)
nm	Nanometre(s)
pH	Power of hydrogen
r/s	Revolutions per second
s	Second(s)
U	Unit(s)
µg	Microgram(s)
µl	Microlitre(s)
µm	Micrometre(s)
µM	Micromolar(s)
V	Volt(s)
v/v	Volume per volume
w/v	Weight per volume

Chapter 1

General introduction

Maize, wheat and rice are the three main cereal grains produced worldwide. Almost half of the world's grain is produced by The Peoples Republic of China, United States of America (USA) and India (FAOSTAT 2013). Wheat (*Triticum aestivum* L.) plays an important role in the diet of humans and animals because it contains starch, proteins and lipids (Shewry and Halford 2002).

Wheat is vulnerable to both biotic and abiotic stresses at different drought, temperature and growth stages (Mackill et al. 1999; Bray et al. 2000; Seki et al. 2003). These stresses include pathogens which cause diseases and affect plant performance. Disease infections can lead to reduction in kernel yield and quality. These pathogenic organisms have the ability to evolve and overcome resistance. To counter act the problem of ever-evolving pathogens scientists continuously search for new resistance sources to maintain levels of resistance in their germplasm (Bariana et al. 2007). Breeding for disease resistance in wheat is an important factor to maintain wheat production, especially in the light of the increasing world population and higher demands placed on available food sources.

Wheat rust and Fusarium head blight (FHB) are two diseases that are well known in South Africa as well as worldwide. Three types of wheat rust occur, namely leaf (brown), stem (black) and stripe (yellow) rust (Singh et al. 2005). These fungal diseases are caused by different species of *Puccinia*. FHB on the other hand is mainly caused by *Fusarium graminearum* Schwabe in South Africa. Resistance to FHB is regulated by several major and minor quantitative trait loci (QTL) and is influenced by environmental conditions (Buerstmayr et al. 2009). FHB not only result in yield losses but infected seed may contain mycotoxins that are harmful to consumers and livestock (Trail 2009).

Wheat quality is a key factor for milling and baking companies because it has a great influence on baking characteristics such as dough strength and elasticity. Baking quality increases with increased protein levels (Randall et al. 1990). Wheat proteins can be grouped into four groups: albumins, globulins, prolamins and glutelins according to their solubility properties (Osborne 1924). Prolamins consist of glutenins and gliadins which are the main storage proteins (80%) of wheat (Shewry et al. 1994).

Gliadin and glutenins have great influence on the baking quality and they are synthesised in the endosperm during grain development. Higher weight glutenin polymers are associated with increased kernel hardness, gluten strength and loaf volume (Gupta et al. 1991). Protein content of wheat kernels is greatly influenced by two external factors namely: nitrogen (N) fertilisation (Anderson et al. 1998) and temperature (Smith and Gooding 1999; Labuschagne et al. 2009).

To combine disease resistance and dough quality in wheat, conventional breeding could be enhanced using marker-assisted selection (MAS) and biochemical tests. MAS accelerates breeding programmes, if markers for the specific trait are available (Yadav et al. 2010). Markers can be used to evaluate specific traits at seedling stage by eliminating undesirable offspring. MAS is a useful tool when different traits are combined into a single genotype. For example, when different disease resistance genes are combined, phenotypic screening cannot be used to screen for all the diseases simultaneously. However, MAS enables researchers to simultaneously screen for all these traits in the laboratory, MAS is also effective when both minor and major QTL are combined (Xu and Crouch 2008). Markers linked to protein quality traits in wheat have also been developed that can act as a useful supplement to biochemical tests used to determine bread-making qualities.

Breeding improved wheat lines and focusing on two or more characteristics such as disease resistance, protein quality and yield, one must prioritise characteristics within the breeding programme. When a breeding programme is conducted to produce new wheat lines containing two or more improved traits, some of these traits may be less than optimal for its criteria since it is difficult to produce individual lines that contain all favourable traits under optimal conditions (Johnson 1992).

Gene pyramiding of desirable traits in crop species has become an important tool for releasing cultivars with durable resistance to biotic and abiotic stresses (Joshi and Nayak 2010). Some disease resistant genes are only effective against some species of a disease or some isolates and combining genes can improve the effectiveness of the plant’s resistance. When molecular markers are available, gene pyramiding can be done, fast and effectively. A gene pyramiding scheme can be divided into two steps. The first step is the pedigree step, where a root genotype is created that contains all the targeted genes. The second step is called the fixation step, where the targeted genes are fixed into a homozygous state (Joshi and Nayak 2010).

Bread-making ability of wheat is mainly due to the storage proteins found in the endosperm and therefore studies should be undertaken to determine the influence of the different units and subunits of these proteins (Shewry and Halford 2002). Some biochemical methods have been developed to determine the composition of these proteins [sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and reverse phase-high pressure liquid chromatography (RP-HPLC)] and quantify different types of proteins [size exclusion-HPLC (SE-HPLC) and RP-HPLC)].

The main aim of this study was to identify experimental wheat lines with a high number of rust or FHB resistance genes/QTL that also showed good bread-making quality characteristics. This was reached through several objectives. The first objective of the study was to evaluate wheat experimental lines, developed from previous studies, to determine the absence or presence of the selected rust and FHB resistance genes/QTL. The second objective was is to use SDS-PAGE, SE-HPLC and RP-HPLC biochemical tests to determine certain bread-making quality characteristics and to determine the effectiveness of each method used. The third objective was to screen molecular markers linked to protein quality traits to determine the presence or absence of certain high molecular weight-glutenin subunits (HMW-GS) and the 1BL.1RS translocation in the experimental wheat lines.

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Chapter 2

Breeding improved wheat cultivars with both disease resistance and good bread-making qualities

2.1 Wheat history

Wheat is part of the diverse Poaceae (grasses) family (Ijaz and Khan 2009) and various cultivated wheat species are available today. *Triticum boeoticum* Boiss (A^bA^b) and *T. urartu* Johnson (A^uA^u) are two primitive diploid *Triticum* species. These two diploid species can be morphological distinguished from each other based on anther lengths, *T. urartu* has an extra lemma awn and their caryopsis colour differs, but genetically they are very similar. Einkorn (*T. monococcum* Linnean) wheat is a diploid and originated from *T. boeoticum.* Domesticated Einkorn wheat was one of the first crops to be cultivated in the Fertile Crescent (Johnson and Dhalinal 1976). Einkorn wheat production is very little today and serves as feed. The tetraploid and hexaploid wheat species replaced einkorn wheat (Perrino et al. 1996).

Triticum dicoccoides (Körn. ex Aschers. and Graebn.) Schweinf. (BBA^uA^u) and *T. araraticum* (Jakubz.; GGA^uA^u) are tetraploid species. The primary wild type *T. dicoccoides* is the ancestral species of other tetraploid species which are cultivated today. The wild type, *T. araraticum,* is the ancestor for *T. timopheevi* that is domesticated glume wheat (Poyarkova 1988).The diploid *T. urartu* donated it's A genome to the tetraploid species (Dvorak et al. 1993).

Wheat can be classified into three main groups according to their chromosome numbers. The three groups consist of the diploids (einkorn), tetraploids (durum wheat) and the hexaploids (bread wheat). *Triticum aestivum* L. is the modern allohexaploid bread wheat (AABBDD, $2n = 6x = 42$) and is a hybrid of the tetraploid *T. turgidum* L. var. *durum* (AABB, $2n = 4x = 28$) and the diploid grass species, *T. tauschii* (Cross.) Schmalh. (DD, $2n = 2x = 14$; Gupta et al. 2002; Singh and Rajaram 2002).

2.2 Economic importance of wheat

In the year 2012, 661 million ton of wheat was produced worldwide, which was 5.5% lower than 2011 due to drought in eastern Europe and central Asia. Utilisation of wheat in 2012 was 687 million ton of which feed utilisation were 136 million ton. The exceeding 26 million ton wheat utilised were obtained from the world wheat stock and lead to an 11.9% decrease to the world wheat stock (FAOSTAT, 2013). Figure 2.1 indicates a sharp increase of the total wheat production internationally till 1990. The production of wheat increased slower from 1991 to 2011.

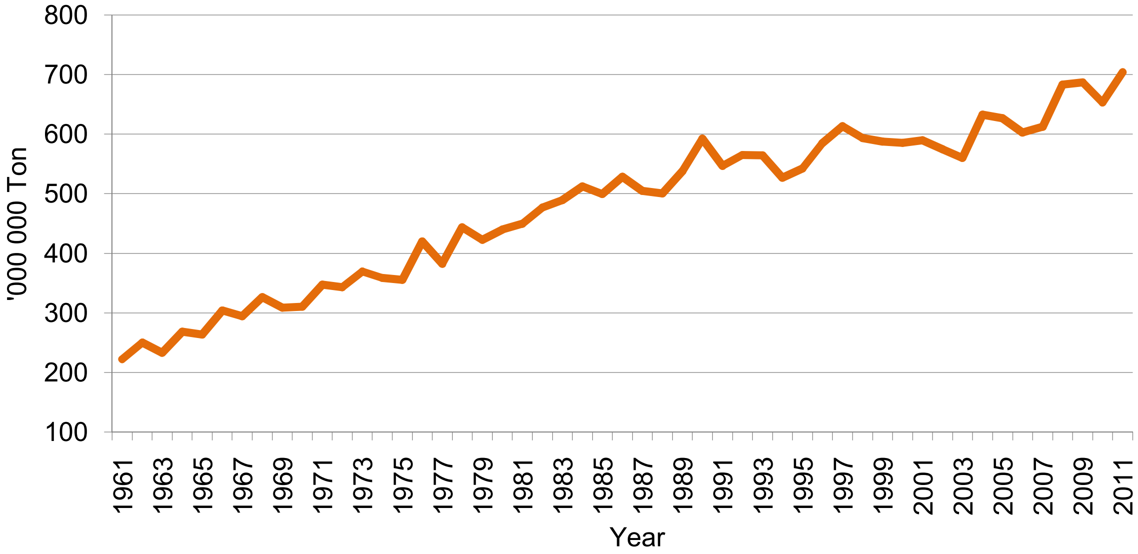

Figure 2.1 Total worldwide yield production of wheat from the year 1963 to 2011 (Grain SA, 2013).

Figure 2.2 indicates wheat production in South Africa from 1990 to 2012 (Grain SA, 2013). The production differed every year, but the linear regression line indicates a steady but small reduction in the production of wheat in South Africa.

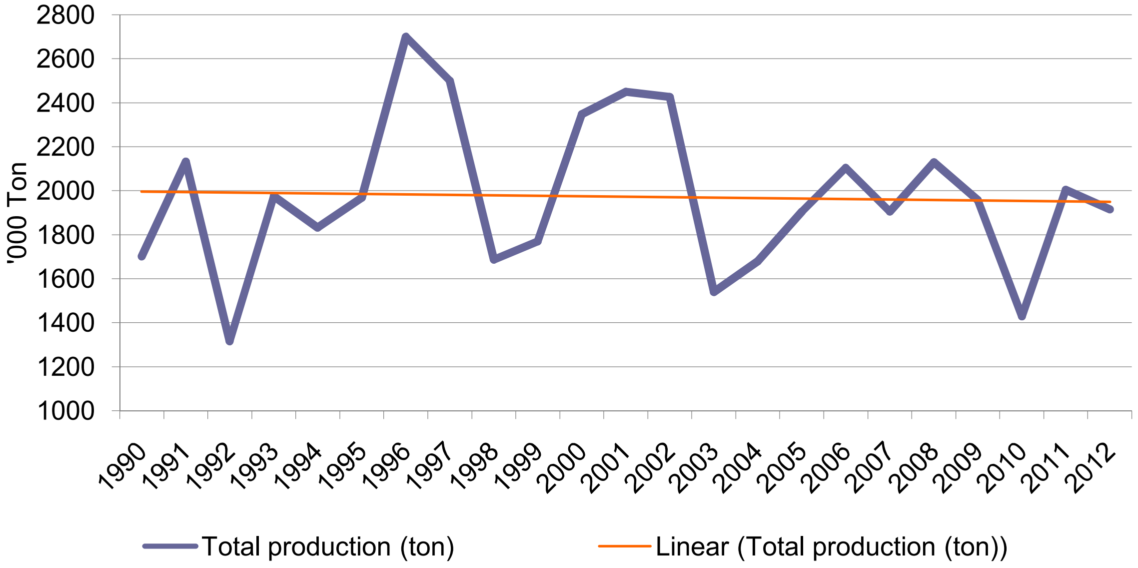

Figure 2.2 Wheat production in South Africa from 1990 till 2012 (Grain SA, 2013).

Figure 2.3 summarises wheat production of every individual province in South Africa for the year 2012 (Grain SA, 2013). All nine provinces of South Africa produce wheat, but the Western Cape, Free State and the Northern Cape are the three main wheat production provinces in South Africa.

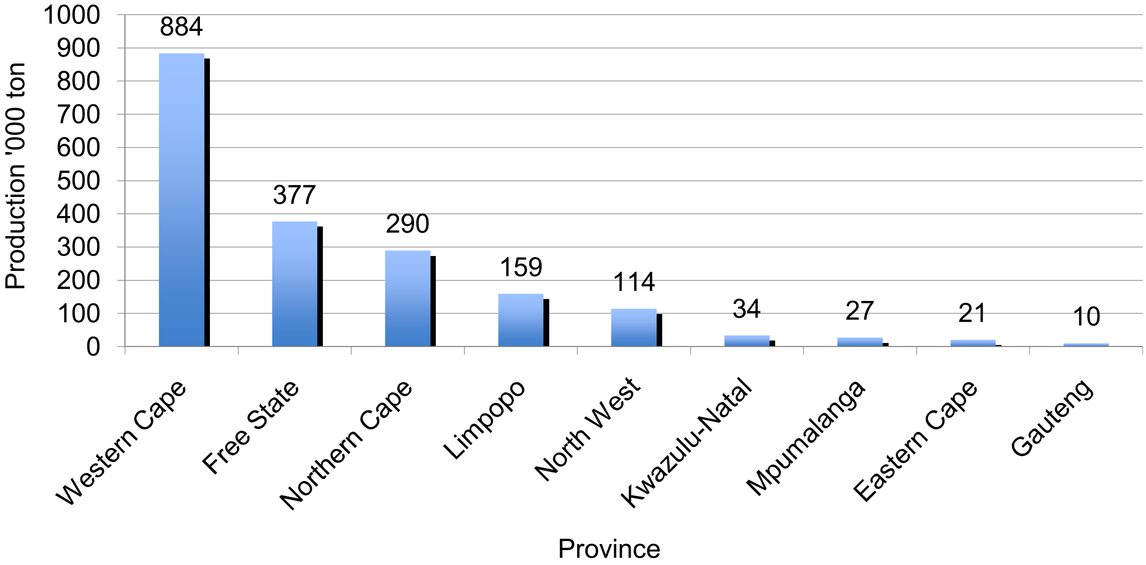

Figure 2.3 Summary of wheat production in 2012 for the nine provinces of South Africa (Grain SA, 2013).

2.3 Wheat quality characteristics

Wheat, an economical important crop, is a basic ingredient for many food types. A need exists for wheat improvement due to the fact that the world population increases daily. Therefore, wheat quality is one of the most important characteristics to select for. Different factors, such as milling and dough properties, have an influence on wheat quality and these factors can be influenced by environmental factors such as soil type and climate (Pasha et al. 2010). Wheat quality characteristics can be divided into two main groups, physical and chemical characteristics. Physical characteristics include kernel colour, shape, weight and hardness (Gaines et al. 1996). Chemical characteristics include protein content, sodium dodecyl sulphate (SDS)-sedimentation and gluten strength (Pasha et al. 2010).

Kernel hardness can be divided into soft and hard and is measured by the particle size index. This system is used worldwide for wheat trading and millers and bakers also use this system to determine for which usage the wheat will be suitable (Morris 2002). Soft wheat flour is used for baking cookies as it has more intact starch granules because it is not necessary to grind it as hard as for hard wheat kernels. Hard wheat flour has a coarser texture and the hard milling of these kernels is responsible for low numbers of intact starch granules. Broken starch granules absorb more water and make it ideal for bread baking (Morris and Rose 1996). Durum wheat is used dominantly for making different types of pastas.

2.4 Wheat resistance breeding

Wheat is an important component of the human diet because it mainly contains protein and starch. Wheat can be grown in different environments, can tolerate cold and therefore it is one of the best adapted crops (Singh and Rajaram 2002). As plant breeders want to improve wheat yield and protein quality they also have the responsibility to incorporate resistance to pests and diseases into wheat.

The evolution rate for some pathogens such as powdery mildew and rust is faster in comparison to other diseases like, smuts and bunts. Facultative parasites evolve even slower than smuts and bunts. Therefore diseases with a higher evolution rates are top priority for breeding resistant cultivars. In order to control continuously evolving pathogens scientists have to keep searching for new resistant gene sources within the wheat germplasm itself and incorporate these sources using conventional breeding, genetic engineering and MAS. Genetic engineering incorporates alien resistant gene segments of other related species, such as rye (*Secale cereale* L.), goatgrasses (*Aegilops speltoides* Tausch), and wild grass (*Haynaldia villosarum ponticum* L.) into the wheat genome (Friebe et al. 1996; Gill et al. 2011; Niu et al. 2011).

Two different types of resistance have been identified and described in plant breeding. The first type is partial resistance (race-specific) and occurs naturally. Complete resistance is race specific and depends on a single gene but due to newly evolving pathogens they can overcome natural resistance. Complete resistance genes have a major impact on disease severity. The second type of resistance is durable resistance (race-nonspecific) and depends on two or more genes and is most of the time durable (Poland et al. 2009). Durable resistance have to be effective for long periods of time and in various environments. Durable resistance is more likely to be effective during adult plant stage than during seedling stage (Johnson 1984).

Adult plant resistance (APR) and seedling resistance are two different resistance types. These two types can show a slow rusting effect, making it more durable. Slow rusting reduces the speed of development of the disease and causes a longer latent period, lower infection frequency, smaller uredial size and reduce the time of sporulation which then directly reduce the number of spores produced (Shaner 1983). The first resistant gene manipulation occurred in 1905 when Biffen found that stripe rust resistance was controlled and inherited by a single recessive gene (Biffen 1905). This was the beginning for breeding resistant cultivars and has become constructive for controlling plant diseases and directly resulted in higher yield.

2.5 Wheat diseases

Wheat diseases are caused by fungal pathogens, bacteria, viruses and insects. Important fungal diseases caused by obligate parasites include the bunts, smuts, powdery mildew and rusts. The facultative parasites are responsible for FHB and the blotches. All these fungal pathogens have various virulence genes which can overcome specific resistance genes of the host organism (Grennan 2006).

2.5.1 Powdery mildew

The fungus *Erysiphe graminis* formae f. sp. *tritici* Marchalis responsible for powdery mildew disease in wheat. Race specific resistance is controlled by the *Pm* genes. Thirty *Pm* genes have been identified which provide resistance to powdery mildew infections (Yahiaoui et al. 2003). The first resistant locus indentified and mapped was the *Pm3* locus which is situated on the short arm of chromosome 1A (Briggle and Sears 1966). Powdery mildew can be identified by small white colonies of cottony mycelia which are the body of the fungus. These colonies can be found on the upper and lower surfaces of the leaves. Aging and sporulation of these colonies change the colour to yellowish gray. During the summer seasons this fungus survives in infested wheat debris. Favourable conditions for powdery mildew are temperatures between 15°C to 22°C and cloudy and humid conditions. An increase of nitrogen (N) fertilisation and frequent irrigation will improve the growth conditions of the fungus.

2.5.2 Karnal bunt

Karnal bunt is a wheat disease caused by a smut fungus, *Tilletia indica* Mitra, which was discovered in 1930 in north-west India (Mitra 1931). Today, this disease is commonly found in the Punjab region in India but is also found in other parts of the world such as Pakistan, Mexico and Nepal. Karnal bunt has a negative effect on yield and flour quality as it gives flour a fishy odour and taste (Aujla et al. 1980; Bansal et al. 1984). This fungus is a basidiomycetous pathogen which look like black cottony teliospores found on the infected seeds of wheat. These teliospores can live for two to five years in the ground and take up to nine months to germinate if the environmental conditions are favourable. Environmental conditions include temperatures of 15°C to 25°C, moisture soil and high humidity for growth. The germinated teliospores release primary sporidia (basidiospores) which then germinate and form mycelia which produce secondary sporidia. The primary and secondary sporidia move to the glumes during the flowering stages of wheat, where they start to grow and produce teliospores in the pericarp (Bonde et al. 1997).

2.5.3 Loose smut

Loose smut is caused by the biotrophic seed borne pathogen, *Ustilago tritici* (Pres.) Rostrup. Growing conditions for this pathogen are high humidity, free moisture for long periods and the wheat plant must have reached the floret opening stage (Loria et al. 1982). The mycelium penetrates through the ovary wall (pericarp) to enter the ovary during flowering stage. After 11 days of penetration it enters the testa and nucellus regions and grows to the scutellum after 26 days. From the scutellum the mycelium grows to the embryo, the growing partof the grain. There are no visible symptoms of infection during vegetative states but brown black spikes (teliospores) appear later in the grain heads. The result is that every infected seed will lead to an infected offspring plant (Batts 1995).Loose smut is present in all wheat producing areas but more humid climates are favourable. This disease is treated by planting resistant wheat cultivars or by treating infected seeds with fungicide (Mau et al. 2004).

2.5.4 Flag smut

Another disease that infects wheat is called flag smut and is caused by *Urocystis agropyri* (Preuss) Schroet. Both susceptible and resistant plants get infected but only susceptible plants show symptoms. Symptoms include twisted coleoptiles of seedlings with bleached spots on the coleoptiles. During the heading developmental stage, long black stripes develop between the veins of blades, which later release teliospores (Griffiths 1924). Optimal growth temperatures for this fungus are between 18°C to 24°C and relatively dry soil (El-Helaly 1948). Teliospores in the ground serve as inoculum for infection of the host plant. Seed treatment with a fungicide or planting of resistant cultivars will prevent yield losses (Mitra 1935; Duveiller et al. 2007).

2.5.5 Black chaff

Black chaff, also known as bacterial leaf streak or bacterial leaf stripe, is caused by the bacterial seed borne pathogen, *Xanthomonas campestris* pv. *undulosa* Smith (Smith et al. 1919).This disease is called black chaff due to the infected black glumes. Moisture and temperatures between 15°C to 30°C is necessary for releasing the pathogen from the seed and lead to leaf colonisation. Bacteria enter the leaves through the stomata and grow in the parenchyma. Multiplication of bacteria in the parenchyma gives rise to elongated light brown lesions which later grow together to form infected areas. Sticky milky or yellow exudates form from these lesions which spread through the field by rain and wind, and lead to infection of culms, leaves, rachis, glumes and awns. The optimal growth temperatures are 26°C and higher. Hail and other injuries to the plant can increase penetration of the bacteria (Duveiller et al. 1997).

2.5.6 Glume blotch

Glume blotch is also known as stagonospora nodorum blotch or septoria nodorum blotch. Glume blotch is caused by the fungus *Stagonospora nodorum* (Berk.) and can infect wheat as well as barley (*Hordeum vulgare* L.; Osbourn et al. 1986). Spores penetrate the host plants through their cuticle and stomata and lead to the swelling of hyphal tips and leaves. The swelling results in oval shaped necrotic leaf blotches and discolouration of the hyphal tips. After a week the pycnidia form in the blotches which rapidly darken and expand and set pycidiospores free. After total chlorosis of the leaf, the fungus starts with asexual reproduction and spreads throughout the whole plant (Bird and Ride 1981).

2.5.7 Common root rot

Common root rot is caused by *Cochliobolus sativus* (Ito and Kurib.) and infects both barley and wheat plants. The pathogen infects seedlings since seeds are infected due to soil borne conidia. Dark brown lesions appear on the roots and outer tissue of the leaf base and lesions coalesce into long necrotic brown tissue. The seedling can die or it will keep growing if it is able to developed new roots but the plant will be underdeveloped. The fungus does not form definite anamorphic fruiting bodies, but brown conidia are visible on the necrotic plant tissue (Mathre et al. 2003).

2.5.8 Fusarium head blight

FHB also known as scab, is responsible for wheat losses worldwide with a great economical impact on production. During the 1990s the United States and Canada have lost over $3 billion due to FHB infections of wheat and barley (McMullen et al. 1997). Outbreaks also occurred in Asia, Europe and South America (Goswami and Kistler 2004). Infected wheat plants are negatively influenced as FHB reduces yield, seed quality and the grain can be infected by mycotoxins (Jansen et al. 2005). Other susceptible grain cereals beside wheat are barley, rye, oats, rice and maize. Researchers struggle to find an effective control system for FHB outbreaks (Parry et al. 1995).

The first FHB outbreak in South Africa has been reported in 1980 in the North-West province (Scott et al. 1988). In 1983 an outbreak occurred in George in the Southern

Cape province and although it was limited to one pivot it spread through the district over time (De Jager 1987). In 1985 and 1986 the susceptible cultivar, Zaragosa, was under heavy attack in the Northern parts of KwaZulu-Natal and the eastern Free State (De Jager 1987). Boshoff (1996) reported of an outbreak in the Swellendam region in 1987. In the early 1990s epidemics have been reported in the Northern Cape (Kriel and Pretorius 2006). It is clear that effective systems to control FHB disease have to be developed and incorporated in problematic areas.

FHB is caused by different species of *Fusarium*. *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* Schwein. (Petch)] is known as the main fungus causing FHB on wheat. FHB infects the flowering parts of their host and effects kernel development, especially in regions where maize, wheat and barley crop rotation is practised (Mihuta-Grimm and Foster 1989). Maize residue can serve as a host for fungi to help it survive when wheat and barley are not available (Calpas et al. 2003).

Environmental conditions play an important role in FHB epidemic outbreaks. *Fusarium* species' optimal growth conditions are high humidity, warm temperatures (22°C to 26°C) and rainy periods. Wheat is most susceptible for FHB during the flowering stage until the end of vegetation (Teich 1989). Different species may have different optimal growth conditions. The main causal agents of FHB in South Africa are *F. graminearum* (warmer climates), *F. Crookwellense* (Burgess, Nelson and Toussoun) and *F. culmorum* [(Wm. G. Sm.) Sacc.; more temperate regions; Parry et al. 1995; Minnaar-Ontong 2011].

2.5.8.1 *Fusarium graminearum*

Fusarium graminearum is a filamentous fungus with a genome size of 36.1 million base pairs (Mb) and four chromosomes and expresses 13937 genes. *Fusarium graminearum*'s genome sequence was released in 2003 and resulted in great research activity of the fungus which is important for resistance studies (Trail 2009).

Fusarium graminearum is haploid for most of its life cycle. Conidia (asexual spores) are produced on infected plants when weather conditions are optimal. Conidia are slimy masses on the hyphal structure of the sporodochia. Conidia are associated with short distance and rain-splash dispersal (Trail 2009). Spores can be dispersed over long and short distances with the help of animals, birds and wind.

Fusarium graminearum belongs to the phylum Ascomycota and the sexual development starts with the formation of hyphea or binucleate cells which are called the dikaryotic phase. This phase forms genetically identical new cells when two genetically diverse nuclei pair. These cells, found in the perithecia, are filled with asci which contain the ascospores which are then released into the air. Ascospores (sexual spores) are the primary inoculum of FHB and are monocyclic. *Fusarium graminearum* does not need a sexually distinct partner to develop ascospores (Trail 2009).

Initiation of FHB infection in the field is caused by airborne spores which fall on flowering spikelets of the host and germination starts after 6-12 hours. Germtubes start growing and hyphae are formed which penetrate the plant through the lemma, glume and palea to form a mycelium network. The mycelium will spread to the head through vascular bundles and cortical parenchyma tissue. The spreading mycelium network will lead to clogging and results in premature bleaching of heads and shrivelled grain due to absence of water and nutrition to the plant (Trail et al. 2005).

2.5.8.2 Fusarium head blight infections

Infection starts immediately after flowering and symptoms will be visible shortly after. Infection by the fungus will lead to decreased spikelets and will be visible due to premature bleaching of spikelets. As the fungus spreads and grows through the head, more spikelets become infected, resulting in partial or complete discolouration of the head as can be seen in Figure 2.4. Therefore the entire head may become bleached over time. In moist and warm weather conditions, light pink/orange coloured spore bearing structures will developed on the rachis and glumes of individual spikelets. Black/blue round bodies may be seen on the surface of infected spikelets later in the season. These bodies are known as perithecia, which are the sexual structures of the fungus. The fungus colonises on the developing grain and therefore the seed shrink and are wrinkled during development in the head. Infected seeds can have colours ranging from pink, soft grey to light brown. (Fernado et al. 1997; Singh and Rajaram 2002). Infections by the fungus can reduce the quality and yield of wheat and can be toxic to animals and humans due to the production of mycotoxins which are secondary metabolites (Nelson et al. 1994).

Mycotoxins also have an influence on the milling, baking, malting and brewing quality characteristics of wheat. *Fusarium graminearum* infections can lead to the production of all kinds of mycotoxins with different grades of toxicity. One of these mycotoxins is Deoxynivalenol (DON) or vomitoxin which is one of a few end products of trichothecenes (Geraldo et al. 2006). Trichothecens are toxic to many plants and their presence in plants can result in wilting, chlorosis and necrosis (Goswami and Kistler 2004). Trichothecenes bind to eukaryotes’ 60S subunit and inhibit protein synthesis (Rocha et al. 2005). DON has a low grade of toxicity to animals but it may lead to feed consumption reduction or complete refusals known as feed refusal and emetic syndromes (Nelson et al. 1994; Calpas et al. 2003).

Other trichothecenes include fusarenon-X, diacetoxyscirpenol, neosolaniol and nivalenol (NIV). The presence of trichothecenes in food can have side effects on humans and animals such as inhibition of protein and starch production, skin irritation, haemorrhage, diarrhoea, nausea, food reflux and vomiting (Calpas et al. 2003). Another mycotoxin, which is responsible for Estrogenic syndrome, is zearalenone and causes reproductive disorders in animals (Nelson et al. 1994; Geraldo et al. 2006). DON accumulation (85%) is higher in South African grains in comparison to NIV accumulation (14%; Minnaar-Ontong 2011) whereas for European grains, NIV have higher accumulation levels than DON (Bottalico and Perrone 2002).

Figure 2.4 Wheat infected by *F. graminearum* (Photo W.M. Kriel).

2.5.8.3 Fusarium head blight resistance breeding

FHB is one of the most commonly known diseases of wheat and is responsible for great economic losses in the wheat industry. FHB is a complex disease and resistance to FHB is quantitatively inherited and environmental effects play a role on disease expression, complicating resistance breeding (Bai and Shaner 1994). Due to the complexity of the disease a QTL mapping approach is being followed to analyse FHB resistance. Five different resistance mechanisms have been described: Type I refers to the rate of resistance to disease incidence, Type II is resistance towards disease severity and spread of the pathogen within the head, Type III is resistance to kernel infection, Type IV is resistance towards Fusarium damaged kernels or yield tolerance and Type V is resistance to DON accumulation (Mesterházy 1995). Type II is a stable resistance which occurs in wheat (Bai and Shaner 1994) and Type I is a major type of resistance for barley (Fetch et al. 2003).

The most economically and effective way to control FHB outbreaks and epidemics is by genetic resistance breeding. FHB resistance breeding programmes must take into account that resistance QTL and genes are quantitatively inherited and that the environment plays a big role in the expression of these genes. The disease can first be screened and detected in matured plants and the aggressiveness of the fungus may vary for location, year and genotype. A single resistant gene would not be enough to ensure resistance and environmental conditions can result in severe FHB epidemic outbreaks. A combination of major and minor genes and QTL will ensure a high level of resistance and will increase the genetic diversity of the FHB resistant gene pool. To help maintain this diversity more effectively, resistant genes and QTL should be identified and bred into wheat lines (Li et al. 2011).

Morphological characteristics of resistant plants include a dark brown discolouration of the inoculated spikelet or visible dark brown spots on the lemma (Bai and Shaner 1994). The biochemical responses of the resistant plants include the production of phenolic compounds and the lignification process which initiates inhibition of rapid growth of the mycelium within the spike (Nicholson and Hammerschmidt 1992; Siranidou et al. 2003). Plants release phenols and triticens which are toxic to the fungus. The lignification process entails physical barriers which thickens cell walls and prevents the cell wall to degrade and the plant nutrients cannot become accessible for the pathogen (Ribichich et al. 2000).

2.5.8.4 Fusarium head blight resistance sources and genes/quantitative trait loci

The pathogen, *F. graminearum,* leads to infections which affect wheat yield and grain quality that have a negative influence on bread-making characteristics. One of the main concerns of FHB infections is the production of mycotoxins which can be harmful to consumers. One way of controlling FHB and mycotoxin infections is to breed resistant cultivars. The wheat gene pool consists of a wide range of FHB tolerance/resistance genes/QTL but crops which are agronomically well adapted and highly productive are often susceptible to FHB. Resistant QTL for FHB only provide partial resistance and therefore more than one QTL are necessary for high level of tolerance/resistance. Therefore a great challenge exists for plant breeders as they have to breed well adapted cultivars with FHB resistance together with high and stable yield and good quality

(Buerstmayr et al. 2009). FHB resistant cultivars have been identified together with the resistant QTL and chromosome locations in several studies.

Sumai 3, a Chinese cultivar, serves as the common source of genetic resistance for FHB (Bai and Shaner 1994). A restriction fragment length polymorphism (RFLP) mapping study detected five QTL for type II resistance, with one major type II FHB QTL for resistance, *Qfhs.ndsu-3BS*, on the short arm of chromosome 3B, detected in a Sumai 3 x Stoa population (Waldron et al. 1999). The *Qfhs.ndsu-3BS* QTL is near simple sequence repeat (SSR)marker Barc133 and was initially identified with the use of RFLP mapping (Waldron et al. 1999) and later confirmed with SSR analysis (Anderson et al. 2001). The *Qfhs.ndsu-3BS* QTL was also detected in a double haploid (DH) population of CM-82036 x Remus (Buerstmayr et al. 2002). The *Qfhs.ndsu-3BS*QTL encodes for some type of enzyme which has the ability to convert DON to DON-3-O-glycoside which is less toxic (Lemmens et al. 2005). The *Qfhs.ndsu-3BS* QTL was re-designated as *Fhb1* (Liu et al. 2006).

In 2003, Buerstmayr and co-workers reported another major QTL, *Qfhs.ifa-5A,* which is located nearby the centromere of chromosome 5A. This QTL is related to type I resistance (initial infection). A study done by Salameh and co-workers (2011) indicated that *Qfhs.ifa-5A* had a smaller impact on FHB resistance compared to *Fhb1*. The study also indicated that lines containing both QTL were slightly more resistant than lines containing only *Fhb1*.

A major type II FHB QTL for resistance was detected in Sumai 3 on the short arm of chromosome 6B close to the centromere (synonym *Fhb2*; Waldron et al. 1999). This QTL region is flanked by markers Gwm133 and Gwm644 (Cuthbert et al. 2007).

A significant QTL was detected on the short arm of chromosome 2D in two populations [Sumai 3 x Nobeokabozu komugi (resistant variety) and Sumai 3 x Gamenya (susceptible variety)] but are close to the semi dwarfing gene locus *Rht8* which can have a negative effect on plant height. Both alleles from the two varieties showed reduced accumulation of DON in comparison to the Sumai 3 allele. Sumai 3 possesses of the *Rht8* locus as plant height is decreased by 10 cm (Handa et al. 2008).

Due to this negative trait of Sumai 3, scientists have started to use Wangshuibai, a Chinese cultivar, for searching for additional FHB resistance QTL. This cultivar have the same 3BS QTL as Nyu Bai (McCartney et al. 2004) but differs from the allele size of Sumai 3 (Liu and Anderson 2003). Liu and co-workers (2006) have found the same gene sequence for the FHB gene for resistance in the *Fhb1* QTL for Sumai 3, Nyu Bai and Wangshuibai.

Additional QTL for resistance are necessary for better reliance of FHB resistance. Two minor QTL for resistance have been identified from Ning 7840 which are located on the long arm of chromosome 2B and the short arm of chromosome 2A (Zhou et al. 2002). Two QTL with smaller effect to FHB resistance was detected on two different regions on the short arm of chromosome 6B from the Sumai 3 cultivar and two others include Stoa derived FHB QTL for resistance located on chromosomes 2A and 4B, respectively (Waldron et al. 1999). Several QTL have been found on different chromosomes (2D, 4B and 5A) which contribute to FHB type I resistance (Lin et al. 2006). Type I QTL for resistance have also been found on different chromosomes of the Wangshuibai x Alondra's DH population (Jia et al. 2005). Chokwang is a Korean cultivar and have different FHB QTL for resistance than Sumai 3 (Yang et al. 2005). Frontana, the Brazilian cultivar, contribute mainly to type I resistance and QTL for resistance were found on chromosomes 2B, 3A, 5A and 6B of DH lines from the Frontana x Remus cross (Steiner et al. 2004). The 3AL QTL was confirmed and additional QTL was detected on chromosome 7AS (Mardi et al. 2006).

2.5.9 Wheat rust

All three rust types (leaf, stem and stripe rust) have become economical important diseases. Rust, a worldwide spread disease, is due to infection of fungi known as *Puccinia* which have the ability to multiply rapidly (McIntosh et al. 1995). The success of these biotrophs (obligate parasites) depends on three factors, namely host susceptibility, weather conditions and the crop's growth stage. Every rust species needs favourable weather conditions which include optimal temperatures for each specific rust type and high humidity which is necessary for dew formation (Singh and Rajaram 2002). New virulence of these fungi is generated through migration, mutation and recombination of virulence genes (Burdon and Silk 1997).

Rust fungi are biotrophs and therefore need a primary host (e.g. wheat) and a secondary host (other grass species) to survive (Singh and Rajaram 2002). Fungicides are one way to control these diseases but due to safety to the environment and cost effectiveness, the most effective control strategy is to develop and grow resistant cultivars (Smale et al. 1998). Continued evolution of the fungi and its ability to travel long distances are the two main reasons why plant breeders continually search for new resistance sources in various crop species (Pretorius et al. 2007). Resistant genes from wild *Triticeae* species can be transferred into wheat species through genetic engineering to increase the number of available resistant genes in wheat (Friebe et al. 1996).

2.5.9.1 Leaf rust

Leaf rust is caused by the fungus *Puccinia triticina* Erikss. [syns. *P. recondita* Rob. ex Desm. f. sp. *tritici* (Erikss. and E. Henn.) D.M. Henderson] and is one of the most important diseases on wheat worldwide. This disease results in great yield and economical losses worldwide due to its frequent and widespread occurrence (Huerta-Espino et al. 2011). Abdel and co-workers (1980) reported wheat yield loss of up to 50% in Egypt. In 1992, in Western Australia more than 100 000 ha was infected and have resulted in 37% yield loss (McIntosh et al. 1995). In Iran leaf rust is an endemic disease each year and in 1993, 1.5 million ton of wheat was lost due to leaf rust infections (Torabi et al. 1995). In South Africa, regional epidemics have occurred in the Western Cape and other provinces in the 1980s due to susceptible cultivars. Irrigation systems have created favourable conditions for this fungus and have led to increased disease outbreaks (Pretorius et al. 1987). In 1987, a leaf rust epidemic in the Free State has led to high yield loss of winter wheat (Pretorius and Le Roux 1988). Under favourable conditions, the disease can cause 30% to 50% yield losses in susceptible cultivars (Rattu et al. 2009).

Breeding for resistant cultivars is a practical technique to control leaf rust diseases. However, these cultivars have to be resistant against regional races of *P. triticina* to be effective (Elyasi-Gomari and Lesovaya 2009). Breeding for durable resistant leaf rust cultivars are difficult because these fungus populations differ (different races or virulence pathotypes) and they easily overcome present resistance and adapt quickly to climatic conditions (Kolmer 2001). At present, 71 leaf rust resistance genes have been mapped to specific chromosome locations in wheat (Kolmer 2013). Most of the leaf rust (*Lr*) resistance gens have been found in the wheat genome itself, but some genes have been obtained from other species such as *T. tauschii* (*Lr21*), *Thinopyrum elongatum* Host. (*Lr24*), *Th. elongatum* Zhuk. (*Lr19*) and *Secale cereal* L. (*Lr 26*; Browder 1980). Different types of resistance genes have been identified in wheat. Most of these resistance genes are effective during seedling and/or adult plant stage. Some race specific resistance genes were also identified which were either effective during seedling or adult plant stage, but these genes are more likely to be overcome by virulent races (Kolmer 2013). Adult-plant partial resistance is not effective during seedling stage but are effective against all known races of *P. triticina.* This type of resistance does not provide complete resistance but regulates the pathogen's effectiveness by producing fewer and smaller uredinia which are surrounded by chlorosis (Huang et al. 2003). Adult-plant partial resistance provide long-term durable resistance and the most commonly known gene is *Lr34* (Dyck 1987). The *Lr34* gene for resistance has been cloned and sequenced (Lagudah et al. 2006). Other adult-plant partial genes for resistance are *Lr46*, *Lr67* and *Lr68* (Singh et al. 1998; Hiebert et al. 2010; Herrera-Foessel et al. 2012).

The uredinial stage (asexual cycle) is present on the primary hosts. Infection starts with the development of orange/brown circular uredinia which are 1.5 mm in diameter and visible on the upper and lower surfaces of the host leaves (Figure 2.5). The uredinia produce brown spores (urediniospores) which are on average 20 µm in diameter. Symptoms include chlorosis or necrosis of the host leave material. Susceptible cultivars have large uredinia while resistant cultivars show smaller uredinia lesions (Bolton et al. 2008). Black spots develop on the infected leaves and release teliospores. Teliospores germinate and produce basidiospores which infect the secondary host where sexual recombination takes place to produce aeciospores which infect the primary host. Urediniospores are then released from the uredinia which initiate germination under optimal conditions. The urediniospores developed germ tubes on the plant and lead to round orange lesions on the leaf.

Figure 2.5 Leaf rust infection on wheat (Photo Z.A. Pretorius).

The different spores can be spread by wind and result in a great diversity of races and pathotypes. These pathotypes can be distinguished by determining their virulence or avirulence to a specific host types (Kolmer 2013). Rust intensity depends on inoculum density, weather conditions and the susceptibility level of the cultivar. Optimal conditions for leaf rust infections are viable spores, susceptible wheat cultivars and moisture on the leaves, therefore long periods of dew are necessary. Optimal temperature conditions are temperatures between 15°C to 20°C for disease infection and for disease development temperatures between 20°C to 25°C are necessary with relatively cold nights and warm days. The primary host for *P. triticina* include all the wheat types, *A. speltoides*, goatgrass and *triticale* (hybrid species of wheat and rye). The alternative hosts are *Thalictrum speciosissimum* L. (dusty meadow rue) and *Isopyrum fumaroides* (Bolton et al. 2008). Leaf rust infected plants result in fewer kernels per head and lower kernel weights (Elyasi-Gomari and Lesovaya 2009).

2.5.9.2 Stem rust

Stem rust (Sr), also known as black rust, is caused by *P. graminis* Pers. f. sp. *tritici* Eriks and Henn. Stem rust infects the stems as well as leaf sheaths and blades and sometimes infection will occur on the heads by forming uredinia structures which developed from aeciospores. The morphological characteristics of the uredinia are oval shaped or elongated lesions with an orange-red colour (Figure 2.6). Lesions are visible on both sides of the infected leaf and are randomly distributed. Stem rust lesions are larger than those of leaf and stripe rust. The uredinia structures tear the epidermal tissue when infecting the plant. The uredinia release urediniospores which start to germinate one to three hours after they have been in contact with water. After germination of the spores germ tubes occur and aspresoriums develop and penetrate the host which leads to stem rust infection. The uredinia structures will later on change to telia structures which release black teliospores. The teliospores germinate and form basidium, which are mycelium, and produce basidiumspores. Basidiumspores spread by wind and infect alternative hosts for overwintering of the pathogen. These infections of basidiumspores on the alternative hosts form aecia and release aeciospores which give rise to stem rust uredinia structures on the primary host. Infections lead to weaken stems of the host plants, lodging and shrivelled prematurely ripened seed (Schumann and Leonard 2000).

Figure 2.6 Stem rust infection on wheat (Photo Z.A. Pretorius).

Urediniospore infection needs temperatures of 15°C to 29°C. For infection development after germination of the urediniospores temperatures of 26°C to 30°C are necessary for the pathogen. Stem rust depends on long dew periods to survive (six to eight hours). Reduction of light will decrease the infection level of these fungi. The infection ability of stem rust is lower than leaf rust. For survival of the fungi, the availability of suitable host plants is one of the most important factors. The primary hosts for *P. graminis* are wheat, barley and triticale and some of the closely related species. One of the secondary hosts is *Berberis vulgaris* L. (European barberry; Singh and Rajaram 2002).

In 1993, about 70% of wheat fields in Bale and Arsi in Ethiopia were planted with the wheat cultivar Enkoy which were susceptible to stem rust. The stem rust epidemic has resulted in 65% to 100% yield losses (Shank 1994). Another recent stem rust epidemic was due to the new stem rust race Ug99 which was identified in Uganda in 1998 by William Wagoire (Pretorius et al. 2000).

The 1B.1R translocation from Petkus rye was widely incorporated into different breeding programmes and contained the *Lr26, Sr31* and *Yr9* gene complex and also had a positive effect on yield. Stem rust was under control for a long time due to several resistant genes until Ug99 and its derivatives appeared and overcome stem rust resistance. Therefore, stem rust is a great concern to the world. Breeders now have to breed cultivars with effective stem rust resistant genes such as *Sr2* and *Sr26*, to overcome the situation. Ug99 and its derivatives have overcome resistant genes *Sr21, Sr24, Sr31* and *Sr38.* A Ug99 race, TTKSF, was discovered in 2000 in South Africa and later detected in Zimbabwe, with virulence for only *Sr21.* TTKST was identified in Kenya in 2006 with virulence for *Sr31, Sr21* and *Sr24* (Jin et al. 2008). In 2007, race TTTSK was identified in Kenya with virulence for *Sr21, Sr31* and *Sr36* (Jin et al. 2009). Another race, TTKSP, with virulence for *Sr21* and *Sr24* was discovered in South Africa in 2007. The race PTKSK has virulence for *Sr31* and was identified in 2007 in Ethiopia but already discovered in 1998 in Uganda and seen in Kenya in 2009. The PTKST race shows virulence for *Sr31* and *Sr24* and was identified in 2008 and detected in Ethiopia in 2007, Kenya in 2008 and in South Africa in 2009. In 2010 the race TRTTF was identified and detected in South Africa and Zimbabwe and shows virulence for *Sr13, SrTmp* and *Sr1A.1R* (Sharma et al. 2013).

2.5.9.3 Stripe rust

Stripe rust, also known as yellow rust (Yr), is caused by *P. striiformis* Westend f. sp. *tritici* Eriks. and Henn. Stripe rust mostly infects leaf blades and infection in the head, leaf sheaths or stems are rare. Initial symptoms of stripe rust are chlorotic lesions on the leaves of wheat (primary host) and will not appear on seedlings. The yellow-orange uredinia develop in these lesions. The individual uredinia merge together to form stripes which can cover the entire leaf (Figure 2.7). The uredinia produce urediniospores (20 µm to 30 µm in diameter) during the growing season of wheat and spread by wind and rain. Later in the season these lesions darken due to dark brown telia which are produced in the epidermis of leaves and release teliospores in the following growing season. The fungus overwinters as mycelium or urediniospores on wheat crops.

Figure 2.7 Stripe rust infection on wheat (Photo Z.A. Pretorius).

Stripe rust mainly effects wheat production during cooler temperatures and in winter growing regions which include more than 60 countries over all the continents, except for Antarctica (Chen 2005). In South Africa a stripe rust epidemic has occurred in the Western Cape in August 1996 when the fungus infected spring wheat (Pretorius et al. 1997). Stripe rust can cause 100% yield loss if environmental conditions are optimal, highly susceptible cultivars are planted and disease development are fast and present for a long time (Chen 2005). Stripe rust infections can be inhibited to some extend using fungicides, but it can be harmful to the environment and are expensive, which make it impossible for developing countries to apply. Therefore resistant cultivars are necessary to ensure crop sustainability (Bux et al. 2011).

Triticum species have been the major host of stripe rust and other primary hosts are barley, rye and triticale. Barberry can serve as an alternative host for the stripe rust fungus. When the alternative host are present, the fungus will complete its life cycle otherwise it will end by releasing urediniospores. Stripe rust has a lower optimal growth temperature in comparison to leaf and stem rust. Optimum infection temperatures range between 7°C to 12°C and moisture on the leaves are necessary for infection (Rapilly 1979). Temperatures of 10°C to 15°C are optimal for disease development. Uredinia will develop 7 to 10 days after disease infection and optimal conditions are available. Infection can lead to yield reduction as the grains have a shrivelled structures and the quality are also negatively affected (Chen 2005).

To date, 53 rust genes for resistance had been identified, mapped and used in different breeding programmes, these genes are name *Yr1*-*Yr53* (Zhang et al. 2013). The spread of the CYR32 *Pst* race has result in the ineffectiveness of some genes (Yang et al. 2003). APR stripe rust resistant genes, *Yr18* (Krattinger et al. 2009) and *Yr36* (Fu et al. 2009) are the only two cloned genes. Another stripe rust resistant gene that is widely used in breeding programmes is *Yr26* (Zhang et al. 2013). These major APR stripe rust genes for resistance can be used in combination with other effective genes (*Yr9, Yr10, Yr15, Yr17, Yr24 and Yr34)* to improve stripe rust resistance in new developed wheat lines (Bariana et al. 2006).

2.5.9.4 Rust resistant genes and markers

Since rust in wheat is caused by three different rust species it is difficult to breed cultivars that are resistant against stem, stripe and leaf rust. A number of markers linked to genes for resistance for all three rusts have been developed. Developing cultivars with genetic resistance to rust is cost effective, efficient and safe to the environment. Since there are many different rust genes for resistance with linked molecular markers available, only the rust resistant genes used in this study will be discussed.

2.5.9.4.1 Gene *Lr19*

The *Lr19* gene was introgressed from *Agropyron elongatum* (Host) P. Beauv. [synonym *Thinopyrum elongatum* (Host) D.R. Dewey] to the wheat line Argusand is located on the long arm of chromosome 7D (Autrique et al. 1995). The effectiveness of the *Lr19* gene was first described by Huerta-Espino and Singh (1994) in Mexico. However, linked to this alien segments were the undesirable gene such as the endosperm yellow pigment gene *Y* which is responsible for yellow coloured flour which reduced its appeal for baking and is undesirable for many countries, together with the desirable stem rust resistance gene *Sr25* (Knott 1968). The *Lr19* leaf rust gene for resistance is effective against all leaf rust pathotypes in South Africa (Prins et al. 1997)

Homologous translocation to the T4-translocation was found in Indis. This translocation contains the *Lr19*, *Sr25*, *Sd1*, *Wsp-D1c* and *Y* genes. The translocation inherited as a single block and caused overlapping at the distal end of chromosome 7DL of heterozygous lines (Marais and Marais 1990). Gamma irradiation was applied to develop deletion lines for this translocation and resulted in a line contain only *Lr19-Sr25-Y* combination (Marais 1992a). Allosynthetic pairing and crossover were used to break the linkage between *Lr19* and *Y* and resistant recombinant lines with white endosperm were obtained (Marais 1992b). The white-endosperm recombinant line consists of the *Lr19* gene that was relocated to chromosome 7BL. No negative effects on yield and quality have been detected for this segment (Prins et al. 1997). The sequence-tagged-sites (STS) marker, STSLr19$_{130}$, have been developed from a conversion of an amplified fragment length polymorphism (AFLP) fragment, to screen for the presence or absence of gene *Lr19*. This dominant marker amplifies a 130 basepair (bp) fragment if the gene for resistance is present (Prins et al. 2001).

2.5.9.4.2 Gene complex *Lr34/Yr18/Sr57*

The *Lr34/Yr18/Sr57* gene complex is controlled by a single gene which encodes the ATP-binding cassette (ABC) transporter (Krattinger et al. 2009). This gene complex is race non-specific and has been effective for more than 50 years. This partial resistance gene complex is effective during adult plant stage which is more effective as seedling stage as grain developed in adult plant stage. This gene complex offers partial resistance to leaf rust, yellow rust and stem rust infections. In the presence of the *Lr34* gene for resistance, flag leaves will developed necrotic tips due to the co-inheritance of the *Ltn1* gene which also can be used as a phenotypic marker for detection of the *Lr34* gene (Singh 1992). The *Lr34/Yr18/Sr57* gene complex encodes for a slow rusting effect and leads to slower rates of rust infections (Lagudah et al. 2006). This gene combination is located on the short arm of chromosome 7D (Krattinger et al. 2009). A study done by Singh (1992) has indicated that cultivars only containing the *Lr34* gene have a 40% disease severity but when one or two minor genes are added the disease severity level drops to 10%-15%. Cultivars with two or three extra minor genes together with *Lr34* have a disease severity level of 1%-5%.

Reduced rust infections in plants with *Lr34* are due to a reduced rate of haustorium infection in the early stages of infection together with little to no cell necrosis (Rubiales and Niks 1995). Resistant plants with the *Lr34* gene accumulate an unknown electro-dense substance in the infected plant cell, where the fungi try to form the haustoria and prevent digestion of the plant mesophyll cell walls. This unknown electro-dense substance causes thickening of the plant cell walls and reduces the speed of the haustorial tube formation. If the haustorial tube has already formed the mycelia will grow slowly because the fungus struggles to move from one cell through to another as a result of the thickening cell walls (Alvarez-Zamorano 1995).

The first two molecular markers linked to the *Lr34/Yr18/Sr57* gene complex were developed by Bossolini and co-workers in 2006 (marker Swm10) and Lagudah and co-workers in 2006 (csLV34, a STS marker). Due to limitations of the previous markers, Lagudah and co-workers (2009) developed markers cssfr1 to cssfr7. Markers cssfr1 to cssfr5 are allele specific markers. Five different oligonucleotides were selected based on sequence changes in exon 11 which differentiated in the presence or absence of *Lr34.* Markers cssfr1 and cssfr2 were developed to determine the presence or absence of the *Lr34* gene, respectively. Two individually multiplexed PCR reactions were performed using the two allele specific primer pairs used to develop cssfr1 and cssfr2 together with the primer pair of marker csLV34 to ensure amplification and these two multiplexed markers were named cssfr3 and cssfr4. Marker cssfr5 was developed as a multiplexed co-dominant marker to determine the state of the *Lr34* gene in one reaction. For the cssfr5 marker, primer pairs of cssfr1 and cssfr2 were combined. Marker cssfr6 detects the *Lr34* sequence polymorphism in exon 12. Marker cssfr7 detects the mutation in exon 22 which has been detected in the cultivar Jagger (Lugadah et al. 2009).

2.5.9.4.3 Gene *Sr2*

The *Sr2* recessive gene is well known and widely used in breeding against stem rust. It provides durable but partial resistance at adult plant stage to stem rust (Sunderwirth and Roelfs 1980), including to race Ug99 and its derivatives. The recessive gene has been transferred from the tetraploid emmer wheat Yaroslalv (*Triticum dicoccum* Schronk) to the bread wheat Hope and H44-24 (McFadden 1930) and is located on the short arm of chromosome 3B (Mago et al. 2010). The *Sr2* gene is in combination with the expression of pseudo black chaff (PBC) which occurs in the glumes and nodes of the stem. The expression of PBC can be used as phenotypic marker for the presence of *Sr2* (McFadden 1939). The *Sr2* gene without the association of PBC is not effective against stem rust (Rajaram et al. 1988). Up until today *Sr2* has provided durable resistance for more than 60 years. Stem rust disease symptoms are influenced by the genetic background of genotypes and the environment which complicate phenotypic scoring. The *Sr2* gene will not offer enough resistance under severe pressure, but in combination with minor stripe rust genes for resistance, it is more effective.

The first developed molecular marker linked to the *Sr2* gene was Gwm533, which amplifies a 120 bp allele fragment in resistant genotypes (Spielmeyer et al. 2003). After screening different lines, scientists detected a second 120 bp fragment at the same locus, with different sequence. The new locus did not contain the *Sr2* gene and therefore results can be misleading since false positive fragments also get amplified. New sequenced tagged microsatellite (STM) markers were developed (stm598tcac and stm559tgag) which can differentiate between the two different loci. Marker, stm598tcac indicates the presence of the *Sr2* gene while marker stm559tgag is not associated with the *Sr2* gene. Polymerase chain reaction (PCR) amplification using these primers resulted in different banding patterns and fragment sizes in different cultivars which complicated interpretation of results (Hayden et al. 2004). Therefore, a cleaved amplified polymorphic site (CAPS) marker, csSr2, has been developed by Mago and co-workers (2010) and amplification and digestion result in three fragments: non-*Sr2*, *Sr2* with the *Bsp*HI restriction site and without the restriction site. No recombination between the marker and the gene has been observed as it is more closely linked to the gene compaired to the Gwm533 marker (Kota et al. 2006). Therefore the CAPS marker is more accurate in determining the presence or absence of the *Sr2* gene. The success rate of the CAPS marker for determining the presence of absence of the *Sr2* gene is 95% whereas the Gwm533 marker has a success rate of 84% (Mago et al. 2010).

2.5.9.4.4 Gene *Sr26*

The alien segment 6Ae#1L carrying the *Sr26* gene was translocated from *Agropyron elongatum* (2*n*=10*x*=70) to the wheat genome. The alien chromosome segment is located on the long arm of chromosome 6A of the wheat genome (Knott 1961; 1968). The original translocation had a negative impact on yield due to *Agropyron* chromatin. Newly developed sources of the *Sr26* translocation are however available with reduced amount of *Agropyron* chromatin (Dundas et al. 2007).

The *Sr26* gene is still effective against race Ug99 and its derivatives and therefore plays an important role in breeding for wheat cultivars with effective resistance to stem rust races. Sr26#43 is a dominant STS marker which amplifies a fragment linked to the gene for resistance with a size of 207 bp (Mago et al. 2005). In 2010, Liu and co-workers tried to develop a co-dominant marker for *Sr26.* They developed 16 STS markers from wheat expressed sequence tags (EST's) which were mapped in the deletion bin 6AL-0.90-1.100 but no co-dominant marker for *Sr26* were obtained. Then they have identified chromosome 6A-specific markers and one of them, BE518379, together with Sr26#43, serves as a co-dominant marker. These markers amplify a 207 bp fragment for homozygous lines containing *Sr26* and a 303 bp fragment for homozygous lines without *Sr26.* Both the 207 bp as well as 303 bp fragments are amplified in heterozygous lines (Liu et al. 2010).

2.5.9.4.5 Quantitative trait loci *QYr.sgi.2B-1*

APR is durable and effective for disease resistance but has a negative influence on yield and plants can still be infected at seedling stage (Brown 2002). Kariega, a South African spring wheat cultivar, shows APR against stripe rust and shows good baking qualities with no negative effect on yield (Prins et al. 2005). Stripe rust resistance in Kariega was identified on four QTL, two minor and two major QTL. The functions of the two minor QTL are still unknown but increase the sensitivity which enabled QTL to be expressed at different developmental stages and under different environmental conditions. The two major QTL, *QYr.sgi-7D* and *QYr.sgi.2B-1* were originally located on chromosomes 7D and 2BS, respectively (Ramburan et al. 2004). *QYr.sgi-7D* was later shown to be the APR gene *Yr18* which are inherited together with *Lr34* and *Sr57. QYr.sgi.2B-1* is associated with chlorotic and/or necrotic response which is a hypertensive response for disease infections (Ramburan et al. 2004). The QTL is relatively large [about 23 centiMorgan (cM) long] with flanking markers Gwm148 and Gwm501 linked to the QTL. The QTL is situated on the long arm of chromosome 2B. The smaller QTL interval namely *QYr.sgi.2B-1a* and the large QTL are both significant for field infections. *QYr.sgi.2B-1a* explains 45% of resistance in comparison of the 32% of *QYr.sgi-7D* (Prins et al. 2011).

2.6 Marker-assisted selection

The genetic make-up of an organism together with environmental factors determine the organism’s phenotype. MAS enables scientists to do selection based on the genotype instead of the phenotype. Molecular markers are associated with different traits of interest e.g. physiological, morphological and behavioural traits. The potential of MAS was limited during the developmental stages due to a lack of sustainable and reliable molecular markers (Ribaut et al. 2010). Today, many markers have been developed and improved to detect the presence of allelic variation and improve the efficiency and precision of breeding programmes worldwide (Collard and Mackill 2008).

Different types deoxyribonucleic acid (DNA)-based molecular markers are available such as RFLP, random amplified polymorphic DNA (RAPD), AFLP, microsatellites or SSR, diversity array technology (DArT), sequence characterised amplified regions (SCARs), CAPS, ESTs, inter-simple sequence repeats (ISSRs), STSs and single nucleotide polymorphism (SNP). Molecular markers are used to identify specific gene fragments which are linked to certain characteristics of an organism (Ruane and Sonnino 2007).

2.6.1 Restriction fragment length polymorphism

RFLP is a hybridisation-based molecular marker technique and was first used for genetic mapping of temperature-sensitive mutations of adeno-virus by identifying DNA sequence polymorphisms (Grodzicker et al. 1975). RFLP was then used for mapping of the human genome (Botstein et al. 1980). This technique makes use of restriction enzymes to detect differences in DNA fragment sizes between individual organisms. Differences in fragment sizes are due to mutations, deletions or insertions, translocations, inversion and duplication. This technique is highly reproducible even between laboratories and co-dominant inheritance can be detected, synteny studies can be performed, no sequence information is necessary and the gels are easy to score. The few disadvantages of RFLP markers are the high quality and quantity of DNA necessary for this technique, specific probe libraries for every species have to be developed, the technique cannot be automated, low levels of polymorphism are detected, it is time-consuming and expensive and requires labelled probes (Roy et al. 1992). RFLP studies based on genetic variation have been done on *Lycopersicon* (tomato; Miller and Tanksley 1990), *Hordeum vulgare* ssp. *Spontaneum* Koch. (wild barley; Zhang et al. 1993) and *Triticum aestivum* (wheat; Siedler et al. 1994)

2.6.2 Random amplified polymorphic DNA

This technique is one of three techniques which have been used for the first time to amplify DNA fragments from any species without any DNA sequencing (Welsh and McClelland 1990; Williams et al. 1990). The other two similar techniques are arbitrarily primed-polymerase chain reaction (AP-PCR) and DNA amplification fingerprinting (DAF). RAPD uses a single arbitrary oligonucleotide primer and amplifies random pieces of the genome. The low cost and simplicity of the agarose gel electrophoresis detection has made RAPD more popular than AP-PCR or DAF. Advantages of RAPD are that it is a simple and fast technique with many possible primers available which cover many loci, no sequencing is necessary, it is inexpensive and small amounts of DNA is necessary for RAPD analysis. However, this technique has three main limitations: it is not highly reproducible, it is a dominant marker and one cannot detect heterozygous alleles and homology (Semagn et al. 2006). This method had been applied to genetic diversity studies of various plants such as *Amaranthus* (Chan and Sun 1997) and wheat (Mukhtar et al. 2002).

2.6.3 Amplified fragment length polymorphism

AFLP is a PCR-based method and ligates adaptors (primer recognition sequences) to restricted DNA for simultaneous screening of randomly distributed DNA regions within the whole genome (Vos et al. 1995). This method is highly reproducible and reliable

(Mueller et al. 1996), no prior sequence information is necessary and detects large numbers of polymorphic loci (Powell et al. 1996). The limitations of this method include many steps to produce results, the need for good quality DNA which is free of restriction enzymes and inhibitors, it is expensive due to polyacrylamide gels and labelling system needed for detection and it is a dominant marker, which will not detect heterozygous alleles (Vos et al. 1995). Various AFLP studies have been performed to determine genetic diversity in various plant species like wheat (Roy et al. 2004) and potatoes (Nunziata et al. 2010).

2.6.4 Microsatellites

Microsatellites are also known as SSRs or short tandem repeats (STRs). Microsatellites are simple repetitive DNA sequence motifs and due to slipped-strand mispairing mutations it leads to polymorphisms (Tautz et al. 1986). The slipped-strand mispairing mutations can lead to gain or loss of repeats (Eisen 1999). SSR allelic differences are due to the size and number of the repeat motif. SSRs can be dominant or co-dominant and have a high throughput which makes it ideal for the use of population genetics studies and mapping (Jarne and Lagoda 1996; Goldstein and Pollock 1997). Disadvantages include expensive primer designs and genomic libraries are necessary for primer design.

2.6.5 Diversity array technology

This method have been developed recently and has originally been used in rice (Jaccoud et al. 2001), barley (Wenzl et al. 2004; Varshney et al. 2012), eucalyptus (Lezar et al. 2004; Steane et al. 2011), *Arabidopsis* (Wittenberg et al. 2005), wheat (Akbari et al. 2006; Semagn et al. 2006), pigeon-pea (Yang et al. 2006) and tomatoes (Van Schalkwyk et al. 2012). This is an open source technique and is useful for genetic diversity and mapping studies. This is a microarray hybridisation-based method which results in several hundred polymorphic loci covering the entire genome of an organism (Jaccoud et al. 2001). This technique does not need prior sequencing, has a high throughput and is highly reproducible. This method is cost-effective based on data points compared to SSR markers and is not covered by exclusive patent rights. However, this technique involves several steps and need skilled labour for the operation of laboratory equipment and software systems and it is a dominant marker.

2.6.6 Sequence characterised amplified regions or Sequence tagged sites

The SCAR and STS techniques make use of two oligonucleotide primers which amplify a specific DNA fragment using PCR. The two ends of the RFLP/RAPD/AFLP marker, linked to a diagnostic characteristic, are cloned and sequenced. New primers are developed based on sequence information. SCARs detect a single locus and the amplification reaction conditions are less sensitive, in comparison to for example RAPD markers and they can be converted into co-dominant markers (Paran and Michelmore 1993). STS markers have been developed for DNA landmarks for physical mapping of the human genome (Olsen et al. 1989). STS marker is co-dominant, highly reproducible and is a simple technique due to automisation (Reamon-Buttner and Jung 2000). Different STS and SCAR markers have been developed to screen for various traits in wheat, such as leaf rust (Blaszczyk et al. 2004) and powdery mildew (Liu et al. 1999).

2.6.7 Cleaved amplified polymorphic sequences

This method amplifies the targeted DNA sequence and after amplification the PCR product gets digested by restriction enzymes (Konieczny and Ausubel 1993). Digestion of PCR products result in polymorphism between samples. CAPS are easier and less time-consuming than AFLP markers. Markers are more useful for comparative mapping studies as it can be developed from ESTs and is most of the time co-dominant (Matsumoto and Tsumura 2004). One limitation of CAPS is the low polymorphism level and marker development is limited because it has to create a restriction enzyme recognition site through mutations (Lezar et al. 2004). CAPS markers have been applied to various molecular breeding programmes and research studies of different species including wheat (Mammadov et al. 2010; Okoǹ et al. 2012).

2.6.8 Expressed sequence tags

ESTs are short complementary DNA (cDNA) sequences applied for discovering and identification of genes and gene transcripts and to determine gene sequences (Liang et al. 2008). Extracted messenger RNA (mRNA) is unstable but converting of mRNA to cDNA using an enzyme called reverse transcriptase can overcome this problem. This cDNA only consist of expressed DNA sequences. Scientists can create 5’ESTs and 3’ESTs by sequencing a few hundred nucleotides from either the 5’ or 3’ end (Jongeneel 2000). Over 6 million ESTs are available in GenBank. ESTs are used for identification of gene transcripts, gene discovery, gene expression and regulation, sequence determination and developing of molecular markers and probes (Semagn et al. 2006). EST’s are used in different studies for wheat improvement (Mullan et al. 2005; Xu et al. 2012).

2.6.9 Inter-simple sequence repeats

ISSRs amplify DNA segments which are present in-between two identical microsatellite repeat regions which are orientated in opposite directions. This method is the reversed version of microsatellites as it uses microsatellites as primers targeting and amplify inter simple sequence repeats of different sizes between the two microsatellites primers used (Zietkiewicz et al. 1994). ISSR does not need sequence information for primer synthesis as the microsatellites sequences are already known. This method is simple and fast with high levels of polymorphism (Kojima et al. 1998) and is highly reproducible (Fang and Roose 1997). ISSR is a dominant marker (Gupta et al. 1994) and co-migration of fragments can cause problems during interpretation (Sanchez et al. 1996).

2.6.10 Single nucleotide polymorphism

SNPs markers are one of the newest molecular marker methods used in different studies of crop species. SNPs are abundant in the genome of various species and these SNPs are due to the change of a single nucleotide or small insertions and deletions (Indels). Yu et al. (2002) detected a SNP in every 170 bp and an Indel in every 540 bp, by comparing the sequences of a Japonica rice cultivar to an Indica cultivar. This method can be applied for mapping, molecular breeding and map-based cloning (Gupta et al. 2001; Rafalski 2002; Batley et al. 2003). The accessibility of genome sequences of different organisms has generated the ability to study sequence variation between individuals, cultivars and subspecies (Semagn et al. 2006). This method has been widely used in different marker-trait association studies, MAS breeding studies and genetic maps of different species (Lai et al. 2012). This method has been applied to various crop species such as wheat (Matsuda et al. 2012), rice (McCouch et al. 2010), maize (Lump et al. 2011) and barley (Sato et al. 2011).

2.7 Wheat protein

Cereal grain (maize, wheat and rice) provide over 200 million ton of proteins annually for humans and animals, which are three times more than for protein rich legumes (Shewry and Halford 2002). Wheat research is important for milling and baking companies as well as consumers. Research will help to improve wheat grain quality, which is important for end use products such as bread, flour and other flour products. The quality and quantity of wheat proteins are determined by the expression of genes. Expressions of these genes are influenced by different environmental factors. Therefore it is necessary to include biochemical analyses to determine the quality and quantity of expressed proteins in the grain itself.

Protein forms part of a healthy and balanced humans' diet. Animal protein sources such as milk, eggs and meat are higher in protein than cereal protein sources. However, in many countries animal protein sources are a luxury and do not form part of an everyday meal. People in developing countries rely on cereals and vegetables to obtain their daily proteins (Matta et al. 2009). One of the components of wheat flour is gluten, which form part of the storage proteins found in the endosperm, and allow flour to form dough for the production of bread (Gianibelli et al. 2001). Bread forms an important part of the modern human society's diet. Already in 1948, Finney and Barmore have discovered that both the concentration and composition of gluten are important for bread-making quality characteristics.

2.7.1 Wheat proteins classification

The three components of wheat kernels are starch, proteins and lipids. Starch constitutes 65% to 75% of dry grain whereas proteins constitute 8% to 20% (Miyazaki and Morita 2003; Saint Pierre et al. 2008). Plant proteins can be classified into four groups: albumins which are soluble in water, globulins which are soluble in a diluted salt solution, prolamins which are soluble in aqueous alcohol and lastly, glutelins which are soluble in diluted acids and alkali. Albumins are the metabolically active proteins also known as enzymes (Osborne 1924).

Globulins, prolamins and glutelins are the storage proteins of plants. Prolamins are the main storage protein group of wheat and serve as the secretory proteins. These proteins are synthesised on the rough endoplasmic reticulum and move into the lumen where they fold and combine with other proteins (Shewry and Halford 2002). The prolamins constitute of 50% gluten and can be divided into polymeric glutenins and monomeric gliadins. Glutenins accumulate in the lumen of the endoplasmic reticulum whereas gliadins move into the vacuole by the Golgi (Rubin et al. 1992). Glutenins and gliadins can be divided into three main groups within the prolamin group according to their characteristics. The first group is the HMW prolamins and include only the HMW-GS and constitute about 6%-10% of the prolamins. The second prolamin group are the sulphur-rich prolamins and include the α-, β- and γ-gliadins and the low molecular weight-glutenin subunits (LMW-GS). The sulphur-rich prolamins constitute 70% to 80% of the total prolamins. The third group is the sulphur-poor prolamins and include the ω-gliadins (Shewry and Halford 2002).

Glutens make up 50% of the total proteins in wheat flour. Gluten forms when flour and water are mixed and is responsible for dough rheological properties which include dough strength and extensibility. Wheat gluten proteins can be divided into two groups of proteins namely the monomeric gliadins which are responsible for extensibility and the polymeric glutenins which are responsible for elasticity. These proteins are ideal for bread-making qualities due to their inter- and intra-molecular covalent and non-covalent bonding (Butow et al. 2004).

2.7.2 Glutenins

Polymeric glutenins are multi-chained polypeptide structures that are held together by disulphide bonds and can be sub-divided into HMW-GS and LMW-GS. The size of HMW-GS range between 80-140 kilodalton (kDa) whereas LMW-GS range between 30-50 kDa. Glutenins are responsible for the visco-elastic properties of dough especially the HMW-GS (Payne et al. 1985).

2.7.2.1 High molecular weight glutenin subunits

HMW-GS are encoded by three genes (*Glu-A1, Glu-B1* and *Glu-D1*) which are located on the homologous group one chromosomes and are synthesised during grain due to their molecular weight development (Huebner and Gaines 1992). The HMW-GS can be divided into two types namely x-type or y-type, based on their slower and faster eletrophoretic mobility, respectively, (Liu et al. 2008). Only the x-type gene is expressed at the *Glu-A1* locus in hexaploid wheat due to specific gene silencing (Shewry et al. 2001). Thus, two alleles are present for every loci on the three different chromosomes for an individual wheat line except for the A chromosome's loci which only expressed the x-type HMW-GS. Every x- and y-type allele have a number which were given according to their electrophoretic mobility. HMW-GS are identified numerically which include the chromosome, whether it is an x- or y-type subunit and the electrophoretic number (e.g. Dx5 or By9). The numbering system has been developed by Payne and Lawrence in 1983 and it is still used today. HMW-GS are less abundant than the LMW-GS but the HMW-GS have a major impact on the dough quality and analysis methods have been established and applied to identify HMW-GS (Shewry and Halford 2002).

2.7.2.2 Influence of high molecular weight glutenin subunits on protein quality

Dough properties that make it possible to bake bread include dough strength and extensibility and are complex traits. Expression of these complex traits depends on the composition and quantity of the LMW-GS, HMW-GS and gliadins. A study done by Payne (1987) has discovered that HMW-GS Dx5+Dy10 is associated with strong dough strength whereas Dx2+Dy12 is associated with weaker dough strength. It was confirmed by a study done by Békés et al. (1994) that the extra cysteine present in Dx5 is responsible for improving dough strength.

HMW-GS Ax1 and Ax2* are positively associated with bread-making quality characteristics whereas a null allele have weaker dough quality characteristics. The HMW-GS expression on the 1B chromosome, Bx7+By8 and Bx17+By18, positively influences the dough strength and extensibility compared to Bx7+By9, Bx20+By20 (negative effect on dough extensibility) and Bx6+By8 (D'Ovidio and Anderson 1994).

The over-expressed *Bx7* alleles also contribute to strong dough strength. Although expression of these alleles leads to strong dough strength, they lead to a lower extensibility in comparison with other alleles (Butow et al. 2003). As mentioned earlier, the combination and ratio of glutenin and gliadin allele's present, rather than single alleles, are responsible for the composition of dough (Békés et al. 2006).

Protein quality directly influences dough and is primarily determined by the grain hardness and protein content, which are largely due to the molecular structures of the storage protein, especially the HMW-GS (Shewry 1999). The HMW-GS have been studied more in comparison to the LMW-GS because the HMW-GS are more accessible for analysis than the LMW-GS (Shewry et al. 1992). Analysis results of LMW-GS are difficult to interpret because they co-migrate with some of the gliadins (Gianibelli et al. 2001).

2.7.2.3 Low molecular weight glutenin subunits

About 33% of the total seed protein is represented by the LMW-GS which form disulphide-linked aggregates and constitute up to 60% of the total glutenins present in grain (Bietz and Wall 1972). The LMW-GS are controlled by genes at the *Glu-A3*, *Glu-B3,* and *Glu-D3* loci on the short arms of chromosomes 1AS, 1BS and 1DS, respectively. LMW-GS form part of the prolamins because of their proline and glutamine amino acid content. Prolamins serve as nutrients during seed germination and seedling growth. The LMW-GS determine the number of cysteine residues which is available to form inter-molecular disulphide bonds with other LMW-GS or HMW-GS to form polymers. The polymer size is correlated to dough strength (Butow et al. 2004). LMW-GS can be divided into B-, C- and D-subunits according to their electrophoretic mobility on SDS-PAGE (Gupta and Shepherd 1990).

2.7.3 Gliadins

Gliadins are the most abundant type of proteins of the prolamin group in wheat and are responsible for viscosity and extensibility of wheat dough. Gliadins have a high content of glutamine and proline. Monomeric gliadins are single peptide chains with intra-molecular disulphide bonds and the size range between 30-80 kDa. Gliadins can be divided into four main types based on mobility, namely alpha (α, fastest mobility), beta- (β), gamma- (γ) and omega- (ω) gliadins (slowest mobility). The α-, β- and γ- gliadins are poor in lysine, arginine and histidine and are responsible for poor nutritional quality in wheat. The role of gliadins is not well understood because they are encoded by multigene families and co-dominantly inherited in blocks which make it difficult to determine their properties (Shewry et al. 2002). Tightly linked genes encoding gliadins are located on the short arms of group 1 (*Gli-1* loci) and 6 (*Gli-2* loci) chromosomes. The *Gli-1* genes code for all the ω- and most of the γ-gliadins. The *Gli-2* genes code for all α-, most of the β- and some of the γ-gliadins (Metakovsky et al. 1986).

2.8 Protein detection methods

Various methods have been developed to determine the quality of wheat based on proteins. These methods include SDS-PAGE, SE-HPLC and RP-HPLC, which are based on different principles. These biochemical tests are based on different characteristics of proteins such as molecular weight (size), charge and hydrophobicity of the surface. These methods have been developed to analyse certain characteristics of these proteins and to determine correlations between proteins.

2.8.1 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Scientists have made use of starch gels in the early days, but in the early 1960s they started to use polyacrylamide gels as an alternative. Shapiro et al. (1967) were one of the first research groups which have made use of SDS-PAGE. In 1970 a scientist with the name Laemmli (1970) found that protein fractions could be separated on the gel using tris(hydroxymethyl)aminomethane (Tris) buffer which is the method as scientist's know it today.

Sodium-dodecyl sulphate (SDS) is an anionic detergent and is responsible for the dissociation of proteins. Dihiothreitol (DTT) act as a disulfide reducing agent and in the presence of both DTT and SDS the protein unfolds completely into polypeptide chains. Separation in the polyacrylamide gel is thus based on differences in molecular weights of each polypeptide. The gel consists of two types of gels namely a stacking gel on top of the separating gel. When an electrical current is applied, the SDS-proteins migrate between the highly mobile chloride ions and the slower glycinate ions. When proteins reach the separating gel it experience a change to its movement due to restrictive pore size of the gel. At this stage the glycinate ions overtake the proteins and the proteins start with the separation process (Garfin 1990).

Proteins are separated based on their molecular weights thus the higher the molecular weight the slower the protein will migrate through the gel. The glutenins and gliadins of whole wheat samples will have comparable patterns between gels, where HMW-GS will be found in the top part of the gel followed by the ω-gliadins, LMW-GS and then the α-, β- and γ-gliadins.

2.8.2 Size-exclusion high performance liquid chromatography

Analysis of cereal proteins, which are important due to their bread-making ability, was difficult to obtain in the past. Cereal proteins are heterogeneous and part of multigene families, making it difficult to separate. Cereal proteins are rich in glutamine, proline and hydrophobic amino acids, making it different from other plant and animal proteins. Another challenge for separation is the interaction of cereal proteins with the lipids and carbohydrates available in the endosperm (Bietz and Wall 1972). Jones and co-workers (1959) discovered that cereal proteins can be separated by moving boundary electrophoresis. Another method for protein separation is chromatography using different columns. One type of chromatography is SE-HPLC which separate proteins based on protein size (Bietz 1979).

SE-HPLC separates samples according to their hydrodynamic size, diffusion coefficient and surface properties. SE-HPLC consists of two phases. The first phase is the stationary phase where the column is packed with particles to form a matrix with a specific pore size. The second phase is the mobile phase which is a liquid and can either be water, buffer or an organic solvent. Large particles will not enter the matrix as the pore sizes are too small and therefore it will elute first. Smaller particles will penetrate the matrix and how fast and deep they penetrate the matrix will determine the elution time (Tayyab et al. 1991).

Wheat gluten proteins consist of a mixture of monomeric as well as polymeric proteins which have an influence on bread-making quality. By separating these proteins and determining the presence of proteins scientists can determine the importance of these proteins individually. Results can be incorporated in breeding programmes to select lines with good bread-making characteristics (Tsilo et al. 2010). Wheat proteins are either SDS-extractable or -unextractable and the unextractable proteins need to be degraded by using sonification. Large- and small-polymeric or monomeric proteins are detectable in both the SDS-extractable and -unextractable proteins. During SE-HPLC proteins elute in the following order: large polymeric proteins (LPP), small polymeric proteins (SPP), large monomeric proteins (LMP) and small monomeric proteins (SMP).

The unextractable polymeric protein percentage (UPP%) of the total polymeric proteins correlates with gluten strength (Marchylo et al. 1989). The UPP% can be used as an indirect guideline in breeding programmes and quality evaluation. A low UPP% indicates weak dough and high UPP% strong dough.

A study done by Gupta and co-workers (1993) indicated that dough strength was significantly correlated with the total UPP. SDS-unextractable proteins with high molecular weight had a higher correlation to the mixograph peak time. These results indicated that HMW unextractable proteins had a more positive effect on dough strength and loaf volume than the other protein fractions. This indicated that the UPP fraction contains more large polymers that are favourable for improved dough strength (Tsilo et al. 2010).

2.8.3 Reverse phase high performance liquid chromatography

RP-HPLC is an automated technique that separates cereal proteins based on their surface hydrophobicity (Bietz 1983). The application of this technique can be utilised for identification, isolation, characterisation and comparison of proteins. Proteins with higher hydrophobicities elute faster than proteins with lower hydrophobicities. HMW-GS elute first followed by LMW-GS whereas gliadins elute last. To determine the HMW-GS composition, wheat albumins, globulins and gliadins must be removed as they can co-elute with the glutenins which will influence the results (Burnouf and Bietz 1989).

HMW-GS are directly related to dough strength (Burnouf and Bouriquet 1980) and RP-HPLC is an advanced technique developed for separating cereal proteins. Burnouf and Bietz (1989) developed a procedure to obtain a high level of pure glutenin using 90%-100% dimethyl sulfoxide (DMSO) as a pre-extracting method to remove non-glutenin proteins. The pure levels of glutenin showed better separation of the glutenin subunits on the chromatogram and advance analysis and interpretation of results.

In a comparative analysis of RP-HPLC and SDS-PAGE, Dong and co-workers (2009) have seen that alleles *By8* and *By8**, *Bx7* and *Bx7** and *Ax2** and *Dx2* had similar motilities and good separation of these alleles could not be obtained on SDS-PAGE and they struggled to identify these alleles. These alleles, with the same mobility, showed different hydrophobicities and eluted at different times. However, they struggled to separate and identify the *Dy10* and *Dy12* alleles as they almost the same hydrophobicity and eluted simultaneously. The x- and the y-type subunits eluting times indicated that the y-type subunits were more hydrophobic than the x-type subunits. The subunits have eluted from the fastest to slowest time in the following order: *Ax*, *Bx*, *Dx*, *By* then *Dy*.

HMW-GS are less hydrophobic in comparison to LMW-GS and therefore elute faster. Gliadins have similar hydrophobicity to LMW-GS and elute in the same time range. This fact and the large diversity among LWM-GS make it difficult to study their role using RP-HPLC analyses. RP-HPLC can also be used for quantification of the different protein groups. Quantitative analyses of these proteins can be used in correlation studies with other rheological and baking characteristics to determine bread-making quality.

2.9 Wheat quality markers

Dough quality in general is more influenced by the number and composition of the HMW-GS than the gliadins and LMW-GS. However, SDS-PAGE has been used traditionally for the detection of HMW-GS and LMW-GS (Radovanovic and Cloutier 2003). SDS-PAGE is time-consuming and scoring of subunits can be difficult. Therefore different PCR-based markers have been developed due to the great influences of HMW-GS on dough. These PCR-based markers can be used to identify the presence of various HMW-GS in a line (D'Ovidio and Anderson 1994). Advantages of PCR-based markers include being a straight forward method that does not require specialised knowledge to interpret results and it is not time consuming. It is important for breeding programmes that focus on quality to identify the HMW-GS of the wheat lines for further selection (Liang et al. 2010). Some of the molecular markers used in the current study and linked to HMW-GS will be discussed further.

2.9.1 Ax2* marker

The dominant Ax2* PCR-based marker amplifies a fragment linked to the *Glu-A1b* (subunit *Ax2**) allele with a fragment size of 1319 bp and was developed by Ma and co-workers (2003). A previous study indicated that this subunit has positive effects on bread quality (Payne and Lawrence 1983). The forward primer is positioned within the coding region whereas the reverse primer is positioned in the central repetitive domain of the gene (Ma et al. 2003).

2.9.2 Dx5 marker

The *1Dx5* gene, located on the long arm of chromosome 1D, is expressed in the absence of such as *1Dx2.* The *1Dx5* and *1Dx2* genes have a high degree of homology and therefore it made it difficult to developed two primers for the *1Dx5* gene. The DNA sequence that had been used for primer development was restricted to the terminal non-repetitive coding region and the immediate flanking non-coding sequences. Due to the use of limited regions only two oligonucleotide primers specific to the *1Dx5* gene were developed due to a single nucleotide change in both regions. The amplification product size is 450 bp and is positioned just 3' of the TATA box to the beginning of the repetitive domain (D'Ovidio and Anderson 1994). The marker detects the *1Dx5* gene which is linked to the *1Dy10* gene (Payne 1987). The *Dx5+Dy10* allele combination is related to good bread-making quality characteristics which is not the case for the *Dx2+Dy12* allele combination which is also expressed by the *1D* locus (Payne 1987).

2.9.3 BxFp marker

Different HMW-GS allele combinations can be expressed at the *1B* locus such as *Bx7+By8*, *Bx6+By8* or *Bx17+By18*, depending on genetic background of the line or cultivar. Marker BxFp detects the *1Bx17* gene and can differentiate between different expression levels of *1Bx7* genes. The BxFp marker was developed by Ma and co-workers (2003) and is a co-dominant marker. The forward primer is positioned in the central repetitive domain and the reverse primer in the C-terminal coding region of the gene. The forward primer has two binding sites in the central domain of the gene and therefore two fragments are amplified. The amplification fragments for the presence of the *1Bx7* gene when it has a normal expression level are 650 bp and 750 bp. When the gene is over expressed in wheat is 670 bp and 770 bp fragments are amplified in size due to a duplication of 18 bp towards the end of the C-terminal of the central repetitive domain. When the *1Bx17* gene is present a fragment of 675 bp is amplified (Butow et al. 2003). The BxFp marker can distinguish between the most dominant allele combinations of the *Glu-1B* locus which are *Bx7+By8* or *Bx17+By18*. It can be used to distinguish between the over-expressed *Bx7* allele which normally combines with the *By8** allele or the normal *Bx7* allele (Ma et al. 2003). Increased expression of *Bx7* is associated with improved dough strength (Marchylo et al. 1992).

2.9.4 MAR marker

This co-dominant marker was developed by Butow and co-workers (2004). This marker amplifies the Bx matrix-attachment region (MAR) region which is located 750 bp upstream of the coding region. The marker will detect the presence of the over-expressed *Bx7* allele or it will ensure the line is without the allele combination *Bx7+By8** (*Glu-B1al* gene). A 563 bp fragment is associated with the over-expressed allele and includes a 43 bp insertion (possible gene duplication) in the MAR. A 520 bp fragment is associated with the *Bx7+By8** allele combination. Another amplification fragment of 800 bp in size indicates expression of the *Bx20* allele (Butow et al. 2004). It is important to detect the presence of the over-expressed *Bx7* allele as it improves dough quality. Due to the larger fragment size differences this marker is better to use than methods that cannot differentiate between the *Bx7* and the over-expressed *Bx7* (Butow et al. 2004). Expression of the *Bx7+By8* allele combination results in good bread-making quality (Payne 1987), while expression of *Bx17+By18* is better than *Bx6+By8* which is less frequently expressed (Morgunov et al. 1990).

2.9.5 ZSBy8F5/R5 marker

This allele specific primer pair was developed by Lei and co-workers in 2006. Forty different primer pairs were designed for the *By* loci based on SNPs which have been detected. The primer pair ZSBy8FS/R5 amplifies the *Glu-By8* allele when present. The forward primer was developed based on a C to T change found at position 983 of the accession X61026 in GenBank. This dominant marker amplifies a fragment size of 527 bp when the *Glu-By8* allele is present. This was the first dominant marker available for the *Glu-B1* allele and can discriminate between *By8* and *By8** which cannot be achieved by SDS-PAGE. The ability to discriminate between the *By8* and *By8** genes can also serve to discriminate between the *Bx7* or the over-expressed *Bx7* gene as the over-expressed gene is usually expressed in combination with the *By8** gene (Lei et al. 2006).

2.9.6 ZSBy9aF1/R3 marker

This primer pair was developed by Lei and co-workers 2006 during the same study of the previously described primer pair. This primer pair was initially used to amplify the *By* gene segment of different wheat cultivars. It was however later found that this primer pair can serve as a co-dominant marker to determine the presence or absence of the *Glu-By9* allele. This primer pair, ZSBy9aF1/R3, amplifies a 662 bp fragment which indicates the presence of the Glu-By9 allele, or a 707 bp fragment shows the absence of the *Glu-By9* allele. The difference in size between the two amplified fragments is due to a 45 bp deletion within the *By9* gene (Lei et al. 2006). Expression of the Bx7+By9 in combination with Ax2* and Dx5+Dy10 had the best baking quality of all lines tested in a study done by Horvat and co-workers (2009).

2.9.7 Glu-B3j marker

Rye is a source of alien genes often used to improve bread wheat. The 1BL.1RS translocation contains different disease resistant genes and is associated with increased yield potential. The dominant PCR-based marker has been developed by Francis and co-workers (1995) from a PCR product used in RAPD analysis. The marker detects whether or not the rye chromatin (1BL.1RS translocation) is present in different wheat lines. End sequencing of the PCR product amplified during RAPD analysis made it possible to develop two oligomer primers namely AF1, that includes bases 2 to 10 of the OPH20 sequence, and AF4, which is situated at the 3’ of the second OPH20 site. This dominant marker amplifies a 1500 bp fragment when the translocation is present. The presence of the translocation has a negative influence on bread-making qualities (Francis et al. 1995).

2.10 Environmental effects on protein expression in wheat

Grain end use quality is determined mainly by the storage proteins which are affected by the genotype and environmental conditions. The genotype affects the qualitative variation which is the different types of units and subunits expressed. The environment influences the qualitative variation which includes the total units and subunits. Environmental conditions, particularly temperature and fertilisation, have a great influence on protein content (Triboï et al. 2000). As environmental conditions cannot be regulated in field trails, great variation has been observed in studies as additional environmental conditions can also influence the examined trait.

Gluten expression takes place during the grain filling phase after anthesis and environmental conditions have a direct influence on the qualitative expression. Variation of gluten expression in wheat varieties was observed when planted in different seasons and localities and was mainly due to temperature differences. Higher mean temperatures (25°C to 32°C) during grain filling (1 to 29 days after heading) showed better gluten quality whereas lower mean temperatures were positively correlated with weaker gluten quality. No significant correlation was detected during later stadiums of grain development (Moldestad et al. 2011). Heat stress (35°C to 40°C) can shorten the grain filling phase and affects the accumulation of proteins and disturbs the balance between them (Triboï et al. 2003).

Nitrogen (N) fertilisation is the environmental factor that has the biggest influence on wheat protein content but the degree of influence is enhanced by other environmental conditions such as water availability and the amount of N in the soil. Wheat protein content is affected by N fertiliser rate, timing and method of application and therefore N fertilisation have to be well managed. N fertilisation does not only affect protein content but also yield and these two characteristics are negatively correlated. A study showed that late N fertilisation had the most positive effect on increased protein content. While N fertilisation during the vegetative growing phase had the most positive effect on yield, over fertilisation of N, which exceeds the plant's requirements, will lead to nitrate leaching and N gaseous emissions which can be harmful to the environment and has no positive effect for the producers (Abedi et al. 2011).

2.11 Conclusions

It is clear that breeders need to develop new wheat cultivars which are resistant to rust and FHB as these diseases are major threats to the world and epidemics can have terrible effects on food demand worldwide. As breeders want to improve disease resistance, they have to improve protein quality as well since it is one of the most important factors for the milling and baking industries as well as for consumers. Different methods have been developed to determine these characteristics which enhance the selection process of a breeding programme. Therefore a need exists to breed wheat lines with good rust and FHB resistance as well as good bread-making quality characteristics.

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Chapter 3

Molecular characterisation of rust and FHB resistant experimental wheat lines

3.1 Introduction

Plant breeding programmes generally aim to improve plant traits which matter to society. During the developmental stage of cultivars the genetic material is not stable due to recombination of parental chromosomes. Recombination can lead to the loss of genes/traits originally selected for if the trait was in a heterozygous state (Collard and Mackill 2008). Therefore it is necessary to follow the presence or absence of the originally selected trait during the breeding process. If markers have been developed that are linked to traits of interest, MAS can be used to determine the presence of the gene/QTL, otherwise plants have to be screened phenotypically (Winter and Kahl 1995). Molecular markers shorten the breeding cycle and selection of lines can be done from the first generation which increases the possibility that the selected traits are present in the offspring.

Breeding populations are developed by gene introgression which is a process where the donor line with the selected trait is crossed with a desirable line that does not contain the selected trait of interest. Offspring can then be backcrossed to the recurrent parent (recipient line) to eliminate sections of the donor parent genome associated with negative traits. The aim of backcrossing is to recover almost the entire genome of the recurrent parent without eliminating the trait of interest transferred from the donor parent. Offspring can also go through several rounds of self-pollination to produce nearly homozygous lines. However, offspring need to be screened and evaluated between generations to ensure the presence of the trait(s) of interest (Winter and Kahl 1995).

Wheat rust and FHB are two of the most threatening fungal diseases of wheat and contribute to food and economic losses worldwide. History has shown that pathogens responsible for these two diseases are capable of overcoming resistance which is a risk to food security. These fungi need to be monitored continuously to determine evolution patterns and new virulence patterns. Breeding new resistant cultivars is one way to ensure continuous resistance and to fight the threat of epidemics due to these diseases. New resistance sources need to be identified and these resistant genes/QTL need to be incorporated into breeding programmes. Resistant genes/QTL can be obtained from naturally occurring genetic variation in wheat or from wild types of wheat or other related species (Collard et al. 2005).

Genetic resistance in plants can be race-specific or race non-specific. Race-specific (qualitative) resistance is controlled by single genes with major to intermediate effects and is sometimes called seedling resistance. When the plant is challenged by the pathogen carrying the avirulence alleles, the race-specific genes for resistance activate a hypersensitive response. The effectiveness of the gene can be influenced by a single mutation of the pathogen and therefore resistance can easily be overcome. Race non-specific (quantitative) resistant genes/QTL are inherited quantitatively and have minor to moderate and additive effects and provide durable resistance and is also called APR (Kou and Wang 2010). Expression of resistance genes/QTL has different levels of effectiveness and is influenced by external factors such as the plant growth stage, climate conditions and inoculum pressure (Coram et al. 2008).

Rust resistance can be inherited through single genes, gene combinations or QTL. There are many rust resistance sources available and the main aim is to incorporate durable resistance into cultivars with good agronomic and quality traits (Vanzetti et al. 2011). Combining different genes using MAS for developing rust resistance cultivars is one of the fastest methods to use since many markers linked to rust resistance genes are available (Samsampour et al. 2010). Newly developed cultivars should contain both APR genes, which should be the first priority, as well as seedling genes for resistance to improve the durability in wheat cultivars. By combining these two types of resistance mechanisms, cultivars will have race and race-nonspecific resistance (Lowe et al. 2011).

FHB resistance is governed by minor and major QTL and the environment plays a big role in the severity levels of FHB. Each FHB QTL for resistance contributes only a certain percentage to resistance (Winter and Kahl 1995). The presence of FHB resistant QTL can be screened phenotypically (inoculation) or genotypically (MAS). Major QTL have a greater impact on controlling the severity levels of FHB than minor QTL. Salameh et al. (2011) stated that major QTL should first be incorporated and selected for in a FHB resistance breeding programme using MAS. These lines should then be evaluated phenotypically to determine the influence of minor QTL. Based on both MAS and phenotypic evaluation, the best FHB resistant lines can then be selected (Cuthbert et al 2007).

Over the last few years, wheat lines containing different combinations of wheat rust (stem, stripe and leaf rust) as well as FHB resistance genes/QTL have been developed at the University of the Free State (UFS). The aim of this study was to genotypically evaluate some of these lines using existing molecular markers linked to the rust and FHB resistance genes/QTL and to use this data to select and identify the best lines containing the highest number of resistance genes. The aim was also to determine the effect of self-pollination on the segregation of resistance genes/QTL, thus evaluation was done for two consecutive years.

3.2 Materials and methods

The main aim of this MSc study was to evaluate lines with MAS to test for the presence of rust and FHB resistant genes/QTL but also to determine the quality of these lines using different protein methods as well as markers linked to quality. Therefore lines/cultivars with known good and weak baking qualities were included to serve as controls. The initial parental lines which were used to developed the rust and FHB experimental lines were also included.

3.2.1 Development of rust resistant lines used in the current study

In the current study two main populations were used for analysis. The two main populations were rust and FHB resistant populations, which had been developed during previous studies done at the UFS. These populations contained different lines with different levels of either rust or FHB resistance. Based on molecular evaluation of these two different populations the best resistant lines in each of these populations, respectively, were identified for use in the on-going breeding programme at the UFS.

The rust resistant population has been previously developed by crosses made between four parental lines which contributed different rust resistance genes/QTL. The first cross was made between Kariega (*Lr34/Yr18/Sr57* and *QYr.sgi.2B-1*) and Avocet*YrSp* (*YrSp* and *Sr26*). The second cross was made between Blade (*Sr2* and *Sr26*) and CS-*Lr19*-149-299 (*Lr19* and *Lr34/Yr18/Sr57*). Offspring (F_1) of these two crosses were then crossed to produce a double cross population. Lines of the double cross population were then evaluated using MAS to identify these lines' resistance gene/QTL composition (Sydenham 2007).

After MAS evaluation, two lines namely line S16 containing *Lr34/Yr18/Sr57*, *QYr.sgi.2B-1*, *Lr19* and *Sr2* as well as line S726 containing *Lr34/Yr18/Sr57*, *QYr.sgi.2B-1*, *Lr19*, *Sr26* and *Sr2* have been identified based on the total number of markers present as well as the level of homosygosity. Seed of lines S16 and S726 were planted and self-pollinated to overcome the heterozygous state of some genes/QTL. However, self-pollination can result in the loss of some genes/QTL and therefore lines had to be evaluated again using MAS to determine the resistance gene/QTL status of the evaluated lines. After self-pollination, two lines were selected namely lines S16(7.3) and

S726(3.2) containing the following rust resistance gene/QTL profiles: *Lr34/Yr18/Sr57*, *QYr.sgi.2B-1*, *Lr19* and *YrSp* for line S16(7.3) and *Lr34/Yr18/Sr57*, *Lr19*, *Sr26* and *Sr2* for line S726(3.2). MAS indicated the absence of the *Sr2* stem rust gene for resistance in the S16(7.3) line and the absence of *QYr.sgi.2B-1* in line S726(3.2). Another promising line identified in the previous study was line S346 (*Lr34/Yr18/Sr57, QYr.sgi.2B-1, Sr26, Sr2* and *Lr19*; Sydenham 2007).

As part of another MSc study at the UFS, seed of lines S16(7.3) and S726(3.2) were planted and self-pollinated and sampled for MAS. The best lines identified after evaluation were line S16(7.3)P1.5.1 containing *Lr34/Yr18/Sr57, QYr.sgi.2B-1* and *Lr19* and line S726(3.2)P1.3.1 containing *Lr34/Yr18/Sr57, Sr2, Lr19* and *Sr26*. During the same study other lines were also evaluated with the same molecular markers as summarised in Table 3.1 (K.J. Senoko, unpublished data).

Table 3.1 Additional rust resistant lines and their gene/QTL combinations developed during a previous study

Line name	Rust resistance genes/QTL
S16(1.2)	*QYr.sgi.2B-1, Lr34/Yr18/Sr57, Lr19, Sr2*
S16(7.3)P3.5.1	*QYr.sgi.2B-1, Lr34/Yr18/Sr57, Lr19*
S16(7.3)P3.6.1	*QYr.sgi.2B-1, Lr34/Yr18/Sr57, Lr19*
S16(7.3)P3.2.1	*QYr.sgi.2B-1, Lr34/Yr18/Sr57, Lr19*
S178(7.2)	*QYr.sgi.2B-1, Lr34/Yr18/Sr57, Sr2, Sr26*
S726(3.2)	*Lr34Yr18/Sr57,Lr19, Sr2,Sr26*

The two markers used to screen for the presence of the *Lr19* and *Sr26* genes are dominant markers and therefore it could not be determined whether the *Lr19* or *Sr26* genes identified in these individual lines were homo- or heterozygous. Presence for the *YrSp* gene could only be detected phenotypically and therefore it was not possible to determine whether the gene was homo- or heterozygous when present. The *Lr34/Yr18/Sr57* gene complex was homozygous in all lines, due to its presence in both F_1 populations obtained after the initial crosses between the parental lines. The two markers, Gwm501 and Gwm148, are co-dominant markers flanking the QTL region and therefore the homo-or heterozygous state for the *QYr.sgi.2B-1* QTL could be determined in the tested wheat lines. After the first round of self-pollination of line S726 the *QYr.sgi.2B-1* QTL was lost but it was still expressed in a homozygous state in line S16(7.3).

Based on the evaluation of data generated during of the two previous studies, best lines were selected based on the number of markers present within each line, highest levels of homozygosity or different combinations of rust genes present, especially lines containing the *Sr2* gene as it has been eliminated during the self-pollination process. Another selection criteria was the number of seeds available for planting. Table 3.2 summarises the lines which have been selected for the current study for further MAS analysis in order to determine the status of the rust resistance genes/QTL as well as the protein quality of these lines. It is important to note that the last three experimental lines in Table 3.2 have not been self-pollinated during previous studies and therefore genes/QTL evaluated were still in a heterozygous state. Figure 3.1 illustrates the breeding scheme and line development for the two best rust resistance lines identified in the first study. Therefore, 14 rust resistant experimental lines were selected for the current study (Table 3.2). Different numbers of seed were planted for the 14 selected rust lines based on seed availability. For most lines 18 seeds were planted but for two lines only two or four seeds were planted. Since not all seed germinated only 203 plants were sampled during the first year for MAS and biochemical analysis. For the second year of analysis, the best 22 self-pollinated lines were selected based on data produced from the 203 lines analysed in the first year. One to six seeds of each of the selected 22 lines were planted based on seed availability and resulted in 88 lines being analysed during the second year.

3.2.2 Development of FHB resistant lines used in the current study

The experimental FHB resistant lines used in the current study have been developed by S.L. Sydenham (unpublished data) for a PhD study at the UFS. Lines were developed by an initial cross between CM-82036 (FHB resistant donor line) and the South African cultivar, Krokodil (recipient line and FHB susceptible). The offspring (F_1) were backcrossed to Krokodil to create a backcross one (BC_1) population. Krokodil was selected as recurrent parent because it is a South African cultivar which is well adapted but lack FHB resistance. The BC_1 lines were backcrossed to Krokodil to produce a BC_2 population. The BC_2 lines were planted and self-pollinated to produce a BC_2F_2 population which were evaluated using MAS to determine the number of FHB resistance QTL present within the individual lines. MAS evaluation of these lines indicated that these lines contained between one and four QTL (one chromosomes 3B, two on 5A, one each on 6B and 7A) associated with FHB resistance.

For the current study, 11 of the BC_2F_2 lines containing four FHB QTL each were selected and planted (Table 3.3). Depending on the number of seed available, between 10 and 18 seeds were planted per BC_2F_2 line and a total of 152 individuals were obtained from the selected 11 lines.

Table 3.2 Summary of selected lines used for rust resistance analysis in the current study

Line name	Line background	Expected resistance/characteristic
Kariega	Rust resistance parental line	*Lr34/Yr18/Sr57, QYr.sgi.2B-1*
Avocet*YrSp*	Rust resistance parental line	*YrSp, Sr26*
Blade	Rust resistance parental line	*Sr2, Sr26*
CS-*Lr19*-149-299	Rust resistance parental line	*Lr19, Lr34/Yr18/Sr57*
Krokodil	Recurrent parent, FHB susceptible	South African commercially planted cultivar
CM-82036	Donor parent, FHB resistant	FHB resistance (Type II)
Frontana	Control line	FHB resistance (Type I)
Sumai 3	Control line	FHB resistance (Type II)
PAN3434	Control line	South African commercially planted cultivar
SST835	Control line	South African commercially planted cultivar
Duzi	Control line	South African commercially planted cultivar
Elands (SGI)	Control line	High baking quality
Kariega (SGI)	Control line	High baking quality
Tugela-DN (SGI)	Control line	High baking quality
Scheepers69 (SGI)	Control line	Low baking quality
Picaflor	Control line	Ug99 resistance
Damphe	Control line	Ug99 resistance
Kingbird	Control line	Ug99 resistance
LineT6-1 (*SrR+Sec*$^{-}$)	Control line	Ug99 resistance
Line 2S#/163 (*Sr39*)	Control line	Ug99 resistance
S16(7.3)	Experimental rust resistant line	*Lr34/Yr18/Sr57, QYr.sgi.2B-1, Lr19*
S16(7.3)P1.5.1	Experimental rust resistant line	*Lr34/Yr18/Sr57, QYr.sgi.2B-1, Lr19*
S16(7.3)P3.5.1	Experimental rust resistant line	*Lr34/Yr18/Sr57, QYr.sgi.2B-1, Lr19*
S16(7.3)P3.6.1	Experimental rust resistant line	*Lr34/Yr18/Sr57, QYr.sgi.2B-1, Lr19*
S16(7.3)P3.2.1	Experimental rust resistant line	*Lr34/Yr18/Sr57, QYr.sgi.2B-1, Lr19*
S16(1.2)	Experimental rust resistant line	*QYr.sgi.2B-1, Lr34/Yr18/Sr57, Sr2, Lr19*
S726(3.2)	Experimental rust resistant line	*Sr26, Sr2, Lr34/Yr18/Sr57*
S726(3.2)P1.3.1	Experimental rust resistant line	*Sr26, Lr34/Yr18/Sr57, Lr19*
S726(3.2)P2.4.1	Experimental rust resistant line	*Sr26, Lr34/Yr18/Sr57, Lr19*
S726(3.2)P3.6.1	Experimental rust resistant line	*Sr26, Lr34/Yr18/Sr57, Lr19*
S726(2.2)	Experimental rust resistant line	*Sr26, Lr34/Yr18/Sr57*
S178(7.2)	Experimental rust resistant line	*Sr26, Lr34/Yr18/Sr57, Sr2, QYr.sgi.2B-1*
S346	Experimental rust resistant line	*Lr19, QYr.sgi.2B-1, Sr26, Sr2, Lr34/Yr18/Sr57*
S157	Experimental rust resistant line	*Sr26, Lr34/Yr18/Sr57, QYr.sgi.2B-1*

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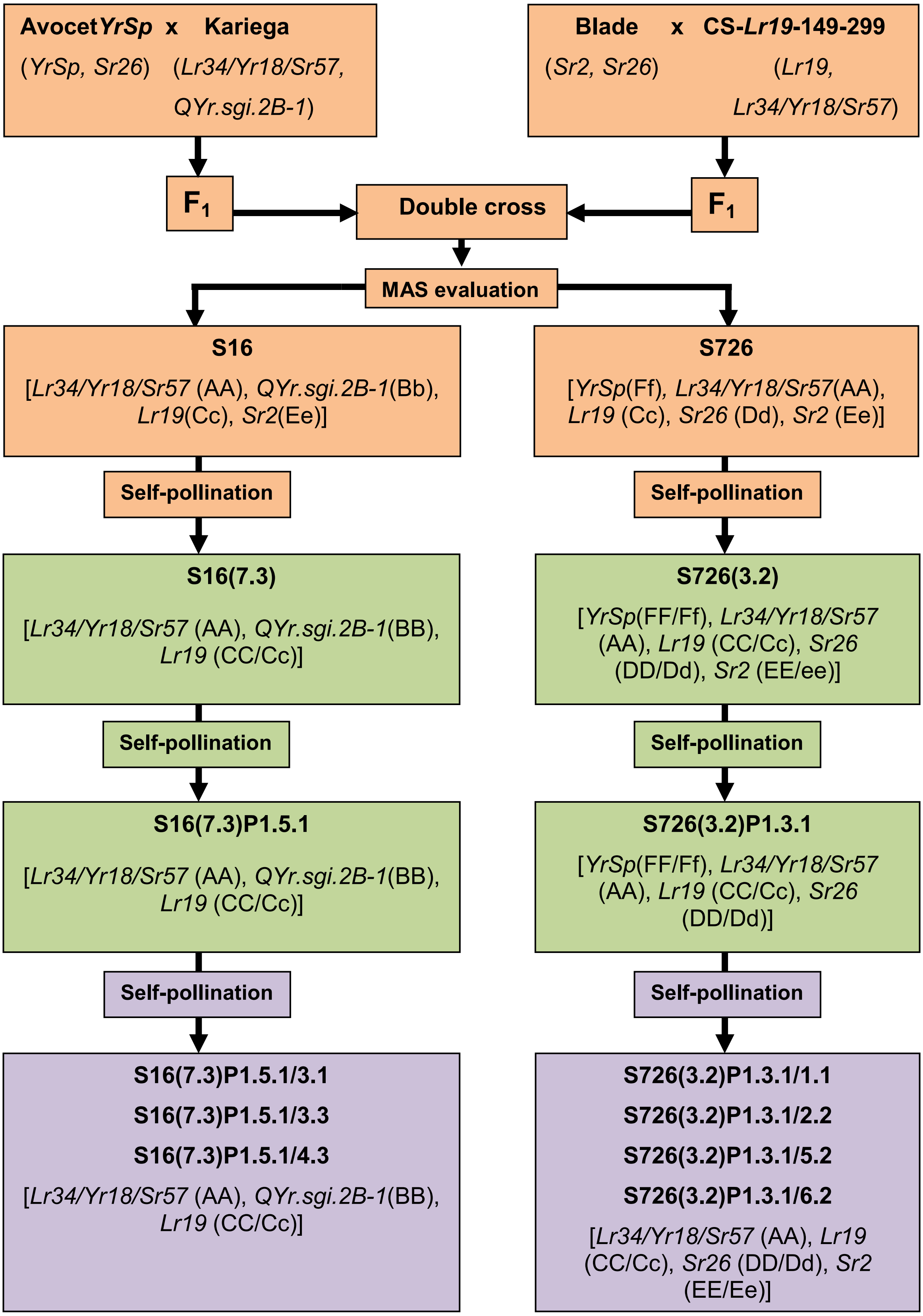

Figure 3.1 **Illustration of the development of the best rust experimental lines used in the study.** The diagram also includes the homozygosity level of the genes/QTL within lines as obtained from MAS analysis. The different colours indicate the different studies. The purple colour indicates the starting point of the current study. The orange (S.L. Sydenham) and green (K.J. Senoko) colours indicate the two previous studies done to develop wheat rust resistance lines.

Table 3.3 Summary of selected lines used for the FHB resistance analysis in the current study

Line name	Line background	Expected resistance/characteristic
Kariega	Rust resistance parental line	*Lr34/Yr18/Sr57, QYr.sgi.2B-1*
Avocet*YrSp*	Rust resistance parental line	*YrSp, Sr26*
Blade	Rust resistance parental line	*Sr2, Sr26*
CS-*Lr19*-149-299	Rust resistance parental line	*Lr19, Lr34/Yr18/Sr57*
Krokodil	Recurrent parent, FHB susceptible	South African commercially planted cultivar
CM-82036	Donor parent, FHB resistant	FHB resistance (Type II)
Frontana	Control line	FHB resistance (Type I)
Sumai 3	Control line	FHB resistance (Type II)
PAN3434	Control line	South African commercially planted cultivar
SST835	Control line	South African commercially planted cultivar
Duzi	Control line	South African commercially planted cultivar
Elands (SGI)	Control line	High baking quality
Kariega (SGI)	Control line	High baking quality
Tugela-DN (SGI)	Control line	High baking quality
Scheepers69 (SGI)	Control line	Low baking quality
Picaflor	Control line	Ug99 resistance
Damphe	Control line	Ug99 resistance
Kingbird	Control line	Ug99 resistance
LineT6-1 (*SrR*+*Sec*$^-$)	Control line	Ug99 resistance
Line 2S#/163 (*Sr39*)	Control line	Ug99 resistance
BC_2F_2P2 6.2 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2P2 14.2 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2P1 7.2 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2 4.1 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2P1 7.1 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2P1 7.3 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2P2 2.3 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2P2 3.2 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2P2 12.1 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2P1 5.3 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL
BC_2F_2P1 2.1 (4QTL)	Experimental FHB resistant line	Four FHB resistant QTL

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Leaf material was sampled and plants were left to self-pollinate and seed was harvested. A total of 55 lines out of the152 FHB BC_2F_2 lines were selected for analysis.

These 55 lines consisted of five individuals of each of the 11 selected lines and these 55 lines were used for FHB resistant marker analysis as well as biochemical analysis for protein quality. At total number of 25 FHB resistant BC_2F_2 lines were selected based on the number of FHB resistant markers present as well as the number of seed available for the planting of the second year. Seed of these 25 BC_2F_2 lines were planted and resulted in 121 FHB resistant BC_2F_3 offspring lines that were sampled for the second year marker analysis and lines were left to self-pollinate and seed harvested and used for biochemical analysis. Figure 3.2 illustrates the breeding scheme followed for the development of FHB resistant lines.

Rust resistant, FHB resistant and control genotypes were grown in the greenhouse with temperatures ranging from 18°C to 22°C. Seed was planted in two litre containers filled with red garden soil. Seed was planted one to three seeds per pot, depending on the number of seeds planted per line. Plants were watered once daily. Once a week during the seedling stage of plant growth 100 ml of microelements (2.5 g/l HYPERFEED) were applied to each pot. When the plants have developed flag leaves, macroelements [10 ml/l EFFECTO Wonder Slow Release 3:2:1 (28)] were applied, weekly. Wheat heads were covered with bags to prevent cross-pollination.

3.2.3 Leaf sample collection

Young leaf material was collected seven weeks after planting. Three 10 cm leaves per plant were cut and placed in 15 ml plastic tubes. The scissor and tweezers used were cleaned with 70% (v/v) ethanol between each cutting to prevent contamination. Tubes containing leaf samples were kept on ice during sampling. Leaf samples were freeze-dried using the Viritis Advantage Freeze Mobile II (New York, NY, USA) for three to five days and stored at -20°C till DNA extraction. Seed of individual plants was harvested separately according to the same numbering system as for the sampling of leaf material. Seed was stored in 15 ml tubes in the germplasm bank at 5°C.

3.2.4 Genomic DNA isolation

For each sample, five 1 cm freeze-dried leaf pieces were cut and placed in a 2 ml microcentrifuge tube. Two 5 mm stainless steel ball bearings were added per sample to grind the leaf material to a fine powder using Qiagen'sTissueLyser (Haan, Germany) for

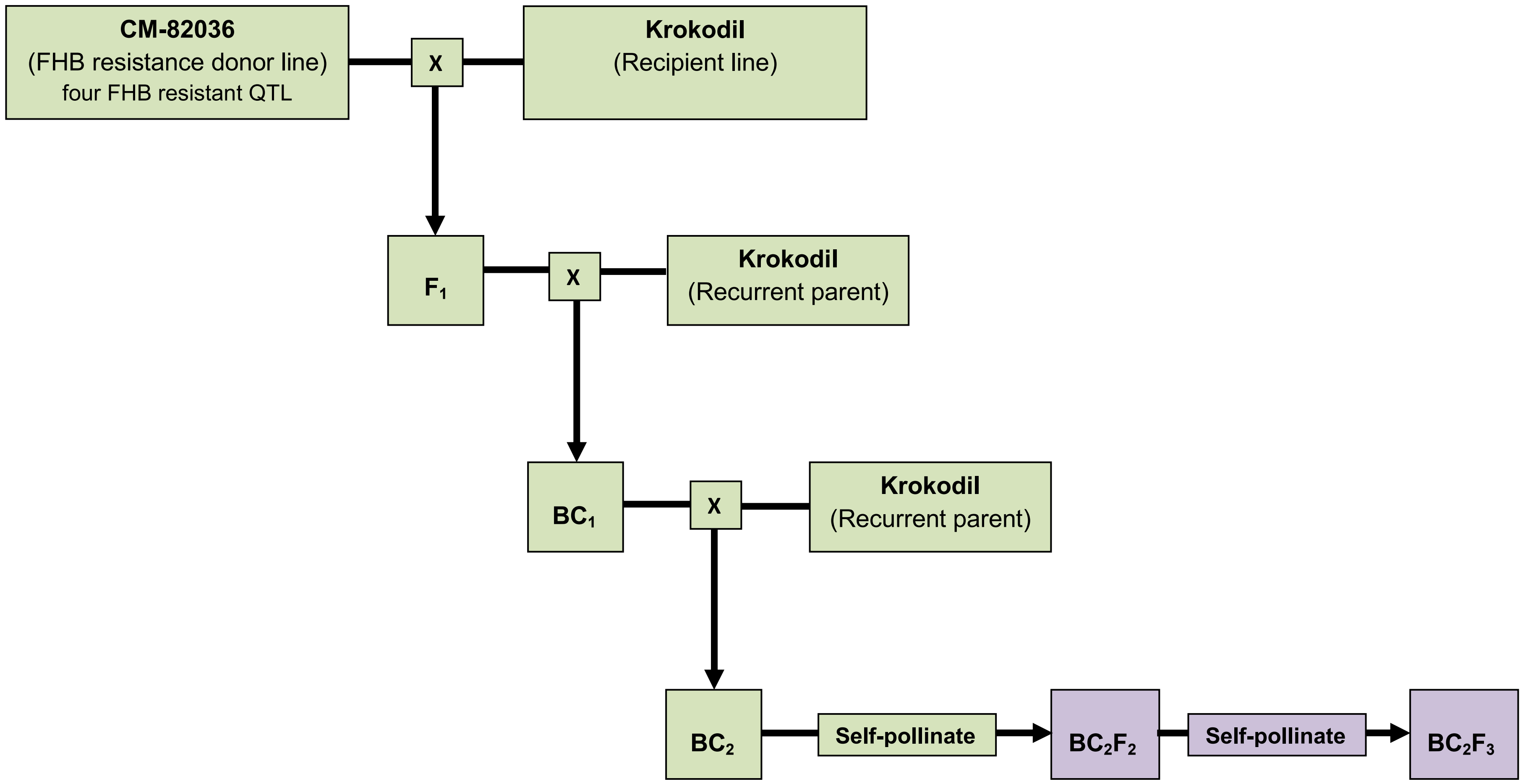

Figure 3.2 Schematic illustration of the development of the FHB resistance backcross two population. The two different colours indicate two separate studies during which the lines were developed. The purple coloured steps indicate the component of the current study, while the green coloured steps indicate the work done by S.L. Sydenham for his PhD study.

1 min at 30 r/s. Genomic DNA (gDNA) was isolated using a modified CTAB (hexadecyltrimethylammonium bromide) method (Saghai-Maroof et al. 1984). A volume of 750 µl CTAB buffer [100 mM tris(hyroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0; 20 mM EDTA (ethylene-diaminetetraacetate), pH 8.0; 1.4 M sodium chloride (NaCl); 2% (w/v) CTAB; 0.2% (v/v) β-mercaptoethanol] was added to the leaf material and mixed well. Samples were incubated in the water bath for 1 h at 65°C. After incubation, 500 µl chloroform:isoamylalcohol [24:1 (v/v)] was added to each sample and mixed well followed by centrifugation at 12000 g for 5 min at 5°C. The supernatant containing the DNA was transferred in a 1.5 ml microcentrifuge tube containing 500 µl (0.66 volumes) 2-isopropanol. DNA was precipitated for 20 min at room temperature (22°C) followed by centrifugation at 12000 g for 5 min at 5°C. The supernatant was discarded and the precipitate washed with 500 µl ice cold 70% (v/v) ethanol for 20 min at room temperature (22°C) and centrifuged at 12 000 g for 5 min at 5°C. The supernatant was discarded and the DNA pellets were air-dried for 1 h at room temperature (22°C). Air-dried DNA pellets were resuspended in 200 µl Tris-Cl/EDTA (TE) buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) and RNase to a final concentration of 200 µl/ml was added followed by incubation in the water bath for 2 h at 37°C. The quality of the gDNA was determined using a 0.8% (w/v) agarose gel and ethidium bromide (EtBr) to visualise the DNA. Electrophoresis was done at 80 V for 1 h in 1x UNTAN buffer (40 mM Tris-HCl; 2 mM EDTA; pH adjusted to pH 7.4 with acetic acid). The DNA quantity was determined with the Helios gamma spectrophotometer (Erlangen, Germany) measuring absorbance at A_{260}, A_{280} and the A_{260}:A_{280} ratio. Isolated gDNA samples were diluted to a final working concentration of 20 ng/µl using 0.1 x TE buffer, pH 8.0 for the screening using SSR markers.

3.2.5 Molecular SSR analysis

All PCR reactions were performed using a DYAD™ (DNA Engine) Peltier Thermal Cycler (Foster City, California, USA).

3.2.5.1 Markers linked to rust resistance

Seven markers linked to rust resistance were selected for screening of the rust resistant lines (Table 3.4). Markers were selected based on previous studies done on the developed lines. PCR were set-up in either a final volume of 20 µl for markers cssfr5 and Gwm501 or in 10 µl for the remaining rust resistant markers. Positive and negative controls were included for every PCR set-up.

Table 3.4 Selected markers linked to rust resistance genes/QTL used in the current study

Targeted trait	Targeted gene/QTL	Marker name	Forward primer(5'-3')	Reverse primer (5'-3')	Reference
Leaf rust resistance	*Lr19*	STSLr19$_{130}$	CATCCTTGGGGACCTC	CCAGTCGCATACATCCA	Prins et al. 2001
Leaf rust resistance	*Lr34/Yr18/Sr57*	cssfr5	L34SPF: GGGAGCATTATTTTTTTCCATCATG L34DINT9F: TTGATGAAACCAGTTTTTTTTCTA	L34DINT13R2: ACTTTCCTGAAAATAATACAAGCA L34MINUSR: TATGCCATTTAACATAATCATGAA	Lagudah et al. 2009
Stem rust resistance	*Sr2*	csSr2	CAAGGGTTGCTAGGATTGGAAAAC	AGATAACTCTTATGATCTTACATTTTTCTG	Mago et al. 2010
Stem rust resistance	*Sr26*	Sr26#43	AATCGTCCACATTGGCTTCT	CGCAACAAAATCATGCACTA	Mago et al. 2005
Stem rust susceptible	*Sr26*	BE 518379	AGCCGCGAAATCTACTTTGA	TTAAACGGACAGAGCACACG	Liu et al. 2010
Stripe rust resistance	*QYr.sgi.2B-1*	Gwm148	GTGAGGCAGCAAGAGAGAAA	CAAAGCTTGACTCAGACCAAA	Röder et al. 1998
Stripe rust resistance	*QYr.sgi.2B-1*	Gwm501	GGCTATCTCTGGCGCTAAAA	TCCACAAACAAGTAGGCGCC	Röder et al. 1998

3.2.5.2 Markers linked to FHB resistance

For screening the selected FHB resistant lines, six markers were selected (Table 3.5) to determine the presence or absence of the FHB resistance QTL. Two of the four FHB genes/QTL for resistance (*Fhb1* and *Qfhs.ifa-5A-1*) tested for, had flanking markers while for the other two genes/QTL (*Qfhs.ifa-5A-2* and *Fhb2*) only one marker each linked to the related FHB resistant genes/QTL was used. Since the other two flanking markers for these two genes/QTL gave unreliable results, they were not included during further tests. PCR reactions for all selected FHB resistant markers were set-up in final volumes of 10 µl. For every PCR set-up controls were included.

3.2.5.3 PCR reaction conditions

Reaction conditions for rust and FHB resistant markers have been optimised and modified based on Sydenham (2007; rust markers) and Sydenham (unpublished data; FHB markers). PCR conditions for all markers, except cssfr5, have been optimised for the use with the ReadyMix of KAPABIOSYSTEMS (Woburn, MA, USA). For cssfr5 GoTaq® Flexi DNA Polymerase was used (Promega, Madison, WI, USA). All PCR primers were synthesised by Intergrated DNA Technologies Inc, (Coralville, IA, USA). All reactions conditions are presented in Table 3.6.

3.2.6 Visualisation of PCR reactions

3.2.6.1 Polyacrylamide gel electrophoresis

PCR products for all markers linked to FHB resistance as well as STSLr19$_{130}$, Sr26#43, Gwm148 and Gwm501 linked to rust resistance were screened using the Gel-Scan 3000 Real-Time DNA Fragment Analysis system with software version 8.00.01 (Corbett Research, Sydney, Australia). The 5% non-denaturing polyacrylamide gel was made to a final volume of 25 ml consisting of 1x Tris-Cl/borate/EDTA (TBE) buffer (89 mM Tris-base; 89 mM boric acid; 2 mM EDTA, pH 8.0), 5% acrylamide:bis-acrylamide (19:1 w/w), 0.12% (v/v) tetramethylethylenediamine (TEMED) and 0.08% (v/v) ammonium persulfate (APS).Gels were poured and left to set overnight. The upper chamber buffer contained 0.5x TBE buffer mixed with deionised water (dH_2O). The bottom chamber contained 0.5x TBE buffer and 1% (v/v) EtBr mixed with dH_2O. The PCR products were mixed with deionised formamide loading dye (98% (v/v) formamide; 10 mM EDTA, pH8.0; 0.05% (w/v) bromophenol blue).

The volume of loading dye added varied from PCR reaction to PCR reaction and from run to run, depending on the signal strength of reactions. Before the samples were loaded on the gel, a pre-run was done at 800 V for 45 min at 37°C and 1 µl loading dye was loaded on the gel to track the progress of the pre-run.

Table 3.5 Selected markers linked to FHB resistance QTL used in the current study

Targeted trait	Targeted gene/QTL	Marker name	Forward primer(5’-3’)	Reverse primer (5’-3’)	Reference
FHB resistance	*Fhb1* (*Qfhs.ndsu-3BS*)	Barc133	AGCGCTCGAAAAGTCAG	GGCAGGTCCAACTCCAG	Röder et al. 1998
FHB resistance	*Fhb1* (*Qfhs.ndsu-3BS*)	Gwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	Röder et al. 1998
FHB resistance	*Qfhs.ifa-5A-2*	Gwm156	CCAACCGTGCTATTAGTCATTC	CAATGCAGGCCCTCCTAAC	Röder et al. 1998
FHB resistance	*Qfhs.ifa-5A-1*	Gwm293	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	Röder et al. 1998
FHB resistance	*Qfhs.ifa-5A-1*	Gwm304	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	Röder et al. 1998
FHB resistance	*Fhb2*	Gwm133	ATCTAAACAAGACGGCGGTG	ATCTGTGACAACCGGTGAGA	Röder et al. 1998

Table 3.6 Optimised PCR reaction conditions for the selected SSR markers

Marker name and targeted allele	Reaction conditions	PCR cycling conditions	Marker type, controls and fragment sizes
cssfr5 *Lr34*	100 ng DNA 1x GoTaq® Flexi buffer (Promega)[1] 2.25 mM $MgCl_2$ 0.05 mM dNTPs 50 ngL34SPF 50 ng L34DINT13R2 50 ngL34DINT9F 50 ngL34MINUSR 1 U Go Taq® DNA polymerase (Promega)	1 cycle:5 min @ 94°C, 5 cycles:1 min @ 94°C, 1 min @ 58°C, 2 min @ 72°C, 30 cycles:30 sec @ 94°C, 30 sec @ 58°C,50 sec @ 72°C, 1 cycle: 30 sec @ 94°C, 30 sec @ 58°C, 5 min @ 72°C	Co-dominant Resistant: Kariega, Avocet*YrSp* and CS-*Lr19*-149-299 (751 bp) Susceptible: Blade (532 bp)
STSLr19$_{130}$ *Lr19*	80 ng DNA 1x ReadyMix[2] (KAPABIOSYSTEMS) 25 ng Lr19F 25 ngLr19R 0.1 mg/ml BSA	1 cycle:3 min @ 94°C, 45 cycles:1 min @ 94°C, 1 min @ 60°C, 2 min @ 72°C, 1 cycle:10 min @ 72°C	Dominant Resistant:CS-*Lr19*-149-299 (130 bp) Susceptible: Kariega, Avocet*YrSp* and Blade (null allele)
Sr26#43 *Sr26* resistant allele	80 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 50 ng Sr26#43F 50 ng Sr26#43R	1 cycle:3 min @ 94°C, 45 cycles:45 sec @ 94°C, 45 sec @ 60°C, 1.15 min @ 72°C, 1 cycle:10 min @ 72°C	Dominant Resistant: Avocet*YrSp* and Blade (207 bp) Susceptible: Kariega and CS-*Lr19*-149-299 (null allele)
BE518379 *Sr26* susceptible allele	60 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 50 ng BE518379F 50 ng BE518379R	1 cycle: 3 min @ 94°C, 35 cycles:1 min @ 94°C, 1 min @ 58°C, 2 min @ 72°C, 1 cycle: 10 min @ 72°C	Dominant Resistant: Avocet*YrSp* and Blade (null allele) Susceptible: Kariega and CS-*Lr19*-149-299 (303 bp)

Table 3.6 Optimised PCR reaction conditions for the selected SSR markers (continued)

Marker name and targeted allele	Reaction conditions	PCR cycling conditions	Marker type, controls and fragment sizes
Gwm501 *QYr.sgi.2B-1*	100 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 75 ng Gwm501F 75 ng Gwm501R 0.1 mg/ml BSA	1 cycle: 3 min @ 94°C, 35 cycles: 1 min @ 94°C, 30 sec @ 62.7°C, 1 min @ 72°C, 1 cycle: 10 min @ 72°C	Co-dominant Resistant: Kariega (177 bp) Susceptible: Avocet*YrSp* (168 bp), Blade (165 bp) and CS-*Lr19*-149-299 (174 bp)
Gwm148 *QYr.sgi.2B-1*	80 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 25 ng Gwm148F 25 ng Gwm148R	1 cycle: 3 min @ 94°C, 45 cycles: 1 min @ 94°C, 1 min @ 57°C, 2 min @ 72°C, 1 cycle: 10 min @ 72°C	Co-dominant Resistant: Kariega (165 bp) Susceptible: Avocet*YrSp* (145 bp), Blade (142 bp) and CS-*Lr19*-149-299 (162 bp)
csSr2 *Sr2*	100 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 75 ng csSr2F 75 ng csSr2R	1 cycle: 3 min @ 93°C, 40 cycles:45 sec @ 95°C, 45 sec @ 56.7°C, 45 sec @ 72°C, 1 cycle: 10 min @ 72°C	Co-dominant Positive and false positives (337 bp)
CAPS reaction	1x NEB Buffer 4 10 U *Bsp*HI	Incubate for 60 min @ 37°C	Resistant: Picaflor and Kingbird (172, 112 and 53 bp) Susceptible: Kariega, Avocet*YrSp*, Blade and CS-*Lr19*-149-299 (225 and 112 bp)

Table 3.6 Optimised PCR reaction conditions for the selected SSR markers (continued)

Marker name and targeted allele	Reaction conditions	PCR cycling conditions	Marker type, controls and fragment sizes
Barc133 *Qfhs.ndsu-3BS/Fhb1*	40 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 0.5 mM $MgCl_2$ (additionally) 25 ng Barc133F 25 ng Barc133R	1 cycle: 5 min @ 94°C, 35 cycles: 1 min @ 94°C, 1 min @ 60°C, 2 min @ 72°C, 1 cycle: 5 min @72°C	Co-dominant Resistant: CM-82036 (125 bp) Susceptible: Krokodil (110 bp)
Gwm533 *Qfhs.ndsu-3BS/Fhb1*	80 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 25 ng Gwm533F 25 ng Gwm533R	1 cycle: 5 min @ 95°C, 35 cycles: 30 sec @ 95°C, 45 sec @ 58°C, 30 sec @ 72°C, 1 cycle: 5 min @72°C	Co-dominant Resistant: CM-82036 (160 bp) Susceptible: Krokodil (120 bp)
Gwm156 *Qfhs.ifa-5A-2*	80 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 0.5 mM $MgCl_2$(additionally) 25 ng Gwm156F 25 ng Gwm156R	1 cycle: 5 min @ 94°C, 35 cycles: 1 min @ 94°C, 1 min @ 52°C, 2 min @ 72°C, 1 cycle: 5 min @72°C	Co-dominant Resistant: CM-82036 (330 bp) Susceptible: Krokodil (295 bp)
Gwm293 *Qfhs.ifa-5A-1*	80 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 0.5 mM $MgCl_2$ (additionally) 25 ng Gwm293F 25 ng Gwm293R	1 cycle: 3 min @ 94°C, 45 cycles: 45 sec @ 94°C, 45 sec @ 55°C, 75 sec @ 72°C, 1 cycle: 10 min @72°C	Co-dominant Resistant: CM-82036 (207 bp) Susceptible: Krokodil (174 bp)
Gwm304 *Qfhs.ifa-5A-1*	40 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 0.5 mM $MgCl_2$ (additionally) 25 ng Gwm304F 25 ng Gwm304R	1 cycle: 3 min @ 94°C, 40 cycles: 30 sec @ 94°C, 45 sec @ 60°C, 75 sec @ 72°C, 1 cycle: 10 min @72°C	Co-dominant Resistant: CM-82036 (219 bp) Susceptible: Krokodil (174 bp)
Gwm133 *Fhb2*	80 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 0.5 mM $MgCl_2$ (additionally) 25 ng Gwm133F 25 ng Gwm133R	1 cycle: 5 min @ 94°C, 35 cycles: 1 min @ 94°C, 1 min @ 56.7°C, 2 min @ 72°C, 1 cycle: 10 min @72°C	Co-dominant Resistant: CM-82036 (192 bp) Susceptible: Krokodil (133 bp)

[1] 1 x Promega buffer: 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 0.1% (v/v) Triton X-100; [2] 1 x ReadyMix contains 1.5 mM magnesium chloride ($MgCl_2$); dNTP - deoxynucleotide triphosphate; BSA - Bovine serum albumin; NEB - New England Biolabs

Samples (total of 1 µl) were loaded on the gel and run at 1200 V for 45 min at 37°C. The 25 bp HyperLadder™V ladder (Bioline, Taunton, MA, USA) was loaded on both sides of the gel and used to determine the sizes of the amplified fragments.

3.2.6.2 Agarose gel electrophoresis

The PCR products of primer pairs cssfr5, csSr2 and BE518379 were analysed through agarose gel electrophoresis on 1.5%, 2.5% and 2% agarose gels, respectively. PCR products were mixed with Ficoll loading dye (15% (w/v) Ficoll; 0.24% (w/v) bromophenol blue) and loaded on different percentage agarose gels and visualised using EtBr staining. Gels were run at 100 V for 1 hour in 1x UNTAN buffer. Fragment sizes were determined by comparison with lambda DNA cut with *Eco*RI and *Hin*dIII or a 100 bp DNA ladder (Promega).

3.2.7 Data analysis

Data were generated from two data sets. For the first data set, resistant and control lines were planted in January 2011 and leaves sampled for genotypic analysis from 14-day old seedlings. These lines were self-pollinated and seed harvested. The second data set was generated from leaf material sampled from the self-pollinated seed planted in July 2012. The SSR markers were screened on all experimental lines for both years. Lines were scored based to the type of SSR marker used. For dominant rust and FHB markers, only the presence (1) or absence (0) of the amplified fragment was scored. Co-dominant markers were scored by indicating whether it was homozygous resistant (1), heterozygous resistant (0.5) or homozygous susceptible (0). For the determination of the number of rust or FHB resistant genes/QTL present per line, markers that were heterozygous were scored as a gene/QTL present.

3.3 Results

3.3.1 Rust genotyping

3.3.1.1 Data generated from first year of screening

During the first year a total of 203 experimental wheat lines and 20 parental and control lines or cultivars were screened and scored using the seven rust resistant markers described in Table 3.4. The complete MAS analysis data set of the rust markers tested on all 203 experimental first year rust resistant lines is given in Appendix I. Most of the tested lines contained three markers linked to rust resistance, while a few lines with only one marker linked to rust resistance were observed.

The four parental lines contained the expected markers, except for Blade that tested negative for the expected resistant gene *Sr2*. This result confirmed previous results obtained by Sydenham (2007) that indicated the Blade seed source of the UFS was segregating for the *Sr2* gene. The marker, cssfr5, detected the *Lr34/Yr18/Sr57* gene for resistance in Avocet*YrSp* which also confirmed results obtained by Sydenham (2007). The presence of the *Lr34/Yr18/Sr57* gene complex in three of the four rust parental lines contributed to the high homozygosity of this resistance gene found in the offspring lines. Kariega (*Lr34/Yr18/Sr57, QYr.sgi.2B-1*) and Kariega (SGI; *Lr34/Yr18/Sr57*) showed different marker combinations and it is due to Kariega that was still segregating when seed was distributed from the Agricultural Research Council - Small Grain Institute (ARC-SGI) to the UFS. The control lines Picaflor, Damphe and Kingbird, tested positive for the *Sr2* stem rust resistance gene which can in future be used to incorporate this gene into UFS experimental lines. Sumai3, Elands (SGI), Scheepers69 (SGI) and line 2S#/163 did not test positive for any rust resistance genes linked to the markers tested.

Of these 203 rust resistant lines tested, only three lines [S346(1.3), S346/5.3 and S346(6.3)] tested positive for all five tested markers linked to rust resistance, the maximum number of markers per line. The level of homo- and heterozygosity of the tested markers varied between the offspring of the 14 rust resistant lines selected. All offspring from each of the 14 rust resistant lines selected were seen as a subpopulation. Marker STSLr19$_{130}$, linked to the leaf rust resistance gene *Lr19*, was still segregating in eight of the subpopulations [S16(7.3), S16(7.3)P1.5.1, S16(7.3)P3.5.1, S16(7.3)P3.6.1, S16(7.3)P3.2.1), S726(3.2), S726(3.2)P1.3.1 and S726(3.2)P3.6.1]. The remaining six populations [S16(1.2), S726(3.2)P2.4.1, S726(2.2), S178(7.2), S346 and S157] were all homozygous susceptible. Since marker STSLr19$_{130}$ is a dominant marker and heterozygous and homozygous resistant lines cannot be differentiated, it is therefore difficult to be sure that the selected line was homozygous resistant for the specific marker. Marker cssfr5 was homozygous resistant in all 14 populations as expected because the *Lr34/Yr18/Sr57* gene complex was present in both first generation (F_1) populations used to develop the initial double cross population at the beginning of the breeding programme.

Marker Gwm148 linked to the stripe rust QTL *QYr.sgi.2B-1*, was homozygous resistant for two subpopulations [S16(7.3) and S16(1.2)] and segregated in seven subpopulations [S16(7.3)P1.5.1, S16(7.3)P3.5.1, S16(7.3)P3.6.1, S16(7.3)P3.2.1), S178(7.2), S346 and S157]. The remaining five subpopulations [S726(3.2),S726(3.2)P1.3.1, S726(3.2)P2.4.1, S726(3.2)P3.6.1 and S726(2.2)] were homozygous susceptible. The other marker linked to the *QYr.sgi.2B-1* QTL, Gwm501, was only homozygous resistant in one subpopulation [S178(7.2)] and segregated in four subpopulation [S16(7.3), S16(7.3)P1.5.1, S16(1.2) and S346]. The other eight subpopulations [S16(7.3)P3.5.1, S16(7.3)P3.6.1, S16(7.3)P3.2.1, S726(3.2), S726(3.2)P2.4.1, S726(3.2)P3.6.1, S726(2.2) and S157] were homozygous susceptible.

The stem rust resistant marker, csSr2, linked to the *Sr2* gene, was homozygous susceptible in all 14 subpopulations. Four subpopulations [S726(3.2), S726(3.2)P1.3.1, S726(3.2)P2.4.1 and S346] segregated for marker Sr26#43, linked to the stem rust resistance gene *Sr26* and only one subpopulation [S726)2.2] tested homozygous resistant for this marker. The other nine subpopulations [S16(7.3), S16(7.3)P1.5.1, S16(7.3)P3.5.1, S16(7.3)P3.6.1, S16(7.3)P3.2.1, S16(1.2), S726(3.2)P3.6.1, S178(7.2) and S157] tested homozygous susceptible. It was expected that the S16 subpopulation should be homozygous susceptible since the marker was absent in the original S16 line selected after the double cross.

Within the segregating subpopulations, the level of homozygosity varied between lines within the specific subpopulation. Homozygous susceptibly for a specific marker within a subpopulation indicated that individuals with the subpopulation lost the marker linked to the gene of interest and indicated that the gene was present in a heterozygous state in the parental line if that specific subpopulation was developed from (e.g. for marker Sr26#43).

Data from the previous study gave an indication of which markers to expect in the offspring lines. The offspring lines within sub population S16(7.3)/1.1 were expected to contain markers Gwm148, Gwm501, STSLr19$_{130}$ and cssfr5 and they were indeed detected in the subpopulation although markers Gwm501 and STSLr19$_{130}$, were still segregating. The two stem rust markers csSr2 and Sr26#43, were homozygous susceptible as expected in the offspring for some subpopulations since marker Sr26#43 was absent in some of the initial lines while csSr2 was lost in some subpopulations through self-pollination.

Lines of subpopulation S16(7.3)P1.5.1 contained the expected markers (Gwm148, Gwm501, STSLr19$_{130}$ and cssfr5) and only marker cssfr5 was homozygous resistant while the other three markers were still segregating. These lines were all homozygous susceptible for markers csSr2 and Sr26#43.

Lines within the subpopulation S16(7.3)P3.5.1 tested positive for markers Gwm148, STSLr19$_{130}$ and cssfr5. Only cssfr5 was homozygous resistant within these lines while the other two marker were still segregating. Marker Gwm501 was homozygous susceptible which indicated that the marker was heterozygous in line S16(7.3) and was confirmed by the data obtained. Markers csSr2 and Sr26#43 were homozygous susceptible as expected.

Lines of subpopulation S16(7.3)P3.6.1 contained markers Gwm148, STSLr19$_{130}$ and cssfr5. Marker cssfr5 was homozygous while the other two markers were segregating. These lines were homozygous susceptible for markers Gwm501, csSr2 and Sr26#43.

The last S16(7.3) subpopulation, S16(7.3)P3.2.1 was homozygous resistant for marker cssfr5 and was segregating for markers Gwm148 and STSLr19$_{130}$. Markers Gwm501, csSr2 and Sr26#43 were absent in lines within this subpopulation. Markers Gwm501 and csSr2 were originally present in the S16(7.3) parental line but were lost through self-pollination, while marker Sr26#43 was not present in line S16 and therefore could not be passed on to the offspring.

It was expected that lines within subpopulation S16(1.2) should contain markers Gwm148, Gwm501, STSLr19$_{130}$ and cssfr5. Since only cssfr5 was homozygous resistant in line S16 it was expected that this marker should be homozygous resistant in all S16 offspring lines and this was indeed the case for line S16(1.2). Markers Gwm148 and STSLr19$_{130}$ were segregating for lines within the S16(1.2) subpopulation and therefore these markers should have been heterozygous in line S16. Markers Gwm501 and csSr2 were homozygous susceptible in lines within the S16(1.2) subpopulation, indicating that these two markers must also been heterozygous within line S16 and through self-pollination these two markers have been lost.

Line S726 was one of the best lines identified during the developmental stages of the lines. Previous MAS analysis indicated that markers cssfr5, Gwm148, Gwm501, STSLr19$_{130}$, Sr26#43 and csSr2 were present in this line. Lines within subpopulation S726(3.2) lost the Gwm148 and Gwm501 markers as well as marker csSr2. This indicated that these markers were heterozygously present in line S726(3.2) and through self-pollination these markers were lost. Marker cssfr5 tested homozygous resistant in these lines and this indicated that the marker was homozygous resistant in line S726(3.2), supporting data obtained in previous studies. Marker Sr26#34 was heterozygous for lines in the S726(3.2) subpopulation and therefore it is clear that the marker was heterozygous in line S726(3.2).

After another round of self-pollination of line S726(3.2), three different subpopulations were obtained which varied from one another in terms of marker combinations. Offspring of subpopulation S726(3.2)P1.3.1 had the same marker combination as S726(3.2). Lines within subpopulation S726(3.2)P2.4.1 were homozygous for marker STSLr19$_{130}$ which resulted from self-pollination of this marker that was heterozygous in line S726(3.2). Marker cssfr5 was homozygous resistant in all lines in the subpopulation as expected. Marker S26#43 was segregating within the subpopulation supporting data that the marker was heterozygous in line S726(3.2). The last subpopulation, S726(3.2)P3.6.1, was homozygous resistant for marker cssfr5 but homozygous susceptible for marker Sr26#43 which can be explained by the heterozygous state of marker Sr26#43 in line S726(3.2). This subpopulation was segregating for marker STSLr19$_{130}$ due to the heterozygous state of the marker in line S726(3.2). Markers Gwm148, Gwm501 and csSr2 were absent in all lines of these three subpopulations. Markers Gwm148 and Gwm501 were thus lost after the first round of self-pollination and these markers must both have been heterozygous in the original line S726. After the third round of self-pollination lines have lost the csSr2 marker which was heterozygous resistant in line S726.

Lines within subpopulation S726(2.2) was homozygous resistant for markers cssfr5 and Sr26#43. The homozygous state of marker Sr26#43 was confirmed with marker BE518379 that detects the susceptible allele. These results were possible because marker cssfr5 was homozygous resistant in line S726 and marker Sr26#43 was heterozygous for line S726 and this supported data obtained for line S726(3.2). The rest of the markers were homozygous susceptible for the S726(2.2) subpopulation, indicating that all these markers were heterozygous in line S726.

Lines within subpopulation S178(7.2) were the only lines that were homozygous resistant for marker Gwm501 out of all 14 selected subpopulations. Marker cssfr5 was also homozygous resistant in these lines. Marker Gwm148 was still segregating within the subpopulation. Markers STSLr19$_{130}$, Sr26#43 and csSr2 were homozygous susceptible for this subpopulation. Markers STSLr19$_{130}$ and Sr26#43 were absent in the original line S178 but marker csSr2 was present in line S178(7.2) and therefore it must have been heterozygous in line S178(7.2).

Line S346 contained all markers (Gwm148, Gwm501, STSLr19$_{130}$, cssfr5, csSr2 and Sr26#43) and is one of the original lines from the double cross population and thus has not been self-pollinated before. After the first round of self-pollination for subpopulation S346, it was observed that only marker cssfr5 was homozygous resistant in the subpopulation as expected. Markers STSLr19$_{130}$, Gwm148, Gwm501 and Sr26#43 were segregating in the subpopulations of line S346, as expected. Marker csSr2 was homozygous susceptible and must have been heterozygous in line S346.

Line S157 was evaluated during a previous study (Sydenham 2007) and results indicated that the line contained markers STSLr19$_{130}$, Gwm148, Sr26#43 and cssfr5. After the first round of self-pollination of line S157, the offspring revealed that two markers, Gwm148 and cssfr5, were present and both were segregating in lines within this subpopulation. The rest of the markers were homozygous susceptible.

After MAS analysis for the 203 rust resistant lines, the 42 best lines were selected based on the number of resistance markers present within each line. The MAS data of the 42 lines as well as the parental and control lines are summarised in Table 3.7. Only these 42 lines were used for the first round of biochemical and MAS analyses linked to protein quality, as described in Chapter 4.

3.3.1.2 Data generated from second year of screening

The 22 lines indicated by grey in Table 3.7 were selected to be planted again, sampled and left to self-pollinate. These 22 lines were selected based on the total number of resistant genes/QTL present, protein quality genes (Chapter 4) and the large unextractable polymeric proteins percentage (LUPP%; Chapter 4) after the first round of analysis. These 22 selected lines resulted in 88 offspring lines which were sampled and analysed using MAS analysis linked to the rust resistance markers.

After MAS using markers linked to rust resistance, the data of the 88 lines analysed during the second year showed that more markers were in a homozygous state compared to lines analysed during the first year (Appendix II). Marker cssfr5, linked to leaf rust resistant gene *Lr34/Yr18/Sr57*, was still in a homozygous state for all lines which was expected since it was already homozygous before self-pollination.

The STSLr19$_{130}$ marker was either segregating or homozygous susceptible in the first year's subpopulations but after another round of self-pollination it seemed that the marker was homozygous resistant in 13 of the 22 second year's subpopulations screened [S16(7.3)P1.5.1/1.3, S16(7.3)P1.5.1/3.1, S16(7.3)P1.5.1/3.3, S16(7.3)P1.5.1/4.2, S16(7.3)P1.5.1/4.3, S16(7.3)P1.5.1/5.1, S16(7.3)P3.6.1/5.3, S16(7.3)P3.6.1/6.1, S726(3.2)P1.3.1/1.1, S16(7.3)P3.2.1/1.1, S16(7.3)P3.2.1/1.2, S16(7.3)P3.2.1/2.1 and S16(7.3)P3.2.1/3.2] and was also thus probably homozygous resistant in the subpopulation from the first year it was developed from. Four second year subpopulations [S16(1.2)/2.1, S178(7.2)/2.3, S178(7.2)/4.1 and S178(7.2)/5.1] were homozygous susceptible, similar to the lines they were developed from. Two of the second year subpopulations [S726(3.2)/1 and S726(3.2)P1.3.1/2.2] were now homozygous susceptible and were thus developed from heterozygous resistant lines.

Table 3.7 Rust resistant marker data of the parental, control and selected rust lines obtained during the first year of screening

Line name	STSLr19$_{130}$ (*Lr19*)	cssfr5 (*Lr34/Yr18/Sr57*)	Gwm148 (*QYr.sgi.2B-1*)	Gwm501 (*QYr.sgi.2B-1*)	csSr2 (*Sr2*)	Sr26#43 (*Sr26*)	Total rust resistant markers
Kariega	0	1	1	1	0	0	3
Avocet*YrSp*	0	1	0	0	0	1	2
Blade	0	0	0	0	0	1	1
CS-*Lr19*-149-299	1	1	0	0	0	0	2
Krokodil	0	0	1	0	0	1	2
CM-82036	0	0	0	0	0	1	1
Frontana	0	1	0	1	0	1	3
Sumai3	0	0	0	0	0	0	0
PAN3434	0	0	1	1	0	0	2
SST835	0	1	0	1	0	0	2
Duzi	0	0	1	1	0	1	3
Elands (SGI)	0	0	0	0	0	0	0
Kariega (SGI)	0	1	0	0	0	0	1
Tugela-DN (SGI)	0	1	0	0	0	0	1
Scheepers69 (SGI)	0	0	0	0	0	0	0
Picaflor	0	0	1	0	1	0	2
Damphe	0	0	0	1	1	0	2
Kingbird	0	1	1	0	1	0	3
Line T6-1 (*SrR*+*Sec*-)	0	0	1	0	0	0	1
2S#2/163 (*Sr39*)	0	0	0	0	0	0	0
S16(7.3)/1.1	0	1	1	1	0	0	3
S16(7.3)P1.5.1/1.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/2.2	0	1	1	1	0	0	3
S16(7.3)P1.5.1/3.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/4.2	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.1	1	1	1	1	0	0	4
S16(7.3)P3.5.1/2.3	0	1	1	0	0	0	2
S16(7.3)P3.5.1/3.2	1	1	0	0	0	0	2
S16(7.3)P3.6.1/5.3	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.3	1	1	1	0	0	0	3

Table 3.7 Rust resistant marker data of the parental, control and selected rust lines obtained during the first year (continued)

Line name	STSLr19$_{130}$ (*Lr19*)	cssfr5 (*Lr34/Yr 18/Sr57*)	Gwm148 (*QYr.sgi-2B-1*)	Gwm501 (*QYr.sgi-2B-1*)	csSr2 (*Sr2*)	Sr26#43 (*Sr26*)	Total rust resistant markers
S16(7.3)P3.2.1/1.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.3	1	1	1	0	0	0	3
S16(7.3)P3.2.1/6.3	1	1	1	0	0	0	3
S16(1.2)/2.1	0	1	1	1	0	0	3
S16(1.2)/3.2	0	1	1	1	0	0	3
S16(1.2)/5.2	0	1	1	1	0	0	3
S726(3.2)/1	1	1	0	0	0	1	3
S726(3.2)P1.3.1/1.1	1	1	0	0	0	1	3
S726(3.2)P1.3.1/2.2	1	1	0	0	0	1	3
S726(3.2)P1.3.1/5.2	1	1	0	0	0	1	3
S726(3.2)P1.3.1/6.2	1	1	0	0	0	1	3
S178(7.2)/2.3	0	1	1	1	0	0	3
S178(7.2)/3.1	0	1	1	1	0	0	3
S178(7.2)/3.2	0	1	1	1	0	0	3
S178(7.2)/4.1	0	1	1	1	0	0	3
S178(7.2)/4.2	0	1	1	1	0	0	3
S178(7.2)/5.1	0	1	1	1	0	0	3
S178(7.2)/5.2	0	1	1	1	0	0	3
S178(7.2)/6.1	0	1	1	1	0	0	3
S346(1.3)	1	1	1	1	0	1	5
S346(3.1)	0	1	1	1	0	1	4
S346(5.2)	0	1	1	1	0	1	4
S346(5.3)	1	1	1	1	0	1	5
S346(6.1)	0	1	1	1	0	1	4
S346(6.3)	1	1	1	1	0	1	5

Lines marked in grey were lines selected after the first year to be planted for the second year of screening; SGI - Small Grain Institute; 1 - Homozygous resistant; 0.5 - Heterozygous; 0 - Homozygous susceptible

Three of the second year subpopulations [S346(1.3), S346/5.3 and S346(6.3)] were still segregating as their related first year lines were heterozygous.

The number of homozygous resistant lines for marker Gwm148 increased in the second year's subpopulations in comparison to the first year's subpopulations (Appendix II). Nineteen second year subpopulations [S16(1.2)/2.1, S16(7.3)P1.5.1/1.3, S16(7.3)P1.5.1/3.1, S16(7.3)P1.5.1/3.3, S16(7.3)P1.5.1/4.2, S16(7.3)P1.5.1/4.3, S16(7.3)P1.5.1/5.1, S16(7.3)P3.6.1/5.3, S16(7.3)P3.6.1/6.1, S178(7.2)/2.3, S178(7.2)/4.1, S178(7.2)/5.1, S346(1.3), S346/5.3, S346(6.3), S16(7.3)P3.2.1/1.1, S16(7.3)P3.2.1/1.2, S16(7.3)P3.2.1/2.1 and S16(7.3)P3.2.1/3.2] were homozygous resistant as expected because their related first year lines were also homozygous resistant. Three second year subpopulations [S726(3.2)/1, S726(3.2)P1.3.1/1.1 and S726(3.2)P1.3.1/2.2] were homozygous susceptible as expected because their related first year lines were also homozygous susceptible.

Marker Gwm501 linked to the stripe rust resistance QTL also showed an increased number of homozygous lines present in the subpopulations (Appendix II). The second year's subpopulations S16(7.3)P1.5.1/1.3, S16(7.3)P1.5.1/3.1, S16(7.3)P1.5.1/3.3, S16(7.3)P1.5.1/4.3, S16(7.3)P1.5.1/5.1, S16(1.2)/2.1, S178(7.2)/2.3, S178(7.2)/4.1, S178(7.2)/5.1, S346(1.3) and S346(6.3) were all homozygous resistant, similar to their related first year lines. The second year subpopulation S346/5.3 was the only subpopulation that showed segregation for this marker. The remaining second year subpopulations [S16(7.3)P1.5.1/4.2, S726(3.2)/1, S726(3.2)P1.3.1/1.1, S726(3.2)P1.3.1/2.2, S16(7.3)P3.2.1/1.1, S16(7.3)P3.2.1/1.2, S16(7.3)P3.2.1/2.1 and S16(7.3)P3.2.1/3.2] were homozygous susceptible like their related first year lines.

Since marker csSr2 was absent in all the lines selected after the first year of screening, it was also absent in all subpopulations screened during the second year.

As expected, marker Sr26#43 was homozygous susceptible in 16 of the 22 second year subpopulations because this marker was absent in the lines they were developed from. Lines in three second year subpopulations [S726(3.2)/1, S726(3.2)P1.3.1/1.1 andS726(3.2)P1.3.1/2.2] were all resistant like their related first year lines. The second year subpopulations S346(1.3) and S346(5.3) were segregating for this marker and their related first year lines were thus heterozygous. The second year lines of subpopulation S346(6.3) were all homozygous susceptible for the marker indicating that the first year line [S346(6.3)] must have been heterozygous since it tested positive for the dominant marker (Appendix II).

Most of the second year lines had the same number of markers as their related first year lines and for most the level of homozygosity improved due to another round of self-pollination. The number of markers present in the different second year lines of the three S346 subpopulations varied. This is because these lines have not been self-pollinated as many times as the other rust subpopulations and therefore the markers were still segregating. The number of markers present in these three S346 subpopulations varied from two markers (line S346/5.3.3) to five markers per line as seen in lines S346(1.3).1 and S346/5.3.4.

All of the lines screened during the second year except for the S346 subpopulation, have been self-pollinated three times up until this point and it was clear from the results that most markers were in a homozygous state and segregation seldomly occurred in the second year lines in comparison to their related first year lines. The second year lines of the S346 groups have only been self-pollinated once and therefore variation was observed in terms of number of markers present within a group and most markers except for csffr5 and Gwm148 were still segregating within these lines.

The best lines were selected based on the number of markers present and the level of homozygosity and are present in Table 3.8.The best selected rust resistant lines indicated in Table 3.8 were also used for biochemical and MAS analysis linked to protein quality during the second year. The complete MAS data linked to rust resistance for all the second year lines are summarised in Appendix II.

3.3.2 FHB genotyping

3.3.2.1 Data from first year of screening

During the first year of the current study, 152 experimental FHB resistant lines were developed from 11 selected BC_2F_2 lines. From these 152 offspring, five lines were selected per BC_2F_2 line for MAS analysis linked to FHB resistance (Appendix III). These 55 offspring lines were also used for biochemical and MAS analysis linked to protein quality (Chapter 4). The six markers linked to FHB resistance summarised in Table 3.5 were used to evaluate these 55 first year experimental FHB lines.

Markers linked to the FHB QTL for resistance on chromosome 7A were not included because it is not a major QTL (Buerstmayr et al. 2009). The MAS data for the six FHB resistant markers is summarised in Table 3.9 and includes the 20 parental and control lines as well as the 55 first year experimental lines.

Table 3.8 **Rust resistant marker data of the best rust resistant lines selected for biochemical analysis and MAS analysis linked to protein quality done during the second year**

Line name	STSLr19$_{130}$ (*Lr19*)	cssfr5 (*Lr34/Yr 18/Sr57*)	Gwm148 (*QYr.sgi. 2B-1*)	Gwm501 (*QYr.sgi. 2B-1*)	csSr2 (*Sr2*)	Sr26#43 (*Sr26*)	Total number of rust resistant markers*
S16(7.3)P1.5.1/1.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.2	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.4	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.5	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.6	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.1.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.1.2	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.3.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/4.2	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.2.1	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.2.2	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.2.3	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.2.4	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/4.3.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.1.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.1.2	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.1.3	1	1	1	1	0	0	4
S16(7.3)P3.6.1/5.3	1	1	1	0	0	0	3
S16(7.3)P3.6.1/5.3.1	1	1	1	0	0	0	3
S16(7.3)P3.6.1/5.3.2	1	1	1	0	0	0	3
S16(7.3)P3.6.1/5.3.3	1	1	1	0	0	0	3
S16(7.3)P3.6.1/5.3.4	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.1	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.2	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.3	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.4	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.5	1	1	1	0	0	0	3

Table 3.8 Rust resistant marker data of the best rust resistant lines selected for biochemical analysis and MAS analysis linked to protein quality done during the second year (continued)

Line name	**STSLr19$_{130}$ (*Lr19*)**	**cssfr5 (*Lr34/Yr 18/Sr57*)**	**Gwm148 (*QYr.sgi. 2B-1*)**	**Gwm501 (*QYr.sgi. 2B-1*)**	**csSr2 (*Sr2*)**	**Sr26#43 (*Sr26*)**	**Total number of rust resistant markers***
S16(7.3)P3.2.1/1.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.3	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.4	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.5	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.6	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.3	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.4	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.5	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.3	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.4	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.5	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.6	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2.3	1	1	1	0	0	0	3
S726(3.2)/1	1	1	0	0	0	1	3
S726(3.2)/1.1	0	1	0	0	0	1	2
S726(3.2)/1.4	0	1	0	0	0	1	2
S726(3.2)P1.3.1/1.1	1	1	0	0	0	1	3
S726(3.2)P1.3.1/1.1.1	1	1	0	0	0	1	3
S346(1.3)	1	1	1	1	0	1	5
S346(1.3).1	1	1	1	1	0	0.5	5
S346/5.3	1	1	1	1	0	1	5
S346/5.3.5	1	1	1	0	0	0.5	4

Lines indicated in grey were the related first year lines of the selected second year lines selected for biochemical analysis and marker-assisted selection linked to protein quality; * - Represents total number of markers present irrespective if the marker was homo- or heterozygous; 1 - Homozygous resistant; 0.5 - Heterozygous; 0 - Homozygous susceptible

Table 3.9 FHB resistant marker data for the parental, control and selected FHB lines tested during the first year

Line name	Barc 133 (3B)	Gwm 533 (3B)	Gwm 304 (5A)	Gwm 293 (5A)	Gwm 156 (5A)	Gwm 133 (6B)	Total number of resistant markers*
Kariega	0	0	0	0	1	1	2
Avocet*YrSp*	0	1	0	0	1	1	3
Blade	1	0	0	0	0	1	2
CS-*Lr19*-149-299	0	1	1	1	1	1	5
Krokodil	0	0	0	0	0	0	0
CM-82036	1	1	1	1	1	1	6
Frontana	0	0	1	1	1	0	3
Sumai3	1	1	0	0	1	1	4
PAN3434	0	0	M	0	1	0	1
SST835	0	0	M	0	0	1	1
Duzi	0	0	M	0	0	M	0
Elands (SGI)	0	0	M	0	1	0	1
Kariega (SGI)	0	0	M	0	1	1	2
Tugela-DN (SGI)	1	1	M	0	1	1	4
Scheepers69 (SGI)	0	1	M	0	0	1	3
Picaflor	1	0	0	0	0	0	1
Damphe	0	0	0	0	0	0	0
Kingbird	1	0	0	0	0	0	1
Line T6-1 (*SrR*+*Sec*-)	1	0	0	0	0	0	1
2S#2/163 (*Sr39*)	0	0	M	0	1	1	2
BC$_2$F$_2$P2.6.2/1.1	0.5	0.5	0.5	1	1	1	6
BC$_2$F$_2$P2.6.2/1.2	1	1	0.5	1	0.5	0.5	6
BC$_2$F$_2$P2.6.2/2.1	0.5	0.5	0.5	0.5	0.5	0.5	6
BC$_2$F$_2$P2.6.2/3.1	0	0	0	0.5	0	0.5	2
BC$_2$F$_2$P2.6.2/4.1	1	1	0.5	0.5	1	0.5	6
BC$_2$F$_2$P2.14.2/1.1	0.5	0.5	1	0	0	0	3
BC$_2$F$_2$P2.14.2/2.1	0.5	0.5	1	0	0	0	3
BC$_2$F$_2$P2.14.2/3.1	0	0	0	0	0	0.5	1
BC$_2$F$_2$P2.14.2/4.2	0.5	0.5	0	0	0	0.5	3
BC$_2$F$_2$P2.14.2/5.2	0	0	0	0	0	1	1
BC$_2$F$_2$P1.7.2/1.2	0	0	0	0	0	0.5	1
BC$_2$F$_2$P1.7.2/2.2	0	0	0	1	0.5	0.5	3
BC$_2$F$_2$P1.7.2/3.2	0	0	0	0	0.5	0.5	2
BC$_2$F$_2$P1.7.2/4.2	0	0	0	0	0.5	0.5	2
BC$_2$F$_2$P1.7.2/5.2	0	0	0	0	0.5	0	1
BC$_2$F$_2$ 4.1/1.3	1	1	0.5	0.5	0.5	0.5	6
BC$_2$F$_2$ 4.1/2.3	0.5	0.5	0.5	0.5	0	0	4
BC$_2$F$_2$ 4.1/3.3	0.5	0.5	0.5	0.5	0.5	1	6
BC$_2$F$_2$ 4.1/4.3	0.5	0.5	0.5	0.5	0.5	1	6
BC$_2$F$_2$ 4.1/5.3	0.5	1	0.5	1	0.5	0.5	6
BC$_2$F$_2$P1.7.1/1.2	0.5	0.5	1	1	1	1	6

Table 3.9 FHB resistant marker data for the parental, control and selected FHB lines tested during the first year (continued)

Line name	Barc 133 (3B)	Gwm 533 (3B)	Gwm 304 (5A)	Gwm 293 (5A)	Gwm 156 (5A)	Gwm 133 (6B)	Total number of resistant markers*
BC_2F_2P1.7.1/2.2	0	0	1	1	1	0	3
BC_2F_2P1.7.1/3.2	1	1	0.5	1	1	0.5	6
BC_2F_2P1.7.1/4.2	0.5	0.5	0.5	1	1	0.5	6
BC_2F_2P1.7.1/5.2	1	0.5	1	1	1	0	5
BC_2F_2P1.7.3/1.3	0	0	0.5	0.5	0.5	0.5	4
BC_2F_2P1.7.3/2.3	0.5	0.5	0	0.5	0.5	0	4
BC_2F_2P1.7.3/3.3	0	0	0	0	0	0	0
BC_2F_2P1.7.3/4.3	0.5	0.5	0.5	0.5	0.5	0.5	6
BC_2F_2P1.7.3/5.3	0.5	0.5	0.5	0.5	0.5	0.5	6
BC_2F_2P2.2.3/1.1	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_2P2.2.3/2.1	0.5	0.5	0	0	0	0	2
BC_2F_2P2.2.3/3.1	0.5	0.5	0.5	0	0	0	3
BC_2F_2P2.2.3/4.1	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_2P2.2.3/5.1	0.5	0.5	0	0	0.5	0	3
BC_2F_2P2.3.2/1.2	0.5	0.5	0	0	0.5	0	3
BC_2F_2P2.3.2/2.2	0	0.5	0.5	0.5	0	0	3
BC_2F_2P2.3.2/3.2	0.5	0.5	0	0.5	0	0	3
BC_2F_2P2.3.2/4.2	0.5	0.5	0.5	0.5	0	1	5
BC_2F_2P2.3.2 /5.3	0.5	0.5	0.5	0.5	0	0.5	5
BC_2F_2P2.12.1/1.1	0.5	0.5	1	1	1	0	5
BC_2F_2P2.12.1/2.1	0.5	0.5	0.5	0.5	1	0	5
BC_2F_2P2.12.1/3.1	0	0	0.5	0.5	0.5	0	3
BC_2F_2P2.12.1/4.1	0.5	0.5	0.5	0	0	0	3
BC_2F_2P2.12.1/5.1	0	0	0.5	0	0	0	1
BC_2F_2P1.5.3/1.1	0	0	0.5	0	0	0.5	2
BC_2F_2P1.5.3/2.1	0	0	0	0	0	1	1
BC_2F_2P1.5.3/3.1	0	0	0.5	0	0	0.5	2
BC_2F_2P1.5.3/3.2	0	0	0.5	0	0	0	1
BC_2F_2P1.5.3/4.1	0	0	0	0	0	0	0
BC_2F_2P1.2.1/1.2	0.5	0.5	0.5	0.5	1	0	5
BC_2F_2P1.2.1/2.2	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_2P1.2.1/3.2	0	0	1	0.5	1	0	3
BC_2F_2P1.2.1/4.2	1	1	0	0	0	0	2
BC_2F_2P1.2.1/6.2	0	0	0.5	0.5	0.5	0	3

Lines marked in grey were the selected first year lines which were planted for the second year; * - Represents total number of markers present, irrespective if the marker was homo- or heterozygous; 1 - Homozygous resistant; 0.5 - Heterozygous; 0 - Homozygous susceptible; M - Missing value; SGI - Small Grain Institute

Krokodil, the recipient parent used to develop the BC_2 population is a South African cultivar developed at the ARC-SGI and did not contain the major FHB resistance genes/QTL *Fhb1* (*Qfhs.ndsu-3BS*) on chromosome 3B, *Qfhs.ifa-5A-1* or *Qfhs.ifa-5A-2* on chromosome 5A or *Fhb2* (Chromosome 6B) which correlated with known pedigree data. The FHB donor parent, CM-82036, is a CIMMYT line and was developed from the Sumai 3/Thornbird-'S' cross and based on literature contains three FHB resistant genes/QTL namely *Fhb1*, *Qfhs.ifa-5A-1* or *Qfhs.ifa-5A-2* and *Fhb2* (Buerstmayr et al. 2002). The six markers (Barc133, Gwm533, Gwm156, Gwm293, Gwm304 and Gwm133) linked to the four FHB resistance QTL were homozygous resistant for the CM-82036 line which confirmed the presence of the QTL. The Brazilian spring wheat cultivar, Frontana, contained the *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2* FHB resistance QTL on chromosome 5A (Steiner et al. 2004) and markers Gwm156, Gwm293 and Gwn304 were present which are linked to the specific QTL. Sumai 3, an Asian spring wheat cultivar, is a good source of FHB resistance since all three major FHB resistant genes/QTL namely *Fhb1* (Waldron et al. 1999; Anderson et al. 2001; Liu and Anderson 2003; Liu et al. 2006; Cuthbert et al. 2006), *Qfhs.ifa-5A* (Yu 1982) and *Fhb2* (Waldron et al. 1999; Anderson et al. 2001; Yang et al. 2003; Cuthbert et al. 2007) have been detected previously by various research studies.

Five of the six markers (Barc133, Gwm533, Gwm133, Gwm156 and Gwm293) linked to the four different FHB QTL were homozygous resistant within Sumai 3, which agreed with other research.

Five of the six markers (Gwm533, Gwm133, Gwm156, Gwm293 and Gwm304) were homozygous resistant for one of the rust parental lines, CS-*Lr19*-149-299. The FHB QTL for resistance detected in this line might be attributed to the Asian background of this line (Chinese Spring derived line). One of the high protein quality control lines, Tugela-DN (SGI), contained four of the six markers (Barc133, Gwm533, Gwm133 and Gwm156) linked to the four main FHB QTL for resistance.

MAS data based on the six FHB markers linked to resistance indicated that the number of markers present in the 55 experimental lines tested during the first year ranged from null to six. Most of these lines tested positive for five markers whereas the average was 3.5 markers per line. Marker Gwm533 was present (either homozygous or heterozygous) in 64% of the 55 experimental lines tested. Marker Gwm133 was present in 49% of the experimental lines which was the lowest percentage. The originally selected 11 BC_2F_2 lines all contained four FHB QTL for resistance, but after these lines had been self-pollinated some of these QTL were lost due to segregation.

Four of the five offspring lines in subpopulation $BC_2F_2P2.6.2$ contained all six markers linked to FHB QTL for resistance on chromosomes 3B, 5A and 6B, respectively. The fifth line, line $BC_2F_2P2.6.2/3.1$ did not test positive for any marker linked to the FHB QTL for resistance on chromosome 3B and tested positive for only two markers, the minimum number of markers present within this subpopulation. All six markers in line $BC_2F_2P2.6.2/2.1$ were present in a heterozygous state. Markers were mostly heterozygous in this subpopulation. Marker Gwm133 was present in five lines within the subpopulation.

Lines within subpopulation $BC_2F_2P2.14.2$ only tested positive for one to three markers. Markers Gwm293 and Gwm156 were absent in all five tested lines.

Four of the five offspring tested in subpopulation $BC_2F_24.1$ tested positive for all six markers. The other line, line $BC_2F_24.1/2.3$, tested positive for only four markers and results indicated the absence of the FHB QTL for resistance on chromosome 6B. Most markers in the subpopulation were heterozygous. Markers Barc133, Gwm293, Gwm304 and Gwm533 were present in all five lines.

Based to the FHB resistant marker data offspring of line $BC_2F_24.1$ were the best in terms of the number of markers present within the subpopulation and four offspring contained markers linked to all four FHB QTL for resistance. The second best subpopulation was developed from line $BC_2F_2P2.6.2$ and four of the five offspring lines contained all four QTL linked to FHB resistance. The two weakest subpopulations were developed from lines $BC_2F_2P1.5.3$ and $BC_2F_2P1.7.2$.

Thirteen tested lines contained all six FHB resistant markers and therefore all four FHB QTL for resistance. Seven offspring lines ($BC_2F_2P2.14.2/3.1$, $BC_2F_2P2.14.2/5.2$, $BC_2F_2P1.7.1/1.2$, $BC_2F_2P1.7.1/5.2$, $BC_2F_2P1.5.3/2.1$, $BC_2F_2P1.5.3/3.2$ and $BC_2F_2P2.12.1/5.1$) contained only one marker each. Two lines ($BC_2F_2P1.7.3/3.3$ and $BC_2F_2P1.5.3/4.1$) did not test positive for any of the FHB resistant QTL present. The *Qfhs.ifa.5A-2* QTL was absent in all offspring of line $BC_2F_2P2.14.2$ while the *Fhb1* gene was absent in all offspring of line $BC_2F_2P1.7.2$. Offspring of the second best subpopulation ($BC_2F_2P1.7.1$) were homozygous resistant for markers linked to the two FHB QTL for resistance on chromosome 5A.

3.3.2.2 Data from second year of screening

Based on the molecular and biochemical data (Chapter 4) generated during the first year on the 55 experimental FHB lines, 25 offspring lines were selected for analysis during the second year. These 25 lines are marked grey in Table 3.9. Between four and six seeds were planted for each of the 25 selected lines. A total of 121 offspring lines from the selected 25 lines were sampled for MAS using markers linked to FHB resistance. The FHB resistance marker data for these121 lines is summarised in Appendix IV.

Nineteen of the second year offspring lines tested contained all six markers linked to FHB resistance and therefore indicated the presence of all four FHB QTL for resistance. The subpopulation of line BC_2F_2P1.7.1/1.2 had the highest number of offspring containing all six FHB resistant markers (five of the six lines tested) while the sixth line contained four markers. The second best subpopulation was offspring of line BC_2F_2P2.6.2/1.2 where three of the six lines contained all six FHB resistance markers. The other two lines contained five of the six markers.

The best subpopulations originated from lines BC_2F_2P2.6.2/1.2 and BC_2F_2P1.7.1/1.2 and most of these BC_2F_3 lines contained five or six markers. The number of markers for the 121 tested lines ranged between null and six. Nineteen of the 121 FHB resistant lines screened during the second year contained six markers. Six of these nineteen lines contained five homozygous markers while three lines had six heterozygous markers. Of these 121 FHB second year lines, 47 lines contained five markers that were either homo- or heterozygous. Only one line, line BC_2F_3P2.2.3/1.1.1 tested negative for all the FHB markers tested. Most offspring from the original line BC_2F_1P2.6.2 contained five or six FHB resistant markers. Marker Gwm293 was present in 62 lines (83%) out of the 121 FHB resistant second year lines which was the highest for all six tested FHB resistant markers. Marker Gwm304 was present in 78 lines (64%) and marker Gwm156 was present in 79 lines (64%). These results indicated that QTL *Qfhs.ifa.5A-1* and *Qfhs.ifa.5A-2* had the highest presence in the 121 tested second year lines compared to the other markers while the *Fhb2* gene had the lowest presence in these lines. Most markers that were heterozygous in the BC_2F_2 lines were homozygous resistant or susceptible in the BC_2F_3 lines.

Comparison of the MAS data linked to FHB resistance for year one and year two's analyses indicated that after an additional round of self-pollination, the level of segregation decreased in the offspring. For the first year a total of 66 groups [11 lines (represented by five individuals each) x 6 markers] were analysed. Within these 66 groups two groups (3%) were homozygous resistant for the specific marker tested. Four groups (6%) were heterozygous, twelve groups (18%) were homozygous susceptible and the remaining 48 groups (73%) were still segregating for the specific markers tested. For the second year a total of 150 groups (25 lines x 6 markers) were analysed. Within these 150 groups, 36 groups (24%) were homozygous resistant, 13 groups (9%) were heterozygous, 19 groups (13%) were susceptible and 82 groups (55%) still showed segregation for the different markers tested. Homozygous resistant groups thus increased from 3% to 24% after an additional round of self-pollination. Homozygous susceptible groups decreased from 18% in the first year to 13% in the second year. This decrease was expected since selection was done against homozygous susceptible individuals. The overall level of segregation decreased from 79% in the first year to 64% in the second year.

Data generated from MAS analysis linked to FHB resistance on the 121 offspring lines was used to select 50 lines with the best combinations of markers present and the highest levels of homozygosity. These 50 lines were used for MAS and biochemical analysis linked to protein quality (Chapter 4). The data generated during MAS analysis linked to the FHB resistance of the top 50 selected second year offspring lines and their related first year lines is summarised in Table 3.10.

3.4 Discussion

Wheat is an important grain crop and is a staple food for most people living in developing countries. However, rust and FHB epidemics can be detrimental to wheat production which will have a negative effect on wheat consumers. Therefore, breeding for resistant wheat cultivars against the major diseases, which include all three rust types (leaf, stem and stripe rust) and FHB, will secure wheat production. Application of MAS to breed new and improved cultivars will enhance the process of releasing new cultivars.

MAS is an effective process for breeding improved wheat cultivars. Collard and Mackill (2008) discussed five factors which can affect the effectiveness of MAS breeding. These factors include the reliability of the marker, quality and quantity of DNA, level of polymorphism, technical procedures and the cost effectiveness of markers. SSR markers are widely used in various rust and FHB resistance breeding programmes because of its high levels of polymorphism and because many markers linked to rust resistant genes and FHB QTL in wheat are available (Mammadov et al. 2012). One of the advantages of MAS breeding programmes includes the ability to select a single plant with the desirable trait or combination of traits. Furthermore, multi-gene traits can be fixed in a homozygous state by the end of a breeding programme and it is easy to evaluate recessive genes and/or genes that cannot be screened easily phenotypically (Koebner and Summers 2003).

Table 3.10 FHB resistant marker data of the best FHB resistant lines selected for biochemical analysis and MAS analysis linked to protein quality during the second year

Line name	Barc 133 (3B)	Gwm 533 (3B)	Gwm 293 (5A)	Gwm 304 (5A)	Gwm 156 (5A)	Gwm 133 (6B)	Total number of resistant markers*
BC_2F_2P2.6.2/1.1	0.5	0.5	0.5	1	1	1	6
BC_2F_3P2.6.2/1.1.1	0.5	0.5	1	1	1	0	5
BC_2F_3P2.6.2/1.1.2	0.5	0.5	1	1	1	0	5
BC_2F_3P2.6.2/1.1.3	0.5	1	1	1	1	0	5
BC_2F_3P2.6.2/1.1.5	0.5	1	1	1	1	0.5	6
BC_2F_2P2.6.2/1.2	1	1	0.5	1	0.5	0.5	6
BC_2F_3P2.6.2/1.2.1	1	1	1	1	1	0.5	6
BC_2F_3P2.6.2/1.2.2	1	1	1	1	0.5	0.5	6
BC_2F_3P2.6.2/1.2.3	1	1	1	1	1	0	5
BC_2F_3P2.6.2/1.2.4	1	1	1	1	0.5	0.5	6
BC_2F_3P2.6.2/1.2.5	1	1	1	0	0.5	0.5	5
BC_2F_2P2.6.2/2.1	0.5	0.5	0.5	0.5	0.5	0.5	6
BC_2F_3P2.6.2/2.1.1	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_3P2.6.2/2.1.5	1	1	1	1	1	0	5
BC_2F_2P2.6.2/4.1	1	1	0.5	0.5	1	0.5	6
BC_2F_3P2.6.2/4.1.1	0.5	1	0.5	0.5	1	0	5
BC_2F_3P2.6.2/4.1.3	1	1	0.5	0.5	0.5	0	5
BC_2F_3P2.6.2/4.1.5	1	1	0.5	0.5	0.5	0	5
$BC_2F_2$4.1/4.3	0.5	0.5	0.5	0.5	0.5	1	6
$BC_2F_3$4.1/4.3.1	0.5	0	0.5	0.5	0.5	0.5	5
$BC_2F_3$4.1/4.3.2	0.5	0.5	0.5	0.5	1	0.5	6
BC_2F_2P1.7.1/1.2	0.5	0.5	1	1	1	1	6
BC_2F_3P1.7.1/1.2.1	1	1	1	1	1	0.5	6
BC_2F_3P1.7.1/1.2.2	0.5	0.5	1	1	1	0.5	6
BC_2F_3P1.7.1/1.2.3	1	1	1	1	1	0.5	6
BC_2F_3P1.7.1/1.2.4	0.5	0.5	1	1	1	0.5	6
BC_2F_3P1.7.1/1.2.5	0	0	0.5	1	1	0.5	4
BC_2F_3P1.7.1/1.2.6	1	1	1	1	1	0.5	6
BC_2F_2P1.7.1/2.2	0	0	1	1	1	0	3
BC_2F_3P1.7.1/2.2.1	0	0	1	1	1	0	3
BC_2F_3P1.7.1/2.2.3	0	0	1	1	1	0	3
BC_2F_3P1.7.1/2.2.5	0	0	1	1	1	0	3
BC_2F_2P1.7.1/3.2	1	1	0.5	1	1	0.5	6
BC_2F_3P1.7.1/3.2.1	1	1	1	0.5	1	0	5
BC_2F_3P1.7.1/3.2.2	1	1	1	0.5	1	0	5

Table 3.10 FHB resistant marker data of the best FHB resistant lines selected for biochemical analysis and MAS analysis linked to protein quality during the second year (continued)

Line name	Barc 133 (3B)	Gwm 533 (3B)	Gwm 293 (5A)	Gwm 304 (5A)	Gwm 156 (5A)	Gwm 133 (6B)	Total number of resistant markers*
BC_2F_3P1.7.1/3.2.3	1	1	1	1	1	0	5
BC_2F_3P1.7.1/3.2.4	1	1	1	0.5	1	0	5
BC_2F_3P1.7.1/3.2.5	1	1	0.5	1	1	0	5
BC_2F_2P1.7.1/5.2	1	0.5	1	1	1	0	5
BC_2F_3P1.7.1/5.2.1	1	1	1	1	1	0	5
BC_2F_3P1.7.1/5.2.2	1	1	0.5	0.5	1	0	5
BC_2F_3P1.7.1/5.2.3	1	1	1	1	1	0	5
BC_2F_2P2.2.3/1.1	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_3P2.2.3/1.1.3	0	0	0.5	0.5	1	0	3
BC_2F_2P2.12.1/1.1	0.5	0.5	1	1	1	0	5
BC_2F_3P2.12.1/1.1.2	0.5	0.5	1	1	1	0	5
BC_2F_3P2.12.1/1.1.3	0.5	0.5	1	1	1	0	5
BC_2F_3P2.12.1/1.1.4	1	1	1	1	1	0	5
BC_2F_3P2.12.1/1.1.5	1	1	1	1	1	0	5
BC_2F_3P2.12.1/1.1.6	0.5	0.5	1	1	1	0	5
BC_2F_2P2.12.1/2.1	0.5	0.5	0.5	0.5	1	0	5
BC_2F_3P2.12.1/2.1.1	0.5	0	0.5	1	1	0	4
BC_2F_3P2.12.1/2.1.2	0.5	0.5	1	1	1	0	5
BC_2F_3P2.12.1/2.1.3	0.5	0.5	0.5	0.5	1	0	5
BC_2F_2P1.2.1/1.2	0.5	0.5	0.5	0.5	1	0	5
BC_2F_3P1.2.1/1.2.2	0.5	0.5	0.5	0.5	1	0	5
BC_2F_3P1.2.1/1.2.3	0.5	0	0.5	0.5	1	0	4
BC_2F_3P1.2.1/1.2.4	0.5	0.5	1	1	1	0	5
BC_2F_2P1.2.1/2.2	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_3P1.2.1/2.2.1	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_3P1.2.1/2.2.2	1	1	1	1	1	0	5
BC_2F_3P1.2.1/2.2.3	0.5	0.5	1	1	0.5	0	5
BC_2F_2P1.2.1/3.2	0	0	1	0.5	1	0	3
BC_2F_3P1.2.1/3.2.1	0	0	1	1	1	0	3
BC_2F_3P1.2.1/3.2.2	0	0	1	1	1	0	3

Lines indicated in grey were the related parental first year lines of the selected second year offspring lines which were selected for further analysis; * - Represents total number of markers present, irrespective if the marker was homo- or heterozygous; 1 - Homozygous resistant; 0.5 - Heterozygous; 0 - Homozygous susceptible

MAS has already been applied in a number of studies on various crops to pyramid genes or QTL for resistance within a single line [wheat (Cox et al. 1994; Liu et al. 2000)], cotton (Gahan et al. 2005), rice (Huang et al. 1997), pea (Schneider 2002) and barley (Werner et al. 2005)]. Pyramiding of resistance genes/QTL in wheat lines is an effective strategy in breeding programmes. Durable resistance genes (Lr34/Yr18/Sr57 and Sr2) have been identified and scientists can use this information to improve wheat lines. Resistant FHB QTL have also been identified and classified into major and minor QTL in terms of their contribution to FHB resistance. With MAS it is possible to detect which QTL are present within a line and in a breeding population the best lines can then be selected in terms of the QTL combinations (Collard and Mackill 2008).

The best identified rust resistant lines in this study mostly contained different combinations of selected rust resistance genes/QTL. Only offspring of line S346 contained five markers linked to rust resistant gene/QTL. This may be attributed to the fact that it was the first time these lines have been self-pollinated.

The only marker that was not present in any of the lines was csSr2, linked to stem rust resistance gene *Sr2.* This result was expected since this marker was also absent in Blade, the supposed donor parent for this specific gene. Previous results indicated that the Blade seed source used by the UFS segregated for the *Sr2* gene. Although Sydenham (2007) only used Blade individuals that tested positive for the *Sr2* gene in crosses, this gene was lost somewhere during the breeding scheme.

The stem rust resistant gene *Sr26* is race non-specific and is one of the major stem rust gene for resistance which is effective against the *Sr31*-virulent race Ug99 (TTKSK) and *Sr24*-virulent derivative (TTKST; Jin et al. 2007; Paroda et al. 2012). The stem rust resistant gene, *Sr26*, was only present in a few lines. Marker Sr26#43, linked to the *Sr26* gene, is a dominant marker, thus lines selected throughout the breeding scheme could have been either homozygous or heterozygous resistant. The small percentage of lines testing positive during this study for carrying the *Sr26* gene thus indicated that the lines selected after the first double cross and during some selections after self-pollination, must have been heterozygous resistant. The problem of using a dominant marker has been overcome with the use of an additional marker (BE518379) linked to the susceptible allele for *Sr26.* The combination of these two markers can act as a co-dominant marker where homozygous and heterozygous resistant individuals can be distinguished from one another. Lines containing the *Sr26* gene should be crossed with the best identified lines that do not contain the *Sr26* gene.

The *Lr34/Yr18/Sr57* gene is a race non-specific and slow-rusting gene which is effective to leaf rust infections during adult plant stage (Lagudah et al. 2009). This gene was homozygous resistant in all selected offspring lines tested in all developed lines during the second year. This gene will provide effective and more durable resistance in comparison to the race specific genes. The complete adult plant stripe rust resistant QTL, *QYr.sgi.2B-1*, (Ramburan et al. 2004) was homozygous resistant in most of the second year lines which will contribute to the stripe rust effectiveness within these lines.

The introgressed gene, *Lr19*, is effective against all pathotypes in South Africa (Prins et al. 1997) and the gene is used effectively in Asia, Europe and Australia in combination with other leaf rust genes to provide long lasting resistance (Pink 2002). The *Lr19* gene was present in almost all lines tested during the second year of screening. The *Lr19* gene was always present together with the *Lr34/Yr18/Sr57* gene. The combination with other genes will provide long lasting leaf rust resistance in the developed rust resistant lines.

Gene-pyramiding of these genes within lines is necessary to produce lines which are effective against all three rust types, ensuring effectiveness against any type of rust epidemic. Race specific rust genes for resistance are not effective against a broad spectrum of rust pathotypes and can easily be overcome and lead to rust outbreaks. Therefore breeders have to implement race non-specific genes or QTL in their breeding programmes to improve the effectiveness of their newly released cultivars (Duveiller et al. 2007). The newly developed rust resistant lines tested in the current study contained both race and race non-specific gene for resistance which should make them effective. The best selected rust resistant lines can be further used in breeding programmes. These lines can be improved further through additional rounds of self-pollination to obtain all markers/traits in a homozygous state. The best lines containing different gene combinations can also be crossed with each other in order to combine more different genes into a single line. These lines could also be used in other breeding programmes to transfer the rust resistant gene/QTL into commercially adapted cultivars that are susceptible to rust. These lines should also be crossed with a *Sr2* donor in order to try to incorporate the missing *Sr2* gene into these lines as it is a durable stem rust gene for resistance and has been effective against the stem rust pathogen for over 50 year (Pretorius et al. 2000).

The most important difference between rust resistant lines tested during the first and second year was the level of segregation of traits between these two groups. Rust resistant marker data indicated higher levels of segregation in lines that underwent one less round of segregation. An additional round of self-pollination increased the number of homozygous resistant markers observed. This showed that the inclusion of an additional self-pollination step was an effective method to increase the level of homozygosity in these lines. MAS was effectively used to select lines with the highest level of homozygosity for the selected genes/QTL.

For the experimental FHB resistant population lines with different numbers of markers and different marker combinations were observed. The highest number of markers present in the BC_2F_3 populations was six although most lines had five markers present. The FHB breeding programme was successful in terms of obtaining a high number of markers within single lines within the best BC_2F_3 lines and the high level of homozygosity which has been achieved. Although all six FHB resistant markers were still present in some of the BC_2F_3 lines, marker Gwm133 linked to the *Fhb2* gene for resistance showed the lowest level of homozygosity within the selected BC_2F_3 lines. The six FHB markers used in this study are linked to four major FHB resistant genes/QTL, namely *Fhb1*, *Qfhs.ifa-5A-1, Qfhs.ifa-5A-2* and *Fhb2*. The *Fhb1* FHB gene for resistance is considered to be the most important QTL linked to type II FHB resistance (Waldron et al. 1999). The *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2* FHB resistance QTL are major QTL and contribute more towards type I FHB resistance (Buerstmayr et al. 2002). The fourth FHB QTL for resistance tested for in the FHB resistant population was the *Fhb2* gene found on chromosome 6B. This QTL contributes towards type II FHB resistance (Waldron et al. 1999). No FHB QTL for resistance on its own is 100% effective against FHB pathogens but each contributes a certain percentage towards the wheat line's effectiveness against FHB resistance. Therefore, major and minor QTL should be combined to improve FHB resistance in wheat. Wheat lines with only one FHB QTL for resistance are more vulnerable to infection than lines with two or more QTL, depending on the effectiveness of the QTL (Li et al. 2011). The FHB resistant lines obtained from the current study can be screened with other FHB resistant markers which are linked to minor FHB QTL which can contribute towards the effectiveness level for FHB resistance in these lines.

Markers were efficiently used to screen for the specific gene's presence after each generation and can in future be used until all genes are present in a homozygous state and fixed within a desirable line. Some advantages of MAS over conventional breeding include that MAS is not influenced by factors such as environmental conditions and disease pressure that can influence the expression of resistance genes. MAS is less time-consuming and can be performed at seedling stage without having to wait for adult plant expression. Single plants with desired characteristics can be selected and lines with undesirable characteristics can be discarded which will accelerate the breeding programme (Mohan et al. 1997). Recessive traits, like *Sr2* resistance, are not phenotypically visible when present in a heterozygous state and additional rounds of self-pollination are necessary to detect the gene. Furthermore, some of the genes are race-specific and with the increasing number of pathotypes present for a certain disease, it would be difficult to create an acceptable and effective inoculum.

For the two FHB populations, the BC_2F_2 and BC_2F_3 populations, various numbers of markers were detected within each line. The main difference between the BC_2F_2 and BC_2F_3 populations was that marker data indicated that progeny of the BC_2F_3 populations were more stable (showed less segregation) than the BC_2F_2 lines within each group. Therefore it was necessary and effective for lines to have been self-pollinated for a second time. In order to efficiently transfer a QTL from one generation to a next every QTL needs two flanking markers to ensure that the entire QTL was transferred from one generation to the next and that part of the QTL was not lost during recombination. Unfortunately due to some markers not giving reliable and repeatable results, flanking markers could not be used for all QTL screened. The presence of specific FHB QTL for resistance cannot be determined using only phenotypical analysis since each FHB QTL contributes a certain level of resistance to FHB and this can vary due to environmental factors and disease pressure. Therefore, it would be difficult to determine the presence of a specific FHB QTL for resistance within a line if only conventional breeding was used.

MAS will be applied differently when selection is done for single genes compared to QTL. A single gene or gene complex is inherited as a block, implying that an entire gene or gene complex will be inherited by the progeny although alleles may vary according to the state of the gene or gene complex inherited from the parents. Therefore, a single marker linked to the gene or gene complex will identify the presence of the gene. However, this is not the case for QTL, since recombination can occur within these QTL which will interrupt the expression of the QTL. A single marker is not efficient to determine the presence of the QTL. Therefore the problem has been overcome by using flanking markers which span the entire region of the QTL and when both markers are present it is most likely that the QTL is also present (Collard and Mackill 2008).

Genetic markers can either be dominant or co-dominant. One disadvantage of dominant markers is that they cannot distinguish between homozygous and heterozygous alleles in contrast to co-dominant markers that amplify both alleles. Co-dominant makers are thus more informative for application in a breeding programme. Markers $STSLr19_{130}$ and Sr26#43 used in the current study are dominant markers. These markers complicated selection of lines containing the *Lr19* or *Sr26* genes because it was not possible to distinguish between heterozygous and homozygous resistant individuals. Individuals were thus only selected based on the presence of the allele and not because of the homozygosity level of the trait. Co-dominant markers on the other hand can sometimes be difficult to score since they amplify both the resistant and susceptible alleles in heterozygotes and sometimes the size difference between the two alleles are small, making interpretation of data difficult.

In the current study different rust and FHB resistant wheat lines have been evaluated using MAS in order to determine the presence or absence of the resistance genes/QTL linked to these makers. The best rust and FHB resistant lines could be selected respectively, based on the number of markers present within each line as well as the level of homozygosity of these markers. Although lines with high levels of resistance to either rust of FHB have been selected, if the quality of these lines does not meet standards set by the milling and baking industry these lines cannot be used further in breeding programmes. Therefore, these selected rust and FHB resistant lines should also be evaluated for protein quality characteristics.

For future studies, these lines should undergo phenotypic screening to rust and FHB resistance to confirm that the genes/QTL indicated to be present based on MAS analysis are indeed present. Therefore lines need to be screened phenotypically both in the field and in the greenhouse. Phenotypic screening of FHB lines to determine disease severity will increase the possibility to identify the presence of minor FHB resistance QTL and will help to select the best lines based on disease severity. For the rust resistant lines, these lines should be screened to determine the presence or absence of the *YrSp* gene since due to the lack of a molecular marker, it can only be determined phenotypically. A new source containing the durable stem rust gene, *Sr2*, can be used in crosses to incorporate this gene into these lines to improve their resistance. For example, Kingbird should be used, that tested positive for the *Sr2* gene, but also confers resistance to the stem rust race Ug99 and all it derivatives.

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Chapter 4

Molecular marker and biochemical analysis linked to protein quality for selected rust or FHB resistant wheat lines

4.1 Introduction

The aims of many wheat plant breeding programmes are to improve yield, resistance to biotic and abiotic stress and bread-making quality. Various methods have been developed over the years that allow breeders to determine certain characteristics in experimental lines. These methods can either detect differences at DNA (molecular) or protein (biochemical) level or can improve the quality and furthermore decrease the time-frame of such a breeding programme (Moose and Mumm 2008). It is necessary for a breeder to understand the experimental traits under investigation, know how they are being inherited and if environmental conditions play a role in the expression of the trait/genes (Peterson et al. 1998). This knowledge will help breeders with the planning of their breeding programme.

One of the main aims of a wheat breeding programme is the improvement of protein quality. Protein quality, a characteristic of gluten proteins (glutenins and gliadins), determines and influences the end use of wheat, which can be bread, pasta or biscuits (Gupta et al. 1989). Quality characteristics include extensibility (dough strength) which prevents the dough from tearing when it rises and are linked to monomeric gliadins. The other protein characteristic is visco-elasticity that is responsible for elasticity of dough and is linked to polymeric glutenin (HMW-GS and LMW-GS; Lindsay and Skerritt 1999). Therefore, dough with both extensibility and elasticity are ideal for bread products. High extensibility dough is used for the production of flat breads and pasta. Additional HMW-GS will increase dough strength, while gliadins and LMW-GS will decrease dough strength (Sissons et al. 2007).

Glutenins make up 85% of the total polymeric proteins and due to their high levels of occurrence; they have been studied extensively to determine their function within wheat (Gupta et al. 1992). Studies have shown that the polymeric proteins have a great influence of the functionality properties of flour quality. The HMW-GS constitutes only 12% of common wheat endosperm proteins, but explain 45% to 70% of the variation seen in European wheat cultivars for bread-making performance (Branlard and Dardevet 1985). The HMW-GS affect dough strength more than LMW-GS and therefore the amount of polymeric proteins do not affect dough strength as much at the size distribution of the polymeric protein which is expressed (MacRitchie 1992). Higher size distribution has a positive effect on dough strength. Therefore, the HMW-GS, Dx5+Dy10, expressed by the gene, *Glu-D1,* has a higher molecular size distribution than Dx2+Dy12 and the presence of Dx5+Dy10 will increase dough strength (Gupta and MacRitchie 1994).

Various techniques have been developed in the past to determine different baking-qualities. The alveograph determine flour strength and has been developed by Launay in 1987. The mixograph can be applied to classified wheat types to determine end-use qualities of wheat lines (Wikström and Bohlin 1996). Results obtained from SDS-sedimentation correlate with bread-baking qualities and the method has been developed by Zeleny in 1947. However, sufficient amounts of seed material are required for the methods mentioned above. Other methods for identifying proteins using single seeds include SDS-PAGE (Laemmli 1970), SE-HPLC (Bietz 1979) and RP-HPLC (Bietz 1983).

SDS-PAGE can be used to separate the HMW-GS and LMW-GS and to determine the profiles of experimental lines. The HMW-GS are expressed by genes located on the group 1 chromosomes (*Glu-1A*, *Glu-1B* and *Glu-1D*). Each chromosome locus expresses two tightly linked genes, the x- (higher size distribution) and the y-type (lower size distribution) except for the loci of *Glu-1A*, which express a null allele for the Ay-allele (Shewry et al. 2001). However, some HMW-GS have the same electrophoretic mobilities and therefore cannot be separated on a SDS-PAGE gel. Effective separation can influence the correctness of the obtained profiles. For example, *By8* and *By8** have relatively similar electrophoretic mobilities, making it difficult to distinguish the two from one another on a SDS-PAGE gel (Butow et al. 2004). It is also difficult to determine whether a line contains the *Bx7*^{OE} allele that has a positive influence on dough strength (Butow et al. 2003). PCR-based markers linked to the alleles that code for the HMW-GS and their translocations, which effect bread-making quality, have been developed. These developed PCR-based markers enable researchers to distinguish between different alleles which have the same mobilities on SDS-PAGE gels and to determine the presence or absence of the *Bx7*^{OE} allele as well as translocations. But one should ask: How good is the correlation between the SDS-PAGE profiles, which are obtained from separating wheat proteins, compared to SSR marker profiles that are based on DNA of wheat lines?

The SE-HPLC test is a biochemical method that can be used to determine the size distributions of the polymeric or total protein in wheat (Gupta et al. 1993). Therefore, four different protein fractions can be obtained based on results of SE-HPLC profiles, namely LPP, SPP, LMP and SMP. Retention times can differ from study to study since the running time, type of column and protocol can influence retention times. Protein fractions can then be divided into SDS-extractable and SDS-unextractable since some proteins need to be sonicated to break down. The SDS-unextractable fractions will be referred to as large or small unextractable poly- or monomeric proteins (LUPP, SUPP, LUMP or SUMP). It was shown that the percentage of total polymeric proteins is correlated with dough strength but the total LUPP was more strongly positively correlated with dough strength than LPP. Therefore, when Gupta et al. (1993) ran the LUPP and LPP fractions on a SDS-PAGE gel it indicated that the LUPP-fraction had a higher ratio of HMW-GS to LMW-GS compared to the LPP fraction. Therefore they concluded that the total LUPP percentage can be used to determine dough strength (Gupta et al. 1993).

Of all proteins in wheat, gluten has the biggest influence on the bread-making ability characteristics of dough. Especially the well-studied HMW-GS of gluten play an important role in bread-making ability characteristics (Sliwinski et al. 2004). Different studies have shown that the *Ax1* and *Ax2** alleles have a positive influence on bread-making characteristics compared to a null allele at this locus on chromosome 1A of wheat (Kolster et al. 1991). Allele pairs *Bx7+By8* and *Bx17+By18* are more positively associated with bread-making characteristics compared to other allelic combinations on chromosome 1B (Morgunov et al. 1990). As for the locus on chromosome 1D, allelic pair *Dx5+Dy10* has a more positive influence on dough quality than allelic pair *Dx2+Dy12*. The $Bx7^{OE}$ allele is strongly associated with dough strength, also located on chromosome 1B (Butow et al. 2003). The rye 1BL.1RS translocation, where the short arm of the 1B wheat chromosome is replaced with the short arm of the 1R rye chromosome, has been used as a resistance source as well as for increasing yield (Moreno-Sevilla et al. 1995). However this translocation has a negative effect on bread-making characteristics and results in sticky and weak dough (Dhaliwal and MacRitchie 1990). This information provides knowledge to breeders to help them select HMW-GS which are associated with good bread-making quality characteristics.

RP-HPLC has been applied as a tool to determine the quality and quantity of wheat proteins and to isolate gliadins and glutenins (Huebner and Bietz 1985; Marchylo et al. 1989; Wieser et al. 1998). The protein composition and their molecular weight distribution have shown correlation between percentage protein, loaf volume and grain hardness of wheat (Huebner et al. 1995). Other studies indicted little variation between the protein composition and baking qualities, but loaf volume was not included in the study (Wieser et al. 1994).

The main aim of this study was to evaluate rust and FHB resistant lines for bread-making qualities. This was done by using different biochemical and molecular marker assays. Since the selected rust and FHB resistant lines formed part of segregating populations, all biochemical and molecular analyses were based on single seed analysis. To reach the aim of the study, biochemical analyses included SDS-PAGE, SE-HPLC and RP-HPLC to determine various protein quality characteristics. Furthermore, seven quality related PCR-based markers were screened to see if there existed a correlation between molecular data (DNA level) and SDS-PAGE profiles (protein level). The last aim of the study was to determine which of the biochemical and molecular analyses methods could be used effectively in the rust and FHB resistance breeding programme to determine certain bread-making qualities. Selection of the best lines based on all traits evaluated throughout the study as well as comparison of the different techniques will be discussed in the next chapter.

4.2 Materials and methods

4.2.1 Plant material

Seed from plant material developed in Chapter 3 (sections 3.2.1 and 3.2.2) was used for biochemical and molecular analyses. Molecular analysis was done using the same DNA extracted as in section 3.2.4. However, biochemical tests were done on single seeds from each selected line. Since the rust and FHB resistant lines formed part of segregating populations, single seed analysis was performed on three seeds of each selected line.

For the current study, 14 rust resistant lines developed from previous studies at the UFS have been selected and planted as described in Chapter 3 (section 3.2.1). From these 14 rust resistant lines, 203 offspring rust resistant lines were obtained which were screened with markers linked to rust resistance. For the development of the FHB resistant lines, 11 selected lines were planted as described in Chapter 3 (section 3.2.2). Of these 11 FHB resistant lines, 152 offspring lines were obtained. Based on the SSR results linked to rust or FHB resistance markers, the best lines were selected based on the number of rust or FHB resistance markers present within an offspring line. For the rust resistant population, 42 offspring lines were selected from the 203 first year rust resistant offspring lines, for PCR-based analysis linked to protein quality. For the FHB resistant population, 55 lines have been selected for further SSR analysis linked to protein quality. These 42 rust and 55 FHB resistant offspring lines were self-pollinated and seed harvested. The harvested seed of these offspring were used for SE-HPLC and RP-HPLC analyses. Seed of 13 of the 42 rust resistant offspring lines and 21 of the 55 FHB resistant lines have been used for SDS-PAGE analysis. Sixteen parental and control lines were also included for SDS-PAGE analysis. Thus, seed of a total of 50 selected lines were used for SDS-PAGE to determine if there existed a correlation between the data obtained from the PCR-based markers linked to protein quality and SDS-PAGE profiles in terms of the HMW-GS. Since PCR-based markers linked to protein quality were tested with the same DNA as for the SSR marker analysis linked to rust and FHB resistance but biochemical tests were performed on the seed of the best selected lines, biochemical tests were performed on the seed that was one generation ahead of the leave material tested. Results obtained from PCR-based markers and biochemical tests played a role in the selection of lines for analysis during the second year.

Only 22 of the 42 first year's rust resistant lines were selected for a second round of analysis and 88 second year offspring lines were obtained and analysed using PCR-based markers linked to rust resistance and protein quality. A total of 50 of the 88 second year offspring lines were selected for SE-HPLC based on PCR-based marker analysis linked to rust resistance and protein quality. For the FHB resistant lines, 25 of the 55 FHB resistant first year lines were selected for the second round of analysis. From these 25 first year FHB resistant offspring lines, 121 second year offspring lines have been obtained which were screened with PCR-based markers linked to FHB resistance and protein quality. Only 50 second year FHB resistant offspring lines were selected for SE-HPLC analysis based on the data obtained from the PCR-based marker analysis linked to FHB resistance and protein quality.

4.2.2 PCR analysis

Six PCR-based markers linked to protein quality were used to screen 42 first year rust resistant offspring lines selected based on rust resistant marker data. For the FHB resistant lines, 55 FHB resistant first year offspring lines were screened with the six PCR-based markers linked to protein quality. For the second year of screening, 88 rust resistant second year offspring and 121 FHB second year offspring lines were screened with the six markers linked to protein quality. All twenty parental and controls lines were included.

4.2.2.1 Protein quality markers

All protein quality markers' PCR reactions were performed using a DYAD™ (DNA Engine) Peltier Thermal Cycler. Five markers were selected to determine the HMW-GS profile and another marker to determine the presence or absence of the 1BL.1RS translocation for every line within the two populations (Table 4.1). All PCR reactions of

Table 4.1 Selected PCR-based markers linked to protein quality

Targeted trait	Targeted gene/QTL	Marker name	Forward primer(5’-3’)	Reverse primer (5’-3’)	Reference
HMW-GS linked to protein quality	*Ax2**	Ax2*	ATGACTAAGCGGTTGGTTCTT	ACCTTGCTCCCCTTGTCTTT	Ma et al. 2003
HMW-GS linked to protein quality	*Dx5*	Dx5	GCCTAGCAACCTTCACAATC	GAAACCTGCTGCGGACAAG	D’Ovidio and Anderson 1994
HMW-GS linked to protein quality	*Bx7*and *Bx17*	BxFp	CGCAACAGCCAGGACAATT	AGAGTTCTATCACTGCCTGGT	Butow et al. 2003
HMW-GS linked to protein quality	*Bx7*^{OE} *Bx20* without *Bx7+By8**	MAR	CCTCAGCATGCAAACATGCAGC	CTGAAACCTTTGGCCAGTCATGTC	Butow et al. 2004
HMW-GS linked to protein quality	*By9*	ZSBy9aF 1/R3	TTCTCTGCATCAGTCAGGA	AGAGAAGCTGTGTAATGCC	Lei et al. 2006
HMW-GS linked to protein quality	1BL.1RS translocation	Glu-B3j	GGAGACATCATGAAACATTTG	CTGTTGGGCAGAAAG	Francis et al. 1995

the protein quality markers had a final volume of 10 µl. Specific controls were added for each specific PCR reaction. The PCR conditions were standardised for each protein quality marker and are summarised in Table 4.2, including controls used and the expected fragment sizes of the PCR product.

4.2.2.2 Agarose gel electrophoresis

PCR products of the protein quality markers were visualised on agarose gels as described in Chapter 3 (section 3.2.6.2). All PCR products of the protein quality markers were separated and visualised on 1.2% agarose gels except for marker ZSBy9aF1/R3 that was visualised on a 1.5% agarose gel.

4.2.3 Biochemical analysis

Biochemical analyses were done to determine certain bread-making qualities of selected wheat lines. Biochemical tests were selected to suit single seed samples. The HMW-GS profile for selected wheat line was determined using SDS-PAGE. SE-HPLC was performed to determine the LUPP%. RP-HPLC was performed to determine the quantity of the gluten sub-units (gliadin and glutenin) for the selected lines.

4.2.3.1 SDS-PAGE analysis

Fifty selected wheat lines were separated on SDS-PAGE, consisting of parental lines, controls and experimental wheat lines of both the rust and FHB resistant populations. SDS-PAGE analysis was performed according to the method of Singh et al. (1991). Electrophoresis was performed on a C.B.S. DSG-200-02 System (Del Mar, CA, USA) connected to a Labcon circulating water bath (Johannesburg, RSA). Glutenins were extracted from single seeds and each line was analysed in triplicate. Seeds were individually crushed using a mortar and pestle, which was sprayed and wiped with 70% (v/v) ethanol between samples to prevent contamination. The crushed seed material was placed into a 1.5 ml microcentrifuge tube.

The crushed seed samples were washed with 1 ml 50% (v/v) n-propanol and pulse vortexed and placed into a water bath at 60°C for 30 min and pulse vortexed after every 15 min. Samples were centrifuged for 2 min at 10 000 revolutions per minute (rpm) and the supernatant discarded. The pellet was resuspended in 85 µl extraction buffer [0.08 M Tris-HCl; 50% (v/v) n-propanol (pH 8.0)] containing 1.25% DTT, freshly prepared. Samples were vortexed and placed in a water bath for 30 min at 60°C. Extraction buffer (85 µl) containing 1.68% (v/v) 4-vinyl pyridine was added, vortexed and incubated for 60 min at 60°C. Samples were centrifuged at 10 000 rpm for 5 min and the supernatant transferred to a new 1.5 ml

Table 4.2 Optimised PCR conditions for the selected PCR-based markers linked to protein quality

Marker name	Reaction conditions	PCR cycling conditions	Marker type, controls and fragment sizes
Ax2*	40 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 75 ng Ax2*F 75 ng Ax2*R	1 cycle:5 min @ 94°C; 38 cycles:30 s @ 94°C; 30 s @ 58°C; 2 min @ 72°C; 1 cycle:5 min @72°C	Dominant _Ax2*_(+): Kariega and Tugela-DN (1319 bp) _Ax2*_(-): Elands and Scheepers69 (null allele)
Dx5	40 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 75 ng DxF 75 ng DxR	1 cycle:5 min @ 94°C; 38 cycles:30 s @ 94°C; 30 s @ 58°C; 2 min @ 72°C; 1 cycle:5 min @72°C	Dominant *Dx5*(+): Elands and Tugela-DN (450 bp) *Dx5*(-): Kariega and Scheepers69 (null allele)
BxFp	40 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 75 ng BxF 75 ng BxR	1 cycle:5 min @ 94°C; 38 cycles:30 s @ 94°C; 30 s @ 58°C; 2 min @ 72°C; 1 cycle:5 min @72°C	Co-dominant *Bx7*(+): Elands and Tugela-DN (750 bp) *Bx17*(+): Kariega and Scheepers69 (675 bp)
MAR	40 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 75 ng MARF 75 ng MARR	1 cycle:5 min @ 94°C; 38 cycles:30 s @ 94°C; 30 s @ 58°C; 2 min @ 72°C; 1 cycle:5 min @72°C	Co-dominant *Bx7*^{OE}(+): Tugela-DN (560 bp) _Bx7+By8*_(-): Elands, Kariega and Scheepers69 (523bp)
ZSBy9aF1/R3	40 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 100 ng ZS*By9*aF1 100 ng ZS*By9*aR3	1 cycle:5 min @ 95°C; 38 cycles:30 s @ 94°C; 30 s @ 59°C, 90 s @ 72°C; 1 cycle:90 s @72°C	Co-dominant *By9*(+): Elands (662 bp) *By9*(-): Kariega, Tugela-DN and Scheepers69 (707 bp)
Glu-B3j	40 ng DNA 1x ReadyMix (KAPABIOSYSTEMS) 75 ng AF1 75 ng AF4	1 cycle:5 min @ 94°C; 35 cycles:30 s @ 94°C; 30 s @ 60°C; 30 s @ 72°C; 1 cycle:5 min @72°C	Dominant 1BL.1RS translocation(+): CM-82036 (1500 bp) 1BL.1RS translocation(-): Elands, Kariega, Tugela-DN and Scheepers69 (null allele)

microcentrifuge tube containing 160 µl sample buffer [0.05% (w/v) Tris-HCl (pH 8.0); 1.25% (w/v) glycerol; 2% (w/v) SDS; 0.02% (w/v) bromophenol blue (pH 8.0)].

Samples were then placed in the water bath (60°C) for 15 min to ensure binding of the SDS with reduced and alkylated glutenin polypeptides. Samples were loaded (10 µl) per well, on the SDS-PAGE gel, that consisted of a stacking [3.03% (w/v) Tris-HCl (pH 6.8); 0.2% (w/v) SDS; acrylamide:bis-acrylamide (66:1 w/w); 5% (w/v) APS; 2% (v/v) TEMED] and separating gel [acrylamide:bis-acrylamide (20:1 w/w); 38% (v/v) Tris-HCl (pH8.8); 3% (w/v) SDS; 0.75% (w/v) APS; 0.05% (v/v) TEMED). Gels were run at 90 V using a Pharmacia Biotech EPS 1000 power supply (Québec, Canada) for 16 h at 18°C in a running buffer [1.4% (w/v) glycine; 0.3% (w/v) Tris; 0.1% (w/v) SDS].

Afterwards the gel was immersed in a fixing solution [80% (v/v) methanol; 20% (v/v) glacial acetic acid] for 3 h and rinsed with distilled water and stained overnight [15% (v/v) trichloroacetic acid; 0.05% (w/v) Coomassie brilliant blue R250; 5% (v/v) methanol] and destained with distilled water. Payne and Lawrence’s (1983) nomenclature was used to identify the HMW-GS.

4.2.3.2 SE-HPLC analysis

SE-HPLC analyses were done at the ARC-SGI at Bethlehem, SA. The method of Gupta et al. (1993) was used with modifications for the extraction of the SDS-extractable and -unextractable proteins. Seeds were individually ground and sieved to collect white flour for extractions. Each wheat line was analysed in duplicate and a single seed per reaction was used.

For the extraction of the SDS-extractable proteins, 17 mg flour was suspended in1.5 ml SDS-phosphate buffer [0.5% (w/v) SDS; 0.7% (w/v) monosodium phosphate (pH 6.9)]. Samples were pulsed vortexed to loosen the pellet, followed by shaking for 5 min at 1400 rpm and centrifuged for 30 min at 10000 rpm. The supernatant of each sample was filtered through a 0.45 µm HT Tyffryn Acrodisc filter into a glass vial. Glass vials were placed into a water bath of 80°C for 2 min to inactivate proteases (Larroque et al. 2000).

The SDS-unextractable proteins were extracted from the pellet left by the extraction of the SDS-extractable proteins. Pellets were resuspended in1.5 ml SDS-phosphate buffer [0.5% (w/v) SDS; 0.7% (w/v) monosodium phosphate (pH 6.9)] and vortexed for 10 s. Each sample was sonicated with an ultrasonic disintegrator (Branson B12 Sonifier Schwäbisch Gmünd, Germany) fitted with a 3 mm experimental tip, at amplitude 5 for 30 s. Samples were centrifuged at 10000 rpm for 30 min. The supernatant of each sample was filtered through a 0.45 µm HT Tyffryn Acrodisc syringe filter into a glass vial. Glass vials were placed into a water bath at 80°C for 2 min to inactivate proteases (Larroque et al. 2000).

Samples were loaded into the Thermo Finnigan™ Surveyor Plus (Thermo Electron, San Jose, CA, USA) HPLC system with a photodiode array detector (PDA) and equipped with a ChromQuest™ 4.2 chromatography data system for integration events. A narrow bore column (300x4.6 mm BioSep-SEC-S 4000 Phenomenex®) was used for the analyses. Samples were individually separated by injecting 20 µl per sample followed by a run-time of 15 min. The mobile phase consisted of trifluoroacetic acid (TFA; 0.1% v/v) in system A and acetonitrile [ACN; ROMIL-SpS™ acetonitrile 200 far ultraviolet (UV)] with TFA (99.9/0.1% v/v) in system B. The eluent consisted of 50% of buffer B with a flow rate of 0.4 ml/min at ambient temperature. The wavelength for detection of proteins was 210 nm.

4.2.3.3 RP-HPLC analysis

Gliadins and glutenins were extracted using a modified method of Marchylo et al. (1989). Whole flour of a single crushed seed was used for both the gliadin and glutenin extractions. Extractions were done in triplicate for every line tested. For gliadin extraction, flour of a single seed was washed with1 ml of 70% (v/v) ethanol and pulse vortexed until the flour was completely suspended and shaken for 30 min at room temperature (22°C). Samples were centrifuged at 14000 rpm for 5 min. The supernatant was filtered through a 0.45 µm filter into a glass vial and stored at 4°C.

For glutenin extractions, pellets from the gliadin extractions were washed twice with 1 ml 50% n-propanol and shaken for 30 min and 10 min respectively. Samples were centrifuged at 14000 rpm for 1 min and the supernatant discarded. Pellets were resuspended in 1ml RP-buffer [50% n-propanol, 2M urea, 0.2M Tris-HCl (pH 6.6)] containing 1% (w/v) DTT. Samples were pulse vortexed and incubated in a waterbath at 60°C for 60 min and pulse vortexed after every 20 min. Finally, 10 µl 4-vinylpyridine was added to every sample, vortexed and incubated in a waterbath at 60°C for 15 min. Samples were centrifuged at 14000 rpm for 5 min. The supernatant was filtered into a glass vial through a 0.45 µm filter and stored at 4°C.

RP-HPLC analysis was performed on a Shimadzu™ Prominence HPLC (Columbia, MD, USA) using a Jupiter C18(300 Å pore size, 5 µm particle size and 250 x 4.6 mm Phenomenex®, Torrance, CA, USA). Samples (25 µl) were auto-injected and separated at a flow rate of 1 ml/min and column temperature of 50°C. Two eluants (A and B) were used. A consisted of 95% water containing 5% (v/v) ACN and 0.1% (v/v) TFA and B 95% ACN containing 0.1% (v/v) TFA.

For gliadin analysis, a linear elution gradient of 100% of A, 0-10 min; 76% of A and 24% of B, 10-40 min; 44% of A and 56% of B, 40-41 min; 10% of A and 90 of B, 41-46 min and 100% of A, 46-60 min. The wavelength for detection was 210 nm (PDA detector) and the different gliadins present were calculated using the areas of the chromatogram using the Class-VP™ software.

For glutenin analysis, a linear elution gradient of 100% of A, 0-2 min; 90% of A and 10% of B, 2-3 min; 80% of A and 20% of B, 3-10 min; 72% of A and 28% of B, 10-20 min; 65% of A and 35% of B, 20-40 min; 55% of A and 45% of B, 40-50 min; 44% of A and 56% of B, 50-51 min; 30% of A and 70% of B, 51-55 min and 10% of A and 90% of B, 55-70 min. The wavelength for detection was 210 nm (PDA detector) and the different glutenin fractions were calculated using the areas of the chromatogram using the Class-VP™ software.

4.2.4 Data analysis

Only lines with the highest number of genes for resistance/QTL were selected for biochemical analyses. Only 50 lines, including parental lines and controls, were initially used for SDS-PAGE since 50 lines should give an accurate indication whether there existed a correlation between the SDS-PAGE profiles and the PCR-based marker data. For SE-HPLC analyses, the selected experimental lines for both populations and year groups were analysed to determine the LUPP%, which gives an indirect indication of good or poor bread baking quality. RP-HPLC was only done for the first set of samples because correlations in this study were not significant for bread-making characteristics.

4.2.4.1 PCR marker analysis

The dominant quality makers were scored for the presence (1) or absence (0) for the specific HMW-GS or translocation. For the co-dominant markers, the presence of the allele was scored as 1, irrespective if the allele was homo- or heterozygous. The total desirable alleles were summed to give the total alleles linked to protein quality. The absence of the 1BL.1RS translocation were seen and counted as a favourable allele to the total alleles linked to protein quality.

4.2.4.2 SDS-PAGE

Samples were individually scored to determine if lines were segregating or not for their HMW-GS SDS-PAGE profiles. The HMW-GS present in every genome were scored using Payne and Lawrence (1983) nomenclature. Figure 4.1 illustrates and explains how the HMW-GS were scored.

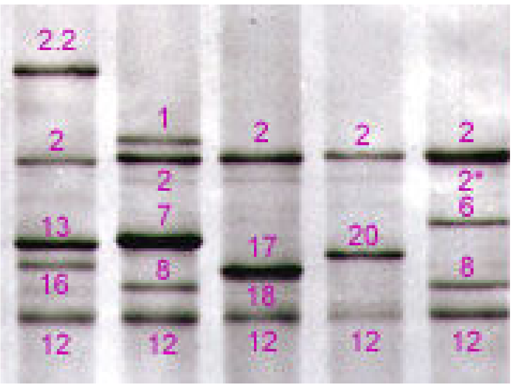

Figure 4.1 Illustration of the separation patterns for HMW-GS and numbering system used (Pfluger 2013).

4.2.4.3 SE-HPLC

The LUPP% is an indirect parameter to evaluate the bread-making quality characteristics of different wheat lines. The SE-HPLC results were used to determine the large unextractable polymeric protein percentage (LUPP%). The LUPP% = 100[SDS-unextractable polymeric protein/(SDS-unextractable polymeric protein + SDS-extractable polymeric protein)]. The first peak on the chromatogram represents this polymeric protein fraction. Figure 4.2 illustrates the four peaks on the chromatogram obtained from SE-HPLC analysis.

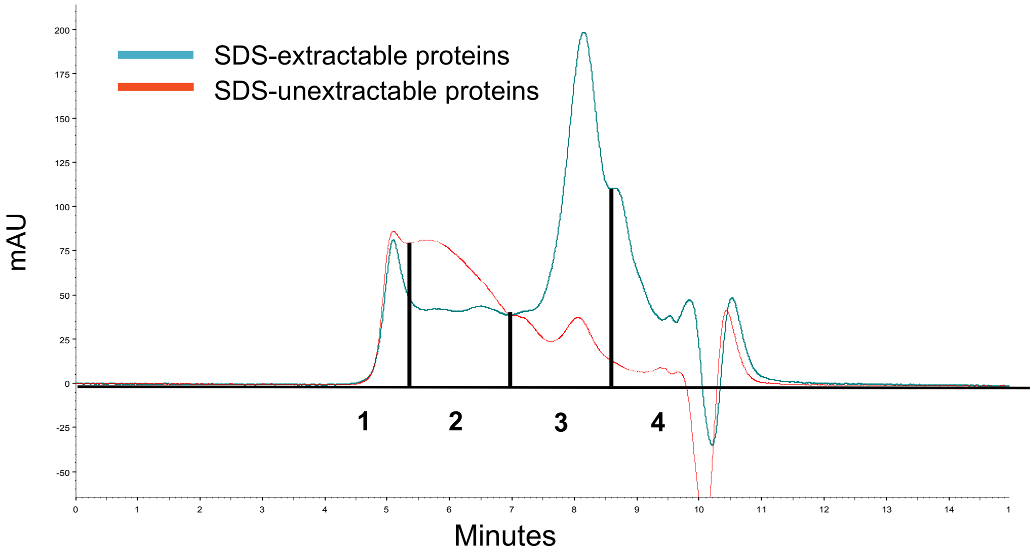

Figure 4.2 Size exclusion-high pressure liquid chromatography profile for sodium dodecyl sulphate-extractable and -unextrable fractions. The first fraction is the large polymeric proteins, the second fraction is the small polymeric proteins, the third fraction is the large monomeric proteins and the fourth fraction is the small monomeric proteins.

4.2.4.4 RP-HPLC

Areas of the chromatogram were integrated at specific time intervals to determine the amount of gliadins and glutenins present in the samples. The ω-, α- and γ-gliadins were measured approximately from 13 min to 25 min, 25 min to 30 min and 30 min to 45 min, respectively. HMW-GS and LMW-GS quantities were measured approximately from 19 min to 26 min and 26 min to 42 min, respectively. The quantity percentages were determined for every protein group to see if there existed any correlation between the LUPP% and/or number of markers linked to protein quality. Figures 4.3 and 4.4 represents chromatograms obtained from the RP-HPLC analysis for gliadins and glutenins respectively.

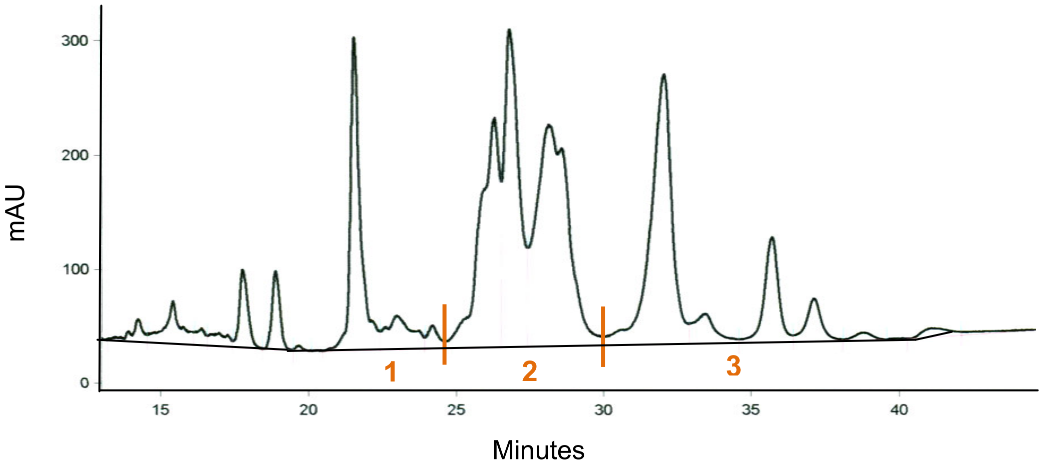

Figure 4.3 **RP-HPLC profile of gliadins.** The first group is the ω-gliadins, the second group is the α-gliadins and the third group is the γ-gliadins.

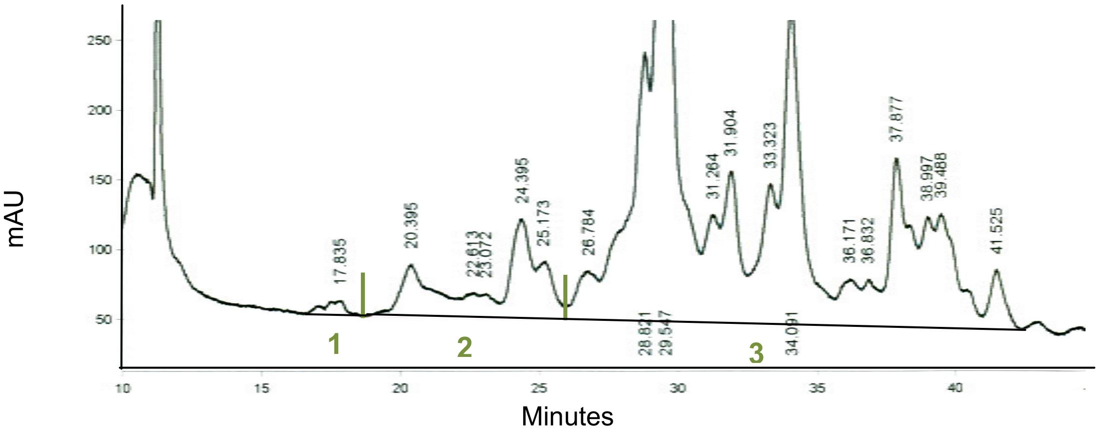

Figure 4.4 **RP-HPLC profile of glutenins.** The first group is the remaining ω-gliadins, the second group is the HMW-GS and the third group is the LMW-GS.

4.3 Results

4.3.1 Genotyping of lines using PCR-based markers linked to quality traits

4.3.1.1 Data obtained during first year of screening

The 42 rust resistant lines, 55 FHB resistant lines and 20 parental and control lines of the populations screened during the first year for the presence of rust and FHB resistance markers (Tables 3.2 and 3.3) were also screened with the protein quality markers. Table 4.3 summarises the data obtained from screening of the parental and control lines with the protein quality markers.

Table 4.3 Summary of the data obtain for protein quality markers evaluated on the parental and control lines

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5
Kariega	**1**	**17**	≠	0	0	0
Avocet*YrSp*	0	**7**	≠	0	0	0
Blade	**1**	**17**	≠	0	0	0
CS-*Lr19*-149-299	0	**7**	≠	0	0	**1**
Krokodil	**1**	**7**	**7^{OE}**	0	0	0
CM-82036	**1**	**17**	≠	0	1	0
Frontana	0	**7**	≠	0	0	0
Sumai 3	**1**	**7**	≠	0	0	0
PAN3434	**1**	**17**	≠	0	0	0
SST835	0	**7**	**7^{OE}**	0	0	0
Duzi	**1**	**17**	≠	0	0	0
Elands (SGI)	0	**7**	≠	1	0	**1**
Kariega (SGI)	**1**	**17**	≠	0	0	0
Tugela-DN (SGI)	0	**7**	**7^{OE}**	0	0	**1**
Scheepers69 (SGI)	0	**17**	≠	0	0	0
Picaflor	0	**17**	≠	0	0	**1**
Damphe	0	**17**	≠	0	0	**1**
Kingbird	**1**	**17**	≠	0	0	**1**
Line T6-1 (*SrR*+*S*-)	**1**	**17**	≠	0	0	0
2S#2/163 (*Sr39*)	**1**	**7**	≠	0	0	**1**

Alleles marked in bold indicate alleles linked to good bread-making qualities; 1 - Gene present; 0 - Gene absent; 7 - *Glu-Bx7* present; 17 - *Glu-Bx17* present; 7^{OE} - *Glu-Bx7*^{OE} present; ≠ - Did not contain the *Bx7+By8** allele combination; SGI - Small Grain Institute

Marker Ax2* scored positively for two of the four rust parental lines (Kariega and Blade) while the other two, Avocet*YrSp* and CS-*Lr19*-149-299 could either have the null allele or the *Ax1* allele. For the BxFp marker Kariega and Blade had the *Bx17* allele while Avocet*YrSp* and CS-*Lr19*-149-299 had the *Bx7* allele. None of the four rust resistant parental lines contained the *Bx7+Bx8** allele combination, the *By9* allele or the 1BL.1RS translocation. Only CS-*Lr19*-149-299 of the rust parental lines tested positive for the *Dx5* allele.

Krokodil and CM-82036, the FHB population's parental lines, tested positive for the *Ax2** allele but negative for *By9* and *Dx5.* Krokodil tested positive for the *Bx7* and *Bx7*^{OE} alleles and CM-82036 for the *Bx17* allele. None of the FHB parental lines tested positive for the *Bx7+By8** allele combination. Of the FHB parental lines only CM-82036 tested positive for the 1BL.1RS translocation.

Off the four ARC-SGI control lines, three (Elands, Kariega and Tugela-DN) were included as high baking quality control lines while Scheepers69 was linked to low baking quality. The three high baking quality control lines, had either an allele or allele combination that is linked to good bread-making quality on the 1A or 1D chromosome. It is not clear whether Elands and Tugela-DN have a null or *Ax1* allele for chromosome 1A, the *Ax2** marker is dominant and only detect the *Ax2** allele. Scheepers69 is a low quality control line and did not contain major alleles linked to bread-making quality.

Data obtained from screening the protein quality markers on the 42 rust resistant lines during the first year are summarised in Table 4.4. Of the 42 rust resistant lines tested, 36 lines tested positive for the *Ax2** allele. The other six lines (all progeny of line S346) could either have a null allele or the *Ax1* allele on the chromosome 1A. All progeny of line S16 were segregating for the *Bx7* and *Bx17* alleles and contained the *Bx7* or *Bx17* allele or were heterozygous (*Bx7/Bx17*) while all progeny of the line S726 tested positive for only *Bx7*. Progeny of line S178 showed segregation for the *Bx7* and *Bx17* alleles while all progeny of line S346 tested positive for the *Bx17* allele. As expected, none of the lines tested positive for the *Bx7+By8** or *By9* alleles or the 1BL.1RS translocation. These segregation patterns were expected based on alleles detected in the four parental lines used to create these lines. Screening of marker Dx5 indicated that 24 rust resistant lines contained the *Dx5* allele while 18 lines tested negative. The *Dx5* allele in these progeny was inherited from CS-*Lr19*-149-299, the only rust parental line that tested positive for the *Dx5* allele.

A total of 55 FHB resistant lines were screened during the first year with protein quality markers and the data obtained are summarised in Table 4.5. Results obtained from the 55 FHB resistant lines correlated with expected results, based on the alleles present in the two parental lines. Both Krokodil and CM-82036 tested positive for the *Ax2** allele and as expected, all 55 progeny tested positive for the *Ax2** allele. Krokodil tested positive for the *Bx7* and *Bx7*^{OE} alleles while CM-82036 tested positive for the *Bx17* allele and negative for the *Bx7*^{OE} allele.

Table 4.4 Summarised data of the protein quality markers tested on the rust resistant lines during the first year of screening

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5
S16(7.3)/1.1	1	7/17	≠	0	0	1
S16(7.3)P1.5.1/1.3	1	7/17	≠	0	0	1
S16(7.3)P1.5.1/2.2	1	7/17	≠	0	0	0
S16(7.3)P1.5.1/3.1	1	7	≠	0	0	1
S16(7.3)P1.5.1/3.3	1	7	≠	0	0	1
S16(7.3)P1.5.1/4.2	1	7	≠	0	0	1
S16(7.3)P1.5.1/4.3	1	7	≠	0	0	1
S16(7.3)P1.5.1/5.1	1	7	≠	0	0	1
S16(7.3)P3.5.1/2.3	1	7/17	≠	0	0	1
S16(7.3)P3.5.1/3.2	1	7	≠	0	0	1
S16(7.3)P3.6.1/5.3	1	7/17	≠	0	0	1
S16(7.3)P3.6.1/6.1	1	7/17	≠	0	0	1
S16(7.3)P3.6.1/6.3	1	7	≠	0	0	1
S16(7.3)P3.2.1/1.1	1	7/17	≠	0	0	1
S16(7.3)P3.2.1/1.2	1	7/17	≠	0	0	1
S16(7.3)P3.2.1/2.1	1	7	≠	0	0	1
S16(7.3)P3.2.1/2.2	1	7	≠	0	0	0
S16(7.3)P3.2.1/3.2	1	7	≠	0	0	1
S16(7.3)P3.2.1/3.3	1	7	≠	0	0	0
S16(7.3)P3.2.1/6.3	1	7	≠	0	0	0
S16(1.2)/2.1	1	7/17	≠	0	0	0
S16(1.2)/3.2	1	7/17	≠	0	0	0
S16(1.2)/5.2	1	7	≠	0	0	0
S726(3.2)/1	1	7/17	≠	0	0	0
S726(3.2)P1.3.1/1.1	1	7	≠	0	0	0
S726(3.2)P1.3.1/2.2	1	7	≠	0	0	0
S726(3.2)P1.3.1/5.2	1	7	≠	0	0	0
S726(3.2)P1.3.1/6.2	1	7	≠	0	0	0
S178(7.2)/2.3	1	7/17	≠	0	0	1
S178(7.2)/3.1	1	7/17	≠	0	0	0
S178(7.2)/3.2	1	7/17	≠	0	0	1
S178(7.2)/4.1	1	7/17	≠	0	0	1
S178(7.2)/4.2	1	17	≠	0	0	1
S178(7.2)/5.1	1	7	≠	0	0	1
S178(7.2)/5.2	1	17	≠	0	0	1
S178(7.2)/6.1	1	17	≠	0	0	0
S346(1.3)	0	17	≠	0	0	0
S346(3.1)	0	17	≠	0	0	0
S346(5.2)	0	17	≠	0	0	0
S346(5.3)	0	17	≠	0	0	1
S346(6.1)	0	17	≠	0	0	0
S346(6.3)	0	17	≠	0	0	1

1 - Gene present; 0 - Gene absent; 7 - *Glu-Bx7* present; 17 - *Glu-Bx17* present; 7^{OE} - *Glu-Bx7*^{OE} present; ≠ - Did not contain the *Bx7+By8** allele combination

Table 4.5 Summarised data of the protein quality markers tested on the FHB resistant lines during the first year of screening

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5
$BC_2F_2P2.6.2/1.1$	1	7/17	7^{OE}	0	0	0
$BC_2F_2P2.6.2/1.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.6.2/2.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.6.2/3.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.6.2/4.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.14.2/1.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.14.2/2.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.14.2/3.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.14.2/4.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.14.2/5.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.7.2/1.2$	1	7	7^{OE}	0	1	0
$BC_2F_2P1.7.2/2.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.7.2/3.2$	1	7	7^{OE}	0	1	0
$BC_2F_2P1.7.2/4.2$	1	7	7^{OE}	0	1	0
$BC_2F_2P1.7.2/5.2$	1	7	7^{OE}	0	1	0
BC_2F_2 4.1/1.3	1	17	≠	0	1	0
BC_2F_2 4.1/2.3	1	17	≠	0	1	0
BC_2F_2 4.1/3.3	1	17	≠	0	1	0
BC_2F_2 4.1/4.3	1	7/17	≠	0	1	0
BC_2F_2 4.1/5.3	1	17	≠	0	1	0
$BC_2F_2P1.7.1/1.2$	1	7/17	7^{OE}	0	0	0
$BC_2F_2P1.7.1/2.2$	1	7/17	7^{OE}	0	0	0
$BC_2F_2P1.7.1/3.2$	1	7/17	7^{OE}	0	0	0
$BC_2F_2P1.7.1/4.2$	1	17	≠	0	0	0
$BC_2F_2P1.7.1/5.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.7.3/1.3$	1	7/17	≠	0	1	0
$BC_2F_2P1.7.3/2.3$	1	17	≠	0	1	0
$BC_2F_2P1.7.3/3.3$	1	17	≠	0	1	0
$BC_2F_2P1.7.3/4.3$	1	7/17	≠	0	1	0

Table 4.5 Summarised data of the protein quality markers tested on the FHB resistant lines during the first year of screening (continued)

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5
$BC_2F_2P1.7.3/5.3$	1	17	≠	0	0	0
$BC_2F_2P2.2.3/1.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.2.3/2.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.2.3/3.1$	1	7/17	7^{OE}	0	0	0
$BC_2F_2P2.2.3/4.1$	1	17	≠	0	1	0
$BC_2F_2P2.2.3/5.1$	1	17	≠	0	1	0
$BC_2F_2P2.3.2/1.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.3.2/2.2$	1	7/17	7^{OE}	0	0	0
$BC_2F_2P2.3.2/3.2$	1	7/17	≠	0	1	0
$BC_2F_2P2.3.2/4.2$	1	7/17	≠	0	1	0
$BC_2F_2P2.3.2/5.3$	1	17	≠	0	1	0
$BC_2F_2P2.12.1/1.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.12.1/2.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.12.1/3.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.12.1/4.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P2.12.1/5.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.5.3/1.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.5.3/2.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.5.3/3.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.5.3/3.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.5.3/4.1$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.2.1/1.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.2.1/2.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.2.1/3.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.2.1/4.2$	1	7	7^{OE}	0	0	0
$BC_2F_2P1.2.1/6.2$	1	7	7^{OE}	0	0	0

1 - Gene present; 0 - Gene absent; 7 - *Glu-Bx7* present; 17 - *Glu-Bx17* present; 7^{OE} - *Glu-Bx7*^{OE} present; ≠ - Did not contain the *Bx7+By8**allele combination

As expected, progeny segregated for the *Bx7*, *Bx17* and *Bx7^{OE}* alleles. Most of the FHB resistant lines contained the *Bx7* allele (33 out of 55 lines tested) and the *Bx7^{OE}* allele (39 out of the 55 lines tested). This was also expected, since the F_1 was backcrossed twice with Krokodil to produce the BC_2F_2 population. Since the 1BL.1RS translocation was only present in the donor line CM-82036, it was expected for the translocation. Since *Dx5* and *By9* were absent in both the FHB parental lines, these markers tested negative in all 55 tested lines. Lines with the best protein marker combinations were selected and progeny of these lines were tested during the second year. The best FHB offspring lines according to their protein quality marker data are lines that contained the *Bx7^{OE}* allele and tested negative for the 1BL.1RS translocation

4.3.1.2 Data obtained during second year of screening

For the second year of screening a total of 209 lines that consisted of 88 rust resistant and 121 FHB resistant lines, were screened with the protein quality markers. These lines were progeny of the best lines tested and identified during the first year of screening. For comparison reasons, lines selected based on data generated during the first year of screening (22 rust resistant lines and 25 FHB resistant lines) were included together with their offspring in Tables 4.6 and 4.7.

As expected, based on the lines selected from the first year, none of the offspring of the 22 selected rust resistant lines contained the *Bx7+By8** allele combination, *By9* allele or 1BL.1RS translocation. Ax2* and Dx5 were present in all offspring of lines that contained the allele during the first round of screening, indicating that Ax2* and Dx5 were homozygous in all these selected lines. Ax2* was absent in all offspring of the three selected lines [S346(1.3), S346(5.3) and S346(6.3)] that did not contain this allele during the first year of screening. Segregation for the *Bx7* and *Bx17* alleles in offspring corresponded to the homozygous or heterozygous state of these alleles in selected lines from the first year of screening. It was homozygous for either the *Bx7* or *Bx17* allele in most offspring. However, as expected, it was still segregating in offspring of lines S16(7.3)P1.5.1/1.3, S16(7.3)P3.2.1/1.1, S16(1.2)/2.1, S726(3.2)/1, S178(7.2)/2.3 and S178(7.2)4.1. The best lines indicated in bold in Table 4.6 were selected for SE-HPLC analysis.

Data generated from screening the 25 FHB resistant lines selected after the first year screening and their 121 offspring using the protein quality markers are summarised in Table 4.7. All offspring contained the *Ax2** allele, as expected, since it was present in all the selected FHB resistant lines screened during the first year.

Table 4.6 Summarised data of the protein quality markers tested on selected first year lines and their offspring of the rust resistant population

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5	Total
S16(7.3)P1.5.1/1.3	2*	7/17	≠	0	0	1	4
S16(7.3)P1.5.1/1.3.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/1.3.2	**2***	**7/17**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/1.3.3	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/1.3.4	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/1.3.5	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/1.3.6	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/3.1	2*	7	≠	0	0	1	4
S16(7.3)P1.5.1/3.1.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/3.1.2	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/3.3	2*	7	≠	0	0	1	4
S16(7.3)P1.5.1/3.3.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/4.2	2*	7	≠	0	0	1	4
S16(7.3)P1.5.1/4.2.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/4.2.2	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/4.2.3	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/4.2.4	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/4.3	2*	7	≠	0	0	1	4
S16(7.3)P1.5.1/4.3.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/5.1	2*	7	≠	0	0	1	4
S16(7.3)P1.5.1/5.1.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/5.1.2	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P1.5.1/5.1.3	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.6.1/5.3	2*	7/17	≠	0	0	1	4
S16(7.3)P3.6.1/5.3.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.6.1/5.3.2	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.6.1/5.3.3	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.6.1/5.3.4	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.6.1/6.1	2*	7/17	≠	0	0	1	4
S16(7.3)P3.6.1/6.1.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.6.1/6.1.2	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.6.1/6.1.3	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.6.1/6.1.4	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.6.1/6.1.5	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/1.1	2*	7/17	≠	0	0	1	4
S16(7.3)P3.2.1/1.1.1	2*	17	≠	0	0	1	4
S16(7.3)P3.2.1/1.1.2	**2***	**7**	**≠**	**0**	**0**	**1**	**4**

Table 4.6 Summarised data of the protein quality markers tested on selected first year lines and their offspring of the rust resistant population (continued)

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5	Total
S16(7.3)P3.2.1/1.1.3	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/1.1.4	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/1.1.5	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/1.1.6	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/1.2	2*	7/17	≠	0	0	1	4
S16(7.3)P3.2.1/1.2.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/1.2.2	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/1.2.3	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/1.2.4	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/1.2.5	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/2.1	2*	7	≠	0	0	1	4
S16(7.3)P3.2.1/2.1.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/2.1.2	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/2.1.3	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/2.1.4	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/2.1.5	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/2.1.6	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/3.2	2*	7	≠	0	0	1	4
S16(7.3)P3.2.1/3.2.1	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/3.2.2	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/3.2.3	**2***	**7**	**≠**	**0**	**0**	**1**	**4**
S16(7.3)P3.2.1/3.2.4	2*	7	≠	0	0	1	4
S16(7.3)P3.2.1/3.2.5	2*	7	≠	0	0	0	3
S16(1.2)/2.1	2*	7/17	≠	0	0	0	3
S16(1.2)/2.1.1	2*	7	≠	0	0	0	3
S16(1.2)/2.1.2	2*	7/17	≠	0	0	0	3
S16(1.2)/2.1.3	2*	7	≠	0	0	0	3
S16(1.2)/2.1.4	2*	7	≠	0	0	0	3
S16(1.2)/2.1.5	2*	7	≠	0	0	0	3
S726(3.2)/1	2*	7/17	≠	0	0	0	3
S726(3.2)/1.1	**2***	**7**	**≠**	**0**	**0**	**0**	**3**
S726(3.2)/1.2	2*	7	≠	0	0	0	3
S726(3.2)/1.3	2*	7	≠	0	0	0	3
S726(3.2)/1.4	**2***	**7/17**	**≠**	**0**	**0**	**0**	**3**
S726(3.2)/1.5	2*	7	≠	0	0	0	3
S726(3.2)/1.6	2*	7	≠	0	0	0	3
S726(3.2)P1.3.1/1.1	2*	7	≠	0	0	0	3
S726(3.2)P1.3.1/1.1.1	**2***	**7**	**≠**	**0**	**0**	**0**	**3**

Table 4.6 Summarised data of the protein quality markers tested on selected first year lines and their offspring of the rust resistant population (continued)

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5	Total
S726(3.2)P1.3.1/2.2	2*	7	≠	0	0	0	3
S726(3.2)P1.3.1/2.2.1	2*	7	≠	0	0	0	3
S178(7.2)/2.3	2*	7/17	≠	0	0	1	4
S178(7.2)/2.3.1	2*	17	≠	0	0	1	4
S178(7.2)/2.3.2	2*	7/17	≠	0	0	1	4
S178(7.2)/2.3.3	2*	17	≠	0	0	0	3
S178(7.2)/2.3.4	2*	17	≠	0	0	0	3
S178(7.2)/4.1	2*	7/17	≠	0	0	1	4
S178(7.2)/4.1.1	2*	7/17	≠	0	0	1	4
S178(7.2)/4.1.2	2*	7	≠	0	0	1	4
S178(7.2)/4.1.3	2*	7/17	≠	0	0	1	4
S178(7.2)/4.1.4	2*	17	≠	0	0	1	4
S178(7.2)/4.1.5	2*	7	≠	0	0	1	4
S178(7.2)/4.1.6	2*	7/17	≠	0	0	1	4
S178(7.2)/5.1	2*	7	≠	0	0	1	4
S178(7.2)/5.1.1	2*	7	≠	0	0	1	4
S178(7.2)/5.1.2	2*	7	≠	0	0	1	4
S178(7.2)/5.1.3	2*	7	≠	0	0	1	4
S178(7.2)/5.1.4	2*	7	≠	0	0	1	4
S346(1.3)	0	17	≠	0	0	0	2
S346(1.3).1	**0**	**17**	**≠**	**0**	**0**	**0**	**2**
S346(1.3).2	0	17	≠	0	0	0	2
S346(1.3).3	0	17	≠	0	0	0	2
S346(1.3).4	0	17	≠	0	0	0	2
S346(1.3).5	0	17	≠	0	0	0	2
S346(5.3)	0	17	≠	0	0	1	3
S346(5.3).1	0	17	≠	0	0	1	3
S346(5.3).2	0	17	≠	0	0	1	3
S346(5.3).3	0	17	≠	0	0	1	3
S346(5.3).4	0	17	≠	0	0	1	3
S346(5.3).5	**0**	**17**	**≠**	**0**	**0**	**1**	**3**
S346(6.3)	0	17	≠	0	0	1	3
S346(6.3).1	0	17	≠	0	0	1	3
S346(6.3).2	0	17	≠	0	0	1	3
S346(6.3).3	0	17	≠	0	0	1	3

Lines marked grey are the related first year lines; Lines marked in bold are the selected lines used for SE-HPLC analysis; 1 - Gene present; 0 - Gene absent; 7 - *Glu-Bx7* present; 17 - *Glu-Bx17* present; 7^{OE} - *Glu-Bx7*^{OE} present; ≠ - Did not contain the *Bx7+By8** allele combination

Table 4.7 Summarised data of the protein quality markers tested on the selected FHB resistant lines of the first year and their offspring

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5	Total
BC$_2$F$_2$P2.6.2/1.1	2*	7/17	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.6.2/1.1.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/1.1.2	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/1.1.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/1.1.5	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_2$P2.6.2/1.2	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.6.2/1.2.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/1.2.2	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/1.2.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/1.2.4	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/1.2.5	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_2$P2.6.2/2.1	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.6.2/2.1.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/2.1.2	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.6.2/2.1.3	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.6.2/2.1.4	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.6.2/2.1.5	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/2.1.6	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_2$P2.6.2/4.1	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.6.2/4.1.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/4.1.2	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.6.2/4.1.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.6.2/4.1.4	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.6.2/4.1.5	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_2$4.1/1.3	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/1.3.1	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/1.3.2	2*	17	≠	0	1	0	2
BC$_2$F$_2$4.1/2.3	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/2.3.1	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/2.3.2	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/2.3.3	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/2.3.4	2*	17	≠	0	1	0	2
BC$_2$F$_2$4.1/3.3	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/3.3.1	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/3.3.2	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/3.3.3	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/3.3.4	2*	17	≠	0	0	0	2

Table 4.7 Summarised data of the protein quality markers tested on the selected FHB resistant lines of the first year and their offspring (continued)

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5	Total
BC$_2$F$_2$4.1/4.3	2*	7/17	≠	0	1	0	2
BC$_2$F$_3$4.1/4.3.1	**2***	**7/17**	**≠**	**0**	**0**	**0**	**3**
BC$_2$F$_3$4.1/4.3.2	**2***	**7/17**	**≠**	**0**	**0**	**0**	**3**
BC$_2$F$_3$4.1/4.3.3	2*	7	≠	0	0	0	3
BC$_2$F$_3$4.1/4.3.4	2*	7/17	≠	0	0	0	3
BC$_2$F$_2$4.1/5.3	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/5.3.1	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/5.3.2	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/5.3.3	2*	17	≠	0	0	0	3
BC$_2$F$_3$4.1/5.3.4	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/5.3.5	2*	17	≠	0	1	0	2
BC$_2$F$_3$4.1/5.3.6	2*	17	≠	0	1	0	2
BC$_2$F$_2$P1.7.1/1.2	2*	7/17	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.7.1/1.2.1	**2***	**7/17**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/1.2.2	**2***	**7/17**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/1.2.3	**2***	**17**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/1.2.4	**2***	**7/17**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/1.2.5	**2***	**7/17**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/1.2.6	**2***	**17**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_2$P1.7.1/2.2	2*	7/17	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.7.1/2.2.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/2.2.2	2*	17	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.7.1/2.2.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/2.2.4	2*	7/17	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.7.1/2.2.5	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_2$P1.7.1/3.2)	2*	7/17	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.7.1/3.2.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/3.2.2	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/3.2.3	**2***	**7/17**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/3.2.4	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/3.2.5	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/3.2.6	2*	17	7^{OE}	0	0	0	4
BC$_2$F$_2$P1.7.1/4.2	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.1/4.2.1	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.1/4.2.2	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.1/4.2.3	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.1/4.2.4	2*	17	≠	0	0	0	3

Table 4.7 Summarised data of the protein quality markers tested on the selected FHB resistant lines of the first year and their offspring (continued)

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5	Total
BC$_2$F$_3$P1.7.1/4.2.5	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.1/4.2.6	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.1/4.2.7	2*	17	≠	0	0	0	3
BC$_2$F$_2$P1.7.1/5.2	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.7.1/5.2.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/5.2.2	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/5.2.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.7.1/5.2.4	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_2$P1.7.3/4.3	2*	7/17	≠	0	1	0	2
BC$_2$F$_3$P1.7.3/4.3.1	2*	7	≠	0	0	0	3
BC$_2$F$_3$P1.7.3/4.3.2	2*	7/17	≠	0	1	0	2
BC$_2$F$_3$P1.7.3/4.3.3	2*	7/17	≠	0	1	0	2
BC$_2$F$_3$P1.7.3/4.3.4	2*	7/17	≠	0	1	0	2
BC$_2$F$_3$P1.7.3/4.3.5	2*	7/17	≠	0	1	0	2
BC$_2$F$_3$P1.7.3/4.3.6	2*	17	≠	0	1	0	2
BC$_2$F$_3$P1.7.3/4.3.7	2*	7/17	≠	0	1	0	2
BC$_2$F$_2$P1.7.3/5.3	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.3/5.3.1	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.3/5.3.2	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.3/5.3.3	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.3/5.3.4	2*	17	≠	0	0	0	3
BC$_2$F$_3$P1.7.3/5.3.5	2*	17	≠	0	0	0	3
BC$_2$F$_2$P2.2.3/1.1	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.2.3/1.1.1	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.2.3/1.1.2	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.2.3/1.1.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.2.3/1.1.4	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_2$P2.2.3/4.1	2*	17	≠	0	1	0	2
BC$_2$F$_3$P2.2.3/4.1.1	2*	17	≠	0	1	0	2
BC$_2$F$_3$P2.2.3/4.1.2	2*	17	≠	0	1	0	2
BC$_2$F$_3$P2.2.3/4.1.3	2*	17	≠	0	1	0	2
BC$_2$F$_3$P2.2.3/4.1.4	2*	17	≠	0	1	0	2
BC$_2$F$_2$P2.3.2/4.2	2*	7/17	7^{OE}	0	1	0	3
BC$_2$F$_3$P2.3.2/4.2.1	2*	17	7^{OE}	0	1	0	3
BC$_2$F$_3$P2.3.2/4.2.2	2*	7/17	7^{OE}	0	1	0	3
BC$_2$F$_3$P2.3.2/4.2.3	2*	7/17	7^{OE}	0	1	0	3
BC$_2$F$_3$P2.3.2/4.2.4	2*	7/17	7^{OE}	0	1	0	3
BC$_2$F$_3$P2.3.2/4.2.5	2*	7/17	7^{OE}	0	1	0	3

Table 4.7 Summarised data of the protein quality markers tested on the selected FHB resistant lines of the first year and their offspring (continued)

Line name	Ax2*	BxFp	MAR	ZSBy9a F1/R3	Glu-B3j	Dx5	Total
BC$_2$F$_3$P2.3.2/4.2.6	2*	7	7^{OE}	0	1	0	3
BC$_2$F$_2$P2.3.2/5.3	2*	17	≠	0	1	0	2
BC$_2$F$_3$P2.3.2/5.3.1	2*	17	≠	0	1	0	2
BC$_2$F$_3$P2.3.2/5.3.2	2*	17	≠	0	1	0	2
BC$_2$F$_3$P2.3.2/5.3.3	2*	17	≠	0	1	0	2
BC$_2$F$_3$P2.3.2/5.3.4	2*	17	≠	0	1	0	2
BC$_2$F$_3$P2.3.2/5.3.5	2*	17	≠	0	1	0	2
BC$_2$F$_2$P2.12.1/1.1	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.12.1/1.1.1	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P2.12.1/1.1.2	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.12.1/1.1.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.12.1/1.1.4	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.12.1/1.1.5	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.12.1/1.1.6	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_2$P2.12.1/2.1	2*	7	**7^{OE}**	0	0	0	4
BC$_2$F$_3$P2.12.1/2.1.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.12.1/2.1.2	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P2.12.1/2.1.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_2$P1.2.1/1.2	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.2.1/1.2.1	2*	7	**7^{OE}**	0	0	0	4
BC$_2$F$_3$P1.2.1/1.2.2	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.2.1/1.2.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.2.1/1.2.4	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.2.1/1.2.5	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_2$P1.2.1/2.2	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.2.1/2.2.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.2.1/2.2.2	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.2.1/2.2.3	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.2.1/2.2.4	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_2$P1.2.1/3.2	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.2.1/3.2.1	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.2.1/3.2.2	**2***	**7**	**7^{OE}**	**0**	**0**	**0**	**4**
BC$_2$F$_3$P1.2.1/3.2.3	2*	7	7^{OE}	0	0	0	4
BC$_2$F$_3$P1.2.1/3.2.4	2*	7	7^{OE}	0	0	0	4

Lines marked grey are the related first year lines; Lines marked in bold are the selected lines used for SE-HPLC analysis; 1 - Gene present; 0 - Gene absent; 7 - *Glu-Bx7* present; 17 - *Glu-Bx17* present; 7^{OE} - *Glu-Bx7*^{OE} present; ≠ - Did not contain the *Bx7+By8** allele combination

As expected, By9 and Dx5 were absent in all offspring, due to the absence of these alleles in the selected FHB resistant lines they were developed from. Offspring were either homozygous for the *Bx7* or *Bx17* allele or heterozygous (*Bx7*/*Bx17*) and segregation patterns corresponded to the lines they were developed from. Almost all of the FHB resistant lines that contained the *Bx7* allele, tested positive for the $Bx7^{OE}$ allele. Results for the 1BL.1RS translocation indicated that the translocation was homozygous in offspring of lines $BC_2F_24.1/1.3$, $BC_2F_24.1/2.3$, $BC_2F_2P2.2.3/4.1$, $BC_2F_2P2.3.2/4.2$ and $BC_2F_2P2.3.2/5.3$ but heterozygous in offspring of lines $BC_2F_24.1/3.3$, $BC_2F_24.1/5.3$ and $BC_2F_2P1.7.3/4.3$. The best FHB resistant lines (marked in bold in Table 4.7) were selected for SE-HPLC analysis.

Continuous selection of lines based on molecular and biochemical data has influenced the number of lines containing favourable and unfavourable alleles. Table 4.8 summarises the percentages of lines containing quality markers at different stages of selection. Data is presented for the parental lines used to create the rust resistant population, the 42 rust resistant lines selected and tested during the first year of screening, the best 22 selected lines based on molecular and biochemical data from the first year, the 88 offspring of the best 22 rust resistant lines that were tested during the second year of screening and finally the 50 best lines selected based on data generated during the second year of screening. The *Ax2**, *Bx7* and *Dx5* alleles were present in more than 90% of the best lines selected at the end of the study. This increased from between 25% to 50% in the original parental lines. However, the presence of the *Bx17* allele decreased from 50% to only 4%

Table 4.9 summarises the percentages of FHB resistant lines containing quality markers at different stages of selection. Data is presented for the two parental lines used to create the FHB resistant population, the 55 FHB resistant lines selected and tested during the first year of screening, the best 25 lines based on molecular and biochemical data from the first year, the 121 offspring of the best 25 FHB resistant lines that were tested during the second year of screening and finally the 50 best lines selected based on data generated during the second year of screening. The *Ax2** and *Dx5* allele percentages did not change during the entire study, as expected. However, both the *Bx7* and $Bx7^{OE}$ allele percentages increased from 50% in the parental lines to more than 80% in the best lines selected at the end of the study. However, the *Bx17* allele percentage decreased from 50% to 4%.

Table 4.8 Percentage of rust resistant lines containing different protein quality molecular markers during different stages of selection

Marker name	**Targeted allele**	**Parental lines**	**42 Lines selected for 1st year of screening**	**22 Best lines selected based on 1st year data**	**88 Offspring tested during 2nd year**	**50 Best lines selected based on 2nd year data**
Ax2*	**Ax2***	**50%**	**86%**	**86%**	**85%**	**100%**
BxFp	***Bx7***	**50%**	**41%**	**45%**	**72%**	**92%**
	Bx17	**50%**	**21%**	**14%**	**20%**	**4%**
	***Bx7*/Bx17**	**0%**	**38%**	**41%**	**8%**	**4%**
MAR	***Bx7***$^{\mathrm{OE}}$	**0%**	**0%**	**0%**	**0%**	**0%**
	Without *Bx7+By8**	100%	100%	100%	100%	100%
ZSBy9a F1/R3	*By9*	0%	0%	0%	0%	0%
Glu-B3j	1BL.1RS translocation	0%	0%	0%	0%	0%
Dx5	**Dx5**	**25%**	**57%**	**73%**	**76%**	**92%**

Data indicated in bold represents favourable bread-making qualities

Table 4.9 Percentage of FHB resistant lines containing different protein quality molecular markers during different stages of selection

Marker name	**Targeted allele**	**Parental lines**	**55 Lines selected for 1st year of screening**	**25 Best lines selected based on 1st year data**	**121 Offspring tested during 2nd year**	**50 Best lines selected based on 2nd year data**
Ax2*	***Ax2****	**100%**	**100%**	**100%**	**100%**	**100%**
BxFp	***Bx7***	**50%**	**60%**	**45%**	**50%**	**82%**
	Bx17	**50%**	**20%**	**14%**	**35%**	**4%**
	Bx7/Bx17	**0%**	**20%**	**41%**	**15%**	**14%**
MAR	***Bx7***$^{\mathrm{OE}}$	**50%**	**71%**	**56%**	**60%**	**96%**
	Without *Bx7+By8**	50%	29%	44%	40%	4%
ZSBy9a F1/R3	*By9*	0%	0%	0%	0%	0%
Glu-B3j	1BL.1RS translocation	50%	33%	36%	29%	0%
Dx5	***Dx5***	**0%**	**0%**	**0%**	**0%**	**0%**

Data indicated in bold represents favourable bread-making qualities.

4.3.2 SDS-PAGE

Most of the HMW-GS separated well and were easy to determine, except for 1Ax2* and 1Dx2 that had similar electrophoretic mobilities, making it difficult to separate and identify. Three seeds per line were tested to determine if the line was homozygous or heterozygous for the protein traits. Most of the lines were homozygous for the three samples tested within a line since the HMW-GS profiles were identical for all three samples (Figure 4.5). Controls included for SDS-PAGE were Sappo (2*, 14+15, 2+12), Elands (1, 7+9, 5+10) and Marquis (1, 7+9, 5+10).

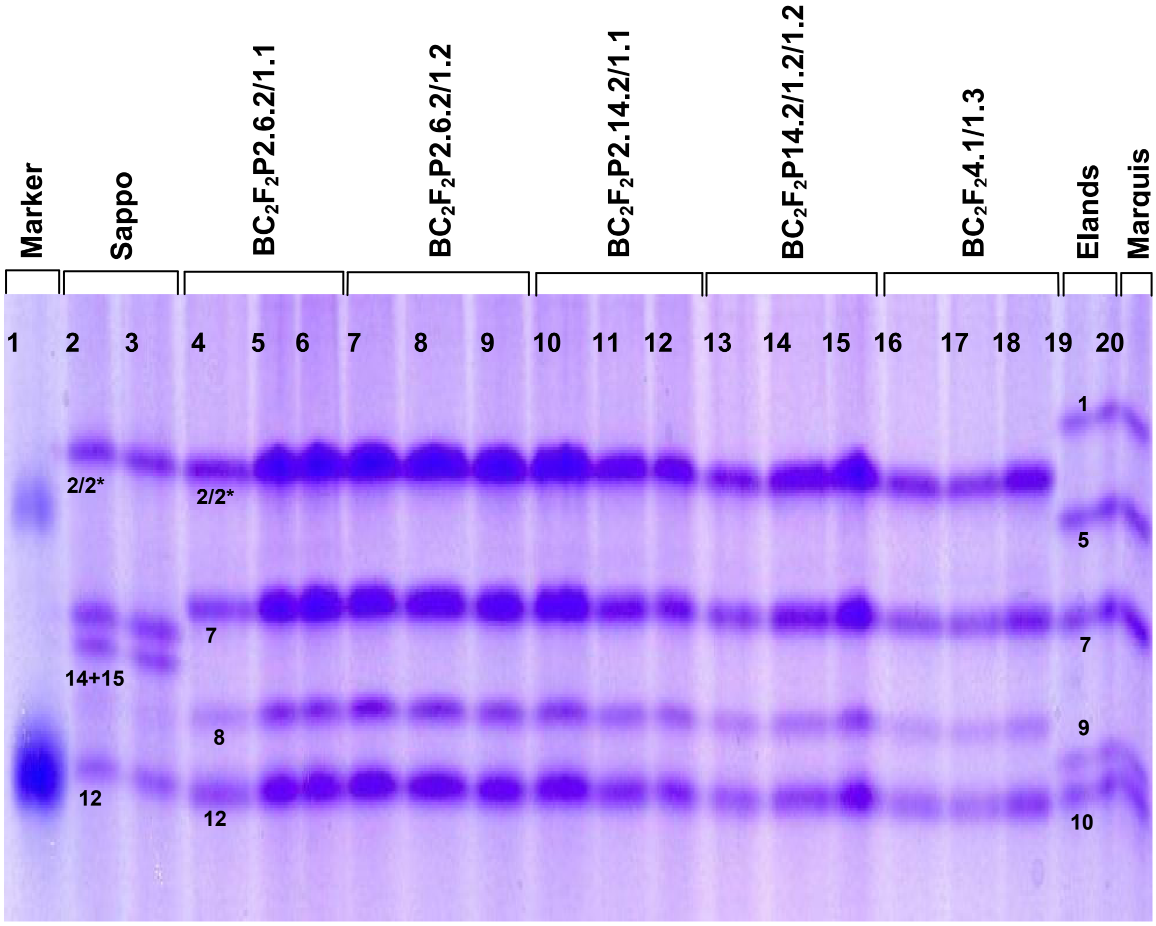

Figure 4.5 SDS-PAGE gel of five homozygous rust resistant lines. Line 1 contains the protein standard, lines 2 and 3 Sappo (control), lines 4-6 individuals of line $BC_2F_2P2.6.2/1.1$, lines 7-9 individuals of line $BC_2F_2P2.6.2/1.2$, lines 10-12 individuals of line $BC_2F_2P2.14.2/1.1$, lines 13-15 individuals of line $BC_2F_2P2.14.2/1.2$, lines 16-18 individuals of line $BC_2F_24.1/1.3$ and lines 19 and 20 Elands (control) and Marquis (control) respectively.

Five of the tested experimental wheat lines (one rust and four FHB resistant lines) displayed segregation for HMW-GS compositions at the *Glu-B1* locus within the three individual seed samples tested per line. These lines have not been self-pollinated enough to ensure a state of homozygosity between seed samples (Figure 4.6).

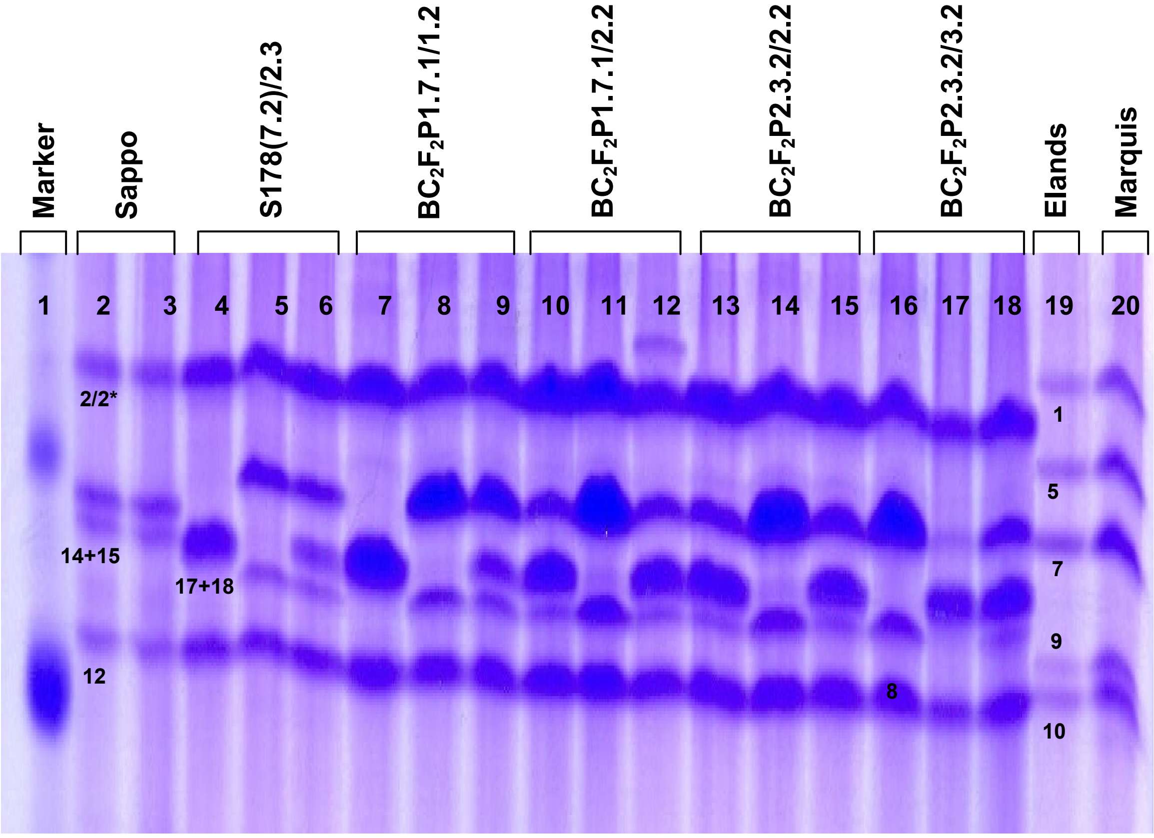

Figure 4.6 **SDS-PAGE gel of five heterozygous lines.** Line 1 contains the protein standard, lines 2 and 3 Sappo (control), lines 4-6 the three samples of line S178(7.2)/2.3, lines 7-9 the three samples of line BC_2F_2P1 7.1/1.2, lines 10-12 the three samples of line BC_2F_2P1 7.1/2.2, lines 13-15 the three samples of line BC_2F_2P2 3.2/2.2, lines 16-18 the three samples of line BC_2F_2P2 3.2/3.2 and lines 19 and 20 Elands (control) and Marquis (control) respectively.

HMW-GS compositions of the 50 rust and FHB resistant, parental and controls lines were identified and summarised in Table 4.10. Since most of the lines were homozygous for their HMW-GS profiles, the data presented in Table 4.10 is the projected profile for

Table 4.10 HMW-GS composition of the parental, control and selected 50 rust and FHB resistant lines based on SDS-PAGE analysis

Cultivar/Line	***Glu-A1***	***Glu-B1***	***Glu-D1***
Kariega	2*	17+18	2+12
Avocet*YrSp*	N	7+8	2+12
Blade	2*	17+18	2+12
CS-*Lr19*-149-299	N	7+8	5+10
Krokodil	2*	7+8	2+12
CM-82036	2*	17+18	2+12
PAN3434	2*	17+18	2+12
SST835	1	7+8	2+12
Duzi	2*	17+18	2+12
Elands (SGI)	1	7+9	5+10
Kariega (SGI)	2*	17+18	2+12
Tugela-DN (SGI)	2*	7+8	5+10
Scheepers69 (SGI)	N	17+18	2+12
Kingbird	2*	17+18	5+10
2S#2/163	2*	7+8	5+10
S16(7.3)/1.1	2*	7+8	5+10
S16(7.3)P1.5.1/1.3	2*	7+8	5+10
S16(7.3)P1.5.1/2.2	2*	7+8	2+12
S16(7.3)P3.5.1/2.3	2*	7+8	5+10
S16(7.3)P3.5.1/3.2	2*	7+8	5+10
S16(7.3)P3.6.1/5.3	2*	7+8	5+10
S16(7.3)P3.6.1/6.1	2*	7+8	5+10
S16(7.3)P3.2.1/1.1	2*	7+8	5+10
S16(7.3)P3.2.1/1.2	2*	7+8	5+10
S16(1.2)/2.1	2*	7+8	2+12
S726(3.2)/1	2*	7+8	2+12
S178(7.2)/2.3	**2***	**7+8/17+18**	**5+10**
S346(1.3)	2*	17+18	2+12
BC$_2$F$_2$P2.6.2/1.1	2*	7+8	2+12
BC$_2$F$_2$P2.6.2/1.2	2*	7+8	2+12
BC$_2$F$_2$P2.14.2/1.1	2*	7+8	2+12
BC$_2$F$_2$P2.14.2/2.1	2*	7+8	2+12
BC$_2$F$_2$P1.7.2/2.2	2*	7+8	2+12
BC$_2$F$_2$P1.7.2/3.2	2*	7+8	2+12
BC$_2$F$_2$ 4.1/1.3	2*	17+18	2+12
BC$_2$F$_2$ 4.1/2.3	2*	17+18	2+12
BC$_2$F$_2$P1.7.1/1.2	**2***	**7+8/17+18**	**2+12**
BC$_2$F$_2$P1.7.1/2.2	**2***	**7+8/17+18**	**2+12**
BC$_2$F$_2$P1.7.3/2.3	2*	17+18	2+12
BC$_2$F$_2$P1.7.3/3.3	2*	17+18	2+12
BC$_2$F$_2$P2.2.3/1.1	2*	7+8	2+12
BC$_2$F$_2$P2.2.3/2.1	2*	7+8	2+12
BC$_2$F$_2$P2.3.2/2.2	**2***	**7+8/7+9**	**2+12**
BC$_2$F$_2$P2.3.2/3.2	**2***	**7+8/7+9/17+18**	**2+12**
BC$_2$F$_2$P2.12.1/2.1	2*	7+8	2+12
BC$_2$F$_2$P2.12.1/3.1	2*	7+8	2+12
BC$_2$F$_2$P1.5.3/2.1	2*	7+8	2+12
BC$_2$F$_2$P1.2.1/2.2	2*	7+8	2+12
BC$_2$F$_2$P1.2.1/3.2	2*	7+8	2+12
BC$_2$F$_2$P1.2.1/4.2	2*	7+8	2+12

Lines marked in bold indicate the segregating lines; N - null allele; SGI - Small Grain Institute the tested lines. The lines marked in bold are the five heterozygous lines. The HMW-GS compositions of these lines were compared to PCR analyses to determine the correlation between the two techniques used. The best characteristics associated with bread-making qualities are the *Ax1* and *Ax2** alleles that are expressed on chromosome 1A, for chromosome 1B both Bx7+*By8* or *Bx17+By18* allele combinations and allele combination *Dx5+Dy10* for chromosome 1D. For the rust and FHB resistant lines tested, most expressed alleles associated with good bread-making qualities except for the FHB resistance lines that expressed the *Dx2+Dy12* allele combination instead of the *Dx5+Dy10* allele combination. Five of the tested (one rust and four FHB resistant lines) segregating for the alleles tested since the three seeds tested within one line varied for the alleles of chromosome 1B. Results obtained for the rust and FHB resistant lines corresponded to their parental lines.

4.3.3 Protein quality marker data versus SDS-PAGE data

As described in the previous section, HMW-GS compositions of 50 wheat lines selected from rust and FHB resistant lines tested during the first year were determined using SDS-PAGE. These same lines were screened using the protein quality PCR-based markers to determine the presence of the HMW-GS alleles and the presence of the 1BL.1RS translocation. A positive correlation was observed between these two methods as indicated in Figure 4.7. Markers *Ax2** and MAR did not show a 100% correlation due to missing values in the data set. The other three markers showed a 100% correlation with the SDS-PAGE data. For this reason it was decided to use only the PCR-based markers to determine the HMW-GS allele composition and the presence of the 1BL.1RS translocation in the rest of the lines tested.

4.3.4 SE-HPLC

4.3.4.1 Parental and control lines

The parental and control lines were included for the SE-HPLC analyses and the LUPP% are summarised in Figure 4.8. A LUPP% of 40% or higher indicates strong dough quality. All rust parental lines, except CS-*Lr19*-149-299, had desirable LUPP%. Krokodil, the recurrent parent for developing the FHB resistant populations had a desirable LUPP% (44.39%) while CM-82036 had a low LUPP% (27.20%). Elands, Kariega and Tugela-DN, included as positive controls for good bread-making qualities, had LUPP% that ranged from 46.90% to 49.53%. Scheepers69, included as the low quality control, had a LUPP% of 29.36%. Tugela-DN had the highest LUPP% of the control lines (49.53%) while Blade had the highest LUPP% of the parental lines (47.01%). The worst performing control line and parental line were 2S#2/163 and CM-82036 respectively, with values of 29.25% and 27.20%.

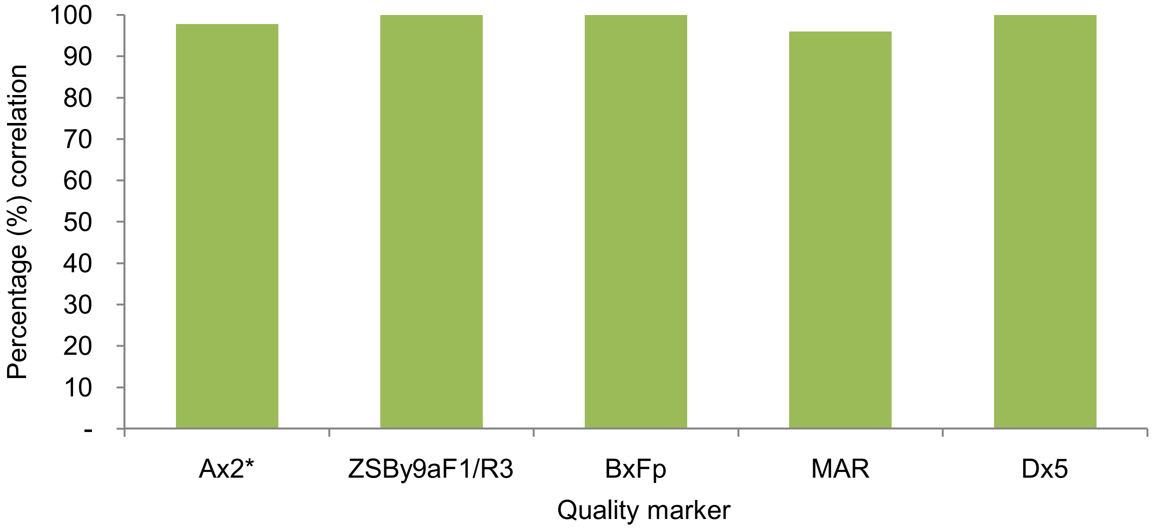

Figure 4.7 Percentage correlation detected between SDS-PAGE data and screening using PCR-based quality markers for the HMW-GS composition of the selected 50 wheat lines tested.

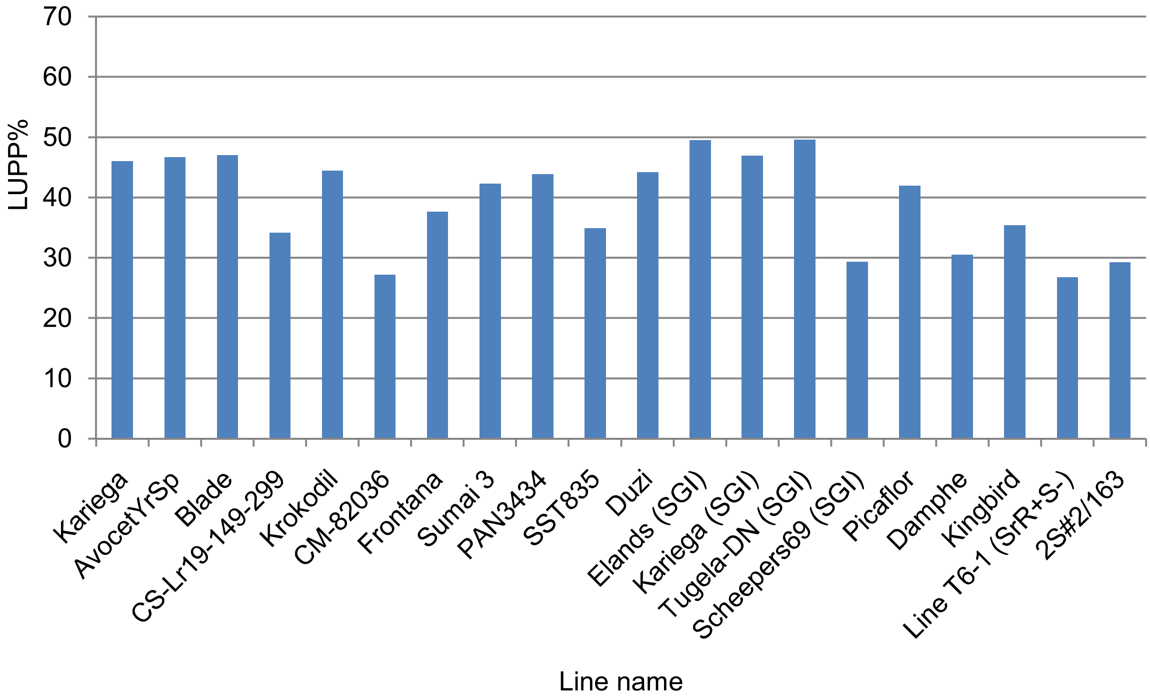

Figure 4.8 LUPP% of the parental and control lines.

4.3.4.2 SE-HPLC data obtained during the first year of screening

Based on molecular marker data for rust genes for resistance/QTL and protein quality data the best 42 rust resistant lines from the 203 resistant lines tested were selected for SE-HPLC analyses during the first year. The LUPP% for the rust resistant lines ranged from 18.79% (red bar, Figure 4.9) to 62.03% (blue bar, Figure 4.9). Only 17 lines had LUPP% higher than 40% and the average LUPP% for the 42 lines was 39.65%. Based on the molecular marker data linked to rust resistance as well as protein quality, the best lines (green bars, Figure 4.9) were selected for further analyses. The two lines with the lowest and highest LUPP% were included in the selected.

The SE-HPLC analyses for the FHB resistant lines were conducted on 55 FHB resistant lines selected from the 152 lines originally planted. Five offspring were randomly selected from each of the 11 FHB resistant lines planted. Figure 4.10 summarises the LUPP% for the FHB resistant experimental wheat lines tested during the first year. The lowest LUPP% was 22.09% (marked red) and the highest was 53.98% (marked blue). The average LUPP% for the FHB resistant lines was 39% and 26 lines had desirable LUPP% of 40% and higher. A total of 24 lines (green bars in Figure 4.10) and the line with the lowest LUPP% (red bar in Figure 4.10) were selected to produce the second experimental FHB population for further analyses based on the number of markers linked to the FHB resistance QTL and the markers linked to the protein quality composition.

4.3.4.3 SE-HPLC data obtained during the second year of screening

For the second year of analysis a total of 100 lines, 50 rust and 50 FHB resistant lines, were analysed using SE-HPLC. The LUPP% are summarised in Figure 4.11 for the rust resistant lines and Figure 4.12 for the FHB resistant lines. Three seeds per selected line were used for SE-HPLC analysis of the rust and FHB resistant lines. The LUPP% for the tested rust resistant lines ranged from 15.36% to 52.98%. High levels of variation for LUPP% were observed for offspring of subpopulation S16(7.3)P1.5.1/1.3 (15.36% to 52.98%) while low levels of variation were observed for offspring of subpopulation S16(7.3)P1.5.1/4.2 (30.25% to 38.96%). The LUPP% for the FHB resistant lines ranged from 23.06% to 48.82%. All offspring of group BC_2F_3P1.7.1/1.2 had low LUPP% while all offspring of subpopulation BC_2F_3P2.12.1/1.1 had LUPP% higher than 40%. Low levels of variation were observed for LUPP% between lines within their respective subpopulations, indicating low levels of segregation within subpopulations.

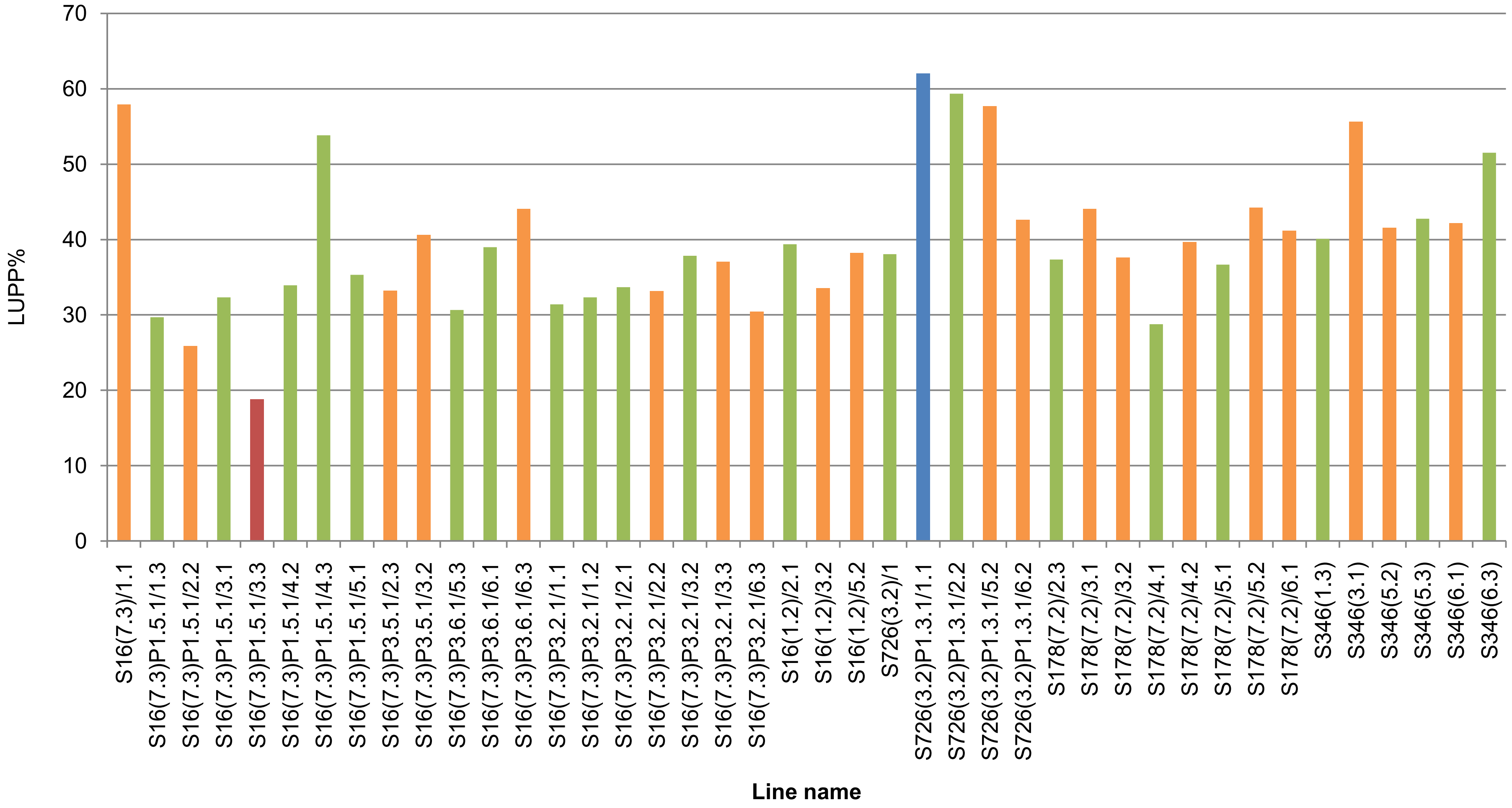

Figure 4.9 LUPP% of the different rust resistant lines selected for SE-HPLC during the first year of screening. The green bars indicate lines selected for the second year of analyses. The red bar indicates the line with lowest LUPP% while the blue bar indicates the line with the highest LUPP% and the orange bars are lines not selected for further analyses.

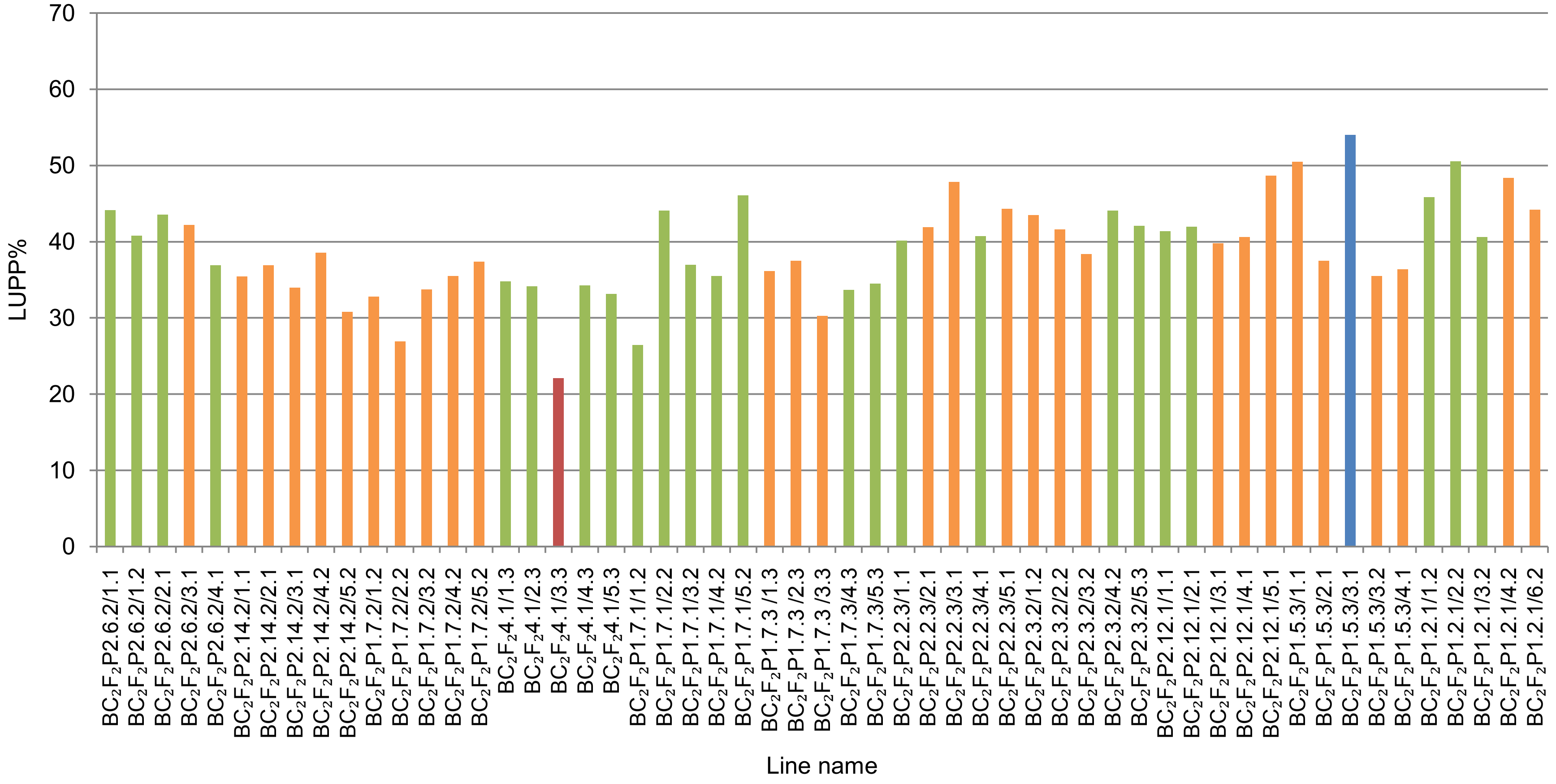

Figure 4.10 LUPP% of the different FHB resistant lines selected for SE-HPLC during the first year of screening. The green bars indicate the lines selected for analyses during the second year. The red bar indicates the line with lowest LUPP% while the blue bar indicates the line with the highest LUPP% and the orange bars lines not selected for further analyses.

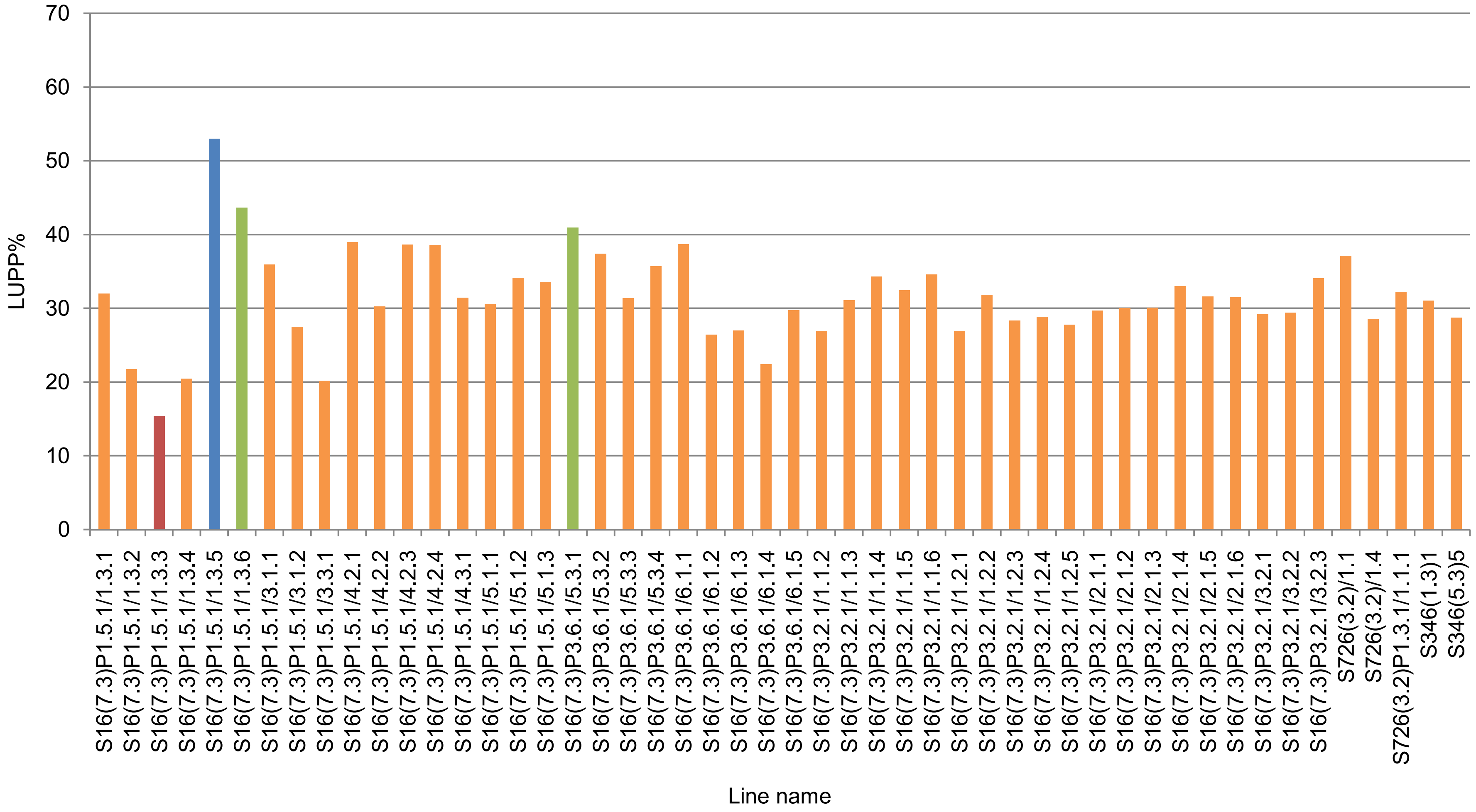

Figure 4.11 LUPP% of the different rust resistant lines selected for SE-HPLC during the second year of screening. The green bars indicate the lines with LUPP% of 40% and higher. The red bar indicates the line with lowest LUPP% while the blue bar indicates the line with the highest LUPP%.

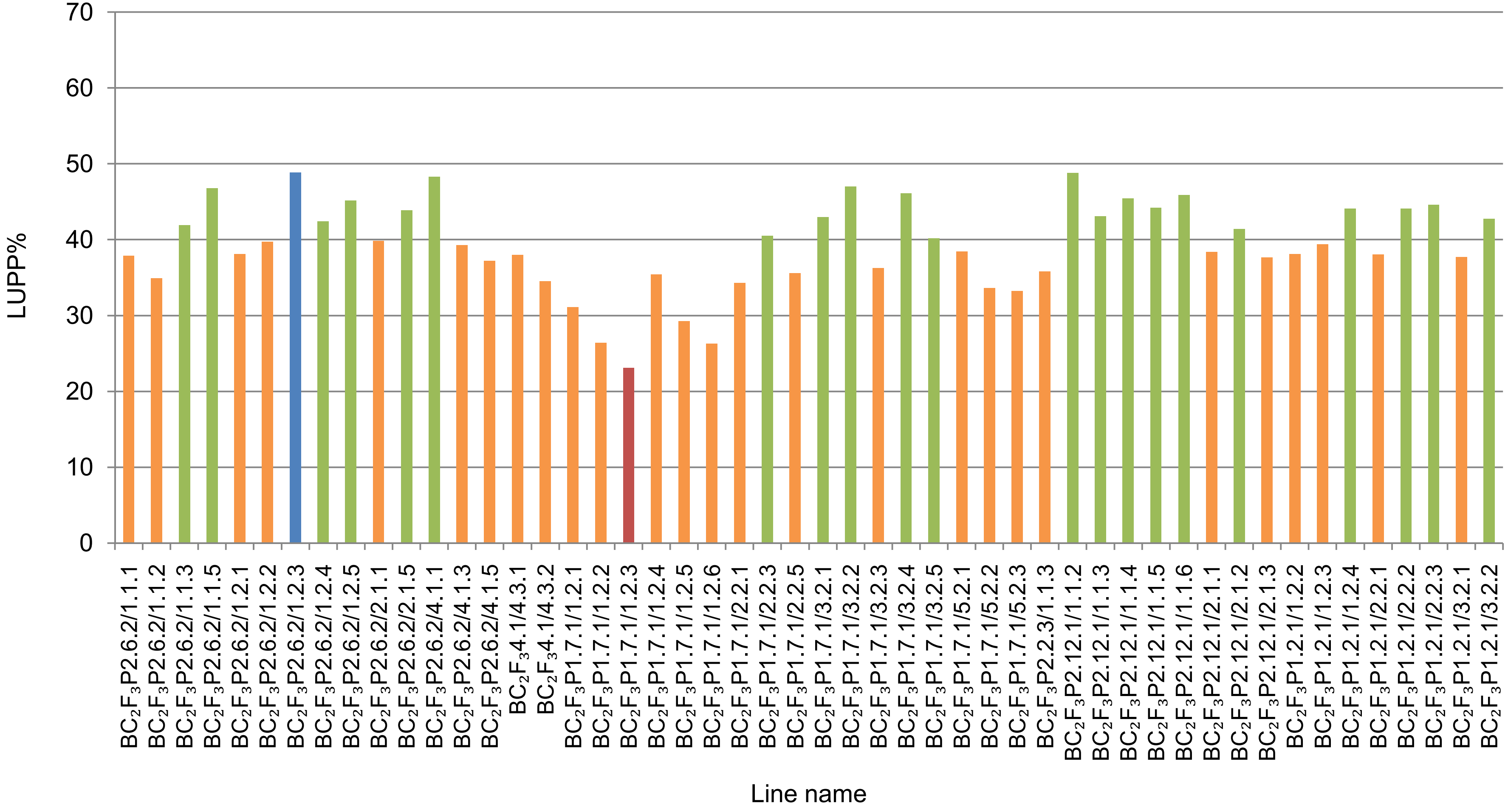

Figure 4.12 LUPP% of the different FHB resistant lines selected for SE-HPLC during the second year of screening. The green bars indicate lines with LUPP% of 40% and higher. The red bar indicates the line with the lowest LUPP% while the blue bar indicates the line with the highest LUPP%.

4.3.5 RP-HPLC

The total quantity percentages of different types of gliadins and glutenins were calculated according to the area on the chromatogram for specific time intervals (Table 4.11). These percentages were used together with the LUPP% to see if there existed any correlation between the protein results, especially the HMW-GS as the LUPP fraction contains the greatest proportion of polymeric proteins. Correlations were not observed (data not shown) between the LUPP% and the quantity of protein (gliadin and glutenin) fractions obtained from RP-HPLC for 50 selected lines tested during the first year. RP-HPLC was therefore not considered for further experimental purposes.

4.4 Discussion

Wheat is one of the most important cereal crops worldwide and therefore breeding programmes should breed for both resistance and quality traits to improve the crop as well as to ensure sustainability (Collard and Mackill 2008). The efficiency level of breeding programmes can be enhanced by using a combination of different analysis methods (Patnaik and Khurana 2001). Throughout this study lines were selected for biochemical analyses or further planting and screening based on available data for these lines, depending on the developmental stage of the experimental lines. Lines were initially selected only based on the number of rust or FHB genes for resistance/QTL present (which was the main focus of the UFS breeding programme) which was a good starting point to eliminate weak lines based on their rust or FHB resistance level. Furthermore, the parental lines used to create the two resistant populations were only selected based on their resistant genes/QTL and not based on their bread-making qualities. Therefore the first round of selection was only based on the presence of the number of resistant genes/QTL. The second round of selection from these identified lines was done based on the number of resistant genes/QTL present, favourable bread-making quality alleles present and the LUPP%. Therefore, since the main aim was to select resistant lines with the best possible bread-making qualities some lines with good quality characteristics were not selected for further analysis due to weak rust or FHB resistance. Obtained results based on the percentages of quality traits present after each round of selection indicated a tendency towards improved selection and accumulation of alleles associated with good quality characteristics in both rust and FHB resistant populations.

According to a study done by Liang et al. (2010), the *Ax1* and *Ax2** glutenin subunits on chromosome 1A have a positive effect on dough strength and elasticity in comparison with a null allele. Results on the parental lines used to create the rust resistant

Table 4.11 Total quantity percentages of the different gliadins and glutenin types and the LUPP% of the selected first year lines tested

Line name	ω-gliadin %	α-gliadin %	γ-gliadin %	ω-gliadin %	HMW-GS %	LMW-GS %	LMW/ HMW	LUPP %
Kariega	28.57	47.63	23.79	0.73	11.09	88.18	7.95	46.04
Avocet*YrSp*	30.86	46.52	22.63	0.79	11.04	88.12	7.98	46.67
Blade	23.97	50.09	25.94	1.15	11.12	88.22	7.94	47.01
CS-*Lr19*-149-299	38.03	37.30	24.66	1.09	11.91	87.34	7.33	34.16
Krokodil	36.24	36.83	26.94	1.18	16.74	82.09	4.93	44.39
CM-82036	35.95	39.98	24.06	0.49	16.08	83.43	5.19	27.20
PAN3434	22.61	46.78	30.61	0.81	10.54	88.64	8.41	43.78
SST835	34.77	43.09	22.14	1.22	14.27	84.51	5.92	34.86
Duzi	26.32	44.79	28.89	0.83	9.82	89.56	9.12	44.19
Elands (SGI)	27.62	45.68	26.70	1.04	9.63	89.26	9.27	47.19
Kariega (SGI)	26.17	46.23	27.59	0.58	9.60	89.79	9.35	44.54
Tugela-DN (SGI)	23.07	47.84	29.09	0.94	12.53	86.89	6.93	49.53
Scheepers69 (SGI)	23.77	45.97	30.26	0.85	10.69	88.40	8.27	29.36
Kingbird	26.41	50.86	22.74	0.93	15.91	83.11	5.22	32.66
2S#2/163	22.45	54.90	22.65	0.89	12.57	86.84	6.91	31.97
S16(7.3)/1.1	40.89	38.95	20.17	0.69	12.84	86.44	6.73	57.89
S16(7.3)P1.5.1/1.3	40.03	40.20	19.77	0.55	12.45	86.92	6.98	29.69
S16(7.3)P1.5.1/2.2	32.80	43.32	23.86	1.19	11.86	87.29	7.36	25.87
S16(7.3)P3.5.1/2.3	32.54	45.69	21.80	0.56	13.49	86.07	6.38	33.21
S16(7.3)P3.5.1/3.2	33.81	46.30	19.90	0.97	13.15	85.88	6.53	40.62
S16(7.3)P3.6.1/5.3	30.65	42.95	26.40	0.64	12.33	86.94	7.05	30.64
S16(7.3)P3.6.1/6.1	36.68	41.855	21.47	0.74	13.16	86.05	6.54	39.00
S16(7.3)P3.2.1/1.1	34.21	41.39	24.41	0.80	11.65	87.50	7.51	31.40
S16(7.3)P3.2.1/1.2	34.93	41.99	23.09	0.77	11.82	87.37	7.39	32.32
S16(1.2)/2.1	29.58	46.02	24.40	0.69	9.94	89.59	9.01	39.38
S726(3.2)/1	26.79	45.50	27.69	1.10	10.05	89.21	8.87	38.07
S178(7.2)/2.3	23.41	47.54	29.05	0.87	10.96	88.38	8.07	37.35

Table 4.11 Total quantity percentages of the different gliadins and glutenin types and the LUPP% of the selected first year lines tested (continued)

Line name	ω-gliadin %	α-gliadin %	γ-gliadi %	ω-gliadi %	HMW-GS %	LMW-GS %	LMW/HMW	LUPP %
S346(1.3)	25.39	45.59	29.01	0.88	8.78	90.61	10.32	40.08
BC$_2$F$_2$P2.6.2/1.1	36.76	40.28	22.74	0.98	15.78	83.24	5.27	44.12
BC$_2$F$_2$P2.6.2/1.2	36.50	39.44	24.06	0.80	14.69	83.62	5.69	40.75
BC$_2$F$_2$P2.14.2/1.1	32.19	42.97	24.84	0.92	12.91	86.11	6.67	35.40
BC$_2$F$_2$P2.14.2/2.1	34.88	39.04	26.07	1.04	15.06	83.90	5.57	36.88
BC$_2$F$_2$P1.7.2/2.2	30.78	41.29	27.95	1.04	12.88	86	6.68	26.90
BC$_2$F$_2$P1.7.2/3.2	36.39	38.24	25.36	1.30	21.45	77.16	3.60	33.70
BC$_2$F$_2$4.1/1.3	42.86	34.65	22.47	0.72	19.49	79.76	4.09	34.79
BC$_2$F$_2$4.1/2.3	40.54	36.03	23.44	0.89	16.59	82.46	4.97	34.15
BC$_2$F$_2$P1.7.1/1.2	39.16	36.60	24.24	0.91	19.90	79.62	4.00	26.39
BC$_2$F$_2$P1.7.1/2.2	37.31	41.33	21.35	1.22	18.99	80.16	4.22	44.04
BC$_2$F$_2$P1.7.3 /2.3	33.37	39.28	27.34	1.35	12.36	86.17	6.97	37.45
BC$_2$F$_2$P1.7.3 /3.3	34.86	36.76	28.37	1.04	14.25	84.60	5.94	30.23
BC$_2$F$_2$P2.2.3/1.1	37.26	40.29	22.45	0.80	16.52	82.67	5.01	40.11
BC$_2$F$_2$P2.2.3/2.1	33.45	39.99	26.57	0.94	15.22	83.84	5.51	41.88
BC$_2$F$_2$P2.3.2/2.2	36.01	39.00	25.00	0.82	13.89	85.25	6.14	41.57
BC$_2$F$_2$P2.3.2/3.2	38.61	37.56	23.83	0.83	17.75	81.40	4.59	38.35
BC$_2$F$_2$P2.12.1/2.1	39.55	38.90	21.54	0.97	16.16	82.87	5.13	41.92
BC$_2$F$_2$P2.12.1/3.1	40.72	37.71	21.58	0.76	18.23	80.98	4.44	39.80
BC$_2$F$_2$P1.5.3/2.1	43.85	36.81	19.36	1.03	16.90	82.05	4.85	37.49
BC$_2$F$_2$P1.5.3/3.2	34.50	39.37	26.12	1.39	14.54	84.08	5.78	35.49
BC$_2$F$_2$P1.2.1/2.2	32.04	41.51	26.45	0.86	15.82	83.25	5.26	50.54
BC$_2$F$_2$P1.2.1/4.2	35.92	39.17	24.91	0.88	15.15	83.93	5.54	48.32

HMW-GS - High molecular weight-glutenin subunits; LMW-GS - Low molecular weight-glutenin subunit; LUPP% - Large unextractable polymeric proteins percentage; SGI - Small Grain Institute population indicated that Kariega and Blade contained the *Ax2** allele while Avocet*YrSp* and CS-*Lr19*-149-299 had a null allele. Selection towards the *Ax2** allele was 100% successful since all of the finally selected rust resistant lines contained the *Ax2** allele. Results on the parental lines used to create the FHB resistant population indicated that both Krokodil and CM-82036 contained the *Ax2** allele which resulted in all FHB experimental lines containing the desirable allele at the *Glu-A1* locus.

Allelic combinations *Bx7+By8* or *Bx17+By18* improve dough strength in comparison to other *Bx* and *By* alleles (Liang et al. 2010). Therefore selection has been done towards the *Bx7+By8* or *Bx17+By18* allele combinations at the *Glu-B1* locus for both the rust and FHB experimental lines. Kariega and Blade contained the *Bx17+By18* allele combination while Avocet*YrSp* and CS-*Lr19*-149-299 had the *Bx7+By8* allele combination. However during the final selection of lines the *Bx7+By8* allele combination has dominated in the rust resistant lines because other traits were also included when lines were selected. Krokodil contained the *Bx7+By8* and CM-82036 the *Bx17+By18* allele combination. Most of the finally selected FHB resistant lines had the *Bx7+By8* allele combination while the rest of the lines were either heterozygous or contained the *Bx17+By18* allelic combination.

The study of Liang et al. (2010) also found that the *Dx5+Dy10* allelic combination on chromosome 5A was associated with higher dough strength in comparison to the *Dx2+Dy12* allelic combination. The Dx5 PCR-based marker only detects the presence of the *Dx5* allele but testing of the parental lines, using SDS-PAGE, indicated that the *Dx5* allele always co-segregated with the *Dy10* allele. Therefore lines containing the *Dx5* allele detected using PCR-based markers were scored in combination with the *Dy10* allele. Lines without the *Dx5* allele were scored *Dx2+Dy12* since it was the only other possible allelic combination for the 1D chromosome of the parental lines. Results indicated that only CS-*Lr19*-149-299 of the four rust parental lines contained the *Dx5+Dy10* allelic combination. However, 92% of the finally selected rust resistant experimental lines contained this favourable allelic combination at the *Glu-D1* locus. Both the parental lines used to develop the FHB resistant populations contained the *Dx2+Dy12* allelic combination and therefore it was not possible for the offspring to contain the favourable *Dx5+Dy10* allelic combination. Future work should focus on incorporating this allelic combination into the FHB resistant population.

According to a study done by Butow et al. (2003), the over-expression of the *Bx7* allele ($Bx7^{OE}$), increases dough strength. Results indicated that none of the rust experimental lines contained the $Bx7^{OE}$ allele because none of the parents contained this allele.

Krokodil, the recurrent parent of the FHB resistant population, contained the *Bx7*^{OE} allele. Only lines with the *Bx7* allele could have the over-expressed *Bx7*^{OE} allele. Since the *Bx7*^{OE} allele has a positive influence on dough strength, it was a desirable characteristic to select for in the offspring lines and 96% of the finally selected FHB resistant lines contained the *Bx7*^{OE} allele.

Although the 1BL.1RS translocation is associated with weak dough strength (Fenn et al. 1994), Liang et al. (2010) found that desirable HMW-GS can stabilise the effect of this translocation. Fortunately none of the rust parental lines contained the translocation. Only CM-82036, the FHB resistant donor line, contained the translocation. However, experimental lines without the translocation were preferably selected and none of the finally offspring lines contained this translocation.

SDS-PAGE is a biochemical method used for allele identification of wheat glutenins (Payne et al. 1983; Johansson et al. 1993; Hussain et al. 2009). SDS-PAGE analysis is a simple technique with inexpensive equipment and is ideal for large scale HMW-GS screening, especially when the glutenin composition for the breeding material is known (Gao et al. 2010). However, the SDS-PAGE system is time-consuming and interpretation of banding patterns can be difficult because some of the subunits have the same electrophoretic mobilities and cannot be separated on the gel making it difficult to distinguish between them. PCR-based markers have been developed previously that are linked to HMW-GS and other alleles linked to protein quality such as the 1BL.1RS translocation and LMW-GS (Liu et al. 2010). Since MAS is based on differences at DNA level it is not influenced by environmental conditions that make it more accurate and experimental plants can be tested at any stage of its life cycle, making MAS an efficient tool to be use in plant breeding (Patnaik and Khurana 2001).

For the current study, 50 lines were selected to do a comparative study between SDS-PAGE and PCR-based marker data linked to HMW-GS. Almost a 100%correlation was observed between these two methods and therefore it was decided to continue with only the molecular markers as they are less time-consuming and easier to score and can be used to distinguish between HMW-GS with similar electrophoretic mobilities. A study done by Liang et al. (2010) has compared SDS-PAGE and MAS analysis and their results also indicated correlation between the two methods as found in this study. Other contributing factors influencing dough quality can also be determined if markers have been developed for the specific traits, such as translocations.

The biochemical SE-HPLC method has been widely used in different studies to determine protein quantity and quality in wheat (Peterson et al. 1992; Johansson and

Svensson 1998; Hussain et al. 2009). The composition and content of wheat proteins contribute mainly to the bread-making quality and can be influenced by genetic as well as environmental factors (Johansson et al. 2001). The LUPP% determined by the SE-HPLC analyses for the rust and FHB resistant populations identified lines with desirable LUPP% between 40% and 49.53%. Desirable LUPP% values were determined based on values obtained for the control lines included in the study. These LUPP% were used as an indirect parameter for selection of lines for further analyses. As for the protein quality markers, the LUPP% was used as a secondary parameter since lines with good resistance had preference in selection. For this reason some of the selected lines had too high or low LUPP% and some lines with favourable LUPP% were excluded on the ground of the absence of rust or FHB genes for resistance/QTL.

Tugela-DN, included as one of the high LUPP% controls, obtained the highest LUPP% (49.53%) under greenhouse conditions but still higher LUPP% have been obtained under field conditions. However, the high LUPP% of Tugela-DN is unacceptable for the milling and baking industry and therefore the LUPP% of Tugela-DN is seen as the cut-off point for the maximum favourable LUPP%. The observed LUPP% of the control lines compared well with cultivar rankings used in industry for LUPP% (B. Wentzel, personal communication).

The average LUPP% for the rust parental lines was 43.47% and only CS-*Lr19*-149-299 had a LUPP% lower than 40%. The average LUPP% for the rust population tested during the first year of screening was 39.65%, which was close to the minimum optimal LUPP%. Only 24% of the rust resistant lines from the first year of screening had favourable LUPP% while the rest of the lines had either lower LUPP% or too high LUPP% (seven lines) according to the optimal LUPP% range of 40% to 49.53%. The selected lines from the first year of screening had an average LUPP% of 38.4% and only 9% of the lines had favourable LUPP% with four lines with too high LUPP%. The rust population tested during the second year of screening had an average LUPP% of 31.5%, which was lower than the average obtained during the first year of screening and only 4% of the subpopulation had favourable LUPP%, with one line having a too high LUPP%. This indicated that lines with a higher number of rust resistant genes/QTL tended to have a lower LUPP% and furthermore confirmed that the LUPP% was a secondary factor when lines were selected.

CM-82036, the resistant donor parent of the FHB resistant population, had a low LUPP% (27.2%), whereas Krokodil, the recurrent parent, had a favourable LUPP% (44.39%). The average LUPP% for the two FHB parental lines was 35.8% and the average LUPP%

for the FHB population screened during the first year was 39% while 42% of the lines had favourable LUPP% while three lines had a too high LUPP%. The FHB resistant population screened during the first year had a higher LUPP% average compared to the LUPP% average of their parents. Lines with a high number of FHB QTL for resistance and high percentage of Krokodil background were selected for this study. Lines screened during the first year were BC_2F_2 lines that were backcrossed twice to Krokodil (LUPP% of 44.39%). Lines selected for the second year of screening had an average LUPP% of 38.72% whereas 52% of lines had favourable LUPP% with only one line with a too high LUPP%. FHB lines selected after the second year of screening had an average LUPP% of 39.3% and 44% of the lines had favourable LUPP%. Although the LUPP% was a secondary trait selected for, the LUPP% increased slightly with 0.3% and the number of lines with favourable LUPP% increased with 2%, which showed that the selected lines were stable for LUPP%.

For future studies, selected seed from the best selected lines should be multiplied for greenhouse and/or field studies in order to perform phenotypic selection to confirm the presence of the genes for resistance/QTL in the rust and FHB resistant lines. Furthermore, baking tests should also be included to confirm results obtained from biochemical tests done during the current study. In order to determine the phenotype of the related line of development more accurately more seeds per line should be tested biochemically. The biochemical tests performed during the current study on single seeds were successfully applied and reliable results were obtained. Results obtained from the SDS-PAGE was usable for determining HMW-GS profiles of the tested lines but MAS dominated the time-consuming method in terms of effectiveness and efficiency. The LUPP% data obtained from SE-HPLC analysis was uncomplicated to use but since the LUPP% was not the main factor of selection it did not really serve its purpose. However, during the final stadiums of the resistance breeding programme, when several lines have been identified with similar levels of resistance, SE-HPLC and LUPP% can be applied to determine which of the resistant lines had the best protein qualities.

The RP-HPLC data obtained from the current study can only be used if additional characteristics obtained from baking tests for and other quality parameters were tested and comparative analyses can be done. Otherwise RP-HPLC should be applied to determine protein profiles but again, after molecular markers have been developed would it really be necessary to perform RP-HPLC as well? Although molecular markers determine the presence or absence of a trait, HMW-GS are the major contributing factors for bread-making quality and can quantitatively be influenced by environmental conditions such as temperature and fertilising. It is therefore important to include molecular markers as well as SE-HPLC analysis when selecting specifically for protein quality. Molecular markers linked to most of the HMW-GS alleles were used in the current study. However, no markers linked to the *Ax1* and null alleles on chromosome 1A and to *Dx2+Dy12* are available. Markers linked to these traits should be included to make the current study more discriminative and affirmative. Different biochemical methods and PCR-based markers linked to protein quality were used to obtain protein characteristics of the listed lines. The value of the data obtained from the different biochemical techniques was determined and it seemed that it was not effective at this stage of the study compared to the molecular markers which has dominated the biochemical tests in terms of efficiency and linkage to higher ranking characteristics.

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Chapter 5

Identification of the best rust and FHB resistant lines based on both molecular and biochemical data

5.1 Introduction

Breeding for disease resistance in wheat is a primary focus to ensure sustainable wheat production and availability. Diseases are mainly due to pests, fungi and bacteria, living organisms that thrive for survival. These living organisms obey the rules of nature and undergo natural selection overtime to ensure their existence (Evans 1997). Since these pathogens evolve over time they overcome resistance, leading to a reduction in the crop's yield and kernel grade (McDonald and Linde 2002). This has a negative influence on the economy and food production worldwide. Conventional breeding combined with marker-assisted resistance breeding can increase the efficiency of wheat breeding programmes and can help to broaden the resistance base (Jauhar and Chibbar 2001; Vasil 2007; Collard and Mackill 2008).

Molecular markers enable scientists to evaluate large breeding populations and enable genotypic selection of individuals at any stage of plant growth. Molecular markers furthermore enable the production of resistant cultivars faster by combining different resistant genes/QTL. Major and minor resistant genes/QTL need to be combined within a cultivar to develop durable resistance (Priyamvada and Tiwari 2011). German and Kolmer (1992) found that the leaf rust resistance gene *Lr34* is an interactive gene that improves the resistance levels of other resistant genes which alone show low levels of resistance. It also improves the resistance of lines which contain minor genes in comparison with lines which only contain of the *Lr34* resistance gene. FHB QTL associated with FHB resistance make different contributions towards FHB resistance and are classified into major and minor QTL. For durable FHB resistance it is important to breed cultivars that contain both major as well as minor QTL to ensure resistance levels that will survive FHB infections (Cuthbert et al. 2007).

Other factors of a disease resistance breeding programme that need to be considered are protein quality, yield and other quality parameters (Šramková et al. 2009). It is important that wheat produced by farmers is useful to the milling and baking industry. Several physical methods have been developed to determine quality characteristics such as grain protein content, kernel hardness, dough strength and loaf volume. Molecular markers have been developed for several of these quality characteristics and results are not influenced by environmental conditions and screening can be done at any plant growth stage. In order to assess bread-making qualities of seed directly, big sample sizes are necessary and this is usually only obtainable during later generations (Koebner and Summers, 2003). Fortunately some biochemical analyses methods exist where only single seeds are necessary for protein quality analyses such as SDS-PAGE, SE-HPLC and RP-HPLC and these can be used during the early stages of a breeding programme since only a small amount of seed per experimental line is sometimes available.

Breeding for different characteristics leads to the release of new (e.g. both resistance as well as good baking quality) cultivars which are acceptable for farmers, the milling and baking industry. Selection for dough quality characteristics in early generations using MAS and indirect methods will decrease the variation in later generations. More physical dough quality tests (e.g. loaf volume, alveograph, mixograph and farinograph) can be performed during later generations of a breeding programme, when sufficient seed material is available, to ensure that bread-making quality characteristics are on the right standard for users (Cho et al 2001).

The aim of this study was to select the best rust and FHB resistant lines for future breeding programmes based on molecular marker data as well as biochemical data generated over a two year period.

5.2 Materials and methods

The best rust and FHB resistant lines of the UFS breeding programme were selected based on molecular data generated using six rust resistant markers (STSLr19$_{130}$, ccfr5, Gwm148, Gwm501, cssr2 and Sr26#43/BE518379), six markers (Barc133, Gwm533, Gwm293, Gwm304, Gwm156 and Gwm133) linked to FHB resistant genes/QTL on chromosomes 3B, 5A and 6B, six markers linked to HMW-GS and the 1BL.1RS translocation and LUPP% determined from SE-HPLC profiles. Methods used to obtain the data for this chapter are described in Chapter 3 (section 3.2) and Chapter 4 (section 4.2).

5.3 Results

5.3.1 Screening of rust resistant lines during the first year

This chapter summarises the resistance data (Chapter 3) combined with the protein quality data (Chapter 4). Figure 5.1 is a graph showing the data based on the number of rust resistant genes/QTL, the LUPP% obtained from the SE-HPLC graph and the protein

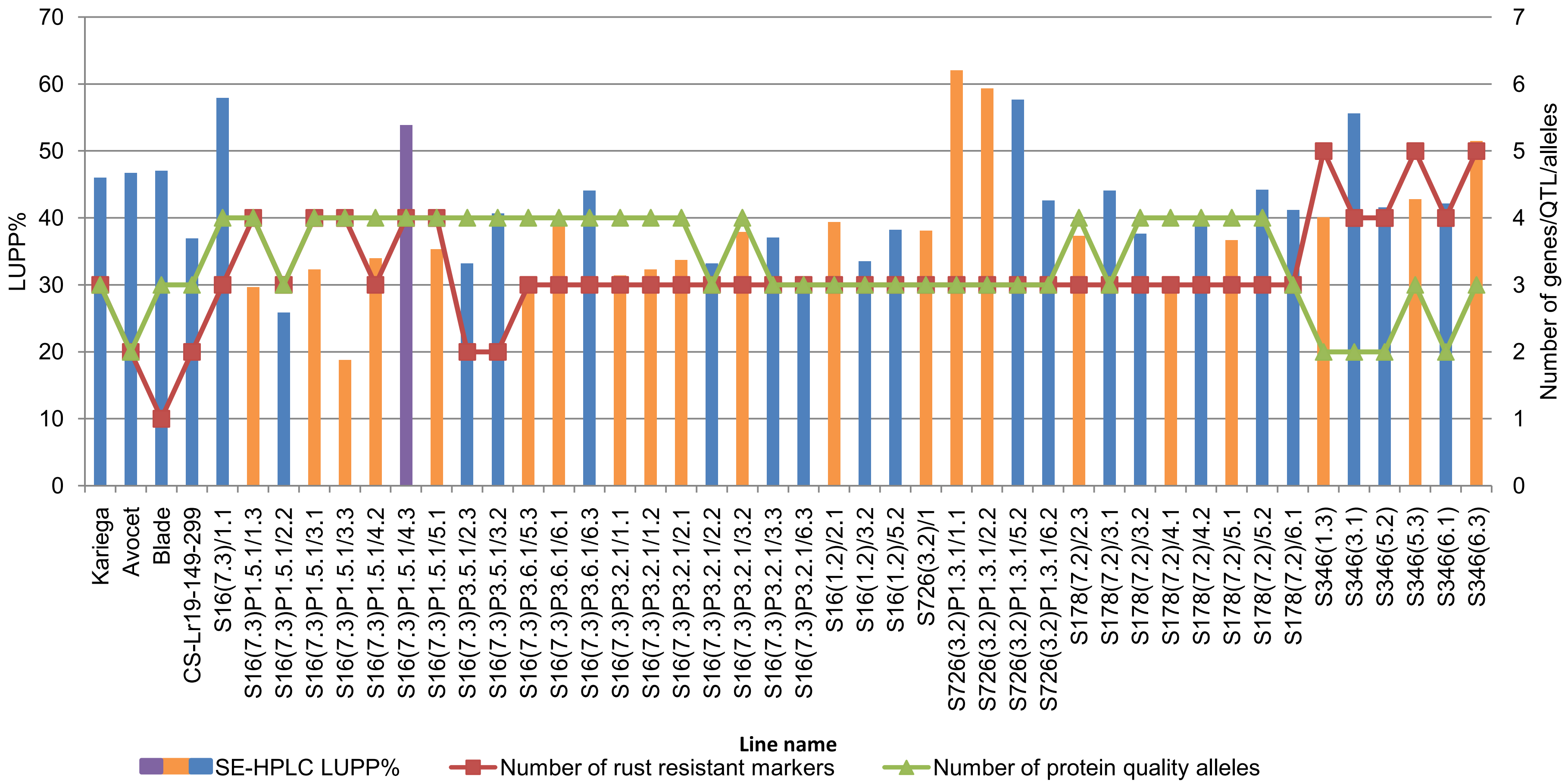

Figure 5.1 **Summary of the LUPP%, number of rust resistant genes/QTL and number of favourable protein quality alleles present in 42 rust resistant lines screened during the first year.** The four parental lines used to develop the rust resistant population are also included. Lines selected for the second year of screening are indicated by orange and purple bars. The best identified line is indicated by the purple bar.

quality alleles present in each of the 42 lines of the rust resistant population screened during the first year. The four parental lines (Kariega, Avocet*YrSp*, Blade and CS-*Lr19*-149-299) were also included in the graph. The bars marked orange are lines that were selected for further analysis to produce the second population that were screened during the second year.

Figure 5.1 is divided into a primary vertical axis which indicates the LUPP% obtained for each entry. The secondary vertical axis indicates the number of rust resistant genes/QTL and number of favourable protein quality alleles. Only two of the lines contained just two rust resistant genes/QTL. Most of the lines contained either three or four rust resistant genes/QTL. Lines showed low levels of segregation for the rust resistant genes/QTL (mostly three or four genes/QTL present) which was expected, since most of these lines have been self-pollinated three or four times already. The six lines of family S346 all contained four rust resistant genes/QTL. These lines have only been self-pollinated once thus should contain a higher number of rust resistant genes/QTL compared to the other lines. For the rust population the highest number of favourable protein quality alleles was four. Line S16(7.3)P1.5.1/3.3 had the lowest LUPP% (18.79%) of the lines analysed during the first year and line S726(3.2)P1.3.1/1.1 had the highest LUPP% (62.03%) and the average LUPP% of the 42 rust experimental lines analysed during the first year was 39.65%. The selection criteria for the selection of experimental lines screened during the first year were three or more rust genes for resistance, two or more favourable protein quality alleles and a LUPP% between 40% and 50%.

The best lines in this group was S16(7.3)P1.5.1/4.3 containing four rust resistant genes/QTL, four protein favourable quality alleles and a LUPP% of 53.84% (which was a bit too high). Unfortunately, most of the other lines that tested positive for four of the rust resistant genes/QTL, had poor bread-making quality (either too high or too low LUPP% or a small number of protein quality alleles). The four lines with the lowest number of protein quality alleles (two each), lines S346(1.3), S346(3.1), S346(5.2) and S346(6.1) all had LUPP% higher than 40%. These four lines were all from the same family and were only self-pollinated once, compared to the three to four self-pollination rounds of the rest of the lines.

5.3.2 Screening of FHB resistant lines during the first year

The data shown in Figure 5.2 summarises the number of FHB resistance markers present in each line, the LUPP% and the number of favourable protein quality alleles of the 55 selected FHB resistant lines tested during the first year of screening. Krokodil (recurrent parent) and CM-82036 (donor parent) were also included in the figure.

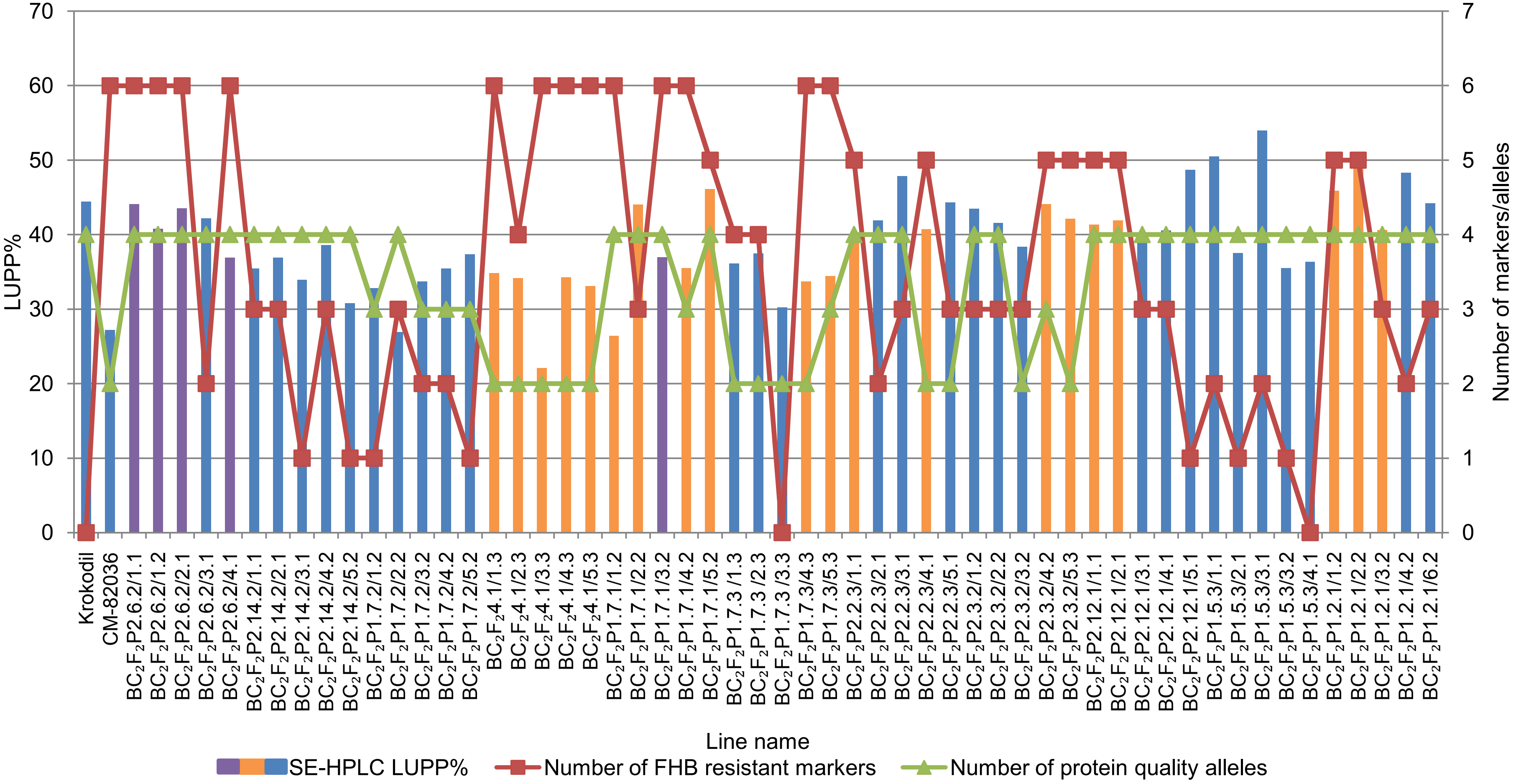

Figure 5.2 **Summary of the LUPP%, number of FHB resistant markers present and number of favourable protein quality alleles present in 55 FHB resistant experimental lines screened during the first year.** The two parental lines used to develop the FHB resistant population are also included. Lines selected for the second year of screening are indicated by orange and purple bars. The best five lines are indicated by the purple bars.

The bars marked in orange represent the first year experimental lines that were selected to produce the experimental lines that were screened during the second year.

Figure 5.2 has a primary y-axis which indicates the LUPP%. The secondary y-axis indicates the number of FHB resistant markers present within each line and the number of favourable protein quality alleles present in each line. The highest number of protein quality alleles present in the FHB resistant population was four. Line $BC_2F_2$4.1/3.3 had the lowest LUPP% of 22.09% and line BC_2F_2P1.5.3/3.1 had the highest LUPP% of 53.98%. The average LUPP% for the 55 selected FHB resistant lines screened during the first year was 39.06%.

The number of markers linked to FHB QTL for resistance present in the 55 lines ranged from null to six. All of these lines were originally selected because it contained all four FHB QTL screened for. However, it is clear that some of these QTL were lost after an additional round of self-pollination. This was expected because these lines were all selected from a BC_2-popluation and all QTL would still have been present in a heterozygous state in the BC_2-population.

The five best FHB resistant lines (BC_2F_2P2.6.2/1.1, BC_2F_2P2.6.2/1.2, BC_2F_2P2.6.2/2.1, BC_2F_2P2.6.2/4.1 and BC_2F_2P1.7.1/3.2) contained all six FHB resistant markers. The five of these lines contained four favourable protein quality alleles. Only the three best lines had a LUPP% higher than 40% while the other two lines had LUPP% of 36.88% and 36.94% respectively. The following five lines were also identified as promising lines: BC_2F_2P1.7.1/5.2, BC_2F_2P2.12.1/1.1, BC_2F_2P2.12.1/2.1, BC_2F_2P1.2.1/1.2 and BC_2F_2P1.2.1/2.2. Although these five lines each contained only five of the six FHB resistant markers, all of them had favourable protein quality markers and LUPP% higher than 40%.

5.3.3 Screening of rust resistant lines during the second year

For the second year of screening 50 rust resistant experimental lines were selected for SE-HPLC analysis based on the number of rust resistant genes/QTL present as well as the quality protein profile. These lines were all offspring of the 16 best lines selected after the first year of screening. Data of the 50 selected rust resistant lines screened during the second year (orange bars) and their parental lines (screened during the first year; blue bars) has been summarised in Figure 5.3. The figure has a primary Y-axis indicating the LUPP% obtained from SE-HPLC analysis. The secondary Y-axis shows the number of rust resistant genes/QTL present as well as the number of favourable protein quality alleles per line. Most of the 50 rust resistant lines selected of the second year of screening had three rust resistant genes/QTL and four favourable quality protein alleles.

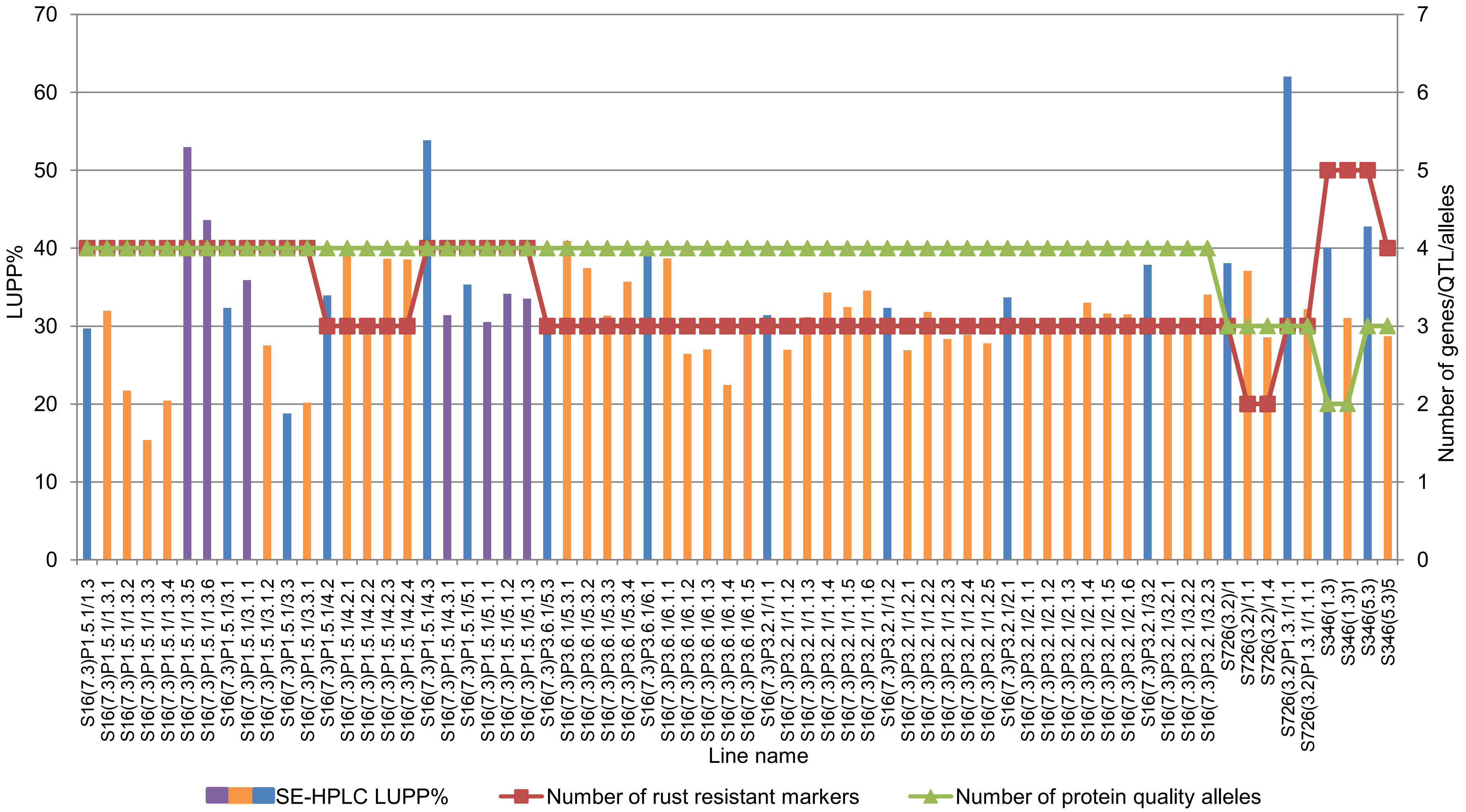

Figure 5.3 Summary of the LUPP%, number of rust resistant genes/QTL present and number of favourable protein quality alleles present in the 50 rust resistant lines (orange/purple bars) and their parental lines (green bars), selected for screening during the second year. The best lines are indicated by purple bars.

Line S16(7.3)P1.5.1/1.3.3 had the lowest LUPP% of 15.36% and line S16(7.3)P1.5.1/1.3.5 had the highest LUPP% of 52.98% with a average of 31.5% for the 50 lines screened during the second year.

The best identified rust resistant line during the second year of screening was line S16(7.3)P1.5.1/1.3.6 with four rust genes for resistance. QTL, three protein quality alleles and a LUPP% of 43.60%, followed by lines S16(7.3)P1.5.1/3.1.1, S16(7.3)P1.5.1/4.3.1, S16(7.3)P1.5.1/5.1.1, S16(7.3)P1.5.1/5.1.2 and S16(7.3)P1.5.1/5.1.3, all with four rust resistant genes/QTL, three protein quality alleles and LUPP% ranging from 30.52% to 35.90%. Although line S16(7.3)P1.5.1/1.3.5 had a LUPP% higher that 50%, this line was also identified as a promising line because it contained four rust resistant genes/QTL and four favourable protein quality alleles. The next best lines were S16(7.3)P1.5.1/4.2.1, S16(7.3)P1.5.1/4.2.3, S16(7.3)P1.5.1/4.2.4, S16(7.3)P3.6.1/5.3.1 and S16(7.3)P3.6.1/6.1.1. Although these five lines only had three rust resistant gene/QTL each, they all had three protein quality markers and LUPP% of almost 40%. Unfortunately line S346(1.3)1 with the highest number of rust genes for resistance (five), showed poor bread-making qualities (two protein quality alleles and LUPP% of 31.02%). Most of the lines tested contained three rust resistant genes/QTL and four protein quality alleles, but were still segregating for LUPP%. For most lines tested, at least one offspring showed improved LUPP%. Self-pollination thus led to improved bread-making qualities in some of the lines.

5.3.4 Screening of FHB resistant lines during the second year

The data of the 50 selected FHB resistant lines screened during the second year are summarised in Figure 5.4 and shows a primary y-axis which indicates the LUPP%. The secondary Y-axis indicates the number of favourable protein quality alleles and the number of FHB resistant markers present for every individual line. The blue bars in the graph point out the parental lines which have been selected from the first year of screening to produce offspring lines that are indicated by the orange and purple bars. The lines indicated by the purple bars were the best offspring lines screened during the second year.

All of the FHB resistant lines tested in the second year had four favourable protein quality alleles except for lines $BC_2F_3$4.1/4.3.1 and $BC_2F_3$4.1/4.3.2 that had three alleles. Most of the FHB resistant lines screened during the second year had five FHB resistance markers.

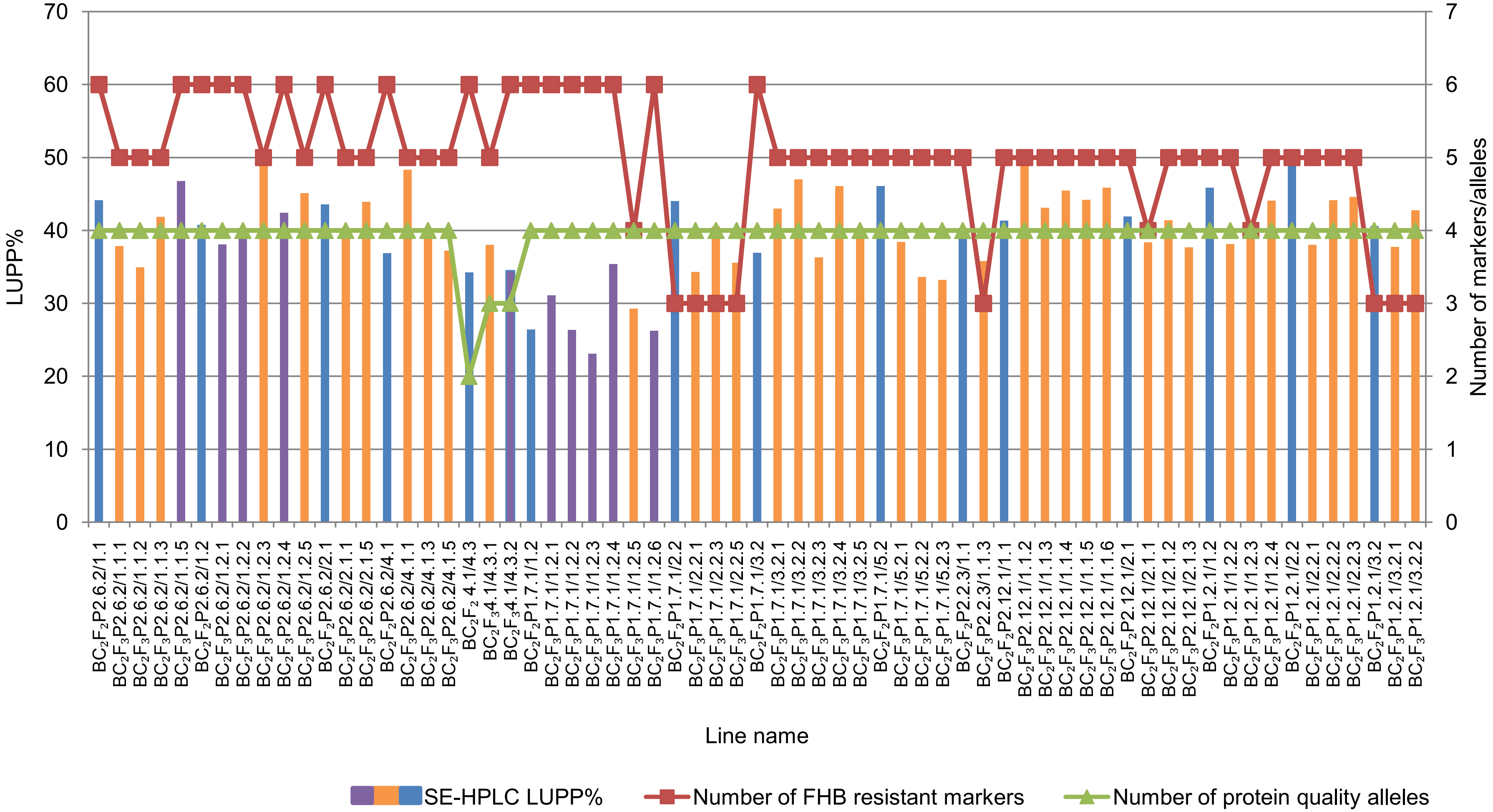

Figure 5.4 Summary of the LUPP%, number of resistant FHB markers present and number of favourable protein quality alleles present in the 50 FHB resistant lines (orange/purple bars) and their parental lines (blue bars), selected for screening during the second year. The best lines are indicated by purple bars.

The number of FHB resistance markers per line had ranged from three to six FHB resistant markers. Line $BC_2F_3P1.7.1/1.2.3$ had the lowest LUPP% of 23.06% while line BC_2F_3P2 6.2/1.2.3 had the highest LUPP% of 48.82%. The average LUPP% for all lines was 39.3%.

Of the ten lines with six FHB resistant markers present, line $BC_2F_3P2.6.2/1.1.5$ was the best performer because it had four protein quality alleles and a LUPP% of 46.78%. The other nine lines that had six FHB resistant markers, four protein quality alleles, except for one line that had three protein quality alleles ($BC_2F_34.1/4.3.2$), and LUPP% ranging from 23.06% to 42.42% were $BC_2F_3P1.7.1/1.2.3$, $BC_2F_3P1.7.1/1.2.6$, $BC_2F_3P1.7.1/1.2.2$, $BC_2F_3P1.7.1/1.2.1$, $BC_2F_34.1/4.3.2$, $BC_2F_3P2.6.2/1.2.1$, $BC_2F_3P1.7.1/1.2.4$, $BC_2F_3P2.6.2/1.2.2$ and $BC_2F_3P2.6.2/1.2.4$. Results indicated that for most of the lines selected after the first year of screening (blue bars in Figure 5.4), one or more of their offspring tested during the second year (orange or purple bars) showed improvement for LUPP%.

5.3.5 Identification of the top ten rust resistant lines

The top ten rust resistant lines selected at the end of the second year's screening process are summarised in Table 5.1. Lines were selected based on the number of rust resistant genes/QTL, number of favourable protein quality alleles and the LUPP%. All lines contained three rust resistant genes/QTL and three favourable protein quality genes. The LUPP% for the ten lines ranged from 21.73% to 52.98%. The top ten lines were all selections from the same original parental line, line S16(7.3)P1.5.1. All top ten lines, except for the last line, line S16(7.3)P1.5.1/1.3.2, that was heterozygous for the Glu-1B allele, has the same protein quality profile (*Ax2**; *Bx7+By8*; *Dx5+Dy10*) and they did not contain the 1BL.1RS translocation. The top ten lines contained *Lr19*, the leaf rust resistance gene or gene complex *Lr34/Yr18/Sr57* and the stripe rust resistance QTL, *QYr.sgi.2B-1.* Since the top ten rust resistant lines all had the same rust resistant gene/QTL and protein quality alleles, lines were ranked based on the LUPP% obtained from the SE-HPLC analysis.

5.3.6 Identification of the top ten FHB resistant lines

The top ten FHB resistant lines selected are summarised in Table 5.2. Lines were selected based on the LUPP% results obtained from SE-HPLC analyses, number of protein quality alleles present and the number of FHB resistant markers present. The LUPP% for the ten selected lines ranged from 23.06% to 46.78%. All ten lines contained four favourable protein quality alleles each that included the absence of the 1BL.1RS translocation that was present in the donor line, CM-82036.

Table 5.1 Top ten rust resistant lines identified after the second year of screening

Ranking order	Line name	LUPP%	Favourable protein quality alleles	Number of rust resistant genes/QTL
1	S16(7.3)P1.5.1/1.3.5	52.98	*Ax2**; *Bx7+By8*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*
2	S16(7.3)P1.5.1/1.3.6	43.60	*Ax2**; *Bx7+By8*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*
3	S16(7.3)P1.5.1/3.1.1	35.90	*Ax2**; *Bx7+By8*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*
4	S16(7.3)P1.5.1/5.1.2	34.11	*Ax2**; *Bx7+By8*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*
5	S16(7.3)P1.5.1/5.1.3	33.50	*Ax2**; *Bx7+By8*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*
6	S16(7.3)P1.5.1/1.3.1	31.97	*Ax2**; *Bx7+By8*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*
7	S16(7.3)P1.5.1/4.3.1	31.41	*Ax2**; *Bx7+By8*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*
8	S16(7.3)P1.5.1/5.1.1	30.52	*Ax2**; *Bx7+By8*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*
9	S16(7.3)P1.5.1/3.1.2	27.52	*Ax2**; *Bx7+By8*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*
10	S16(7.3)P1.5.1/1.3.2	21.73	*Ax2**; *Bx7+By8/Bx17+By18*; *Dx5+Dy10*	*Lr19; Lr34/Yr18/Sr57; QYr.sgi.2B-1*

Table 5.2 Top ten FHB resistance lines identified after the second year screening

Ranking order	Line name	LUPP%	Favourable protein quality alleles	Number of homozygous FHB resistant markers	FHB resistant QTL present
1	BC$_2$F$_3$P2 6.2/1.2.1	38.07	*Ax2**; *Bx7+By8*; *Bx7*^{OE}; *Dx2+Dy12*	5	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*
2	BC$_2$F$_3$P1 7.1/1.2.4	31.10	*Ax2**; *Bx7+By8/Bx17+By18*; *Bx7*^{OE}; *Dx2+Dy12*	5	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*
3	BC$_2$F$_3$P2 6.2/1.1.5	46.78	*Ax2**; *Bx7+By8*; *Bx7*^{OE}; *Dx2+Dy12*	4	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*
4	BC$_2$F$_3$P2 6.2/1.2.4	42.42	*Ax2**; *Bx7+By8*; *Bx7*^{OE}; *Dx2+Dy12*	4	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*
5	BC$_2$F$_3$P2 6.2/1.2.2	39.72	*Ax2**; *Bx7+By8*; *Bx7*^{OE}; *Dx2+Dy12*	4	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*
6	BC$_2$F$_3$P1 7.1/1.2.6	26.25	*Ax2**; *Bx17+By18*; *Bx7*^{OE}; *Dx2+Dy12*	4	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*
7	BC$_2$F$_3$P1 7.1/1.2.3	23.06	*Ax2**; *Bx17+By18*; *Bx7*^{OE}; *Dx2+Dy12*	4	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*
8	BC$_2$F$_3$P1 7.1/1.2.1	35.38	*Ax2**; *Bx7+By8/Bx17+By18*; *Bx7*^{OE}; *Dx2+Dy12*	3	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*
9	BC$_2$F$_3$P1 7.1/1.2.2	26.34	*Ax2**; *Bx7+By8/Bx17+By18*; *Bx7*^{OE}; *Dx2+Dy12*	3	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*
10	BC$_2$F$_3$4.1/4.3.2	34.48	*Ax2**; *Bx7+By8/Bx17+By18*; *Dx2+Dy12*	1	*Fhb1; Fhb2; Qfhs.ifa.5A-1; Qfhs.ifa.5A-2*

All ten lines contained four genes/QTL linked to the six FHB resistant markers tested. The number of homozygous FHB resistant markers ranged from five to one. The top ten FHB lines were developed through backcrossing twice to Krokodil that showed a LUPP% of 44.39% while the donor line, CM-82036 had a low LUPP% (27.20%). In spite of having being backcrossed twice to a Krokodil with a high LUPP%, only two of the top ten lines had a LUPP% over 40%. The top ten lines had a *Ax2**; *Bx7+By8* and/or *Bx17+By18*; $Bx7^{OE}$; *Dx2+Dy12* protein quality allele profile except for line ten that did not contain the $Bx7^{OE}$ allele and none of the ten lines contained the 1BL.1RS translocation. All six FHB resistant markers were present in the top ten lines and therefore, all four FHB QTL for resistance were present within the top ten lines. Lines were ranked based on the number of homozygous FHB resistant markers present within a line. The top ten lines were mainly developed from lines BC_2F_2P2 6.2 and BC_2F_2P1.7.1.

5.4 Discussion

The main aim of the entire study was to evaluate the protein quality of rust and FHB resistant lines from the existing resistance breeding programme at the UFS. The first and main selection criteria was thus to select lines with the highest possible rust or FHB resistant genes/QTL. From these lines a further selection was done based on certain protein quality characteristics. Selection was furthermore done over a two year period where lines tested during the second year were offspring from the best selected lines tested during the first year of screening. This approach made it possible to evaluate the effect of an additional round of self-pollination on the number of resistant genes/QTL present, the number of protein quality alleles present as well as the LUPP%. The rust resistant population was developed from a series of crosses using four parental rust resistant cultivars followed by several rounds of self-pollination. The FHB resistant population on the other hand was developed through a backcross breeding programme using a FHB resistant donor line and two backcrosses to the South-African cultivar Krokodil followed by several rounds of self-pollination. Krokodil processes several good bread-making qualities.

The presence of five rust resistant genes/QTL (*Lr19, Lr34/Yr18/Sr57, Sr2, Sr26* and *QYr.sgi.2B-1*) were followed using molecular markers during the study for both years of screening. Lines were not screened for the presence of the yellow rust gene, *YrSp*, since no molecular marker linked to the *YrSp* gene was available. Rust resistant lines screened during the first year tested positive for different numbers and combinations of rust resistant genes/QTL per line. The highest number of markers present was four per line and none of these lines contained the *Sr2* stem rust resistance gene. Marker cssfr5 linked to the *Lr34/Yr18/Sr57* rust resistance gene complex was present in all lines.

Marker Gwm148, one of the flanking markers linked to the stripe rust QTL for resistance, *QYr.sgi.2B-1*, was present in most of the lines screened during the first year. Rust resistant lines screened during the second year of the study showed variation for the number and combination of rust resistant genes/QTL present. Lines screened during the second year of analysis had a maximum of four rust resistant markers. Rust resistant markers present in most off the lines were STSLr19$_{130}$ and Gwm148 while marker cssfr5 linked to the *Lr34/Yr18/Sr57* gene complex was present in all lines screened during the second year. The level of homozygosity improved after a second round of self-pollination since less segregation was observed between offspring lines. Only offspring of line S346 contained lines that were heterozygous for the stem rust resistant gene, *Sr26*. Offspring of line S346 tested during the first year have only been self-pollinated once. Since only a dominant marker (Sr26#43) was available during the first year of screening, heterozygous individuals were probably selected and segregation in the offspring was thus expected as was seen in offspring tested during the second year. These lines, heterozygous for the rust resistant gene *Sr26,* should be self-pollinated again to obtain the *Sr26* gene in a homozygous state. A marker (BE518379) detecting the susceptible allele can be used to differentiate between homozygous and heterozygous lines for the stem rust resistant gene *Sr26*.

None of the top ten rust resistant lines contained the *Sr2* gene that is an important slow rusting gene against stem rust and especially against the new Ug99 race and its derivatives (McIntosh et al. 1995; Priyamvada and Tiwari 2011). Three of the control lines tested, Picaflor, Damphe and Kingbird, tested positive for the *Sr2* gene. Therefore the top lines should be crossed with these *Sr2* resistant sources to improve these lines' resistance against rust diseases. The top ten lines also did not contain the *Sr26* rust resistance gene. However, offspring lines S346(1.3)1 and S346(5.3)5 were heterozygous for the *Sr26* rust resistance gene and therefore need to be self-pollinated to obtain homozygous resistant lines for*Sr26* rust resistance. These homozygous *Sr26* rust resistant lines can then be crossed with the top ten lines to incorporate *Sr26* stem rust resistance into these lines.

During the current study FHB resistant lines were screened during the first and second year using six molecular markers linked to four different FHB resistant genes/QTL, namely *Fhb1*, *Qfhs.ifa.5A-1*, *Qfhs.ifa.5A-2* and *Fhb2*. Two flanking markers (Barc133 and Gwm533) linked to the *Fhb1* gene, two flanking markers (Gwm293 and Gwm304) linked to the FHB resistant QTL *Qfhs.ifa.5A-1,* marker Gwm156 linked to the FHB resistant QTL *Qfhs.ifa.5A-2* and marker Gwm133 linked to the *Fhb2* gene were used. Each QTL contribute towards the plant's tolerance against FHB infection, therefore major and minor QTL had been described. The two FHB resistant gens/QTL *Fhb1* and *Fhb2* provide tolerance to type II infection, while the FHB QTL for resistance on chromosome 5A provides type I resistance (Buerstmayr et al. 2002). The *Fhb1* gene/QTL on chromosome 3B is the QTL with largest effect (Waldron et al. 1999). The *Fhb2* resistant gene/QTL is located on chromosome 6B and has a smaller effect than the QTL on chromosome 3B (Waldron et al. 1999). The major FHB QTL for resistance found on chromosomes 3B and 5A respectively have shown to improved FHB resistance levels in wheat lines (Wilde et al. 2007). Some of the FHB resistant lines screened during the first year tested positive for all six markers and the best line, BC_2F_2P1.7.1/3.2, had four homozygous resistant markers present while the two flanking markers of the FHB QTL for resistance on chromosome 3B were heterozygous. Offspring screened during the second year also tested positive for six markers linked to the four FHB QTL for resistance and the best line, BC_2F_3P2 6.2/1.2.1 had five homozygous FHB resistant markers. The FHB resistant line, BC_2F_2P2 6.2/1.2, screened during the first year only tested positive for three homozygous FHB resistant markers whereas the offspring line, BC_2F_3P2.6.2/1.2.1 showed an improved level of homozygosity due to additional round of self-pollination. From this result it is clear that additional rounds of self-pollination is necessary to improve the homozygosity level of offspring and that molecular markers enable researchers to follow the presence of the resistance genes/QTL in self-pollinated lines. All top ten lines tested positive for six FHB resistant markers but some lines had higher levels of homozygosity for the FHB markers. However, one of the top ten lines only had one homozygous marker and therefore another round of self-pollination is necessary to improve the level of homozygosity. Lines with missing FHB QTL for resistance but good bread-making characteristics can be crossed with lines containing the missing QTL in a homozygous state in order to improve these lines.

The LUPP% was determined using protein profiles of each line generated using SE-HPLC. The average LUPP% of the rust resistant population tested during the second year was lower than the average LUPP% obtained during the screening done in the first year. This might be because to the fact that the number of rust resistant genes/QTL present was the main selection criteria during the first year. The variation might also be due to different environmental conditions during the two year's plantings in the greenhouse. Environmental conditions such as water availability (Brooks et al. 1982), temperature (Randall and Moss 1990; Guedira et al. 2002), light intensity (Sofield et al. 1977) and fertilisers (Morris and Paulsen 1985) can affect the expression of proteins. Protein content, including the LUPP, can vary between 6% to 25% due to environmental conditions (Blackman and Payne 1987) although genotypic factors mainly determine protein content (Fowler et al.1990). High levels of variation for LUPP% were observed during the second year of testing. Lines with the highest and lowest LUPP% were both direct offspring of the same parental line S16(7.3)P1.5.1/1.3 which had a LUPP% of 29.69%. High levels of variation for LUPP% were detected both within and between populations. Although self-pollination of lines led to an overall decrease in LUPP%, some individual offspring had higher LUPP% than the lines they were developed from.

None off the rust parental lines tested positive for the 1BL.1RS translocation or for the *By9* allele or the *Bx7+By8** allele combination, thus these traits were absent in their offspring. Although all protein quality markers were tested on the rust resistant experimental lines, only the scores of four of the six markers were used to determine the number of favourable protein quality alleles present in the rust resistant population. Favourable protein quality alleles segregating in the rust resistant population included the presence of the *Ax2**, *Bx7* or *Bx17* and *Dx5* alleles and the absence of the 1BL.1RS translocation as it is linked to poor bread-making quality and result in sticky dough with low dough strength (Fenn et al. 1994).

The average LUPP% for the first and second year of screening of the FHB resistant populations was more or less similar. Although the maximum LUPP% (48.82%) obtained during the second year lines of screening were lower than the maximum LUPP% obtained during the first year of screening (50.54%), it was more favourable because it was within the acceptable range of 40% to 50%. The variation obtained for the LUPP% correlated with work of Belderok and co-workers (2000) which stated that protein content is weakly inherited and greatly influenced by environmental factors such as soil nitrogen and water availability during the growing season.

Four favourable protein quality markers were detected between the two parental lines of the FHB resistant population. While the *By9* and *Dx5* alleles were absent in both Krokodil and CM-82036. Favourable protein quality alleles included the presence of the *Ax2**, *Bx7+By8* or *Bx17+By18*, $Bx7^{OE}$ and the absence of the 1BL.1RS translocation (which was present in CM-82036). Most of the selected 55 lines tested during the first year contained the four favourable protein quality alleles and 48 of the 50 lines tested during the second year contained four favourable protein quality alleles.

Although the number of FHB resistant markers present per line increased from the BC_2F_2 to BC_2F_3 population, the number of quality protein alleles and average LUPP% stayed more or less the same. This might be due to the fact that the primary selection criteria for selection for further analyses were FHB resistance. Since the *Dx5+Dy10* allele was absent in the parental lines and thus all offspring of the FHB resistant population, the best lines should be crossed with a genotype that contains the *Dx5+Dy10* allele combination that is associated with strong dough (Vasil and Anderson 1997).

With every round of self-pollination the homozygosity level of genes/QTL increases (Sapir 2009). Offspring lines of both the rust and FHB resistant populations screened during the first year showed high levels of segregation between lines within a subpopulation and some of the resistant genes/QTL was still in a heterozygous state. After another round of self-pollination, offspring lines screened during the second year of analysis showed lower levels of segregation between lines within a subpopulation and the level of homozygous FHB resistant markers increased. Data obtained from the screening during the second year can be useful to select lines that contain homozygous resistant genes/QTL.

Selection of the best rust and FHB resistant lines indicated that lines with the best resistance to either rust or FHB did not necessarily have the best bread-making qualities and vice versa. Some lines with a high number of resistant genes/QTL and good protein quality characteristics resulted in low yield. For some lines no seed or very low number of seed were obtained and this limited further studies due to a lack of seed to analyse. Blackman and Payne (1987) found that higher grain yield is associated with lower protein concentration which corresponds to a study done by Balla et al. (2011). Brown (2002) also found that farmers rather plant cultivars with lower resistance to diseases as the yield is higher in comparison to cultivars with high disease resistant levels. Lines identified with high levels of disease resistance in the current study should also be evaluated agronomically for yield potential.

Since the current breeding programme is in its beginning phase and have only been planted in the greenhouse, limited seed per line was available and therefore no baking tests could be done to evaluate the bread-making ability of rust and FHB resistant lines. Therefore seed must be multiplied to perform baking tests to determine different bread-making quality characteristics such as loaf volume, extensibility and water absorption which will help to obtain data to select more accurately for bread-making quality. Baking tests is necessary as Fredriksson et al. (1997; 1998) determined that the protein content correlates positively with gluten content, farinograph dough stability and bread loaf volume. Field and greenhouse tests should also be done on the rust and FHB resistant lines in order to evaluate them phenotypically for disease resistance and to select the best performing lines under abiotic stress conditions. Genomic evaluation should not replace conventional breeding but it can improve the efficiency and precision of a breeding programme (Varshney et al. 2007). Several rounds of self-pollination can lead to the loss of some originally selected genes/QTL as in this case with the rust and FHB resistant genes/QTL and protein quality alleles. Therefore the doubled haploid (DH) technique could be a better option in comparison to the self-pollination option since with the DH technique homozygous levels of all genes/QTL is reached must faster (Li et al. 2013).

The best lines based on the number of resistant genes/QTL and favourable protein quality alleles have been identified for both the rust and FHB resistant populations. Results indicated that the number of resistant genes/QTL was the main selection criteria at this stage of the study since these top ten lines did not have desirable LUPP%. This conclusion is supported by the selection funnel in pedigree wheat breeding that indicates that selection for disease resistance should take place during the F_2 and F_3 generations due to high levels of segregation. Quality selections should start during the F_4 and F_5 generations. At the F_6 generation selection should be initiated for yield as genotypes should have reached a homozygosity level of 97% on average (Koebner and Summers, 2003).

For future studies, lines with different combinations of homozygous resistant genes/QTL can be crossed with one another to try and incorporate missing genes/QTL that are present in other lines. Another possibility is to cross the best rust resistant line with the best FHB resistant line to obtain lines with combined rust and FHB resistance.

5.5 References

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Chapter 6

General conclusions and recommendations

Biological studies include the interaction between pathogens and their hosts. Understanding these complex interactions are important for the development of new strategies for disease and pest management in the field. There are two main reasons for plant breeders to continue breeding new food crop cultivars. The first reason is to improve and add new disease genes for resistance/QTL because ever-evolving pathogens overcome plant resistance. The second reason is to improve crop quality and quantity since the world population increases daily.

The primary aim of this study was to improve wheat lines by pyramiding gene for resistances/QTL to produce resistance levels which are effective against the three rust types (leaf, stripe and stem rust) and FHB these are well known diseases worldwide and have resulted in major epidemics. Gene pyramiding of rust and FHB, gene for resistances/QTL can be achieved through MAS since many markers linked to these genes have been developed over the years. Durable disease resistance is the most desirable type of resistance since it is not easily overcome because it depends on the expression of two or more genes or race non-specific resistance. Genes for resistance/QTL have certain percentages of effectiveness and the disease severity is influenced by environmental conditions and the pathotype. Different pathotypes of a disease can overcome resistance to specific genes/QTL and destroy the crop or have a negative effect on the production of the crop.

The secondary aim of this study was to evaluate the protein quality of the disease resistant lines. Only methods which can analyse single seed samples were selected due to a limitation of available seed during the early generation stages of the breeding programme. Selection of lines for further analyses was more lenient towards higher resistance as to quality. Due to segregation of genes in the F_2 and F_3 generations variation of the examined factors were detected between experimental lines. Segregation made it possible to select lines which met the requirements for this breeding programme. When two or more examined traits are involved in a breeding programme, it is possible that all traits will not be optimal in all examined lines. Examined traits need to be prioritised when selection is applied to experimental lines and the importance of each trait at that stage need to be established.

In this study, experimental lines with good resistance levels to FHB and rust have been identified as these were primary traits selected for. These selected lines also contained the best possible HMW-GS. When protein analysis is included in a breeding programme one must consider external factors which will have an influence on results when results are quantified. The type of protein which is expressed is determined at DNA level and cannot be influenced by external factors. Quantity expressions of proteins are influenced by external factors and contribute to the variation between experimental lines within a population. When protein quality characteristics are the primary trait for selection, the breeding programme must consider including more than one locality with precise instructions to be followed at each locality. It is then necessary to monitor these external factors in order to incorporate drastical deviations into the data set.

Results from this study indicated the MAS was successfully applied in the UFS wheat resistance breeding programme. With the application of MAS, targeted traits were successfully tracked from one generation to the next. MAS enabled the selection at the end of each breeding step, of the best lines in terms of both disease resistance and protein quality traits. The application of MAS accelerated the breeding process and ensured that only the best lines in terms of resistance and protein quality were selected and taken forward from generation to generation. If only conventional breeding steps were followed this would not have been possible and the number of lines carried over from generation to generation would have grown exponentially with no guarantee that the best lines were included in the process.

This study furthermore confirmed that each additional round of self-pollination added to the breeding scheme by improving the homozygosity level of the targeted traits. Although self-pollination of heterozygous lines also lead to the loss of some traits due to recombination events, MAS enabled the selection of the best lines that still carried the highest numbers of targeted traits. However, for the rust resistant population, results indicated that two of the gene for resistances present in the original parental lines, *Sr2* and *Sr26*, were lost somewhere during the breeding programme. Results indicated that the original source for *Sr2* resistance, Blade, still segregated for this trait and it might be that the *Sr2* gene was not even present at the beginning of the study. Results also indicated that *Sr2* was present in three of the control lines screened (Kingbird, Damphe and Picaflor). Of these three cultivars, Kingbird showed the highest levels of resistance to Ug99 race of stem rust, including all derivatives of this race, in greenhouse experiments performed at the UFS. It is thus recommended that Kingbird should be included in the current rust resistance breeding programme and should thus be crossed with the best rust resistant lines identified during the current study. This will enable the transfer of both *Sr2* resistance and resistance to Ug99 and all its derivatives, to the best lines from this study.

The best rust resistant lines that also showed good protein quality characteristics, contained three rust gene for resistances/QTL, namely *Lr19, Lr34/Yr18/Sr57* and *QYr.sgi.2B-1*. Although two of the original genes for resistance, *Sr2* and *Sr26*, were not present in these lines, the best selected lines still contain a combination of slow-rusting, non-host specific APR genes as well as specific seedling resistance. *Lr19* will provide leaf rust specific seedling resistance to these lines. It has been shown that *Lr19* provides durable resistance if it is combined with other slow rusting genes, which is the case in the best lines, since *Lr34/Yr18/Sr57* is also present in all these lines. *Lr34/Yr18/Sr57* will provide durable APR to leaf rust and is race non-specific. *QYr.sgi.2B-1* will provide complete APR to stripe rust.

On the other hand, even after the additional rounds of self-pollination in the FHB resistant population, lines were identified that still carried all four FHB QTL for resistance originally selected for. The best FHB resistant line identified contained five of the six markers tested for in a homozygous state. Another round of self-pollination followed by MAS, will enable the selection of lines that carry six FHB resistant markers in a homozygous state.

The correlation of molecular markers linked to certain protein quality characteristics and the LUPP% (obtained from SE-HPLC analysis) enabled the selection of disease resistant lines with good potential bread-making quality characteristics. Because analyses could be done on single seeds, selections for bread-making quality characteristics could be done during early generations. Traditionally, this would not have been possible, because selection would only have been done at the end of the breeding programme when quality tests could be performed a large amount of seeds. Early selection ensured that the best resistant lines that also show good bread-making quality characteristics, will be carried forward in the breeding programme.

For future research, the best identified lines of this study should be planted for seed multiplication to obtain enough seed for baking testes as well as for phenotypical disease screening under field and greenhouse conditions. Lines without important rust genes/QTL such as stem rust gene for resistances can be improved by crossing these lines with lines that contain these genes in an effort to improve these lines. FHB lines can be tested for minor FHB QTL for resistance and try to incorporate more of these QTL into lines to improve the FHB tolerance of these lines. The best identified rust and FHB resistant lines can also be crossed with one another to obtain lines with combined rust and FHB resistance.

Summary

Wheat is one of the most important food crops and consumption in the past year was higher than production worldwide, which indicates improved wheat cultivars need to be developed to maintain the demand for wheat. One of the biggest threats of wheat is ever-evolving pathogens that overcome resistance. To overcome threatening diseases, breeders need to incorporate new resistant genes/QTL into improved cultivars. Incorporating different resistant genes/QTL into a single line using gene-pyramiding and MAS can enhance the breeding process. However, pyramiding of genes for resistance can lead to a decrease in baking quality characteristics, which are important for the milling and baking industry as well as for consumers. To overcome this problem, lines should also be tested for bread-making quality characteristics such as HMW-GS and the LUPP% which are directly linked to protein quality.

The aim of the study was to identify rust and FHB resistant lines with good protein content. Rust and FHB resistant wheat lines were developed during previous studies. The best rust and FHB resistant lines were planted and self-pollinated. Lines were evaluated for the presence of five rust resistant genes/QTL (*Lr19, Lr34/Yr18/Sr57, QYr.sgi.2B-1, Sr2* and *Sr26*) and four FHB resistant genes/QTL (*Fhb1, Qfhs.ifa-5A-1, Qfhs.ifa-5A-2* and *Fhb2*) for two consecutive years. These selected rust and FHB resistant lines were also subjected to three biochemical tests namely SDS-PAGE, SE-HPLC and RP-HPLC. PCR-based markers linked to HMW-GS alleles and the IBL.1RS translocation, associated with weak dough strength, were also tested. Since the breeding programme was still in an early stage, only few seeds per line were available and therefore biochemical tests that can be performed using single seeds were selected. Results from SDS-PAGE and molecular markers linked to HMW-GS were similar and therefore lines were further evaluated using only molecular markers. No correlations were detected for the RP-HPLC data of lines therefore it was excluded during further analysis.

The rust and FHB resistant genes/QTL co-segregated based on their related parental lines. An additional round of self-pollination either led to higher levels of homozygosity for the selected traits or to the loss of traits due to recombination. MAS enabled the selection and enhancement of homozygous lines.

Only a few offspring of one of the rust resistant lines contained the *Sr26* gene, while the *Lr34/Yr18/Sr57* gene was present in all tested lines. None of the rust resistant lines contained the *Sr2* gene which is a major gene for resistance against stem rust and especially effective against the threatening Ug99 race. The top ten rust resistant lines all had the same rust resistant gene/QTL present (*Lr19, Lr34/Yr18/Sr57* and *QYr.sgi.2B-1*) as well as the same protein quality alleles (*Ax2**, *Bx7+By8* and *Dx5+Dy10*). The LUPP% of these lines showed high levels of variation and was optimal (40% to 50%) for only one line.

High levels of variation were detected for the FHB resistant markers in the FHB resistant populations. The top ten lines contained all six markers although the level of homozygosity varied. Four of these lines expressed both the *Bx7+By8* and *Bx17+By18* alleles and only one line did not express the $Bx7^{OE}$ allele. None of these lines expressed the 1BL.1RS translocation. Two of these lines showed a desirable LUPP%.

Results indicated the preference towards rust or FHB resistance selection followed by selection for protein quality alleles and lastly the LUPP%. The top ten rust and FHB lines can serve as resistance sources in further breeding programmes.

Key words: LUPP%, marker-assisted selection, resistance breeding, SDS-PAGE, SE-HPLC, wheat protein, quality.

Opsomming

Koring is een van die belangrikste voedselgewasse en die afgelope jaar was die verbruik van koring wêreldwyd hoër as produksie wat daarop dui dat verbeterde koring kultivars ontwikkel moet word om aan die behoefte vir koringte kan voorsien. Een van die grootste bedreigings vir koring is patogene wat steeds bly verander om weerstand te kan oorkom. Ten einde hierdie bedreiging te kan oorkom moet planttelers nuwe weerstandsgene en kwantitatiewe eienskap lokusse (QTL) in verbeterde kultivars inbou. Die teelproses kan aansienlik versterk word deur van geenstapeling en merker-ondersteunde seleksie gebruik te maak om verskillende weerstandsgene en QTL in ‘n enkele lyn in te bou. Die stapeling van gene kan egter tot ‘n afname in bakkwaliteitseienskappe, wat belangrik vir die meulenaars en die bakindustrie asook verbruikers is, lei. Om hierdie probleem te oorkom behoort lyne ook vir broodkwaliteits-eienskappe soos hoë molekulêre gewig gluten sub-eenhede (HMW-GS) en groot onekstraheerbare polimeriese proteïen persentasie (LUPP%) wat direk aan proteïenkwaliteit gekoppel is, getoets te word.

Die doel van die studie was om roes en fusariumaarskroei (FHB) weerstandbiedende lyne met goeie proteïeninhoud te identifiseer. Roes en FHB weerstandbiedende lyne is gedurende vorige studies ontwikkel. Die beste roes en FHB weerstandbiedende lyne is geplant en selfbestuif. Lyne is vir die teenwoordigheid van vyf roesweerstandsgene of QTL (*Lr19, Lr34/Yr18/Sr57, QYr.sgi.2B-1, Sr2* en *Sr26*) en vier FHB weerstandsgene of QTL (*Fhb1, Qfhs.ifa-5A-1, Qfhs.ifa-5A-2* en *Fhb2*) gedurende twee opeenvolgende jare, geëvalueer. Hierdie geselekteerde roes en FHB weerstandbiedende lyne was ook aan drie biochemiese toetse, naamlik natrium dodesielsulfaatpoliakrielamiedgelelektroforese (SDS-PAGE), grootte uitsluitings-hoë prestasie vloeistof chromatografie (SE-HPLC) en omgekeerde-fase-hoë prestasie vloeistof chromatografie (RP-HPLC) onderwerp. Polimerase kettingreaksie (PCR) gebaseerde merkers gekoppel aan HMW-GS allele en die 1BL.1RS translokasie, wat met swak deegsterkte geassosieer is, is ook getoets. Aangesien die teelprogram nog in ‘n vroeë stadium van ontwikkeling was, was slegs ‘n klein aantal saad van elke lyn beskikbaar en daarom is biochemiese toetse wat op enkel sade gedoen kan word, geselekteer. Dieselfde resultate is verkry deur van SDS-PAGE en molekulêre merkers gekoppel aan die HMW-GS gebruik te maak en daarom is die res van die lyne slegs getoets deur van die molekulêre merkers gebruik te maak. Geen korrelasies is vir die RP-HPLC data van die verskillende lyne verkry nie en daarom is die tegniek nie verder gebruik nie.

Die roes en FHB weerstandsgene of QTL het ko-segregasie getoon gebaseer op hul onderskeie ouers. ‘n Addisionele rondte van selfbestuiwing het tot of hoër vlakke homosigositeit van die geselekteerdeeienskappe, of tot ‘n verlies van van die eienskappe weens rekombinasie gelei. MAS het die seleksie en verhoging van homosigotiese lyne moontlik gemaak.

Slegs ‘n klein aantal lyne afkomstig van een van die roesweerstandbiedende lyne het die *Sr26* geen bevatterwyl die *Lr34/Yr18/Sr57* geen in al die getoetste lyne teenwoordig was. Geen van die roesweerstandbiedende lyne het die *Sr2* geen wat ‘n belangrike weerstandsgeen teen stamroes en veral die Ug99 patogeen is, bevat nie. Die top 10 roesweerstandbiedende lyne het almal dieselfde roesweerstandsgene of QTL (*Lr19, Lr34/Yr18/Sr57* en *QYr.sgi.2B-1*) asook dieselfde proteïen kwaliteitsallele(*Ax2**, *Bx7+By8* en *Dx5+Dy10*) bevat. Die LUPP% van hierdie lyne het hoë vlakke van variasie getoon en was slegs optimaal (40% tot 50%) in een van die lyne.

Hoë vlakke van variasie is vir die FHB weerstandsmerkers in die FHB weerstandbiedende populasies opgespoor. Die top tien lyne het almal ses merkers bevat alhoewel die vlak van homosigositeit gevarieer het. Vier van hierdie lyne het beide die *Bx7+By8* en *Bx17+By18* allele uitgedruk en slegs een van hierdie lyne het nie die $Bx7^{OE}$ alleel bevat nie. Geen een van hierdie lyne het die 1BL.1RS translokasie uitgedruk nie. Twee van die lyne het aanvaarbare LUPP% gehad.

Resultate het aangetoon dat daar tydens seleksie voorkeur verleen is aan roes en FHB weerstand gevolg deur seleksie vir proteïenkwaliteitallele en laastens vir LUPP%. Die top tien roes en FHB lyne kan in die toekoms as weerstandbronne in ander teelprogramme gebruik word.

Sleutelwoorde: Koring proteïen, kwaliteit, LUPP%, merker-ondersteunde seleksie, SDS-PAGE, SE-HPLC, weerstandsteling.

Appendix I

Rust resistant marker data for the rust resistant lines tested during the first year

Line name	STSLr19$_{130}$ (*Lr19*)	cssfr5 (*Lr34/Yr 18/Sr57*)	Gwm148 (*QYr.sgi. 2B-1*)	Gwm501 (*QYr.sgi. 2B-1*)	csSr2 (*Sr2*)	Sr26#43 (*Sr26*)	Total number of rust resistant markers
S16(7.3)/1.1	0	1	1	1	0	0	3
S16(7.3)/1.2	1	1	1	0	0	0	3
S16(7.3)/2.1	0	1	1	0	0	0	2
S16(7.3)/3.1	0	1	1	0	0	0	2
S16(7.3)/4.1	1	1	1	0	0	0	3
S16(7.3)/4.2	1	1	1	0	0	0	3
S16(7.3)/5.1	1	1	1	0	0	0	3
S16(7.3)/5.2	0	1	1	1	0	0	3
S16(7.3)/5.3	0	1	1	1	0	0	3
S16(7.3)P1.5.1/1.1	0	1	1	1	0	0	3
S16(7.3)P1.5.1/1.2	0	1	1	0	0	0	2
S16(7.3)P1.5.1/1.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/2.1	1	1	1	0	0	0	3
S16(7.3)P1.5.1/2.2	0	1	1	1	0	0	3
S16(7.3)P1.5.1/2.3	0	1	1	0	0	0	2
S16(7.3)P1.5.1/3.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.2	0	1	0	0	0	0	1
S16(7.3)P1.5.1/3.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/4.1	0	1	1	0	0	0	2
S16(7.3)P1.5.1/4.2	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.2	0	M	1	1	0	0	2
S16(7.3)P1.5.1/5.3	1	1	1	0	0	0	3
S16(7.3)P1.5.1/6.1	1	1	0	0	0	0	2
S16(7.3)P1.5.1/6.2	1	1	0	0	0	0	2
S16(7.3)P1.5.1/6.3	0	M	0	1	0	0	1
S16(7.3)P3.5.1/1.1	0	1	1	0	0	0	2
S16(7.3)P3.5.1/1.2	1	1	0	0	0	0	2
S16(7.3)P3.5.1/2.1	1	1	0	0	0	0	2
S16(7.3)P3.5.1/2.2	1	1	0	0	0	0	2
S16(7.3)P3.5.1/2.3	0	1	1	0	0	0	2
S16(7.3)P3.5.1/3.1	1	1	0	0	0	0	2
S16(7.3)P3.5.1/3.2	1	1	0	0	0	0	2
S16(7.3)P3.5.1/3.3	0	1	1	0	0	0	2
S16(7.3)P3.5.1/4.1	0	1	0	0	0	0	1
S16(7.3)P3.5.1/4.2	1	1	0	0	0	0	2
S16(7.3)P3.5.1/4.3	0	1	1	0	0	0	2
S16(7.3)P3.5.1/5.1	1	M	0	0	0	0	1
S16(7.3)P3.5.1/5.2	1	1	0	0	0	0	2
S16(7.3)P3.5.1/5.3	0	M	1	0	0	0	1
S16(7.3)P3.5.1/6.1	0	1	0	0	0	0	1
S16(7.3)P3.5.1/6.2	0	1	0	0	0	0	1
S16(7.3)P3.5.1/6.3	1	1	0	0	0	0	2
S16(7.3)P3.6.1/1.1	0	1	0	0	0	0	1
S16(7.3)P3.6.1/1.2	0	1	1	0	0	0	2
S16(7.3)P3.6.1/1.3	1	1	0	0	0	0	2
S16(7.3)P3.6.1/2.1	1	1	0	0	0	0	2
S16(7.3)P3.6.1/2.2	1	1	0	0	0	0	2
S16(7.3)P3.6.1/2.3	0	0	1	1	0	0	2
S16(7.3)P3.6.1/3.1	1	0	0	1	0	0	2
S16(7.3)P3.6.1/3.2	0	0	0	1	0	0	1
S16(7.3)P3.6.1/3.3	0	0	0	1	0	0	1
S16(7.3)P3.6.1/4.1	0	0	0	1	0	0	1
S16(7.3)P3.6.1/4.2	1	0	0	1	0	0	2
S16(7.3)P3.6.1/4.3	0	0	1	1	0	0	2

Rust resistant marker data for the rust resistant lines tested during the first year (continued)

Line name	**STSLr19$_{130}$ (*Lr19*)**	**cssfr5 (*Lr34/Yr 18/Sr57*)**	**Gwm148 (*QYr.sgi. 2B-1*)**	**Gwm501 (*QYr.sgi. 2B-1*)**	**csSr2 (*Sr2*)**	**Sr26#43 (*Sr26*)**	**Total number of rust resistant markers**
S16(7.3)P3.6.1/5.1	0	0	0	1	0	0	1
S16(7.3)P3.6.1/5.2	1	0	0	1	0	0	2
S16(7.3)P3.6.1/5.3	1	0	1	1	0	0	3
S16(7.3)P3.6.1/6.1	1	0	1	1	0	0	3
S16(7.3)P3.6.1/6.2	0	0	0	1	0	0	1
S16(7.3)P3.6.1/6.3	1	0	1	1	0	0	3
S16(7.3)P3.2.1/1.1	1	0	1	1	0	0	3
S16(7.3)P3.2.1/1.2	1	0	1	1	0	0	3
S16(7.3)P3.2.1/1.3	1	0	0	1	0	0	2
S16(7.3)P3.2.1/2.1	1	0	1	1	0	0	3
S16(7.3)P3.2.1/2.2	1	0	1	1	0	0	3
S16(7.3)P3.2.1/2.3	1	0	0	1	0	0	2
S16(7.3)P3.2.1/3.1	M	0	0	1	0	0	1
S16(7.3)P3.2.1/3.2	1	0	1	1	0	0	3
S16(7.3)P3.2.1/3.3	1	0	1	1	0	0	3
S16(7.3)P3.2.1/4.1	1	0	0	M	0	0	1
S16(7.3)P3.2.1/4.2	1	0	0	1	0	0	2
S16(7.3)P3.2.1/4.3	0	0	0	M	0	0	0
S16(7.3)P3.2.1/5.1	1	0	0	1	0	0	2
S16(7.3)P3.2.1/5.2	0	0	1	1	0	0	2
S16(7.3)P3.2.1/5.3	1	0	0	1	0	0	2
S16(7.3)P3.2.1/6.1	1	0	0	1	0	0	2
S16(7.3)P3.2.1/6.2	0	0	1	1	0	0	2
S16(7.3)P3.2.1/6.3	1	0	1	1	0	0	3
S16(1.2)/1.1	1	0	0	1	0	0	2
S16(1.2)/1.2	1	0	0	1	0	0	2
S16(1.2)/1.3	1	1	0	M	0	0	2
S16(1.2)/2.1	1	1	0	1	0	0	3
S16(1.2)/2.2	1	0	0	1	0	0	2
S16(1.2)/2.3	1	0	0	1	0	0	2
S16(1.2)/3.1	1	0	0	1	0	0	2
S16(1.2)/3.2	1	1	0	1	0	0	3
S16(1.2)/3.3	1	0	0	1	0	0	2
S16(1.2)/4.1	1	0	0	1	0	0	2
S16(1.2)/4.2	1	0	0	M	0	0	1
S16(1.2)/4.3	1	0	0	M	0	0	1
S16(1.2)/5.1	1	0	0	1	0	0	2
S16(1.2)/5.2	1	1	0	1	0	0	3
S16(1.2)/5.3	1	0	0	1	0	0	2
S16(1.2)/6.1	1	0	0	1	0	0	2
S16(1.2)/6.2	1	0	0	1	0	0	2
S16(1.2)/6.3	1	0	0	1	0	0	2
S726(3.2)/1	0	0	1	1	1	0	3
S726(3.2)/2	0	0	0	1	0	0	1
S726(3.2)P1.3.1/1.1	0	0	1	1	1	0	3
S726(3.2)P1.3.1/1.2	0	0	0	1	1	0	2
S726(3.2)P1.3.1/1.3	0	0	0	M	0	0	0
S726(3.2)P1.3.1/2.1	0	0	1	1	0	0	2
S726(3.2)P1.3.1/2.2	0	0	1	1	1	0	3
S726(3.2)P1.3.1/2.3	0	0	1	1	0	0	2
S726(3.2)P1.3.1/3.1	0	0	0	1	1	0	2
S726(3.2)P1.3.1/3.2	0	0	0	M	0	0	0

Rust resistant marker data for the rust resistant lines tested during the first year (continued)

Line name	STSLr19$_{130}$ (*Lr19*)	cssfr5 (*Lr34/Yr 18/Sr57*)	Gwm148 (*QYr.sgi. 2B-1*)	Gwm501 (*QYr.sgi. 2B-1*)	csSr2 (*Sr2*)	Sr26#43 (*Sr26*)	Total number of rust resistant markers
S726(3.2)P1.3.1/3.3	0	0	0	M	1	0	1
S726(3.2)P1.3.1/4.1	0	0	0	M	0	0	0
S726(3.2)P1.3.1/4.2	0	0	0	1	1	0	2
S726(3.2)P1.3.1/4.3	0	0	0	M	1	0	1
S726(3.2)P1.3.1/5.1	0	0	0	1	1	0	2
S726(3.2)P1.3.1/5.2	0	0	1	M	1	0	2
S726(3.2)P1.3.1/5.3	0	0	0	M	1	0	1
S726(3.2)P1.3.1/6.1	0	0	0	1	1	0	2
S726(3.2)P1.3.1/6.2	0	0	1	1	1	0	3
S726(3.2)P1.3.1/6.3	0	0	0	M	1	0	1
S726(3.2)P2.4.1/1.1	0	0	0	1	0	0	1
S726(3.2)P2.4.1/1.2	0	0	0	1	0	0	1
S726(3.2)P2.4.1/1.3	0	0	0	1	1	0	2
S726(3.2)P2.4.1/2.1	0	0	0	1	1	0	2
S726(3.2)P2.4.1/2.2	0	0	0	1	0	0	1
S726(3.2)P2.4.1/2.3	0	0	0	1	1	0	2
S726(3.2)P2.4.1/3.1	0	0	0	1	1	0	2
S726(3.2)P2.4.1/3.2	0	0	0	1	1	0	2
S726(3.2)P2.4.1/3.3	0	0	0	M	1	0	1
S726(3.2)P2.4.1/4.1	0	0	0	1	0	0	1
S726(3.2)P2.4.1/4.2	0	0	0	1	0	0	1
S726(3.2)P2.4.1/4.3	0	0	0	1	1	0	2
S726(3.2)P2.4.1/5.1	0	0	0	1	1	0	2
S726(3.2)P2.4.1/5.2	0	0	0	1	1	0	2
S726(3.2)P2.4.1/5.3	0	0	0	1	0	0	1
S726(3.2)P2.4.1/6.1	0	0	0	1	1	0	2
S726(3.2)P2.4.1/6.2	0	0	0	1	1	0	2
S726(3.2)P2.4.1/6.3	0	0	0	1	1	0	2
S726(3.2)P3.6.1/1.1	0	0	0	1	0	0	1
S726(3.2)P3.6.1/1.2	0	0	0	1	0	0	1
S726(3.2)P3.6.1/1.3	0	0	0	1	0	0	1
S726(3.2)P3.6.1/2.1	0	0	0	M	0	0	0
S726(3.2)P3.6.1/2.2	0	0	1	1	0	0	2
S726(3.2)P3.6.1/2.3	0	0	1	1	0	0	2
S726(3.2)P3.6.1/3.1	0	0	0	1	0	0	1
S726(3.2)P3.6.1/3.2	0	0	0	M	0	0	0
S726(3.2)P3.6.1/3.3	0	0	1	1	0	0	2
S726(3.2)P3.6.1/4.1	0	0	1	1	0	0	2
S726(3.2)P3.6.1/4.2	0	0	0	1	0	0	1
S726(3.2)P3.6.1/4.3	0	0	1	1	0	0	2
S726(3.2)P3.6.1/5.1	0	0	0	1	0	0	1
S726(3.2)P3.6.1/5.2	0	0	1	1	0	0	2
S726(3.2)P3.6.1/5.3	0	0	1	1	0	0	2
S726(3.2)P3.6.1/6.1	0	0	1	1	0	0	2
S726(3.2)P3.6.1/6.2	0	0	0	M	0	0	0
S726(3.2)P3.6.1/6.3	0	0	0	M	0	0	0
S726(2.2)/1	0	0	0	1	1	0	2
S726(2.2)/2	0	0	0	M	1	0	1
S178(7.2)/1.1	1	1	0	M	0	0	2
S178(7.2)/2.1	0	1	0	1	0	0	2
S178(7.2)/2.2	0	1	0	1	0	0	2
S178(7.2)/2.3	1	1	0	1	0	0	3
S178(7.2)/3.1	1	1	0	1	0	0	3
S178(7.2)/3.2	1	1	0	1	0	0	3
S178(7.2)/4.1	1	1	0	1	0	0	3
S178(7.2)/4.2	1	1	0	1	0	0	3
S178(7.2)/4.3	1	1	0	1	0	0	3
S178(7.2)/5.1	1	1	0	1	0	0	3
S178(7.2)/5.2	1	1	0	1	0	0	3

Rust resistant marker data for the rust resistant lines tested during the first year (continued)

Line name	STSLr19$_{130}$ (*Lr19*)	cssfr5 (*Lr34/Yr 18/Sr57*)	Gwm148 (*QYr.sgi. 2B-1*)	Gwm501 (*QYr.sgi. 2B-1*)	csSr2 (*Sr2*)	Sr26#43 (*Sr26*)	Total number of rust resistant markers
S178(7.2)/6.1	1	1	0	1	0	0	3
S346/1.1	0	0	0	1	0	0	1
S346/1.2	0	0	0	1	1	0	2
S346/1.3	1	1	1	1	1	0	5
S346/2.1	1	0	0	1	1	0	3
S346/2.2	1	1	0	1	0	0	3
S346/2.3	0	0	0	1	1	0	2
S346/3.1	1	1	0	1	1	0	4
S346/3.2	1	0	0	1	1	0	3
S346/3.3	0	0	0	1	1	0	2
S346/4.1	0	0	0	1	1	0	2
S346/4.2	0	0	0	1	0	0	1
S346/4.3	1	0	0	1	0	0	2
S346/5.1	0	1	0	1	0	0	2
S346/5.2	1	1	0	1	1	0	4
S346/5.3	1	1	1	1	1	0	5
S346/6.1	1	1	0	1	1	0	4
S346/6.2	1	1	0	1	1	0	4
S346/6.3	1	1	1	1	1	0	5
S157/1.1	0	0	0	0	0	0	0
S157/1.2	1	0	0	1	0	0	2
S157/1.3	1	0	0	1	0	0	2
S157/2.1	1	0	0	1	0	0	2
S157/2.2	1	0	0	1	0	0	2
S157/3.1	1	0	0	1	0	0	2
S157/3.2	0	0	0	0	0	0	0
S157/3.3	1	0	0	1	0	0	2
S157/4.1	1	0	0	1	0	0	2
S157/4.2	0	0	0	0	0	0	0
S157/4.3	1	0	0	1	0	0	2
S157/5.1	0	0	0	1	0	0	1
S157/5.2	0	0	0	0	0	0	0
S157/5.3	1	0	0	1	0	0	2
S157/6.1	0	0	0	1	0	0	1
S157/6.2	1	0	0	1	0	0	2
S157/6.3	1	0	0	1	0	0	2

1 - homozygous resistant present; 0 - homozygous susceptible present; M - Missing value

Appendix II

Rust resistant marker data for the rust resistant lines tested during the second year

Line name	STSLr19$_{130}$ (*Lr19*)	cssfr5 (*Lr34/Yr18/Sr57*)	Gwm148 (*QYr.sgi. 2B-1*)	Gwm501 (*QYr.sgi. 2B-1*)	csSr2 (*Sr2*)	Sr26#43 (*Sr26*)	Total number of rust resistant markers*
S16(7.3)P1.5.1/1.3.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.2	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.3	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.4	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.5	1	1	1	1	0	0	4
S16(7.3)P1.5.1/1.3.6	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.1.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.1.2	1	1	1	1	0	0	4
S16(7.3)P1.5.1/3.3.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/4.2.1	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.2.2	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.2.3	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.2.4	1	1	1	0	0	0	3
S16(7.3)P1.5.1/4.3.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.1.1	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.1.2	1	1	1	1	0	0	4
S16(7.3)P1.5.1/5.1.3	1	1	1	1	0	0	4
S16(7.3)P3.6.1/5.3.1	1	1	1	0	0	0	3
S16(7.3)P3.6.1/5.3.2	1	1	1	0	0	0	3
S16(7.3)P3.6.1/5.3.3	1	1	1	0	0	0	3
S16(7.3)P3.6.1/5.3.4	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.1	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.2	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.3	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.4	1	1	1	0	0	0	3
S16(7.3)P3.6.1/6.1.5	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.3	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.4	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.5	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.1.6	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.3	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.4	1	1	1	0	0	0	3
S16(7.3)P3.2.1/1.2.5	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.2	1	1	1	0	0	0	3

Rust resistant marker data for the rust resistant lines tested during the second year (continued)

Line name	$STSLr19_{130}$ (*Lr19*)	cssfr5 (*Lr34/Yr18/Sr57*)	Gwm148 (*QYr.sgi.2B-1*)	Gwm501 (*QYr.sgi.2B-1*)	csSr2 (*Sr2*)	Sr26#43 (*Sr26*)	Total number of rust resistant markers*
S16(7.3)P3.2.1/2.1.3	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.4	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.5	1	1	1	0	0	0	3
S16(7.3)P3.2.1/2.1.6	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2.1	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2.2	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2.3	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2.4	1	1	1	0	0	0	3
S16(7.3)P3.2.1/3.2.5	1	1	1	0	0	0	3
S16(1.2)/2.1.1	0	1	1	1	0	0	3
S16(1.2)/2.1.2	0	1	1	1	0	0	3
S16(1.2)/2.1.3	0	1	1	1	0	0	3
S16(1.2)/2.1.4	0	1	1	1	0	0	3
S16(1.2)/2.1.5	0	1	1	1	0	0	3
S726(3.2)/1.1	0	1	0	0	0	1	2
S726(3.2)/1.2	0	1	0	0	0	1	2
S726(3.2)/1.3	0	1	0	0	0	1	2
S726(3.2)/1.4	0	1	0	0	0	1	2
S726(3.2)/1.5	0	1	0	0	0	1	2
S726(3.2)/1.6	0	1	0	0	0	1	2
S726(3.2)P1.3.1/1.1.1	1	1	0	0	0	1	3
S726(3.2)P1.3.1/2.2.1	0	1	0	0	0	1	2
S178(7.2)/2.3.1	0	1	1	1	0	0	3
S178(7.2)/2.3.2	0	1	1	1	0	0	3
S178(7.2)/2.3.3	0	1	1	1	0	0	3
S178(7.2)/2.3.4	0	1	1	1	0	0	3
S178(7.2)/4.1.1	0	1	1	1	0	0	3
S178(7.2)/4.1.2	0	1	1	1	0	0	3
S178(7.2)/4.1.3	0	1	1	1	0	0	3
S178(7.2)/4.1.4	0	1	1	1	0	0	3
S178(7.2)/4.1.5	0	1	1	1	0	0	3
S178(7.2)/4.1.6	0	1	1	1	0	0	3
S178(7.2)/5.1.1	0	1	1	1	0	0	3
S178(7.2)/5.1.2	0	1	1	1	0	0	3
S178(7.2)/5.1.3	0	1	1	1	0	0	3
S178(7.2)/5.1.4	0	1	1	1	0	0	3
S346(1.3)1	1	1	1	1	0	0.5	5
S346(1.3)2	0	1	1	1	0	0.5	4
S346(1.3)3	0	1	1	1	0	0.5	4
S346(1.3)4	0	1	1	1	0	0	3
S346(1.3)5	1	1	1	1	0	0	4

Rust resistant marker data for the rust resistant lines tested during the second year (continued)

Line name	STSLr19$_{130}$ (*Lr19*)	cssfr5 (*Lr34/Yr 18/Sr57*)	Gwm148 (*QYr.sgi. 2B-1*)	Gwm501 (*QYr.sgi. 2B-1*)	csSr2 (*Sr2*)	Sr26#43 (*Sr26*)	Total number of rust resistant markers*
S346(5.3)1	0	1	1	1	0	0.5	4
S346(5.3)2	0	1	1	1	0	0.5	4
S346(5.3)3	0	1	1	0	0	0	2
S346(5.3)4	1	1	1	1	0	0.5	5
S346(5.3)5	1	1	1	0	0	0.5	4
S346(6.3)1	1	1	1	1	0	0	4
S346(6.3)2	1	1	1	1	0	0	4
S346(6.3)3	0	1	1	1	0	0	3

* - Represents total number of markers present irrespective if the marker was homo- or heterozygous; 1 - Homozygous resistant; 0.5 - Heterozygous; 0 - Homozygous susceptible

Appendix III

FHB resistant marker data for the rust resistant lines tested during the first year

Line name	Barc 133	Gwm 533	Gwm 304	Gwm 293	Gwm 156	Gwm 133	Total number of FHB resistant markers*
BC$_2$F$_2$P2.6.2/1.1	0.5	0.5	1	0.5	1	1	6
BC$_2$F$_2$P2.6.2/1.2	1	1	1	0.5	0.5	0.5	6
BC$_2$F$_2$P2.6.2/2.1	0.5	0.5	0.5	0.5	0.5	0.5	6
BC$_2$F$_2$P2.6.2/3.1	0	0	0.5	0	0	0.5	2
BC$_2$F$_2$P2.6.2/4.1	1	1	0.5	0.5	1	0.5	6
BC$_2$F$_2$P2.14.2/1.1	0.5	0.5	0	1	0	0	3
BC$_2$F$_2$P2.14.2/2.1	0.5	0.5	0	1	0	0	3
BC$_2$F$_2$P2 14.2/3.1	0	0	0	0	0	0.5	1
BC$_2$F$_2$P2.14.2/4.2	0.5	0.5	0	0	0	0.5	3
BC$_2$F$_2$P2.14.2/5.2	0	0	0	0	0	1	1
BC$_2$F$_2$P1.7.2/1.2	0	0	0	0	0	0.5	1
BC$_2$F$_2$P1.7.2/2.2	0	0	1	0	0.5	0.5	3
BC$_2$F$_2$P1.7.2/3.2	0	0	0	0	0.5	0.5	2
BC$_2$F$_2$P1.7.2/4.2	0	0	0	0	0.5	0.5	2
BC$_2$F$_2$P1.7.2/5.2	0	0	0	0	0.5	0	1
BC$_2$F$_2$ 4.1/1.3	1	1	0.5	0.5	0.5	0.5	6
BC$_2$F$_2$ 4.1/2.3	0.5	0.5	0.5	0.5	0	0	4
BC$_2$F$_2$ 4.1/3.3	0.5	0.5	0.5	0.5	0.5	1	6
BC$_2$F$_2$ 4.1/4.3	0.5	0.5	0.5	0.5	0.5	1	6
BC$_2$F$_2$ 4.1/5.3	0.5	1	1	0.5	0.5	0.5	6
BC$_2$F$_2$P1.7.1/1.2	0.5	0.5	1	1	1	1	6
BC$_2$F$_2$P1.7.1/2.2	0	0	1	1	1	0	3
BC$_2$F$_2$P1.7.1/3.2	1	1	1	0.5	1	0.5	6
BC$_2$F$_2$P1.7.1/4.2	0.5	0.5	1	0.5	1	0.5	6
BC$_2$F$_2$P1.7.1/5.2	1	0.5	1	1	1	0	5
BC$_2$F$_2$P1.7.3/1.3	0	0	0.5	0.5	0.5	0.5	4
BC$_2$F$_2$P1.7.3/2.3	0.5	0.5	0.5	0	0.5	0	4
BC$_2$F$_2$P1.7.3/3.3	0	0	0	0	0	0	0
BC$_2$F$_2$P1.7.3/4.3	0.5	0.5	0.5	0.5	0.5	0.5	6
BC$_2$F$_2$P1.7.3/5.3	0.5	0.5	0.5	0.5	0.5	0.5	6
BC$_2$F$_2$P2.2.3/1.1	0.5	0.5	0.5	0.5	0.5	0	5
BC$_2$F$_2$P2.2.3/2.1	0.5	0.5	0	0	0	0	2

Rust resistant marker data for the rust resistant lines tested during the first year (continued)

Line name	Barc 133	Gwm 533	Gwm 304	Gwm 293	Gwm 156	Gwm 133	Total number of FHB resistant markers*
BC_2F_2P2.2.3/3.1	0.5	0.5	0	0.5	0	0	3
BC_2F_2P2.2.3/4.1	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_2P2.2.3/5.1	0.5	0.5	0	0	0.5	0	3
BC_2F_2P2.3.2/1.2	0.5	0.5	0	0	0.5	0	3
BC_2F_2P2.3.2/2.2	0	0.5	0.5	0.5	0	0	3
BC_2F_2P2.3.2/3.2	0.5	0.5	0.5	0	0	0	3
BC_2F_2P2.3.2/4.2	0.5	0.5	0.5	0.5	0	1	5
BC_2F_2P2.3.2/5.3	0.5	0.5	0.5	0.5	0	0.5	5
BC_2F_2P2.12.1/1.1	0.5	0.5	1	1	1	0	5
BC_2F_2P2.12.1/2.1	0.5	0.5	0.5	0.5	1	0	5
BC_2F_2P2.12.1/3.1	0	0	0.5	0.5	0.5	0	3
BC_2F_2P2.12.1/4.1	0.5	0.5	0	0.5	0	0	3
BC_2F_2P2.12.1/5.1	0	0	0	0.5	0	0	1
BC_2F_2P1.5.3/1.1	0	0	0	0.5	0	0.5	2
BC_2F_2P1.5.3/2.1	0	0	0	0	0	1	1
BC_2F_2P1.5.3/3.1	0	0	0	0.5	0	0.5	2
BC_2F_2P1.5.3/3.2	0	0	0	0.5	0	0	1
BC_2F_2P1.5.3/4.1	0	0	0	0	0	0	0
BC_2F_2P1.2.1/1.2	0.5	0.5	0.5	0.5	1	0	5
BC_2F_2P1.2.1/2.2	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_2P1.2.1/3.2	0	0	0.5	1	1	0	3
BC_2F_2P1.2.1/4.2	1	1	0	0	0	0	2
BC_2F_2P1.2.1/6.2	0	0	0.5	0.5	0.5	0	3

* - Represents total number of markers present irrespective if the marker was homo- or heterozygous; 1 - Homozygous resistant; 0.5 - Heterozygous; 0 - Homozygous susceptible

Appendix IV

FHB resistant marker data for the FHB resistant lines tested during the second year

Line name	Barc133	Gwm533	Gwm293	Gwm304	Gwm156	Gwm133	Total number of FHB resistant markers*
BC_2F_3P2 6.2/1.1.1	0.5	0.5	1	1	1	0	5
BC_2F_3P2 6.2/1.1.2	0.5	0.5	1	1	1	0	5
BC_2F_3P2 6.2/1.1.3	0.5	1	1	1	1	0	5
BC_2F_3P2 6.2/1.1.5	0.5	1	1	1	1	0.5	6
BC_2F_3P2 6.2/1.2.1	1	1	1	1	1	0.5	6
BC_2F_3P2 6.2/1.2.2	1	1	1	1	0.5	0.5	6
BC_2F_3P2 6.2/1.2.3	1	1	1	1	1	0	5
BC_2F_3P2 6.2/1.2.4	1	1	1	1	0.5	0.5	6
BC_2F_3P2 6.2/1.2.5	1	1	1	0	0.5	0.5	5
BC_2F_3P2 6.2/2.1.1	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_3P2 6.2/2.1.2	0.5	0.5	0	0	0.5	0	3
BC_2F_3P2 6.2/2.1.3	0.5	0.5	0	0	0	0	2
BC_2F_3P2 6.2/2.1.4	0.5	0.5	0	0	0.5	0	3
BC_2F_3P2 6.2/2.1.5	1	1	1	1	1	0	5
BC_2F_3P2 6.2/2.1.6	0	0	0.5	0.5	0.5	0	3
BC_2F_3P2 6.2/4.1.1	0.5	1	0.5	0.5	1	0	5
BC_2F_3P2 6.2/4.1.2	0.5	1	0	0	0	0	2
BC_2F_3P2 6.2/4.1.3	1	1	0.5	0.5	0.5	0	5
BC_2F_3P2 6.2/4.1.4	1	1	0.5	0.5	0	0	4
BC_2F_3P2 6.2/4.1.5	1	1	0.5	0.5	0.5	0	5
$BC_2F_3$4.1/1.3.1	1	1	1	1	1	0.5	6
$BC_2F_3$4.1/1.3.2	1	1	0	0	0.5	0.5	4
$BC_2F_3$4.1/2.3.1	0	0	0.5	0.5	0	0	2
$BC_2F_3$4.1/2.3.2	0	0	0.5	0.5	0	0	2
$BC_2F_3$4.1/2.3.3	0.5	0.5	0.5	0.5	0	0	4
$BC_2F_3$4.1/2.3.4	0.5	1	0.5	0	0	0	3
$BC_2F_3$4.1/3.3.1	0.5	0.5	0.5	0.5	0.5	1	6
$BC_2F_3$4.1/3.3.2	0.5	0.5	0	0	0	1	3
$BC_2F_3$4.1/3.3.3	0.5	0	0	0	0	1	2
$BC_2F_3$4.1/3.3.4	0.5	1	0.5	0.5	0	1	5
$BC_2F_3$4.1/4.3.1	0.5	0	0.5	0.5	0.5	0.5	5

FHB resistant marker data for the FHB resistant lines tested during the second year (continued)

Line name	Barc133	Gwm533	Gwm293	Gwm304	Gwm156	Gwm133	Total number of FHB resistant markers*
$BC_2F_3$4.1/4.3.2	0.5	0.5	0.5	0.5	1	0.5	6
BC_2F_3 4.1/4.3.3	0.5	0.5	0.5	0.5	0.5	0.5	6
BC_2F_3 4.1/4.3.4	0.5	0	0.5	0	0	0.5	3
$BC_2F_3$4.1/5.3.1	0.5	0.5	0.5	0.5	0.5	0.5	6
$BC_2F_3$4.1/5.3.2	0.5	0.5	0	0	0	0.5	3
$BC_2F_3$4.1/5.3.3	0.5	0.5	0	0	0	0.5	3
$BC_2F_3$4.1/5.3.4	0.5	1	1	0.5	1	0.5	6
$BC_2F_3$4.1/5.3.5	0.5	1	0	0	0.5	0.5	4
$BC_2F_3$4.1/5.3.6	0	0	1	1	1	0.5	4
BC_2F_3P1 7.1/1.2.1	1	1	1	1	1	0.5	6
BC_2F_3P1 7.1/1.2.2	0.5	0.5	1	1	1	0.5	6
BC_2F_3P1 7.1/1.2.3	1	1	1	1	1	0.5	6
BC_2F_3P1 7.1/1.2.4	0.5	0.5	1	1	1	0.5	6
BC_2F_3P1 7.1/1.2.5	0	0	0.5	1	1	0.5	4
BC_2F_3P1 7.1/1.2.6	1	1	1	1	1	0.5	6
BC_2F_3P1 7.1/2.2.1	0	0	1	1	1	0	3
BC_2F_3P1 7.1/2.2.2	0	0	1	1	1	0	3
BC_2F_3P1 7.1/2.2.3	0	0	1	1	1	0	3
BC_2F_3P1 7.1/2.2.4	0	0	1	1	1	0	3
BC_2F_3P1 7.1/2.2.5	0	0	1	1	1	0	3
BC_2F_3P1 7.1/3.2.1	1	1	1	0.5	1	0	5
BC_2F_3P1 7.1/3.2.2	1	1	1	0.5	1	0	5
BC_2F_3P1 7.1/3.2.3	1	1	1	1	1	0	5
BC_2F_3P1 7.1/3.2.4	1	1	1	0.5	1	0	5
BC_2F_3P1 7.1/3.2.5	1	1	0.5	1	1	0	5
BC_2F_3P1 7.1/3.2.6	1	1	1	0.5	1	0	5
BC_2F_3P1 7.1/4.2.1	0	0	1	1	1	0	3
BC_2F_3P1 7.1/4.2.2	0	0	1	1	1	0	3
BC_2F_3P1 7.1/4.2.3	1	1	1	1	1	0	5
BC_2F_3P1 7.1/4.2.4	1	1	1	1	1	0.5	6
BC_2F_3P1 7.1/4.2.5	1	1	1	1	1	0.5	5
BC_2F_3P1 7.1/4.2.6	0.5	0.5	1	1	1	0	5
BC_2F_3P1 7.1/4.2.7	0.5	0.5	1	1	1	0	5
BC_2F_3P1 7.1/5.2.1	1	1	1	1	1	0	5
BC_2F_3P1 7.1/5.2.2	1	1	0.5	0.5	1	0	5

FHB resistant marker data for the FHB resistant lines tested during the second year (continued)

Line name	Barc133	Gwm533	Gwm293	Gwm304	Gwm156	Gwm133	Total number of FHB resistant markers*
BC$_2$F$_3$P1 7.1/5.2.3	1	1	1	1	1	0	5
BC$_2$F$_3$P1 7.1/5.2.4	1	1	1	0.5	1	0	5
BC$_2$F$_3$P1 7.3/4.3.1	0	0	0.5	0.5	0.5	0.5	4
BC$_2$F$_3$P1 7.3/4.3.2	0	0.5	1	0.5	0.5	0.5	5
BC$_2$F$_3$P1 7.3/4.3.3	0	0.5	0.5	0.5	0.5	0.5	5
BC$_2$F$_3$P1 7.3/4.3.4	1	1	0	0	0	0.5	3
BC$_2$F$_3$P1 7.3/4.3.5	0.5	0.5	0.5	0.5	0.5	0.5	6
BC$_2$F$_3$P1 7.3/4.3.6	0	0.5	0	0	0.5	0	2
BC$_2$F$_3$P1 7.3/4.3.7	0	0	1	0.5	1	0.5	4
BC$_2$F$_3$P1 7.3/5.3.1	0	0	1	0.5	1	0	3
BC$_2$F$_3$P1 7.3/5.3.2	1	1	0.5	0.5	0.5	0.5	6
BC$_2$F$_3$P1 7.3/5.3.3	0.5	0.5	0	0	0	0.5	3
BC$_2$F$_3$P1 7.3/5.3.4	0.5	0.5	0.5	0.5	0.5	0.5	6
BC$_2$F$_3$P1 7.3/5.3.5	0	0.5	0.5	0.5	0.5	0	4
BC$_2$F$_3$P2 2.3/1.1.1	0	0	0	0	0	0	0
BC$_2$F$_3$P2 2.3/1.1.2	0	0	1	0.5	1	0	3
BC$_2$F$_3$P2 2.3/1.1.3	0	0	0.5	0.5	1	0	3
BC$_2$F$_3$P2 2.3/1.1.4	0.5	0.5	0	0	0	0	2
BC$_2$F$_3$P2 2.3/4.1.1	1	1	0.5	0.5	0.5	0	5
BC$_2$F$_3$P2 2.3/4.1.2	0	0	0.5	0.5	0.5	0	3
BC$_2$F$_3$P2 2.3/4.1.3	0.5	0.5	0	0	0	0	2
BC$_2$F$_3$P2 2.3/4.1.4	0.5	0.5	1	1	1	0	5
BC$_2$F$_3$P2 3.2/4.2.1	0	0	1	1	0	1	3
BC$_2$F$_3$P2 3.2/4.2.2	0.5	0.5	0.5	0.5	0	1	5
BC$_2$F$_3$P2 3.2/4.2.3	0.5	0	1	1	0	1	4
BC$_2$F$_3$P2 3.2/4.2.4	0.5	1	1	1	0	1	5
BC$_2$F$_3$P2 3.2/4.2.5	0.5	0.5	0.5	0.5	0	1	5
BC$_2$F$_3$P2 3.2/4.2.6	0.5	0	1	0.5	0	1	5
BC$_2$F$_3$P2 3.2/5.3.1	1	1	0	0	0	0.5	3
BC$_2$F$_3$P2 3.2/5.3.2	0	0	1	1	0	0.5	3
BC$_2$F$_3$P2 3.2/5.3.3	1	1	0.5	1	0	0.5	5
BC$_2$F$_3$P2 3.2/5.3.4	0	0	0	0	0	0.5	1
BC$_2$F$_3$P2 3.2/5.3.5	1	1	0	0	0	0.5	3
BC$_2$F$_3$P2 12.1/1.1.1	0	0	1	1	1	0	3
BC$_2$F$_3$P2 12.1/1.1.2	0.5	0.5	1	1	1	0	5

FHB resistant marker data for the FHB resistant lines tested during the second year (continued)

Line name	Barc133	Gwm533	Gwm293	Gwm304	Gwm156	Gwm133	Total number of FHB resistant markers*
BC_2F_3P2 12.1/1.1.3	0.5	0.5	1	1	1	0	5
BC_2F_3P2 12.1/1.1.4	1	1	1	1	1	0	5
BC_2F_3P2 12.1/1.1.5	1	1	1	1	1	0	5
BC_2F_3P2 12.1/1.1.6	0.5	0.5	1	1	1	0	5
BC_2F_3P2 12.1/2.1.1	0.5	0	0.5	1	1	0	4
BC_2F_3P2 12.1/2.1.2	0.5	0.5	1	1	1	0	5
BC_2F_3P2 12.1/2.1.3	0.5	0.5	0.5	0.5	1	0	5
BC_2F_3P1 2.1/1.2.1	0	0	1	1	1	0	3
BC_2F_3P1 2.1/1.2.2	0.5	0.5	0.5	0.5	1	0	5
BC_2F_3P1 2.1/1.2.3	0.5	0	0.5	0.5	1	0	4
BC_2F_3P1 2.1/1.2.4	0.5	0.5	1	1	1	0	5
BC_2F_3P1 2.1/1.2.5	0	0.5	0.5	0.5	1	0	4
BC_2F_3P1 2.1/2.2.1	0.5	0.5	0.5	0.5	0.5	0	5
BC_2F_3P1 2.1/2.2.2	1	1	1	1	1	0	5
BC_2F_3P1 2.1/2.2.3	0.5	0.5	1	1	0.5	0	5
BC_2F_3P1 2.1/2.2.4	0	0	0.5	1	1	0	3
BC_2F_3P1 2.1/3.2.1	0	0	1	1	1	0	3
BC_2F_3P1 2.1/3.2.2	0	0	1	1	1	0	3
BC_2F_3P1 2.1/3.2.3	0	0	1	1	1	0	3
BC_2F_3P1 2.1/3.2.4	0	0	1	1	1	0	3

* - Represents total number of markers present irrespective if the marker was homo- or heterozygous; 1 - Homozygous resistant; 0.5 - Heterozygous; 0 - Homozygous susceptible