Development of wheat lines with complex resistance to rusts and Fusarium head blight

by

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Declaration

I, Ansori Maré, declare that the thesis that I herewith submit for the Doctoral Degree in Plant Breeding at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

I, Ansori Maré, hereby declare that I am aware that the copyright is vested in the University of the Free State.

_____ _____

Ansori Maré Date

Dedication

This PhD thesis is dedicated to my husband,

Frikkie Maré,

who taught me less than your best is not enough.

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List of abbreviations

2.4-D	2,4-Dichlorophenoxyacetic acid
$2DCF_1$	Second double cross first generation
α	Alpha
AAA	ATPases associated with diverse cellular activities
ABC	ATP-binding cassette
ADP	Adenosine diphosphate
AFLP	Amplified fragment length polymorphism
AKA	Acrylamide:bis-acrylmide
APR	Adult plant resistance
APS	Ammonium persulfate
ARC-SGI	Agricultural Research Council – Small Grain Institute
ASR	All stage resistance
ATP	Adenosine triphosphate
β	Beta
BC_1	Backcross one
BC_2	Backcross two
bp	Base pairs
CAPS	Cleaved amplified polymorphic sequences
CI	Coefficient of infection
CIMMYT	International Maize and Wheat Improvement Centre
Cq	Quantification cycle
CTAB	Hexadecyltrimethylammonium bromide
cv	Cultivar
DCF_1	Double cross first generation
DCF_2	Double cross second generation
ddH_2O	Doubled distilled water
DEPC	Diethyl pyrocarbonate
DH	Doubled haploid
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
dpi	Days post inoculation
EDTA	Ethylene-diaminetetraacetate

ELISA	Enzyme linked immunosorbent assay
EtBr	Ethidium bromide
F_1	First generation
FAO	Food and Agriculture Organization
FDK	Fusarium damaged kernels
FHB	Fusarium head blight
FMF_1	Rust and FHB resistant crosses first generation
FMF_2	Rust and FHB resistant crosses second generation
FMF_3	Rust and FHB resistant crosses third generation
f. sp.	formae specialis
GA_3	Gibberellic acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
gDNA	Genomic DNA
HG	Housekeeping gene
HMW-GS	High-molecular-weight glutenin subunits
hpi	Hours post inoculation
HTAP	High-temperature adult plant
IT	Infection type
kDa	Kilodalton
LC-MS/MS	Liquid chromatography-tandem mass spectrometric assay
LMW-GS	Low-molecular-weight glutenin subunits
Lr	Leaf rust resistance gene
MAS	Marker-assisted selection
MES	2-(N-morpholino) ethanesulfonic acid
MIQE	Minimum Information for publication of quantitative real-time PCR experiments
MS	Murashige and Skoog
NaCl	Sodium chloride
NIV	Nivalenol
PCR	Polymerase chain reaction
Pgt	*Puccinia graminis* f. sp. *tritici*
PPM	Plant preservative mixture
PSB	Potato sucrose broth
psi	Pounds per square inch
Pst	*Puccinia striiformis* f. sp. *tritici*
Pt	*Puccinia triticina*
qPCR	Quantitative-polymerase chain reaction

QTL	Quantitative trait loci
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
RNA	Ribonucleic acid
RSA	Republic of South Africa
rpm	Revolutions per minute
RT-PCR	Real-time polymerase chain reaction
®	Registered
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE-HPLC	Size exclusion high performance liquid chromatography
SNP	Single nucleotide polymorphisms
Sr	Stem rust resistance gene
SSR	Simple sequence repeat
STS	Sequence-tagged site
TAE	Tris/Acetate/EDTA
TBE	Tris-HCl/Borate/EDTA
TE	Tris-HCl/EDTA
TEF1	*Translation elongation factor 1 alpha-subunit*
TEF	*Translation elongation factor*
TEMED	N,N,N',N-Tetramethylethylenediamine
™	Trade mark
Tris-HCl	Tris(hydroxymethyl) aminomethane hydrochloride
UFS	University of the Free State
USA	United States of America
Yr	Stripe rust resistance gene

List of SI units

%	Percentage
°C	Degrees Celsius
cm	Centimetre(s)
g	Gram(s)
g	Gravity
h	Hour(s)
l	Litre(s)
M	Molar(s)
mg	Milligram(s)
min	Minutes
ml	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar(s)
ng	Nanogram(s)
pH	Power of hydrogen
pmol	Picomole
r/s	Revolutions per second
sec	Second(s)
µl	Microlitre(s)
µm	Micrometre(s)
V	Volt(s)
v/v	Volume per volume
w/v	Weight per volume

Abstract

Wheat is the second most important food crop worldwide. The world human population is increasing daily. Sustainable food production is necessary to ensure sufficient future food supply. Wheat production is negatively affected by various pathogens that challenge breeders to develop more disease resistant wheat cultivars. Leaf-, stem- and stripe rust as well as Fusarium head blight (FHB) are considered some of the most destructive fungal pathogens of wheat. Resistance breeding is considered the most economic and eco-friendly solution to control wheat diseases in the long-term. Combining of several effective resistance genes/quantitative trait loci (QTL) into a single wheat line is necessary to increase the probability of obtaining durable resistance. Molecular markers linked to resistance genes/QTL can assist breeders in selecting the best offspring throughout their breeding scheme.

The main aim of this study was to develop a wheat line with combined resistance to the rusts and FHB. Both wheat rust and FHB resistant lines used in this study were developed in previous studies. The best resistant lines developed in these studies were identified using marker-assisted selection (MAS) and were then used in a breeding scheme to combine wheat rust and FHB resistance genes/QTL. Offspring from crosses was evaluated for the presence of six rust resistance genes/QTL: *Lr19, Lr34/Yr18/Sr57, Sr2/Yr30, Sr26, Sr39* and *QYr.sgi.2B-1*, as well as for three FHB resistance genes/QTL: *Fhb1*, *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2*. The best identified lines obtained from a series of crosses were selected to develop two double-cross populations as well as self-pollinated populations. Selected pre-breeding lines were also used to optimise and apply the doubled haploid (DH) technique to develop lines with fixed genotypes. Molecular markers linked to the high-molecular-weight glutenin subunits (HMW-GS) and the 1BL.1RS translocation were used for screening the final populations developed because the primary focus for selection was rust and FHB resistance genes/QTL and not HMW-GS and the 1BL.1RS translocation.

Validation of the phenotypic expression of the rust and FHB resistance genes/QTL was done through greenhouse inoculation of the parental, control and experimental lines/cultivars containing different resistance gene/QTL combinations. Seedling and adult plants were inoculated with the three rusts respectively, firstly for the timeline study to determine the optimal day's post inoculation (dpi) to collect sample material for quantitative-polymerase chain reaction (qPCR) analysis and secondly to determine fungal gene expression in the experimental lines. Both point and spray inoculations for

FHB were used and disease development was evaluated at 4, 7, 10, 14, 18 and 21 dpi. Sample collection for qPCR analysis was done at 21 dpi. Good correlations between the data obtained from the phenotypic evaluations, qPCR and MAS were obtained for both rust and FHB resistance evaluations. Therefore, markers linked to rust and FHB resistance genes were phenotypically expressed.

Lines with combined wheat rust and FHB resistance were obtained from the self-pollinated populations but are still segregating for some of the genes/QTL. Lines with combined rust and/or FHB resistance genes/QTL were also developed through the DH technique. No markers linked to stripe rust resistance were used in this study except for genes with multiple disease resistance such as *Lr34/Yr18/Sr57* and *Sr2/Yr30*. Additional stripe rust resistance genes are however present in the genetic background of the experimental lines as they were present in the parental cultivars/lines originally used to develop these lines like Kariega, Avocet*YrSP*, 2S#/163 and CM-82036. Valuable pre-breeding wheat lines with combined rust and FHB resistance have been developed which can be used in future disease resistance breeding programmes.

Key words: Disease resistance breeding, doubled haploids, Fusarium head blight, leaf-, stem- and stripe rust, marker-assisted selection, phenotypic evaluation, quantitative-polymerase chain reaction, *Triticum aestivum*/hexaploid wheat

Chapter 1

General introduction

Wheat is an economically important crop and is the main ingredient for food types such as bread, pasta and biscuit products frequently used in many households around the world. Sustainability and increased wheat production is necessary to keep up with the annual growth in the human population and the corresponding increase in demand. Figure 1.1 indicates the worldwide production and utilisation of wheat for the last 10 years (FAO 2016). For most years wheat production was higher than utilisation. The question remains: Will wheat production continue to increase and keep up with the growing human population in future? Wheat breeders are continually challenged to improve characteristics of this widely planted and utilised crop species.

Wheat is the second most important cereal crop produced in South Africa. South Africa is not self-sufficient and need to import wheat to supplement domestic production. An average of 1.85 million tonnes of wheat were produced by the South African farming industry for the last ten years (Figure 1.1; Grain SA 2017). Stress related factors such as wheat diseases, drought and increasing winter temperatures can cause severe losses in wheat production and negatively affect the industry and economy. The negative impact on the wheat industry directly affect consumers as wheat products are consumed on a daily basis in many South African households.

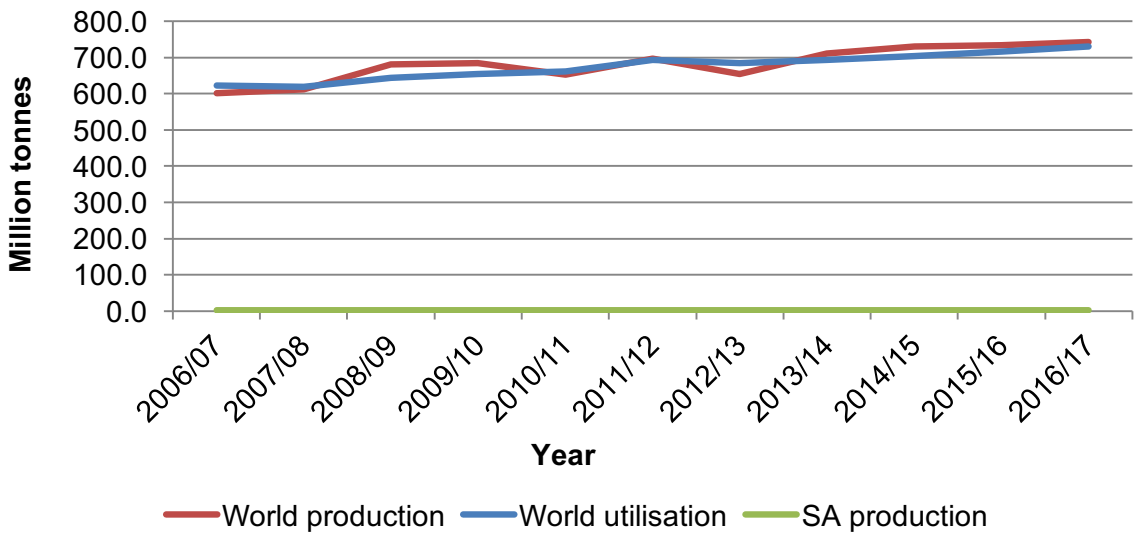


Figure 1.1 World wheat production and utilisation compared to South African (SA) wheat production (FAO 2016; Grain SA 2017).

Wheat diseases, including existing pathogens, the development of new more virulent pathotypes as well as newly introduced diseases, continue to threat wheat production and therefore plant breeders and researchers in other disciplines need to continuously improve wheat cultivars to overcome this challenge. The major fungal diseases of wheat include FHB and the three wheat rusts (leaf-, stem- and stripe rust). Fusarium head blight infections, mainly caused by *Fusarium graminearum* Schwabe [anamorph *Gibberella zeae* Schwein (Petch)], lead to decreased yield, shrivelled seed and the accumulation of mycotoxins (secondary metabolites). High levels of mycotoxins can be harmful to humans and animals when infected wheat products are consumed (Jansen et al. 2005). Rust infections are caused by different *Puccinia* species and have a negative influence on yield (McIntosh et al. 1995). Fusarium head blight and rust disease outbreaks need to be limited in order to maintain optimal wheat production and produce mycotoxin free wheat.

One way of limiting rust and FHB disease outbreaks is to breed wheat cultivars with durable disease resistance through combining different resistance genes (Joshi and Nayak 2010). Although many disease resistance genes/QTL have been discovered and deployed over the past years, some resistance genes/QTL were overcome by these fungi e.g. stem rust race 'Ug99' (Pretorius et al. 2000). Fungicides can be used to control rust and FHB pathogens but this increases input costs and has a negative impact on the environment (Chen 2014). Research on biofungicides to control diseases is in progress but a limited number of products have been released on the market (Palazzini et al. 2016).

A number of effective rust resistance genes against the different wheat rust pathogens have been identified. Rust resistance genes/QTL can act as all stage resistance genes (ASR; normally race-specific) or adult plant resistance genes (APR, often more durable; Collard and Mackill 2008). One of the strategies to achieve durable resistance includes combining of APR and ASR genes. Combining of resistance genes will decrease the risk of severe epidemics as genes can support one another, should one of the genes be overcome by the pathogen (Mundt 2014).

Major and minor FHB resistance genes/QTL have been identified in different wheat cultivars and was used in several FHB resistance breeding efforts (Buerstmayr et al. 2013). Five different FHB resistance types have been identified over the years and different FHB resistance genes/QTL are linked to different FHB resistance types. FHB resistance genes/QTL are inherited quantitatively and need to be combined to increase resistance against FHB infections (Miedaner et al. 2006).

Molecular markers are available for most of the identified rust and FHB resistance genes/QTL and can be used to keep track of the corresponding genes/QTL and enable breeders to make better selections based on the gene of interest (Hou et al. 2015). Phenotypic evaluation can be used in cases where molecular markers have not been developed yet or when it is easier and less costly compared to molecular markers. It can also assist breeders to validate molecular marker data under greenhouse or field conditions, as some traits are greatly influenced by environmental conditions (Cobb et al. 2013).

Combining disease resistance genes/QTL can have a negative impact on yield as some resistance genes/QTL have yield penalties. The *Lr34/Yr18/Sr57* gene showed a 9% yield penalty under some environments (Singh and Huerta-Espino 1997). Cultivars with durable rust resistance need to compete with high yielding cultivars as yield is considered the most important trait by many farmers. Release of disease resistant cultivars will thus only be successful if they can compete with other commercially available high yielding cultivars. The second trait that wheat lines with improved disease resistance need to compete with is acceptable milling and baking characteristics, as determined by the milling and baking industry, before they can be considered for release as a cultivar. Yield, milling and baking quality and disease resistance need to be in balance to satisfy different groups involved in the food chain, from the farmer to the consumer (Patil et al. 2014).

Currently in South Africa most wheat cultivars do have resistance to the dominating pathotypes of at least one or two of the rust pathogens. In contradiction only a limited number of cultivars show FHB tolerance with the source of tolerance in these cultivars being uncharacterised. No cultivars adapted to South African growing conditions have been released with combined durable rust and FHB resistance and this need to be addressed.

The main aim of this study was to combine durable rust and FHB resistance into a single wheat line. This was achieved by setting several objectives:

(i) To evaluate offspring lines for the presence of molecular markers known to be linked to different rust and FHB resistance genes/QTL.

(ii) To use molecular marker data linked to rust and FHB resistance genes/QTL as a selection tool before making crosses to combine rust- and FHB resistance genes into a single wheat line.

(iii) To phenotypically validate molecular marker data linked to resistance genes/QTL by inoculating different wheat lines with the wheat rust- or FHB pathogens.

(iv) To standardise and apply a qPCR method to various lines with different rust- or FHB resistance gene combinations, after inoculation with the three wheat rusts and FHB, respectively.

(v) To develop DH elite lines containing both FHB and/or rust resistance genes/QTL to ensure that lines are homozygous.

(vi) To evaluate elite lines with molecular markers linked to HMW-GS and the 1BL.1RS translocation.

(vii) Finally, to identify the best breeding lines based on the number of resistance genes/QTL linked to rusts and FHB together with their HMW-GS genotypes. The identified lines will be distributed to wheat breeding companies or used in a backcross breeding programme to further improve their agronomic characteristics in line with South African requirements.

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Chapter 2

Literature review: Rust and Fusarium head blight resistance wheat breeding

2.1 Introduction

The value of bread wheat (*Triticum aestivum* L.) is attributed to the wide range of products it offers the consumer. Wheat is one of the top three cereal crops globally (maize and rice) and serves as staple food for some parts of the world (Khan and Iqbal 2011). With the human population increasing annually, higher wheat production is a main priority worldwide to ensure food security. Wheat seed is a good carbohydrate source and also contains proteins and lipids and contributes to the fibre content of the human diet. The endosperm of matured seed contains proteins and gives wheat its unique bread-making ability. The value of wheat relies on high yield (for the farmer) and good bread-making quality (millers and bakers as well as the consumer). A great need exists to breed wheat cultivars with suitable characteristics for farmers, millers and bakers and finally consumers (Nestel et al. 2006).

Wheat is challenged by various biotic and abiotic stresses that lead to the development of and research on new wheat cultivars. Wheat grows under various climatic and agronomical conditions, but new traits need to be incorporated to ensure that better adapted and improved cultivars are developed. New environments can physically challenge plant growth and have a negative effect on development and wheat maturity. Yield and dough quality are the two major agronomically- and economically important traits of bread wheat. These traits can be influenced by fungal-, bacterial- and viral diseases, insects, nematodes, physiologic and genetic disorders as well as mineral and environmental stresses (Duveiller et al. 2012). The availability of genetic diversity in wheat as well as related plant species and ancestral plants helped breeders to overcome these biotic stresses (Van den Wouw et al. 2010; Buchanan-Wollaston et al. 2017).

2.2 Wheat taxonomy and classification

Wheat is part of the Poaceae (grass) family and consists of different species within this family. Domesticated and wild type species share the same genus name, *Triticum*. Wheat species are diploid (einkorn; 2n=2x=14), tetraploid (durum wheat; 2n=4x=28) or hexaploid (bread wheat; 2n=6x=42). All hexaploid wheat developed through domestication with *Aegilops tauschii* (Coss.) Schmal. (D genome donor), contributing characteristics such as round glumes and cold hardiness (Peng et al. 2011).

Different wheat species are responsible for different end-use products. Durum wheat is used for the production of semolina flour and pasta. Bread wheat can be categorised based on different characteristics. The first classification of bread wheat cultivars is based on the planting season, namely winter or spring wheat. Another classification is based on the gluten content, namely hard wheat (high protein content) or soft wheat (high starch content). Bread wheat can also be classified based on grain colour that differs between red, white or amber. Hard red winter or spring wheat cultivars are mainly used for bread products. Hard white cultivars are used for bread baking and brewing, while soft wheat cultivars are used for cakes, biscuits, pastries, muffins and piecrusts. Hard wheat contains less intact starch fibres compared to soft wheat because additional milling of hard wheat breaks the starch fibres. Grain texture is an important quality characteristic and influences the baking and milling characteristic parameters (Rogers et al. 1993).

2.3 Wheat history and domestication

Plant domestication, especially of food related plants, is an old and frequently used method to improve wild species to obtain higher yields and improved quality. Plant domestication is the process where wild plants evolved into crop plants through artificial selection (Meyer and Puruggan 2013). This usually includes an early hybridisation event followed by selective breeding. Only specific traits are improved during plant domestication, known as the domestication syndrome. Traits include larger fruit or grain size, more robust plants with higher apical dominance, loss of natural seed dispersal, decrease in bitterness of edible parts, synchronisation of flowering time and changes in photoperiod sensitivity (Doebley et al. 2006).

Bread wheat domestication occurred approximately 10 000 years ago and resulted in a change from nomadic and hunter-gather lifestyles to more established agricultural societies, called the Neolithic Revolution. The first cultivated wheat forms were the diploid einkorn wheat (*T. monococcum* L.; AA genome) and the tetraploid emmer wheat (AABB genome). Wheat cultivation started in the southern-eastern parts of Turkey and reached the Near East approximately 9 000 years ago and gave rise to hexaploid bread wheat (AABBDD). Hexaploid bread wheat has evolved from tetraploid emmer wheat (AABB genome) and *A. tauschii*. The two most important wheat domestication traits were increased grain size and non-shattering of seeds to allow humans to harvest seed (Eckardt 2010). Norman Borlaug, the father of the Green Revolution, identified the semi-dwarf genes of wheat (*Rht1* and *Rht2*) that provide better grain production as stems were stronger, leading to better water and fertiliser absorption (Hedden 2003).

Although cultivation made bread wheat a better adapted crop to various environmental conditions and soil types, domestication and cultivation made cultivars human-dependant and unable to outgrow adapted wild species. Wheat cultivation led to a genetic bottleneck and made cultivated wheat more vulnerable to diseases and pests (Mondal et al. 2016). Plant breeders now need to continuously search for new genes to overcome diseases. New genetic material can be obtained from wheat progenitors, related wheat species and mutations (Van den Wouw et al. 2010; Kole 2011; Wulff and Moscou 2014).

2.4 Plant breeding

The aim of plant breeding is to develop parental lines with favourable genetic variation needed for a specific breeding programme (Shimelis and Liang 2012). These parental lines, together with various breeding techniques, are used to improve crops' traits. Pre-breeding and applied breeding are two distinct breeding phases that are essential for cultivar development. Pre-breeding is the process where new parental lines are developed, while applied breeding focuses on development and release of new cultivars (Collard and Mackill 2008).

Plant breeding was the starting point of crop species domestication to improve agriculturally important traits (Jiang 2013). Plant breeding is a co-evolutionary process between people and plants. Plant breeding focuses on the selection of better traits among variants, e.g. yield, quality, resistance and tolerance to stresses. Plant breeding can be divided into four main criteria: (i) plant breeding based on observing variant selection, disregarding their origin, (ii) generation and selection of expanded variation by controlled mating, (iii) monitoring the inheritance of within-genome variation and selection of recombinants and (iv) the creation and introduction of novel variation into the genome through genetic engineering (Breseghello 2013).

The conventional breeding technique is the direct origin of plant breeding. Conventional breeding relies on the phenotype of plants and visual assessment using different measuring techniques for different traits of selection (Milc et al. 2011). Conventional breeding is an efficient method that provides improved cultivars. One example of successful conventional breeding was the discovery of the semi-dwarf genes in wheat with higher yield combined with non-lodging (Hedden 2003). Conventional breeding relies on the diversity of landraces or closely related species and Mendelian- and quantitative genetic approaches for phenotypic selection (Prohens 2011).

Despite the success of conventional breeding, limitations are present. The phenotype is the direct expression of the genotype within a specific environment. Traits linked to polygenic inheritance and high genotype x environment interaction make it difficult to select plants with the best breeding value (Prohens 2011). To overcome this problem, more environments need to be tested which are difficult as it leads to labour, time and cost increases (Moose and Mumm 2008). Quantitative complex traits contain additional alleles with small effects and can be masked and become unnoticed (Morgante and Salamini 2003). Plants with small effective additional alleles may be discarded and will be lost in the gene pool, leading to a decrease in genetic diversity (Ragimekula et al. 2013).

Although phenotypic evaluations have limitations and molecular breeding was introduced to breeders, phenotypic evaluation methods cannot be totally eliminated from breeding programmes as it is still considered useful in many breeding programmes worldwide. Every individual breeding programme needs to be optimised in terms of cost, time and resource availability (Peng et al. 2014). In a study done by Agostinelli et al. (2012), results showed a similar decrease in Fusarium damaged kernels (FDK) and deoxynivalenol (DON) using phenotypic selection compared to genotypic selection. Some important traits are still phenotypically evaluated, as molecular markers are not yet developed or it is cheaper and easier to do phenotypical evaluations. Phenotypic evaluation is also combined with molecular breeding programmes for greenhouse and field evaluation for confirmation of traits linked to molecular markers (Mulualem and Bekeko 2016).

A conventional wheat breeding programme (self-fertilising crop) can take a minimum of nine years or typically 12 years to release a new cultivar (Campbell et al. 2000; Shimelis and Liang 2012; Figure 2.1). The time interval needed to release a new cultivar can be shortened by developing DH lines and/or using MAS. Therefore, breeders need to strategise and manage their breeding programmes because of the prolonged process.

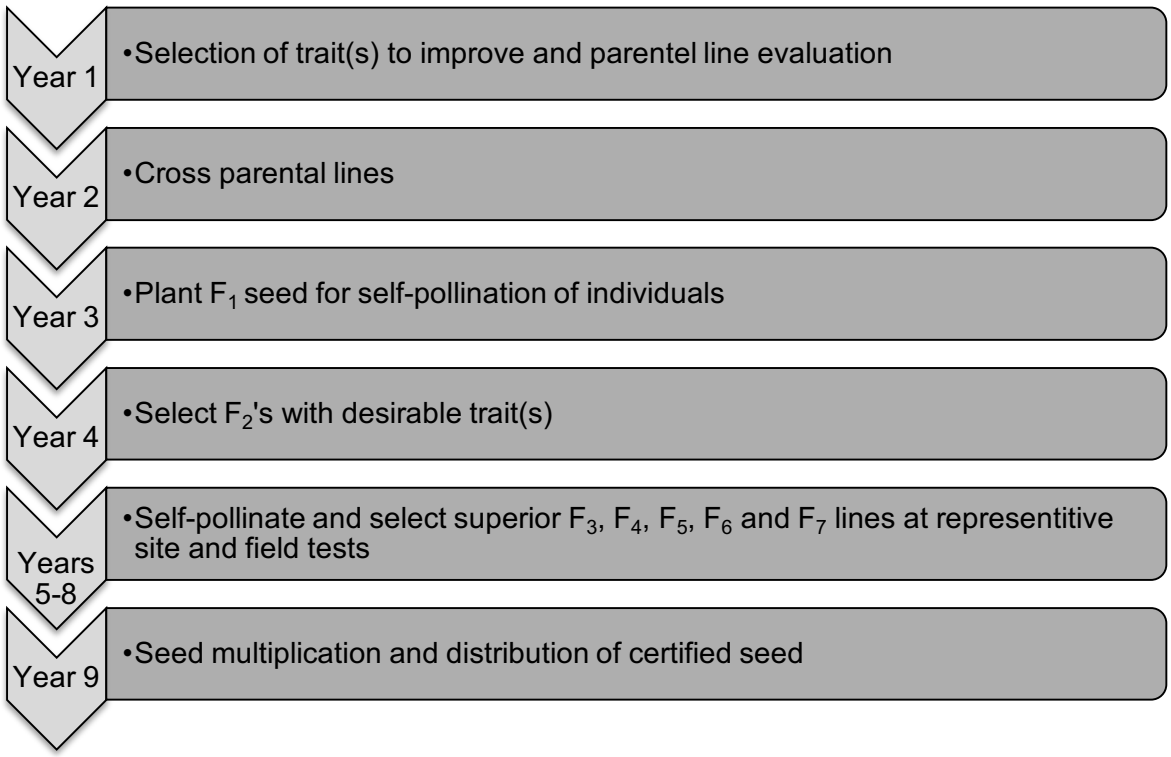


Figure 2.1 Schematic illustration of a breeding programme for the development of a new wheat cultivar (Shimelis and Liang 2012).

2.5 Doubled haploids

The DH breeding method has the ability to accelerate breeding programmes and release new cultivars faster (Guzy-Wróbelska and Szarejko 2003). The DH method is an *in vitro* breeding method with various advantages compared to other breeding methods. Firstly, homozygosity is reached in the first generation after crossing the selected parents whereas other breeding programmes require six or more self-pollinated generations to obtain homozygosity. Secondly, all genes of DH lines are fixed in a homozygous state which prevent loss of genes due to a heterozygous genetic background or unpredictable selection due to segregation. Thirdly, the DH method preserves valuable genetic variation better compared to conventional methods. Segregating populations that are planted in a single environment mask certain genotypes and lead to the disposal of plants containing valuable genes (Choo et al. 1985).

Haploid plants are a natural occurrence and Blakeslee identified the first natural haploids of *Datura stramonium* L. (Jimson weed) in 1921. Following this event, various scientists identified other haploid plant species. The value of haploid plants was known but the number and availability of naturally produced haploid plants were not sufficient for use in

plant breeding programmes. Many attempts were made to produce haploid plants, but in 1964 the group of Professor SC Meheshwari of Delhi University in India released the first protocol for haploid production of *D. innoxia* Mill. using the anther culture technique (Hu 1997).

Haploid plants are called sporophytes and consist of the gametic chromosome number (*n* instead of *2n*). The chromosome doubling process of haploid plants result in DH plants (Germanà 2011). Various crop species have been improved through DH breeding programmes such as oats (*Avnea sativa* L.; Ferrie et al. 2014), Chinese cabbage (*Brassica rapa* L.; Lee et al. 2014), cauliflower (*B. oleracea* L.; Gu et al. 2014), maize (*Zea mays* L.; Lübberstedt and Frei 2012) and barley (*Hordeum vulgare* L.; McGrann et al. 2014). Wheat is a crop species frequently used in DH breeding programmes. Haploid wheat plants are produced either by anther culture (microspore culture), egg cell culture (megaspore culture) or interspecific crosses (intergeneric crosses; Wessels and Botes 2014).

The interspecific cross technique is considered more successful in producing haploid plants than the anther or egg cell culture techniques (Khan et al. 2012). The two main problems with anther and cell cultures are the production of albino plants and the success of DH is genotypic dependant (Hassain et al. 2012). Although the anther culture technique is not the most favourable technique for DH production in wheat breeding programmes, new wheat cultivars were released using this method, for example a wheat variety Florin, in 1978 (De Buyser et al. 1987).

Various plant species are used to develop haploid wheat plants through the interspecific cross technique. In 1970 reports showed that bulbous barley was successfully used in the development of DH barley plants (Kasha and Kao 1970). In 1975 pollen of bulbous barley (*H. bulbosum* L.) was used to develop DH bread wheat plants (Barclay 1975) but could not be successfully used for durum wheat (O'Donoughue and Bennett 1994). In 1984, haploid wheat embryos were produced for the first time using interspecific crosses between hexaploid wheat and maize (Zenkteler and Nitzsche 1984). Maize and wheat interspecific crosses are a popular and superior technique to induce wheat haploid embryos and are widely used in various breeding programmes (Laurie and Bennett 1986; Campbell et al. 2000; Wessels and Botes 2014). Other plants used to induce wheat haploid embryo's are pearl millet [*Pennisetum glaucum* (L.) R. Br.; Laurie 1989; Ahmad and Comeau 1990], eastern gamagrass [*Tripsacum dactyloides* (L.) L.; Riera-Lizarazu and Mujeeb-Kazi 1993; Li et al. 1996], teosinte (*Z. mays* spp. *mexicana*; Ushiyama et al. 1991; Suenaga et al. 1998), Job's tears (*Coi lachrymal-jobi* L.; Mochida

and Tsujimoto 2001) and sorghum (*Sorghum bicolor* (L.) Moench; Inagaki and Mujeeb-Kazi 1995; Laurie and Bennett 1988).

During interspecific crosses, two different parental genomes are combined within the same nucleus and hybrid embryos develop (Khan et al. 2012). Parental genomes combine after successful fertilisation and deoxyribonucleic acid (DNA) elimination start in the early allopolyploidisation stages. Two different elimination types occur during interspecific crosses. The first type is partial somatic chromosome elimination from one parental species. The second type is complete uniparental chromosome elimination and occurs between closely and remotely related species (Gernand et al. 2005). Complete uniparental chromosome elimination occurs between wheat and maize crosses and is successfully used to develop homozygous DH wheat plants.

After successful interspecific crosses and chromosome elimination of the pollen donor plant, haploid embryos develop from the female plant. These haploid embryos are placed on a rescue medium [Murashige and Skoog (MS) medium with sucrose; Murashige and Skoog 1962] to germinate haploid embryos. Grown haploid plants from the germinated embryos are treated with a chromosome doubling agent to produce homozygous plants. Chromosome doubling agents include caffeine (Thomas et al. 1997), nitrous oxide (Hansen et al. 1988), antimicrotubule herbicides trifluralin, amiprophos-methyl (Hansen and Andersen 1998) or most commonly colchicine (Jensen 1974). These chromosome doubling chemical agents prevent spindle fibres to develop and disrupt polar chromosomal migration and therefore the disrupted mitosis process results in chromosome doubling (Jensen 1974). One problem with the DH technique is that it produces a low number of haploid embryos and therefore a high number of interspecific crosses need to be done to obtain a sufficient number of embryos. Only a third of the developed seed after interspecific crosses develop into embryos (Bains et al. 1998). Disadvantages of the interspecific cross method to obtain DH plants include being time consuming because of the cultivation of the pollen donor parent to ensure pollen availability and the technique requires a lot of space, technical skill and is time consuming (Alikina et al. 2016).

2.6 Application of marker-assisted selection

Molecular markers linked to the traits of interest speed up breeding processes and ensure incorporation of intended gene combinations and assist plant breeders in developing new and enhanced crop species in terms of quality and quantity (Lagudah 2011). The three biggest challenges in recent years for breeders are food security,

climate change and drought stress (Ronald 2014). Marker-assisted selection is a modern genetic technology that has developed over the past 20 years. Other modern genetic technologies used for crop development include genetic engineering and other techniques used to induce mutations and genetic editing (Nogué et al. 2016).

Plant breeders work with biotic and abiotic challenges on a daily basis and this leads to new technology development to enhance cultivar and crop development and improvement. Molecular markers are an independent and reliable evaluation method and shorten breeding periods of new crop species (Wessels and Botes 2014; Shah et al. 2017). Marker-assisted backcrossing is widely applied to transfer one or more genes into an elite or well-adapted line. Marker-assisted pyramiding combines several genes into one genotype using different parental lines. Marker-assisted selection is an early generation application where undesirable gene combinations are eliminated at an early stage of the breeding programme. Molecular markers and phenotypic evaluation in breeding programmes can be used to evaluate additional unidentified QTL that control a specific trait (Collard and Mackill 2008).

2.7 Real time-polymerase chain reaction

Real-time polymerase chain reaction (RT-PCR) also known as qPCR is based on the PCR method developed by Kary Mullis in the 1980's. The RT-PCR is a technically improved version of the PCR method because it quantifies small amounts of specific nucleic acid sequences. Real-time-PCR can be applied to measure and assess changes in gene expression related to biological processes (Valasek and Repa 2005).

Real-time-PCR measures the fluorescence during the reaction. The detected amount of fluorescence is proportional to the amount of PCR product (Jensen 2012). Four main types of qPCR assays were developed. The first type does not need additional fluorescent-labelling of oligonucleotides and is based on the interaction between double-stranded DNA and binding dyes. This assay amplifies specific and non-specific DNA and therefore optimisation of primers are important (Klein 2002). The other three qPCR types make use of additional fluorescent-labelling of oligonucleotides. In the second type of qPCR fluorescence is produced during the cleavage of hydrolysis probes (Heid et al. 1996). The third type emits fluorescence during hybridisation of one molecular beacon. Molecular beacons acts as probes that bind to specific nucleic acid sequence and once it binds to the specific target sequences it will undergo spontaneous fluorogenic conformational change and elicit a response (Tyagi and Kramer 1996). The fourth type of qPCR emits fluorescence during hybridisation of two hybridisation probes (Wittwer et al.

1997). Other qPCR technologies besides the four main types have been developed such as scorpion primers with an attached fluorescence labelled tail (Thelwell et al. 2000). Two new fluorescence reagents have been developed for quantifying PCR products. The first reagent is SYBR®Green which is a binding dye and detects PCR products as it accumulates during PCR (Pandey et al. 2011). The second reagent is TaqMan® which uses fluorogenic probes and quantifies the target gene as it accumulates during PCR (Nagy et al. 2017).

Analysis of qPCR results is a valuable breeding tool and assists plant breeders to understand host-pathogen interactions. Quantitative-PCR is applied in resistance breeding to detect primary inoculum, evaluate the general infection levels, for quantitative disease assessment and to determine fungicide efficiency (Sameh et al. 2011). Quantitative-PCR is a sensitive technique and needs to be standardised before analysis can be done. Reference genes are used to normalise qPCR data to reduce gene quantification errors. Ideal reference genes show stable expression under various environmental conditions and in different plant tissues. Therefore, housekeeping genes (HGs) are most likely selected because they are involved in basic cellular processes of organisms (Thellin et al. 1999). The traditional HGs used for qPCR studies of wheat are alpha (α)-tubulin, beta (β)-tubulin, Ubiquitin, Actin, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ribosomal protein and histone, but more stable HGs have lately been discovered (cell division control protein, ATPases (adenosine triphosphate) associated with diverse cellular activities (AAA) superfamily of ATPases, adenosine diphosphate (ADP)-ribosylation factor and RNase L inhibitor-like protein) and should be considered for use in future studies (Poalocci et al. 2009).

High sensitivity and reproducibility of qPCR analysis make it a favourable technique to quantify gene expression (Scholtz and Visser 2013). Gene expression studies are relevant to a number of research divisions including life science, agriculture and medicine (Bustin et al. 2009). In the past, no rules and requirements were set for qPCR analyses, leading to studies with unreliable and irreproducible results. Therefore, Bustin and co-workers (2009) determined the minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines that explain the minimum information needed for evaluating qPCR studies to improve the consistency of results between laboratories.

2.8 Wheat disease resistance breeding

Although many disease resistance genes/QTL have been discovered and deployed over the past years, some of these resistant genes/QTL were overcome by pathogens. One way of limiting rust and FHB disease outbreaks is to breed wheat cultivars with durable disease resistance. Durable disease resistance can limit disease epidemics as resistance remains effective while the cultivar possessing durable resistance is widely cultivated (Johnson 1978). Qualitative and quantitative resistance are two defined resistance mechanisms and both are present in wheat.

Qualitative resistance refers to a single gene that acts in a gene-for-gene relationship (Flor 1942). The host plant contains effective ASR genes that lead to a hypersensitive response with initial infection by pathogens. A hypersensitive response of the host plant leads to cell death and prevents further spread of the pathogen (Kolmer 2005). This gene-for-gene relationship between host and pathogen can easily be overcome by a single mutation in the pathogen, leading to ineffective ASR genes (Kolmer et al. 2013). Recent examples are the stem rust 'Ug99' races TTKST and TTTSK that overcame resistance towards the stem rust resistance genes *Sr24* and *Sr36,* respectively (Jin et al. 2009). Host plants obtain better resistance when effective ASR genes are combined within a single plant. Wheat lines with combined ASR genes need to be well managed as stepwise breakdown of these genes can occur and lead to major epidemics (Priyamvada et al. 2011).

Non-specific APR is a quantitative resistance mechanism. The APR mechanism consists of multiple genes and reduces disease severity (Rutkoski et al. 2011). Adult plant resistance genes do not give hypersensitive response reactions and are race non-specific. Adult plant resistance genes are quantitatively inherited and depend on non-genetic effects such as host, growth stage and the environment (Geiger and Heun 1989). The additive effect of multiple genes results in durable resistance (Singh et al. 2000). Genotypic screening for APR genes are difficult using MAS due to multiple gene effects where most minor genes are overshadowed by major genes (Lee 1995). Phenotypic evaluation of APR genes is also difficult because infection is masked by the presence of ASR genes (Rutkoski et al. 2011). Race non-specific genes/QTL can be used as fundamental genes in a pre-breeding programme together with specific major and minor seedling genes.

2.9 Wheat diseases

Wheat diseases are caused by fungal, viral and bacterial infections as well as insects and pests. Wheat diseases can be prevented or managed to some extend with the use of chemical pesticides or fungicides but an ideal solution is to breed resistant cultivars (Gilligan 2008). There is a continuous challenge with releasing resistant wheat cultivars as pathogens overcome resistance because they are ever-evolving. Development and release of new cultivars can take up to ten years should resistance genes be available. New cultivar development is an on-going challenge for plant breeders. Disease outbreaks can result in yield losses and lead to a negative effect on the economy and food production worldwide (Tilman et al. 2002). The economically most important wheat diseases include FHB, wheat rusts, leaf spotting diseases and wheat curl mite (Graf et al. 2015).

2.9.1 Wheat rust diseases

Infections of the three wheat rusts lead to the reduction of yield and quality of grain. Epidemics of all three rust types were reported over the past years with tremendous economic impacts. Stem rust is the most destructive rust type because it infects all the above ground parts of the wheat plant. Yield losses of 100% were reported due to stem rust infections (Leonard and Szabo 2005). Leaf rust is the most widely spread rust type over the world and primarily infects leaf blades. Depending on the development stage of the infected wheat plant leaf rust infections can result in 7-30% yield losses (Roelfs et al. 1992; Huerta-Espino et al. 2011). Although leaf rust is less destructive than stem rust, leaf rust outbreaks happen more frequently and therefore result in a greater total loss compared to the other rust types (Huerta-Espino et al. 2011). Stripe rust infections flourish under lower temperatures and the availability of susceptible wheat cultivars. Stripe rust epidemics showed yield penalties of 10-70% but yield penalties up to 100% were reported on highly susceptible cultivars (Chen 2005).

All three rust types have the potential to cause severe yield losses in South Africa as the differentiation in temperatures for the different wheat production areas do favour their development during at least some stage of crop development. Stripe rust outbreaks occur most of the time on winter wheat in the Free State province, due to favourable lower temperatures during vegetative growth stages which can proceed up to flowering. Leaf- and stem rust incidence are more prominent on spring wheat in the Western Cape province although sporadic outbreaks have been recorded on winter wheat in the Free State province (Tolmay et al. 2016).

Wheat rusts are fungal wheat diseases and act as biotic stressors to wheat plants. These biotic stresses limit yield production and negatively affect grain quality (Gowda et al. 2014). Wheat rust diseases are caused by different species of the obligate biotrophic fungus *Puccinia*. *Puccinia* is a genera in the Pucciniaceae family and more than 3 000 described species exist. Three wheat rust types exist: leaf-, stem- and stripe rust. These rusts are heteroecious and need a telial/uredinal (primary) and a pycnial/aecial host (secondary) to complete their lifecycles. The lifecycle of wheat rust fungi consists of an asexual and a sexual (secondary host is needed) cycle. Rust fungi need a living host for growth and reproduction but in the absence of a host fungi survive as spores. The three wheat rusts are macrocyclic and follow all five spore stages to complete their lifecycle (Hiratsuka and Sato 1982; Knott 1989). The five spore stages include basidiospores, pycniospores, aeciospores, urediniospores and teliospores.

2.9.2 General life cycle of *Puccinia* species

Life cycles of the three wheat rusts consist of the same basic principles but some minor differences exist, for example variation of the secondary hosts and temperatures. The uredinial stage is the asexual cycle and urediniospores keep developing for as long as the climatic conditions are favourable. Teliospore (diploid) production is the beginning of the sexual stage as the primary host matures. Teliospore germination gives rise to four haploid basidiospores, two + and two - mating types. Basidiospores infect the secondary host and produce haploid pycnia. Mating between a pycniospore and a flexuos hypha of the opposite mating type results in fertilisation and gives rise to a dikaryotic aecium structure that releases dikaryotic aeciospores and again infect the primary host to complete the lifecycle (Roelfs 1985; Leonard and Szabo 2005).

2.9.3 Leaf rust

Leaf rust, also known as brown rust, is caused by *Puccinia triticina* Erikss. (*Pt*) [earlier described as *P. recondita* Rob. ex. Desm. f. sp. *tritici* (Erikss. and E. Henn.; D.M. Henderson); Figure 2.2]. Wheat is the primary host whereas *Thalictrum speciosissimum* L. (dusty meadow rue), *Isopyrum fumaroides* L., Anchusa and Clematis serve as secondary hosts (Bolton et al. 2008; Chai et al. 2016). Disease symptoms include large uredinia without chlorosis and necrosis of susceptible plant tissue. Resistant plants show small hypersensitive flecks or various sizes of uredinia surrounded by chlorosis and necrosis. Uredinia burst through the epidermis of the host plant, develop a round to ovoid shape pustule and grow up to 1.5 mm in diameter. Orange to brown uredinia appear on the upper and lower leaf surface of the host during infection. The uredinia produce

urediniospores (average diameter of 20 µm) during the asexual phase and will keep re-infecting the host while favourable conditions last. Favourable conditions include free water on the host leaf surface and temperatures between 15-25˚C (Roelfs 1989). Urediniospores are spread naturally by wind and rain. After four to eight hours urediospores start to germinate and develop germ tubes penetrating the stomata when moisture levels from either dew or rain water are 100% and the temperature is at ±20˚C (Hu and Rijkenberg 1998; Zhang and Dickinson 2001; Zhang et al. 2003).



Figure 2.2 Wheat leaf infected by *Puccinia triticina.*

Puccinia triticina is present worldwide and can be found where ever hexaploid wheat is grown. Leaf rust is the most prevalent of the three wheat rust types and causes regular economic losses due to infections that lead to smaller kernel size grain and reduces kernel production and negatively affects the quality of wheat flour (Liu et al. 2014a). Leaf rust epidemics in South Africa have been reported in the Western- and Eastern Cape provinces, KwaZulu-Natal and the Northern Cape. More recently lower leaf rust incidences were observed which have been attributed to low inoculum levels resulting from fungicides that are applied to control stripe rust infections as well as the use of resistant cultivars (Terefe et al. 2009).

Spraying fungicides can control *Pt* but planting of resistant cultivars is more cost-effective and eco-friendly (Ellis et al. 2014). Over the years, leaf rust resistance genes (*Lr*) *Lr1* to *Lr75* have been identified and are randomly present on all chromosomes of hexaploid wheat. The most recently identified *Lr* genes are *Lr70* on chromosome 5DS (Hiebert et al. 2014), *Lr71* on chromosome 1BL (Singh et al. 2013), *Lr72* on chromosome 7BS (Herrera-Foessel et al. 2014), *Lr73* on chromosome 2BS (Park et al. 2014) and *Lr75* on chromosome 1BS (Singla et al. 2017). Only 72 *Lr* genes have been officially designated as *Lr1* to *Lr75*, *Lr21* is similar to *Lr40*, *Lr39* is similar to *Lr41* and *Lr43* has been removed from the designation list (Li et al. 2016). Most *Lr* genes are ASR genes and therefore resistance is easily overcome by *Pt*. Only a few APR *Lr* genes have been identified with partial resistance and therefore a need exists to keep on searching for new APR genes to combine with effective ASR genes to ensure durable resistance against leaf rust (Nsabiyera et al. 2016). Examples of leaf rust APR genes are *Lr34/Yr18/Sr57, Lr46/Yr29*, *Lr67/Yr47* and *Lr68* (Martínez et al. 2001; Singh and Huerta-Espino 2003; Hiebert et al. 2010; Herrera-Foessel et al. 2012).

Identified *Lr* genes were obtained from various resistant sources and transferred to wheat germplasm. The resistant sources are divided into three gene pools. The primary gene pool contains germplasm of wheat and progenitor species. The secondary gene pool contains germplasm of *A. speltoides* Tausch, while the tertiary gene pool contains germplasm of *Aegilops umbellulata* Zhuk. Transferred *Lr* genes from the secondary and tertiary gene pools contain linkage drag that can negatively influence other traits (e.g. yield and flour colour; Hiebert et al. 2014).

The *Lr16* gene is a widely deployed ASR gene and provides effective resistance when combined with *Lr34/Yr18/Sr57* (Kassa et al. 2017). A study done by Draz et al. in 2015 found that *Lr19*, *Lr29* and *Lr34/Yr18/Sr57* could be used for breeding wheat cultivars with higher levels of leaf rust resistance. Effective leaf rust ASR genes, *Lr16*, *Lr19, Lr21*, *Lr25*, *Lr29, Lr41* and *Lr47* provided durable leaf rust resistance in Argentina when combined with leaf rust APR genes, *Lr34/Yr18/Sr57* and *Lr46/Yr29* (Vanzetti et al. 2011).

2.9.4 Stem rust

Stem rust is caused by *P. graminis* Pers. f. sp. *tritici* Eriks. & Henning (*Pgt*) and infects wheat species as the primary host (Figure 2.3). The secondary host of *Pgt* is *Berberis* species (Jin 2011). Stem rust infections on wheat can occur on the stem, leaf sheaths and are often found on the leaf blades and glumes. Urediniospore germination needs free water and infections occur through the stomata of the host plant. The first symptoms

appear as a small chlorotic fleck a few days after infection. After 8-10 days of infection, small pastules develop that rapture the epidermis of the host due to pressure of mass production of brick-red urediniospores. Pastules are linear or diamond shaped and grow up to 10 mm long. Teliospore production follows when urediniospore production ceases after several days of infection (Roelfs and Groth 1980; Leonard and Szabo 2005). The production of teliospores towards the end of the season results in darker black lesions and therefore, stem rust is also known as black rust (Leonard and Szabo 2005). Stem rust develops optimally when susceptible cultivars are planted in areas with day temperatures of 25-30˚C and night temperatures of 15-20˚C.



Figure 2.3 Wheat plant infected by *Puccinia graminis* f. sp. *tritici.*

The pathogenic variability and long distance wind dispersal ability of stem rust make it one of the most destructive wheat diseases and cause epidemics that lead to significant yield and financial losses (Dean et al. 2012). Frequent stem rust epidemics have occurred between 1920 and 1960 in America with total losses of up to 50% (Leonard and Szabo 2005). The deployment of effective stem rust resistance genes has reduced stem rust epidemics. Stem rust epidemics are present worldwide wherever wheat is planted,

namely Canada (Kolmer 2001), Europe (Zadoks 1963), Asia (Joshi and Palmer 1973; Knott 1989; Roelfs et al. 1992), Australia (Rees 1972; Park 2007), South America (German et al. 2007), East Africa (Wanyera et al. 2006) and South Africa (Pretorius et al. 2007).

In wheat, more than 60 distinct stem rust resistance (*Sr*) genes have been reported (Yu et al. 2017). Some of the *Sr* genes were found in hexaploid wheat while others were transferred from tetraploid wheat, diploid wheat, rye and other wild relatives (Yu et al. 2014). The most recent *Sr* gene (*Sr59*) was transferred from the T2DS·2RL rye translocation (Rahmatov et al. 2016).

One of the most widely deployed stem rust resistance genes in history was *Sr31* which was effective for many years. The rye segment carrying *Sr31* as well as genes associated with increased adaptability and yield and additional resistance genes to other rust types were transferred from rye to wheat. In 1999 *Sr31* resistance was overcome by an African race 'Ug99' (TTKSK) found in Uganda (Pretorius et al. 2000). Currently 13 variants within the Ug99 group have been identified with differences in virulence for *Sr9h*, *Sr21*, *Sr24*, *Sr30*, *Sr31*, *Sr36* and *SrTmp* (Singh et al. 2011; Pretorius et al. 2012; Patpour et al. 2015; Singh et al. 2015; Fetch et al. 2016). The outbreak caused by race *'*Ug99*'* and its derivatives pressured the wheat industry to breed new cultivars with resistance to stem rust as the universal resistance gene *Sr31* was no longer effective.

Only a few of the designated *Sr* genes are still effective against 'Ug99'. Only five APR *Sr* genes have been identified (*Sr2/Yr30*, *Sr55*, *Sr56*, *Sr57* and *Sr58*) and should be combined with effective ASR *Sr* genes (*Sr26* and *Sr39*) to control stem rust epidemics (Singh et al. 2008; Yu et al. 2017). One of the most widely used APR *Sr* genes is *Sr2/Yr30* that only provides partial resistance and should be combined with other effective stem rust resistance genes (McIntoch et al. 1995).

Effective 'Ug99' resistance genes are *Sr25*, *Sr26* (Mago et al. 2005; Liu et al. 2010), *Sr43* (Niu et al. 2014), *Sr44* (Liu et al. 2013) and *Sr52* (Qi et al. 2011). These effective *Sr* genes were all obtained from additional sources such as rye or species from the tertiary gene pool of wheat. Some of the effective genes against all new 'Ug99' races are *Sr22*, *Sr25*, *Sr26, Sr35, Sr39, Sr40 and Sr44* but these genes are not widely deployed in breeding programmes as they are associated with linkage drag that are linked to yield penalties and yellow flour colour (Singh et al. 2006; Jin et al. 2007; Singh et al. 2011; Yu et al. 2011). However, reduced segments, still carrying the desirable *Sr* gene but without additional linkage drag were developed for some of these effective *Sr* genes (Dundas et

al. 2007; Mago et al. 2009; Olson et al. 2010). Effective *Sr* genes against races in South Africa are *Sr2*, *Sr13*, *Sr14*, *Sr22*, *Sr25*, *Sr26*, *Sr29*, *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr39*, *Sr42* and *Sr43* (Figlan et al. 2014).

2.9.5 Stripe rust

Wheat stripe rust, also known as yellow rust, is caused by *P. striiformis* Westend f. sp. *tritici* Erikss. (*Pst*; Figure 2.4). The secondary hosts of *P. striiformis* are different *Berberis* species and *Mahonia aquifolium* (Pursh) Nuttall (Chen et al. 2014). Stripe rust infections occur in wheat growing areas with cooler climates and moist weather conditions during the growing season. Urediniospores of stripe rust are able to travel long distances by wind. Symptoms of stripe rust on susceptible plants include uredinia pastules arranged in long stripes, usually between the veins of the leaf sheaths, glumes and awns. The uredinia produce a mass of yellow urediniospores that breaks through the epidermis of the leaf tissue. Resistant plants represent various infection types; no visible symptoms, small hypersensitive flecks and uredinia surrounded by necrosis or chlorosis with limited urediniospore production (Hovmøller et al. 2011). Urediniospores can germinate rapidly in the presence of free rain or dew at relatively cool temperatures between 7-12˚C (Chen et al. 2014). After urediniospore germination infection occurs through the stomata. Chlorotic areas are visible from 6-7 days and pastules from 10-14 days after infection.

Stripe rust can be controlled through fungicide application but it will increase input costs and chemicals can have a negative influence on the environment (Chen 2014). Stripe rust disease epidemics in the past resulted in significant yield losses and negatively affected the wheat industry and economy. Regular regional stripe rust epidemics were reported in North- and South America, Asia, Africa, Australia, New Zeeland and Europe with yield losses up to 5% but in some cases as high as 25% (Wellings 2011; Ziyaev et al. 2011; Solh et al. 2012). Individual yield losses up to 80% were recorded in the Middle East and North America in 2010 due to new strains that overcame the *Yr27* resistance gene. A new stripe rust race with increased fitness and heat-tolerance was discovered in Mexico in 2002 and is a significant problem because it spread and became the predominant race in various countries (Ali et al. 2014). Severe stripe rust outbreaks with resultant yield losses can occur when susceptible cultivars are planted and infection take place at early growth stages (Solh et al. 2012).

The stripe rust resistance (*Yr)* genes can either be ASR or APR. Some APR genes need high temperatures to be expressed and are only effective during the later growth stages when day temperatures rise. Resistance expressed by these genes is referred to as

high-temperature adult plant resistance (HTAP). At present, 70 *Yr* genes are described, and most of these *Yr* genes are race-specific (Rosewarne et al. 2013; Li et al. 2014).



Figure 2.4 Wheat leaf infected by *Puccinia striiformis* f. sp. *tritici.*

A total of 13 APR *Yr* genes are described namely *Yr16* (Devos et al. 1993), *Lr34/Yr18/Sr57* (Lagudah et al. 2006), *Lr46/Yr29/Pm39/Sr58* (Rosewarne et al. 2006), *Sr2/Yr30* (Lagudah 2011), *Yr36* (Uauy et al. 2005), *Yr39* (Lin and Chen 2007), *Lr67/Yr46/Pm46/Sr55* (Herrera-Foessel et al. 2011), *Yr48* (Lowe et al. 2011), *Yr49* (McIntosh et al. 2011), *Yr52* (Ren et al. 2012), *Yr54* (Basnet et al. 2013), *Yr59* (Zhou et al. 2014) and *Yr62* (Lu et al. 2014). Some *Yr* genes were transferred from common wheat relatives or tetraploid wheat accessions. Durum wheat cultivars are a valuable source of *Yr* genes (Xu et al. 2013). Some of the widely deployed *Yr* genes (*Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17* and *Yr27*) were defeated by the emergence of new stripe rust races (Zegeye et al. 2014; Jighly et al. 2015; Maccaferri et al. 2015).

Combining different effective HTAP and APR *Yr* genes will allow breeders to breed cultivars with complete and long-lasting resistance (Chen 2014). Most of the stripe rust

ASR genes have become ineffective to at least some *Pst* races, but in combinations with other effective *Yr* genes, these genes can still be deployed and contribute towards genetic control (Liu et al. 2015). Examples of these genes include *Yr5*, *Yr7*, *YrSP*, *YrV23*, *YrQz*, *YrTp1*, *YrCN19* and the *Yr* gene in YW243 (Luo et al. 2009). Major and minor resistance QTL for stripe rust resistance with additive or epistatic effects were identified (Rosewarne et al. 2013). The *YrSP* stripe rust resistance gene was originally obtained from wheat cv. Spaldings Prolific and provides broad spectrum resistance and is used to differentiate between races (Johnson et al. 1972; Stubbs 1988; Wellings and McIntoch 1990). The *YrSP* gene is located on the 2BL chromosome (Guan et al. 2005). Major and minor stripe rust resistance QTL can be used in combination with other effective *Yr* genes to decrease disease severity (Ramburan et al. 2004). Two major *Yr* QTL (*QYr.sgi-2B.1* and *QYr.sgi-4A.1*) were identified in the South African wheat cultivar, Kariega (Agenbag et al. 2014). The Kariega *Yr* QTL on chromosome 2B is used to incorporate stripe rust resistance in newly developed South African breeding lines (Tolmay et al. 2016).

2.9.6 Wheat rusts phenotypic screening

Different rust phenotypic screening methods were developed over the past years to evaluate plant resistance. Field and greenhouse screening methods were developed to monitor disease expression of breeding lines and cultivars (Forster et al. 2014). Controlled greenhouse conditions are used to simulate the perfect environmental conditions to enhance rust infection of either seedlings or adult plants. Both seedling and adult plant growth stages are evaluated as rust resistance genes are expressed as ASR or APR genes (Ellis et al. 2014). Different scales were developed over the past years to assess different infection types (ITs) and host response levels that are linked to specific symptoms.

The Stakman scale is generally used to score leaf- and stem rust ITs of seedlings in the greenhouse (Table 2.1; Stakman et al. 1962). This scale was originally developed to identify rust races but was later used to discriminate between different degrees of resistance of wheat varieties. The Stakman scale was used in various rust screening studies to determine the ITs of different wheat varieties (Niu et al. 2011; Nzuve et al. 2012; Riaz et al. 2016). In 1975, Browder and Young published a three digit 0-7 scale for leaf rust but this scale is not often used by other researchers and is not widely adapted. Stripe rust infections on seedlings are classified using scales developed by Gassner and Straib in 1932 and McNeal et al. in 1971 (Table 2.2).

The McNeal scale is more commonly used in stripe rust and describe more ITs compared to the Gassner and Straib scale. The McNeal scale was developed to evaluate pastule formation on infected leaves and therefore is not optimal to evaluate variation of pastules on the leaf area (Dawson et al. 2015). For most of the studies that use the McNeal scale, plants with an IT score of 0-3 are considered as resistant, 4-6 as intermediate and 7-9 susceptible (Kertho et al. 2015).

Table 2.1 The Stakman scale for basic infection types to classify rusts in the greenhouse (Stakman et al. 1962)

Infection type[a]	Host response	Disease symptoms
0	Immune	No uredia nor other indications of infections
0;	Nearly immune	No uredia, but few faint flecks present
;	Resistant	No uredia, but hypersensitive necrotic or chlorotic flecks present
1	Resistant	Uredia minute; surrounded by distinct necrotic areas
2	Moderately resistant	Uredia small to medium: usually in green islands surrounded by a decidedly chlorotic or necrotic border
3	Moderately susceptible	Uredia medium in size; coalescence infrequent; no necrosis, but chlorotic areas may be present, especially under unfavourable growing conditions
4	Very susceptible	Uredia large, and often coalescing; no necrosis, but chlorosis may be present under unfavourable growing conditions
X	Heterogeneous	Uredia variable, sometimes including all infection types and intergradations between them on the same leaf; no visual separation possible; on reinoculation small uredia may produce large ones, and *visa versa*
Y		Variable uredia sizes with larger uredia towards the tip of the leaf
Z		Variable uredia sizes with larger uredia towards the leaf base

[a] Variation within a given infection type can be indicated by plus and minus signs. The highest and lowest limits of the infection type can be indicated by ++ and -- respectively. A plus-minus sign symbolises a mixture between the + an – infection types. Pronounced chlorosis is indicated by the symbol “c”, whereas “b” symbolises browning with a tendency towards necrosis and “n” symbolises necrosis.

Table 2.2 The basic infection types for classifying stripe rust in the greenhouse

Infection type		Host response	Disease symptoms
Grassner[a]	McNeal[b]		
i	0	Immune	No visible infection
00	1	Very resistant	Necrotic/chlorotic flecks, without sporulation
0	2	Resistant	Necrotic/chlorotic stripes, without sporulation
I	3	Moderately resistant	Trace sporulation, necrotic/chlorotic stripes
I	4	Light moderate	Light sporulation, necrotic/chlorotic stripes
	5	Moderate	Intermediate sporulation, necrotic/chlorotic stripes
II	6	High moderate	Moderate sporulation, necrotic/chlorotic stripes
	7	Moderate susceptible	Abundant sporulation, necrotic/chlorotic stripes
III	8	Susceptible	Abundant sporulation, with chlorosis
IV	9	Very susceptible	Abundant sporulation, without chlorosis

[a] McNeal et al. (1971); [b] Grassner and Straib (1932)

The McNeal and Stakman scales can be used for adult plant rust infection assessments of all rust types, but a modified Cobb scale are more often used for adult plant assessments under field conditions (Peterson et al. 1948). The modified Cobb scale quantifies disease severity where 0% is immune and 100% completely susceptible. The host infection response (reaction) of adult plants can be evaluated with a scale that was developed by Roelfs et al. (1992). To determine the coefficient of infection (CI), the disease severity according to the Cobb scale is multiplied with a constant response value linked to the corresponding host's reaction response (Table 2.3). The CI has the best correlation to crop loss but the two variables used to calculate the CI is dependent (McIntosh et al. 1995). In the case of adult plant assessments, disease severity and host response reactions for leaf- and stripe rust are measured on the upper side of the flag leafs, while stem rust is assessed based on the infection of the stem sheath.

Table 2.3 Response values of major host response classes for wheat rusts of adult plants

Host response	Observation	Response value
Immune	0	0.0
Resistant	R	0.2
Resistant to moderately resistant	RMR	0.3
Moderately resistant	MR	0.4
Moderately resistant to moderately susceptible	MRMS	0.6
Moderately susceptible	MS	0.8
Moderately susceptible to susceptible	MSS	0.9
Susceptible	S	1.0

2.10 Fusarium head blight

Fusarium head blight, also known as scab, is mainly caused by *F. graminearum* Schwabe [anamorph *Gibberella zeae* Schwein (Petch)] and infects wheat spikes (Figure 2.5). *Fusarium graminearum* infects oats, barley and is responsible for ear and stalk rot in maize (Trail 2009). Fusarium head blight infections of wheat are associated with negative effects including yield losses, production of mycotoxins and the presence of FDK in grain (Tamburic-Ilincic 2012). Favourable weather conditions include warm moist periods during wheat anthesis (Parry et al. 1995). Fusarium head blight symptoms include water-soaked brownish spots in the middle of the glume or rachis, premature bleaching of spikelets and FDK (Parry et al. 1995).



Figure 2.5 Wheat infected by *Fusarium graminearum*.

Later on, infection starts to spread in the wheat head and salmon pink to orange fungal growth becomes visible. Infected grain shrinks and discolours to grey-brown as infection leads to premature death and bleaching of infected wheat spikelets (Wiese 1987). Development of blue/black perithecia during extended periods of warm and moist climatic conditions leads to a scab appearance (Mathre 1982). *Fusarium graminearum* infection occurs with the germination of airborne ascospores in the flowering spikelets of wheat plants. After infection intercellular growth of the fungus follows and spreads through the xylem and pith tissue resulting in colonisation and necrosis. After FHB infection trichothecene type B mycotoxins such as DON, nivalenol (NIV) and their acetylated derivatives are produced and can be harmful when consumed (Arunachalam and Doohan 2013).

Sexual development of the FHB causing fungus starts with the formation of hyphae, consisting of binucleate cells (overwintering structures; Guenther and Trail 2005). During the extended binucleate phase, known as the dikariotic phase, two genetically distinct nuclei pair and produce new binucleate cells with identical nuclei. These cells produce small coiled cells, the fruiting bodies initials (Trail and Common 2000). Initials develop into flask shaped perithecia that are filled with asci. Asci are tubular sacs filled with ascospores. Ascospores are the result of meiosis and serve as the primary source of inoculum during moist periods. These spores develop into a slimy mass on infected plant surfaces or crop residues.

Sprorodochia are cushion shaped hyphal structures that produce asexual conidia spheres during moist periods. These spores develop into a slimy mass on infected plant surfaces of crop residues. Conidia spores are fusiform shaped and form slimy masses to help with rain-splash dispersal. Therefore, conidia spores are associated with short distance dispersal (Shaner 2003) in comparison to ascospores that are associated with long-distance dispersal by wind (Trail 2009).

Severe FHB infections resulted in significant economic losses to wheat and barley grain production as well as quality in the 1990's and the early 2000's (McMullen et al. 1997). The low market value of FHB infected grains causes growers to shift their production to other crops (McMullen et al. 2012). Fusarium head blight epidemics were observed in the United States (McMullen et al. 1997), Canada (Gilbert and Tekauz 2000), Europe (Parry et al. 1995), Australia (Burgess et al. 1987), China (Bai and Shaner 1996) and South Africa (Kriel and Pretorius 2008). The double cropping system where maize is planted in summer and wheat in winter has favoured FHB infections especially under irrigation (Kriel and Pretorius 2008).

Breeding for FHB resistance is complicated by the five different resistance types that are controlled by different loci (Tamburic-Ilincic 2012). The five FHB resistance types are: Type I, resistance to primary infection; Type II, resistance to disease spread; Type III, resistance to DON accumulation; Type IV, resistance to kernel infection and Type V, resistance to yield loss (Mesterhazy 1995). Major FHB resistance QTL have been identified, namely *Fhb1* (*Qfhs.ndsu-3BS*) on chromosome 3B, *Fhb2* on chromosome 6B (Waldron et al. 1999) and *Qfhs.ifa-5A* on chromosome 5A (Buerstmayr et al. 2003). Two major QTL, *Fhb1* and *Fhb2,* confers Type II resistance, while the QTL on chromosome 5A confers Type I resistance. The Brazilian cultivar Frontana carries a major QTL on chromosome 3A that confers Type I resistance (Steiner et al. 2004). Every FHB resistance QTL contributes a certain percentage to FHB tolerance. A QTL is described as a major or minor QTL according to the percentage of tolerance it provides towards FHB infection (Winter and Kahl 1995). Environmental conditions greatly influence the interaction between wheat and FHB infections (Mesterhazy 1995).

The Chinese wheat landrace, Wangshuibai, has good resistance against FHB but lacks good agronomical traits. Sumai3 and Ning are two Chinese wheat cultivars with high levels of FHB resistance and are frequently used in breeding programmes as source of FHB resistance. The FHB resistant cultivar CM-82036 originated from the Sumai3'/'Thornbird-S' cross from a breeding programme between the International Maize and Wheat Improvement Centre (CIMMYT) of Mexico and South America (Buerstmayr et al. 2002). This resistant cultivar also contains the *Fhb1* gene that encodes for an enzyme with the ability to transform DON to DON-3-O-glycoside that is less toxic (Lemmens et al. 2005). CM-82036 contains two major FHB resistance genes with Type I and Type II resistance (*Fhb1* and *Qfhs.ifa-5A*) and is therefore, a popular cultivar to use in breeding programmes as a FHB resistance source (Buerstmayr et al. 2002).

2.11 Fusarium head blight phenotypic screening

Natural infection by FHB is inconsistent over years due to environmental conditions that play a vital role in spore germination and infection progress (Mesterhazy 2003). Therefore, different inoculation methods were developed and used over the past years to evaluate FHB disease infection.

With the grain spawn method, FHB infected grain is spread on the soil surface in the field. Mist irrigation is applied to ensure high levels of humidity for perithecial and inoculum development. This method expands the time period of inoculum availability and

simulates natural infection. All types of resistance can be measured with this convenient and low cost method. However, inoculum and disease development depend on environmental conditions that make disease escapes possible which may negatively influence data and results (Bai and Shaner 1994).

The spray inoculation method is used for field and greenhouse trials and can measure Type I and Type II resistance. This method is influenced by environmental factors and it is difficult to optimise conditions for spore germination especially under field conditions. Environmental factors that contribute to successful germination of spores are long periods of optimal temperatures, high levels of humidity and flowering time of spikes. The amount of viable inoculum present also affects the germination rate of *Fusarium* species (Parry et al. 1995).

The third inoculation method is called point inoculation. With this method only one spikelet per head is inoculated and the spread of disease, also known as type II resistance, is measured. This method reduces the probability of disease escape and an equal amount of inoculum is used for every inoculation. Inoculated heads are covered with plastic bags or mist irrigation is applied to obtain the optimal humidity levels for spore germination (Mesterhazy et al. 1999). This technique is more labour intensive than the grain spawn method, but is a reliable method to evaluate Type II resistance as the disease escape level is low.

Different types of data can be obtained from phenotypic FHB trials that can be used for resistance studies such as levels of secondary metabolites, factors linked to agronomic traits and grain quality. The severity of infection is measured as the average percentage of diseased spikelets. Incidence is the percentage of spikes that are infected. The FHB index (%) can be calculated as follows: Fusarium head blight index (%) = (Severity x Incidence)/100 (Stack and McMullen 1994). Several studies indicated a negative correlation between the FHB index and FHB resistance (Kalih et al. 2015; Osman et al. 2015).

Disease severity is obtained for both the spray and point inoculation methods. Poor correlation between these two methods was observed in studies (Miedaner et al. 2003). These poor correlations can be explained by the different QTL that govern Type I and Type II resistance (Schroeder and Christensen 1963). Evaluation of FHB severity takes place 18-21 dpi (Bai and Shaner 1996).

2.12 Combined rusts and Fusarium head blight resistance breeding

Resistance genes linked to morphological traits and MAS breeding methods are applied towards disease resistance breeding in wheat (Somers et al. 2007). Numerous molecular markers linked to different resistance genes are developed which enable breeding programmes to speed up the breeding process through continuous selection of the best possible resistant line(s) based on molecular markers linked to traits of interest.

A study by Zeng et al. (2013) developed substitution and translocation bread- and durum wheat lines with resistance to FHB, leaf- and stem rust. They obtained this resistance from two durum wheat - *Thinopyrum intermedium* Host (wheatgrass) partial amphiploid lines. In 2005, Šíp et al. reported on the identification of wheat lines with combined resistance to eight different pests including the three rust types and FHB, but they struggled to find lines with high or moderate resistance to all eight diseases. Somers et al. (2007) have managed to combine *Lr21* and orange wheat blossom midge (*Sm1*) resistance genes into Canadian wheat lines together with various combinations of FHB resistance QTL. The Austrian winter wheat cultivar Capo has QTL on chromosomes 2AL, 2BL and 2BS with resistance to leaf- and stripe rust. The long lasting resistance of Capo is due to a combination of minor- and major genes and can be used as a source in rust resistance breeding (Buerstmayr et al. 2014).

Although previous studies succeeded in combining different disease resistance genes/QTL some combinations might be more challenging to achieve. One example is the combination of the *Fhb1* gene (major FHB resistance gene) and *Sr2* gene (durable stem rust resistance gene) into a single cultivar. Both these genes are located on the short arm of chromosome 3B, the largest chromosome of wheat (Hare and McIntoch 1979; Anderson et al. 2001; Paux et al. 2008). Due to their close proximity on 3B and repulsion linkage, they are difficult to combine into a single wheat line. It is thus important to be informed on gene location and linkage when planning specific gene combinations in a breeding scheme.

2.13 Wheat proteins and quality

Although breeding aimed at improving disease resistance is important to ensure sustainable wheat production, end use quality and yield are two important factors not to be neglected. Wheat flour forms dough due to the gluten content in the endosperm of wheat seed. The visco-elastic properties of gluten include elasticity and dough strength. These two properties need to be in fine balance to obtain satisfactory dough for the

milling- and baking industries (Singh et al. 1990). Wheat proteins are categorised into four groups based on solubility; globulin in salt solutions; albumin in water; gliadin in alcohol and glutenin in acid solution (Osborne 1907; Hubbard et al. 1997; Erickson 2009).

Gluten is the major storage protein in the endosperm and contributes 80-85% towards total flour proteins. Gluten is mainly responsible for the processing qualities of wheat (Shewry et al. 1995). Gluten is a macropolymer and consists of inter- and intra-molecular disulphide bonds of polymeric glutenins and momomeric gliadins. Polymeric glutenins are divided into HMW-GS and low molecular-weight-glutenin subunits (LMW-GS) and are multimeric aggregates with disulphide bonds. HMW-GS and LMW-GS are poorly soluble in alcohols and are responsible for viscosity and dough elasticity (Payne 1987).

The HMW-GS are expressed by genes on the short arm of the group 1 chromosomes. HMW-GS express only 10% of the total storage proteins and are responsible for dough viscosity and bread-making quality (Shewry et al. 1992). HMW-GS are further divided into two tightly linked x- and y-type alleles per loci. The x-type alleles range from 80-88 kilodalton (kDa), whereas the y-type alleles are smaller and range from 67-73 kDa (Payne and Lawrence 1983). The *Glu-1A* locus expresses only an x-type allele, whereas the *Glu-1B* and *Glu-1D* loci express two tightly linked x- and y-type alleles (Payne and Lawrence 1983; Gupta et al. 1996).

The HMW-GS molecular structure consists of three domains. A long central repetitive domain flanked by the short N- and C-terminal non-repetitive domains (Payne and Lawrence 1983). The central repetitive domain consists of tandem and interspersed repeats of tri- (only in x-type alleles), hexa- and nonapeptide motifs. These motifs are rich in amino acids glutamine, glycine and proline. Differences between HMW-GS alleles are due to the variation in the number of tri- and hexapeptides of the central repetitive domain. HMW-GS are responsible for 30-70% of the genetic variation for wheat dough properties (Shewry et al. 1992).

The LMW-GS are encoded by the genes at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci on the short arm of the homoeologous group 1 chromosomes (Gupta and Shepherd 1990). LMW-GS range in size from 32-45 kDa and are divided into B-, C- and D groups according to their mobility during sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; D'Ovidio and Masci 2004). The LMW-GS represent about 40% of the total gluten fraction and are studied less due to their corresponding molecular

weights with gliadins (Gianibelli et al. 2001). Allelic variation for both LMW-GS and HMW-GS are associated with different rheological properties (Payne 1987).

The momomeric gliadins are soluble in alcohol and are divided into four distinct groups, alpha-, beta-, gamma- and omega-gliadins (Gupta and Shepherd 1990). Genes for the alpha- and beta gliadins are located on the short arms of group 6 chromosomes. The gamma- and omega gliadins are expressed by genes located on the short arms of the group 1 chromosomes (Gupta and Shepherd 1990).

Research over the past years investigated the role of wheat proteins in the bread-making process. Various methods and tests are being developed to evaluate wheat protein quality and properties associated with bread-making characteristics. Bread-making characteristic parameters include grain hardness, protein content percentage, starch content percentage, wet gluten and zeleny sedimentation value. Farinograph parameters determine dough consistency, water absorption, developing time, dough stability, degree of softening and farinograph quality number. Dough mixing parameters measured with a reomixer include area under centre line, initial slope, peak time, peak height and bandwidth. Bread-making parameters measure bread yield, loaf volume and bread crumb grain (Hussain et al. 2012; Salmanowicz et al. 2012).

Wheat protein research indicates that gliadins (monomeric proteins) are responsible for dough elasticity while glutenins (polymeric proteins) are responsible for dough viscosity (Khatkar and Schofield 1997). A glutenin/gliadin ratio is a good indicator of dough visco-elastic properties. One constraint of wheat quality tests is the large seed sample size required to perform quality tests, especially in pre-breeding programmes, as a small amount of seed per individual line is available. Size exclusion high performance liquid chromatography (SE-HPLC) and SDS-PAGE are two techniques used for single seed analyses. One problem associated with applying these techniques in a pre-breeding programme is the segregation in offspring. Molecular markers linked to these protein traits are advantageous to analyse leaf material of segregating plants that are still in the field or greenhouse. Molecular markers are ideal for direct selection and are used extensively in breeding programmes (Liang et al. 2010).

2.14 High molecular weight-glutenin subunit molecular markers

Several studies were done on HMW-GS due to the large contribution on genetic variation for dough properties (Gianibelli et al. 2001; He et al. 2005; Liu et al. 2005). Many HMW-GS are associated with either good or poor bread-making quality. Most HMW-GS genes

are cloned and molecular markers developed are frequently used in pre-breeding and breeding programmes. One advantage of HMW-GS molecular markers is the ability to discriminate between different subunits with the same molecular weight or mobility (D'Ovidio and Anderson 1994). Two allele specific markers (MAR and BxFp) have been developed to discriminate between allele variants on the *Glu-B1* locus (Butow et al. 2003 and 2004). Molecular marker MAR is used to discriminate between the Glu-*Bx7*^{OE}, *Glu-Bx20* and without *Glu-Bx7+By8**. Lines carrying the Glu-*Bx7*^{OE} have a higher dough strength compared to the other *Glu-B1* alleles (Ragupathy et al. 2008). The BxFp molecular marker discriminates between *Glu-Bx7+By8* and *Glu-Bx17+By18*. Three diagnostic sequence-tagged site (STS) markers, Umn19, Umn25 and Umn26 were developed to discriminate between the *Glu-Ax2** and other *Glu-Ax* alleles, *Glu-Dx2* and *Glu-Dx5*, as well as between *Glu-Dy10* and *Glu-Dy12*, respectively (Liu et al. 2008). Wheat lines with *Glu-Ax1* or *Glu-Ax2** have better dough strength compared to the null allele. The *Glu-Dx2+Dy12* alleles are more common in soft wheat and *Glu-Dx5+Dy10* is associated with better bread-making qualities (Liu et al. 2014b). Markers linked to glutenin subunit genes are used in various breeding programmes worldwide (Jin et al. 2011; Liu et al. 2014b). Using HMW-GS markers linked to good bread-making quality can assist to select for improved wheat gluten quality from an early generation (Liang et al. 2015).

2.15 Conclusions

Food security will remain a challenge in future as the human population continue to increase. Therefore, increasing food production will remain a main priority worldwide. Biotic and abiotic stresses negatively affect grain yield and quality. Although chemicals are available to prevent epidemic outbreaks of wheat diseases, a more environmental friendly solution is to breed for resistance. Leaf-, stem- and stripe rust as well as FHB are considered important fungal diseases of wheat in South Africa, known for their negative influence on yield potential and grain quality. Currently no South African wheat cultivar has sufficient levels of FHB tolerance or resistance and although some cultivars contain rust resistance no cultivar with combined resistance to the three rusts and FHB is registered. Various resistance genes/QTL linked to FHB- and rust resistance were discovered over the past years that are effective against these local pathogens and pathogen races. These resistance genes/QTL can be combined through a breeding programme to identify lines with combined resistance to FHB and all three rust diseases. The presence of resistance genes/QTL can be monitored in early generations using molecular markers to improve resistance. To confirm that markers correlate with the

linked gene(s), lines should be phenotypically evaluated. Although disease resistance is important for cultivar development, yield and end use quality is evenly important and therefore selection for these traits should preferably also be included in a disease resistance breeding programme. The HMW-GS can assist breeders to select for better bread-making qualities. Molecular markers linked to HMW-GS were developed and can be used to determine bread-making qualities from an early generation to prevent limited genetic diversity at later generations. Combined resistance to FHB and rusts will decrease the farmer's input costs and lower their risk of yield losses. Disease free grain will result in improved grain quality and lower toxin levels.

2.16 References

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Chapter 3

Molecular characterisation of combined rusts and Fusarium head blight resistant wheat lines

3.1 Introduction

Improving characteristics of wheat is an on-going process as it is challenged by biotic and abiotic stresses. New resistance against stressors are constantly being sought and bred into wheat to overcome limitations. Resistance breeding against diseases is one of the main priorities of wheat breeders (Todorovska et al. 2009). Numerous disease resistance genes have been identified that can be used to improve and release new relevant wheat cultivars by applying different breeding techniques (Gupta et al. 2010).

To develop cultivars resistant to wheat diseases, a resistant source should be identified and incorporated using a breeding technique to achieve the goal. In the past, before molecular markers were available, resistant cultivars were developed using conventional breeding techniques that rely on phenotypic evaluation. The first molecular markers, restriction fragment length polymorphism (RFLP) were only developed and used in the 1970's (Botstein et al. 1980). Thereafter, PCR based markers namely amplified fragment length polymorphism (AFLP) or simple sequence repeats (SSRs) were developed and frequently used in breeding programmes to develop wheat lines with desirable traits. Different types of molecular markers are used in many wheat breeding programmes (Nelson et al. 1997; Shi et al. 1998; Gold et al. 1999; Prins et al. 2001; Wang et al. 2002; Helguera et al. 2003; Thapa et al. 2016).

Advantages of molecular markers are that they can be applied to seedlings and therefore selection of the most desirable plants can be done at an early stage and undesirable plants can be discarded. Molecular markers are not influenced by environmental conditions. Co-dominant markers can distinguish between homo- and heterozygous genotypes (Winter and Kahl 1995). Molecular markers can identify genotypes of traits that are difficult to evaluate phenotypically and therefore a higher number of traits that can be identified using MAS are available to the breeder (Zhu et al. 2012).

Molecular markers should preferably be closely linked to the gene of interest. Therefore, the ideal marker is one that is located within the targeted gene sequence. Two flanking markers need to be screened when the marker is not located within the targeted gene sequence to minimise the chance of crossovers and the loss of the gene (Collard and Mackill 2008). One possible disadvantage of molecular markers is that their use in

breeding programmes (cross- and backcross breeding) can decrease the genetic diversity of the breeding population due to the strong selection pressure applied to select desirable traits (Govindaraj et al. 2015).

Marker-assisted selection can be used to enhance resistance breeding where several genes need to be combined in one genotype. Marker-assisted selection can be applied to young plants using leaf material to determine their genotypes before flowering and crosses between segregating lines can be done more resourcefully (Miedaner and Korzun 2012). Although genotypes of a segregating population can be known by the time crosses are made, the lack of synchronisation of flowering dates between identified female and male lines often determine whether all preferred crossing combinations based on genotype are possible.

Major wheat diseases include FHB and the three rusts; leaf-, stem- and stripe rust (Dill-Macky 2010; Goutam et al. 2015). These diseases are responsible for great economical losses worldwide and in South Africa. Spraying of fungicides can control the respective pathogens, especially the rusts but increase input costs and negatively affects the environment. Another way to overcome this problem is to use breeding techniques and tools to develop resistant cultivars (Olivier 2014).

Numerous rusts and FHB resistance genes have been identified and several cultivars have been released with resistance. Some resistance genes have been overcome which in the past led to epidemics such as the stem rust outbreak caused by ‘Ug99’ (Pretorius et al. 2000). New resistance genes are still being identified and successfully crossed into newly developed cultivars. Newly discovered resistance sources are mainly identified through natural genetic variation, exploiting variation occurring in wheat landraces and species closely related to wheat (Collard et al. 2005; Mondal et al. 2016).

To obtain durable rust resistance, APR and ASR genes should preferably be combined when developing resistant wheat lines (Ellis et al. 2014). Different rust isolates have overcome different rust resistance genes and therefore selection of rust resistance genes are often location specific and based on the avirulence/virulence ratio of dominating pathogens towards available resistance genes (Collard and Mackill 2008). When developing rust resistant wheat lines for South Africa, ASR genes such as *Lr19*, *Sr26*, *Sr39* and *YrSP* are still effective and should be favourably considered. These should preferably be combined with the APR resistance genes *Lr34/Yr18/Sr57*, *Sr2*/*Yr30* and *QYr.sgi.2B-1* in an effort to achieve durability as well as to set a higher level of

residual resistance should one of the ASR genes fail due to a virulence shift in the pathogen population.

Fusarium head blight resistance genes/QTL act quantitatively and every gene/QTL contributes a certain percentage to resistance (Buerstmayr et al. 2009). Major and minor FHB resistance genes/QTL have been identified and used in different breeding programmes (Snijders 1990; Steiner et al. 2004; Steed et al. 2005; Lin et al. 2006). Two major FHB genes that refer to Type I and Type II resistance are *Qfhs.ifa-5A* and *Fhb1*, respectively (Waldron et al. 1999; Buerstmayr et al. 2003). These two major FHB resistance genes are present in the cultivar CM-82036 that is used as the donor parent in several breeding programmes (Wilde et al. 2007).

To combine all these rusts and FHB resistance genes/QTL into a single wheat line would have been difficult and time consuming using conventional breeding methods. However, with molecular markers linked to rusts and FHB resistance genes/QTL the breeding process can be more accurate with accelerated breeding cycles (Goutam et al. 2015). Using molecular markers the best genotypes, based on the number of rusts and FHB resistance genes present, can be used in a double cross breeding scheme to incorporate lacking genes (Moose and Mumm 2008). Self-pollination cycles and development of DH lines can be used to achieve lines homozygous for the targeted genes. With both methods, a chance exists to lose heterozygous genes and therefore many lines need to be produced to ensure the desirable genotype.

The breeding process of wheat cultivars can take up to 12 years before the new cultivar is released for commercial use (Shimelis and Liang 2012). After selecting wheat lines with the desirable resistance genes, lines need to be evaluated for end-use quality and yield. The HMW-GS explain up to 70% of the variation obtained in bread-making characteristics. Over the past years molecular markers linked to HMW-GS have been developed and can be used in breeding programmes to assist breeders to select for good bread-making characteristics from an early stage in their breeding programmes (Liu et al. 2008a). Molecular markers linked to HMW-GS can be used in segregating populations as it only requires DNA, while other techniques such as SDS-PAGE and SE-HPLC require flour samples that are difficult to obtain in segregating populations (Liang et al. 2010). Several studies identified specific HMW-GS with a positive influence on bread-making characteristics. Alleles *Ax1*, *Ax2**, *Bx7+By8*, *Bx17+By18* and *Dx5+Dy10* are associated with better bread-making characteristics and stronger dough (Payne et al. 1987). The $Bx7^{OE}$ allele is also associated with stronger dough (Butow et al. 2003; Vawser and Cornish 2004). The 1BL.1RS translocation from rye is a good source of

disease resistance and improves yield but negatively influence bread-making characteristics such as dough stickiness and dough strength (Radovanovic et al. 2002).

In previous breeding projects at the UFS, numerous wheat lines containing different resistance gene/QTL combinations against the rusts and FHB were developed. The aim of this study was to combine these rusts and FHB resistance genes/QTL into a single wheat line. By making crosses and genotypically evaluating the lines with molecular markers linked to resistance genes/QTL, it will be possible to monitor the genes/QTL and identify the best lines with combined resistance to rusts and FHB. The HMW-GS composition of lines in the final population will be determined using molecular markers.

3.2 Materials and methods

One of the main objectives for the study was to develop a wheat population with combined durable resistance to leaf-, stem- and stripe rust as well as FHB. Molecular markers linked to rusts and FHB resistance genes/QTL were used to determine the genotypes of the wheat lines. The best genotypes according to the highest number of resistance genes/QTL were used to make crosses to firstly combine rust- and FHB resistance, secondly to improve the best identified lines by further adding missing target genes/QTL and lastly to produce lines homozygous for the targeted genes/QTL.

3.2.1 Selection of plant material

Wheat lines from previous studies were used to develop the breeding populations for the current study. Rust resistant lines with durable resistance to leaf-, stem- and stripe rust were available as well as lines with combined FHB resistance QTL. Both APR and ASR genes/QTL were present in the selected rust resistant lines. The FHB resistant lines were selected based on MAS for major FHB resistant QTL linked to both Type I and Type II FHB resistance.

3.2.1.1 Selection of rust resistant female parental lines

The rust resistant population was previously developed using four parental lines, Kariega (*Lr34/Yr18/Sr57* and *QYr.sgi.2B-1*), Avocet*YrSP* (*Lr34/Yr18/Sr57, YrSP* and segregating for *Sr26*), Blade (*Sr26*) and a derivative of Chinese Spring, CS*Lr19*-149-299 (*Lr19* and *Lr34/Yr18/Sr57*; Figure 3.1). The seed source of Blade used in this breeding programme was segregating for the *Sr2*/*Yr30* gene and therefore the gene was not present in the offspring. Kariega was crossed with Avocet*YrSP* while Blade was crossed with CS*Lr19*-149-299.

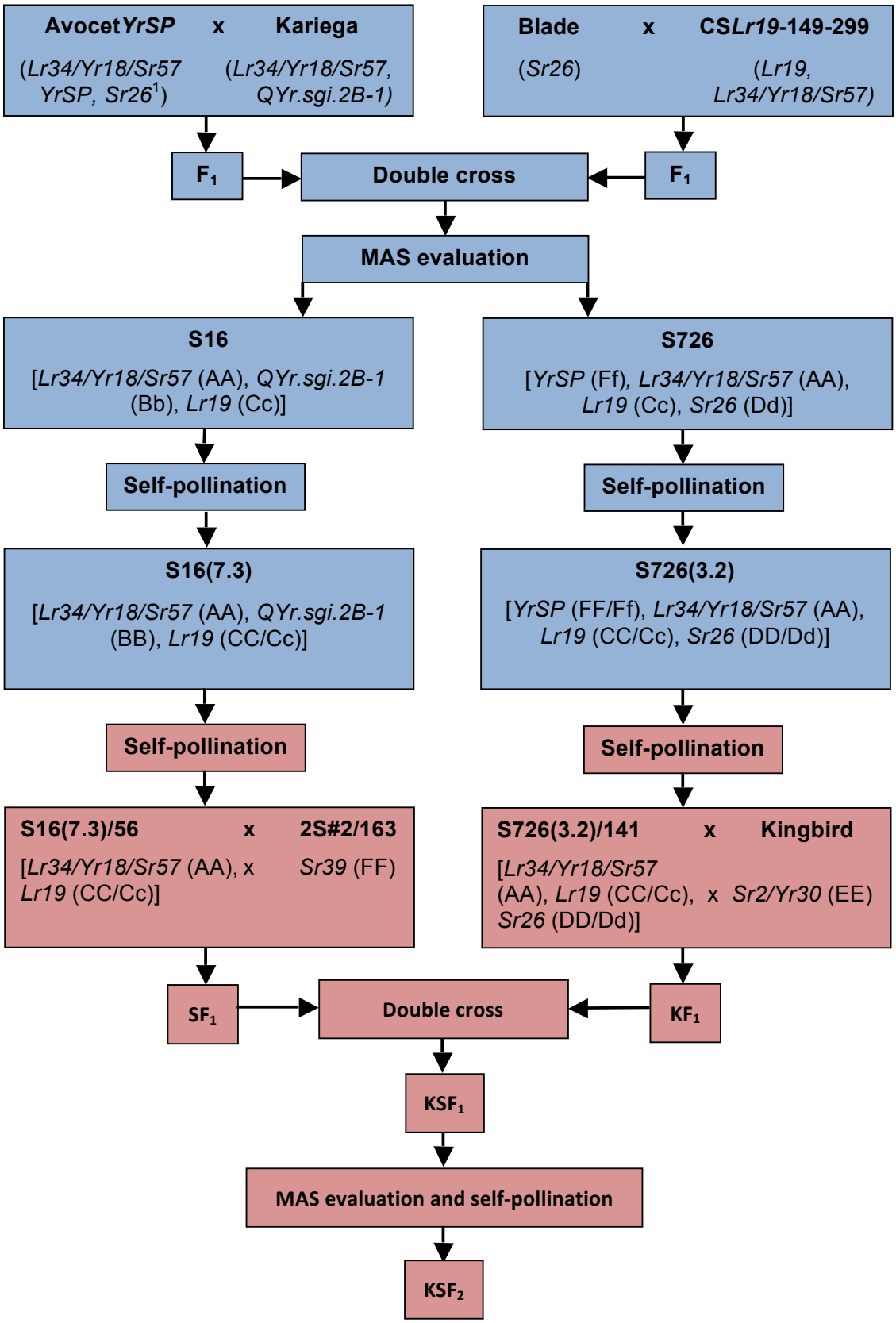


Figure 3.1 **Breeding scheme used to develop the rust resistant parental lines used in this study.** The homozygosity level of each gene is indicated in brackets. The blue colour indicates the work of Sydenham 2007 and the red colour the work of MJP van der Linde (unpublished data). [1]*Sr26* is segregating for Avocet*YrSP;* MAS-marker-assisted selection.

Offspring (F_1) from the two crosses were planted and crossed to develop a double cross population. The double cross offspring were screened using MAS, with markers linked to the stated rust resistance genes/QTL, except for *YrSP,* since no molecular markers were available at that stage. Through MAS lines S16 (*Lr34/Yr18/Sr57, QYr.sgi.2B-1, Lr19* and *Sr2*/*Yr30*) and S726 (*Lr34/Yr18/Sr57, QYr.sgi.2B-1, Lr19, Sr2*/*Yr30* and *Sr26*) were identified as the best genotypes based on the highest number of rust resistance genes present. Seed generated after self-pollinating lines S16 and S726 were planted and seedlings subjected to MAS to determine their genotypes. Offspring were once again self-pollinated to improve their level of homozygosity. Lines S16(7.3) and S726(3.2) were identified as the best lines based on genotypic evaluation. The latter two lines and others were planted for another cycle of self-pollination and MAS application. MAS screening indicated the loss of the *Sr2*/*Yr30* resistance gene in line S726(3.2), which can be attributed to its heterozygous status in the previous generation (Figure 3.1; Sydenham 2007).

As part of another MSc study at the UFS, the best four S726(3.2) offspring lines, based on the highest number of resistant markers present, were crossed with Kingbird (*Lr34/Yr18/Sr57* and *Sr2*/*Yr30*) and the best three S16(7.3) offspring lines were crossed with line 2S#2/163. The offspring (KF_1 and SF_1 respectively) were subjected to MAS. Genotypes with the highest number of rust resistance markers were selected to develop a double cross population. The double cross offspring (KSF_1) were planted, subjected to MAS and the offspring self-pollinated (Figure 3.1; MJP van der Linde, unpublished data).

For the current study, 17 out of 808 KSF_1 lines were selected based on marker data and re-screened with molecular markers linked to rust resistance genes (Figure 3.2). The best three KSF_1 lines were identified based on the highest number of rust resistance genes/QTL present. A total of 63 seeds per selected KSF_1 line were planted over a period of three weeks, using six different planting dates (two per week). The rust resistant lines were used as female lines in a crossing scheme as they were still an early segregating population compared to the more stable male lines. Leaf material was collected from seedlings and used for MAS to determine/confirm each line's rust resistance marker profile and to identify the best lines to be used in crosses.

3.2.1.2 Selection of Fusarium head blight resistant male parental lines

A FHB resistant wheat donor line, CM-82036, was crossed with a South African commercially well adapted wheat cultivar, Krokodil, susceptible to FHB.

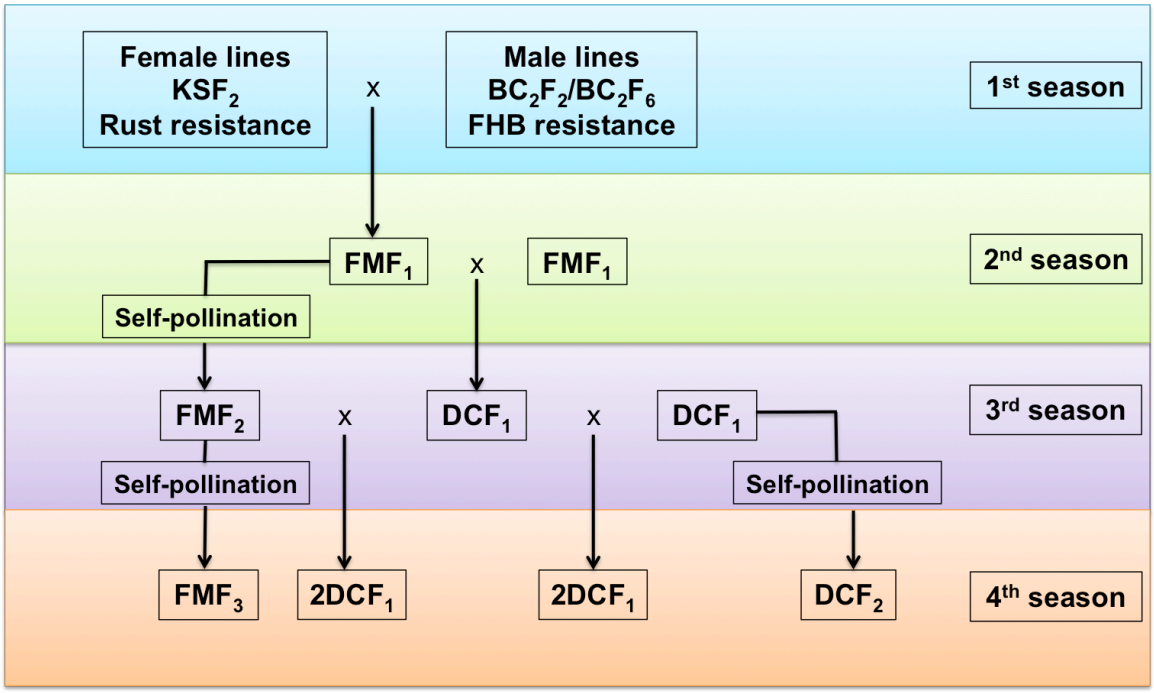


Figure 3.2 Breeding scheme used in this study to combine rusts and Fusarium head blight (FHB) resistance genes/QTL into a single wheat line over four seasons (indicated by different colours).

Offspring were backcrossed twice to Krokodil (recurrent parent) to develop the BC_2F_1 population (Sydenham 2014; Figure 3.3). Offspring were self-pollinated and lines were evaluated with molecular markers linked to FHB resistance genes/QTL (*Fhb1*, *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2*). For the purpose of the current study, the *Qfhs.ifa-5A* QTL was divided into two sub-QTL, as a background marker (Barc165) mapped within the *Qfhs.ifa-5A* QTL (*Qfhs.ifa-5A*) region according to Sydenham (2014; Figure 3.4). Since FHB resistant male lines for the current study were developed from the study of Sydenham (2014), markers linked to both sub-QTL (*Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2*) were used and reported in this study. The percentage of Krokodil background was determined for each BC_2F_2 offspring line. Based on these results, 40 lines were selected to develop a recombinant inbred line (RIL) population up to BC_2F_6 under the guidance of two honours students, SW Meintjies and S Kassier. Offspring were evaluated after every self-pollination cycle using molecular markers linked to FHB resistance QTL.

Based on results obtained by Sydenham (2014), 10 lines of the BC_2F_2 population were selected based on both the highest percentage Krokodil background and number of FHB resistance QTL present.

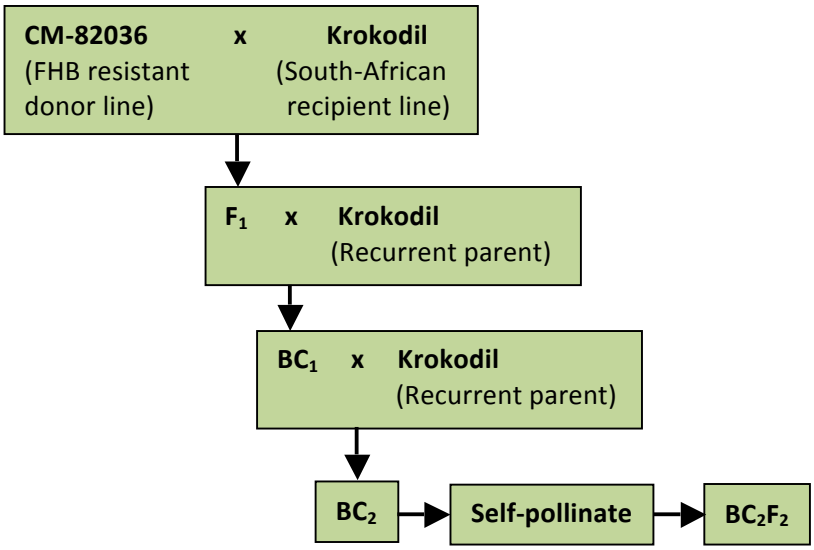


Figure 3.3 **Backcross breeding scheme used by Sydenham (2014) to develop the FHB resistant wheat lines used as male parents in this study.**

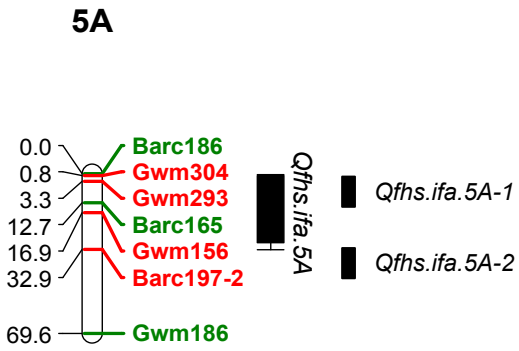


Figure 3.4 **Partial linkage map of targeted chromosome 5A.** Left: distance between markers given in centiMorgans (cM). Right: specific marker and quantitative trait loci (QTL) names; Markers linked to Fusarium head blight (FHB) resistance genes/QTL are indicated in red, mapped polymorphic background markers in green and FHB resistance gene/QTL regions by black bars.

For the current study, the 10 lines were re-screened with molecular markers linked to the FHB resistance QTL. Three of these 10 BC_2F_2 wheat lines were selected to serve as male parents in the crossing scheme (Figure 3.2). As a limited number of seed was available additional lines were selected from the RIL population developed by SW Meintjies and S Kassier. Seven of the BC_2F_6 RILs were selected as additional male parental lines, mainly based on the homozygosity level of markers linked to some QTL (Figure 3.2). A total of 185 seeds were planted using seven different planting dates, six days after the planting date of the female plants to achieve synchronised flowering dates.

3.2.2 Development of wheat lines with combined rusts and Fusarium head blight resistance

The best three rust resistant lines (female parents) were crossed with the best FHB resistant lines (male parents) to develop the FMF_1 population (Figure 3.2). The primary heads of the female parents were emasculated and used for crosses. Before and after emasculation pollinated wheat heads were covered with white paper bags to prevent cross-pollination. Un-emasculated heads were also covered with white paper bags to ensure self-pollination.

Crosses were produced by transferring pollen of the selected male lines to the preferred emasculated female lines based on the best rust resistance marker profile. Male lines were selected based on the highest possible number of genes/QTL present. Only the best female lines according to the highest number of genes/QTL present were used to developed crosses. In total 127 crosses were made between the female and male lines to combine rusts and FHB resistance genes/QTL. Overall, 1 229 FMF_1 seeds were produced and harvested.

3.2.3 Greenhouse growing conditions

All lines used in the crossing schemes were grown in the greenhouse under controlled temperatures ranging between 18˚C to 22˚C. Two to three seeds were planted per two litre pot filled with red garden top soil. Plants were watered daily and once a week fertilised using 100 ml of micro-elements (2.5 g/l HYPERFEED) during the vegetative stage. After four weeks plants were fertilised using 100 ml of a mixture of micro-elements (92.5 g/l HYPERFEED) and macro elements [10 ml/l EFFECTO Wonder Slow Release 3:2:1 (28)] on a weekly basis.

3.2.4 Collection of leaf material

Leaf material, used for DNA extraction, of each female and male line was collected four weeks after planting. Three 10 cm long leaves per plant were cut and placed in a 15 ml plastic tube. Scissors and tweezers used for collecting leaf material were cleaned with 70% (v/v) ethanol between each sample to prevent contamination. Plastic tubes containing leaf samples were placed directly on ice in a cooler box after sampling. Leaf samples were freeze-dried using the Viritis Advantage Freeze Mobile II (New York, NY, USA) for three days and stored at -20˚C.

3.2.5 Genomic DNA isolation for genotyping

For all freeze-dried leaf samples, five 1 cm pieces of freeze-dried material were placed in a 2 ml micro centrifuge tube containing two 5 mm stainless steel balls. All leaf material samples were ground to a fine powder using Qiagen's TissueLyser (Haan, Germany) for 1 min at 30 r/s. A modified hexadecyltrimethylammonium bromide (CTAB) method of Saghai-Maroof et al. (1984) was used for genomic DNA (gDNA) isolation. For every sample, 750 µl CTAB buffer [100 mM Tris-HCl [tris(hydroxymethyl) aminomethane hydrochloride], pH 8.0; 20 mM EDTA (ethylene-diaminetetraacetate) pH 8.0; 1.4 M NaCl (sodium chloride); 2% (w/v) CTAB; 0.2% (v/v) β-mercaptoethanol] was added and mixed until all the dry tissue was completely liquefied. Samples were incubated in a water bath for 1 h at 65˚C and lightly shaken every 15 min. A volume of 500 µl chloroform:isoamylalcohol [24:1 (v/v)] was added to each sample after incubation and mixed, followed by centrifugation for 5 min at 12 000 *g* at 4˚C. After centrifugation, the supernatant of each sample was transferred to a new 1.5 ml micro centrifuge tube containing 500 µl (0.66 volumes) 2-isopropanol. DNA was precipitated for 20 min at room temperature (22˚C) followed by centrifugation for 5 min at 12 000 *g* at 4˚C. The supernatant was discarded and the DNA pellet washed with 500 µl ice cold 70% (v/v) ethanol and incubated for 20 min at room temperature (22˚C). Samples were again centrifuged for 5 min at 12 000 *g* at 4˚C. The supernatant was discarded and the DNA pellets air-dried for 1 h on a bench (22˚C). DNA pellets were resuspended in 200 µl TE (Tris-HCl/EDTA) buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) and 200 µl/ml RNase and incubated for 2 h in a water bath at 37˚C.

After gDNA extraction, samples were run on a 0.8% (w/v) agarose gel to determine the quality of the gDNA. Ethidium bromide (EtBr) was used to visualise the gDNA. Electrophoresis was done at 100 V for 30 min in 1x UNTAN buffer (40 mM Tris-HCl, pH 8.0; 2 mM EDTA pH 8.0; pH was adjusted to pH 7.4 with acetic acid). Gels were visualised under ultra violet light. DNA quantity of each sample was measured using the Helios gamma spectrophotometer (Erlangen, Germany), measuring the absorbance at A_{260}, A_{280} and the A_{260}:A_{280} ratio. All gDNA samples were diluted to a working concentration of 20 ng/µl using 1 x TE buffer, pH 8.0.

3.2.6 Genotyping using polymerase chain reaction

All PCR reactions for genotyping the female and male wheat lines were performed using a DYAD™ (DNA Engine) Peltier Thermal Cycler (Foster City, CA, USA). Female lines were genotyped using nine molecular markers linked to rust resistance alleles (Table

3.1). These markers were selected as they were previously used to genotype the selected female lines and their donor parents.

Male lines were genotyped using five molecular markers linked to different FHB resistance genes/QTL (Table 3.2). All primers were synthesised by Intergrated DNA Technologies Inc (Coralville, IA, USA). Reaction conditions of the molecular markers used are presented in Table 3.3. Only the original six rust resistant parental cultivars (section 3.2.1.1) were included as PCR controls of the female lines while only CM-82036 and Krokodil were included as PCR controls for the male lines. All eight original parental lines were included as PCR controls during genotyping the double cross populations and self-pollinated lines with combined resistance to rusts and FHB. Additionally, molecular markers linked to HMW-GS and the 1BL.1RS translocation were screened (Tables 3.4 and 3.5). These quality markers were only applied in the final populations ($2DCF_1$, DCF_2 and FMF_3; Figure 3.2) of this study as they were not considered when lines were selected to make crosses because selection focussed on resistance genes/QTL.

3.2.7 Visualisation of polymerase chain reactions

3.2.7.1 Polyacrylamide gel electrophoresis

Polymerase chain reaction products for all markers used except products from the multiplex markers Sr39 and BE500705, cssfr5 and Glu-B3j were screened using the GelScan 3000 Real-Time DNA Fragment Analysis system with software version 8.00.01 (Corbett Research, Sydney, Australia). The 5% (w/v) non-denaturing polyacrylamide gel was prepared to a final volume of 25 ml consisting of 1 x TBE (Tris-HCl/Borate/EDTA) buffer (89 mM Trisbase; 89 mM Boric acid; 2 mM EDTA, pH 8.0), 5% AKA (acrylamide:bis-acrylmide; 19:1 w/w), 0.12% (v/v) TEMED (N,N,N',N-tetramethylethylenediamine) and 0.08% (v/v) APS (ammonium persulfate). Polyacrylamide gels were prepared and left to polymerise overnight at room temperature (22˚C). The vertical system consisted of an upper and bottom chamber. The upper chamber buffer contained 0.5x TBE buffer mixed with doubled distilled water (ddH_2O). The bottom chamber buffer contained 0.5x TBE and 1% (v/v) EtBr mixed with ddH_2O. Polymerase chain reaction products were mixed with deionised formamide loading dye [98% (v/v) formamide; 10 mM EDTA, pH 8.0; 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 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 3.1 Molecular markers linked to rust resistance genes/QTL used in this study

Marker name	Targeted trait	Targeted gene/QTL	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
$STSLr19_{130}$	Leaf rust resistance	*Lr19*	CATCCTTGGGGACCTC	CCAGTCGCATACATCCA	Prins et al. 2001
cssfr5	Leaf rust resistance	*Lr34/Yr18/Sr57*	L34SPF: GGGAGCATTATTTTTTTCCATCATG L34DINT9F: TTGATGAAACCAGTTTTTTTTCTA	L34DINT13R2: ACTTTCCTGAAAATAATACAAGCA L34MINUSR: TATGCCATTTAACATAATCATGAA	Lagudah et al. 2009
csSr2	Stem rust resistance	*Sr2*/*Yr30*	CAAGGGTTGCTAGGATTGGAAAAC	AGATAACTCTTATGATCTTACATTTTTCTG	Mago et al. 2011
Sr26#43	Stem rust resistance	*Sr26*	AATCGTCCACATTGGCTTCT	CGCAACAAAATCATGCACTA	Mago et al. 2005
BE518379	Stem rust susceptible	*Sr26*	AGCCGCGAAATCTACTTTGA	TTAAACGGACAGAGCACACG	Liu et al. 2010
Sr39	Stem rust resistance	*Sr39*	AGAGAGAGTAGAAGAGCTGC	AGAGAGAGAGCATCCACC	Gold et al. 1999
BE500705	Stem rust susceptible	*Sr39*	ATCTGTGGCAGTGTGCTCCT	TCCTGCAAATGCTTGTCGTT	Mago et al. 2009

QTL-Quantitative trait loci.

Table 3.2 Molecular markers linked to FHB resistance genes/QTL used in this study

Marker name	Targeted trait	Targeted gene/QTL	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
Umn10	FHB resistance	*Fhb1* (*Qfhs.ndsu-3BS*)	CGTGGTTCCACGTCTTCTTA	TGAAGTTCATGCCACGCATA	Liu et al. 2008b
Gwm156	FHB resistance	*Qfhs.ifa-5A-2*	CCAACCGTGCTATTAGTCATTC	CAATGCAGGCCCTCCTAAC	Röder et al. 1998
Gwm293	FHB resistance	*Qfhs.ifa-5A-1*	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	Röder et al. 1998
Gwm304	FHB resistance	*Qfhs.ifa-5A-1*	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	Röder et al. 1998

FHB-Fusarium head blight; QTL-Quantitative trait loci.

Table 3.3 Polymerase chain reaction conditions for the molecular markers used in this study

Marker name and targeted gene/QTL	Reaction conditions and total reaction volume	PCR cycling conditions	Marker type, controls and fragment sizes
STSLr19$_{130}$ *Lr19*	80 ng DNA 1x KAPATaq ReadyMix DNA polymerase 25 ng Lr19F primer 25 ng Lr19R primer 0.1 mg/ml Bovine serum albumin (BSA) Total reaction volume: 10.1 µl	1 cycle: 3min @ 94˚C, 45 cycles: 1 min @ 94˚C, 1min @ 60˚C, 2 min @ 72˚C, 1 cycle: 10 min @ 72˚C	Dominant marker Resistant control: CS-*Lr19*-149-299 (130 bp) Susceptible controls: Kariega, Avoset*YrSP* and Blade (null allele)
Cssfr5 *Lr34/Yr18/Sr57*	60 ng DNA 1x KAPA2G Fast ReadyMix PCR Kit 25 ng L34SPF primer 25 ng L34DINT9F primer 25 ng L34DINT13R2 primer 25 ng L34MINUSR primer Total reaction volume: 10 µl	1 cycle: 5 min @ 94˚C, 5 cycles: 1 min @ 94˚C, 1 min @ 58˚C, 2 min @ 72˚C, 35 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 marker Resistant controls: Kariega, Avoset*YrSP*, CS-*Lr19*-149-299 (751 bp) Susceptible control: Blade (532 bp)
csSr2 *Sr2/Yr30*	100 ng DNA 1x KAPATaq ReadyMix DNA polymerase 75 ng csSr2F primer 75 ng csSr2R primer Total reaction volume: 20 µl	1 cycle: 3 min @ 93˚C, 40 cycles: 45 sec @95˚C, 45 sec @ 57.6˚C, 45 sec @ 72˚C, 1 cycle: 10 min @ 72˚C	Co-dominant marker Positives and false positives (337 bp)
Sr2/*Yr30* CAPS reaction	1x CutSmart® buffer 10 U *Bsp*HI	Incubate for 60 min @ 37˚C	Resistant controls: Picaflor and Kingbird (172 bp, 112 bp, 53 bp) Susceptible controls: Kariega, Avoset*YrSP*, Blade, CS-*Lr19*-149-299 (225 bp and 112 bp)

Table 3.3 Polymerase chain reaction conditions for the molecular markers used in this study (continued)

Marker name and targeted gene/QTL	Reaction conditions and total reaction volume	PCR cycling conditions	Marker type, controls and fragment sizes
Sr26#43 *Sr26* resistant allele	80 ng DNA 1x KAPATaq ReadyMix DNA polymerase 50 ng Sr26#43F primer 50 ng Sr26#43R primer Total reaction volume: 10 µl	1 cycle: 3 min @ 94˚C, 45 cycles: 45 sec @ 94˚C, 45 sec @ 60˚C, 75 sec @ 72˚C, 1 cycle: 10 min @ 72˚C	Dominant marker Resistant controls: Avocet*YrSP* and Blade (207 bp) Susceptible controls: Kariega and CS-*Lr19*-149-299 (null allele)
BE518379 *Sr26* susceptible allele	60 ng DNA 1x KAPATaq ReadyMix DNA polymerase 50 ng BE518379F primer 50 ng BE518379R primer Total reaction volume: 10 µl	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 marker Resistant controls: Kariega and CS-*Lr19*-149-299 (303 bp) Susceptible controls: Avocet*YrSP* and Blade (null allele)
Sr39 and BE 500705 *Sr39*	60 ng DNA 1x KAPATaq ReadyMix DNA polymerase 50 ng Sr39F primer 50 ng Sr39R primer 25 ng BE500705F primer 25 ng BE500705R primer Total reaction volume: 11 µl	1 cycle: 3 min @ 94˚C, 30 cycles: 30 sec @ 92˚C, 30 sec @ 56˚C, 40 sec @ 72˚C, 1 cycle: 10 min @ 72˚C	Co-dominant marker Resistant control: 2S#/163 (900 bp) Susceptible controls: Kariega, Avocet*YrSP*, Blade and CS-*Lr19*-149-299 (166 bp)

Table 3.3 Polymerase chain reaction conditions for the molecular markers used in this study (continued)

Marker name and targeted gene/QTL	Reaction conditions and total reaction volume	PCR cycling conditions	Marker type, controls and fragment sizes
Umn10 *Fhb1*/*Qfha.ndsu-3BS*	80 ng DNA 1x KAPATaq ReadyMix DNA polymerase 25 ng Umn10F primer 25 ng Umn10R primer Total reaction volume: 10 µl	1 cycle: 3 min @ 94˚C, 35 cycles: 1 min @ 94˚C, 1 min @ 62˚C, 2 min @ 72˚C, 1 cycle: 5 min @ 72˚C	Co-dominant marker Resistant control: CM-82036 (239 bp) Susceptible control: Krokodil (236 bp)
Gwm156 *Qfhs.ifa-5A-2*	80 ng DNA 1x KAPATaq ReadyMix DNA polymerase 0.5 mM $MgCl_2$ (additionally) 25 ng Gwm156F primer 25 ng Gwm156R primer Total reaction volume: 10 µl	1 cycle: 5 min @ 94˚C, 35 cycles: 1 min @ 94˚C, 1 min @ 52˚C, 2 min @ 72˚C, 1 cycle: 10 min @ 72˚C	Co-dominant marker Resistant control: CM-82036 (330 bp) Susceptible control: Krokodil (295 bp)
Gwm293 *Qfhs.ifa-5A-1*	80 ng DNA 1x KAPATaq ReadyMix DNA polymerase 0.5 mM $MgCl_2$ (additionally) 25 ng Gwm293F primer 25 ng Gwm293R primer Total reaction volume: 10 µl	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 marker Resistant control: CM-82036 (207 bp) Susceptible control: Krokodil (174 bp)
Gwm304 *Qfhs.ifa-5A-1*	40 ng DNA 1x KAPATaq ReadyMix DNA polymerase 0.5 mM $MgCl_2$ (additionally) 25 ng Gwm304F primer 25 ng Gwm304R primer Total reaction volume: 10 µl	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 marker Resistant control: CM-82036 (219 bp) Susceptible control: Krokodil (174 bp)

QTL-Quantitative trait loci; PCR-Polymerase chain reaction; BSA-Bovine serum albumin; bp-base pairs; CAPS-Cleaved amplified polymorphic sequences, $MgCl_2$-Magnesium chloride

Table 3.4 Molecular markers linked to protein quality used in this study

Marker name	Targeted trait	Targeted gene/QTL	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
Umn19	HMW-GS	*Ax2**	CGAGACAATATGAGCAGCAAG	CTGCCATGGAGAAGTTGGA	Liu et al. 2008a
BxFp	HMW-GS	*Bx7* and *Bx17*	CGCAACAGCCAGGACAATT	AGAGTTCTATCACTGCCTGGT	Butow et al. 2003
MAR	HMW-GS	$Bx7^{OE}$, *Bx20* and without *Bx7+By8**	CCTCAGCATGCAAACATGCAGC	CTGAAACCTTTGGCCAGTCATGTC	Butow et al. 2004
Umn25	HMW-GS	*Dx2* and *Dx5*	GGGACAATACGAGCAGCAAA	CTTGTTCCGGTTGTTGCCA	Liu et al. 2008a
Umn26	HMW-GS	*Dy10* and *Dy12*	CGCAAGACAATATGAGCAAACT	TTGCCTTTGTCCTGTGTGC	Liu et al. 2008a
Glu-B3j	Translocation	1BL.1RS	GGAGACATCATGAAACATTTG	CTGTTGGGCAGAAAG	Francis et al. 1995

HMW-GS-High-molecular-weight glutenin subunits; QTL-Quantitative trait loci.

Table 3.5 Polymerase chain reaction conditions for molecular markers linked to protein quality used in this study

Marker name and targeted gene/QTL	Reaction conditions and total reaction volume	PCR cycling conditions	Marker type, controls and fragment sizes
Umn19 *Ax2**	80 ng DNA 1x KAPATaq ReadyMix DNA polymerase 25 ng Umn19F 25 ng Umn19R Total reaction volume: 10 µl	1 cycle: 5 min @ 94°C; 35 cycles: 1min @ 94°C; 1min @ 60°C; 2 min @ 72°C; 1 cycle: 5 min @72°C	Co-dominant *Ax2**(+): Kariega and Blade (344 bp) *Ax1; Ax-null* (+): Avocet*YrSP* and CS-*Lr19*-149-299 (362 bp)
BxFp *Bx7* or *Bx17*	40 ng DNA 1x KAPATaq ReadyMix DNA polymerase 75 ng BxF 75 ng BxR Total reaction volume: 10 µl	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* (+): Avocet*YrSP* and CS-*Lr19*-149-299 (750 bp) *Bx17* (+): Kariega and Blade (675 bp)
MAR $Bx7^{OE}$, *Bx20* or without *Bx7+By8**	40 ng DNA 1x KAPATaq ReadyMix DNA polymerase 75 ng MARF 75 ng MARR Total reaction volume: 10 µl	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 $Bx7^{OE}$ (+): Krokodil (560 bp) *Bx7+By8** (-): Kariega and Avocet*YrSP* (523 bp)

Table 3.5 Polymerase chain reaction conditions for molecular markers linked to protein quality used in this study (continued)

Marker name and targeted gene/QTL	Reaction conditions and total reaction volume	PCR cycling conditions	Marker type, controls and fragment sizes
Umn25 *Dx2* or *Dx5*	80 ng DNA 1x KAPATaq ReadyMix DNA Polymerase 25 ng Umn25F 25 ng Umn25R Total reaction volume: 10 µl	1 cycle: 5 min @ 94°C; 35 cycles: 1min @ 94°C; 1min @ 60°C; 2 min @ 72°C; 1 cycle: 5 min @72°C	Co-dominant *Dx2* (+): Kariega and Avocet*YrSP* (299 bp) *Dx5* (+): Kingbird and 2S#/163 (281 bp)
Umn26 *Dy10* or *Dy12*	80 ng DNA 1x KAPATaq ReadyMix DNA Polymerase 25 ng Umn26F 25 ng Umn26R Total reaction volume: 10 µl	1 cycle: 5 min @ 94°C; 35 cycles: 1min @ 94°C; 1min @ 60°C; 2 min @ 72°C; 1 cycle: 5 min @72°C	Co-dominant *Dy12* (+): Kariega and Avocet*YrSP* (415 bp) *Dy5* (+): Kingbird and 2S#/163 (397 bp)
Glu-B3j 1BL.1RS translocation	40 ng DNA 1x KAPATaq ReadyMix DNA Polymerase 75 ng AF1 75 ng AF4 Total reaction volume: 10 µl	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 (1 500 bp) 1BL.1RS translocation (-): Kariega and Avocet*YrSP* (null allele)

QTL-Quantitative trait loci; PCR-Polymerase chain reaction; bp-base pairs.

After the pre-run, 1 µl of the PCR samples mixed with loading dye were loaded on the gel and run at 1 200 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 to determine amplified fragment sizes.

3.2.7.2 Agarose gel electrophoresis

The PCR products of the multiplex marker Sr39 and BE 500705 were separated on a 2% (w/v) agarose gel while the PCR products of markers cssfr5 and Glu-B3j were separated on a 1.5% (w/v) and 1% (w/v) agarose gel, respectively.

PCR products of markers Sr39, BE 500705 and Glu-B3j were mixed with Ficoll loading buffer [15% (w/v) Ficoll, 0.24% (w/v) bromophenol blue], while PCR products of cssfr5 already contain loading buffer present that is present in the 1x KAPA2G Fast ReadyMix PCR Kit used and loaded on the gel and visualised using EtBr. Gels were run at 100 V for 30 min in 1x UNTAN buffer. Fragment sizes were determined by loading a 100 bp DNA ladder (Promega, Madison, WI, USA).

3.2.8 Data analyses

Samples were scored based to the type of molecular marker used. For dominant markers, 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 determining the total number of rust or FHB resistant genes/QTL present per line, heterozygous markers were scored as if the gene/QTL was present, as the resistance allele can be carried over when used in crossing or self-pollination.

Protein quality markers were also screened on the final populations ($2DCF_1$, DCF_2 and FMF_3) of this study and scored using abbreviations of the allele name except for the dominant marker linked to the 1BL.1RS translocation that was scored present (1) or absent (0). Marker Umn19 was used to detect the presence of the HMW-GS alleles on locus *Glu-A* and distinguished between *Glu-Ax2** (2*) or *Glu-Ax1* (1) and *Glu-Ax0* (0). Markers BxFp and MAR amplified alleles present on the *Glu-B* locus. The BxFp marker distinguished between the *Glu-Bx7* (7), *Glu-Bx17* (17) alleles or being heterozygous (7/17). Marker MAR distinguished between alleles of the over expressed *Glu-Bx7* (7^{OE}), lines without *Glu-Bx7*+*By8** (≠) or heterozygous (7^{OE}/≠). Marker Umn25 and Umn26 amplified the HMW-GS alleles on *Glu-D*. Marker Umn25 amplifies *Glu-Dx2* (2), *Glu-Dx5*

(5) or heterozygous (2/5), while marker Umn26 amplifies the *Glu-Dy12* (12), *Glu-Dy10* (10) or heterozygous (12/10).

All molecular marker data analysis was done using Microsoft Excel (version 14.1.0). The percentages calculated and given in the result section were calculated for the specific gene/QTL/allele present in the relevant population or offspring group.

3.2.9 Selection of FMF_1 seed

Based on the genotypes of the female (F) and male (M) lines used in the crosses to produce offspring of the first cross in the breeding scheme (FMF_1 population), 800 FMF_1 seeds were selected and planted. Genotypes of the female lines were given higher priority when the FMF_1 seeds were selected as the female lines (that contributes the rust resistance genes/QTL) had higher levels of segregation compared to the FHB resistant male lines that were more homozygous. Three seeds were planted per pot. Planting dates were set for every Monday and Thursday for three consecutive weeks. A total of 800 selected FMF_1 seeds were planted over a period of three weeks, using six different planting dates (two per week).

Leaf material was sampled for DNA extraction from 765 FMF_1 plants. Genotyping of the FMF_1 plants was done using all rusts and FHB resistant markers. As some genes were heterozygous in some lines used during crosses, it was necessary to determine the genotypes of all FMF_1 plants to be used in the next crosses before crosses were made. The best FMF_1 lines according to their rusts and FHB resistant genotypes were crossed with one another. When certain markers were missing in a female line, a male line carrying this specific marker was selected as the opposite parent in the cross. Higher emphasis was once again placed on selecting female parents with good rust resistance genotypes (still segregating compared to the more homozygous FHB resistance genotypes). The higher level of homozygosity in FHB resistance genotypes increased the number of suitable FHB resistant lines available and made it easier to find a suitable male plant. Most male lines contained all FHB resistance markers as well as the rust resistance marker(s) missing in the female line.

Mainly the primary heads of the female parents were used for emasculation and crosses. Before and after emasculation pollinated wheat heads were covered with white paper bags to prevent cross-pollination. Un-emasculated wheat heads were also covered with white paper bags to ensure self-pollination. Self-pollinated seed (FMF_2) were harvested separately for each individual plant and stored in the germplasm bank at 10˚C. In total 94

double crosses were made and 1 787 double cross (DCF_1) seeds were produced and harvested.

3.2.10 Selection of double cross DCF_1 seed and self-pollinated FMF_2 seed

Based on the genotypes of the FMF_1 plants used to create the DCF_1 population, 513 DCF_1 seeds were planted (Figure 3.2). Only seed produced from the best crosses made, based on the possible gene combinations, were selected. Additionally, three self-pollinated lines (FMF_2-60.2, FMF_2-60.7 and FMF_2-60.9), selected based on the genotypes of the FMF_1 plants, were selected and 84 FMF_2 seeds were planted per line (Figure 3.2). Leaf material from 497 DCF_1 and 244 FMF_2 four week old seedlings were collected for DNA extraction and MAS. All 741 (497 DCF_1 and 244 FMF_2) lines were genotyped using both the rusts and FHB resistance markers. This genotypic data was used to select parental lines for the production of the second double cross population ($2DCF_1$). Mainly the primary heads of the female parents were used for emasculation and crosses. Before and after emasculation pollinated wheat heads were covered with white paper bags to prevent cross-pollination. Un-emasculated wheat heads were also covered with white paper bags to ensure self-pollination. Self-pollinated DCF_2 and FMF_3 seed was harvested and stored in the germplasm bank at 10˚C. In total 44 double crosses were made and 493 $2DCF_1$ seed harvested. Of the 44 double crosses made, 33 crosses were made between DCF_1 x DCF_1 lines while 11 of the double crosses were made between DCF_1 x FMF_2 lines.

3.2.11 Selection of second double cross $2DCF_1$ and self-pollinated seed

After a strict selection process, based on the presence of all markers linked to rusts and FHB resistance, only 80 $2DCF_1$ seeds originating from seven crosses were planted together with seeds from four self-pollinated lines (DCF_1-18.30, DCF_1-50.9, DCF_1-51.16 and FMF_2-60.7.78; Figure 3.2). A total of 25, 15, 15 and 15 seeds were planted from these selected self-pollinated lines, respectively. Leaf material were harvested from 148 four week old seedlings and screened using molecular markers linked to rusts and FHB resistance genes/QTL and to HMW-GS as well as the 1BL.1RS translocation. Wheat heads were covered with white paper bags to ensure self-pollination. Once dried the $2DCF_2$ seed was harvested and stored in the germplasm bank at 10˚C.

3.3 Results

3.3.1 Molecular marker data generated of selected female rust resistant parental lines

The 17 selected KSF_1 lines were re-screened to identify the best lines in terms of the number of rust resistance markers and the level of homozygosity (Table 3.6). The 17 KSF_1 lines were selected as they had none or only one rust resistance gene missing and genes/QTL in a heterozygous state were considered as present as they can still contribute the resistance allele. As expected from a segregating population, co-dominant marker data indicated a high level of heterozygosity in these lines. All 17 KSF_1 lines did contain the *Lr34/Yr18/Sr57* gene (either homozygous resistant or heterozygous). Homozygous susceptible markers were detected in some of the 17 lines for some markers. Markers linked to the *Lr19* and *Sr2*/*Yr30* genes are dominant markers and therefore homozygous resistance alleles could not be differentiated from the heterozygous alleles. No homozygous resistance genes were detected for the *Sr26* and *Sr39* genes. Results indicated that the yellow rust QTL from Kariega, *QYr.sgi.2B-1,* was absent in all 17 offspring. Further investigation of allele composition and segregation ratios indicated that markers linked to this QTL was wrongly scored as present in the S16(7.3) offspring. Silver stained PAGE was used to evaluate offspring in the work done by MJP van der Linde (unpublished data) and it can be more difficult to interpret segregation patterns in gels as polymerisation may result in uneven gels. Fragments amplified by flanking markers, Gwm148 and Gwm501, have a three base pair difference in allele sizes between alleles from Kariega (resistant allele) and alleles from CS*Lr19*-149-299 (susceptible allele). Therefore, uneven gels can influence the scoring of gels. Because results indicated that the QTL was lost in the selected lines the two flanking markers, Gwm148 and Gwm501, were not evaluated further during the study.

Only three KSF_1 lines (KSF_1-159, KSF_1-182 and KSF_1-682) contained all five rust resistance genes (*Lr19*, *Lr34/Yr18/Sr57*, *Sr2/Yr30*, *Sr26* and *Sr39*). Line KSF_1-682 was the only line of the three best selected KSF_1 lines that were heterozygous for the *Lr34/Yr18/Sr57* gene. The remaining 14 KSF_1 lines indicated the loss of one marker while KSF_1-328 lost two rust resistance genes. Since the three selected lines (KSF_1-159, KSF_1-182 and KSF_1-682) were identified as the best lines out of the 17 KSF_1 lines, they were selected as the female parent population contributing rust resistance genes.

Table 3.6 Rust resistance marker data for the KSF_1 lines

Line name	$STSLr19_{130}$ (*Lr19*)	Cssfr5 (*Lr34/Yr 18/Sr57*)	csSr2 (*Sr2*/*Yr 30*)	Sr26#43 & BE518379 (*Sr26*)	Sr39 & BE500705 (*Sr39*)	Gmw148 (*QYr.sgi.2 B-1*)	Gwm501 (*QYr.sgi.2 B-1*)
KSF_1-52	1[1]	1	1	0[2]	0.5	CS[4]	CS
KSF_1-111	1	1	1	0.5[3]	0	CS	CS
KSF_1-126	1	1	1	0.5	0	KB[5]	CS
KSF_1-128	1	1	0	0.5	0.5	KB	CS
KSF_1-138	1	0.5	1	0.5	0	CS	CS
KSF_1-159	1	1	1	0.5	0.5	KB	CS
KSF_1-170	1	1	1	0.5	0	KB	KB
KSF_1-176	1	0.5	0	0.5	0.5	KB	KB
KSF_1-182	1	1	1	0.5	0.5	KB	KB
KSF_1-188	1	0.5	1	0	0.5	CS	CS
KSF_1-198	0	0.5	1	0.5	0.5	CS	CS
KSF_1-290	0	0.5	1	0.5	0.5	CS	CS
KSF_1-328	0	0.5	0	0.5	0.5	KB	KB
KSF_1-386	1	1	1	0.5	0	CS	CS
KSF_1-476	0	0.5	1	0.5	0.5	KB	KB
KSF_1-658	1	0.5	1	0.5	0	CS	CS
KSF_1-682	1	0.5	1	0.5	0.5	KB	KB

[1]Homozygous resistant gene/QTL; [2]Homozygous susceptible gene/QTL; [3]Heterozygous gene/QTL; markers Gmw148 and Gwm501 were scored based on allele composition [4]CS=CS-*Lr19*-149-299; [5]KB=Kingbird. Lines highlighted in grey were selected as the rust resistant female lines.

A total of 185 KSF_2 female wheat lines were obtained from seed planted of lines KSF_1-159, KSF_1-182 and KSF_1-682. These 185 KSF_2 female lines were screened using nine molecular markers linked to rust resistance (Table 3.3). The complete marker data set for these 185 KSF_2 female lines is given in Appendix A. All nine rust resistance markers segregated in the KSF_2 lines. Marker $STSLr19_{130}$ linked to the *Lr19* leaf rust resistance gene is a dominant marker but molecular data of the KSF_2 offspring of the three selected KSF_1 lines indicated that *Lr19* was heterozygously present in the three KSF_1 lines. Due to the dominant nature of this marker the homozygosity level of *Lr19* in these lines could only be determined based on the segregation data of the offspring. Only one line (KSF_1-682) of the selected three KSF_1 lines was heterozygous for the *Lr34/Yr18/Sr57* gene and this was confirmed through segregation in the offspring. All three selected KSF_1 lines contained the resistance allele for *Sr2*/*Yr30* using the dominant cleaved amplified polymorphic sequences (CAPS) marker. Segregation for the *Sr2*/*Yr30* gene in the KSF_2 offspring indicated that the *Sr2*/*Yr30* gene was heterozygous present in the three selected KSF_1 lines. A high number of heterozygous KSF_2 lines were observed for both the *Sr26* and *Sr39* genes. All three selected KSF_1 lines were heterozygous for the *Sr39* gene, as indicated by a 1:2:1 segregation ratio after self-pollination for markers linked to the *Sr39* resistance gene in the KSF_2 offspring. However, although results indicated that the *Sr26* gene was heterozygous in all KSF_1 lines, a distorted segregation was observed. The segregation ratios of the KSF_2 lines for the different rust resistance markers used

are summarised in Table 3.7, where the percentages were calculated including data for all the KSF_2 lines.

The best lines for use in crosses were selected based on the highest number of rust resistance markers present per line. Wheat heads of these lines were emasculated and served as female lines. The highest number of rust resistance genes/QTL detected in the offspring was five, however most lines contained four rust resistance genes/QTL. In total, 127 crosses were made between the female and male lines. Of these 127 crosses made, 32 offspring from line KSF_1-159, 49 offspring from line KSF_1-182 and 46 offspring from line KSF_1-682 were used as female lines.

3.3.2 Molecular data generated of selected male Fusarium head blight resistant parental lines

The 10 FHB resistant lines (three BC_2F_2 and seven BC_2F_6) lines were evaluated with molecular markers linked to the FHB QTL on chromosomes 3B and 5A (Table 3.8). Marker data confirmed higher levels of segregation in the BC_2F_2 compared to BC_2F_6 lines. No heterozygous genotypes were observed in the FHB resistance data of the seven BC_2F_6 lines. For the three BC_2F_2 lines, only line BC_2F_2-39 showed a homozygous resistant genotype for the *Fhb1* gene. Line BC_2F_2-39 was the best line of the three BC_2F_2 lines because it contained both genes/QTL, with *Fhb1* being homozygous resistant and the QTL on chromosome 5A being heterozygous. The other two lines were homozygous susceptible for either one or two markers. The BC_2F_6 population was homozygous for all markers. Three of the seven BC_2F_6 lines (BC_2F_6-4.3, BC_2F_6-6.3 and BC_2F_6-21.1) were homozygous susceptible for *Fhb1*, but all seven BC_2F_6 lines were homozygous resistant for all the markers linked to the FHB resistance QTL on chromosome 5A. Four of the ten lines (BC_2F_2-39, BC_2F_6-10.2, BC_2F_6-12.3 and BC_2F_6-37.1) were selected as the best lines and prioritised in selecting the male populations.

In total 185 male lines were screened with molecular markers linked to FHB resistance genes/QTL (Table 3.2). The complete data set generated from the four FHB resistance molecular markers screened is presented in Appendix B. Data of the offspring from the BC_2F_2 lines showed higher levels of genetic variation due to being segregating lines. Offspring from the BC_2F_6 lines were stable and low levels of segregation were visible for them. Offspring of lines BC_2F_6-37.1, BC_2F_6-10.2 and BC_2F_6-12.3 contained all four FHB resistance markers tested. The segregation ratios of markers linked to the FHB resistance genes/QTL are summarised in Table 3.7.

Table 3.7 Segregation percentages of molecular markers present in the offspring of the different populations

Marker name	Allele status	KSF_2	BC_2F_2/BC_2F_6	FMF_1	DCF_1	FMF_2-60.2	FMF_2-60.7	FMF_2-60.9	$2DCF_1$	DCF_2	FMF_3
$STSLr19_{130}$	R%[1]	62		59	40	35	39	38	35	33	27
	S%[2]	38		41	60	65	61	62	65	67	73
Cssfr5	HR%[3]	81		0	27	29	23	23	50	78	100
	HE%[4]	12		93	51	49	50	50	38	16	0
	HS%[5]	7		7	23	22	27	28	12	6	0
csSr2	R%	70		50	64	79	83	72	79	75	67
	S%	30		50	36	21	17	28	21	25	33
Sr26#43 and BE518379	HR%	2		0	11	6	10	5	37	27	13
	HE%	64		36	53	52	50	50	38	53	67
	HS%	38		64	36	41	40	44	25	20	20
Sr39 and BE 500705	HR%	20		0	4	0	18	0	33	36	100
	HE%	52		62	50	0	61	79	54	47	0
	HS%	28		38	45	100	21	21	13	17	0

Table 3.7 Segregation percentages of molecular markers present in the offspring of the different populations (continued)

Marker name	Allele status	KSF_2	BC_2F_2/BC_2F_6	FMF_1	DCF_1	FMF_2-60.2	FMF_2-60.7	FMF_2-60.9	$2DCF_1$	DCF_2	FMF_3
	HR%		44	0	11	16	17	16	19	13	0
Umn10	HE%		8	78	47	46	46	58	60	64	67
	HS%		48	22	42	38	37	26	21	23	33
	HR%		92	0	35	20	16	33	12	24	0
Gwm156	HE%		0	100	61	70	67	46	69	65	73
	HS%		8	0	4	10	17	21	19	11	27
	HR%		89	0	22	32	14	29	45	58	33
Gwm293	HE%		4	95	49	36	54	25	38	31	27
	HS%		6	5	29	32	32	46	17	11	40
	HR%		89	0	22	32	14	29	45	58	33
Gwm304	HE%		4	95	49	36	54	25	38	31	27
	HS%		6	5	29	32	32	46	17	11	40

[1]R% resistant gene/quantitative trait loci (QTL) present; [2]S% susceptible gene/quantitative trait loci (QTL) present; [3]HR% is the percentage lines that were homozygous resistant for the marker, [4]HE% is the percentage lines that were heterozygous resistant for the marker and [5]HS% is the percentage of lines that were homozygous susceptible for the marker.

Table 3.8 Marker data linked to FHB resistance QTL of the three BC_2F_2 lines and seven BC_2F_6 lines selected as male plants for the crossing scheme

Line name	Umn10 *Fhb1*	Gwm156 *Qfhs.ifa-5A-2*	Gwm293 *Qfhs.ifa-5A-1*	Gwm304 *Qfhs.ifa-5A-1*
BC_2F_2-7	0.5[1]	0	0.5	0.5
BC_2F_2-20	0[2]	0	0.5	0.5
BC_2F_2-39	1[3]	0.5	0.5	0.5
BC_2F_6-4.3	0	1	1	1
BC_2F_6-6.3	0	1	1	1
BC_2F_6-10.2	1	1	1	1
BC_2F_6-12.3	1	1	1	1
BC_2F_6-21.1	0	1	1	1
BC_2F_6-37.1	1	1	1	1

FHB-Fusarium head blight; QTL-Quantitative trait loci; [1]Heterozygous resistant gene/QTL; [2]Homozygous susceptible gene/QTL; [3]Homozygous gene/QTL; Lines highlighted in grey were selected as the best Fusarium head blight (FHB) resistant male lines.

Based on genotypic data generated for each putative FHB resistant parental line as well as pollen availability, lines were selected to pollinate female lines and donate the FHB resistance QTL. The 127 crosses were made using pollen from 41 offspring of line BC_2F_2-39, 22 offspring of line BC_2F_6-37.1, 57 offspring of line BC_2F_6-12.3 and seven offspring of line BC_2F_6-6.3. Offspring from only these four lines could be utilised for crosses as their flowering dates synchronised well with the flowering dates of the female lines. They were also identified as preferred genotypes based on FHB resistance genes/QTL. A total of 800 FMF_1 seed from 83 crosses made were selected and planted to develop the double cross population. Selections were done based on the genotypic profiles of the female and male lines.

3.3.3 Molecular data generated of the FMF_1 lines

From the selected 800 FMF_1 seed planted, 765 germinated and leaf samples were collected for MAS. All 765 FMF_1 lines were screened using the nine markers linked to rust resistance genes/QTL and the four markers linked to the FHB resistance genes/QTL (Appendix C). The segregation ratios off all markers linked to rusts and FHB resistance for all the FMF_1 lines are summarised in Table 3.7.

The FMF_1 lines were genotyped and evaluated to identify the best lines for use in crosses. Lines with the highest number of markers linked to FHB and rust resistance genes/QTL were used to develop a double cross (DCF_1) population. The genotypic data,

identifying the best FMF_1 line considering the number of rust and FHB resistance genes/QTL as well as the presence of the missing gene/QTL in the female line and pollen availability determined which lines were selected to pollinate the female line and thereby also donating the missing gene/QTL in the female line. Due to the homozygous nature of the BC_2F_6 male lines, the FMF_1 offspring mainly segregated and differed based on the rust resistance genes/QTL present and were more similar for FHB resistance genes/QTL.

Marker $STSLr19_{130}$ was not present in CM-82036 or Krokodil (used to develop the male parental lines) and therefore could not be present in a homozygous state in the offspring. The heterozygous state of *Lr19* in the FMF_1 lines was confirmed by a 1:1 (present/absent) segregation ratio in the offspring lines. The *Lr34/Yr18/Sr57* gene was detected in CM-82036 that was used to develop the male lines. However, the *Lr34/Yr18/Sr57* gene was heterozygous in 93% of the FMF_1 lines. This indicated that the *Lr34/Yr18/Sr57* gene was not present in any of the male lines selected and used to develop the FMF_1 lines as no homozygous resistant lines was detected in the offspring. The high percentage of offspring lines containing the *Lr34/Yr18/Sr57* gene correlates with the high percentage (93% homozygous or heterozygous resistant) obtained in the female KSF_2 lines. The *Sr2*/*Yr30* gene was not present in CM-82036 or Krokodil and therefore the male lines could not contribute to the presence of the gene. The 1:1 (present/absent) segregation ratio in the FMF_1 lines indicated that this dominant marker was heterozygous present in the KSF_2 lines. The *Sr26* resistance allele was heterozygous in all FMF_1 lines it was detected in. This correlates with the absence of the *Sr26* stem rust resistance gene in the two cultivars used to develop the male lines. The presence of the stem rust resistance gene *Sr39* decreased in the FMF_1 lines compared to the female lines, due to the absence of this gene in CM-82036 and Krokodil. Umn10 was not present in any of the six rust resistant cultivars and therefore was expected to be heterozygous in the offspring. Marker Gwm156 linked to the *Qfhs.ifa-5A-2* FHB resistance QTL was heterozygous in all FMF_1 offspring. The two flanking markers, Gwm293 and Gwm304, linked to the FHB resistance QTL *Qfhs.ifa-5A-1* showed good co-segregation in the FMF_1 offspring.

Based on genotypic data of the 765 FMF_1 lines, the best lines based on the highest number of rust and FHB resistant genes/QTL were identified and selected for crosses. A total of 94 crosses were made for the production of first double cross population. Spikes not used for crosses were allowed to self-pollinate to produce FMF_2 seed. In total, 1 787 DCF_1 seed was harvested as well as self-pollinated FMF_2 seed. Based on the genotypes

of the FMF_1 lines used during crosses, 513 DCF_1 seeds from 30 crosses were selected and planted. Additionally, seed from three FMF_2 lines (FMF_2-60.2, FMF_2-60.7 and FMF_2-60.9) were also planted based on the putative high number of resistance genes present (selected based on the genotypes of the FMF_1 lines).

3.3.4 Molecular data generated of the DCF_1 lines

Only 494 of the 513 DCF_1 seeds planted germinated and leaf material of seedlings was collected for MAS using 10 markers linked to rusts and FHB resistance. The complete data set of the genotypes of the DCF_1 lines is presented in Appendix D. After MAS the best lines based on the highest number of markers present in each line were selected to develop the second double cross ($2DCF_1$) population. The segregation rations of the markers screened in the DCF_1 population are summarised in Table 3.7.

Considering the molecular data obtained for the DCF_1 offspring segregation between the offspring was observed, but the level of homozygosity (resistant or susceptible) have increased compared to the high level of heterozygous lines obtained in the FMF_1 population for all markers. The increased levels of homozygosity are due to the presence of the same marker in most of the parental lines used to develop the double cross.

A total of 44 crosses were made to develop the second double cross ($2DCF_1$) population for this study. Of these 44 crosses, 33 were made between DCF_1 x DCF_1 lines and 11 between DCF_1 x FMF_2 lines. Spikes not used for crosses were allowed to self-pollinate to produce DCF_2 and FMF_3 seed. In total 493 $2DCF_1$ seed was harvested. Based on the genotypes of the DCF_1 and FMF_2 lines used during crosses, 80 $2DCF_1$ seed from seven crosses were selected and planted. Additionally, seed from three DCF_2 lines (DCF_2-18.30, DCF_2-50.9 and DCF_2-51.16) were also planted based on the putative high number of resistance genes present (selected based on the genotypes of the DCF_1 lines).

3.3.5 Molecular data generated from the FMF_2 lines

A total of 84 FMF_2 seed was planted from each of the following lines: FMF_1-60.2, FMF_1-60.7 and FMF_1-60.9. Leaf material was collected for MAS using molecular markers linked to both rusts and FHB resistance. The complete data set is summarised in Appendix E. Observed segregation in the FMF_2 offspring of these three lines is presented in Table 3.7. Molecular data of the FMF_2-60.2 offspring indicated the absence of *Sr39*. The maximum number of markers linked to resistance alleles in the three FMF_2-llines were as follows: eight markers in four of the FMF_2-60.2 offspring, nine in three offspring of line FMF_2-60.2 and nine in two offspring of line FMF_2-60.9.

Based on molecular data linked to the rusts and FHB resistance genes/QTL of the FMF_2 lines, line FMF_2-60.7.78 was identified as one of the best lines and 15 FMF_3-60.7.78 self-pollinated seeds planted together with selected second double cross self-pollinated $2DCF_1$ seed and DCF_2 seed mentioned in Section 3.3.6.

Leaf material of seedlings was harvested and screened using molecular markers linked to rusts and FHB resistance as well as markers linked to HMW-GS and the 1BL.1RS translocation. Wheat heads were covered with white paper bags to ensure self-pollination.

3.3.6 Molecular data generated from the $2DCF_1$, DCF_2 and FMF_3 lines

Leaf material of 78 (out of 80 seed that germinated) of the second double cross ($2DCF_1$) plants was collected for MAS using molecular markers linked to rusts and FHB resistance genes/QTL. A total of 55 DCF_2 and 15 FMF_3-60.7.78 lines were also screened. These lines were also evaluated with molecular markers linked to HMW-GS and the 1BL.1RS translocation. The complete data set of the $2DCF_1$, DCF_2 and FMF_3 offspring is represented in Appendix F, and the segregation ratios are displayed in Table 3.7.

High levels of segregation were still present in the $2DCF_1$ offspring because of the heterozygous nature of the DCF_1 population it was developed from (Table 3.7). Although homozygous resistance genes/QTL were obtained for some markers in the $2DCF_1$ offspring, improvement of homozygosity levels was difficult to obtain because flowering dates did not synchronise or the genotype with the specific homozygous resistance gene(s)/QTL was not the best genotype when the entire genotypic profile was considered.

Offspring of line $2DCF_1$-20 was homozygous resistant for the *Lr34/Yr18/Sr57* gene as both parental lines used in the cross were homozygous resistant. Offspring of line $2DCF_1$-30 were homozygous resistant for markers Gwm293 and Gwm304 linked to the FHB resistance QTL *Qfhs.ifa-5A-1*. Offspring of line $2DCF_1$-31 was homozygous resistant for *Lr34/Yr18/Sr57* and *Sr2*/*Yr30* while offspring of line and $2DCF_1$-34 was homozygous resistant for *Lr34/Yr18/Sr57*.

As expected, offspring of three DCF_2 lines were still segregating for markers that were heterozygous in the DCF_1 line (Table 3.7). Offspring of line DCF_1-18.30 were homozygous resistant for markers linked to the *Lr34/Yr18/Sr57* gene and the *Qfhs.ifa-*

5A-2 QTL. Offspring of lines DCF_1-50.9 and DCF_1-51.16 were homozygous resistant for *Sr2*/*Yr30* (based on 100% resistance in all offspring) and *Lr34/Yr18/Sr57*, respectively.

The offspring (FMF_3) of line FMF_2-60.7.78 were homozygous resistant for markers linked to *Lr34/Yr18/Sr57* and *Sr39* (Table 3.7). Markers linked to the other genes were still segregating and no homozygous resistant data points were obtained for markers Umn10 and Gwm156.

Molecular data linked to the HMW-GS and 1BL.1RS translocation showed segregation for some of these markers in the $2DCF_1$, DCF_2 and FMF_3 offspring (Table 3.9 and Appendix G). No segregation was observed for markers Umn19 and Glu-B3j linked to *Glu-Ax2** gene and the 1BL.1RS translocation in any of the lines tested. All lines contained the *Glu-Ax2** gene and none the 1BL.1RS translocation. No segregation was observed in the other four HMW-GS markers (MAR, BxFp, Umn25 and Umn26) tested in the DCF_2-18.30 and DCF_2-51.6 offspring. Offspring of line FMF_3-60.7.78 showed only segregation for markers Umn25 and Umn26 that are linked to the HMW-GS *Glu-Dx2* or *Glu-Dx5* and *Glu-Dy10* or *Glu-Dy12*, respectively. Table 3.9 summarises allele compositions and segregation ratios for markers linked to the HWM-GS and the 1BL.1RS translocation for selected $2DCF_1$, DCF_2 and FM_3 offspring. Most of the $2DCF_1$, DCF_2 and FMF_3 offspring were homozygous for *Glu-Bx7* instead of *Glu-Bx17* that was evaluated with marker BxFp. None of the lines contained the *Glu-Bx20* (alternative allele tested with marker BxFp) allele, because the allele was not present in any of the eight original parental lines.

3.3.7 Molecular data generated of repulsion linkage between *Sr2/Yr30* and *Fhb1*

The *Fhb1* and *Sr2*/*Yr30* resistance genes are linked in repulsion phase on the short arm of chromosome 3B and are thus difficult to combine into a single genotype/line. Lines containing both these genes were detected in offspring lines throughout the study screened using molecular markers Umn10 (*Fhb1*) and csSr2 (*Sr2*/*Yr30*). Due to the dominant nature of the csSr2 marker, only the presence or absence of the gene could be determined and not the heterozygosity level. For all populations tested, *Sr2*/*Yr30* was detected in combination with the homozygous resistant *Fhb1* in a small number of lines (Table 3.10). Offspring of lines FMF_1, DCF_2-18.30, DCF_2-51.16 and FMF_3-60.7.78 did not have any individuals containing *Fhb1* in a homozygous state due to the genotypes of the parental lines.

Table 3.9 Allele composition percentages based on markers linked to quality traits for the offspring of populations $2DCF_1$, DCF_2 and FMF_3

Offspring group	Umn19[1]	BxFp			MAR			Umn25			Umn26			1BL.1RS
	Ax2%*	*Bx7%*	7/17%	*Bx17%*	$Bx7^{OE}$%	7^{OE}/ ≠*7+8*%*	≠*Bx7+ By8*%*	*Dx2%*	2/5%	*Dx5%*	*Dy12%*	12/10%	*Dy10%*	Absent%
$2DCF_1$-12	100	100	0	0	38	52	10	100	0	0	100	0	0	100
$2DCF_1$-20	100	100	0	0	0	0	100	0	100	0	0	100	0	100
$2DCF_1$-28	100	100	0	0	15	40	45	30	50	20	30	50	20	100
$2DCF_1$-30	100	100	0	0	0	100	0	0	44	56	0	44	56	100
$2DCF_1$-31	100	57	43	0	0	57	43	0	43	57	0	43	57	100
$2DCF_1$-32	100	100	0	0	0	100	0	0	0	100	0	0	100	100
$2DCF_1$-34	100	67	33	0	0	67	33	0	100	0	0	100	0	100
DCF_2-18.30	100	100	0	0	0	0	100	0	0	100	0	0	100	100
DCF_2-50.9	100	100	0	0	47	33	20	27	67	6	27	67	6	100
DCF_2-51.16	100	100	0	0	0	0	100	0	0	100	0	0	100	100
FMF_3-60.7.78	100	100	0	0	100	0	0	20	60	20	20	60	20	100

[1]Markers were scored as indicated in section 3.2.8.

Table 3.10 Number of lines containing both the *Fhb1* and *Sr2*/*Yr30* resistance genes in the different populations used throughout the study

Populations	*Sr2*/*Yr30*[1] and *Fhb1* (AA)%	*Sr2*/*Yr30* and *Fhb1* (Aa)%	Total
FMF_1	0	41	41
DCF_1	2	30	32
FMF_2-60.2	4	39	43
FMF_2-60.7	10	44	54
FMF_2-60.9	3	48	51
$2DCF_1$	12	47	59
DCF_2-18.30	0	40	40
DCF_2-50.9	13	80	93
DCF_2-51.16	0	27	27
FMF_3-60.7.78	0	33	33
Total	44	429	473

[1]*Sr2/Yr30* can be either homozygous or heterozygous resistant.

The number of lines where *Sr2*/*Yr30* was present in combination with *Fhb1* in a homozygous resistant state was lower (44) compared to the number of lines that contained the *Sr2*/*Yr30* gene and the heterozygous resistant *Fhb1* gene (429). Data generated on offspring of line DCF_2-50.9 indicated that 93% contained both the *Fhb1* and *Sr2*/*Yr30* resistance genes that is much higher compared to the other populations. The presence of both *Fhb1* and *Sr2*/*Yr30* was detected in all populations screened.

3.3.8 Best identified lines with combined rusts and FHB resistance

Different populations with combined rusts and FHB resistance were developed during this study and in every population lines with a high number of resistance markers were identified and/or used in crosses. Five main categories were generated to group the best identified lines with combined rusts and FHB resistance. These five categories are (1) leaf rusts and FHB resistance genes/QTL; (2) stem rusts and FHB resistance genes/QTL; (3) leaf- and stem rusts and *Fhb1* resistance genes/QTL; (4) leaf- and stem rusts and *Qfhs.ifa-5A* resistance genes/QTL and (5) all target genes present. Genes that were heterozygous resistant was grouped as resistant genes/QTL. Table 3.11 summarises the percentages of lines within each category for the different populations developed in this study. A small number of lines were present in each category for all different populations. Three of the populations did not have lines in category 1. The FMF_3 population (the smallest population) did not have any lines in categories 1, 2 or 5. The $2DCF_1$ population contained the highest and population DCF_1 the lowest total percentage of lines present in all five categories.

Table 3.11 Percentages of lines with combined rusts and FHB resistance for the different populations developed during this study

Population	Category 1[1] (%)	Category 2 (%)	Category 3 (%)	Category 4 (%)	Category 5 (%)
FMF_1	3.3	0.7	0.0	1.3	5.2
DCF_1	0.4	0.8	0.0	3.2	2.4
FMF_2	1.2	2.9	0.4	1.2	2.0
$2DCF_1$	0.0	2.6	2.6	2.6	7.7
DCF_2	0.0	1.8	1.8	1.8	7.3
FMF_3	0.0	0.0	6.7	6.7	0.0

FHB-Fusarium head blight; QTL-Quantitative trait loci; [1]The five categories are (1) leaf rusts and FHB resistance genes/QTL; (2) stem rusts and FHB resistance genes/QTL; (3) leaf- and stem rusts and *Fhb1* resistance genes/QTL; (4) leaf- and stem rusts and *Qfhs.ifa-5A* resistance genes/QTL and (5) all target genes present

Table 3.12 summarises the genotypes of the best identified lines based on the number of markers present for the different populations developed. Only the best lines (with the highest possible number of markers) were included. Of the 10 listed populations in Table 3.12, a maximum of eight markers linked to resistance alleles were detected in four populations compared to a maximum of nine markers linked to resistance alleles in the remaining six populations. The highest number (40) of offspring containing nine markers each was detected in the FMF_1 population. The 10 most important lines based on lines with the most homozygous resistance markers are highlighted in grey in Table 3.12. The line with the highest number of homozygous resistance markers present was line DCF_2-51.16.2 with seven. However, line DCF_2-51.16.2 was homozygous susceptible for Umn10 linked to *Fhb1* and heterozygous for *Sr39*. The best lines according to the number of homozygous resistance markers present was obtained from the self-pollinated populations and only one of the ten lines was developed from the $2DCF_1$ population. Only two (FMF_2-60.9.36 and $2DCF_1$-28.3) of the ten lines still contained all nine markers linked to the rusts and FHB resistance genes.

The 10 best lines identified in Table 3.12 are summarised in Table 3.13 together with the genotypes obtained from the quality markers. The data linked to the 1BL.1RS translocation was not included as the translocation was not present in any of the selected lines. Low levels of variation and segregation was observed between these lines based on the quality marker data, indicating the decrease in genetic diversity due to selection. Segregation for quality markers was only observed in line FMF_3-60.7.78.13 for markers Umn25 and Umn26 that differentiate between HMW-GS alleles *Glu-Dx2*+*Dy12* and *Glu-Dx5*+*Dy10*. All lines tested with marker MAR expressed the *Glu-Bx7* allele and only two lines expressed the over-expressed *Glu-Bx7*^{OE} allele as indicated by marker BxFp. Most lines expressed the *Glu-Dx5*+*Dy10* genotype.

Table 3.12 Best lines identified during the study with combined resistance to rusts and Fusarium head blight

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF$_1$ population (nine markers present)									
FMF$_1$-3.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-36.8	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-36.10	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-39.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-40.6	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-40.7	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-44.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-44.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-60.1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-60.7	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-60.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-60.10	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-67.3	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-67.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-67.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-67.13	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-76.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-76.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-77.1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-77.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-77.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-77.6	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_1$-78.3	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5

Table 3.12 Best lines identified during the study with combined resistance to rusts and Fusarium head blight (continued)

Line name	STSLr19$_{130}$ (*Lr19*)	Cssfr5 (*Lr34/Yr 18/Sr57*)	csSr2 (*Sr2*/*Yr30*)	Sr26#43 and BE518379 (*Sr26*)	Sr39 and BE500705 (*Sr39*)	Umn10 (*Fhb1*)	Gwm156 (*Qfhs.ifa-5A-2*)	Gwm293 (*Qfhs.ifa-5A-1*)	Gwm304 (*Qfhs.ifa-5A-1*)
FMF_1-78.6	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-79.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-81.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-81.11	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-81.13	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-81.15	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-87.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-89.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-89.19	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-97.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-99.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.15	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.16	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-106.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1 population (nine markers present)									
DCF_1-18.30	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-19.11	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-35.3	1	0.5	1	1	0.5	0.5	0.5	0.5	0.5
DCF_1-36.17	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-36.20	1	0.5	1	1	0.5	0.5	0.5	1	1
DCF_1-43.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5

Table 3.12 Best lines identified during the study with combined resistance to rusts and Fusarium head blight (continued)

Line name	STSLr19$_{130}$ (*Lr19*)	Cssfr5 (*Lr34/Yr18 /Sr57*)	csSr2 (*Sr2*/*Yr30*)	Sr26#43 and BE518379 (*Sr26*)	Sr39 and BE500705 (*Sr39*)	Umn10 (*Fhb1*)	Gwm156 (*Qfhs.ifa-5A-2*)	Gwm293 (*Qfhs.ifa-5A-1*)	Gwm304 (*Qfhs.ifa-5A-1*)
DCF$_1$-43.10	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_1$-50.2	1	0.5	1	0.5	1	0.5	0.5	0.5	0.5
DCF$_1$-50.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_1$-51.16	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_1$-51.20	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF$_2$-60.2 population (eight markers present)									
FMF$_2$-60.2.42	1	0.5	1	0.5	0	0.5	0.5	1	1
FMF$_2$-60.2.59	1	1	1	0.5	0	0.5	0.5	1	1
FMF$_2$-60.2.62	1	1	1	0.5	0	0.5	0.5	0.5	0.5
FMF$_2$-60.2.80	1	0.5	1	0.5	0	1	0.5	0.5	0.5
FMF$_2$-60.7 population (nine markers present)									
FMF$_2$-60.7.30	1	0.5	1	0.5	1	0.5	0.5	0.5	0.5
FMF$_2$-60.7.34	1	0.5	1	0.5	0.5	1	0.5	0.5	0.5
FMF$_2$-60.7.78	1	1	1	0.5	1	0.5	0.5	0.5	0.5
FMF$_2$-60.9 population (nine markers present)									
FMF$_2$-60.9.36	1	1	1	0.5	0.5	1	0.5	1	1
FMF$_2$-60.9.62	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
2DCF$_1$ population (nine markers present)									
2DCF$_1$-12.4	1	0.5	1	1	1	0.5	0.5	0.5	0.5
2DCF$_1$-12.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-28.3	1	1	1	0.5	1	0.5	0.5	1	1
2DCF$_1$-28.10	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-31.11	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-31.15	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5

Table 3.12 Best lines identified during the study with combined resistance to rusts and Fusarium head blight (continued)

Line name	STSLr19$_{130}$ (*Lr19*)	Cssfr5 (*Lr34/Yr18 /Sr57*)	csSr2 (*Sr2*/*Yr30*)	Sr26#43 and BE518379 (*Sr26*)	Sr39 and BE500705 (*Sr39*)	Umn10 (*Fhb1*)	Gwm156 (*Qfhs.ifa-5A-2*)	Gwm293 (*Qfhs.ifa-5A-1*)	Gwm304 (*Qfhs.ifa-5A-1*)
DCF$_2$-18.30 population (eight markers present)									
DCF$_2$-18.30.4	0	1	1	1	0.5	0.5	1	1	1
DCF$_2$-18.30.6	0	1	1	0.5	0.5	0.5	1	1	1
DCF$_2$-18.30.9	1	1	0	1	1	0.5	0.5	1	1
DCF$_2$-18.30.13	1	1	1	0.5	0	0.5	0.5	1	1
DCF$_2$-18.30.16	0	1	1	0.5	1	0.5	0.5	1	1
DCF$_2$-18.30.18	0	1	1	1	0.5	0.5	0.5	1	1
DCF$_2$-18.30.19	1	1	0	0.5	1	1	0.5	1	1
DCF$_2$-18.30.23	1	1	0	0.5	1	1	0.5	1	1
DCF$_2$-18.30.24	0	1	1	1	1	0.5	0.5	1	1
DCF$_2$-50.9 population (nine markers present)									
DCF$_2$-50.9.1	1	0.5	1	0.5	0.5	1	0.5	1	1
DCF$_2$-50.9.2	1	1	1	0.5	1	0.5	0.5	0.5	0.5
DCF$_2$-50.9.13	1	1	1	0.5	1	0.5	0.5	0.5	0.5
DCF$_2$-50.9.14	1	0.5	1	0.5	1	0.5	0.5	0.5	0.5
DCF$_2$-51.16 population (eight markers present)									
DCF$_2$-51.16.2	1	1	1	1	0.5	0	1	1	1
DCF$_2$-51.16.6	0	1	1	0.5	0.5	0.5	1	1	1
DCF$_2$-51.16.7	1	1	0	1	0.5	0.5	0.5	0.5	0.5
DCF$_2$-51.16.11	1	1	0	1	0.5	1	0.5	1	1
FMF$_3$-60.7.78 population (eight markers present)									
FMF$_3$-60.7.78.1	0	1	1	0.5	1	0.5	0.5	0.5	0.5
FMF$_3$-60.7.78.4	0	1	1	0.5	1	0.5	0.5	1	1
FMF$_3$-60.7.78.12	0	1	1	0.5	1	0.5	0.5	1	1
FMF$_3$-60.7.78.13	1	1	1	0.5	1	0	0.5	1	1

[1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0). The 10 best lines with the highest number of resistance markers in homozygous state are highlighted in light grey.

Table 3.13 The genotypic profile of the ten best identified rusts and FHB resistant lines, including quality marker data

Line name	STSLr19$_{130}$[1]	Cssfr5[2]	csSr2[1]	Sr26#43 & BE518379[2]	Sr39 and BE500705[2]	Umn10[2]	Gwm156[2]	Gwm293[2]	Gwm304[2]	Umn19[3]	MAR[3]	BxFp[3]	Umn25[3]	Umn26[3]
FMF$_2$-60.9.36	1	1	1	0.5	0.5	1	0.5	1	1	NA	NA	NA	NA	NA
2DCF$_1$-28.3	1	1	1	0.5	1	0.5	0.5	1	1	2*	7	7^{OE}	2	12
DCF$_2$-18.30.4	0	1	1	1	0.5	0.5	1	1	1	2*	7	≠	5	10
DCF$_2$-18.30.9	1	1	0	1	1	0.5	0.5	1	1	2*	7	≠	5	10
DCF$_2$-18.30.19	1	1	0	0.5	1	1	0.5	1	1	2*	7	≠	5	10
DCF$_2$-18.30.23	1	1	0	0.5	1	1	0.5	1	1	2*	7	≠	5	10
DCF$_2$-18.30.24	0	1	1	1	1	0.5	0.5	1	1	2*	7	≠	5	10
DCF$_2$-51.16.2	1	1	1	1	0.5	0	1	1	1	2*	7	≠	5	10
DCF$_2$-51.16.11	1	1	0	1	0.5	1	0.5	1	1	2*	7	≠	5	10
FMF$_3$-60.7.78.13	1	1	1	0.5	1	0	0.5	1	1	2*	7	7^{OE}	2/5	12/10

FHB-Fusarium head blight; NA-data not available; [1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0). [3]Markers were scored based on the HMW-GS allele present (2*-*Glu-Ax2**; 7-*Glu-Bx7*; 7OE-over-expressed *Glu-Bx7*; ≠-do not contain *Glu-Bx7+By8**; 2-*Glu-Dx2*; 5-*Glu-Dx5*; 12-*Glu-Dy12*; 10-*Glu-Dy10*).

No data linked to the quality markers was available for line FMF_2-60.9.36 because these markers were only evaluated on the final populations of this study. Line $2DCF_1$-28.3 was the only line apart from line FMF_2-60.9.36 (with no available quality marker data) from the top 10 lines identified that contained all nine markers linked to rusts and FHB resistance.

Although line $2DCF_1$-28.3 contained all markers linked to rusts and FHB resistance it contained the *Glu-Dx2+Dx12* gene that has been linked with weaker dough strength compared to *Glu-Dx5+Dy10*. Fortunately the data indicated that line $2DCF_1$-28.3 also contained the over-expressed *Glu-Bx7* allele that has been linked to stronger dough properties which can make up for the lower dough strength caused by the *Glu-Dx5+Dy10* allele. Although baking tests, to determine the end use quality, will have to be applied in the future, molecular markers linked to the HMW-GS genes could be helpful to select for genes linked to better end use quality properties from an early generation. The usefulness of marker application will however be determined by the genetic diversity for the trait of interest as lower levels of segregation for HMW-GS genes was observed in early generations of this study. This can be attributed to low genetic diversity available in the female (KSF_2) and male (BC_2F_2 and BC_2F_6) parental lines for HMW-GS genes.

The number of resistance genes/QTL per line for the different populations with combined rusts and FHB resistance indicates high selection pressure towards resistance breeding as most lines of the breeding populations of this study contained a higher number of markers linked to resistance alleles.

The number of markers present per line per breeding population is summarised in Figure 3.5. This was expressed as a percentage as the different populations varied in their number of entries and therefore, the selection pressure would not have been clearly demonstrated in the graph due to the low number of lines in the smaller populations. According to Figure 3.5, most of the lines within six populations contained seven markers, while most of the lines within two populations contained six markers and most of the lines within one population contained eight markers. Populations DCF_2 and $2DCF_1$ had the highest percentage of lines containing nine markers, this is possibly because of the strong selection pressure for resistance genes/QTL in the self-pollinated populations of the first double cross and the second double cross population.

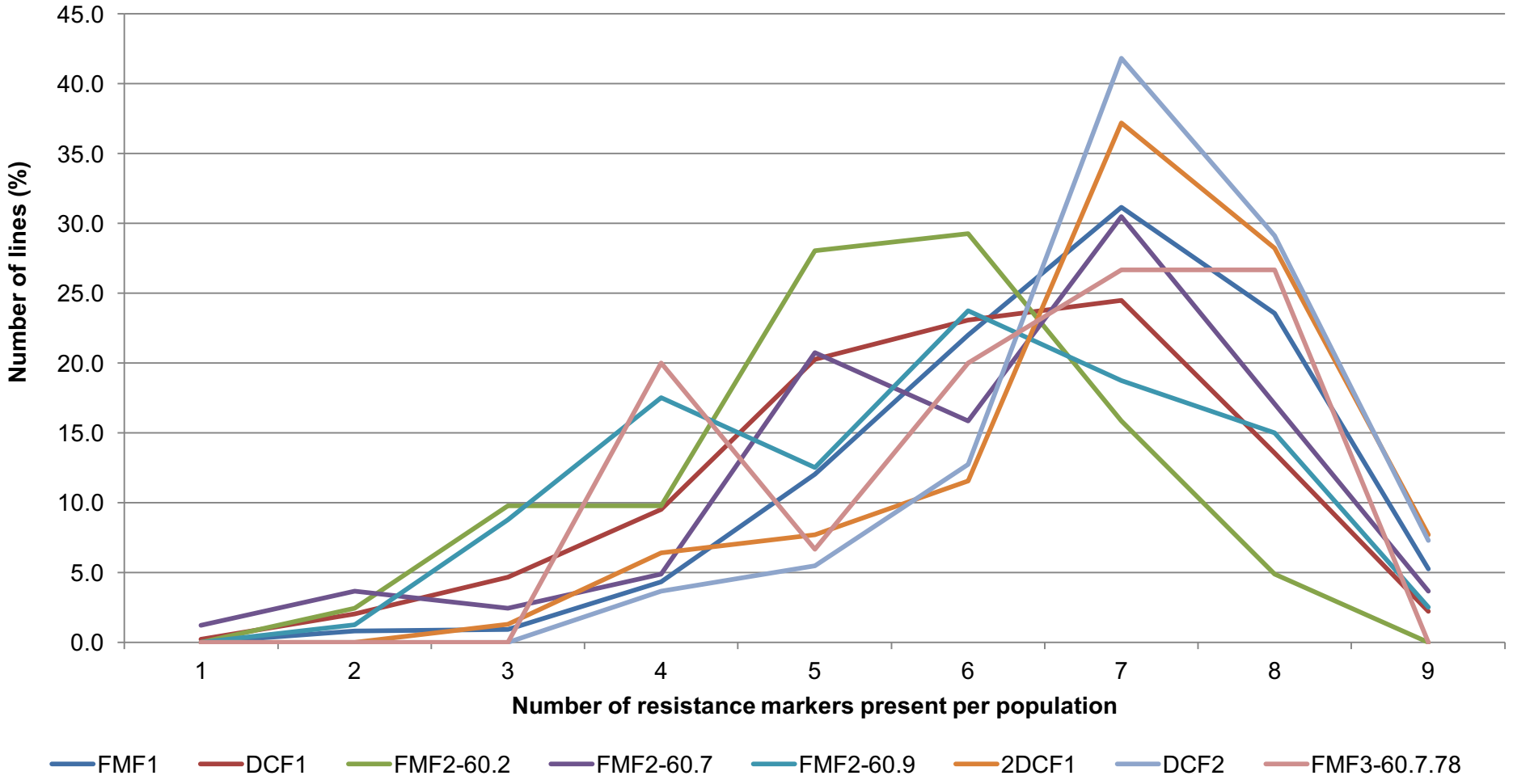


Figure 3.5 Illustration of the selection pressure towards combined rusts and FHB resistance genes/QTL for all different populations developed in this study.

3.4 Discussion

Breeding for durable disease resistance is an ongoing process. Since wheat was domesticated, genetic diversity decreased with a subsequent increase towards disease susceptibility including towards rusts and FHB. Rusts and FHB resistance sources have been identified and used in wheat breeding programmes. Although many of these resistance sources have been overcome through pathogen adaption in the past, new sources of resistance are still being discovered. Combining APR and ASR genes/QTL into a single wheat line can provide durable rust resistance (Roelfs 1988; McIntosh et al. 1995). Adult plant resistance genes providing partial resistance can be difficult to select for under field conditions and are furthermore only visible in adult plants. Marker-assisted selection is a valuable tool to aid breeders in combining resistance genes/QTL into a single cultivar. Two of the most important rust APR genes are *Lr34/Yr18/Sr57* and *Sr2*/*Yr30* that are known for their durable resistance against rust pathogens (Ellis et al. 2014).

The *Lr34/Yr18/Sr57* gene is an ATP-binding cassette (ABC) transporter providing broad-spectrum resistance to *Pt* (leaf rust), *Pgt* (stem rust), *Pst* (stripe rust) and *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal (powdery mildew). The *Lr34/Yr18/Sr57* gene confers partial resistance at the adult plant stage and additional ASR genes need to be added to increase the level of resistance against *Pt*. For example, the leaf rust resistance genes, *Lr19* and *Lr24*, were introgressed from *A. elongatum* and acts as ASR genes and can enhance durable resistance against *Pt* (Singh et al. 2004).

For the current study the ASR gene, *Lr19*, was selected in an effort to develop wheat lines with enhanced durability to leaf rust by combining it with the *Lr34/Yr18/Sr57* gene. The dominant STS molecular marker used to indicate the presence or absence of the *Lr19* gene was found useful in this study despite various wheat genetic backgrounds being combined. Results correspond to results obtained by Singh et al. (2004). A decrease in the number of lines containing the *Lr19* gene was detected from one generation to the next. This decrease can be attributed to the dominant nature of the STS marker and therefore homozygous resistant lines could not be distinguished and favoured for use in crosses over heterozygous lines. Despite of this, most of the top selected lines still contained the *Lr19* gene.

The Cssfr5 microsatellite marker is a co-dominant marker and therefore more lines contained the *Lr34/Yr18/Sr57* gene because informative decisions could be made when selecting parental lines for use in crosses. The co-dominant molecular marker data made

it easy to select the best line as homozygosity levels could be distinguished between lines with the same genotypic profiles. This marker is furthermore a diagnostic marker and no segregation is expected between the marker and the *Lr34/Yr18/Sr57* gene.

The *Sr2*/*Yr30* stem rust resistance gene provides partial resistance to the stem rust pathogens including 'Ug99' races and all their derivatives (Pretorius et al. 2000; Malik et al. 2013). The *Sr2*/*Yr30* gene is still effective since it was first identified and used in the 1920's (McFadden 1930; Yu et al. 2011; Singh et al. 2015). Partial resistance provided by *Sr2*/*Yr30* can be enhanced when it is combined with other effective stem rust resistance genes (Singh et al. 2011a and 2011b). Effective ASR genes include *Sr26* and *Sr39* and were selected in this study to increase resistance and the potential durability thereof against stem rust in the developed wheat lines. Various molecular markers have been developed for rust resistance genes, showing high levels of accuracy. The csSr2 CAPS marker was validated in two other studies with an accuracy level of 95% (Mago et al. 2011; Bernardo et al. 2013). The disadvantage of a CAPS marker is that it is more expensive compared to microsatellites because of the cleaving enzyme. This makes it also more time consuming as an additional cleaving step is added.

One limitation associated with the CAPS marker, csSr2, linked to *Sr2*/*Yr30* is the dominant nature, limiting detection to a mere presence or absence of the gene. However, segregation ratios in the offspring were used to discriminate between homozygous resistant and heterozygous parental lines. *Sr2/Yr30* is a recessive trait and need to be homozygous to be expressed. Only having a dominant marker linked to a recessive trait makes selection more complicated compared to a dominant trait. Although co-dominant markers linked to the *Sr26* and *Sr39* resistance genes were used, most offspring were heterozygous for *Sr26* and *Sr39*. This may be attributed to the fact that within segregating populations it is difficult to find individual lines homozygous for all traits of interest.

As for stripe rust, ASR and APR resistance genes have been identified and used to develop stripe rust resistant wheat cultivars. One such cultivar is Kariega, a South African wheat cultivar developed at the Agricultural Research Council – Small Grain Institute (ARC-SGI), containing the APR QTL, *QYr.sgi.2B-1.* Kariega also contains the *Lr34/Yr18/Sr57* gene and a stripe rust resistance QTL on chromosome 4A, *QYr.sgi-4A.1*. This slow rusting stripe rust QTL *QYr.sgi-4A.1* was first classified as a minor QTL (Ramburan et al. 2004) but other studies revealed that it explains up to 28% of leaf area affected (Prins et al. 2011). Kariega is therefore considered a good source of resistance to use in breeding programmes as it can contribute three slow-rusting QTL against stripe

rust with flanking markers being available to select for these QTL (Agenbag et al. 2014). For the current study only molecular markers linked to the *Lr34/Yr18/Sr57* QTL of the three available slow rusting QTL in Kariega was applied. The presence of the *QYr.sgi.2B-1* QTL was unfortunately wrongly notated during earlier studies in lines selected as female parental lines for the current study. The wrong notation can be attributed to the small base pair differences in the amplified fragments of the two flanking markers Gwm148 and Gwm501 that can be difficult to score using silver stained PAGE. Therefore, none of the lines developed in this study contained the stripe rust resistance QTL, *QYr.sgi.2B-1*. Due to the large number of genes initially targeted to be combined in this study, only markers linked to major QTL were considered and therefore markers linked to the identification of the *QYr.sgi-4A.1* was not included. These markers should however in future be applied on the 10 best selected lines to determine whether the *QYr.sgi-4A.1* QTL is present or absent in these lines.

Avocet*YrSP* was one of the original parental cultivars used to develop the rust resistant wheat lines. This cultivar was selected as it could contribute resistance to stem rust (*Sr26*) and stripe rust (*YrSP*). The *YrSP* gene provides all stage resistance for a wide-range of *Pst* races (Wan and Chen 2014). During the initiation of this study no molecular markers were available to screen for the presence of the *YrSP* gene, and therefore the inheritance and presence of this gene were not followed in the developed populations. In 2015 Feng et al. published a paper identifying three molecular markers that can be used in combination to determine the presence of the *YrSP* gene with a 97.4% accuracy level (Feng et al. 2015). However, after several attempts, these three markers could not be standardised for use in the current study. The *YrSP* gene alone cannot provide durable stripe rust resistance and should be combined with other stripe rust resistance genes. Therefore, the only targeted stripe rust resistance genes in this study were the two *Yr* genes linked to the *Lr34/Yr18/Sr57* and *Sr2*/*Yr30* genes.

With the presence of the destructive ‘Ug99’ stem rust pathogen and its continuous evolved derivatives, resistance breeding towards stem rust and incorporation of effective resistance genes became one of the main priorities for wheat breeders. For this reason, lines developed during this study is relevant as some of them contain both *Sr2/Yr30* and *Sr39* that is effective against ‘Ug99’ and its derivatives. Some of the developed lines carries an additional ASR gene, *Sr26* as well and the *Sr57* gene that is part of *Lr34/Yr18/Sr57*.

Resistance genes/QTL against FHB, caused mainly by *F. graminearum*, act quantitatively and therefore several genes/QTL must preferably be combined to ensure

effective and durable resistance against FHB. Various molecular markers linked to FHB resistance QTL have been developed and used in various breeding programmes worldwide. Miedaner and Korzun concluded in 2012 that a FHB resistance QTL should explain a minimum of 10-20% of the phenotypic variance to be feasible for MAS. Therefore, this study focussed on the two major identified FHB resistance QTL.

The Sumai3 derivative, CM-82036, (contributing the two major FHB resistance QTL) was selected as the donor parent to develop FHB resistant lines in this study. The *Fhb1* resistance QTL is linked to Type I resistance and the QTL located on chromosome 5A, *Qfhs.ifa-5A* is linked to Type II resistance (Waldron et al. 1999; Buerstmayr et al. 2002). The *Fhb1* FHB resistance QTL explains the highest phenotypic variation of all the identified QTL (Buerstmayr et al. 2009) and was therefore considered as the most important FHB resistance QTL for this study.

Molecular markers linked to these FHB resistance QTL were successfully used to evaluate the presence of the QTL in the different populations and to identify the best lines based on their respective genotypes. A near perfect marker, Umn10, was used to screen for the *Fhb1* resistance QTL in the different populations. A 30% increase in lines containing the *Fhb1* QTL was observed from the male population to the second double cross population ($2DCF_1$) compared to a decrease in the number of lines that tested homozygous- and heterozygous resistant for markers linked to *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2*. Four of the selected BC_2F_2 and BC_2F_6 male lines did not contain the *Fhb1* resistance QTL but with the high selection pressure for this QTL it was expected that its presence should increase in consequent progeny. All nine male lines (BC_2F_2 and BC_2F_6) tested positive for the two flanking markers, Gwm293 and Gwm304, linked to *Qfhs.ifa-5A-1.* As expected (due to segregating lines being used for crosses) the number of lines that tested positive for these markers decreased in the next generations. Only two of the nine lines (BC_2F_2 and BC_2F_6) did not test positive for the Gwm156 marker linked to the *Qfhs.ifa-5A-2* QTL, and therefore similar results were obtained as for markers linked to the *Qfhs.ifa-5A-1* QTL, because working with segregating lines a 50-50% chance exist to either gain or loose the trait of interest, therefore a decrease was obtained.

For all populations tested, lines containing all three FHB resistance QTL in combination with rust resistance genes were identified. A total of 66 lines, across all populations develop during this study, were identified that contained the highest possible number of combined rusts and FHB resistance genes/QTL. These 66 lines also contained both the *Sr2*/*Yr30* and *Fhb1* genes that are in repulsion linkage, indicating the strong selection pressure towards FHB and rusts resistance. However, these populations are still

segregating populations and some of the data points obtained for the molecular markers are still heterozygous and therefore the number of lines that contain *Sr2*/*Yr30* and *Fhb1* can decrease. This already became evident as the number of lines that contained the *Sr2/Yr30* gene and the homozygous *Fhb1* gene were significantly lower compared to the number of lines that contained the *Sr2/Yr30* gene and *Fhb1* in a heterozygous state (Table 3.10). But the percentage of lines containing *Sr2/Yr30* and homozygous *Fhb1* did increase from zero in the FMF_1 population to 12% in the $2DCF_1$ population and to 13% in the DCF_2 population. The percentage of lines containing the *Sr2/Yr30* gene and *Fhb1* in a heterozygous state did stay more or less the same from one population to another, with DCF_2-50.9 that contained the highest percentage (80%).

Results further showed that optimised crossing approaches could be successfully implemented to combine a large number of genes/QTL into a single wheat line. The double cross technique improved the level of homozygosity especially when the segregation ratios of the different markers of populations DCF_1 and $2DCF_1$ are compared. Self-pollination also improves the level of homozygosis but can also result in the loss a gene as seen in the FMF_3 population. Therefore, based on results obtained in this study the double cross technique is considered better when aiming to combine a large number of genes into a single line, especially when lines with high levels of homozygous resistance genes/QTL can be selected for use when planning crossing combinations. However, never exclude good self-pollinated lines as they can still contribute value to a breeding programme.

Lines used to make crosses were mainly selected based on the presence of rusts and FHB resistance genes/QTL while quality markers linked to the HMW-GS and the 1BL.1RS translocation was not screened for in all populations. These markers were only evaluated in the final populations ($2DCF_1$, DCF_2 and FMF_3) to determine the genetic diversity for these genes considering bread-making characteristics. The *Glu-Ax2** gene of the *Glu-A* locus was fixed in all populations and the 1BL.1RS translocation that can negatively affect dough strength was not present in any of the populations. Genes at the *Glu-B* and *Glu-D* loci showed variance and segregation in some of the populations. For this study the ten best lines were identified based on the number of homozygous resistance genes/QTL present and their genotypic profile based on the quality markers as provided in Table 3.13. Most lines contained the HMW-GS linked to stronger dough strength, such as *Glu-Ax2**, *Glu-Bx7* and *Glu-Dx5+Dy10*. Molecular markers enable selection for good dough quality characteristics from an early stage in a breeding

programme, especially in segregating populations that cannot be subjected to traditional bread-making quality tests that requires bigger seed samples.

3.5 Conclusions

The main aim of this study was to develop wheat lines with combined durable rusts and FHB resistance. Marker-assisted selection was applied to evaluate and identify the best lines in terms of the number of resistance genes/QTL present. A number of wheat lines were identified with combined leaf-, stem- and stripe rust and FHB resistance. Marker-assisted selection was successfully applied to determine the genotypic profiles of the developed lines, to identify the best lines for targeted crosses to combine rusts and FHB resistance and thereby to achieve the main aim of this study. Unfortunately, the stripe rust resistance QTL *QYr.sgi.2B-1* was not incorporated into these breeding lines. However, other stripe rust resistant sources were present in the genetic background of these populations. The *Yr18* resistance gene forms part of the *Lr34/Yr18/Sr57* gene as well as *Yr30* that is linked to the *Sr2* gene. The *YrSP* and *QYr.sgi-4A.1* resistance gene/QTL might also be present in some of the earlier generation lines as they were present in the original parental cultivars (Avocet*YrSP* and Kariega, respectively) used to develop the rust resistant population.

The genetic diversity of HMW-GS was also determined using MAS and can be used to assist with selection of lines with good baking qualities linked to HMW-GS. Because selection throughout this study was done using only MAS, developed lines should be subjected to phenotypic evaluation to evaluate the expression of the disease resistance genes linked to markers used to identify genotypes as well as bread-making qualities. The best lines identified through this study formed part of early generation pre-breeding populations and therefore high levels of heterozygosity for most genes are still present. These genes might segregate out in future generations following self-pollination. The use of self-pollination to increase the level of homozygosity of the desired traits is a lengthy process that is labour intensive and expensive. To overcome this problem, the best identified lines can be selected for the development of a DH population.

To conclude, the main aim of this study to obtain wheat lines with combined resistance to wheat rusts and FHB was achieved. Molecular markers were successfully applied to determine the genotypic profiles of lines linked to the different genes/QTL selected for during this study. These genotypic profiles were used to determine the best lines to be used to make crosses to develop two double cross populations that delivered lines with combined rusts and FHB resistance. The quality markers linked to HMW-GS and the

1BL.1RS translocation was applied to lines in the final populations and lines with preferred HMW-GS and with combined resistance to FHB and rust were successfully identified.

For future studies, the best identified lines with combined rusts and FHB resistance could be screened using markers linked to the *YrSP* gene (when new markers become available or on successful optimisation of the current markers) and *QYr.sgi-4A.1* QTL to determine whether they are present or not as the original parents did contain these genes/QTL. Lines (F_1) developed from the Avocet*YrSP* and Kariega cross can be evaluated with markers to determine the presence of these two genes/QTL. These identified lines with both *YrSP* and *QYr.sgi-4A.1* can be used in a breeding programme together with the best lines identified in this study to incorporate new and effective stripe rust resistance genes/QTL to further improve the potential durability of stripe rust resistance. The developed lines should also undergo a breeding proses to improve the homozygosity level either by self-pollination or using the DH technique. As lines with improved resistance to rusts and FHB might potentially have a yield and/or baking quality penalties further tests should be done to also evaluate for these characteristics.

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Chapter 4

Development of combined rusts and Fusarium head blight resistance doubled haploid wheat lines

4.1 Introduction

The DH breeding technique helps to speed up cultivar development in breeding programmes of different crop species. It can take up to 15 years before a new wheat cultivar is released. This lengthy process negatively influences the availability and release of seed that is adapted to the changing climate and with resistance to new diseases (Smale et al. 2008). Shortened breeding cycles can help to overcome this problem as new adapted cultivars can be released faster which will support farmers by making improved and adapted wheat cultivars available sooner (Atlin et al. 2017). Different breeding methods have been developed over the years to shorten the breeding cycles of self-pollinated crop species such as wheat. These methods include DH methods, rapid-generation advance and genomic selection (Niu et al. 2014; Arruda et al. 2016; Hickey et al. 2017).

Wheat breeding programmes have been using several of these methods to enhance breeding progress (Dwivedi et al. 2015). Interspecific crosses (wide hybridisation) is a frequently used method to develop DH wheat lines (Niroula and Bimb 2009). Several species were identified and used in different studies to act as pollinators in developing DH wheat lines. These species include barley, sorghum, teosinte, maize and pearl millet (Forster et al. 2007). According to Snape et al. (1986), the DH technique is successful when it meets the following three criteria: (i) efficient production of DH lines of desired genotypes, (ii) random sample representation of parental gametes and (iii) development of genetically stable DH lines. The wide hybridisation maize x wheat system fulfils these criteria and is frequently used to develop DH wheat lines (Karanja et al. 2002; Singh et al. 2004; Niu et al. 2014).

The DH method has been applied in various wheat breeding programmes from which commercialised cultivars were released in many different countries (De Pauw et al. 2011; Săulescu et al. 2012). Using DH technology and MAS in combination will not only shorten the time required for cultivar development but makes it possible to combine a selection of genes/QTL that are stable and constant in the offspring (Mago et al. 2011; Tadesse et al. 2013).

One advantage of a DH breeding programme is the decrease in the number of years to develop and release more relevant cultivars that is resistant to new diseases and adapted to environmental conditions. Higher levels of homozygosity are obtained in DH lines. Another advantage is that it is a useful technique to fix a desirable combination of traits rapidly. The chance of selecting offspring that outperform the parental lines is higher compared to any other breeding technique. One disadvantages of the DH breeding programme is the low effectiveness of DH production due to genotypic dependency of the technique. Segregation distortion is furthermore obtained in the offspring. Hazardous chemicals such as colchicine are used for chromosome doubling and this leads to induction of abnormalities. Limited genetic variation is also obtained in the offspring due to the lack of natural selection (Niemirowicz-Szczytt 1997).

The wide hybridisation wheat x maize method to obtain DH wheat lines is technically difficult as many factors affect the efficiency of DH production. These factors include specific maize and wheat genotypes used, position of the spikelet in the flower, pollination synchronisation, post-pollination treatment, embryo rescue and colchicine treatment (Niu et al. 2014). A higher success rate of haploid development was observed for hexaploid compared to durum wheat (Garcia-Llamas et al. 2004). Winter hexaploid wheat genotypes were reported to be more responsive compared to spring hexaploid genotypes (Sharma et al. 2005). Temperature, light and photoperiod are three environmental factors that have an influence on the efficiency of DH production. These factors are described in detail in a review of the DH production method by Niu et al. (2014).

With so many factors that can have an influence on the success when developing DH lines, optimisation and understanding of the method are necessary for any breeder who wants to apply this method. The aim of this study was to firstly optimise the wide hybridisation wheat x maize DH method in our laboratory. Secondly to apply the DH technique using the pre-breeding lines of the different populations developed in this study to obtain homozygous lines faster without the use of the traditional self-pollination technique.

4.2 Materials and methods

The objectives of the study were to optimise the DH method followed by development of DH lines with combined rusts and FHB resistance. The wheat x maize (pollen donor) wide hybridisation method was selected because of the success rate reported in other breeding programmes.

4.2.1 Selection of wheat plants for optimisation of the doubled haploid procedure

As explained in Chapter 2, section 2.5, the success of the DH technique is linked to genotype and therefore, both rusts and FHB resistance lines were included as these populations were developed using two different breeding schemes. Based on the genotypic data (generated using MAS for both rusts and FHB resistance) which was generated on the parental lines used in the crossing scheme (section 3.2.2), wheat lines with heterozygous resistance genes/QTL were selected for the optimisation of the DH method (Table 4.1).

4.2.2 Optimisation of the doubled haploid method

The DH method, as described by Niu et al. (2014) was used as a starting point to optimise the method for use in this study. A fast growing sweet corn cultivar (Early Sunglow) was selected as the pollinator. This cultivar also produces a high amount of pollen. Seed of Early Sunglow was planted ten days earlier than seed of the wheat lines to synchronise flowering dates. Two sweet corn seeds were planted per pot. Planting dates were set for every Monday and Thursday for four consecutive weeks. Maize seed was planted in 20 l pots filled with red garden top soil, wheat seed was planted as described in section 3.2.3. Pots were watered and fertilised as described in section 3.2.3. Maize plants were grown in the greenhouse under controlled temperatures between 20˚C to 24˚C.

Selected wheat heads were emasculated before their pollen was viable and covered with white paper bags to prevent cross-pollination from other wheat lines. After three to five days, emasculated wheat heads were pollinated with pollen collected from the sweet corn plants. Pollinated wheat heads were again covered with a white paper bag. Crosses were made in the early mornings and late afternoons when fresh pollen is produced by the sweet corn plants. Twenty-four hours after pollination, wheat heads were sprayed with 213.05 mg/l (w/v) 2,4-Dichlorophenoxyacetic acid [(2.4-D), pH 10.36; Sigma, Johannesburg, RSA] and covered with white paper bags for two weeks.

After two weeks, pollinated wheat heads were cut off for rescuing of embryos in the laboratory. Individual seed per wheat head were sterilised in 70% (v/v) ethanol for 1 min and rinsed three times with ddH_2O.

Table 4.1 Genotypic data of lines selected for optimisation of the doubled haploid technique

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 & BE518379 (*Sr26*)[2]	Sr39 & BE500705 (*Sr39*)[2]	Gwm148 (*QYr.sgi.2 B-1*)	Gwm501 (*QYr.sgi.2 B-1*)	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
BC_2F_2-7.2								0.5	0	0	0
BC_2F_2-20.5								0.5	0	0	0
BC_2F_2-39.9								0.5	1	0	0
BC_2F_2-39.1								0.5	0	0.5	0.5
BC_2F_2-39.7								0.5	1	1	1
BC_2F_2-7.5								1	0	0.5	0.5
BC_2F_2-39.5								0.5	0	0	0
BC_2F_2-7.3								0.5	0	0.5	0.5
BC_2F_2-20.4								0.5	0	0	0
BC_2F_2-39.11								0.5	1	1	1
BC_2F_2-39.1								0.5	0	0.5	0.5
BC_2F_2-7.4								0.5	0	0.5	0.5
BC_2F_2-39.2								0.5	1	0.5	0.5
BC_2F_2-39.12								0	1	0.5	0.5
BC_2F_2-39.5								0.5	0	0	0
BC_2F_2-20.2								0	0	0.5	0.5
BC_2F_2-20.2								0	0	0.5	0.5
BC_2F_2-39.9								0.5	1	0	0
BC_2F_2-7.2								0.5	0	0	0
BC_2F_2-20.6								0.5	0	0	0
BC_2F_2-7.2								0.5	0	0	0
BC_2F_2-20.4								0.5	0	0	0
BC_2F_2-39.5								0.5	0	0	0
BC_2F_2-39.9								0.5	1	0	0
BC_2F_2-20.2								0	0	0.5	0.5
BC_2F_2-20.6								0.5	0	0	0
BC_2F_2-7.3								0.5	0	0.5	0.5
BC_2F_2-20.5								0.5	0	0	0

Table 4.1 Genotypic data of lines selected for optimisation of the doubled haploid technique (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 & BE518379 (*Sr26*)[2]	Sr39 & BE500705 (*Sr39*)[2]	Gwm148 (*QYr.sgi.2 B-1*)	Gwm501 (*QYr.sgi.2 B-1*)	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
BC_2F_2-20.2								0	0	0.5	0.5
BC_2F_2-20.6								0.5	0	0	0
BC_2F_2-20.4								0.5	0	0	0
BC_2F_2-20.5								0.5	0	0	0
BC_2F_2-39.12								0	0.5	0.5	0.5
BC_2F_2-20.2								0	0	0.5	0.5
KSF_2-182.60	0	1	0	0.5	0.5	KB[3]					
KSF_2-159.2	0	1	1	0	0.5	KB	CS[4]				
KSF_2-682.20	0	0	1	0.5	0.5	KB					
KSF_2-182.23	0	1	1	0	0.5	KB					
KSF_2-682.6	0	0.5	0	0.5	0	KB					
KSF_2-159.28	0	1	0	0	0.5	KB	CS				
KSF_2-159.35	0	1	0	0.5	0.5	KB	CS				
KSF_2-182.54	0	1	0	0	0.5	KB					
KSF_2-682.62	0	1	0	0.5	0	KB					
KSF_2-682.62	0	1	0	0.5	0	KB					
KSF_2-159.14	1	1	0	0.5	0	KB	CS				
KSF_2-182.38	1	1	0	0	0.5	KB					
KSF_2-182.32	1	1	0	0	0.5	KB					
KSF_2-159.27	0	1	1	0	0.5	KB					
KSF_2-682.49	1	0	0	0.5	0.5	KB					
KSF_2-182.29	0	1	0	0.5	0	KB					

[1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0); Markers Gmw148 and Gwm501 were scored based on allele composition ([3]KB-Kingbird; [4]CS-CS*Lr19*-149-299). Null-alleles were amplified for in line 2S#/163 (marker Gwm148) and for marker Gwm501 in Kingbird and 2S#/163.

Each seed set was cut open, embryos rescued and cultured on MS Basal medium [4.4 g/l MS Basal medium (w/v); 3.4 g/l Phytagel (w/v); 30 g/l sucrose (w/v); Sigma, Johannesburg, RSA]. The pH of the MS basal medium was adjusted to 5.8 using 2-(N-morpholino) ethanesulfonic acid (MES) buffer [19.5 g/l MES (w/v); pH 6.3; Sigma, Johannesburg, RSA]. The MS basal medium was autoclaved for 30 min at 121˚C and 0.075% (v/v) plant preservative mixture (PPM; Plant Cell Technology, Washington, DC, USA) was added. The cultured embryos on the MS basal medium were placed in the dark for 2-8 days at room temperature (22˚C) to stimulate germination. Germinated embryos were placed at continuous room temperature (22˚C) under lights to stimulate plant growth. After plants were 5-6 cm tall, they were replanted in seedling trays filled with potting soil and placed in the greenhouse with temperatures between 18˚C to 22˚C. Seedlings were watered daily and fertilised weekly using microelements (2.5 g/l HYPERFEED). After three weeks, plants were taken out of the soil and treated with a colchicine solution [0.45 g/l (w/v) colchicine; Sigma, Johannesburg, RSA; 20 ml/l (v/v) Dimethyl sulfoxide (DMSO); Sigma, Johannesburg, RSA; 100 mg/l (w/v) Gibberellic acid (GA_3) 100 mg/l (w/v); Sigma, Johannesburg, RSA; 0.3 ml/l (v/v) Tween 80; Sigma, Johannesburg, RSA; pH 5.5] at room temperature (22˚C) for eight hours. Airflow was supplied during the colchicine treatment and plants were covered with a container to keep out any light. The plants' roots were rinsed overnight using tap water and replanted the next morning in the greenhouse in 2 l pots filled with red garden top soil. Plants were watered daily and fertilised as described in section 3.2.3. When heading was reached heads were covered with white paper bags to prevent cross-pollination. Seed was harvested per individual plant and stored in the germplasm bank at 10˚C.

Six DH_1 seeds per seed producing DH_1 plant were planted in the greenhouse and leaf material collected for MAS to determine whether they were true DH or self-pollinated offspring. Leaf material collected from these plants was evaluated using molecular markers linked to rusts and FHB resistance genes/QTL described in section 3.2.6. Additionally two flanking markers linked to the stripe rust resistance QTL, *QYr.sgi.2B-1*, were screened as these markers are co-dominant and amplified different allele sizes in the control lines. Therefore they can indicate heterozygosity in offspring lines and thereby identify self-pollinated lines. The additional two molecular markers and their PCR conditions are summarised in Tables 4.2 and 4.3. Markers were evaluated as described in sections 3.2.7 and 3.2.8.

Table 4.2 Molecular markers linked to the *QYr.sgi.2B-1* stripe rust resistance QTL

Marker name	Targeted trait	Targeted gene/QTL	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
Gwm148	Stripe rust resistance	*QYr.sgi.2B-1*	GTGAGGCAGCAAGAGAGAA	CAAAGCTTGACTCAGACCAAA	Röder et al. 1998
Gwm501	Stripe rust resistance	*QYr.sgi.2B-1*	GGCTATCTCTGGCGCTAAAA	TCCACAAACAAGTAGGCGCC	Röder et al. 1998

QTL-Quantitative trait loci.

Table 4.3 Polymerase chain reaction conditions for molecular markers linked to the *QYr.sgi.2B-1* stripe rust resistance QTL

Marker name and targeted gene/QTL	Reaction conditions and total reaction volume	PCR cycling conditions	Marker type, controls and fragment sizes
Gwm148 *QYr.sgi.2B-1*	80 ng DNA 1x KAPATaq ReadyMix DNA polymerase 25 ng Gwm148F primer 25 ng Gwm148R primer Total reaction volume: 10 µl	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 marker Resistant control: Kariega (165 bp) Susceptible controls: AvocetYrSP (145 bp), Blade (142 bp) CS-*Lr19*-149-299 (162 bp)
Gwm501 *QYr.sgi.2B-1*	100 ng DNA 1x KAPATaq ReadyMix DNA polymerase 75 ng Gwm501F primer 75 ng Gwm501R primer 0.1 mg/ml BSA Total reaction volume: 20 µl	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 marker Resistant control: Kariega (177 bp) Susceptible controls: AvocetYrSP (168 bp), Blade (165 bp) CS-*Lr19*-149-299 (174 bp)

PCR-Polymerase chain reaction; QTL-Quantitative trait loci; BSA-Bovine serum albumin; bp-base pairs.

Different allele sizes were detected for all eight parental control lines for flanking markers Gwm148 and Gwm501 linked to the stripe rust QTL, *QYr.sgi.2B-1,* and therefore abbreviations of the parental lines were used to score the specific alleles from each of these lines [Kariega (KA), Avocet*YrSP* (AV), Blade (BL), CS-*Lr19*-149-299 (CS), Kingbird (KB), 2S#/163 (2S#), CM-82036 (CM) and Krokodil (KR)]. Wheat heads were covered with white paper bags to prevent cross-pollination. Matured seed was harvested and stored in the germplasm bank at 10˚C.

4.2.3 Application of the doubled haploid procedure on selected wheat lines

The DH technique was applied to selected entries of the DCF_1 and FMF_2 offspring developed as described in Chapter 3. A total of 29 interspecific crosses were made between maize and DCF_1 or FMF_2 wheat lines (Table 4.4). The procedure described in section 4.2.2 was followed to produce haploid embryos and DH wheat plants. Seventy-six wheat embryos were rescued, of which 59 germinated. Thirty-one rescued embryos developed into seedlings while the rest formed calli. Seven haploid seedlings did not survive the replanting or greenhouse conditions. Colchicine treatment was applied to 24 haploid seedlings. All 24 colchicine treated seedlings were planted in the greenhouse in 2 l pots filled with red garden top soil. Plants were watered and fertilised as described in section 3.2.2. Wheat heads were covered with white paper bags to prevent cross-pollination. DH_1 seed was harvested and stored in the germplasm bank at 10˚C. In total 1 472 DH_1 seed was harvested from 24 DH_1 lines. Four seeds per DH_1 line were planted in the greenhouse for genotyping using markers linked to rusts and FHB resistance as well as markers linked to HMW-GS and the 1BL.1RS translocation as summarised in Tables 3.1, 3.2, 3.4 and 4.2.

4.3 Results

4.3.1 Molecular data generated from doubled haploid rust and FHB resistant lines

A total of 50 interspecific crosses were made between maize and wheat. Of the 50 interspecific crosses, 34 were made using the FHB resistant parental lines and 16 using the rust resistant lines. A total of 64 embryos were rescued of which 29 were transparent and did not germinate. Only 15 of the remaining 35 embryos germinated and subsequent seedlings planted in seedlings trays. Seven of the 15 seedlings did not survive the greenhouse conditions. The surviving eight seedlings underwent colchicine treatment of which only three produced seed.

Table 4.4 Genotypic data of lines selected for application of the doubled haploid technique

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 & BE518379 (*Sr26*)[2]	Sr39 & BE500705 (*Sr39*)[2]	Gwm148 (*QYr.sgi.2 B-1*)	Gwm501 (*QYr.sgi.2 B-1*)	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-34.4	1	1	1	1	0.5	KB[3]	CS[4]	0	1	1	1
DCF_1-12.8	0	0	1	0.5	0	KB/KR[5]	CS/KR	1	1	0.5	0.5
FMF_2-60.9.64	1	0.5	1	0.5	0.5	KB	CS	0	0.5	1	1
DCF_1-36.9	0	0	1	1	0.5	KB	CS	1	1	0	0
DCF_1-50.4	1	1	0	0.5	0.5	KB	CS	1	0.5	0.5	0.5
FMF_2-60.7.84	1	0.5	1	0.5	1	KR	KR	0.5	0.5	0	0
DCF_1-21.5	1	1	1	0	0.5	KB	CS	0	0.5	0	0
DCF_1-18.23	1	1	1	0.5	0.5	KB	CS	0.5	0.5	0	0
DCF_1-40.10	0	0	0	0.5	0.5	KB	CS	0.5	0.5	1	1
DCF_1-51.4	0	0	0	0.5	0.5	KB	CS	1	1	1	1
FMF_2-60.7.82	1	0.5	0	1	0	KR	KR	1	0.5	1	1
FMF_2-60.2.67	1	1	0	0	0	KB/KR	CS/KR	1	0.5	1	1
FMF_2-60.9.66	0	0	0	0	0	KB/KR	CS/KR	1	1	1	1
DCF_1-48.15	1	1	1	0.5	0	KR/CM[6]	KR/CM	0.5	0.5	0.5	0.5
DCF_1-48.4	0	0.5	0	0	0.5	KR	KR	1	0.5	1	1
DCF_1-48.2	0	1	0	0	0.5	CM	CM	0.5	0.5	0.5	0.5
FMF_2-60.9.52	1	1	1	0	0.5	KR	KR	0.5	1	1	1
DCF_1-40.14	1	0.5	1	0.5	0.5	KR	KR	0.5	0.5	0.5	0.5
DCF_1-43.3	0	0	1	0.5	0.5	KR	KR	0.5	1	1	1
DCF_1-43.5	1	1	1	0.5	0.5	KB	CS	0.5	0.5	0.5	0.5

Table 4.4 Genotypic data of lines selected for application of the doubled haploid technique (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 & BE518379 (*Sr26*)[2]	Sr39 & BE500705 (*Sr39*)[2]	Gwm148 (*QYr.sgi.2 B-1*)	Gwm501 (*QYr.sgi.2 B-1*)	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF$_1$-43.5	1	1	1	0.5	0.5	KB	CS	0.5	0.5	0.5	0.5
DCF$_1$-43.9	1	1	1	0	0	KR	KR	0.5	0.5	0.5	0.5
DCF$_1$-43.10	1	1	1	0.5	0.5	KR	KR	0.5	0.5	0.5	0.5
DCF$_1$-53.12	1	0	1	0.5	0.5	KR	KR	0.5	0.5	0.5	0.5
DCF$_1$-50.15	1	0	0	0	0	KR	KR	1	0.5	0.5	0.5
DCF$_1$-50.9	1	0.5	1	0.5	0.5	KR	KR	0.5	0.5	0.5	0.5
DCF$_1$-50.27	1	0.5	0	0.5	0.5	KB	CS	1	0.5	0.5	0.5
DCF$_1$-42.16	0	0.5	1	0.5	0.5	KB	CS	0.5	1	0.5	0.5
FMF$_2$-60.2.65	0	0.5	1	0.5	0	KB/KR	CS/KR	0.5	0.5	1	1

[1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0); Markers Gmw148 and Gwm501 were scored based on allele composition ([3]KB-Kingbird; [4]CS-CS*Lr19*-149-299; [5]KR-Krokodil; [6]CM-CM-82036). Null-alleles were amplified for in line 2S#/163 (marker Gwm148) and for marker Gwm501 in Kingbird and 2S#/163.

These three plants developed from embryos rescued from the male plants BC_2F_2-39.2 (DH_1-FHB22.3), BC_2F_2-39.12 (DH_1-FHB23.8) and BC_2F_2-7.2 (DH_1-FHB29.1). Lines were named according to a system where DH_1 indicates the first generated plant of an interspecific cross using the FHB resistant wheat lines. The number following FHB is the interspecific cross number and the number after the point indicates the rescued embryo number for the specific cross.

DH_1-FHB22.3 produced 16 seeds, DH_1-FHB23.8 11 seeds and DH_1-FHB29.1 156 seeds. Six seeds per DH_1 line were planted for genotyping to determine the homozygosity level of the lines. Although these lines were the male parental lines, containing only the FHB resistance genes/QTL, molecular markers linked to rust resistance genes/QTL were also included to ensure better coverage of the genome for determining the level of homozygosity. The genotypic data is summarised in Table 4.5. Genotypes for the FHB resistance genes/QTL of the male lines are also displayed in Table 4.5. No rust resistance genes/QTL were found present in the tested DH lines as expected since they were absent in the male lines. Most of the male lines selected for optimisation of the DH procedure were offspring of three BC_2F_2 lines. These three lines were selected based on genotype variation and the presence of heterozygous markers. A small number of rust resistant lines were selected due to poor synchronisation of flowering dates between the maize and rust resistant wheat lines. No DH plants or seed were obtained from the rust resistant wheat lines.

The DH offspring lines of DH_1-FHB22.3 and DH_1-FHB23.8 showed no genetic variation for markers evaluated and no heterozygous markers were detected. Small amounts of seed were also obtained for these two lines. Although these lines did not have good FHB resistance genotypes, markers indicated that the offspring lines were homozygous for all markers tested. Variation was however detected in offspring of line DH_1-FHB29.1 for the three markers linked to the *Fhb1* and *QYr.sgi.2B-1* gene/QTL regions. These results indicated that self-pollination must have occurred during crossing. The large number of seed (156) that was harvested from the DH_1-FHB29.1 line also indicated the possibility of self-pollination. Results thus indicated that only two of the three putative DH wheat lines were true DH lines.

4.3.2 Molecular data generated from doubled haploid DCF_1 and FMF_2 lines

After successful optimisation of the DH technique and the generation of DH lines the technique was applied to specifically selected DCF_1 and FMF_2 lines generated during the pre-breeding programme described in Chapter 3.

Table 4.5 Genotypic data of the DH lines developed from three Fusarium head blight resistant lines

Line name	STSLr19$_{130}$ (*Lr19*)	Cssfr5 (*Lr34/Yr 18/Sr57*)	csSr2 (*Sr2/Yr30*)	Sr26#43 & BE518379 (*Sr26*)	Sr39 & BE500705 (*Sr39*)	Gwm148 (*QYr.sgi.2B-1*)	Gwm501 (*QYr.sgi.2B-1*)	Umn10 (*Fhb1*)	Gwm156 (*Qfhs.ifa-5A-2*)	Gwm293 (*Qfhs.ifa-5A-1*)	Gwm304 (*Qfhs.ifa-5A-1*)
BC_2F_2-39.2								0.5[1]	1[3]	0.5	0.5
DH_2-FHB22.3.1	0[2]	0	0	0	0	CM/CM[4]	CM/CM	0	1	1	1
DH_2-FHB22.3.2	0	0	0	0	0	CM/CM	CM/CM	0	1	1	1
DH_2-FHB22.3.3	0	0	0	0	0	CM/CM	CM/CM	0	1	1	1
DH_2-FHB22.3.4	0	0	0	0	0	CM/CM	CM/CM	0	1	1	1
DH_2-FHB22.3.5	0	0	0	0	0	CM/CM	CM/CM	0	1	1	1
DH_2-FHB22.3.6	0	0	0	0	0	CM/CM	CM/CM	0	1	1	1
BC_2F_2-39.12								0	1	0.5	0.5
DH_2-FHB23.8.1	0	0	0	0	0	KR/KR[5]	KR/KR	0	1	1	1
DH_2-FHB23.8.2	0	0	0	0	0	KR/KR	KR/KR	0	1	1	1
DH_2-FHB23.8.3	0	0	0	0	0	KR/KR	KR/KR	0	1	1	1
DH_2-FHB23.8.4	0	0	0	0	0	KR/KR	KR/KR	0	1	1	1
DH_2-FHB23.8.5	0	0	0	0	0	KR/KR	KR/KR	0	1	1	1
DH_2-FHB23.8.6	0	0	0	0	0	KR/KR	KR/KR	0	1	1	1
BC_2F_2-7.2								0.5	0	0	0
DH_2-FHB29.1.1	0	0	0	0	0	KR/KR	KR/KR	0	0	0	0
DH_2-FHB29.1.2	0	0	0	0	0	CM/KR	CM/KR	0.5	0	0	0
DH_2-FHB29.1.3	0	0	0	0	0	KR/KR	KR/KR	0	0	0	0
DH_2-FHB29.1.4	0	0	0	0	0	KR/KR	KR/KR	0	0	0	0
DH_2-FHB29.1.5	0	0	0	0	0	CM/KR	CM/KR	0.5	0	0	0
DH_2-FHB29.1.6	0	0	0	0	0	CM/KR	CM/KR	0.5	0	0	0

Lines highlighted in grey indicate the Fusarium head blight (FHB) resistant male lines used to develop the doubled haploid (DH) lines. [1]Heterozygous gene/quantitative trait loci (QTL); [2]Homozygous susceptible gene/quantitative trait loci (QTL); [3]Homozygous resistant gene/quantitative trait loci (QTL); Markers Gmw148 and Gwm501 were scored based on allele composition ([4]CM-CM-82036; [5]KR-Krokodil).

A total of 29 interspecific crosses were made using 22 DCF_1 lines and seven FMF_2 lines as female parents. A total of 76 embryos were rescued from these 29 interspecific crosses and colchicine treatment was applied to 24 haploid plants to double their chromosomes (Figure 4.1). The success rate of the DH process was calculated as the number of rescued embryos that survived the colchicine treatment step. Only two interspecific crosses (DH8 and DH27) showed a 100% success rate where all rescued embryos developed into plants that also survived the colchicine treatment. DH14 produced the highest number of embryos (10) and colchicine treated plants (7).

Eight of the 29 interspecific crosses did not develop any embryos. Although nine of the 29 interspecific crosses did produce embryos these embryos did not develop into plants. All 24 plants that developed from the rescued embryos survived the colchicine treatment and produced between 10 and 338 seeds (Table 4.6).

Four DH_1 seeds of each of the 24 DH_1 lines were planted in the greenhouse and leaf material collected for genotyping. The genotypic data is summarised in Table 4.6. Genotypic data of the offspring of the putative 24 DH_1 lines revealed that nine embryos developed through self-pollination as variation was detected between offspring of DH_1 lines. Results indicated that 15 DH_1 lines developed from haploid embryos as they were homozygous for markers tested with no genetic variation between offspring. Only these15 DH_1 lines were used for further analysis.

Only the two DCF_1 lines used to develop DH-18 and DH-26 contained all 11 markers linked to eight different rusts and FHB resistance genes/QTL. The development of DH lines was a secondary objective in this study as the technique was still being optimised. Therefore, the best lines developed during this study were used in crosses rather than for DH production (as described in Chapter 3). Figure 4.2 summarises the evaluation of rusts and FHB resistance markers present in the wheat lines used to develop the interspecific cross in comparison with the offspring of that specific DCF_1 and FMF_2 wheat line for the true DH lines generated.

Results indicated that the number of markers present in the offspring was lower for all lines, except for the offspring line DH_1-27.1 that was similar. The number of markers present in the offspring ranged from seven (DH_1-26.3) to two (DH_1-14.4, DH_1-14.6, DH_1-14.8 and DH_1-18.1). Line DCF_1-48.15 used to develop the DH_1-14 lines was homozygous or heterozygous resistant for eight molecular markers but DH-offspring lines only contained two homozygous resistant markers each while the other six markers were homozygous susceptible.

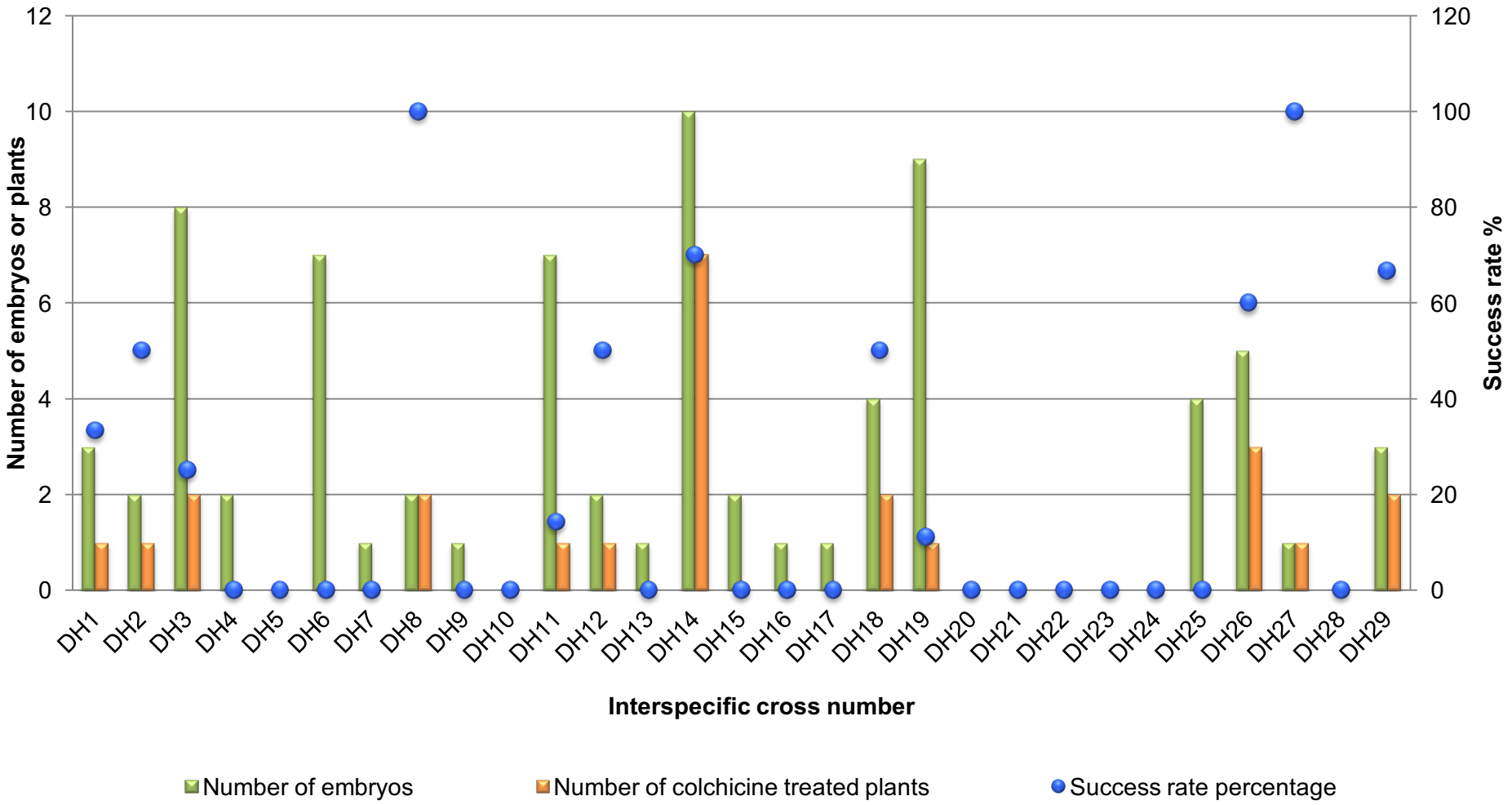


Figure 4.1 Summary of the number of embryos rescued and colchicine treated plants generated from the 29 interspecific crosses made. The success rate percentage is indicated on the secondary vertical axis.

Table 4.6 Molecular marker data for rust and Fusarium head blight resistance markers present in the 24 DH_1 offspring populations developed from DCF_1 and FMF_2 lines

Line name	Number of seed	$STSLr19_{130}$[1]	Cssfr5[2]	csSr2[1]	Sr26#43 and BE518379[2]	Sr39 and BE500705[2]	Gwm148	Gwm501	Umn10[2]	Gwm156[2]	Gwm293[2]	Gwm304[2]
DH_1-1.1 (DCF_1-34.4)	338	1	1	1	1	0.5	KB[3]/KB	CS[4]/CS	0	0.5	1	1
DH_2-1.1.1		1	1	1	1	0.5	KB/KB	CS/CS	0	0	1	1
DH_2-1.1.2		1	1	1	1	0.5	KB/KB	CS/CS	0	0	1	1
DH_2-1.1.3		1	1	1	1	0.5	KB/KB	CS/CS	0	1	1	1
DH_2-1.1.4		1	1	1	1	1	KB/KB	CS/CS	0	1	1	1
DH_1-2.2 (DCF_1-12.8)	123	0	0	1	0.5	0	KB/KR[5]	CS/KR	1	1	0.5	0.5
DH_2-2.2.1		0	0	1	0	0	KB/KB	CS/CS	1	1	0	0
DH_2-2.2.2		0	0	1	0	0	KB/KB	CS/CS	1	1	0	0
DH_2-2.2.3		0	0	1	0	0	KB/KB	CS/CS	1	1	0	0
DH_2-2.2.4		0	0	1	0	0	KB/KB	CS/CS	1	1	0	0
DH_1-3.4 (FMF_2-60.9.64)	27	1	0.5	1	0.5	0.5	KB/KB	CS/CS	0	0.5	1	1
DH_2-3.4.1		1	0	1	1	0.5	KB/KB	CS/CS	0	0	1	1
DH_2-3.4.2		1	0	1	1	0.5	KB/KB	CS/CS	0	0	1	1
DH_2-3.4.3		1	0	1	1	0.5	KB/KB	CS/CS	0	0	1	1
DH_2-3.4.4		1	0	1	1	0.5	KB/KB	CS/CS	0	0	1	1
DH_1-3.6 (FMF_2-60.9.64)	34	1	0.5	1	0.5	0.5	KB/KB	CS/CS	0	0.5	1	1
DH_2-3.6.1		1	0	1	1	1	KB/KB	CS/CS	0	0	1	1
DH_2-3.6.2		1	0	1	1	1	KB/KB	CS/CS	0	0	1	1
DH_2-3.6.3		1	0	1	1	1	KB/KB	CS/CS	0	0	1	1
DH_2-3.6.4		1	0	1	1	1	KB/KB	CS/CS	0	0	1	1
DH_1-8.1 (DCF_1-18.23)	84	1	1	1	0.5	0.5	KB/2S#[6]	CS/(2S#)	0.5	0.5	0	0
DH_2-8.1.1		0	1	1	1	1	2S#/2S#	2S#/2S#	0	0	0	0
DH_2-8.1.2		0	1	1	1	1	2S#/2S#	2S#/2S#	0	0	0	0
DH_2-8.1.3		0	1	1	1	1	2S#/2S#	2S#/2S#	0	0	0	0
DH_2-8.1.4		0	1	1	1	1	2S#/2S#	2S#/2S#	0	0	0	0

Table 4.6 Molecular marker data for rust and Fusarium head blight resistance markers present in the 24 DH_1 offspring populations developed from DCF_1 and FMF_2 lines (continued)

Line name	Number of seed	STSLr19$_{130}$[1]	Cssfr5[2]	csSr2[1]	Sr26#43 and BE518379[2]	Sr39 and BE500705[2]	Gwm148	Gwm501	Umn10[2]	Gwm156[2]	Gwm293[2]	Gwm304[2]
DH_1-8.2 (DCF_1-18.23)	46	1	1	1	0.5	0.5	KB/2S#	CS/(2S#)	0.5	0.5	0	0
DH_2-8.2.1		0	1	0	0	0	KB/KB	CS/CS	1	1	0	0
DH_2-8.2.2		0	1	0	0	0	KB/KB	CS/CS	1	1	0	0
DH_2-8.2.3		0	1	0	0	0	KB/KB	CS/CS	1	1	0	0
DH_2-8.2.4		0	1	0	0	0	KB/KB	CS/CS	1	1	0	0
DH_1-11.7 (FMF_2-60.7.82)	95	1	0.5	0	1	0	KR/2S#	KR/2S#	1	0.5	1	1
DH_2-11.7.1		0	1	0	1	0	KR/KR	KR/KR	1	0	1	1
DH_2-11.7.2		0	1	0	1	0	KR/KR	KR/KR	1	0	1	1
DH_2-11.7.3		0	1	0	1	0	KR/KR	KR/KR	1	0	1	1
DH_2-11.7.4		0	1	0	1	0	KR/KR	KR/KR	1	0	1	1
DH_1-12.1 (FMF_2-60.2.67)	37	1	1	0	0	0	KB/KR	CS/KR	1	0.5	1	1
DH_2-12.1.1		0	1	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_2-12.1.2		1	1	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_2-12.1.3		1	1	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_2-12.1.4		0	1	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_1-14.1 (DCF_1-48.15)	46	1	1	1	0.5	0	KR/CM[7]	KR/CM	0.5	0.5	0.5	0.5
DH_2-14.1.1		0	1	0	1	0	KR/KR	KR/KR	0	0	0	0
DH_2-14.1.2		0	1	0	1	0	KR/KR	KR/KR	0	0	0	0
DH_2-14.1.3		1	1	0	1	0	KR/KR	KR/KR	0	0	0	0
DH_2-14.1.4		0	1	0	1	0	KR/KR	KR/KR	0	0	0	0
DH_1-14.3 (DCF_1-48.15)	43	1	1	1	0.5	0	KR/CM	KR/CM	0.5	0.5	0.5	0.5
DH_2-14.3.1		0	1	0	1	0	CM/CM	CM/CM	0	0	0	0
DH_2-14.3.2		0	1	0	1	0	CM/CM	CM/CM	0	0	0	0
DH_2-14.3.3		1	1	0	0.5	0	CM/CM	CM/CM	0	0	0	0
DH_2-14.3.4		0	1	0	1	0	CM/CM	CM/CM	0	0	0	0

Table 4.6 Molecular marker data for rust and Fusarium head blight resistance markers present in the 24 DH_1 offspring populations developed from DCF_1 and FMF_2 lines (continued)

Line name	Number of seed	STSLr19$_{130}$[1]	Cssfr5[2]	csSr2[1]	Sr26#43 and BE518379[2]	Sr39 and BE500705[2]	Gwm148	Gwm501	Umn10[2]	Gwm156[2]	Gwm293[2]	Gwm304[2]
DH_1-14.4 (DCF_1-48.15)	38	1	1	1	0.5	0	KR/CM	KR/CM	0.5	0.5	0.5	0.5
DH_2-14.4.1		0	1	0	1	0	CM/CM	CM/CM	0	0	0	0
DH_2-14.4.2		0	1	0	1	0	CM/CM	CM/CM	0	0	0	0
DH_2-14.4.3		Seed did not germinate										
DH_2-14.4.4		0	1	0	1	0	CM/CM	CM/CM	0	0	0	0
DH_1-14.6 (DCF_1-48.15)	95	1	1	1	0.5	0	KR/CM	KR/CM	0.5	0.5	0.5	0.5
DH_2-14.6.1		0	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_2-14.6.2		0	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_2-14.6.3		0	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_2-14.6.4		0	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_1-14.8 (DCF_1-48.15)	11	1	1	1	0.5	0	KR/CM	KR/CM	0.5	0.5	0.5	0.5
DH_2-14.8.1		0	1	0	0	0	CM/CM	CM/CM	0	1	0	0
DH_2-14.8.2		0	1	0	0	0	CM/CM	CM/CM	0	1	0	0
DH_2-14.8.3		0	1	0	0	0	CM/CM	CM/CM	0	1	0	0
DH_2-14.8.4		0	1	0	0	0	CM/CM	CM/CM	0	1	0	0
DH_1-14.9 (DCF_1-48.15)	10	1	1	1	0.5	0	KR/CM	KR/CM	0.5	0.5	0.5	0.5
DH_2-14.9.1		0	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_2-14.9.2		0	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_2-14.9.3		1	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_2-14.9.4		0	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_1-14.10 (DCF_1-48.15)	79	1	1	1	0.5	0	KR/CM	KR/CM	0.5	0.5	0.5	0.5
DH_2-14.10.1		0	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_2-14.10.2		0	1	0	0	0	KR/KR	KR/KR	0	1	0	0
DH_2-14.10.3		Seed did not germinate										
DH_2-14.10.4		1	1	0	0	0	KR/KR	KR/KR	0	1	0	0

Table 4.6 Molecular marker data for rust and Fusarium head blight resistance markers present in the 24 DH_1 offspring populations developed from DCF_1 and FMF_2 lines (continued)

Line name	Number of seed	STSLr19$_{130}$[1]	Cssfr5[2]	csSr2[1]	Sr26#43 and BE518379[2]	Sr39 and BE500705[2]	Gwm148	Gwm501	Umn10[2]	Gwm156[2]	Gwm293[2]	Gwm304[2]
DH_1-18.1 (DCF_1-40.14)	47	1	0.5	1	0.5	0.5	KR/2S#	KR/2S#	0.5	0.5	0.5	0.5
DH_2-18.1.1		1	0	0	1	0	KR/KR	KR/KR	0	0	0	0
DH_2-18.1.2		1	0	0	1	0	KR/KR	KR/KR	0	0	0	0
DH_2-18.1.3		1	0	0	1	0	KR/KR	KR/KR	0	0	0	0
DH_2-18.1.4		1	0	0	1	0	KR/KR	KR/KR	0	0	0	0
DH_1-18.3 (DCF_1-40.14)	55	1	0.5	1	0.5	0.5	KR/2S#	KR/2S#	0.5	0.5	0.5	0.5
DH_2-18.3.1		1	0	0	0	1	2S#/2S#	2S#/2S#	0	1	1	1
DH_2-18.3.2		1	0	0	0	1	2S#/2S#	2S#/2S#	0	1	1	1
DH_2-18.3.3		1	0	0	0	1	2S#/2S#	2S#/2S#	0	1	1	1
DH_2-18.3.4		1	0	0	0	1	2S#/2S#	2S#/2S#	0	1	1	1
DH_1-19.1 (DCF_1-43.3)	13	0	0	1	0.5	0.5	KR/2S#	KR/2S#	0.5	1	1	1
DH_2-19.1.1		0	0	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_2-19.1.2		0	0	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_2-19.1.3		0	0	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_2-19.1.4		0	0	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_1-26.2 (DCF_1-50.9)	80	1	0.5	1	0.5	0.5	KR/2S#	KR/2S#	0.5	0.5	0.5	0.5
DH_2-26.2.1		0	1	1	1	0	KR/KR	KR/KR	0	0	0	0
DH_2-26.2.2		0	1	1	1	0	KR/KR	KR/KR	0	0	0	0
DH_2-26.2.3		1	1	1	1	0	KR/KR	KR/KR	0	0	0	0
DH_2-26.2.4		0	1	1	1	0	KR/KR	KR/KR	0	0	0	0
DH_1-26.3 (DCF_1-50.9)	25	1	0.5	1	0.5	0.5	KR/2S#	KR/2S#	0.5	0.5	0.5	0.5
DH_2-26.3.1		0	1	1	0	1	2S#/2S#	2S#/2S#	1	1	1	1
DH_2-26.3.2		0	1	1	0	1	2S#/2S#	2S#/2S#	1	1	1	1
DH_2-26.3.3		0	1	1	0	1	2S#/2S#	2S#/2S#	1	1	1	1
DH_2-26.3.4		Seed did not germinate										

Table 4.6 Molecular marker data for rust and Fusarium head blight resistance markers present in the 24 DH_1 offspring populations developed from DCF_1 and FMF_2 lines (continued)

Line name	Number of seed	STSLr19$_{130}$[1]	Cssfr5[2]	csSr2[1]	Sr26#43 and BE518379[2]	Sr39 and BE500705[2]	Gwm148	Gwm501	Umn10[2]	Gwm156[2]	Gwm293[2]	Gwm304[2]
DH_1-26.4 (DCF_1-50.9)	28	1	0.5	1	0.5	0.5	KR/KR	KR/KR	0.5	0.5	0.5	0.5
DH_2-26.4.1		0	1	1	0	0	KR/KR	KR/KR	0	0	0	0
DH_2-26.4.2		0	1	1	0	0	KR/KR	KR/KR	0	0	0	0
DH_2-26.4.3		1	1	1	0	0	KR/KR	KR/KR	0	0	0	0
DH_2-26.4.4		1	1	1	0	0	KR/KR	KR/KR	0	0	0	0
DH_1-27.1 (DCF_1-50.27)	29	0	0.5	0	0.5	0	KB/KR	CS/KR	0.5	1	0.5	0.5
DH_2-27.1.1		0	1	0	1	0	KB/KB	CS/CS	1	1	1	1
DH_2-27.1.2		0	1	0	1	0	KB/KB	CS/CS	1	1	1	1
DH_2-27.1.3		0	1	0	1	0	KB/KB	CS/CS	1	1	1	1
DH_2-27.1.4		0	1	0	1	0	KB/KB	CS/CS	1	1	1	1
DH_1-29.1 (FMF_2-60.2.65)	39	0	0.5	1	0.5	0	KB/KR	CS/KR	0.5	0.5	1	1
DH_2-29.1.1		0	0	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_2-29.1.2		0	0	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_2-29.1.3		0	0	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_2-29.1.4		0	0	0	0	0	KR/KR	KR/KR	1	1	1	1
DH_1-29.2 (FMF_2-60.2.65)	50	0	0.5	1	0.5	0	KB/KR	CS/KR	0.5	0.5	1	1
DH_2-29.2.1		0	0	1	0	0	KR/KR	KR/KR	0	1	1	1
DH_2-29.2.2		0	0	1	0	0	KR/KR	KR/KR	0	1	1	1
DH_2-29.2.3		0	0	1	0	0	KR/KR	KR/KR	0	1	1	1
DH_2-29.2.4		0	0	1	0	0	KR/KR	KR/KR	0	1	1	1

Lines highlighted in grey indicate the DH cross number and the DCF_1 and FMF_2 line used to develop the DH lines is indicated in brackets. DH_1-first doubled haploid generation; DCF_1-first double-cross generation; FMF_2-second generation of rust and FHB resistance lines; DH_2- second doubled haploid generation. [1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0). Markers Gmw148 and Gwm501 were scored based on allele composition ([3]KB-Kingbird; [4]CS-CS*Lr19*-149-299; [5]KR-Krokodil; [6]2S#-2S#/163; [7]CM-CM-82036).

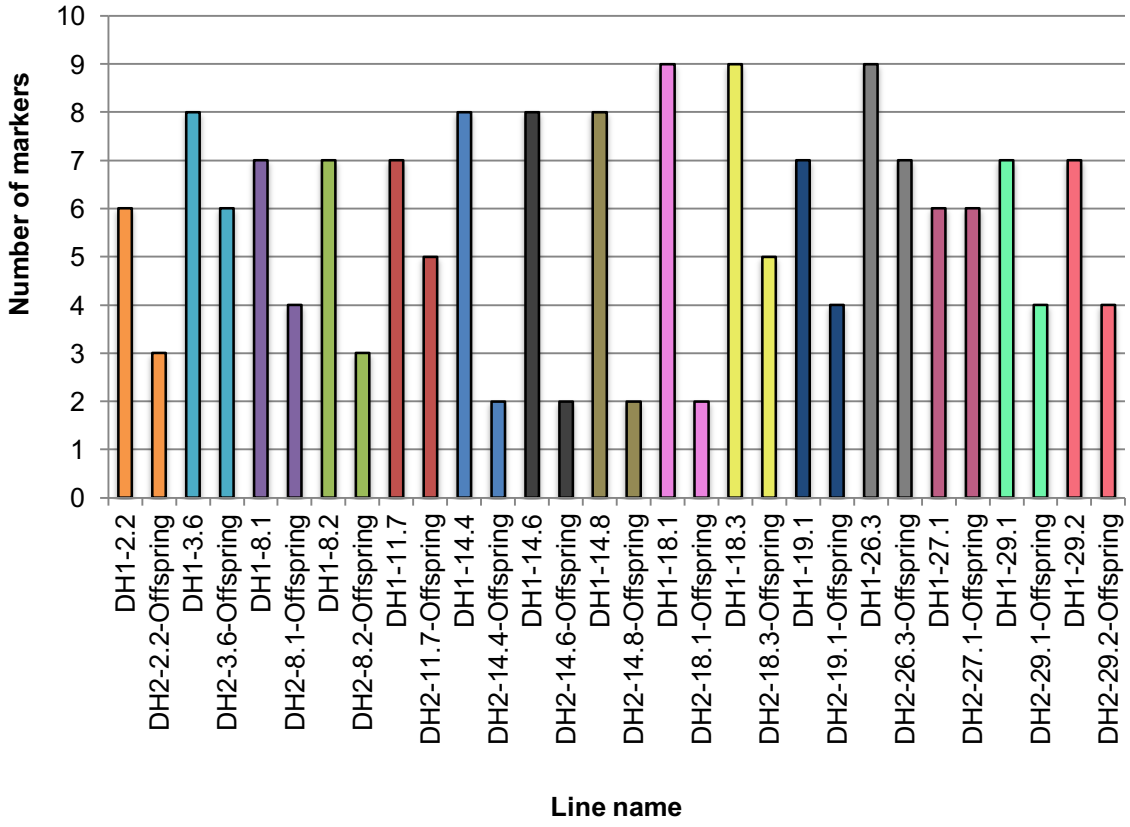


Figure 4.2 Total number of rusts and FHB resistant markers present in the DCF_1 and FMF_2 lines compared to their DH_1 offspring lines.

This big difference may be contributed to the high number of heterozygous markers present in line DCF_1-48.15. Heterozygous markers segregate in a 1:2:1 ratio (AA:Aa:aa) in the offspring and most of these heterozygous markers were thus inherited in the homozygous susceptible state. Only the offspring groups of DH_1-2.2 and DH_1-26.3 were homozygous resistant for both the *Sr2/Yr30* and *Fhb1* resistance genes/QTL that are in repulsion linkage.

All offspring lines were evaluated using molecular markers linked to HMW-GS and the 1BL.1RS translocation (Table 4.7). Marker Umn19 was used to detect the presence of the HMW-GS alleles on locus *Glu-A* and distinguished between *Glu-Ax2** (2*) or *Glu-Ax1* (1) and *Glu-Ax0* (0). As discussed in Chapter 3, all lines were homozygous for the *Glu-Ax2** gene (marker Umn19) and none contained the 1BL.1RS translocation (dominant marker Glu-B3j). This was also observed for the DH offspring lines. Markers BxFp and MAR amplified alleles present on the *Glu-B* locus. The BxFp marker distinguished between the *Glu-Bx7* (7), *Glu-Bx17* (17) or heterozygous alleles (7/17).

Table 4.7 Molecular data of the HMW-GS and 1BL.1RS translocation for the 24 DH_1 offspring populations developed from DCF_1 and FMF_2 lines

Line name	Umn19[1]	BxFp[1]	MAR[1]	Umn25[1]	Umn26[1]	Glu-B3j[2]
DH_2-1.1.1	2*	7	7^{OE}	2	12	0
DH_2-1.1.2	2*	7	7^{OE}	2	12	0
DH_2-1.1.3	2*	7	7^{OE}	2	12	0
DH_2-1.1.4	2*	7	7^{OE}	2	12	0
DH_2-2.2.1	2*	7	≠	5	10	0
DH_2-2.2.2	2*	7	≠	5	10	0
DH_2-2.2.3	2*	7	≠	5	10	0
DH_2-2.2.4	2*	7	≠	5	10	0
DH_2-3.4.1	2*	7	7^{OE}	5	10	0
DH_2-3.4.2	2*	7	7^{OE}	5	10	0
DH_2-3.4.3	2*	7	7^{OE}	5	10	0
DH_2-3.4.4	2*	7	7^{OE}	5	10	0
DH_2-3.6.1	2*	7	7^{OE}	2	12	0
DH_2-3.6.2	2*	7	7^{OE}	2	12	0
DH_2-3.6.3	2*	7	7^{OE}	2	12	0
DH_2-3.6.4	2*	7	7^{OE}	2	12	0
DH_2-8.1.1	2*	7	≠	2	12	0
DH_2-8.1.2	2*	7	≠	2	12	0
DH_2-8.1.3	2*	7	≠	2	12	0
DH_2-8.1.4	2*	7	≠	2	12	0
DH_2-8.2.1	2*	7	≠	5	10	0
DH_2-8.2.2	2*	7	≠	5	10	0
DH_2-8.2.3	2*	7	≠	5	10	0
DH_2-8.2.4	2*	7	≠	5	10	0
DH_2-11.7.1	2*	7	7^{OE}	5	10	0
DH_2-11.7.2	2*	7	7^{OE}	5	10	0
DH_2-11.7.3	2*	7	7^{OE}	5	10	0
DH_2-11.7.4	2*	7	7^{OE}	5	10	0
DH_2-12.1.1	2*	7	≠	2	12	0
DH_2-12.1.2	2*	7	≠	2	12	0
DH_2-12.1.3	2*	7	≠	2	12	0
DH_2-12.1.4	2*	7	≠	2	12	0
DH_2-14.1.1	2*	7	≠	2	12	0
DH_2-14.1.2	2*	7	≠	2	12	0
DH_2-14.1.3	2*	7	≠	2	12	0
DH_2-14.1.4	2*	7	≠	2	12	0
DH_2-14.3.1	2*	7	≠	2	12	0
DH_2-14.3.2	2*	7	≠	2	12	0
DH_2-14.3.3	2*	7	≠/7^{OE}	2\5	12\10	0
DH_2-14.3.4	2*	7	≠	2	12	0
DH_2-14.4.1	2*	7	7^{OE}	2	12	0
DH_2-14.4.2	2*	7	7^{OE}	2	12	0
DH_2-14.4.3			Seed did not germinate			
DH_2-14.4.4	2*	7	7^{OE}	2	12	0
DH_2-14.6.1	2*	7	7^{OE}	2	12	0
DH_2-14.6.2	2*	7	7^{OE}	2	12	0
DH_2-14.6.3	2*	7	7^{OE}	2	12	0
DH_2-14.6.4	2*	7	7^{OE}	2	12	0
DH_2-14.8.1	2*	7	7^{OE}	2	12	0
DH_2-14.8.2	2*	7	7^{OE}	2	12	0
DH_2-14.8.3	2*	7	7^{OE}	2	12	0
DH_2-14.8.4	2*	7	7^{OE}	2	12	0
DH_2-14.9.1	2*	7	7^{OE}	2	12	0
DH_2-14.9.2	2*	7	7^{OE}	2	12	0
DH_2-14.9.3	2*	7	7^{OE}	2	12	0
DH_2-14.9.4	2*	7	7^{OE}	2	12	0

Table 4.7 Molecular data of the HMW-GS and 1BL.1RS translocation for the 24 DH_1 offspring populations developed from DCF_1 and FMF_2 lines (continued)

Line name	Umn19[1]	BxFp[1]	MAR[1]	Umn25[1]	Umn26[1]	Glu-B3j[2]
DH_2-14.10.1	2*	7	≠	2	12	0
DH_2-14.10.2	2*	7	≠	2	12	0
DH_2-14.10.3			Seed did not germinate			
DH_2-14.10.4	2*	7	≠	2	12	0
DH_2-18.1.1	2*	7	≠	2	12	0
DH_2-18.1.2	2*	7	≠	2	12	0
DH_2-18.1.3	2*	7	≠	2	12	0
DH_2-18.1.4	2*	7	≠	2	12	0
DH_2-18.3.1	2*	7	7^{OE}	2	12	0
DH_2-18.3.2	2*	7	7^{OE}	2	12	0
DH_2-18.3.3	2*	7	7^{OE}	2	12	0
DH_2-18.3.4	2*	7	7^{OE}	2	12	0
DH_2-19.1.1	2*	7	7^{OE}	2	12	0
DH_2-19.1.2	2*	7	7^{OE}	2	12	0
DH_2-19.1.3	2*	7	7^{OE}	2	12	0
DH_2-19.1.4	2*	7	7^{OE}	2	12	0
DH_2-26.2.1	2*	7	≠	2	12	0
DH_2-26.2.2	2*	7	≠	2	12	0
DH_2-26.2.3	2*	7	≠	2	12	0
DH_2-26.2.4	2*	7	≠	2	12	0
DH_2-26.3.1	2*	7	≠	5	10	0
DH_2-26.3.2	2*	7	≠	5	10	0
DH_2-26.3.3	2*	7	≠	5	10	0
DH_2-26.3.4			Seed did not germinate			
DH_2-26.4.1			Seed did not germinate			
DH_2-26.4.2	2*	7	7^{OE}	5	10	0
DH_2-26.4.3	2*	7	7^{OE}	5	10	0
DH_2-26.4.4	2*	7	7^{OE}	2\5	12\10	0
DH_2-27.1.1	2*	7	7^{OE}	2	12	0
DH_2-27.1.2	2*	7	7^{OE}	2	12	0
DH_2-27.1.3	2*	7	7^{OE}	2	12	0
DH_2-27.1.4	2*	7	7^{OE}	2	12	0
DH_2-29.1.1	2*	7	≠	2	12	0
DH_2-29.1.2	2*	7	≠	2	12	0
DH_2-29.1.3	2*	7	≠	2	12	0
DH_2-29.1.4	2*	7	≠	2	12	0
DH_2-29.2.1	2*	7	≠	2	12	0
DH_2-29.2.2	2*	7	≠	2	12	0
DH_2-29.2.3	2*	7	≠	2	12	0
DH_2-29.2.4	2*	7	≠	2	12	0

HMW-GS-High-molecular-weight glutenin subunits; DH_1-first doubled haploid generation; DCF_1-first double-cross generation; FMF_2-second generation of rust and FHB resistance lines; DH_2- second doubled haploid generation; [1]Markers scored for the presence of HMW-GS alleles (2*=*Glu-Ax2**; 7=*Glu-Bx7*; 7^{OE}=over-expressed *Glu-Bx7*; ≠=do not contain *Glu-Bx7+By8**; 2=*Glu-Dx2*; 5=*Glu-Dx5*; 12=*Glu-Dy12*; 10=*Glu-Dy10*); [2]Marker Glu-B3j is a dominant marker linked to the 1BL.1RS translocation and were scored as either present (1) or absent (0).

Marker MAR distinguished between alleles of the over expressed *Glu-Bx7* (7^{OE}), lines without *Glu-Bx7+By8** (≠) or heterozygous (7^{OE}/≠). All offspring lines were homozygous for the *Glu-Bx7* gene (marker BxFp). Variation was observed in the offspring for genes linked to *Glu-Bx7*^{OE} or lines that did not contain *Glu-Bx7+By8**. Marker Umn25 and Umn26 amplified the HMW-GS alleles on *Glu-D*. Marker Umn25 amplified *Glu-Dx2* (2), *Glu-Dx5* (5) or heterozygous allele (2/5), while marker Umn26 amplified the *Glu-Dy12*

(12), *Glu-Dy10* (10) or heterozygous alleles (12/10). Markers Umn25 and Umn26 indicated variation between the different offspring populations for *Glu-Dx2+Dy12* and *Glu-Dx5+Dy10*. Doubled haploid offspring DH_2-14.3 and DH_2-26.4 that were indicated to be self-pollinated lines based on marker segregation of the rusts and FHB markers, were also heterozygous for markers linked to HMW-GS.

4.3.3 Comparison of the success rate between the optimisation and application of the DH technique

The DH technique is technically more difficult compared to other breeding methods such as single-seed descent and pedigree selection methods. The percentages of germinated embryos, seedlings and colchicine treated plants were used to compare the success rate between the optimisation and application of the DH method in this study (Table 4.8). Fifty and 29 interspecific crosses were made during the optimisation and application phases, respectively. A total of 64 embryos were rescued from the 50 interspecific crosses during the optimisation phase while 76 embryos were rescued from the 29 interspecific crosses during the application phase. The number of rescued embryos:interspecific cross ratio for the two phases is given in Table 4.8 and was calculated as the number of rescued embryos:the number of interspecific crosses. The expressed percentages of the other factors given in Table 4.8 were calculated as the number of objects divided to the number of rescued embryos. Almost half the amount of interspecific crosses was made during the application phase, yet more embryos were rescued. An increase in percentages of germinated embryos, seedlings, colchicine treated plants, seed producing plants and successful DH plants were observed for the optimisation phase when compared to the application phase. A higher responsiveness to haploid induction was present in the application phase and led to a higher success rate compared to the optimisation phase.

Table 4.8 Comparison between the optimisation and application of the doubled haploid method

Factors	Optimisation phase	Application phase
Rescued embryos:interspecific crosses ratio	1.28:1	2.62:1
Germinated embryos	55%	78%
Seedlings replanted to soil	23%	40%
Colchicine treated plants	13%	32%
Seed producing plants	5%	32%
Doubled haploid plants produced	3%	20%

4.4 Discussion

The DH technique is advantageous to shorten breeding time for cultivar development but the application of the DH technique requires technical skills and knowledge of the process (Inagaki et al. 1998; Mehta and Angra 2000). Niu et al. (2014) gave a detailed description of the complete method as well as additional indicators that can help with the understanding and improvement of the different steps of the DH technique.

Optimisation was applied using similar (with the same genetic background) female (rust resistant) and male (FHB resistant) lines that were used in Chapter 3 to combine rust and FHB resistance genes/QTL. None of the rust resistant lines produced any DH lines and various factors could have contributed to this. The rust resistant lines were only used in 16 of the 50 interspecific crosses made as they were early maturing lines and therefore their early flowering dates did not synchronise with the maize pollen donor. The *Lr19* gene was present in four of the rust resistant lines used in the interspecific crosses. Wessels and Botes (2014) concluded that wild species translocations like the one carrying *Lr19* do not promote DH development. Furthermore, both *Sr26* and *Sr39* present in these lines were also introduced into wheat from other species and present translocations. There were thus three different translocations present in the background of some of the rust resistant lines used in the interspecific crosses.

The FHB resistant wheat plants were planted two weeks earlier and two weeks later than the first and last planting dates of the rust resistant wheat lines to ensure synchronised flowering dates as well as the availability of the pollen for the crosses. The FHB resistant wheat lines matured later compared to the rust resistant female lines and therefore more FHB resistant lines were available for interspecific crosses as the late maturing of the FHB resistant wheat lines synchronised with the pollen availability of maize plants.

As the DH technique was not fully optimised by the time that the second double crosses had to be made, as described in Chapter 3, the application of the DH technique in the breeding scheme was considered as a secondary method to breed for combined rust and FHB resistance. Interspecific crosses using the DCF_1 and FMF_2 lines were thus only made after crosses were made to develop the second double cross population ($2DCF_1$). Only 29 interspecific crosses were made using wheat lines selected based on a high number of combined rusts and FHB resistant markers, or either rust or FHB resistant markers. An increase of percentages linked to all the factors used to measure the success between the optimisation phase and application phase was observed for the

application process and indicated the improvement of the development of DH lines in this study.

No DH line containing all the screened rusts and FHB resistance genes/QTL were obtained because a limited number of the best identified FMF_1 lines (Chapter 3) were used for interspecific crosses. The DH_2-14 populations contained a low number of desirable traits although the DCF_1 line they were developed from contained eight resistance genes/QTL. Markers linked to these genes/QTL were heterozygous, except for Cssfr5 (*Lr34/Yr18/Sr57*) that was homozygous resistant and the two dominant markers, $STSLr19_{130}$ (*Lr19*) and csSr2 (*Sr2/Yr30*) that were present. Markers linked to the *Sr39* resistance gene indicated homozygous susceptibility for the DCF_1-48.15 line that was used to develop the DH_2-14 populations. This indicated the high probability to lose heterozygous genes as there is a 50:50 chance that the heterozygous gene/QTL will either be homozygous resistant or susceptible in the progeny after the DH process. For this study 11 markers linked to eight different rusts and FHB resistance genes/QTL had to be combined. Eight genes/QTL is a large number of desirable traits to combine and therefore a larger DH population is required to increase the probability to produce a DH line with the desirable genotype. The larger the genetic variation of crosses the larger the DH population should be to obtain the desirable genotypes (Inagaki et al. 1998).

Although line DH_2-1.1.4 was not produced by the DH process but was a product of self-pollination, it was the best line identified in the offspring regarding combined resistance to rusts and FHB. All rusts resistance genes were homozygous resistant, due to no segregation in the offspring linked to the dominant markers of *Lr19* and *Sr2/Yr30*, for line DCF_1-34.34 used to developed DH_1-1.1, indicating that these genes are also homozygous resistant. Only *Fhb1* of the FHB genes/QTL screened was absent in line DH_2-1.1.4. Therefore, line DH_2-1.1.4 can be used to make crosses with offspring of line DH_1-26.3 that was homozygous resistant for all the FHB markers and can contribute the *Fhb1* gene to the DH_2-1.1.4 progeny. Line DH_2-1.1.4 contained the *Glu-Ax2** and *Glu-Bx7*^{OE} HMW-GS genes that are linked to stronger dough strength but contain the *Glu-Dx2+Dy12* HMW-GS gene that correlated with weaker dough strength. Offspring of line DH_1-26.3 contained the *Glu-Dx5+Dy10* HMW-GS gene that is linked to stronger dough strength and therefore, can also contribute the *Glu-Dx5+Dy10* HMW-GS gene when used to develop crosses. Lines DH_1-12.1, DH_1-19.1, DH_1-26.3, DH_1-27.1 and DH_1-29.1 are valued lines for a FHB resistance breeding programme because they are homozygous resistant for all the FHB resistance markers.

The DH technique is labour intensive and large population sizes are required depending on the number of traits. To decrease the number of lines to be treated with colchicine and replanted in pots, MAS can be used on seedlings from the germinated rescued embryos to identify plants with the desirable genotypic profile and the rest can be discarded from the population (Humphreys and Knox 2015).

Genotypes of wheat lines used to develop DH lines were a random combination of the rusts and FHB parental lines' genotypes. Not all embryos were haploid and not all haploid embryos developed into DH lines but DH lines were developed successfully with genotypic variation between the DH lines. This illustrates that the genotypes of the wheat lines met the first and second criteria of Snape et al. (1986) to produce DH lines successfully. The genotypic data of the successful DH lines also supported the third criteria linked to genetic stability of offspring although this needs to be confirmed further in offspring of these lines. Therefore, the maize x wheat system was successfully used in this study for haploid induction of wheat lines and to develop DH lines that fulfil the three criteria of Snape et al. (1986) to be successful.

4.5 Conclusions

The maize x wheat DH system was successfully optimised and applied in this study. Although no DH wheat lines containing all eight rust and FHB resistance genes/QTL were obtained, DH lines were successfully generated. Important lines were identified and can be used successfully to combine all rusts and FHB resistance genes/QTL into a single wheat line and contribute better HMW-GS genes linked to stronger dough strength. Therefore, the DH technique and MAS were applied successfully in this study and indicate the significance of these two methods when used in combination.

4.6 References

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Chapter 5

Phenotypic validation and rust fungal gene expression

5.1 Introduction

Leaf-, stem- and stripe rust is caused by *Pt*, *Pgt* and *Pst*, respectively. These three rust types are wheat fungal pathogens and can cause severe yield losses (McIntosh et al. 1995). Resistance breeding and fungicides are used to control rust infections. Resistance breeding against these three rust pathogens is an on-going process as new more virulent pathotypes evolve and overcome resistance genes. Fungicides can also become less effective against these pathogens and together with increased input costs are problematic for farmers, especially in developing countries (Olivier 2014; Lamichhane et al. 2016).

Effectiveness of resistance genes can be evaluated phenotypically and data generated can contribute to predict the potential durability of different resistance sources. Over the years some resistance genes have lost their effectiveness while other genes are still effective and can be incorporated into wheat lines to breed rust resistant cultivars. The effective *Lr34/Yr18/Sr57* gene is one of the most studied APR genes and was mapped to chromosome 7DS. Another effective APR gene is *Sr2/Yr30* that is located on chromosome 3BS. Effective APR genes support ASR genes (German and Kolmer 1992; Vanegas et al. 2008).

Phenotypic evaluation of inoculated plants need technical skills and can be labour intensive when large populations have to be evaluated. Photosynthetic proteins and antioxidant systems are affected by rust infections and can be used to determine infection levels in wheat (Chen et al. 2015). Infected wheat lines can also be evaluated by quantifying genes expressed by the pathogen by using qPCR analyses (Casassola et al. 2015). These techniques can assist breeders and pathologists to understand infection development and the effect of rust resistance genes on disease development.

Many genes are activated in the host (wheat) and the pathogen (*Puccinia* spp.) during rust infections. Different response reactions are activated in different plants because different combinations of disease resistance mechanisms are present in the host plant. The different disease mechanisms also have an influence on gene expression of the pathogen. Two different plant defence responses have been described. (i) Plant recognition receptors activate the basal defence response as soon as the pathogen

makes contact with the plant cell surface. Haustoria (pathogen feeding structure) secrete effector proteins that suppress the basal defence response of the host plant. These effector proteins transform plant cells to accommodate the pathogen. (ii) Plant immune receptors (resistance proteins) recognise effector proteins and activate a second defence response that are responsible for localised cell death also known as a hypersensitive response (Jones and Dangl 2006; Dodds et al. 2009; Dobon et al. 2016). The first defence mechanism confer quantitative resistance and have a small effect on pathogenesis but is more durable because it is multiple genes with minor effects and lead to lower selection pressure because of the complexity (Parlevliet 2002). The second type of disease defence mechanism confer qualitative resistance and is highly effective but race specific and because it is a single gene it can easily be overcome by pathogens as it is less complex compared to quantitative resistance genes (Chung et al. 2010). Therefore both types of resistance genes need to be combined to ensure effective and durable resistance.

The main objective of this study was to determine whether the rust resistance genes/QTL identified in breeding lines developed in this study by using MAS, are phenotypically expressed. The second objective was to quantify fungal gene expression in rust infected wheat lines containing different combinations of rust resistance genes/QTL. The last objective was to subject wheat lines containing different rust resistance genes/QTL combinations to field trials to monitor rust infection and development on these lines.

5.2 Materials and methods

Different techniques were used to reach the objectives of this study. Timeline studies were performed for all three rusts types at both seedling and adult plant stage, because breeding lines evaluated during the study contained both ASR and APR rust resistance genes/QTL. The timeline study was used to determine the best time to harvest samples to isolate ribonucleic acid (RNA) to determine the biomass accumulation of the rust fungi by amplifying the *β-tubulin* gene using qPCR. Leaf material was furthermore harvested to determine the rust resistance genes/QTL genotypes of the screened lines. Field trials were planted for two consecutive years and disease severity determined and MAS applied to obtain both the rust resistance phenotypic and genotypic profiles of these lines.

5.2.1 Timeline studies of wheat lines infected with *Puccinia* species at seedling and adult plant stage

A timeline study was conducted on both seedlings and adult plants using wheat cultivars/lines with rust resistance genes/QTL. These cultivars/lines included the original parents of the breeding lines developed during this study (Kariega, Avocet*YrSP*, Blade, CS*Lr19*-149-299, Kingbird and line 2S#/163). Additional control cultivars (Thatcher*Lr19*, CS Hope 3B and Sumai3) were included. Thatcher*Lr19* was selected as it carries the *Lr19* gene without the presence of *Lr34/Yr18/Sr57*. CS-Hope 3B was included as it carries the *Sr2/Yr30* gene. Sumai3 was used as a susceptible (inoculated) and negative (uninoculated) control. Table 5.1 summarises cultivars used for the timeline study. Leaf material was harvested at different time intervals, to determine the optimal time (dpi) for gene expression studies. Only cultivars/lines with fixed genotypes were considered for planting and use for the gene expression study. Different sampling dates were used to determine the best time (dpi) for gene expression analysis. Insufficient leaf material per seedling was available for the four or five consecutive sampling dates and therefore sampling on the same seedling for each cultivar/line was not possible. Thus more seeds per cultivar/line was planted and inoculated to ensure that there was enough leaf material to harvest for qPCR analysis although it was not harvested from the same plant.

Table 5.1 Cultivars and control lines used for timeline studies of rust infections

Cultivar or line name	All stage resistance gene/QTL	Adult plant resistance gene/QTL
Kariega	None	*Lr34/Yr18/Sr57; QYr.sgi.2B-1*
Avocet*YrSP*	*Sr26*[1]*; YrSP*	*Lr34/Yr18/Sr57*
Blade	*Sr26*	None
CS*Lr19*-149-299	*Lr19*	*Lr34/Yr18/Sr57*
Kingbird	None	*Lr34/Yr18/Sr57; Sr2/Yr30*
2S#/163	*Sr39*	None
Thatcher*Lr19*	*Lr19*	None
CS Hope 3B	None	*Lr34/Yr18/Sr57; Sr2/Yr30*
Sumai3	None	*Lr34/Yr18/Sr57*

QTL-Quantitative trait loci; [1]*Sr26* is segregating in the Avocet*YrSP* seed source used during this study.

5.2.1.1 *Puccinia* species spore multiplication

For rust spore multiplication, ± 40 seed of susceptible cultivars was planted in 500 ml pots filled with Mikskaar professional potting soil 70. Pots were watered daily and placed in the greenhouse at 23˚C. Germinated seed was treated with 0.3 g/l (w/v) 1,2-Dihydropyridazin-3,6-dion (Maleic hydrazide; Sigma, RSA). Seedlings of the susceptible cultivars Morocco, Federation/Kavkas and Morocco were respectively inoculated with *Pt*

(isolate MCDS), *Pgt* (isolate PTKST) and *Pst* (isolate 6E22A+) eight days after planting. Seedlings were spray inoculated using a urediniospore and Soltrol™130 isoparaffin oil solution (Chevron Phillips Chemical Company LP, The Woodlands, TX, USA). Following inoculation, pots were placed in a growth chamber (23˚C) to allow seedlings to dry-off completely. Dried-off seedlings inoculated with *Pt* (isolate MCDS) and *Pgt* (isolate PTKST) were placed in a condensation dew chamber (20˚C) while seedlings inoculated with *Pst* (isolate 6E22A+) were placed in a covered container, containing warm water, inside a cold room (11˚C) for 24 h. The following day, seedlings were placed in a growth chamber (23˚C) to dry-off and transferred to the greenhouse for disease development at 23˚C for those inoculated with *Pt* and *Pgt* and at 17˚C for those inoculated with *Pst*.

5.2.1.2 Timeline study of disease development of wheat seedlings infected with *Puccinia* species

For the seedling timeline study, 20 seeds per cultivar/line were planted in 500 ml pots filled with Mikskaar professional potting soil 70. Three pots were planted per cultivar/line, one for each of the three different rust diseases. After planting, pots were watered and placed in a growth chamber at 23˚C under 24 h of light to promote even seed germination. Following seed germination, pots were placed in the greenhouse (23˚C) until seedlings reached the two leaf stage. All nine cultivars/lines were spray inoculated with *Pt* [isolate MCDS; 1 mg/ml spores in Soltrol™130 solution (w/v)], *Pgt* [isolate PTKST; 1 mg/ml spores in Soltrol™130 solution (w/v)] and *Pst* [isolate 6E22A+; 1mg/ml spores in Soltrol™130 solution (w/v)], respectively. Fresh spores were collected on the day of spray inoculation from the infected susceptible cultivars (section 5.2.1.1). Inoculated seedlings were placed in a growth chamber for 1 h at 23˚C to dry-off. Inoculated seedlings were treated as described in section 5.2.1.1. Leaf samples from different seedlings of the same cultivar or line were collected at 0, 5, 10 and 15 dpi from *Pt* and *Pgt* inoculated seedlings. For *Pst* inoculated seedlings, leaf samples were collected at 0, 5, 10, 15 and 20 dpi. Collected leaf material was placed in 15 ml tubes and kept in liquid nitrogen and stored in a freezer at -80˚C until leaf samples were used for RNA isolation and qPCR analyses.

5.2.1.3 Timeline study of disease development of adult wheat plants infected with *Puccinia* species

For the timeline study on infected adult plants, four seeds were planted per pot for every cultivar/line in 2 l pots filled with top soil in the greenhouse. Three pots were planted per cultivar/line, one for each of the three different rust diseases. Planting was repeated

three times, once a week, to synchronise flowering times between cultivars/lines. After seed germination, plants were reduced to two plants per pot. Plants were watered daily and fertilisation applied as described in section 3.2.3.

All cultivars/lines were inoculated on the same day with either *Pt* (isolate MCDS), *Pgt* (isolate PTKST) or *Pst* (isolate 6E22A+). Plants were spray inoculated using 1 mg/ml (w/v) fresh spores mixed with distilled water and a droplet of Tween 20. Plants inoculated with *Pt* (isolate MCDS) and *Pgt* (isolate PTKST) were placed immediately after inoculation in a plastic dew chamber in a greenhouse cubicle (23˚C). The plastic covered frame, serving as the dew camber, was placed in a tray filled with water to a depth of 5 cm to enhance dew development. Plants inoculated with *Pst* (isolate 6E22A+) were placed in a plastic dew chamber, on a metal grid raised above the water line and filled with warm water, inside a cold room (11˚C). Plants were incubated overnight (24 h) and upon removal from the dew chambers placed in greenhouse cubicles set at 23˚C for the leaf- and the stem rust inoculated plants and at 17˚C for the stripe rust inoculated plants.

Leaf material from any of the two plants available for the same cultivar/line of the adult plants was collected at 0, 5, 10 and 15 dpi for each cultivar/line inoculated with *Pt, Pgt, and Pst*, respectively. Additionally, leaf samples were also collected at 20 dpi for cultivars/lines inoculated with *Pst.* Different harvesting dates of infected leaf material were used to determine the best time to differentiate between resistance and susceptibility according to the qPCR analysis that used the *β-tubulin*:*translation elongation factor 1 alpha-subunit* (*TEF1*) gene expression ratios. Flag leaves were collected at the different dpi from plants of all nine cultivars/lines inoculated with *Pt* and *Pst.* Leaf sheaths were collected at the different dpi from the nine cultivars/lines inoculated with *Pgt*. Collected leaf samples were placed in 15 ml tubes and kept in liquid nitrogen and stored in a freezer at -80˚C until they were used for RNA isolation and qPCR analyses.

5.2.2 Optimisation of analysis of fungal gene expression of wheat cultivars infected with *Puccinia* species

5.2.2.1 RNA isolation

Sampled leaf tissue was ground in liquid nitrogen using a mortar and pestle to a fine powder and placed in 1.5 ml microcentrifuge tubes that were placed directly in liquid nitrogen and stored at -80˚C. For RNA isolation, 100 µl of the ground tissue was transferred to a new 1.5 ml microcentrifuge tube. Samples were partially thawed on the

bench and 500 µl Trizol reagent (Invitrogen™, Thermo Fisher Scientific, Johannesburg, RSA) was added to each sample and mixed well. Samples were incubated at room temperature (22˚C) for 10 min. A volume of 100 µl chloroform was added to each sample and tubes inverted 15 times, followed by incubation at room temperature (22˚C) for 5 min. Samples were centrifuged at 4˚C for 15 min at 12 000 *g*. A volume of 250 µl of the clear supernatant was transferred to a new 1.5 ml microcentrifuge tube containing 250 µl isopropanol. Samples were inverted 10 times and incubated for 10 min at room temperature (22˚C). Samples were centrifuged at 4˚C for 10 min at 12 000 *g* to collect RNA. The supernatant was removed using a water jet pump and 500 µl 70% (v/v) ethanol added to every RNA pellet. Samples were centrifuged at 4˚C for 10 min at 7 500 *g*. The supernatant was removed using a water jet pump and samples air-dried for 5 min at room temperature (22˚C). A volume of 200 µl diethyl pyrocarbonate (DEPC) treated water was added to every sample and incubated overnight at 4˚C. The RNA concentrations of samples were determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and was blanked using DEPC water. RNA quality was determined by separating 500 ng of the extracted RNA on a 1% (w/v) agarose gel. Ethidium bromide was used to visualise the RNA. Electrophoresis was done at 80 V for 30 min in 0.5x TAE (Tris-HCl/Acetate/EDTA) buffer (48.4 g/l (w/v) Tris-HCl; 10 mM EDTA; 11.5 ml/l (v/v) glacial acetic acid). RNA was diluted to a final concentration of 5 ng/µl using DEPC water.

5.2.2.2 DNA isolation and marker-assisted selection

Additional leaf material was also collected from inoculated seedlings and adult plants and placed in 1.5 ml tubes. Additional harvested leaf material for DNA isolation was placed on ice and freeze-dried. Seedling leaf material used for DNA isolation was harvested after collection of all samples needed for RNA isolation and phenotypic evaluation was done. Leaf material needed for DNA isolation of the adult plants was collected just before inoculation. DNA was isolated as described in section 3.2.4. All leaf material samples both from seedlings and adult plants of the cultivars/lines were screened using the rust resistance markers presented in Table 3.1 and as described in section 3.2.6.

5.2.3. Optimisation of primers used to detect fungal gene expression

Primers were developed for the amplification of the *β-tubulin* HG of *Pt* and *Pst* based on available sequence data (National Center for Biotechnology Information/Primer-Basic Local Alignment Search Tool). The *TEF1* gene of wheat was included as the HG of wheat that was used as the standard to determine the gene expression ratios. Primers

for the *β-tubulin* gene of *Pgt* and *TEF1* gene of wheat were obtained from HD Castelyn (unpublished data). Leaf samples, 192 hours post inoculation (hpi) obtained from HD Castelyn, infected with *Pt*, *Pgt* and *Pst*, were used for optimisation of the qPCR primers. RNA was isolated from these samples as described in section 5.2.2.2. The MIQE guidelines were used for qPCR analysis (Bustin et al. 2009). The qPCR analysis was done using a Bio-Rad1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) with a CFX96 real-time attachment.

The qPCR reactions contained 10 ng/µl RNA, 1x KAPA™ SYBR® FAST qRT-PCR kit (KAPABIOSYSTEMS, Sigma, Johannesburg, RSA) and 10 pmol of each primer in a total volume of 10 µl. A temperature gradient (50-60°C) was done for all primer pairs to determine the optimal annealing temperatures while a 1:10 dilution series was done to determine their amplification efficiency. The amplification cycles were as follows: denaturation at 95°C for 5 min followed by 40 cycles of 95°C for 3 sec and 30 sec at the specific annealing temperature (Table 5.2). A melt curve was included and ranged from 65-95°C with 0.5°C increments every 5 sec to confirm no amplification of non-specific amplification products occurred. Following optimisation of primers, qPCR analysis was applied to samples used for the timeline study for both seedlings and adult plants. The timeline study was done to determine the best time to harvest leaf material where the biggest differences were observed between fungal gene expression in susceptible versus resistant plants.

Table 5.2 Quantitative polymerase chain reaction primer sequences and annealing temperatures

Gene	Primer sequence (5'-3')	Fragment size (bp)	Annealing temperature
P. triticina β-tubulin (Leaf rust)	F: CCTGAGTTGACCTCGCAAAT R: CGAAACCTTACCACGGAAGTA	106	56°C
P. graminis β-tubulin (Stem rust)	F: CTCGATCGTGATGAGTGGGA R: AGTGCAATCGAGGGAAAGGA	106	56°C
P. striiformis β-tubulin (Stripe rust)	F: CTCGGACGAAACCTTCTGTAT R: CGTAGGTAGGTGTAGCCAATTT	106	58.7°C
Wheat *TEF1*	F: GCCCTCCTTGCTTTCACTCT R: AACGCGCCTTTGAGTACTTG	91	53°C

qPCR-Quantitative polymerase chain reaction; bp-base pair(s); *β-tubulin-beta-tubulin; TEF1-translation elongation factor 1 alpha-subunit*; F-forward; R-reverse

5.2.4 Phenotypic evaluation and fungal gene expression in experimental wheat lines infected with *Puccinia* species

Following optimisation of the qPCR analysis, the phenotypic and fungal gene expression evaluations were applied to experimental lines developed during this study containing different rust resistance gene/QTL combinations. Experimental lines were selected from the rust resistant female lines used in section 3.2.2. Cultivars/lines selected for seedling and adult plant evaluations are summarised in Table 5.3. All parental and control cultivars/lines were evaluated using all three rusts types. The first three experimental lines in Table 5.3 were planted and inoculated with *Pt* (leaf rust). Experimental lines KSF_2-682.2 to KSF_2-682.11 were inoculated with *Pgt* (stem rust) and the last two experimental lines in Table 5.3 were inoculated with *Pst* (stripe rust).

Table 5.3 Parental, control and experimental cultivars/lines selected for phenotypic and fungal gene expression evaluation

Cultivar/line	Background	Rust resistance gene/QTL genotype
Kariega	Rust resistant parental line	*Lr34/Yr18/Sr57; QYr.sgi.2B-1*
Avocet*YrSP*	Rust resistant parental line	*Lr34/Yr18/Sr57; Sr26*[1]; *YrSP*
Blade	Rust resistant parental line	*Sr26*
CS*Lr19*-149-299	Rust resistant parental line	*Lr19*; *Lr34/Yr18/Sr57*
Kingbird	Rust resistant parental line	*Sr2/Yr30*; *Lr34/Yr18/Sr57*
2S#/163	Rust resistant parental line	*Sr39*
CM-82036	FHB resistant donor line	*Lr34/Yr18/Sr57*
Krokodil	FHB susceptible recurrent line	None
CS Hope 3B	Control line	*Lr34/Yr18/Sr57; Sr2/Yr30*
KSF_2-159.11	Experimental line	*Lr34/Yr18/Sr57*
KSF_2-682.9	Experimental line	*Lr19*
KSF_2-182.1	Experimental line	*Lr19; Lr34/Yr18/Sr57*
KSF_2-682.2	Experimental line	*Sr2/Yr30*
KSF_2-182.24	Experimental line	*Sr39*
KSF_2-682.1	Experimental line	*Sr26*
KSF_2-159.1	Experimental line	*Sr2/Yr30; Sr26*
KSF_2-682.25	Experimental line	*Sr2/Yr30; Sr39*
KSF_2-182.3	Experimental line	*Sr26; Sr39*
KSF_2-682.11	Experimental line	*Sr2/Yr30; Sr26; Sr39*
KSF_2-159.25	Experimental line	*Lr19; Lr34/Yr18/Sr57*
KSF_2-182.26	Experimental line	*Lr19; Lr34/Yr18/Sr57; Sr2/Yr30; Sr26; Sr39*

QTL-Quantitative trait loci; [1]*Sr26* is still segregating in Avocet*YrSP.*

5.2.4.1 Inoculation of wheat seedlings with *Puccinia* species

Five seeds per cultivar/line were planted, in a circular order starting at the name tag with seed number one, in a 500 ml pot as described in section 5.2.1.2. Seedlings were inoculated 14 days after planting when two leaves were present per plant. The primary leaf was sampled at 10 dpi for RNA isolation and fungal gene expression analysis. Primary leaves from all five plants planted per experimental line were harvested individually while only one primary leaf, from one of the five plants available, was harvested for the parental or control cultivars/lines. The second leaf of each plant was used to determine the infection type of each cultivar/line at 15 dpi. Leaf samples for DNA extraction were collected from all plants after the infection types were determined. DNA samples were used for MAS using markers linked to rust resistance genes/QTL as summarised in Table 3.1.

5.2.4.2 Inoculation of adult wheat plants with *Puccinia* species

Four seeds per cultivar/line (Table 5.3) were planted in a 2 l pot and inoculated as explained in section 5.2.1.3. Leaf samples for DNA isolation were harvested from each experimental line and parental and control cultivars/lines before inoculation and freeze-dried. DNA samples were used for MAS using markers linked to rust resistance genes/QTL (Table 3.1). Flag leaves were collected 10 dpi from individual plants inoculated with *Pt* and *Pst*. Leaf sheaths of wheat lines inoculated with *Pgt* were collected at 10 dpi. Collected plant material 10 dpi was used for RNA isolation and qPCR analysis as described in sections 5.2.2.1 and 5.2.3. Phenotypic evaluation was done 15 dpi to determine the infection type.

5.2.5 Wheat rust field trials

Rust resistant lines as well as parental and control lines were selected for planting in field trials for two consecutive years, 2015 and 2016. Field trials were planted at Redgates, a PANNAR® seed research farm, outside Greytown South Africa (29°08'16.41"S, 30°37'22.68"E). During the first year (2015), the six parental rust resistant donor cultivars/lines and 11 KSF_2 experimental lines with different rust resistance gene/QTL combinations were planted. In 2016, six rust resistant parental donor cultivars/lines, the two FHB parental lines, the control line, CS Hope 3B, and 12 KSF_2 experimental lines with different rust resistance gene/QTL combinations were planted. Rows were planted serpentine and every tenth entry was a susceptible check. A total of 10 seeds per entry were planted. Spreader head rows were inoculated with leaf-, stem- and stripe rust

spores early in the season to serve as primary inoculum and to enhance epidemic development later in the season. Leaf material was collected in the field from each individual plant of the experimental lines as well as from the parental and control cultivars/lines. Leaf material was used for DNA isolation as described in section 3.2.4 and MAS linked to the rust resistant molecular markers (Table 3.1). All plants were phenotypically evaluated for all three different rust type infection severities.

5.2.6 Data analyses

Fungal biomass accumulation for each of the three rust genes was determined through calculation of the expression ratio (*β-tubulin*:*TEF1*). The *TEF1* gene of wheat was used as the reference gene for all three rust *β-tubulin* genes. Quantitative-PCR analysis for all four primers were done using four technical replications. Following qPCR, quantification cycle (Cq) values were imported into GeNorm-Plus2 software (Biogazelle, Ghent, Belgium) to obtain the average expression stability (M) values. Expression results were determined using qBase^{Plus} software (Biogazelle). Replicate variability was set to 0.5 and samples with a lower or higher replicate variability of 0.5 were excluded from analysis.

MAS data was evaluated as described in section 3.2.8. Infections types were recorded at 15 dpi using the Stakman scale (Table 2.1; Stakman et al. 1962). Phenotypic evaluation of adult plants was done at 15 dpi and the infection response types were recorded according to Stubbs et al. 1986 [resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S)].

The disease severity of experimental lines planted in the field trials in 2015 and 2016 were evaluated per plant as they were still segregating. Disease severity of leaf rust was calculated as the percentage of leaf or stem area affected by rust. Disease severity of stem rust was calculated as the percentage of stem area affected by rust while stripe rust disease severity was calculated as the percentage of leaf area affected by rust.

5.3 Results

5.3.1 Timeline study data of parental and control cultivars/lines

5.3.1.1 Molecular marker data of parental and control cultivars/lines

The parental cultivars used to develop the rust resistant lines used in this study were used to conduct the timeline study because their genotypes are fixed and contained all rust resistance genes/QTL relevant to this study. Additional control lines were also included in the timeline study. Sumai3 was used as a negative control while

Thatcher*Lr19* contains the *Lr19* gene without *Lr34/Yr18/Sr57* and CS Hope 3B contains the *Sr2/Yr30* gene. The same cultivars/lines were used for the timeline study in both the seedling and adult plant inoculation methods. MAS confirmed the genotypes for the rust resistant markers as presented in Table 5.1.

5.3.1.2 Seedling phenotypic data and fungal gene expression ratio of parental and control cultivars/lines

All cultivars/lines were separately inoculated with *Pt*, *Pgt* and *Pst* pathotypes. Leaf material was collected for qPCR evaluation at different time-intervals while cultivars/lines were phenotypically evaluated at 15 dpi. Table 5.4 summarises the phenotypic evaluation data for the three rust types at seedling stage.

Table 5.4 Phenotypic rust evaluation data at seedling stage

Cultivar/line	Leaf rust MCDS	Stem rust PTKST	Stripe rust 6E22A+
Kariega	3++	3	3++
Avocet*YrSP*	4	2+3	0
Blade	3	;1-	3
CS*Lr19*-149-299	;	3-	3++
Kingbird	4	3++	4
2S#/163	4	;1-	;cn
Thatcher*Lr19*	;	1	4
CS Hope 3B	3	4	3
Sumai3	3+	4	3+

Photos of infected leaves were taken 15 dpi and are shown in Figure 5.1. Lines containing rust specific ASR genes (Avocet*YrSP*, Blade, CS*Lr19*-149-299, 2S#/163 and Thatcher*Lr19*) showed low infection types towards the corresponding rust pathotypes. Results indicate that line 2S#/163 carries an *Yr* ASR gene(s) not tested for in this study. Results indicated the absence of the *Sr26* gene in Avocet*YrSP* because a higher stem rust infection type was observed compared to Blade that also contains the *Sr26* gene. Thus even though Avocet*YrSP* tested positive for the *Sr26* gene using MAS, contradictory results were obtained based on the seedling infection rating. The gene thus might be present but is not being expressed, MAS might have given a false positive result because it is not a diagnostic marker or the seed source for Avocet*YrSP* used in this study might still be segregating for *Sr26*. Phenotypic data indicated the presence of

the *YrSP* gene, even though no molecular marker was used to confirm the presence of the *YrSP* gene.

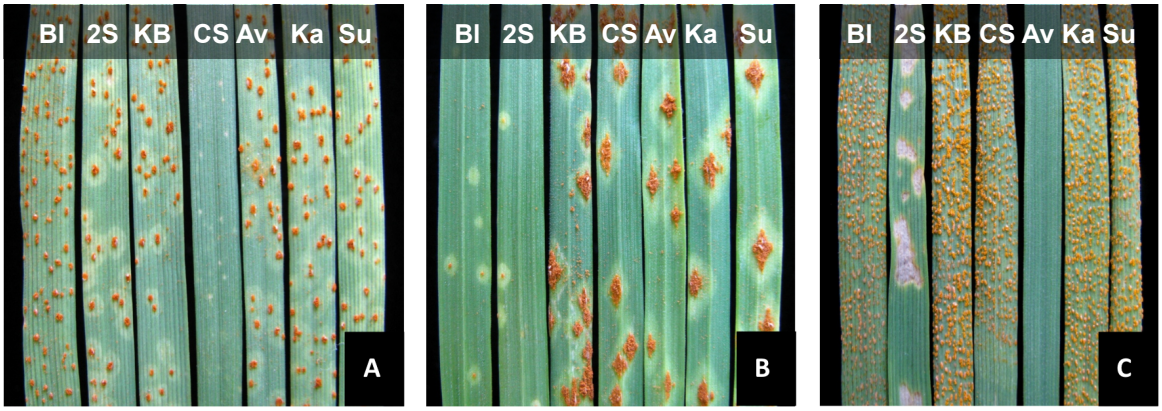


Figure 5.1 Photos of rust infected seedling leaves at 15 days post inoculation. Seedling leaves infected with (A) *P. triticina* (leaf rust), (B) *P. graminis* (stem rust) and (C) *P. striiformis* (stripe rust) pathotypes. Leaves from left to right: Blade (Bl), 2S#/163 (2S), Kingbird (KB), CS-*Lr19*-149-299 (CS), Avocet*YrSP* (Av), Kariega (Ka) and Sumai3 (Su).

The qPCR data for the three different rust types are summarised in Figures 5.2 to 5.4. These graphs indicate the expression ratios of the *β-tubulin* gene relatively to the *TEF1* housekeeping gene of wheat at different time-intervals after infection using the three different rust pathotypes. Due to poor seed germination of Avocet*YrSP* when the timeline study was done, no leaf material could be harvested for qPCR analysis after leaf rust infection. However, molecular marker as well as seedling infection type data was obtained. Most genotypes showed the highest expression ratios at 10 dpi. Genotypes with ASR resistance genes maintained a low expression ratio for all time intervals making it easy to discriminate them from susceptible genotypes with higher expression ratios. CS*Lr19*-149-299 and Thatcher*Lr19*, both containing the *Lr19* gene, showed low (0.025 and 0.310) leaf rust infection ratios, while line 2S#/163 (*Sr39*), Blade (*Sr26*) and Thatcher*Lr19* showed low (0.185, 0.484 and 1.172) stem rust infections and Avocet*YrSP* (*YrSP*) and 2S#/163 (unknown resistance) showed low stripe rust infections (0.076 and 0.288) at 10 dpi. MAS indicated the absence of the *Sr26* gene in Avocet*YrSP*, which was confirmed by the host response and the infection ratio (10.484). The biggest differences observed regarding the expression ratio of resistant versus susceptible cultivars/lines were at 10 dpi for all three rust types and therefore leaf material of experimental lines for qPCR analysis was harvested at 10 dpi.

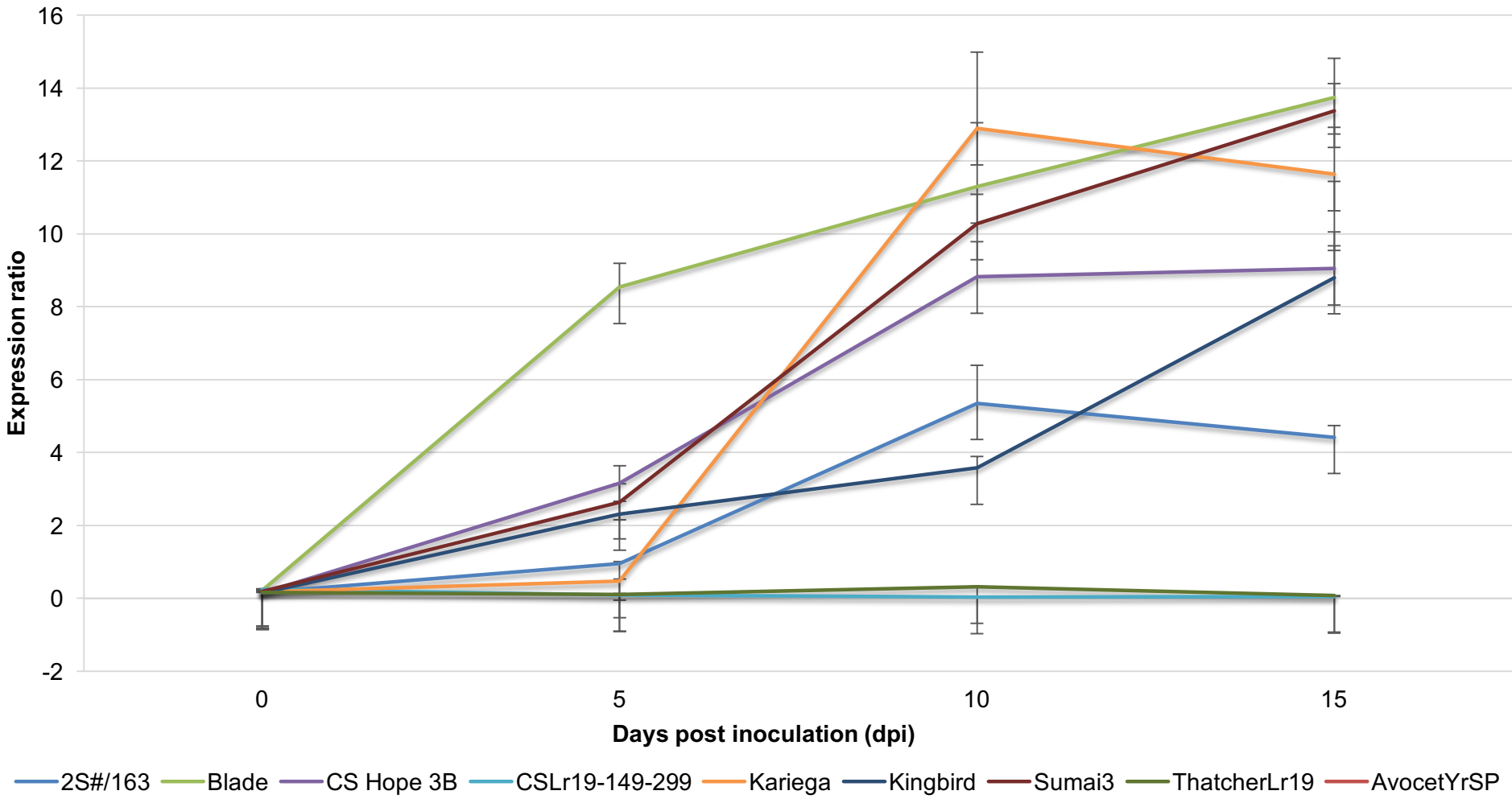


Figure 5.2 Expression ratios obtained for seedling infected cultivars/lines of the *β-tubulin* gene of *P. triticina* (leaf rust) relative to the *translation elongation factor 1 alpha-subunit* gene of wheat. Bars indicate standard error for each data point.

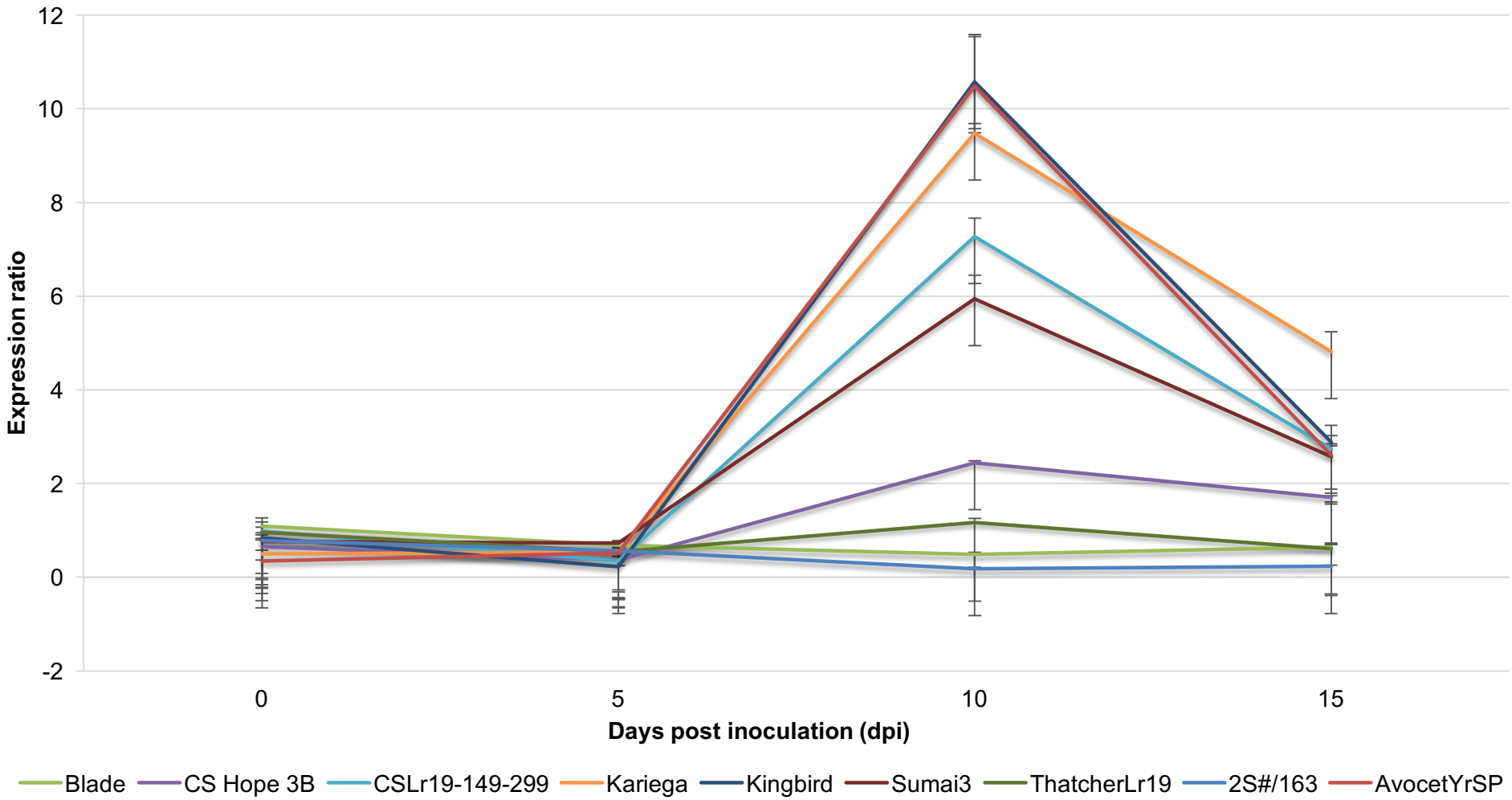


Figure 5.3 Expression ratios obtained for seedling infected cultivars/lines of the *β-tubulin* gene of *P. graminis* (stem rust) relative to the *translation elongation factor 1 alpha-subunit* gene of wheat. Bars indicate standard error for each data point.

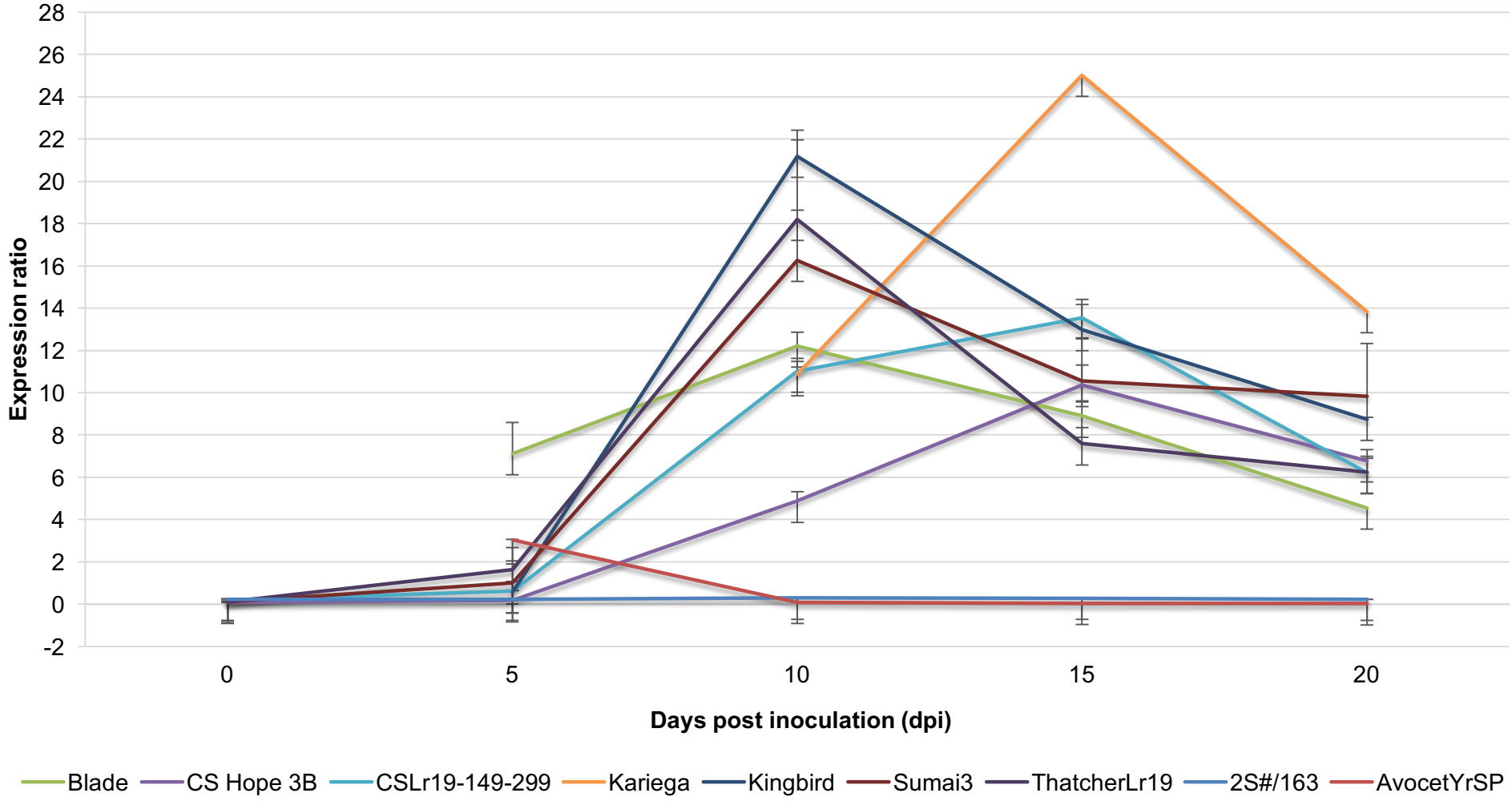


Figure 5.4 **Expression ratios obtained for seedling infected cultivars/lines of the *β-tubulin* gene of *P. striiformis* (stripe rust) relative to the *translation elongation factor 1 alpha-subunit* gene of wheat.** Bars indicate standard error for each data point.

5.3.1.3 Adult plant phenotypic data and fungal gene expression ratio of parental and control lines

A timeline study was also conducted for eight of the nine cultivars/lines at adult plant stage because different APR genes/QTL were present in the parental lines used to develop the rust resistant lines evaluated in this study. Thatcher*Lr19* was excluded during the adult plant stage timeline study due to poor seed germination. All eight cultivars were separately inoculated with the same rust pathotypes used in the seedling timeline study. Leaf- (for leaf- and stripe rust) and leaf sheath (for stem rust) material was collected for qPCR analysis at different time intervals as well as when cultivars/lines was phenotypically evaluated 15 dpi. No data was generated for CS Hope 3B infected with the leaf rust pathotype because insufficient leaf material was available at 15 dpi. The cultivar/lines' response at 15 dpi are summarised in Table 5.5 for all three rust types.

Table 5.5 Phenotypic rust evaluation data at adult plant stage

Cultivar/line	Leaf rust MCDS	Stem rust PTKST	Stripe rust 6E22A+
Kariega	S	S	0R
Avocet*YrSP*	S	R	0R
Blade	S	R	MR
CS*Lr19*-149-299	0R	S	MSS
Kingbird	ZMS	R	RMR
2S#/163	RMR	0R	0R
CS Hope 3B	N/A	MS	RMR
Sumai3	S	S	MS

N/A-Not available; Z-uredia towards the leaf base; Phenotypic evaluation was done according to Table 2.3.

CS*Lr19*-149-299 was the only cultivar/line with a resistance response type to leaf rust due to the presence of the ASR *Lr19* gene as seen in the seedling timeline trial as well. It also contains the APR *Lr34/Yr18/Sr57* gene, but other cultivars/lines (Kariega, Avocet*YrSP* and Sumai3) containing the *Lr34/Yr18/Sr57* gene gave compatible response types towards leaf rust, as this slow rusting gene is known to be associated with slower rust development and severity levels rather than incompatible response types. Kingbird also contains the *Lr34/Yr18/Sr57* gene and was moderately susceptible, with pustules concentrated at the base of the leaf. Kingbird also contains the *Sr2/Yr30* gene that is tightly linked to *Lr27* (Mago et al. 2011), and this genetic background can explain the more resistant response type observed for Kingbird. CS*Lr19*-149-299 and 2S#/163 showed resistant (0R and RMR) response types during phenotypic evaluations for leaf rust (Figure 5.5) and also showed low *β-tubulin:TEF1* ratios during qPCR analysis

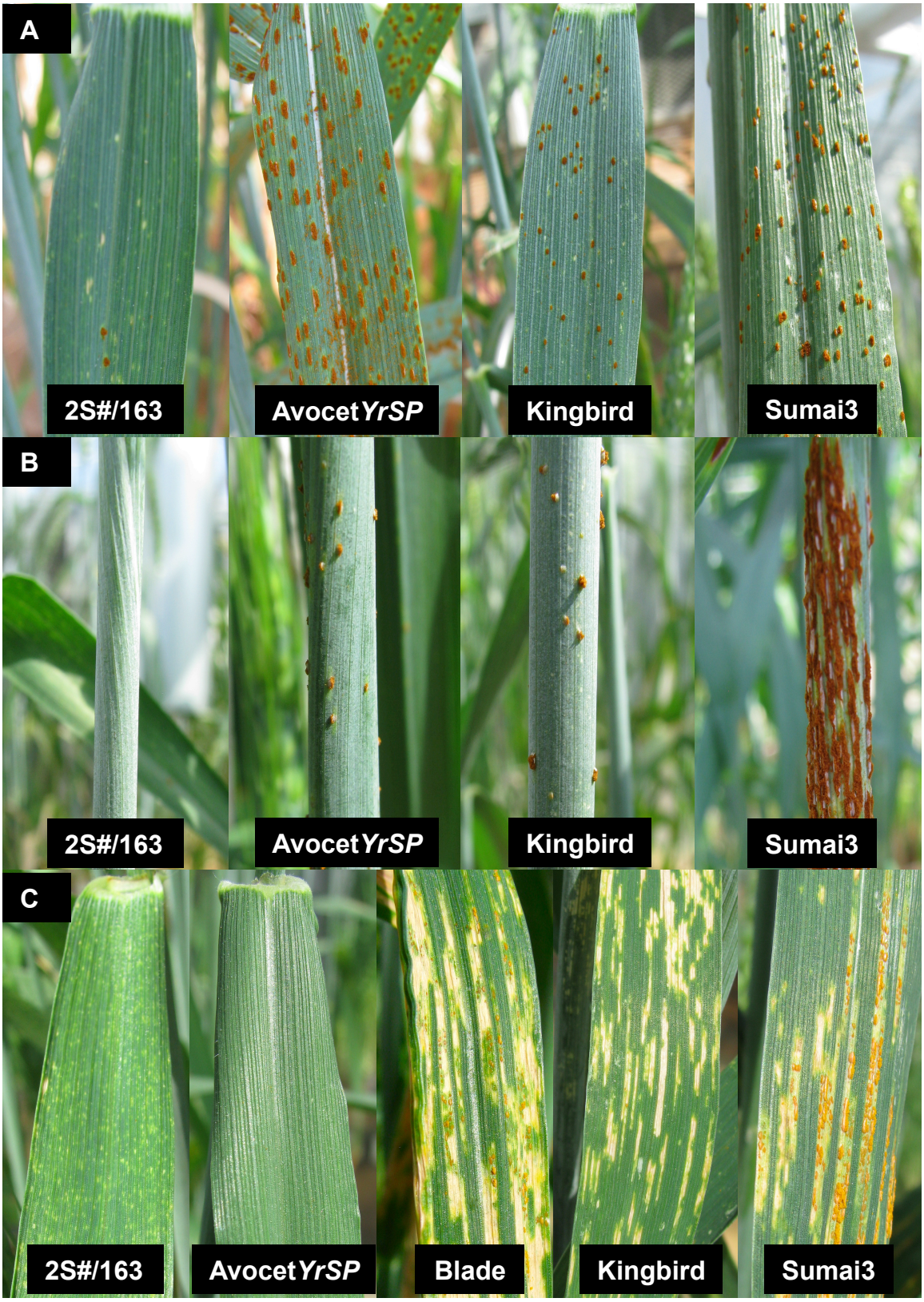


Figure 5.5 Photos of rust infected adult plant leaves/stems at 15 days post inoculation. Adult plant leaves/stems infected with (A) *P. triticina* (leaf rust), (B) *P. graminis* (stem rust) and (C) *P. striiformis* (stripe rust) isolates.

(Figure 5.6). However, Kingbird had a ZMS phenotypic score but low *β-tubulin:TEF1* ratios (value even lower than 2S#/163).

Avocet*YrSP*, Blade and line 2S#/163 showed resistance response types for stem rust infections and low *β-tubulin:TEF1* ratios (Figure 5.7) attributed to the presence of the ASR genes present for the latter two. Contradictory data was obtained for Avocet*YrSP* when considering the seedling infection type and the adult plant response type data. Kingbird contain the *Sr2/Yr30* and *Lr34/Yr18/Sr57* genes, the same as for CS Hope 3B, but the response type for Kingbird (RMR) towards stem rust was more resistant when compared to that of CS Hope 3B (MS), indicating that Kingbird carries an additional resistance source(s) towards stem rust or the specific stem rust pathotype used. Kingbird and CS Hope 3B obtained low *β-tubulin:TEF1* ratios. Kariega, CS*Lr19*-149-299 and Sumai3 were susceptible for stem rust and showed high *β-tubulin:TEF1* ratios. Lines containing *Sr2/Yr30*, *Sr26* and *Sr39* showed low *β-tubulin:TEF1* ratios while lines containing only the *Lr34/Yr18/Sr57* APR gene had high *β-tubulin:TEF1* ratios (Figure 5.7).

Higher levels of variation in the adult plant response was obtained for cultivars/lines inoculated with stripe rust. Kariega and Avocet*YrSP* were resistant towards stripe rust infection which can be attributed to the presence of the APR *QYr.sgi.2B-1* and ASR *YrSP* genes, respectively in combination with the APR *Lr34/Yr18/Sr57* gene and therefore had low *β-tubulin:TEF1* ratios (Figure 5.8). Line 2S#/163 carries a resistance source that was not tested for in this study. Blade was moderately resistant (MR) while Kingbird and CS Hope 3B, containing the *Sr2/Yr30* and *Lr34/Yr18/Sr57* genes, were resistant (R) and resistant to moderately resistant (RMR), respectively. CS*Lr19*-149-299 was moderately susceptible to susceptible (MSS) and Simai3 was moderately susceptible (MS).

Figure 5.5 illustrates plant response levels of the cultivars/lines inoculated with the three different rust pathotypes. The qPCR data of the *β-tubulin* genes expressed relatively to the wheat *TEF1* gene is illustrated in Figures 5.6 to 5.8 for the three rust pathotypes. Expression ratios of the susceptible adult plants infected with leaf- and stem rust mostly peaked at 10 dpi, while the susceptible adult plants infected with stripe rust peaked at 15 dpi. However, the most defined difference was observed between susceptible (high expression ratio) and resistant (low expression ratio) lines at 10 dpi. The phenotypic data correlated well with the expression ratios of the qPCR data, except for stripe rust infected Blade plants that showed a MR reaction response but had a high expression ratio which can be attributed to the high level of chlorosis observed that might have decreased the expression of *TEF1* genes.

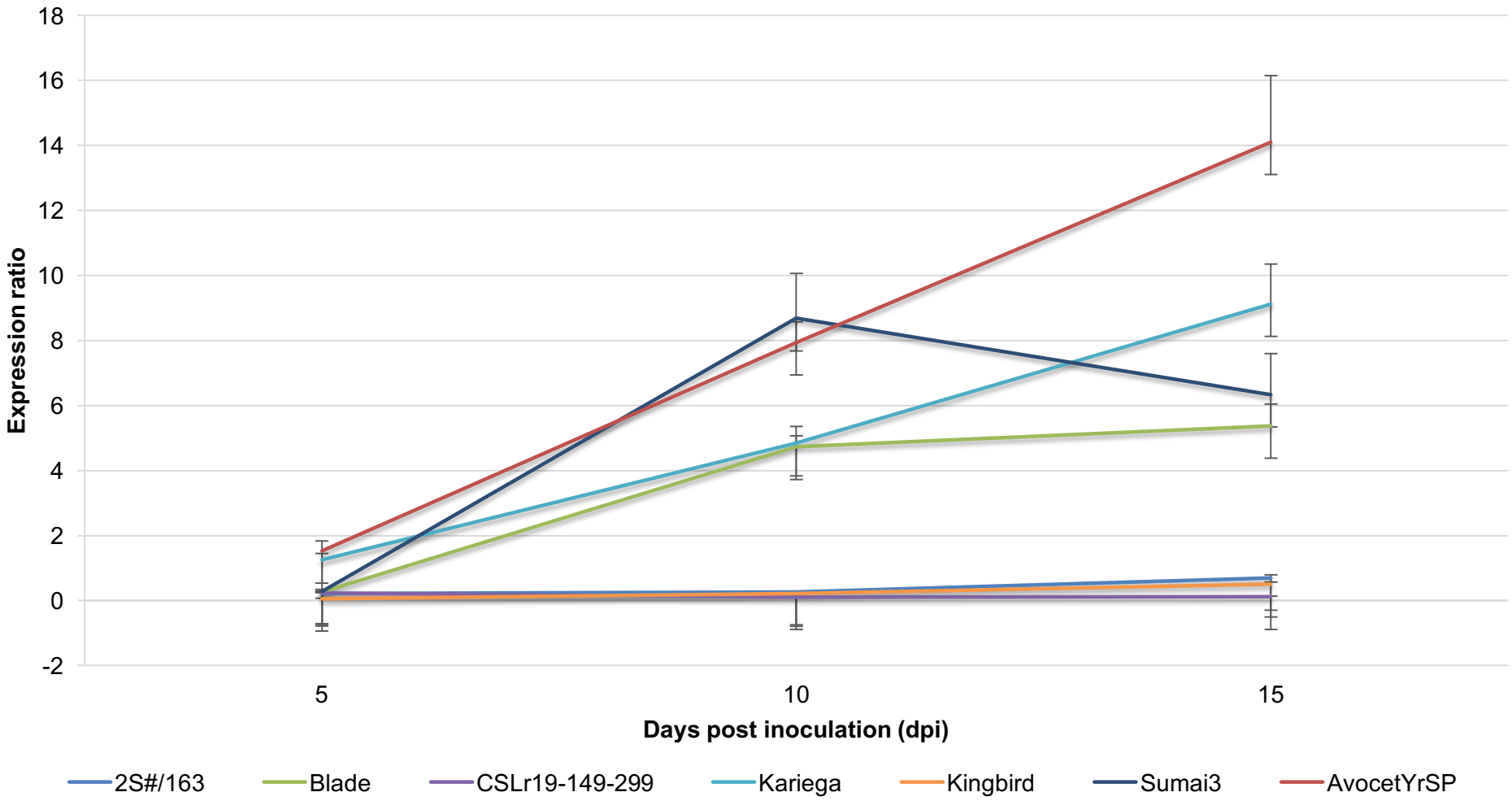


Figure 5.6 **Expression ratio of infected adult plant cultivars/lines of the *β-tubulin* gene of *P. triticina* (leaf rust) relative to the *translation elongation factor 1 alpha-subunit* gene of wheat.** Bars indicate standard error for each data point.

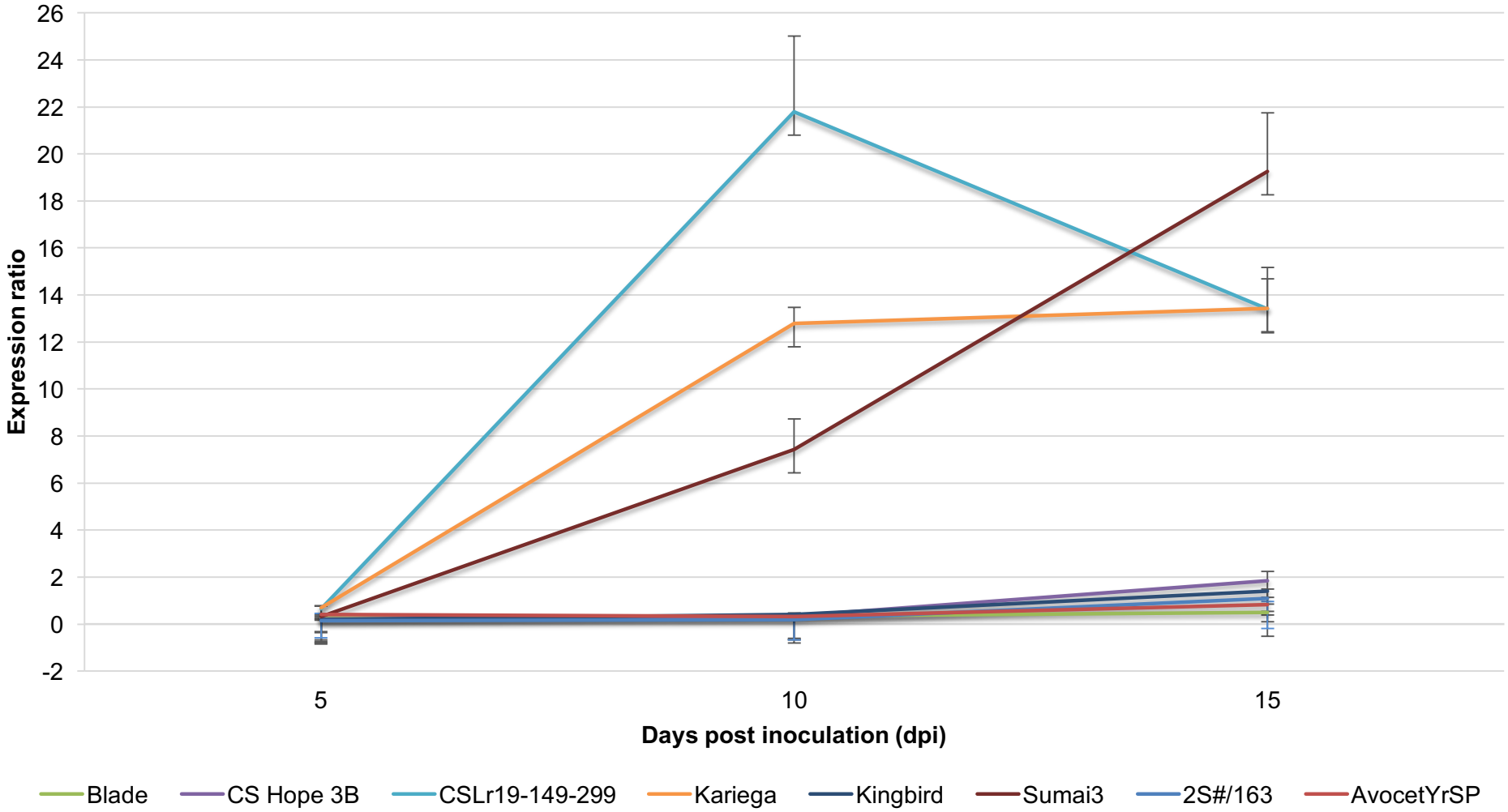


Figure 5.7 Expression ratio of infected adult plant cultivars/lines of the *β-tubulin* gene of *P. graminis* (stem rust) relative to the *translation elongation factor 1 alpha-subunit* gene of wheat. Bars indicate standard error for each data point.

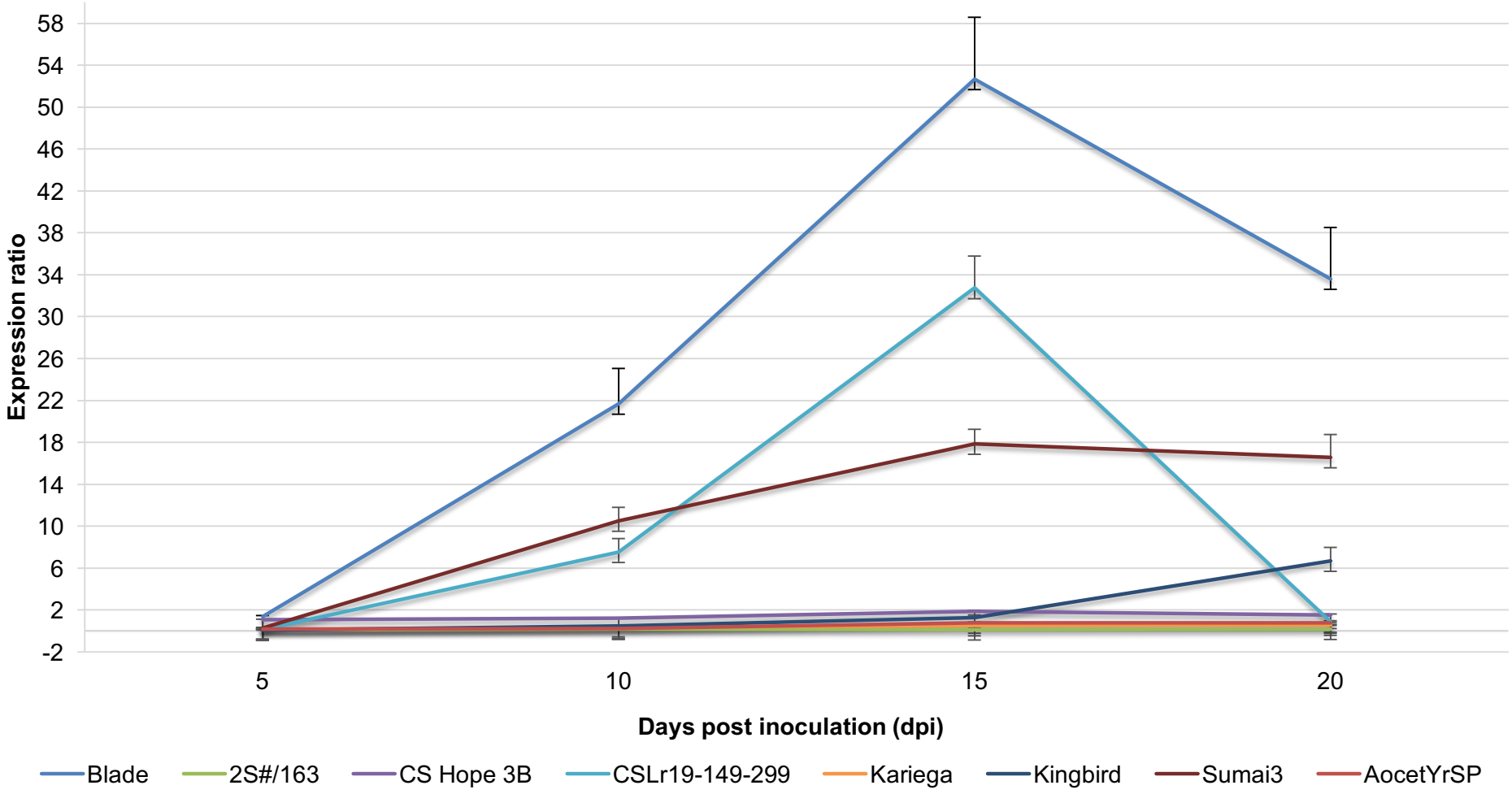


Figure 5.8 Expression ratio of infected adult plant cultivars/lines of the *β-tubulin* gene of *P. striiformis* (stripe rust) relative to the *translation elongation factor 1 alpha-subunit* gene of wheat. Bars indicate standard error for each data point.

The expression ratios of both Blade and CS*Lr19*-149-299 peaked at 15 dpi followed by a radical decrease in the expression ratios at 20 dpi. This decrease can be attributed to the pathogen that reached the end of its disease cycle and less to no *β-tubulin* genes were present at 20 dpi compared to the *TEF1* gene of wheat.

5.3.2 *Puccinia* inoculation data of experimental lines at seedling stage

All eight parental lines used to develop the rust and FHB resistant populations during this study as well as three experimental lines, KSF_2-159.11, KSF_2-682.9 and KSF_2-182.1, containing different combinations of leaf rust genes (Table 5.3), were inoculated at the seedling stage with the leaf rust pathotype, MCDS. Another set of parental lines together with the CS Hope 3B control line and seven experimental lines KSF_2-682.2, KSF_2-182.24, KSF_2-682.1, KSF_2-159.1, KSF_2-682.25, KSF_2-182.3, KSF_2-682.11 containing different combinations of stem rust resistance genes (Table 5.3) were inoculated with the stem rust pathotype, PTKST, eight days after the seed was planted. The stripe rust isolate, 6E22A+, was used to inoculate another set of the parental lines as well as two experimental lines KSF_2-159.25 and KSF_2-182.26 containing different combinations of stripe rust resistance genes (Table 5.3). One seedling per parental cultivar/line was evaluated while all five plants per experimental line were evaluated, because the experimental lines were still considered segregating, thus each individual offspring could have a different genotype. A total of 5 seeds per pot were planted for each line.

5.3.2.1 Molecular marker data of *Puccinia* inoculated experimental lines at seedling stage

All lines were evaluated with rust resistance markers linked to five rust resistance genes to obtain the complete ASR rust resistance genotype. Table 5.6 summarises the genotypes obtained for each line. The experimental lines showed segregation between offspring of the same experimental line and heterozygous genes were recorded for some molecular markers (e.g. *Sr26* and *Sr39*). Various gene combinations were obtained between the experimental lines.

5.3.2.2 Phenotypic evaluation data of *Puccinia* inoculated experimental lines at seedling stage

All rust inoculated seedlings were individually phenotypically evaluated at 15 dpi by determining the infection type towards the three different rust types (Table 5.6). Lines selected based on the presence of different leaf rust resistance gene combinations, were only phenotypically evaluated for leaf rust resistance, etc.

Table 5.6 Rust resistance genotypes as well as phenotypic infection types of parental, control and experimental lines at seedling stage

Rust inoculated groups	Cultivar/line	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Leaf rust MCDS	Stem rust PTKST	Stripe rust 6E22A+
	Kariega	0	1	0	0	0	3	3	3+
	Avocet*YrSP*	0	1	0	0	0	4	4	0;
	Blade	0	0	0	1	0	4	1	4
Parental and	CS*Lr19*-149-299	1	1	0	0	0	;	4	4
control	Kingbird	0	1	1	0	0	3	2++	4
cultivars/lines	2S#/163	0	0	0	0	1	3	;1	;cn
	CM-82036	0	1	0	0	0	2	4	;
	Krokodil	0	0	0	0	0	3	3	4
	CS Hope 3B	0	1	1	0	0	4	4	3
	KSF_3-159.11.1	1	1	1	0	0.5	;		
	KSF_3-159.11.2	0	1	1	0	0	3+		
	KSF_3-159.11.3	1	1	1	0	0.5	;		
Leaf rust	KSF_3-159.11.5	1	1	0	0	0.5	;		
infected	KSF_3-682.9.2	0	0	1	0.5	0.5	3		
experimental	KSF_3-682.9.5	1	0	1	0.5	0	;		
lines	KSF_3-182.1.1	1	1	1	0	0	;		
	KSF_3-182.1.3	1	1	1	0	0	;		
	KSF_3-182.1.4	1	1	1	0	0	;		
	KSF_3-182.1.5	1	1	0	0	0	;		
	KSF_3-682.2.1	1	0	0	0	0		2+	
	KSF_3-682.2.2	0	0	1	0.5	0		1	
	KSF_3-682.2.3	1	0	1	1	0		;1	
	KSF_3-682.2.4	1	0	1	0.5	0		;1	
Stem rust	KSF_3-182.24.1	1	1	0	0	0.5		;1	
infected	KSF_3-182.24.2	1	1	0	0	0.5		;1	
experimental	KSF_3-182.24.3	1	1	0	0	0.5		;1	
lines	KSF_3-182.24.5	1	1	0	0	0.5		;1	
	KSF_3-682.1.1	0	0	0	0.5	0		2	
	KSF_3-682.1.2	0	1	0	0.5	0		1	
	KSF_3-682.1.3	0	0	0	0.5	0		;1	
	KSF_3-682.1.5	0	1	0	0	0		4	

Table 5.6 Rust resistance genotypes as well as phenotypic infection types of parental, control and experimental lines at seedling stage (continued)

Rust inoculated groups	Cultivar/line	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Leaf rust MCDS	Stem rust PTKST	Stripe rust 6E22A+
Stem rust infected experimental lines (continued)	KSF_3-159.1.1	0	1	1	1	0		;	
	KSF_3-159.1.2	1	1	1	0	0.5		;	
	KSF_3-159.1.3	1	1	0	0.5	0.5		;	
	KSF_3-159.1.4	0	1	1	0.5	0.5		;	
	KSF_3-159.1.5	0	1	1	0	0		3++	
	KSF_3-682.25.1	0	0	1	0	0.5		1	
	KSF_3-682.25.3	0	0	1	0	0.5		1	
	KSF_3-682.25.5	0	0	1	0	0.5		1	
	KSF_3-182.3.1	1	0.5	0	0.5	0.5		;1	
	KSF_3-182.3.2	0	1	0	0.5	0.5		1	
	KSF_3-182.3.3	0	1	0	1	0.5		1	
	KSF_3-182.3.5	1	1	0	0.5	0.5		1	
	KSF_3-682.11.1	0	0	1	1	0.5		0	
	KSF_3-682.11.2	0	0	1	1	0.5		0	
	KSF_3-682.11.4	1	0	1	1	0.5		0	
	KSF_3-682.11.5	0	0	1	1	0.5		0	
Stripe rust infected experimental lines	KSF_3-159.25.1	0	1	1	0	0			4
	KSF_3-159.25.2	0	1	1	0	0			3+
	KSF_3-159.25.3	0	1	1	0	0			4
	KSF_3-159.25.5	0	1	1	0	0			2c
	KSF_3-182.26.1	0	1	1	1	0.5			;cn
	KSF_3-182.26.2	0	1	1	0	0.5			;cn
	KSF_3-182.26.3	1	1	0	1	0			;cn
	KSF_3-182.26.4	1	1	1	0	0			;cn

[1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0). Phenotypic evaluation was done according to Table 2.1.

Variation was observed between the offspring of the same experimental line as expected because the lines were still segregating. A good correlation was observed between the seedling infection types of the experimental lines and their molecular marker data except for the stripe rust infected lines. This can be attributed to the presence of the ASR *YrSP* gene as well as the unknown seedling gene(s) in line 2S#/163, as markers was not yet optimised or used for these two resistance sources, respectively. The ASR genes identified as being present using MAS were phenotypically expressed as low rust seedling infection types were observed in the lines containing ASR genes towards the specific rust type.

Avocet*YrSP* does not carry the *Sr26* resistance gene that was assumed to be present based on original MAS data. This was supported by phenotypic evaluations as atypical *Sr26* seedling infection types of 2+3 (Table 5.4) and 4 (Table 5.6), as apposed to the ;1 in Table 5.4 and 1 in Table 5.6 for Blade (*Sr26*), were obtained with the *Sr26* avirulent stem rust pathotype used. Resistance against stripe rust infections was phenotypically observed in 2S#/163 and CM-82036, meaning that ASR genes not tested for in the current study regulate stripe rust resistance as it is being expressed at the seedling stage. Offspring of experimental line KSF_2-126.26 showed good stripe rust resistance, indicating that either the *YrSP* gene or resistance transferred form 2S#/163 was transferred to these lines. Unfortunately MAS was not applied to follow these genes. However, these lines have the same seedling infection type as line 2S#/163 indicating that they may carry the same gene.

5.3.2.3 Fungal gene expression data of *Puccinia* inoculated experimental lines at seedling stage

The rust inoculated seedling parental, control and experimental lines were used to quantify the *β-tubulin* gene of the rust isolates and the wheat *TEF1* gene at 10 dpi. The expression ratio of the *β-tubulin:TEF1* was determined. The expression ratios of lines infected with leaf-, stem- and stripe rust are illustrated in Figures 5.9 to 5.11, respectively.

Good differentiation was obtained between the expression ratios of the leaf rust inoculated susceptible and resistant lines. CS*Lr19*-149-299 was the only parental line that contained a leaf rust resistance ASR gene (*Lr19*) that is expressed at seedling stage. Low expression levels of the *β-tubulin* gene of *Pt* was observed in eight of the experimental lines and only two experimental lines (KSF_3-159.11.2 and KSF_3-682.9.2) had high expression ratios.

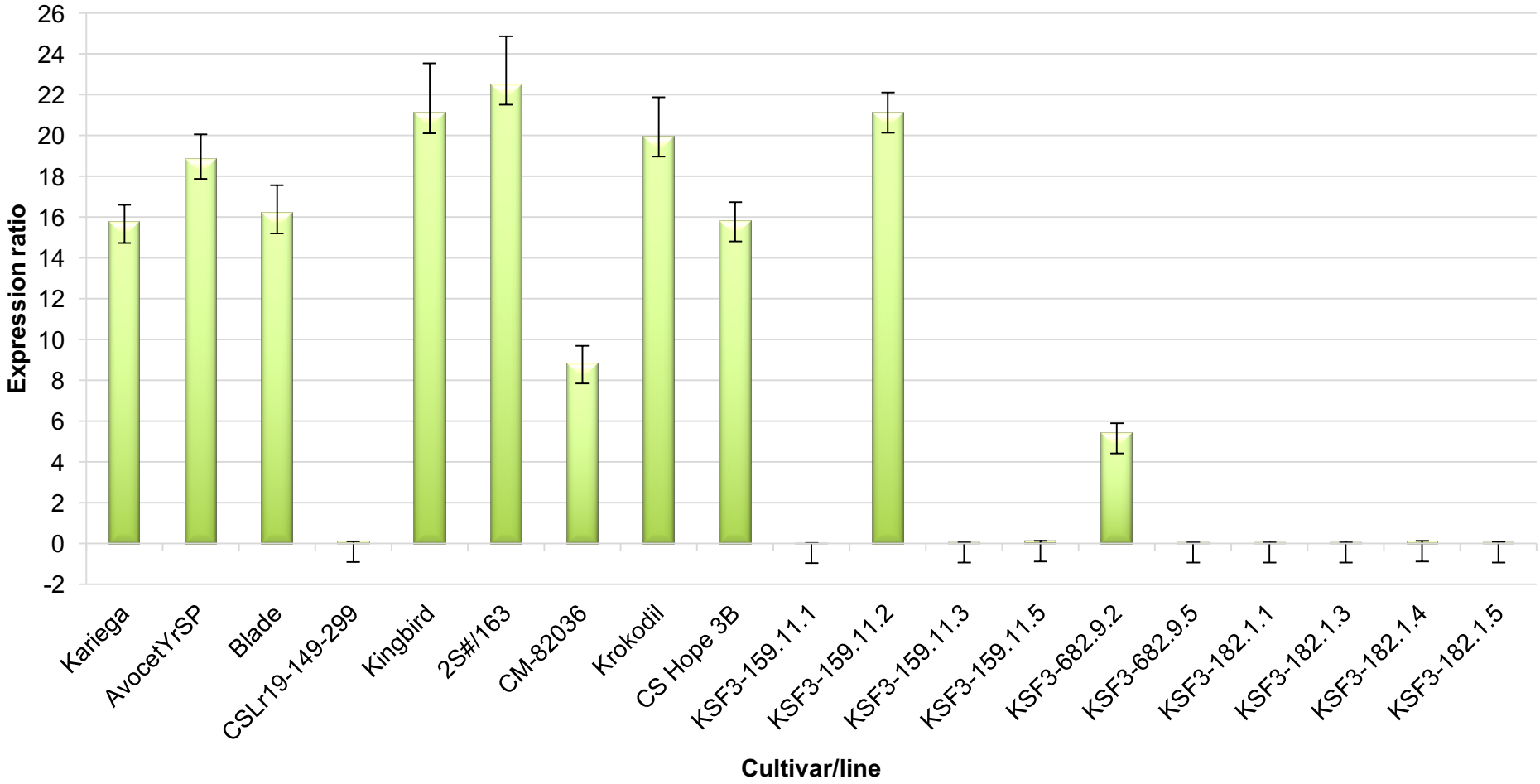


Figure 5.9 Expression ratio of the *β-tubulin* gene of *P. triticina* relative to the wheat *translation elongation factor 1 alpha-subunit* gene for the parental, control and leaf rust resistant experimental cultivars/lines at seedling stage. Bars indicate standard error for each data point.

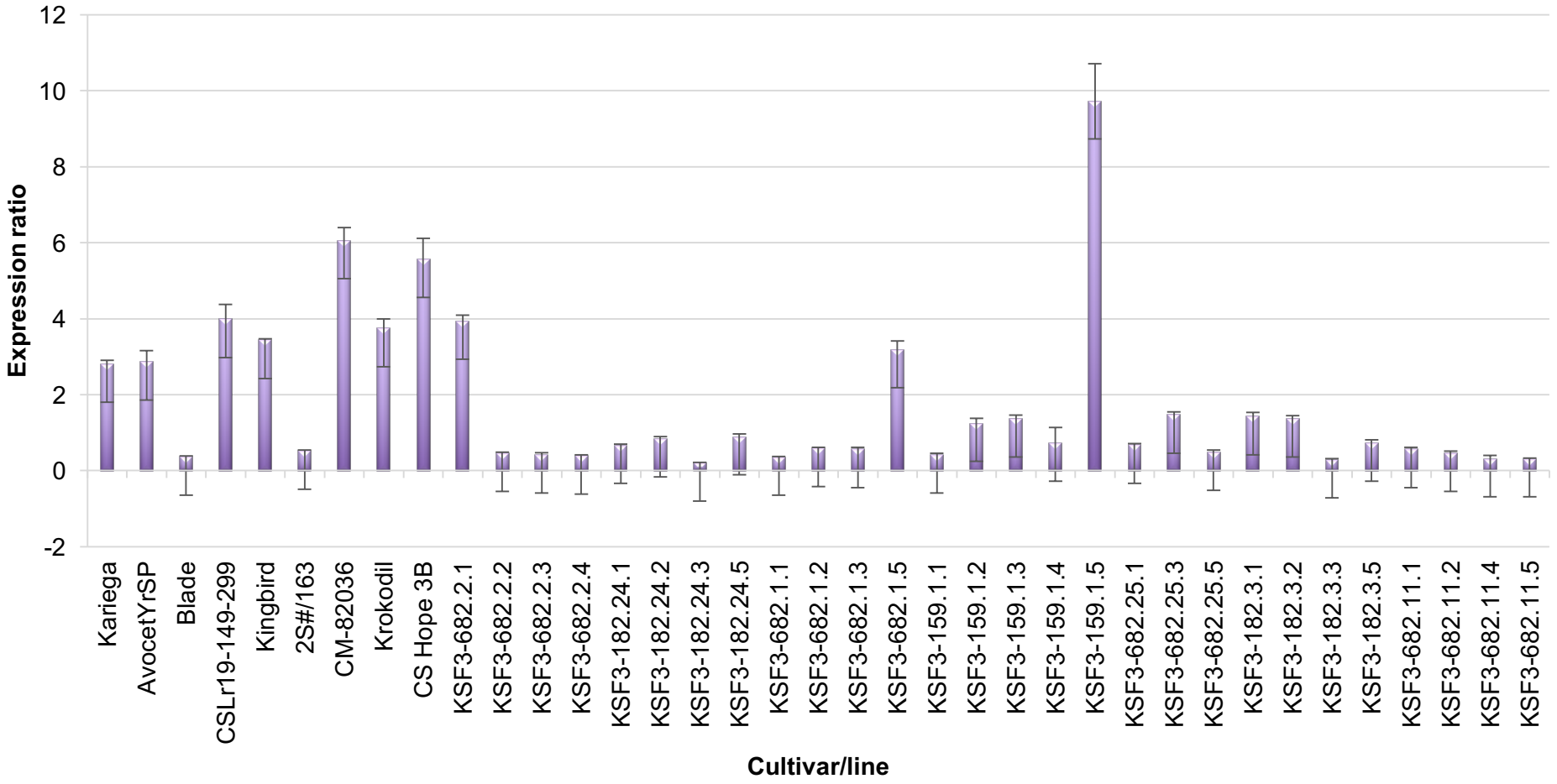


Figure 5.10 Expression ratio of the *β-tubulin* gene of *P. graminis* relative to the wheat *translation elongation factor 1 alpha-subunit* gene for the parental, control and stem rust resistant experimental cultivars/lines at seedling stage. Bars indicate standard error for each data point.

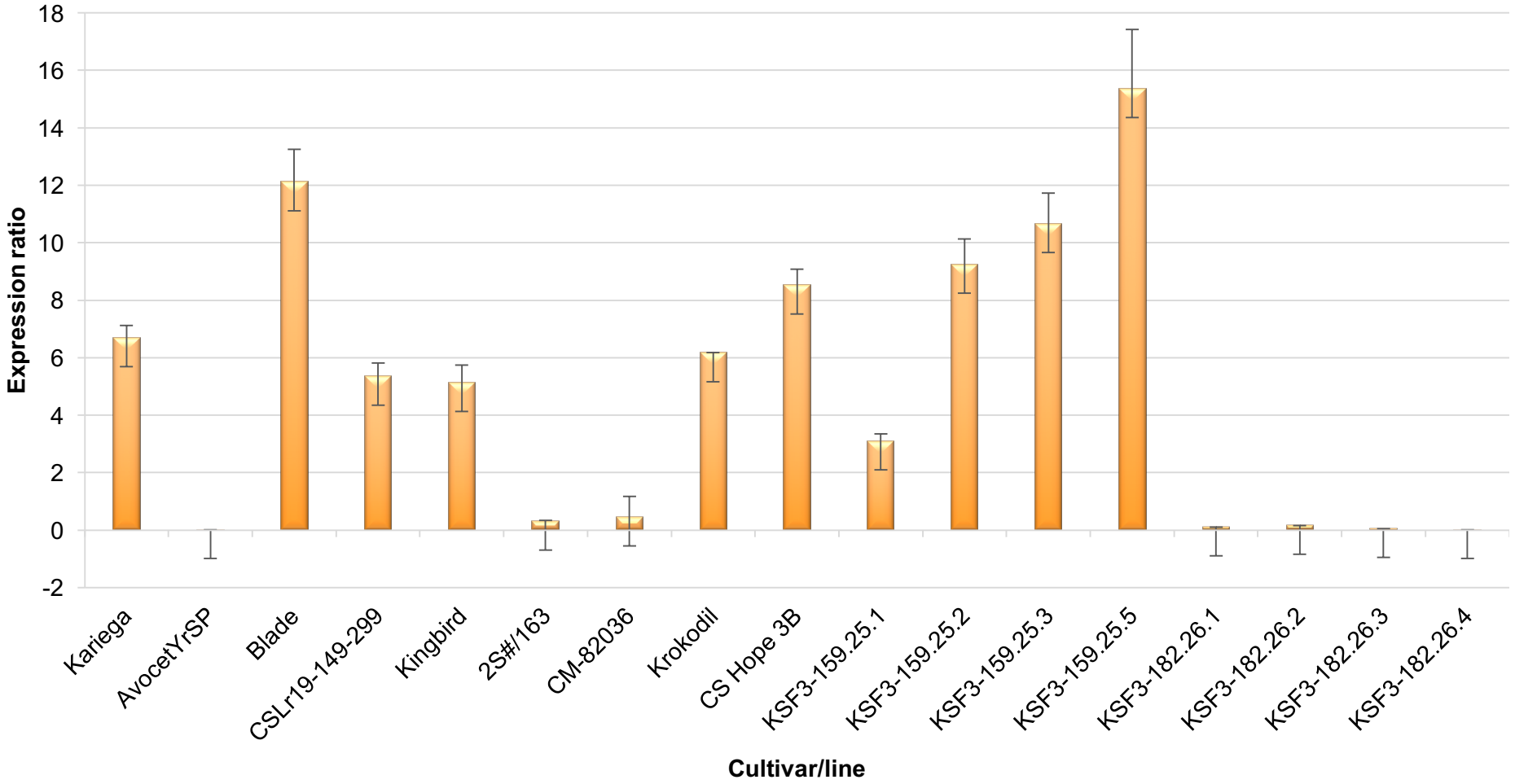


Figure 5.11 Expression ratio of the *β-tubulin* gene of *P. striiformis* relative to the wheat *translation elongation factor 1 alpha-subunit* gene for the parental, control and stripe rust resistant experimental cultivars/lines at seedling stage. Bars indicate standard error for each data point.

Experimental lines with low expression ratios indicate low leaf rust infection and the presence of the ASR leaf rust resistance gene, *Lr19*, that inhibit leaf rust infection. Results indicated a good correlation between marker, phenotypic and rust gene expression data.

Results of the expression ratios of the parental, control and stem rust resistant experimental lines that were inoculated with a *Pgt* pathotypes showed good differentiation between resistant and susceptible lines. Low expression ratios and infection types were obtained for Blade and 2S#/163, which can be explained by the presence of the ASR genes, *Sr26* and *Sr39*, respectively. The rest of the parental and control lines without any stem rust resistance ASR genes showed higher expression ratios. Three experimental lines, KSF_3-682.2.1, KSF_3-682.1.5 and KSF_3-159.1.5, showed high expression ratios, correlating to high phenotypic infection type data, because the two ASR stem rust resistance genes, *Sr26* and *Sr39*, were absent as indicated by molecular marker data. The rest of the experimental lines showed low infection types and expression ratios due to the presence of either the *Sr26* and/or *Sr39* resistance genes. There was no clear correlation between the number of stem rust resistance genes present and fungal gene expression ratios, because some lines containing only one resistance gene, e.g. KSF_3-182.24.3, had the lowest ratio of 0.2 while line KSF_3-159.1.3 that carries both the *Sr26* and *Sr39* genes had a expression ratio of 1.36. However, most lines containing both these stem rust resistance genes had expression ratios of 1.46 or lower.

The parental and stripe rust resistant experimental seedling lines, inoculated with *Pst* showed differentiation in the expression ratios obtained. The three parental cultivars/lines, Avocet*YrSP*, 2S#/163 and CM-82036, showed low fungal gene expression ratios that correlated with the low phenotypic stripe rust seedling infection types.

5.3.3 *Puccinia* inoculation data of experimental lines at adult plant stage

The same experimental lines used for seedling stage inoculations were also inoculated with the three rusts pathotypes to evaluate rust infection at the adult plant stage because two APR resistance genes were present in these experimental lines, namely the *Lr34/Yr18/Sr57* and *Sr2/Yr30*. Only two plants per pot were inoculated per parental, control and experimental line. Rust resistance molecular markers were evaluated on all lines to determine genotypes. Flag leaves or leaf sheaths were collected at 10 dpi to quantify the expression of the *β-tubulin* gene of the *Puccinia* species relative to the *TEF1*

gene of wheat. The phenotypic plant response of the parental, control and experimental lines was evaluated 15 dpi. Because experimental lines were still segregating, flowering dates of nine of the selected experimental lines did not synchronise with the flowering dates of the other selected lines and parental lines. They could therefore not be inoculated because plants were too early and already dying off.

5.3.3.1 Molecular marker data of *Puccinia* inoculated experimental lines at adult plant stage

Genotypic data obtained form MAS linked to rust resistance genes is presented in Table 5.7. Marker data for the parental lines was similar to data obtained during seedling stage (Table 5.6) except for Avocet*YrSP*. The *Sr26* gene was absent during the seedling stage, but heterozygous at adult plant stage. This indicates that the seed source used was not pure and that Avocet*YrSP* obtained was still segregating for the *Sr26* gene. Because only a limited number of lines could be inoculated and evaluated due to a lack of flower synchronisation between the segregating experimental lines, limited data was available. Therefore no genetic variation was detected for markers linked to leaf rust resistance in experimental lines. All three inoculated experimental lines carry both the *Lr19* and *Lr34/Yr18/Sr57* genes. Variation was observed between genotypes of the stem rust inoculated lines although some important gene combinations could not be evaluated due to poor synchronisation of flowering dates. For example lines KSF_3-682.2.6 and KSF_3-682.2.7 that contained only one of the Sr genes, namely *Sr2/Yr30*, were not inoculated and evaluated. Lines evaluated however did not contain the *Sr2/Yr30* gene in combination with the *Sr26* gene, but was present in combination with the *Sr39* gene while only one line, KSF_3-682.11.6, contained all three *Sr* genes.

5.3.3.2 Phenotypic evaluation data of *Puccinia* inoculated experimental lines at adult plant stage

The phenotypic data obtained 15 dpi showed a positive correlation with the genotypic data obtained through MAS. The reaction responses for the three rust types are summarised in Table 5.7. All experimental lines showed resistance while variation was observed for the parental and control lines. As expected Kariega showed stripe rust resistance at adult plant stage, due to the presence of the *QYr.sgi.2B-1* QTL and *Lr34/Yr18/Sr57* gene combination, while it was susceptible to stripe rust in the seedling stage (Table 5.6). Blade was moderately resistant (MR) in the adult plant stage but susceptible in the seedling stage, indicating that an APR stripe rust gene/QTL is present in its genetic background.

Table 5.7 Rust resistant genotypes as well as phenotypic adult plant response types of parental, control and experimental lines

Rust inoculated groups	Cultivar/line	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Leaf rust MCDS	Stem rust PTKST	Stripe rust 6E22A+
Parental and control cultivars/lines	Kariega	0	1	0	0	0	S	S	R
	Avocet*YrSP*	0	1	0	0.5	0	S	R	0R
	Blade	0	0	0	1	0	S	R	MR
	CS*Lr19*-149-299	1	1	0	0	0	0R	S	MSS
	Kingbird	0	1	1	0	0	ZMS	R	RMR
	2S#/163	0	0	0	0	1	S	R	0R
	CM-82036	0	1	0	0	0	MR	S	0R
	Krokodil	0	0	0	0	0	MS	S	S
	CS Hope 3B	0	1	1	0	0	N/A	MS	RMR
Leaf rust infected experimental lines	KSF_3-159.11.6	1	1	1	0	0.5	0R		
	KSF_3-159.11.7	1	1	1	0	1	0R		
	KSF_3-182.1.6	1	1	1	0	0	0R		
Stem rust infected experimental lines	KSF_3-182.24.6	1	1	0	0	1		R	
	KSF_3-182.24.7	1	1	0	0	1		R	
	KSF_3-682.1.6	0	1	0	0.5	0		R	
	KSF_3-682.1.7	0	0.5	0	0.5	0		R	
	KSF_3-682.25.6	0	0	1	0	1		N/A	
	KSF_3-682.25.7	0	0	1	0	1		0R	
	KSF_3-182.3.6	1	1	1	0	0.5		0R	
	KSF_3-682.11.6	1	0	1	1	1		0R	
Stripe rust infected experimental lines	KSF_3-159.25.6	1	1	0	0	0			R
	KSF_3-159.25.7	1	1	1	0	0			0R
	KSF_3-182.26.6	1	1	1	0	0			0R
	KSF_3-182.26.7	1	1	0	0.5	0			0R

N/A-Not available; Z-uredia towards the leaf base; [1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0); Phenotypic evaluation was done according to Table 2.3.

Cultivars containing the APR gene, *Sr2/Yr30*, namely Kingbird and CS Hope 3B, was adult plant resistant for stem rust while they were susceptible in the seedling stage. Lines 2S#/163 and CM-82036 are also stripe rust resistant in the adult plant stage, confirming the compatible seedling infection types obtained during seedling evaluation.

All three leaf rust inoculated experimental lines contained both *Lr19* and *Lr34/Yr18/Sr57* and the presence of both genes were confirmed with phenotypic response scores of 0R indicating immunity to infection. Stem rust inoculated lines containing both the *Sr2/Yr30* and/or the *Sr26* or *Sr39* genes were immune (scores of 0R) compared to lines containing either the *Sr26* or *Sr39* genes (scores of R). All evaluated stripe rust experimental lines showed high levels of resistance (scores of R and 0R) even though the APR QTL, *QYr.sgi.2B-1* from Kariega was absent in these lines. The APR gene *Lr34/Yr18/Sr57* was confirmed in these lines and additional stripe rust resistance were either inherited from Avocet*YrSP* (*YrSP* gene), form the unknown stripe rust resistance present in line 2S#/163 or from Blade which also showed APR.

5.3.3.3 Fungal gene expression data of *Puccinia* inoculated experimental lines at adult plant stage

Fungal gene expression in adult plants inoculated with the three different rust types were determined per individual adult wheat plant at 10 dpi (Figures 5.12 to 5.14). The biomass accumulation of the pathogen was quantified by amplifying the *β-tubulin* gene and expressed relatively to the *TEF1* gene of wheat. Variation in expression ratios for all three rust types were obtained between the different inoculated lines. Low expression levels of the leaf rust *β-tubulin* gene were obtained for CS*Lr19*-149-299 cultivar due to the presence of the ASR *Lr19* gene. A low expression ratio was also detected in Kingbird due to the presence of the *Sr2/Yr30* gene that might have a positive influence on the expression of the *Lr34/Yr18/Sr57* gene when it is combined in the same genotype (Silva et al. 2015). CM-82036 also showed a lower expression ratio compared to the other susceptible parental and control lines and this trend was also observed in seedlings. A high expression ratio was obtained for line 2S#/163 similar to results obtained at seedling stage. However, the opposite was seen during the timeline study indicating that the low expression ratio during optimisation may have been due to insufficient rust infection of line 2S#/163 during the standardisation trial of the adult plants. All three experimental lines showed low expression ratios that correlate well with that of the donor line CS*Lr19*-149-299 that also carries the *Lr19* and *Lr34/Yr18/Sr57* genes, similar to these three lines.

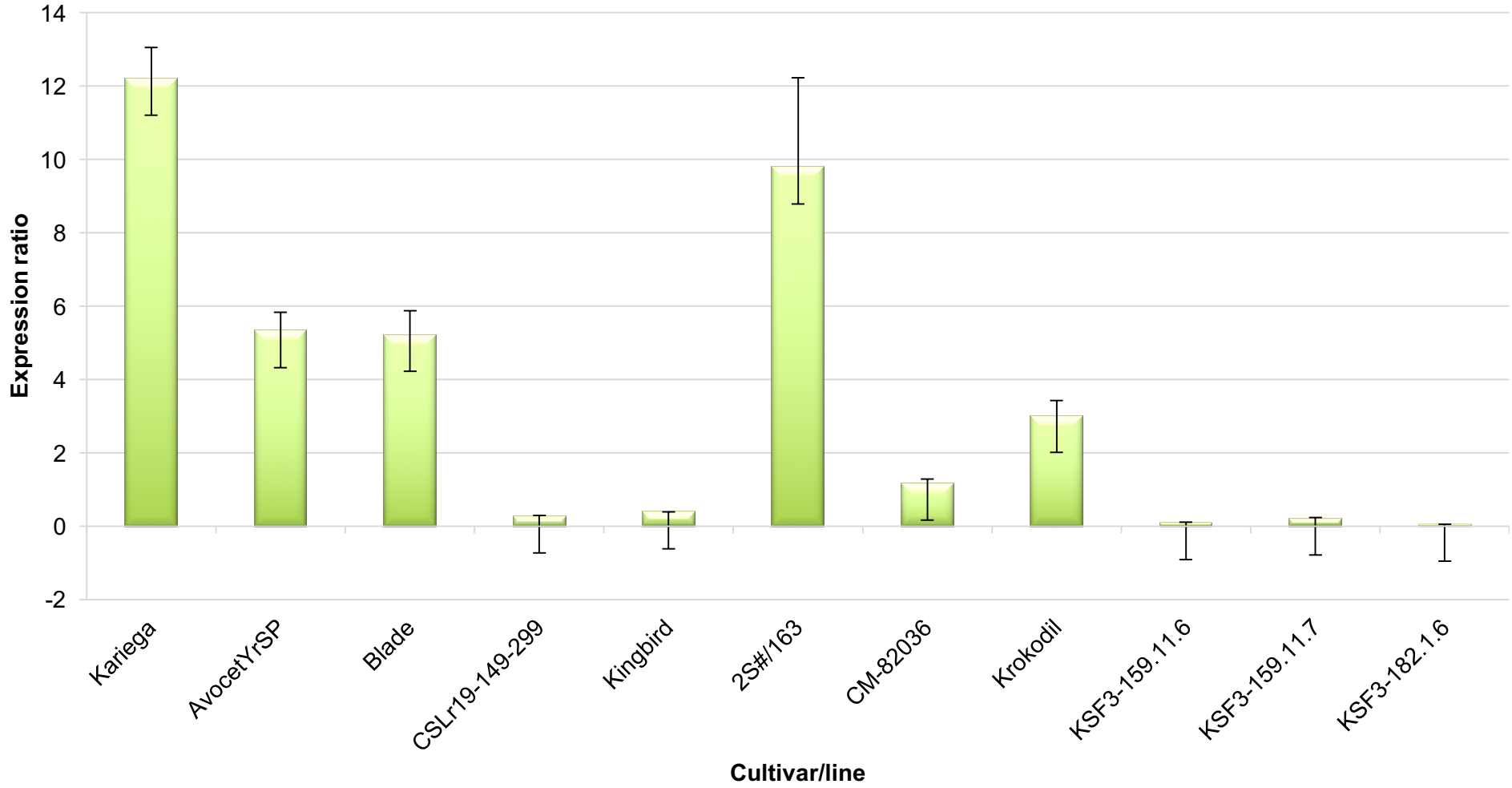


Figure 5.12 Expression ratio of the *β-tubulin* gene of *P. triticina* relative to the wheat *translation elongation factor 1 alpha-subunit* gene for the parental, control and leaf rust resistant experimental cultivars/lines at adult plant stage. Bars indicate standard error for each data point.

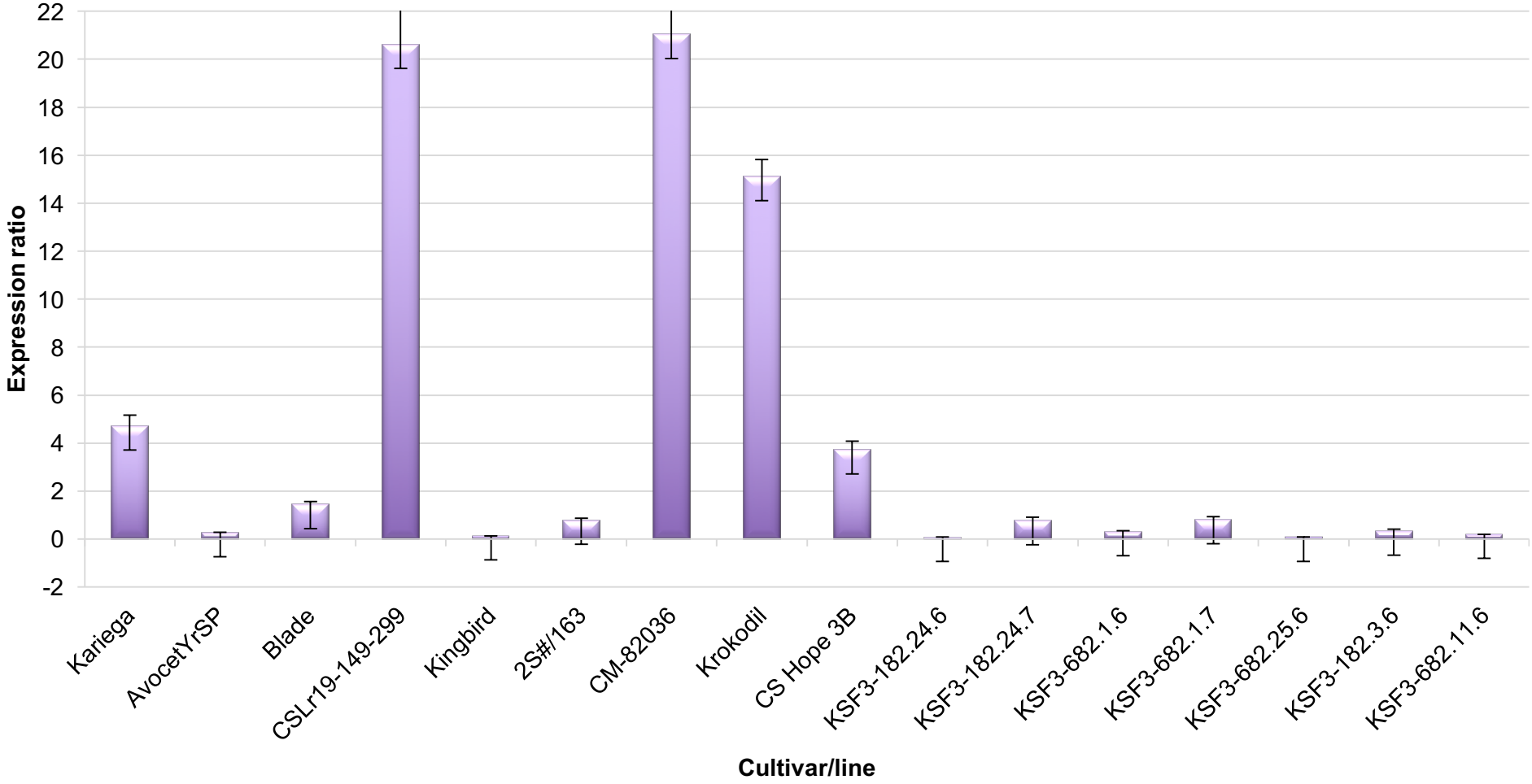


Figure 5.13 Expression ratio of the *β-tubulin* gene of *P. graminis* relative to the wheat *translation elongation factor 1 alpha-subunit* gene for the parental, control and stem rust resistant experimental cultivars/lines at adult plant stage. Bars indicate standard error for each data point.

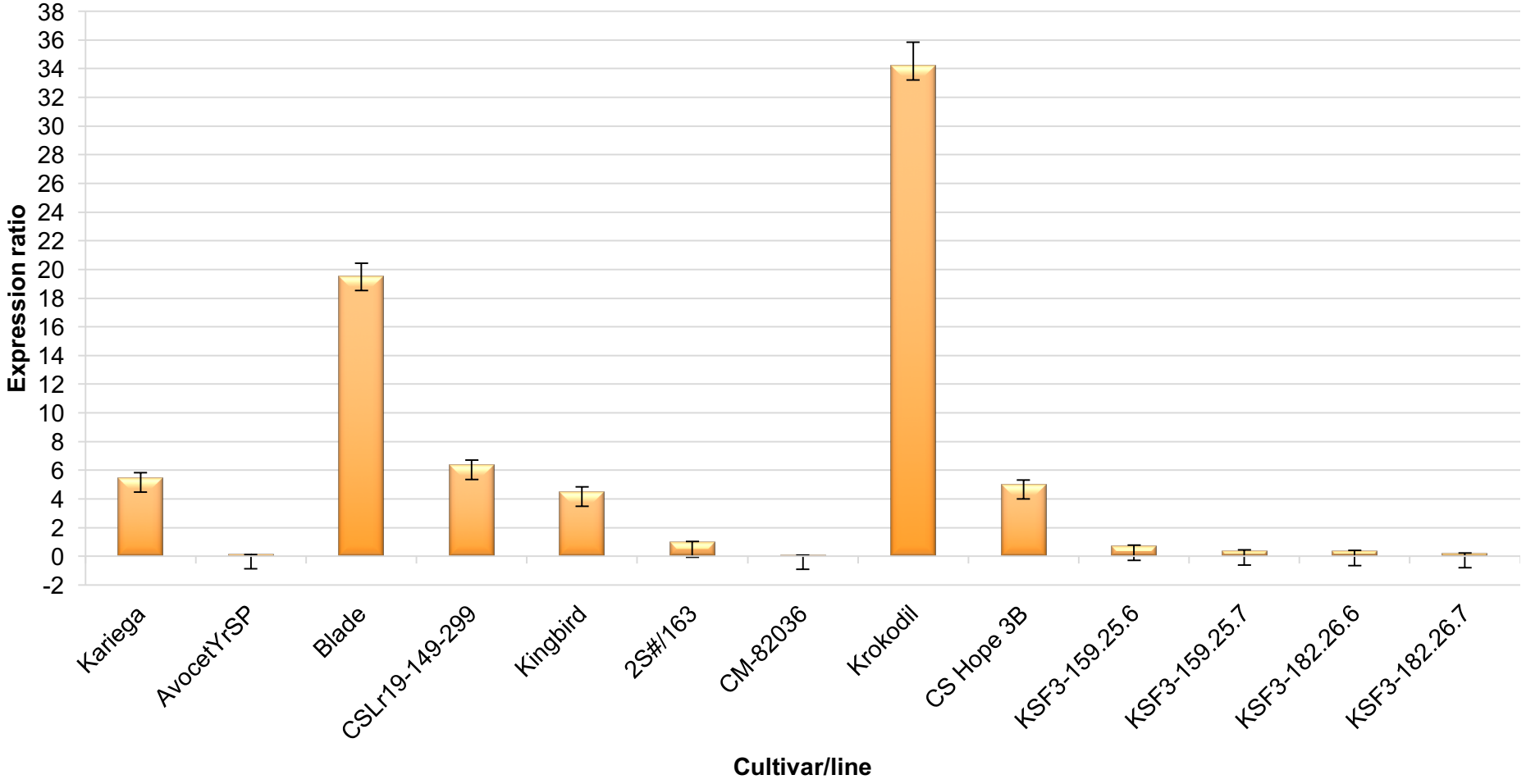


Figure 5.14 Expression ratio of the *β-tubulin* gene of *P. striiformis* relative to the wheat *translation elongation factor 1 alpha-subunit* gene for the parental, control and stripe rust experimental cultivars/lines at adult plant stage. Bars indicate standard error for each data point.

Low expression ratios were observed in the parental and control lines for Avocet*YrSP*, Blade, Kingbird and 2S#/163 inoculated with stem rust due to the presence of different *Sr* resistance genes in these cultivars/lines. The *Sr2/Yr30* gene is present in Kingbird, providing APR, explaining the lower ratio compared to that observed during seedling stage, indicating the effectiveness of the *Sr2/Yr30* gene during adult plant stage. The same was observed for CS Hope 3B and although it was only moderately susceptible during the adult plant stage the *Sr2/Yr30* gene was better expressed during the adult plant stage compared to seedling stage. None of the parental cultivars used to develop the BC_2 FHB resistant lines (CM-82036 and Krokodil) showed any type of stem rust resistance. All experimental lines showed low expression ratios due to the presence of the ASR (*Sr26* and/or *Sr39*) and/or APR (*Sr2/Yr30*) stem rust resistance genes. The expression ratios in the experimental lines confirmed data obtained using MAS and phenotypic evaluation.

No marker data was available to identify the *YrSP* gene in the experimental lines. The ASR gene present in line 2S#/163 is currently unknown and not one of the genes evaluated for during this study. This line was initially selected for the presence of the *Sr39* gene. CM-82036 also had a low *β-tubulin:TEF1* stripe rust expression ratio, indicating the presence of a gene that was not identified using MAS during this current study. CM-82036 however, could not have contributed to any stripe rust resistance in the KSF lines as CM-82036 was used to develop the FHB resistant lines used in this study. It is however possible that CM-82036 can contribute towards stripe rust resistance in the combined rust and FHB resistant lines developed during this study. The rest of the parental and control lines showed high expression ratios. All four experimental lines showed low expression ratios. These lines inherited either the *YrSP* gene from Avocet*YrSP* and/or the stripe rust resistant gene present in line 2S#/163 in combination with the *Lr34/Yr18/Sr57* gene. The low expression ratios in these four lines correlated with low infection types scored during phenotypic evaluation for stripe rust infection.

Three parental and control cultivars/lines, Avocet*YrSP*, 2S#/163 and CM-82036, showed low expression ratios for stripe rust infection. The low expression ratio of Avocet*YrSP* is due to the presence of the ASR stripe rust gene, *YrSP*. Low expression levels in line 2S#/163 and CM-82036 confirmed previous results obtained using phenotypic evaluation and expression at seedling stage that these lines contain yet unidentified *Yr* resistance genes. Genes present in the other two lines are unknown. Kariega carries adult plant stripe rust resistance QTL on chromosome 2B and 4A as well as the *Yr18* gene present in the *Lr34/Yr18/Sr57* gene, explaining the lower expression ratio observed compared to

Blade, CS*Lr19*-149-299 and Krokodil. CS*Lr19*-149-299 and Kingbird also showed lower expression ratios than Blade and Krokodil but higher ratios compared to the resistant cultivars probably due to the presence of APR genes such as *Yr18* and *Yr30*. Blade and Krokodil showed the highest expression ratios. Blade showed high levels of necrosis that influenced the biomass accumulation of *TEF1* genes in wheat and thus the *β-tubulin:TEF1* ratio. Krokodil is susceptible to stripe rust, explaining the high expression ratios. All four experimental lines showed low expression levels, similar to the parental lines, thus confirming the presence of either the *YrSP* gene or the yet unidentified stripe rust resistance gene present in line 2S#/163.

5.3.4 Field data

For the field trial in 2015, all three rust types were present and infection occurred. Disease severity for the three rust types was recorded for individual plants and molecular marker data linked to rust resistance genes was also obtained for these lines. High segregation levels were observed between individual plants per entry because segregating F_2 lines were used for the field trial. Less variation was observed for disease severity between individual plants within an entry compared to between entries. No stripe rust infection was observed for all parental, control and experimental lines although spreader rows showed 100% susceptibility. The experimental lines should show lower levels of infection compared to the susceptible checks because these were all rust resistant breeding lines. Variation in disease severity was also observed between experimental plants containing the same gene combinations, as confirmed using MAS. Disease severity and genotypic data linked to rust resistance genes obtained for the wheat lines planted in 2015 at Redgates are summarised in Table 5.8. Seed of Avocet*YrSP* and CS*Lr19*-149-299 did not germinate, therefore no phenotypic or MAS data is available.

Offspring of line KSF_1-409 did not show any disease severity. All offspring contained *Sr39* in the heterozygous state whereas *Lr34/Yr18/Sr57* was in the homozygous state while *Lr19* was present in some of these lines. A similar trend was observed for offspring of line KSF_1-271. None of the three stem rust resistance genes were present in offspring of lines KSF_1-718 and KSF_1-239, except for line KSF_2-718.1 that was heterozygous for *Sr26*, and therefore higher stem rust severity was detected in these offspring, indicating the effectiveness of the selected stem rust resistance genes in the field. One offspring, KSF_2-239.7, had a 70% leaf rust severity, the highest off all offspring despite of the presence of the *Lr34/Yr18/Sr57* gene.

Table 5.8 Rust resistance genotypes and disease severity scores of wheat lines planted in the field trial at Redgates in 2015

Cultivar/line	Genotype					Disease severity (%)		
	Lr19[1]	*Lr34/Yr18/Sr57*[2]	*Sr2/Yr30*[1]	*Sr26*[2]	*Sr39*[2]	Leaf rust	Stem rust	Stripe rust
Kariega	0	1	0	0	0	30	70	0
Blade	0	0	0	1	0	10	10	0
Kingbird	0	1	1	0	0	1	10	0
2S#/163	0	0	0	0	1	40	15	0
KSF_2-717.1	0	1	1	0	0	30	20	0
KSF_2-717.2	0	1	1	0	0	10	20	0
KSF_2-717.3	1	1	1	0	0	10	20	0
KSF_2-717.4	1	1	1	0	0	2	20	0
KSF_2-717.5	0	1	1	0	0	30	20	0
KSF_2-409.1	0	1	0	0	0.5	0	0	0
KSF_2-409.2	1	1	0	0	0.5	0	0	0
KSF_2-409.3	0	1	0	0	0.5	0	0	0
KSF_2-409.4	1	1	0	0	0.5	0	0	0
KSF_2-409.5	1	1	0	0	0.5	0	0	0
KSF_2-271.1	0	1	0	0	0.5	0	0	0
KSF_2-271.2	0	1	1	0	0.5	2	0	0
KSF_2-271.3	0	1	0	0	0.5	0	0	0
KSF_2-271.4	0	1	0	0	0.5	0	0	0
KSF_2-718.1	0	1	0	0.5	0	0	10	0
KSF_2-718.2	0	1	0	0	0	2	30	0
KSF_2-718.3	0	1	0	0	0	5	30	0
KSF_2-239.1	0	1	0	0	0	30	35	0
KSF_2-239.2	0	1	0	0	0	30	35	0
KSF_2-239.3	0	1	0	0	0	2	35	0
KSF_2-239.4	0	1	0	0	0	10	60	0
KSF_2-239.5	0	1	0	0	0	5	35	0
KSF_2-239.6	0	1	0	0	0	10	35	0
KSF_2-239.7	0	1	0	0	0	70	50	0
KSF_2-342.1	0	1	0	0	0	0	60	0
KSF_2-342.2	0	1	0	0	0.5	2	5	0
KSF_2-473.1	0	0	0	0	0.5	0	5	0
KSF_2-473.2	0	1	0	0	0.5	5	5	0
KSF_2-473.3	1	0.5	0	0	0.5	0	5	0
KSF_2-473.4	0	1	0	0	0.5	0	5	0
KSF_2-473.5	1	0.5	0	0	0.5	0	5	0
KSF_2-473.6	1	0.5	0	0	0.5	0	5	0
KSF_2-473.7	0	1	0	0	0	0	30	0
KSF_2-5.1	0	1	1	0	0	2	20	0
KSF_2-5.2	0	1	1	0	0	2	20	0
KSF_2-5.3	0	1	1	0.5	0	0	15	0
KSF_2-5.4	0	1	1	0	0	2	35	0
KSF_2-5.5	0	1	0	0	0	5	30	0

Table 5.8 Rust resistance genotypes and disease severity scores of wheat lines planted in the field trial at Redgates in 2015 (continued)

Cultivar/line	Genotype					Disease severity (%)		
	Lr19[1]	*Lr34/Yr18/Sr57*[2]	*Sr2/Yr30*[1]	*Sr26*[2]	*Sr39*[2]	Leaf rust	Stem rust	Stripe rust
KSF_2-29.1	0	1	0	0	0.5	0	5	0
KSF_2-29.2	0	1	0	0	0.5	0	5	0
KSF_2-29.3	0	1	0	0	0.5	0	5	0
KSF_2-29.4	0	1	0	0	0.5	0	5	0
KSF_2-29.5	1	1	0	0	0	0	60	0
KSF_2-11.1	1	1	0	0.5	0.5	0	5	0
KSF_2-11.2	0	1	0	0.5	0.5	1	5	0
KSF_2-11.3	0	1	1	0.5	0	2	5	0
KSF_2-11.4	1	1	0	0.5	0	5	5	0
KSF_2-11.5	0	1	1	0.5	0	0	5	0
KSF_2-402.1	0	1	0	0.5	0	2	5	0
KSF_2-402.2	0	1	0	0.5	0.5	2	5	0
KSF_2-402.3	0	1	0	0.5	0.5	0	5	0

[1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0).

The highest stem rust severity of 60% (except for the 70% in the parental cultivar Kariega) was detected in lines that did not carry any of the three stem rust resistance genes (KSF_2-239.4, KSF_2-342.1 and KSF_2-29.5). Most lines showed resistant to moderately resistant disease reactions towards leaf rust. The highest leaf rust severity for lines containing the *Lr19* gene was 10% (KSF_2-717.3) while the severity for the rest of the lines containing the *Lr19* gene ranged from 0-5%. In contrast disease severity scores for lines containing the *Lr34/Yr18/Sr57* gene ranged from 0-70%. Disease severity in plants containing both the *Lr19* and *Lr34/Yr18/Sr57* genes ranged from 0-10%. Higher infection levels were obtained for stem rust infections compared to leaf rust infections.

High stripe rust infection levels were obtained during the field trial at Redgates in 2016. This high infection levels caused severe leaf damage. Because the stripe rust infection occurred earlier compared to leaf- and stem rust in the field because of the cooler temperatures, leaves of entries with high stripe rust infections could not be evaluated for leaf rust later in the season. Therefore, no leaf rust infection results were recorded during 2016. The stripe- and stem rust severity scores as well as genotypic data obtained from MAS linked to rust resistance genes of the wheat lines planted in 2016 at Redgates are presented in Table 5.9.

Table 5.9 Rust resistance genotypes and disease severity scores of wheat lines planted in the field trial at Redgates in 2016

Cultivar/line	Genotype					Disease severity (%)	
	Lr19[1]	*Lr34/Yr18/Sr57*[2]	*Sr2/Yr30*[1]	*Sr26*[2]	*Sr39*[2]	Stem rust	Stripe rust
Kariega	0	1	0	0	0	50	5
Avocet*YrSP*	0	1	0	0	0	90	0
Blade	0	0	0	1	0	5	30
CS*Lr19*-149-299	1	1	0	0	0	70	50
Kingbird	0	1	1	0	0	5	5
2S#/163	0	0	0	0	1	10	5
CM-82036	0	1	0	0	0	80	5
Krokodil	0	0	0	0	0	20	90
CS Hope 3B	0	1	1	0	0	5	10
KSF_3-159.11.1	0	1	1	0	0.5	5	20
KSF_3-159.11.2	0	1	1	0	0.5	5	20
KSF_3-159.11.3	0	1	0	0	0.5	5	25
KSF_3-159.11.4	1	1	1	0	0	70	25
KSF_3-159.11.5	0	1	0	0	1	5	25
KSF_3-159.11.6	1	1	1	0	0	70	30
KSF_3-159.11.7	1	1	1	0	0.5	0	20
KSF_3-682.9.1	1	0	1	1	0.5	0	20
KSF_3-682.9.2	0	0	0	1	0.5	5	20
KSF_3-682.9.3	1	0	1	0	0.5	5	30
KSF_3-682.9.4	1	0	1	0.5	0.5	0	30
KSF_3-682.9.5	0	0	0	0.5	0	10	30
KSF_3-682.9.6	1	0	1	1	0	0	30
KSF_3-682.9.7	1	0	1	0	0.5	0	30
KSF_3-682.9.8	0	0	0	1	0.5	5	20
KSF_3-182.1.1	0	1	1	0	0	5	50
KSF_3-182.1.2	0	1	1	0	0	5	50
KSF_3-182.1.3	0	1	1	0	0	40	50
KSF_3-182.1.4	0	1	1	0	0	70	50
KSF_3-182.1.5	1	1	1	0	0	30	50
KSF_3-182.1.6	1	1	0	0	0	20	50
KSF_3-182.1.7	1	1	1	0	0	20	50
KSF_3-182.1.8	1	1	1	0	0	10	50
KSF_3-682.2.1	1	0	0	0.5	0	5	30
KSF_3-682.2.2	0	0	0	0.5	0	10	30
KSF_3-682.2.3	0	0	1	0.5	0	5	30
KSF_3-682.2.4	0	0	1	0.5	0	5	30
KSF_3-682.2.5	0	0	0	0.5	0	5	30
KSF_3-182.24.1	0	1	0	0	1	5	40
KSF_3-182.24.2	0	1	0	0	1	0	20
KSF_3-182.24.3	0	1	0	0	1	0	20
KSF_3-182.24.4	0	1	0	0	1	5	30
KSF_3-182.24.5	0	1	0	0	1	0	30
KSF_3-682.1.1	0	0.5	0	0.5	0	5	75
KSF_3-682.1.2	0	0	0	0	0	20	90
KSF_3-682.1.3	0	1	0	0.5	0	10	60
KSF_3-682.1.4	0	0.5	0	0.5	0	30	60
KSF_3-682.1.5	0	1	0	0.5	0	5	60
KSF_3-682.1.6	0	0.5	0	0.5	0	5	50

Table 5.9 Rust resistance genotypes and disease severity scores of wheat lines planted in the field trial at Redgates in 2016 (continued)

Cultivar/line	Genotype					Disease severity (%)	
	Lr19[1]	*Lr34/Yr18/Sr57*[2]	*Sr2/Yr30*[1]	*Sr26*[2]	*Sr39*[2]	Stem rust	Stripe rust
KSF_3-159.4.1	0	1	0	0	0.5	5	50
KSF_3-159.4.2	0	1	1	1	0.5	5	60
KSF_3-159.4.3	0	1	0	0	0	70	10
KSF_3-159.4.4	0	1	1	0	0.5	5	10
KSF_3-159.4.5	0	1	0	0.5	0.5	5	10
KSF_3-159.4.6	0	1	0	0.5	0.5	5	5
KSF_3-682.25.1	0	0	1	0	1	5	20
KSF_3-682.25.2	0	0	1	0	1	5	10
KSF_3-682.25.3	0	0	1	0	1	5	20
KSF_3-682.25.4	0	0	1	0	1	5	30
KSF_3-682.25.5	0	0	1	0	1	5	30
KSF_3-682.25.6	0	0	1	0	1	5	40
KSF_3-682.25.7	0	0	1	0	1	5	40
KSF_3-682.25.8	0	0	1	0	1	5	30
KSF_3-182.3.1	1	1	0	0.5	1	0	10
KSF_3-182.3.2	1	1	0	0	1	5	15
KSF_3-182.3.3	1	1	0	1	1	0	10
KSF_3-182.3.4	1	1	0	0.5	1	5	15
KSF_3-182.3.5	1	1	0	0.5	1	5	20
KSF_3-182.3.6	1	1	0	0	1	5	20
KSF_3-682.11.1	0	0	0	1	1	5	40
KSF_3-682.11.2	1	0	1	1	1	5	75
KSF_3-682.11.3	1	0	0	1	1	5	40
KSF_3-682.11.4	1	0	1	1	1	0	75
KSF_3-682.11.5	1	0	1	1	1	0	75
KSF_3-682.11.6	0	0	1	1	1	5	60
KSF_3-682.11.7	1	0	0	1	1	5	40
KSF_3-159.23.1	1	1	1	0	1	0	10
KSF_3-159.23.2	1	1	1	0	1	0	10
KSF_3-159.23.3	1	1	1	0	1	0	20
KSF_3-159.23.4	1	1	1	0	1	0	25
KSF_3-159.31.1	1	1	1	0.5	1	5	30
KSF_3-159.31.2	0	1	1	0.5	1	5	30
KSF_3-159.31.3	0	1	1	0.5	1	5	10
KSF_3-159.31.4	1	1	0	0.5	1	5	10
KSF_3-159.31.5	0	1	0	1	1	5	10
KSF_3-159.31.6	1	1	1	0.5	1	5	10
KSF_3-159.31.7	1	1	0	0.5	1	5	5
KSF_3-159.31.8	1	1	1	0.5	1	5	5

[1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0).

High stem rust severity was obtained for Kariega, Avocet*YrSP*, CS*Lr19*-149-299 and CM-82036 due to the absence of any ASR *Sr* genes. The ASR *Sr26* gene was not present in Avocet*YrSP* based on marker data and field trial data. These results further support the segregation of *Sr26* in the Avocet*YrSP* source used when considering stem rust results in the seedling- and adult plant tests under controlled conditions in this study (presented in Tables 5.4 to 5.7). Low stem rust severity was obtained when *Sr26* and/or *Sr39* were present. Most lines showed a low stem rust severity level, while offspring of line KSF_2-182.1 had higher stem rust severity levels and *Sr26* and *Sr39* were absent in all these offspring. These results indicate the effectiveness of these two ASR stem rust resistance genes in the field. Severity scores for lines containing only the *Sr2/Yr30* gene ranged from 5-70% indicating that the gene was not expressed uniformly in lines where it was present. Experimental lines containing only *Sr26* showed a lower severity ranging from 5-30% compared to lines containing only the *Sr2/Yr30* gene. Severity for lines containing only the *Sr39* gene ranged from 0-5%. Severity for experimental lines with a combination of the *Sr2/Yr30* and *Sr26* and/or *Sr39* ranged from 0-5% and indicate the effectiveness of the ASR genes in combination and that they support the *Sr2/Yr30* gene. In addition severity for experimental lines that contained none of the evaluated stem rust resistance genes ranged from 20-70% indicating that for some of these lines additional *Sr* resistance genes might be present in their genetic background. This can be attributed to the high genetic variation present because these lines were developed from six different parental cultivars/lines originally.

Krokodil is susceptible to stripe rust and had the highest stripe rust severity score of all the parental and control cultivars/lines. No infection was observed for Avocet*YrSP* due to the presence and effectiveness of the ASR gene *YrSP*. Kariega, Kingbird, 2S#/163 and CM-82036 all showed very high levels of stripe rust resistance. The experimental lines differed in their levels of stripe rust severity ranging from 5-90%. No markers linked to stripe rust resistance genes/QTL were evaluated using MAS except for the APR genes present in the gene complexes that are associated with multiple disease resistance such as *Lr34/Yr18/Sr57* and *Sr2/Yr30*.

5.4 Discussion

In order to breed new disease resistant cultivars, it is necessary to effectively evaluate breeding lines for resistance. Disease resistance evaluation can be done through phenotypic evaluation in the field or in the greenhouse, using MAS and/or by quantification of fungal gene expression in the plant. In order to select the best method(s), these methods need to be evaluated and compared. During this study, rust

resistant experimental lines developed throughout this study were evaluated for rust resistance using greenhouse and field phenotypic evaluations, using MAS linked to both ASR and APR genes and by measuring fungal biomass accumulation in infected plants using qPCR.

The initial timeline studies were performed to determine the best time to harvest leaf material after rust inoculation for qPCR analysis. The optimal harvesting time need to be determined because the experimental lines were still segregating for the traits of interest. Segregation limits the opportunity to obtain and screen lines with the same genetic background. Limited leaf material is furthermore available at seedling stage and therefore the optimal harvesting time after inoculation had to be determined through the timeline study using cultivars with fixed genotypes. The timeline studies at seedling and adult plant stage revealed that 10 dpi was the best time interval to distinguish between fungal biomass accumulation in resistant versus susceptible lines. This is five days earlier compared to the visual phenotypic evaluation done at 15 dpi in this study and can be seen as a potential advantage as shorter trial periods are necessary. However, high levels of chlorosis and necrosis can lead to an incorrect susceptibility classification as seen in Blade during the adult plant stripe rust inoculation trial. High levels of chlorosis and/or necrosis decrease the expression and thus biomass produces by the *TEF1* genes. Because the expression ratio is calculated relative to the *TEF1* gene (*β-tubulin:TEF1*), and necrosis/chlorosis decreases the *TEF1* gene, infection seems higher than what it should be. Therefore, the best outcome might be to combine phenotypic evaluation with qPCR analysis to prevent miss-classification of genotypes.

In this study, data gathered through MAS, qPCR and phenotypic evaluation was positively correlated during the seedling stage, adult plant stage and field trials Therefore, these methods were found complementary to each other and can thus be used in combination to validate breeding lines when the necessary resources are available. The limitation of one method can be strengthened by the other method(s) when used in combination. Phenotypic and qPCR data masked the presence of the APR genes when they are combined with effective ASR genes. Therefore MAS using markers linked to the APR genes/QTL is necessary to validate the presence of these genes/QTL especially in a breeding programme where a number of genes/QTL for the same trait need to be combined. Scientists and breeders point out that in order to obtain durable resistance, two or more resistance genes/QTL should be combined and APR genes need to be included to support ASR genes (Hussain et al. 2015).

Phenotypic evaluation can support MAS, by identifying the presence of additional resistance genes that the breeders were not aware off and can be used to locate the gene which would allow tracking thereof in the breeding programme, especially when it is a ASR gene as in the case of the 2S#/163 line that contained an unknown ASR stripe rust resistance gene. The APR genes *Lr34/Yr18/Sr57* and *Sr2/Yr30* are known for their durability and partial resistance. These genes support the effectiveness of other resistance genes. When the *Lr34/Yr18/Sr57* gene was present singly in a genotype (Kariega, Avocet*YrSP*, CS*Lr19*-149-299 and CM-82036) compatible stem rust host response types were obtained compared to more resistant response types obtained when they were combined with other *Sr* resistance genes. This finding was also reported in a study done by Spielmeyer et al. (2013). Therefore, although the *Lr34/Yr18/Sr57* gene is considered a multiple disease resistant gene, it might not give strong enough protection under prolonged epidemic conditions and should preferably being deployed in combination with other resistance sources including effective ASR genes. It is this also important to identify and select for ASR genes in resistance breeding. In this study six different parental lines were used to develop the rust resistant experimental lines and through combining the different resistance genes and types of resistance into a single genotype the outcome of this study should not only be measured in increased levels of resistance but also in the durability thereof.

Phenotypic evaluation or at least end validation is necessary in combination with MAS in a breeding programme especially when only a limited number of genes are tracked by using MAS. During phenotypic evaluation the comparative response types and severity levels become available and therefore when variation in disease response is observed between lines with the same genotype obtained through MAS the breeder can make further progress using phenotypic selection. Therefore phenotypic evaluation and qPCR can differentiate between different levels of resistance, even when the genotypic profile is the same, and better selections can be made on the former. In this study some lines with the same genotypic profile showed differences in their level of resistance or susceptibility. The same tendency was obtained in a study conducted by Wang et al. (2012) which concluded that the homo- or heterozygous state of the resistance genes can alter the level of elicitors produced and affect the timing and the strength of the defence response. Differences found in the expression of resistance can be the result of the heterozygosity level of the corresponding resistance gene (Kolmer 1996, 2005). This can also explain the difference in resistance levels as recorded for the experimental lines in this study because they were still found to segregate.

Experimental lines with combined resistance to leaf-, stem- and stripe rust were developed during this study. Field data furthermore confirmed that some of the experimental lines showed good field resistance against all three rust types. Variation in the level of resistance was observed in different offspring of an entry, possibly because of resistance sources not being fixed in these lines. However, evaluation of segregating lines is more labour intensive, as each plant needs to be evaluated individually compared to genotypically fixed lines that can be evaluated as a group, over different seasons and locations.

5.5 Conclusions

In this study three different techniques (MAS, phenotypic evaluation and qPCR) were used to evaluate the expression of rust resistance genes/QTL. All three techniques were successfully applied in this study on parental, control and rust resistant experimental cultivars/lines. Application of the different methods generated different types of data with a strong positive correlation being observed between the different data sets for the different cultivars/lines used in this study.

Rust inoculations for the three different rust types (leaf, stem and stripe) were conducted successfully in the greenhouse at both seedling and adult plants stages, as ASR and APR genes were present in the genotypes, as well as in field trials for two consecutive years (2015 and 2016). Phenotypic evaluation has revealed that resistance genes identified using MAS was expressed phenotypically which resulted in different levels of resistance or susceptibility. Additional resistance genes were also identified through phenotypic evaluation that would have been missed if only MAS was used in this study. This indicates the benefit of using these methods in combination to complement each other when resources allow.

Several lines with good rust resistance to all three rust types were obtained through successful screening in the greenhouse as well as in the field. Although no markers linked to ASR genes against stripe rust was used in this study, lines with good stripe rust resistance were obtained indicating the importance of genetic diversity in wheat. The importance of international collaboration and exchange of germplasm became evident as the experimental lines developed in this study originated from six different parental cultivars/lines, developed in different breeding programmes based in different countries.

The experimental lines developed in this study indicated the effectiveness of combining ASR and APR genes to develop rust resistant wheat lines. The experimental lines

showed sufficient rust resistance levels and therefore lines used in crosses to combine rust and FHB resistance as described in Chapter 3 can contribute effective rust resistance. The genetic diversity observed in the experimental lines indicated that variation among the best lines regarding rust resistance may still occur and therefore, after genotype fixation of the best combined rust and FHB resistance lines, phenotypic evaluation and qPCR should be done to select the best lines based on phenotypic expression.

5.6 References

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Chapter 6

Phenotypic validation and Fusarium head blight fungal gene expression

6.1 Introduction

The fungal disease FHB, mainly caused by *Fusarium graminearum*, is a major disease that infects wheat and several other small grains worldwide causing yield losses and mycotoxin infected grain (Bai and Shaner 1994; McMullen et al. 1997). There are three main control strategies that can be used against FHB infection; spraying of fungicides, agricultural practices and genetic improvement of host plants (Dill-Macky and Jones 2000; Jones 2000; Ferrigo et al. 2016). Not one of these control strategies is completely effective in controlling FHB infections. FHB resistance breeding can be used to ensure non-toxic grain production and sustainable yields (Bai and Shaner 2004; Buerstmayr and Buerstmayr 2015).

Only a small number of FHB tolerant or resistant wheat germplasm sources have been identified during the past years and successfully used in FHB resistance breeding programmes. Some of these sources with resistance have poor agronomic traits and should be back crossed with cultivars that have good agronomical traits to improve the adaptability and performance of FHB resistant wheat cultivars (Zhang et al. 2011). Sumai3, and its derivatives (e.g. CM-82036), is one of the most frequently and successfully used cultivars in different breeding programmes as the FHB resistant donor parent. The *Fhb1* gene located on chromosome 3BS was mapped in Sumai3 by Waldron et al. (1999) and explains the highest phenotypic variation of FHB resistance for genes/QTL present in Sumai3. Sumai3 also contains the *Fhb2* and *Qfhs.ifa-5A* resistance genes/QTL located on chromosomes 6B and 5A respectively (Buerstmayr et al. 2003; Cuthbert et al. 2007). In a study done by Buerstmayr et al. (2002) they found that the *Fhb2* gene/QTL was not present in CM-82036 and therefore not successfully transferred by crossing with Sumai3.

Five different types of FHB resistance have been identified over the past years. Type I and Type II resistance described by Schroeder and Christensen in 1963, are at present the best characterised sources of resistance. These two resistance types confer resistance to initial infection and resistance to spread of the disease. The *Fhb1* and *Qfhs.ifa-5A* resistance genes/QTL confer Type II and Type I resistance respectively while *Fhb2* is linked to field resistance (Anderson et al. 2001; Buerstmayr et al. 2003; Cuthbert et al. 2007).

Combining of FHB resistance genes/QTL can be validated and followed using molecular breeding and/or conventional breeding. Major and minor FHB resistance QTL were found on all wheat chromosomes except chromosome 7D (Buerstmayr et al. 2009). Several molecular markers linked to FHB resistance genes/QTL are available. Flanking markers are presently used to screen for most FHB resistance genes/QTL. A near-perfect marker, Umn10, was developed for the *Fhb1* resistance gene (Liu et al. 2008).

FHB infections and consequent disease development are favoured by humid and warm climatic conditions during flowering (Parry et al. 1995). Various inoculation methods are available for evaluation of FHB disease severity under both field and greenhouse conditions (Imathiu et al. 2014). Field evaluation of FHB infections can be challenging because it is time consuming and expensive to repeat experiments over several years due to varying environmental conditions that have an influence on disease development (Mesterhazy et al. 2003). Artificial inoculation is important during FHB evaluations because natural infection by the pathogen is not guaranteed during any season (Mesterhazy 1995). The spray inoculation method is used to evaluate large numbers of experimental lines under field and greenhouse conditions for both Type I and Type II resistance. The point inoculation method is mainly used under greenhouse conditions to evaluate Type II resistance (Tamburic-Illincic 2012).

Quantitative evaluation of FHB infection and mycotoxin accumulation is important when new germplasm with improved FHB resistance is being developed (Zhang et al. 2011). The *Fusarium* pathogen penetrates host tissue leading to penetration of the cell wall, membrane, mitochondria and chloroplast causing premature bleaching because photosynthesis cannot take place (Wojciechowski et al. 1995; Miller and Ewen 1997; Zhang et al. 2011). Depending on the FHB resistance level of different cultivars, the host plant will express different levels of hydrolysing enzymes and photosynthetic parameters as a reaction upon infection by the FHB pathogen. These parameters can be used to quantitatively evaluate host-pathogen interactions (Zhang et al. 2011). The clip-dipping inoculation assay can be applied to seedlings by cutting off the coleoptile's apex and dip it into a conidial suspension to determine lesion lengths at 7 dpi (Shin et al. 2014). Other methods for evaluating FHB resistance include the detached leaf assay (Browne and Cooke 2004), seed germination assay (Browne 2009), coleoptiles assay (Li et al. 2010) and inoculation (spray and point) methods (Miedaner et al. 2003). Mycotoxin accumulation can be evaluated using the liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay (Ji et al. 2015; Etzerodt et al. 2016). An alternative

method to determine *Fusarium* and mycotoxin accumulation in infected plants is the enzyme linked immunosorbent assay (ELISA) (Schlang and Duveiller 2011).

Improved technologies have led to the development of PCR based *Fusarium* detection and quantification methods. Different DNA based technologies contributed to sequencing of various DNA fragments of different *Fusarium* species (Knutsen and Holst-Jensen 2004; Laday et al. 2004; Mach et al. 2004; Mulè et al. 2004; Yli-Mattila et al. 2004; De Vos et al. 2014). These sequenced DNA fragments were used to develop species specific PCR primers that can be used for detection, identification and quantification of *Fusarium* species. Studies of the biosynthesis pathways of mycotoxins produced sequence information that led to the development of molecular markers linked to genes in the mycotoxin biosynthesis pathways of *Fusarium*. These markers can be used to distinguish between *Fusarium* species based on their ability to produce mycotoxins (Brunner et al. 2012). These PCR based methods can be used to distinguish between *Fusarium* species and to determine whether identified species are producers or non-producers of mycotoxins (Brunner and Mach 2010). Real-time PCR can furthermore be used to quantify fungal biomass accumulation in plants (McCartney et al. 2003).

The main objective of this study was to validate whether the FHB resistance genes/QTL, identified using MAS in the breeding lines developed during this study, are expressed phenotypically. Another objective was to quantify *F. graminearum* fungal biomass accumulation and the expression of the *Tri5* gene linked to the biosynthetic pathway of mycotoxins, using a reference-gene-based quantitative PCR method. The last objective was to determine whether a correlation existed between visible FHB infection and the reference-gene-based quantitative PCR method.

6.2 Materials and methods

To obtain the set objectives, BC_2F_6 wheat lines (developed in Chapter 3 of this study) with different combinations of FHB resistance genes/QTL were selected and evaluated using FHB resistance molecular markers as described in Chapter 3. Selected lines were also point and spray inoculated and phenotypically evaluated at different dpi. The *Fusarium* biomass accumulation and the *Tri5* gene expression were quantitatively evaluated using qPCR.

6.2.1 Phenotypic evaluation of *Fusarium graminearum* infection

6.2.1.1 Selection of plant material

A total of 19 wheat lines were selected for phenotypic evaluation of FHB infection. Eleven of these lines served as the parental and control lines and the other eight lines were experimental lines (Table 6.1). The parental lines included the six wheat cultivars/lines that were used to develop the rust resistant lines as well as Krokodil, receiver parent and CM-82036, donor parent, that were used to develop the FHB resistant lines used throughout the study. The control lines included cultivars containing additional FHB resistance genes/QTL (Sumai3 and Frontana) and a commercial cultivar SST-835 that showed high infection rates in a previous PhD study done at the UFS (Sydenham 2014). The experimental lines were selected from the BC_2F_6 RIL population based on containing different FHB resistance genes/QTL combinations.

Table 6.1 Wheat lines selected for phenotypic evaluation

Cultivar/line	Background	Expected FHB resistance genotype
Kariega	Rust resistant parental line	[1]None
Avocet*YrSP*	Rust resistant parental line	None
Blade	Rust resistant parental line	None
CS*Lr19*-149-299	Rust resistant parental line	None
Kingbird	Rust resistant parental line	None
2S#/163	Rust resistant parental line	None
CM-82036	FHB resistant donor line	*Fhb1*, *Qfhs.ifa-5A-1, Qfhs.ifa-5A-2*
Krokodil	FHB susceptible recurrent line	None
Sumai3	Control line	*Fhb1*, *Qfhs.ifa-5A-1, Qfhs.ifa-5A-2, Fhb2*
Frontana	Control line	*Qfhs.ndsu-3AS*
SST-835	Control line	None
BC_2F_6-2.3	Experimental line	*Fhb1*
BC_2F_6-1.1	Experimental line	*Qfhs.ifa-5A-1*
BC_2F_6-13.2	Experimental line	*Qfhs.ifa-5A-2*
BC_2F_6-6.3	Experimental line	*Qfhs.ifa-5A-1, Qfhs.ifa-5A-2*
BC_2F_6-32.2	Experimental line	*Fhb1*
BC_2F_6-12.3	Experimental line	*Fhb1, Qfhs.ifa-5A-1, Qfhs.ifa-5A-2*
BC_2F_6-37.1	Experimental line	*Fhb1, Qfhs.ifa-5A-1, Qfhs.ifa-5A-2*
BC_2F_6-25.2	Experimental line	None

FHB-Fusarium head blight; [1]None refers to lines containing none of the FHB resistance quantitative trait loci screened for.

6.2.1.2 Phenotypic evaluation greenhouse trial set-up

The FHB phenotypic evaluation trial was done in a small greenhouse cubicle with temperatures set at 18˚C/24˚C minimum/maximum. The bench in one half of the cubicle was used for the spray inoculation trial and the bench in the other half of the cubicle was used for the point inoculation trial. Nineteen 2 l pots per trial were filled with top soil. Four seeds were planted per pot, but were reduced after seedling emergence to only two plants per pot. Available data on days to flowering for each entry was used to plant the longer growers earlier in an effort to synchronise flowering for all entries.

6.2.1.3 *Fusarium* inoculum preparation

The most aggressive *F. graminearum* isolate identified by De Villiers in 2009 were used to prepare inoculum. Molecular markers were used to categorise the isolate as a DON producing isolate (Minnaar-Ontong 2011). A potato sucrose broth (PSB) was prepared as a growth medium for the DON producing *F. graminearum* isolate. The potato extract was prepared from boiling 400 g peeled and diced potatoes inside muslin in 1 l water for 10 min. The liquid was poured through a sieve and stored in the refrigerator (4˚C). The medium was prepared using 500 ml potato extract, 20 g sucrose and 500 ml ddH_2O. The pH was adjusted to 6.5 and autoclaved at 15 pounds per square inch (psi) for 20 min. A 250 ml flask was filled with 200 ml PSB and a pure culture of the isolate added. The flask was placed on an orbital shaker [250 revolutions per minute (rpm)] at room temperature (22˚C) for spore development. After seven days the fungal culture was filtered through a double layer of cheesecloth and used for inoculation.

6.2.1.4 Spray inoculation method

The spray inoculation method was used to evaluate Type I and Type II resistance. The inoculum was prepared from the filtered 200 ml spore suspension filled with autoclaved ddH_2O to 1 l and 20 µl Tween 20 (Sigma, Johannesburg, RSA) was added and mixed. The inoculum was transferred to a 750 ml spray bottle. Heads beginning to flower were sprayed with inoculum until small droplets were visible on both sides of the head. Inoculated heads were covered with clear plastic zip lock bags after they were allowed to dry for 30 min to ensure a high level of humidity and dew formation. Heads were inoculated early in the mornings. The plastic bags were removed at 2 dpi. A few wheat heads were selected as negative controls and sprayed with only water and Tween 20. The infected spikelets per head were counted 4, 7, 10, 14, 18 and 21 dpi. The inoculated

heads were collected in a 15 ml tube at 21 dpi and freeze-dried (Alpha 1-2 LDplus Entry Freeze Dryer, Lasec, Bloemfontein, RSA) for seven days.

6.2.1.5 Point inoculation method

The point inoculation method was used to evaluate Type II FHB resistance. Autoclaved round cotton wool wads (3 mm in diameter) were soaked in the inoculum suspension. A cotton wool wad was placed inside the floret in the middle of the head. Heads were inoculated when the first anthers were extruded indicating the beginning of flowering. Inoculations were done early in the mornings. The spikelet with the cotton wool wad was covered with a strip of parafilm tape and the whole head was covered with a clear zip lock plastic bag. The parafilm tape and plastic bag was removed two days post inoculation. A few heads were selected as negative controls and a cotton wool wad soaked in ddH_2O was used. The infected spikelets were counted at 4, 7, 10, 14, 18 and 21 dpi. The inoculated head was collected in a 15 ml tube at 21 dpi and freeze-dried for seven days.

6.2.2 DNA isolation for marker-assisted selection

Leaf material was collected from inoculated plants. DNA was isolated as described according to section 3.2.5. All leaf material samples were evaluated using MAS linked to FHB resistance genes/QTL (Table 3.4).

6.2.3 Quantification of *Fusarium graminearum* fungal biomass accumulation and *Tri5* gene expression

6.2.3.1 DNA isolation

The inoculated freeze-dried wheat heads were divided into three parts for DNA isolation: rachis, chaff and seed. After the wheat head was separated into three parts, each part was grounded using a mortar and pestle (to a fine powder). DNA was extracted using 250 µl of plant material of each of the three parts, individually. DNA was extracted as described in section 3.2.5. The concentration of the extracted DNA was determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA). All DNA samples were diluted to a working concentration of 5 ng/µl using TE buffer.

6.2.3.2 Quantitative polymerase chain reaction analysis

The *β-tubilin* gene was selected for real-time PCR analysis to quantify the fungal biomass accumulation of *F. graminearum* while the *Tri5* gene was used to quantity gene

expression linked the DON biosynthesis pathway. The *translation elongation factor* (*TEF*) gene was used as a HG to quantify the wheat DNA. Primers used for qPCR analysis are summarised in Table 6.2. All three parts of the inoculated DNA wheat heads were subjected to qPCR analysis using all three primer pairs.

The MIQE guidelines were used for qPCR analyses (Bustin et al. 2009). The qPCR analyses were done using the Bio-Rad1000 thermal cycler with a CFX96 real-time attachment and KAPA SYBR® FAST qPCR Kit Master Mix (2X) Universal (KAPABIOSYSTEMS; Lasec, Bloemfontein, RSA). A temperature gradient was done for all three primer pairs to determine optimal annealing temperatures. After the annealing temperature was determined a tenfold dilution series was done for all three primer pairs to evaluate the amplification efficiency. A melt curve was included in the cycling conditions, ranging from 65˚C to 95˚C with 0.5˚C increments every 5 sec, to confirm the absence of non-specific amplification products.

6.2.4 Data analysis

6.2.4.1 Phenotypic evaluation

Spikelets per inoculated head were counted and recorded for both the spray and point inoculation methods. Bleached spikelets were counted and recorded at 4, 7, 10, 14, 18 and 21 dpi. The disease percentage score was determined using the following equation:

$$\text{FHB infection } \% \; = \; 100\left(\frac{\text{bleached spikelets}}{\text{total spikelets}}\right).$$

6.2.4.2 Molecular marker evaluation

Molecular markers linked to FHB resistance genes/QTL were used to evaluate all 11 parental and control lines as well as eight experimental lines. Markers are co-dominant markers and were scored according to alleles present: Homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0).

6.2.4.3 Real-time polymerase chain reaction evaluation

The *TEF*, *β-tubilin* and *Tri5* genes were quantified using qPCR and Cq values used to determine expression ratios. The Cq values of the qPCR analysis were imported into GeNorm-Plus2 software (Biogazelle) and used to determine expression ratios. Four technical replications were done of all samples for all three primer pairs. Replicate variability was set to 0.5 and samples out of this range were excluded from the analysis.

Table 6.2 Primers used to determine the expression ratios of the *β-tubilin* and *Tri5* genes of *Fusarium graminearum* as well as the housekeeping *translation elongation factor* gene of wheat in inoculated wheat heads

Gene/ Organism	Primer sequence (5'-3')	qPCR reactions	qPCR cycling conditions	Fragment size	References
β-tubilin/ *F. graminearum*	F: GGTCTCGACAGCAATGGTGTT R: GCTTGTGTTTTTCGTGGCAGT	10 ng/µl DNA 1 x KAPA SYBR® FAST qPCR Kit Master Mix (2X) Universal 25 ng FGtubF 25 ng FGtubR	1 cycle: 3 min 30 sec @ 95˚C; 40 cycles: 15 sec @ 95˚C, 15 sec @ 67˚C, 20 sec @ 72˚C	111 bp	Reischer et al. 2004
Tri5/ *F. graminearum*	F: GARCAGTACAACTTTGGAGG R: ACCATCCAGTTCTCCATCTG	10 ng/µl DNA 1 x KAPA SYBR® FAST qPCR Kit Master Mix (2X) Universal 25 ng Tri5F 25 ng Tri5R	1 cycle: 3 min @ 95˚C; 45 cycles: 20 sec @ 95˚C, 20 sec @ 52˚C, 15 sec @ 60˚C	178 bp	Brunner et al. 2009
TEF/ Wheat	F: AGGTATTAAGCAGTACATTTTCTC R: GGACTAGACTCAAAATTAGTATTT	10 ng/µl DNA 1 x KAPA SYBR® FAST qPCR Kit Master Mix (2X) Universal 25 ng TEFF 25 ng TEFR	1 cycle: 3 min @ 95˚C; 45 cycles: 15 sec @ 95˚C, 20 sec @ 57˚C, 20 sec @ 62.5˚C	181 bp	Brunner et al. 2009

qPCR-quantitative polymerase chain reaction; TEF-translation elongation factor; F-forward; R-reverse; bp-base pairs.

6.2.4.4 Comparison analysis

Correlation analysis of the *β-tubilin:TEF* expression ratio and FHB infection percentage for the spray and point inoculation trials was calculated using GenStat 17th edition.

6.3 Results

6.3.1 Molecular marker evaluation of Fusarium head blight resistance genes/quantitative trait loci

All lines selected for point and spray inoculation were evaluated using four molecular markers linked to three FHB resistance genes/QTL. Results indicated that these lines contained the same molecular markers linked to FHB resistance as predicted based on the genotypes of the lines they were developed from because the evaluated genes/QTL was fixed in the experimental BC_2F_6 lines. Frontana, SST-835 and the rust resistant parental lines did not contain any of the markers linked to FHB resistance genes/QTL. Genotypes based on the evaluated FHB resistance genes/QTL of the inoculated wheat lines are summarised in Table 6.3.

6.3.2 Phenotypic evaluation of *Fusarium graminearum* infection

Type I and Type II FHB resistance were measured using the spray inoculation method. Different FHB infection percentages were observed for the different parental, control and experimental wheat lines that were spray inoculated. None of the lines showed FHB infection at 4 dpi. The first bleached spikelets were observed at 7 dpi. Sumai3, BC_2F_6-6.3 and BC_2F_6-37.1 were the only cultivars/lines that did not show any bleached spikelets after 21 dpi (Figures 6.1 and 6.2). The highest infection of 50% was observed for Kariega. Of the eight experimental lines, line BC_2F_6-25.2 showed the highest percentage of FHB infection (44%). This line does not contain any FHB genes/QTL. Sumai3 and BC_2F_6-37.1 that showed no infection at 21 dpi, contains the *Fhb1*, *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2* gene/QTL while BC_2F_6-6.3, containing only the *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2* QTL, also showed no infection. Of cultivars containing *Fhb1*, *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2*, CM-82036 showed the highest percentage (3%) of infection. Line BC_2F_6-13.2 that only contained *Qfhs.ifa-5A-2* showed only 1% infection. Of the experimental lines, containing FHB resistance genes/QTL, line BC_2F_6-2.3 showed the highest percentage of infection (13%). This line tested positive for only the *Fhb1* gene. The FHB infection rates at 21 dpi of lines spray inoculated are indicated in Figure 6.1. Photos of the spray inoculated wheat heads were taken at 21 dpi and are given in Figure 6.2.

Table 6.3 Fusarium head blight resistance genotypes of the inoculated wheat lines

Cultivar/line	Umn10 *Fhb1*	Gwm156 *Qfhs.ifa-5A-2*	Gwm293 *Qfhs.ifa-5A-1*	Gwm304 *Qfhs.ifa-5A-1*
Kariega	0	0	0	0
Avocet*YrSP*	0	0	0	0
Blade	0	0	0	0
CS*Lr19*-149-299	0	0	0	0
Kingbird	0	0	0	0
2S#/163	0	0	0	0
CM-82036	1	1	1	1
Krokodil	0	0	0	0
Sumai3	1	1	1	1
Frontana	0	0	0	0
SST-835	0	0	0	0
BC_2F_6-2.3	1	0	0	0
BC_2F_6-1.1	0	0	1	1
BC_2F_6-13.2	0	1	0	0
BC_2F_6-6.3	0	1	1	1
BC_2F_6-32.2	1	0	0	0
BC_2F_6-12.3	1	1	1	1
BC_2F_6-37.1	1	1	1	1
BC_2F_6-25.2	0	0	0	0

The point inoculation method is used to evaluate Type II resistance and the *Fhb1* gene provides Type II resistance. Different FHB infection rates were observed for lines that were point inoculated. CM-82036, the FHB resistant donor line used in this study, was the only cultivar that did not show any bleached spikelets at 21 dpi (Figures 6.1 and 6.3). Line BC_2F_6-1.1 had the highest FHB infection of 48%. This line only contained the *Qfhs.ifa-5A-1* QTL. Lines containing the *Fhb1* gene/QTL showed low infection levels ranging from 0% (CM-82036) to 11% (BC_2F_6-37.1). Lines BC_2F_6-1.1 (*Qfhs.ifa-5A-1*) and BC_2F_6-6.3 (*Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2*) showed high levels of infection while line BC_2F_6-13.2, that only contained the *Qfhs.ifa-5A-2* QTL, showed low levels of infection (13%). Figure 6.1 indicates the FHB infection rates at 21 dpi of the different entries subjected to point inoculation. Figure 6.3 shows photos of the point inoculated wheat heads at 21 dpi for the different lines.

6.3.3 Quantitative polymerase chain reaction evaluation

The inoculated wheat heads of the evaluated lines were used to quantify the fungal biomass accumulation of *F. graminearum* using the *β-tubilin* gene and to quantify the *Tri5* gene that is expressed in the DON biosynthesis pathway. Quantification of these two genes was expressed as a ratio to the expression of the *TEF* gene of wheat.

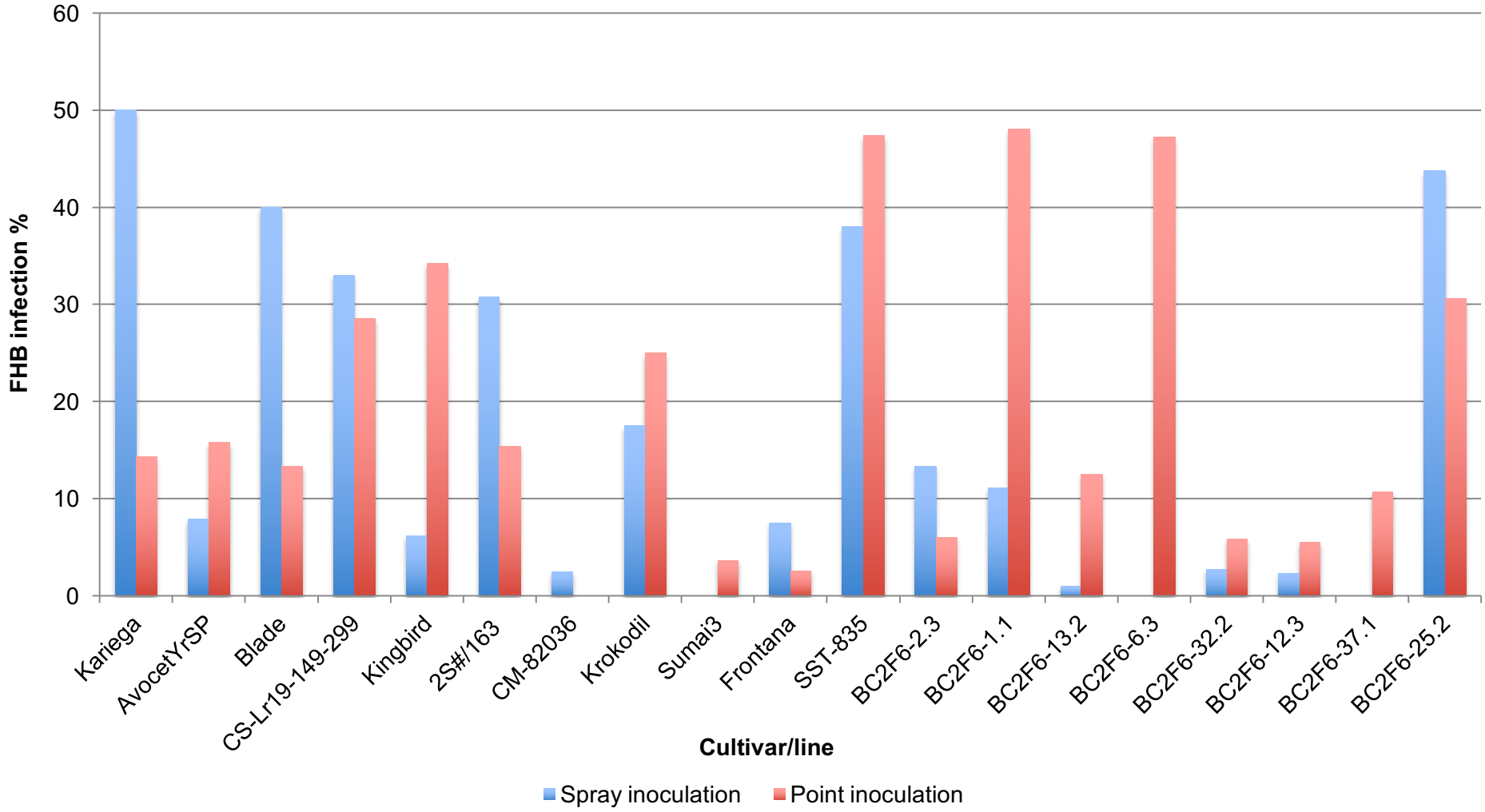


Figure 6.1 **Fusarium head blight (FHB) infection rates of the parental, control and experimental lines used for point and spray inoculation.**



Figure 6.2 **Photos of spray inoculated wheat heads of the parental, control and experimental lines at 21 days post inoculation.**



Figure 6.3 **Photos of point inoculated wheat heads of the parental, control and experimental lines at 21 days post inoculation.**

Expression ratios were calculated for each line for each of the three parts of the head sampled (rachis, chaff and seed). However, after analysis, the three ratios for the different parts of the wheat head were combined, because no significant results were obtained from the three different parts individually. Thus only the combined results are presented in this chapter. The qPCR data obtained from the three different parts individually is given in Appendix H for both the spray and point inoculations.

6.3.3.1 Spray inoculated wheat heads

Higher levels of variation between the different spray inoculated wheat heads were observed for the *β-tubilin:TEF* ratios compared to the *Tri5:TEF* ratios (Figures 6.4 and 6.5). The highest *β-tubilin:TEF* ratio (50.18) was observed for CS-*Lr19*-149-299 (no FHB resistance genes/QTL) and the lowest (0.2) for line BC_2F_6-37.1 that contained all three FHB resistance genes/QTL tested for in this study. Experimental lines BC_2F_6-32.2 (0.4), BC_2F_6-12.3 (0.43) and BC_2F_6-37.1 (0.2; all containing *Fhb1*) showed lower expression ratios than CM-82036 (2.85), their parental line. Line BC_2F_6-2.3 (4.05), containing *Fhb1*, also showed low expression ratio even though it was higher than CM-82036. Line BC_2F_6-6.3 (0.59), although only containing the *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2* QTL and not *Fhb1*, also had a low ratio. The experimental line with no FHB resistance genes/QTL, BC_2F_6-25.2 showed the highest expression ratio of all experimental lines tested.

Low levels of variation was observed for the *Tri5:TEF* ratio for the spray inoculated lines. The *Tri5:TEF* ratios varied between 2.03 and 4.73 compared to the high variation seen for the *β-tubilin:TEF* ratios that varied between 0.20 and 50.18. Experimental lines with the lowest *Tri5:TEF* ratios were BC_2F_6-12.3 (2.03), BC_2F_6-32.2 (2.21), BC_2F_6-2.3 (2.47) and BC_2F_6-6.3 (2.5). Ratios for these lines were lower than for the two FHB resistant sources CM-82036 (2.8) and Sumai3 (2.83). Experimental line BC_2F_6-37.1 that showed the lowest *β-tubilin:TEF* ratio of all experimental lines tested, had the highest *Tri5:TEF* ratio of all experimental lines. The ratio was even higher than for line BC_2F_6-25.2 that contains no FHB resistance genes/QTL and that had the highest *β-tubilin:TEF* ratio of all experimental lines. The *β-tubilin:TEF* and *Tri5:TEF* ratios of the spray inoculated wheat lines are summarised in Figures 6.4 and 6.5, respectively.

6.3.3.2 Point inoculated wheat lines

All lines containing the *Fhb1* gene (CM-82036, Sumai3, BC_2F_6-2.3, BC_2F_6-32.2, BC_2F_6-12.3 and BC_2F_6-37.1) showed low *β-tubilin:TEF* ratios ranging from 0.54 to 2.22

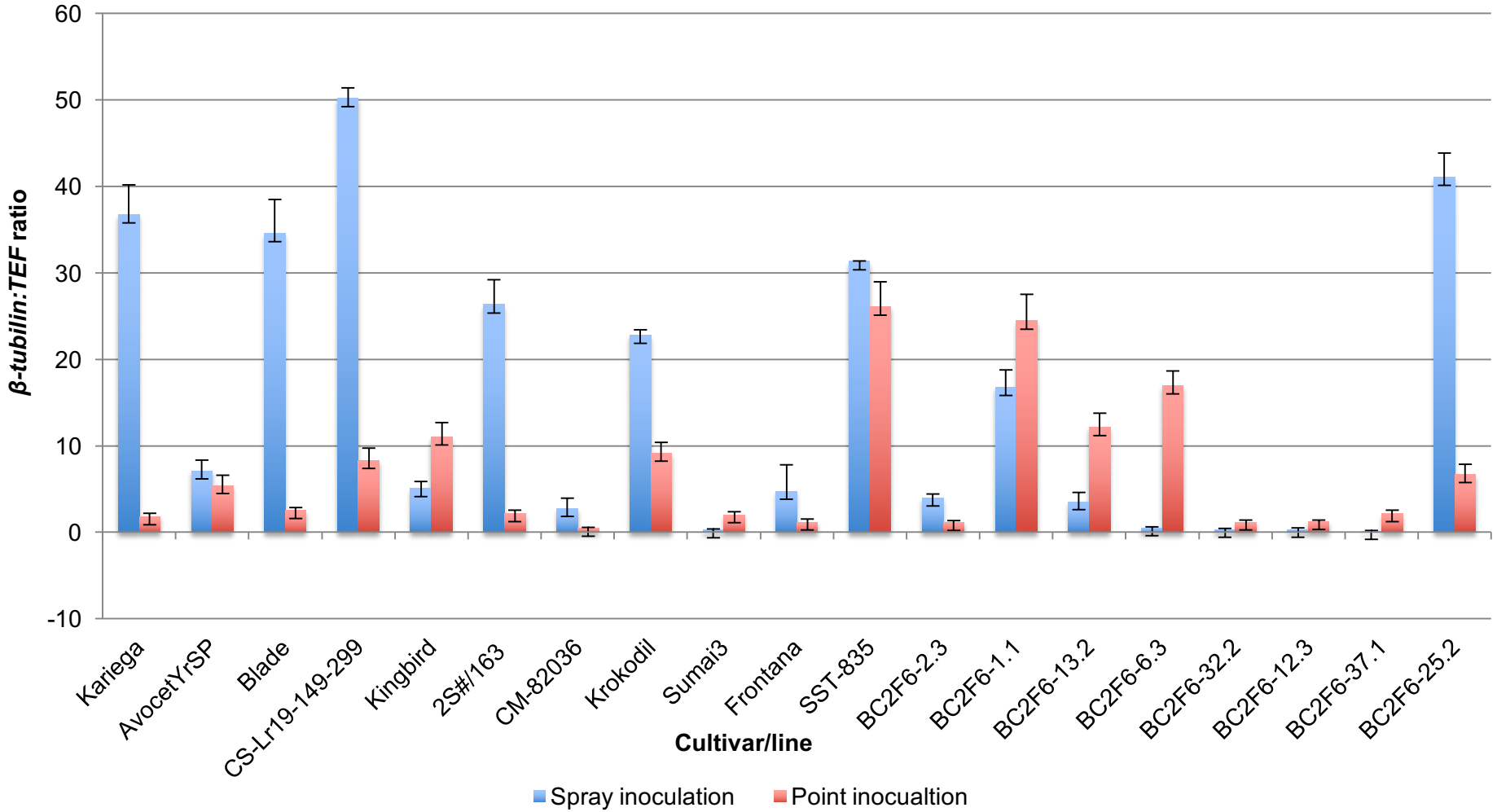


Figure 6.4 The *β-tubilin:translation elongation factor* (*TEF*) expression ratios of the parental, control and experimental wheat lines after spray and point inoculation. Bars indicate standard error for each data point.

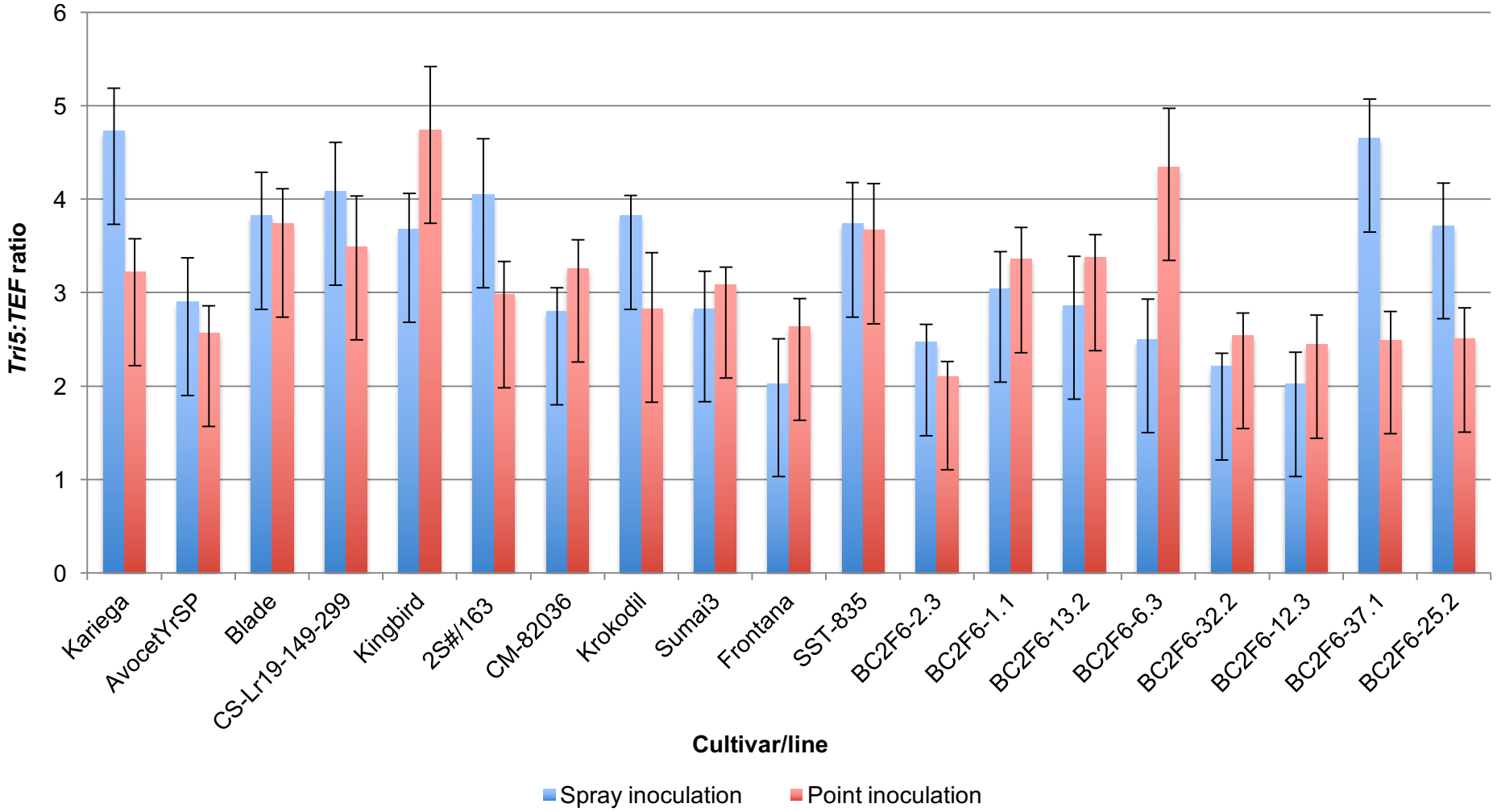


Figure 6.5 The *Tri5:translation elongation factor* (*TEF*) expression ratios of the parental, control and experimental wheat lines after spray and point inoculation. Bars indicate standard error for each data point.

Experimental lines containing only the *Qfhs.ifa-5A-1* and/or *Qfhs.ifa-5A-2* QTL (BC_2F_6-1.1, BC_2F_6-13.2, BC_2F_6-6.3 and BC_2F_6-25.2) showed higher *β-tubilin:TEF* ratios (6.73 to 24.46) compared to lines containing the *Fhb1* gene. The *β-tubilin:TEF* ratios varied from 0.54 to 26.08. Three of the rust resistant parental lines (Kariega, Blade and 2S#/163) unexpectedly also showed low *β-tubilin:TEF* ratios. Frontana, known to have Type II resistance showed a low (1.27) *β-tubilin:TEF* ratio. High levels of variation were observed for the *β-tubilin:TEF* ratios of the point inoculated lines. The highest *β-tubilin:TEF* ratio was observed for SST-835 (26.08) while the lowest *β-tubilin:TEF* ratio was observed for CM-82036 (0.54). The *β-tubilin:TEF* ratios of the point inoculated lines were lower compared to the spray inoculated lines.

Low levels of variation for the *Tri5:TEF* ratios were detected between the different lines that were point inoculated. The *Tri5:TEF* ratios varied between 2.10 (BC_2F_6-2.3) and 4.74 (Kingbird). Experimental line BC_2F_6-2.3, containing *Fhb1*, had the lowest *Tri5:TEF* ratio of both the experimental lines and FHB resistant parent donor lines. The *β-tubilin:TEF* ratios and *Tri5:TEF* ratios of the point inoculated lines are given in Figures 6.4 and 6.5, respectively.

6.3.4 Comparison analyses

The main objective of the study was to phenotypically validate the molecular marker data. Higher FHB infection rates were observed for spray and point inoculated lines containing no markers linked to FHB resistance genes/QTL, and vice versa. The *β-tubilin:TEF* expression ratios obtained based on qPCR results correlated significantly positively with the FHB infection percentages obtained based on phenotypic evaluation for both the spray ($r = 0.93$; $p < 0.001$) and point ($r = 0.87$; $p < 0.001$) inoculation methods (Figures 6.6 and 6.7). The *Tri5:TEF* ratios for both the point and spray inoculated wheat lines did not show any correlation with FHB infection or molecular marker data.

6.4 Discussion

Phenotypic evaluation studies of FHB infection on wheat are used in various breeding programmes to evaluate resistance levels of different wheat lines. Various FHB resistance genes/QTL have been identified and used in different resistance breeding programmes. Over the years, research identified two major FHB resistance genes, *Fhb1* and *Qfhs.ifa-5A*, which are used extensively in FHB resistance breeding programmes (Anderson 2007).

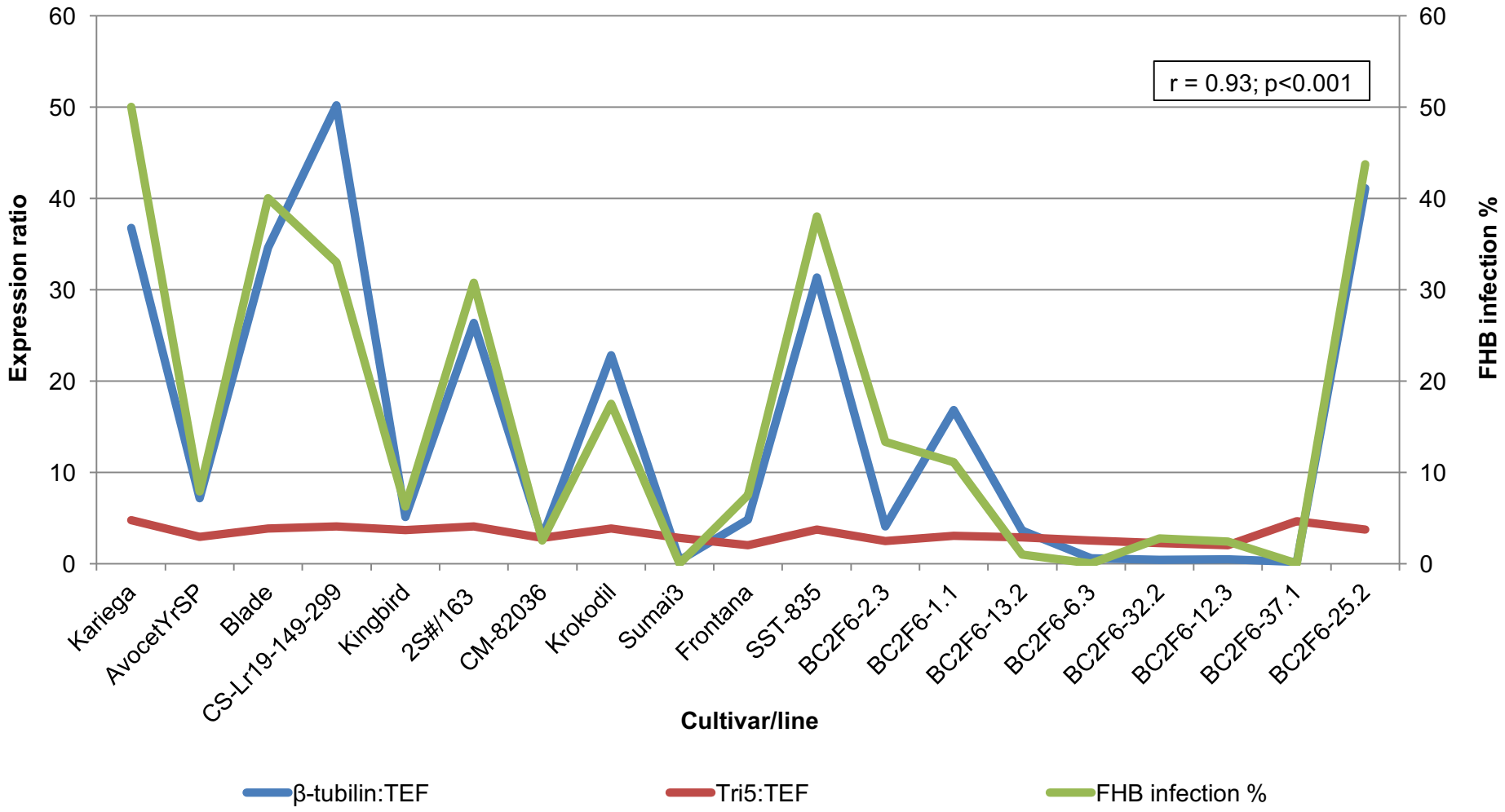


Figure 6.6 The expression ratios and Fusarium head blight (FHB) infection rates of the spray inoculated control, parental and experimental wheat lines. *TEF-translation elongation factor.*

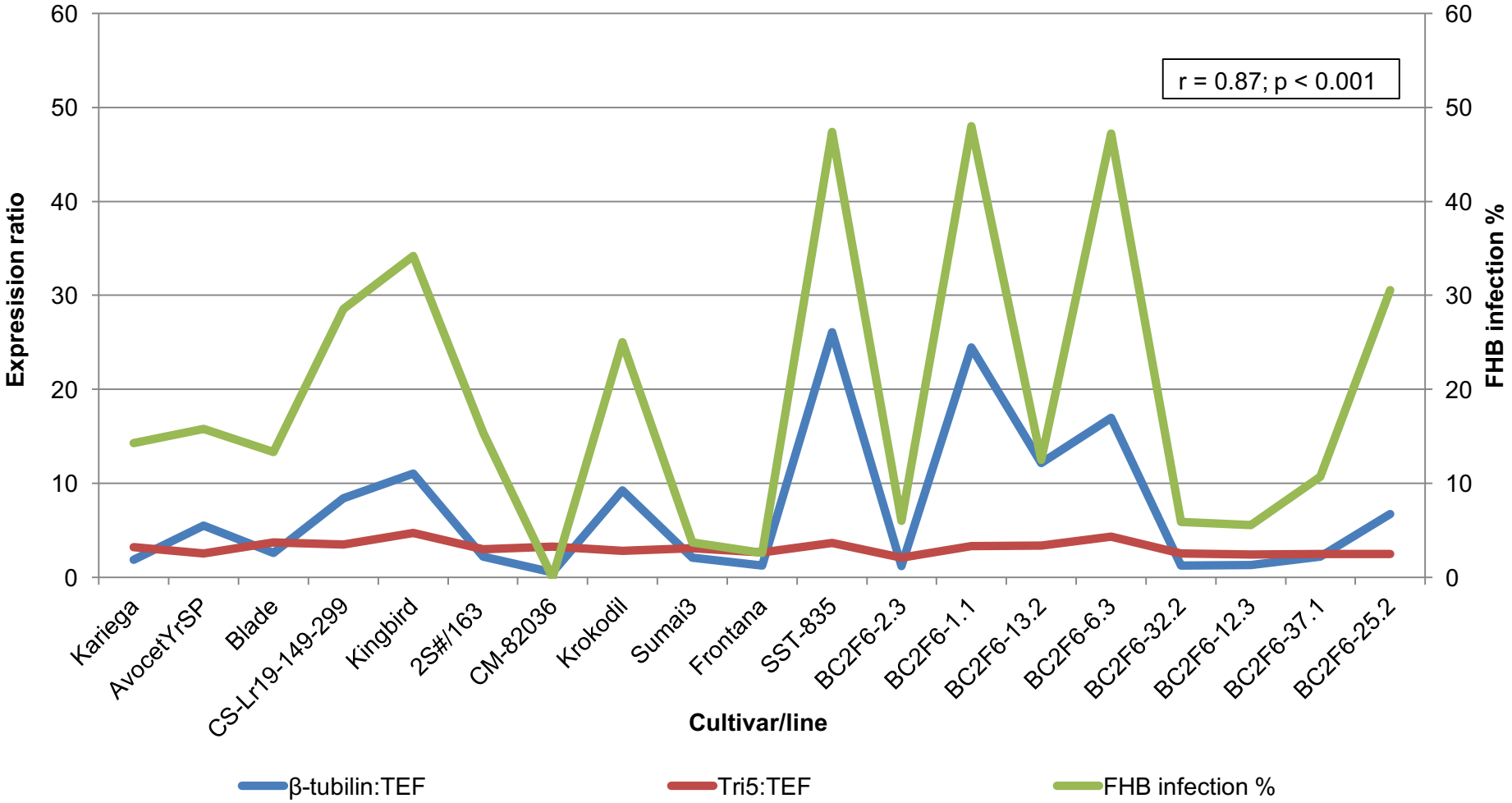


Figure 6.7 The expression ratios and Fusarium head blight (FHB) infection rates of the point inoculated control, parental and experimental wheat lines. *TEF-translation elongation factor.*

The *Fhb1* resistance gene confers Type II resistance and *Qfhs.ifa-5A* Type I resistance. These two major FHB resistance genes are present in CM-82036, a Sumai3 derivative. CM-82036 was selected as donor parental line to develop the FHB resistant lines used in this study. Sumai3 was included as a resistant control line and low levels of FHB infections was observed for both inoculation methods used. Molecular marker data for Sumai3 confirmed the presence of *Fhb1* and *Qfhs.ifa-5A* and low *β-tubilin:TEF* expression ratios was furthermore obtained using qPCR analysis. Similar results were obtained for the FHB resistant donor parent, CM-82036. Krokodil, used as recurrent parent in the development of the BC_2 lines, showed high FHB infection levels for both phenotypic and qPCR results for both inoculation methods. The high infection level can be explained by the absence of the two major FHB resistance genes, *Fhb1* and *Qfhs.ifa-5A*, supported by molecular marker data.

The main aim of this study was to combine rusts and FHB resistance into a single wheat line, therefore cultivars/lines used to develop the rust resistant lines for this study were also included during the FHB infection trials. The FHB infection percentages showed variation between the parental lines for both spray and point inoculations. Variation was also observed between the FHB infection percentages of the two different inoculation methods for the same cultivar/line. For example, Kariega showed high levels of infection but much lower infection for the point inoculation method compared to the spray inoculation method. The opposite was observed for Kingbird. CS*Lr19*-149-299 and SST-835 showed relative high infection levels for both inoculation methods. Even though variation was observed between cultivars/lines and inoculation method used, there was a significant positive correlation between *Fusarium* infection percentage and *β-tubilin:TEF* ratio of the spray and point inoculation methods. Limited information is known about the FHB resistance background for the rust resistant parental lines.

Based on molecular data none of the rust resistant parental lines contained *Fhb1* or *Qfhs.ifa-5A*. Even though infection levels varied, FHB infection was detected for all rust resistant parental lines for both inoculation methods used. Minor uncharacterised FHB resistance QTL may be present in the genetic backgrounds of some of these six rust resistant parental lines that showed some levels of FHB resistance.

Frontana was included as a resistant control as it contains a FHB resistance QTL on chromosome 3A that confers Type I resistance. Frontana showed higher levels of FHB infection (8%) as well as *β-tubilin:TEF* ratios (4.81) when spray inoculated compared to being point inoculated (3%; 1.27). These results were unexpected because the FHB

resistance QTL on chromosome 3A confers Type I resistance that is evaluated using the spray inoculation method. The difference may be associated with environmental conditions during the inoculation trial that might have favoured a type of infection over another.

SST-835 was included as a susceptible control. This is a South African irrigation wheat cultivar considered to be susceptible to FHB. Phenotypic evaluation showed high levels of FHB infection (spray inoculation 38%; point inoculation 47%) for both inoculation methods as well as high *β-tubilin:TEF* ratios (spray inoculation 31.32; point inoculation, 26.08). SST-835 showed the highest infection of all evaluated lines using the point inoculation method.

The first experimental line, BC_2F_6-2.3, contained the *Fhb1* resistance gene that confers Type II resistance. The FHB infection percentage and *β-tubilin:TEF* ratio obtained after point inoculation (18% and 1.23; evaluation of Type II resistance) was lower compared to spray inoculated plants (13% and 4.05; evaluation of both Type I and Type II resistance). This supports other research findings that the *Fhb1* resistance gene contributes to Type II resistance (Anderson et al. 2001). Experimental lines BC_2F_6-1.1, BC_2F_6-13.2 and BC_2F_6-6.3 contained different combinations of molecular markers linked to the *Qfhs.ifa-5A* resistance gene. These lines showed higher FHB infection percentages and *β-tubilin:TEF* ratios for the point inoculated wheat heads compared to the spray inoculated wheat heads. This finding correlates with other studies that have shown that *Qfhs.ifa-5A* confers Type I resistance (Buerstmayr et al. 2003). Line BC_2F_6-6.3 that contained both the *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2* QTL (thus the entire QTL region for *Qfhs.ifa-5A*) showed the lowest point inoculated infection percentage, compared to the other two lines that only contained either the *Qfhs.ifa-5A-1* or *Qfhs.ifa-5A-2* QTL. Both these QTL should thus preferable be present in lines selected to contain Type I resistance. However, more lines containing different combinations of these QTL must be screened to confirm these results.

Molecular marker data indicated that experimental lines BC_2F_6-12.3 and BC_2F_6-37.1 contained markers linked to both the *Fhb1* and *Qfhs.ifa-5A* resistance genes. FHB infection in these lines was low for both the spray and point inoculation methods. The qPCR data of the *β-tubilin:TEF* ratios for both inoculation methods was also lower compared to lines that did not contain markers linked to both these FHB resistance genes. This result indicates the importance of combining FHB resistance genes to improve resistance. However, line BC_2F_6-32.2 that only contained the *Fhb1* gene, showed similar infection percentages than line BC_2F_6-12.3 that contained both the *Fhb1*

and *Qfhs.ifa-5A* genes. This supports previous findings that the *Fhb1* gene is a major FHB resistance QTL (Waldron et al. 1999; Anderson et al. 2001). Overall, the *β-tubilin:TEF* ratios in lines containing *Fhb* genes/QTL for the point inoculated lines were lower than for the spray inoculated lines, which supports data that the *Fhb1* explains the highest phenotypic variation of FHB resistance genes/QTL (Buerstmayr et al. 2003; Cuthbert et al. 2007).

The *Tri5* gene together with eight other genes is responsible for DON and NIV production in the trichothecene biosynthesis pathway. The expression of the *Tri5* gene was evaluated at 21 dpi for both spray and point inoculated wheat lines. *Tri5* expression was detected in all evaluated wheat lines with low levels of variation between the different wheat lines. The *Tri5:TEF* ratios varied from 2.03 to 4.74. The *Tri5:TEF* ratios was evaluated for all three different parts of the wheat head. No correlation was observed between the *Tri5:TEF* and the *β-tubilin:TEF* ratios or FHB infection for both inoculation methods, even when using the combined ratio of the different wheat head parts. This low variation for the expression of the *Tri5* gene for both inoculation methods can be explained by the study done by Amarasinghe and Fernando (2016). They found that *Tri5* expression is initiated at 2 dpi and peak at 4 dpi and then decreases. The early initiation of the *Tri* genes (including *Tri5*) is important to initiate the biosynthesis pathway of DON and NIV. Future *Tri5* expression studies should thus be performed at 2-4 dpi. The *F. graminearum* isolate used should also be subjected to re-evaluation to reconfirm that it is a DON producing isolate.

6.5 Conclusions

Phenotypic evaluation for FHB infection is challenged by various factors that can influence results. These factors include environmental conditions, inoculum type and resistance sources present in experimental wheat lines. FHB infection greatly depends on optimal environmental conditions and available inoculum. No cultivar showing total FHB resistance is available at present. However, combining of major and minor FHB resistance genes/QTL can limit FHB infection to a certain level (Buerstmayr and Buerstmayr 2015).

During this study two major FHB resistance genes were combined with different rust resistance genes/QTL. The presence of these genes/QTL in breeding lines were followed and evaluated using molecular markers and therefore it was necessary to phenotypically evaluate the presence of these genes in these breeding lines. Phenotypic evaluation revealed a lower level of FHB infection in lines that tested positive for one or

more markers linked to FHB resistance genes/QTL. Lines containing the *Fhb1* gene linked to Type II FHB resistance showed lower infection levels after being point inoculated. Lower FHB infection levels were observed for lines that carried the *Qfhs.ifa-5A* gene linked to Type I resistance when evaluated using the spray inoculation technique. Lower FHB infection levels for both inoculation methods were also observed for lines containing both of these genes. Therefore, results indicated a positive correlation between molecular marker data linked to the FHB resistance genes and the phenotypic evaluation of FHB infection.

The FHB infection of the experimental lines was also evaluated using a gene-based method to quantify the fungal biomass accumulation of *F. graminearum* and the *Tri5* gene expression level that are linked to the biosynthesis pathway of mycotoxins. The obtained *β-tubilin:TEF* ratios were positively correlated to FHB infection percentages. No correlation was seen between *Tri5:TEF* ratios and *β-tubilin:TEF* ratios or FHB infection percentages. The *Tri5* gene is apparently expressed during initiation of mycotoxin production that starts at 2 dpi. Therefore, since *Tri5* gene expression was only evaluated at 21 dpi during this study, low expression levels of the *Tri5* gene at 21 dpi confirms that it is not expressed three weeks after infection.

All three parts of the inoculated wheat heads were analysed using qPCR. Results indicated that all three parts did contain the fungal *β-tubilin* and *Tri5* genes. Therefore, all three parts were used for analysis to obtain more differentiation between the data. No comparison in the differentiation between the lines/cultivars was obtained from the individual parts of the inoculated wheat head and therefore the whole wheat head can be used for analysis when quantifying the expression of genes.

Overall, low FHB infection levels of the inoculated wheat heads were observed at 21 dpi for both the spray and point inoculation methods. Environmental conditions have a big influence on FHB development. Although the experiment was done in the greenhouse where conditions can be monitored, environmental conditions such as day length and light intensity can influence greenhouse conditions negatively and prevent optimal conditions for FHB infections. High humidity levels favours FHB infection but it was difficult to obtain optimal humidity levels as the year the experiment was done, was an extremely dry and warm year. This could explain the low FHB infection levels observed during this study. Sprinklers can be used in the future to regulate humidity levels better and improve growing conditions of *F. graminearum*.

Although low infection levels were obtained for FHB infection, a positive correlation was observed between the three different evaluation methods and lines with better resistance could be distinguished from susceptible lines. Although FHB disease severity is usually evaluated at 21 dpi, quantification of expressed genes to determine the infection rate, should be done at an earlier stage as indicated in literature.

For future research, these experimental lines can be used in field trials to evaluate the level of FHB resistance under field conditions as well. Although a positive correlation was observed between the three different methods (MAS, disease severity percentage and *β-tubilin:TEF* expression ratios) low variation between the *Tri5:TEF* expression ratios were observed. The disease severity percentage was determined 21 dpi and therefore the expression of the *Tri5* gene was absent and resulted in the low variation between the expression ratios of the lines/cultivars. Therefore, a timeline study needs to be done to determine the optimal dpi date for expression ratio analysis. MAS is a reliable method to detect the presence of major or minor FHB resistance genes/QTL but should be done in combination with phenotypic evaluation as MAS can decrease genetic diversity and cannot identify FHB resistance QTL for which markers have not been developed.

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Chapter 7

General conclusions and recommendations

Wheat disease resistance breeding is a continuous process aiming to develop cultivars resistant against the most threatening pathogens in a given environment. The ability of some pathogens to overcome deployed resistance genes further necessitates a more advanced breeding approach to the long-term control of these ever-evolving pathogens. Outbreaks of wheat diseases led to major epidemics in the past which resulted in major grain losses that negatively affected the economy. A new era of wheat breeding started with sequencing of the wheat genome. New high-throughput technologies and breeding methods were developed during the last number of years. Although MAS can expedite identification of resistance genes/QTL, cultivar development is still a lengthy process. Resistance genes/QTL need to be fixed in a line and other traits such as yield, agronomic performance and end-use quality should also be considered and improved.

Major fungal wheat diseases affecting grain production worldwide and in South Africa include the wheat rusts (leaf-, stem- and stripe rust) and FHB. The primary aim of this study was to combine rust and FHB resistance genes/QTL into a single wheat line. Durable disease resistance can be achieved by combining effective ASR and APR genes/QTL. Molecular markers linked to selected ASR and APR genes/QTL can be used to identify and follow these traits in progeny. Molecular markers provide breeders the opportunity to select the best breeding lines in terms of the traits of interest. When combining different resistance genes/QTL into a single line, major ASR genes can mask minor genes during phenotypic evaluation, thus limiting information available to a breeder.

When aiming to combine different selected genes/QTL using directed crosses, the probability of losing genes/QTL during the breeding process exists. Heterozygous genes/QTL need to be fixed in a line in order to be stable in the offspring. Fixing of heterozygous genes/QTL can be obtained by self-pollination or by developing DH lines. There is always a chance for the heterogeneous genes/QTL to be homozygous present or absent after genes/QTL are fixed. The chance to obtain a line with all the selected gene/QTL combinations decreases as the number of genes increases. Large breeding populations are therefore required to obtain a line with all the selected gene/QTL combinations. Although both gene fixation processes (self-pollination or DH

development) have the same main aim, DH development is much faster but a more labour intensive and technically difficult method.

Phenotypic evaluation of disease development on wheat breeding lines is essential for determining the efficiency of the selected genes/QTL. Different inoculation methods have been developed and optimised to simulate the infection process. Phenotypic evaluation needs people with experience to differentiate between different infection types and severity levels of infection and to produce reliable data. Therefore, other techniques have been developed to differentiate between different levels of infection. Quantification of biomass accumulation of a pathogen using the qPCR method is an example of one of these techniques.

The main aim of this study was to combine selected rust and FHB resistance genes/QTL in a single wheat line. Molecular markers were available for most of the selected rust and FHB resistance genes/QTL and were successfully applied in this study. Molecular markers used in this study had different differentiation abilities and therefore influenced the ability of selecting the best lines for crosses. Co-dominant markers are more informative as they allow the differentiation between homozygous and heterozygous resistant lines and the ability to select homozygous traits assists in identifying the best offspring. This is a major advantage over dominant markers such as markers linked to the *Lr19* and *Sr2* genes used in this study. The flanking markers linked to the *Qfhs.ifa-5A* FHB resistance QTL were in some cases difficult to evaluate due to the diverse genetic backgrounds of parental lines used in this study, whereas the near perfect co-dominant marker linked to the *Fhb1* gene was much easier to use. However, although the differentiation ability levels of the molecular markers differed, selection of parental lines and best lines to be used in crosses were successfully implemented using MAS.

Although molecular markers were successfully used in this study, screening of so many molecular markers and obtaining data timeous to select the best lines for crosses before flowering was challenging, especially for the two larger populations. Techniques such as single nucleotide polymorphisms (SNPs) that have a higher throughput rate can be a solution when screening large populations but these techniques are costly and must be available to the researcher.

Another objective for this study was to evaluate the presence of selected resistance genes/QTL genotipically and phenotypically. A good correlation between the resistance genes/QTL present, based on molecular marker data and phenotypic evaluation, was obtained for most of the genes/QTL. The *Lr34/Yr18/Sr57* gene did not result in more

resistant response types when evaluated phenotypically, but it is an APR gene with multiple-disease resistance and in combination with the *Sr2/Yr30* gene, a less compatible response type was observed. Therefore phenotypical evaluation revealed informative data about the combination of genes/QTL used. The combined presence of the two major FHB resistance genes/QTL showed a decrease in FHB infection levels, indicating the effectiveness of these two major FHB resistance genes/QTL.

A number of lines containing eight of the selected resistance genes/QTL were identified in different populations developed in this pre-breeding programme. These identified lines with the best genotypes linked to rust and FHB resistance should be considered for use in breeding programmes to increase the number of resistance genes/QTL in future released cultivars. These lines can further be improved by improving the homozygosity level through self-pollination or DH development.

The DH technique was successfully optimised and applied in this study. The low success rate achieved during the optimisation process illustrates that this is a technically more difficult technique to use compared to self-pollination, but remains an attractive method to use for rapid development of homozygous lines. Five valuable DH lines, homozygous resistant for all FHB resistance markers tested, were developed during the application of the DH method.

The breeding scheme applied in this study was effective because lines with combined rust and FHB resistance genes/QTL were obtained. Both double cross and self-pollinated lines were developed using the breeding scheme but more elite lines (with a high number of resistance genes) were obtained during the second double crosses compared to the self-pollinated lines. Heterozygous genes/QTL can segregate out after a generation of self-pollination, therefore a chance exists of losing these genes/QTL. Using the double cross method increased efficiency in this breeding programme as it allowed selection of lines complimenting each other in resistance genes/QTL present to be used in the crosses based on marker data. Lines developed through this breeding programme furthermore have diverse genetic backgrounds as they were originally developed from eight different parental cultivars/lines. Therefore, additional resistance genes/QTL not selected and screened for during this study can potentially still be present in the background of these lines that can add further value when used in future breeding. Although stripe rust resistance was observed in these lines, no markers linked to the genes were used during this study and the transfer of these genes/QTL was thus not followed using MAS. Lines developed during this study also contained only one leaf rust ASR gene (*Lr19*) that can potentially be overcome, leading to high infection rates.

Adding another leaf rust resistance gene can be considered to further increase the likelihood of achieving durable leaf rust resistance. However, the presence of the *Lr19* gene in combination with the *Lr34/Yr18/Sr57* slow rusting gene complex can already contribute towards durable resistance and lower the risk of severe leaf rust outbreaks should the *Lr19* gene be overcome. For FHB resistance, additional markers can be evaluated to determine the potential presence of other FHB genes/QTL, known to occur in the donor parents, to further differentiate between the best identified lines.

Both phenotypic evaluation and gene expression methods can be used to select the best lines in terms of the lowest disease infection levels as a good correlation was observed between these two methods. However, phenotypic evaluation requires someone with experience in rating lines consistently for their phenotype. Therefore, breeding programmes without plant pathologists or skilled personnel in this field can use the gene expression method to more accurately discriminate between resistant and susceptible lines. All three methods (inoculation methods, gene expression and MAS) have their limitations but when combined, complement one another to overcome limitations and together can assist breeders to make informed decisions on line advancement.

For future recommendations, phenotypic evaluation revealed the presence of a stripe rust ASR gene in line 2S#/163. Further research is needed to determine whether this is a current designated stripe rust resistance gene or not and if not to locate the position of this gene. Furthermore, the *QYr.sgi.2B-1* QTL was absent in the developed lines. Incorporation of both the *QYr.sgi.2B-1* QTL as well as the ASR resistance gene present in line 2S#/163 is important because they can contribute to stripe rust resistance in the developed breeding lines. Additionally, markers developed and published in 2015 linked to the *YrSP* gene should be optimised and used to screen the best identified lines in this study for the presence of the *YrSP* gene. Another major stripe rust resistance QTL was discovered in Kariega that is located on chromosome 4A. Since Kariega was one of the original rust resistant parental cultivars/lines used to develop the populations of this study, flanking markers for this QTL should be used to screen the best lines identified in this study for the potential presence of this QTL. As limited detail about the source(s) of stripe rust resistance is available for the lines developed in this study, the observed resistance to stripe rust should be better characterised and investigated further. Doubled haploid lines should be developed from the top identified lines to ensure the homozygous presence of the respective rust and FHB resistance genes/QTL. The developed DH lines can then be considered for further use in a breeding programme to improve the lines by adding desirable traits of interest.

The top identified lines should contain acceptable dough strength characteristics (based on HMW-GS data). Additional end-use quality tests are required to confirm the dough strength of the lines but also to characterise them further for all the other quality characteristics considered important in our local release criteria. As other traits are also important for cultivar development, the best identified lines should undergo evaluation tests regarding their agronomical characteristics and yield potential. DH lines will enable multiple field trials over different locations and seasons because the genotypes are stable compared to segregating lines. The best lines can also be used to transfer resistance genes/QTL to cultivars currently planted by wheat producers that are agronomically well adapted and high yielding.

Although good correlations were observed between phenotypic evaluations, qPCR analysis and MAS, single plants per entry were evaluated because lines were still segregating, leading to the observed genetic variation. To obtain more accurate data, lines with fixed genotypes should be used and samples could be bulked for MAS and qPCR analysis. Although single segregating plants were used, valuable data and information was obtained by these analyses. Because variation between genotypes with the same genotypic profiles were observed, the best identified lines, with both rust and FHB resistance, can be subjected to further phenotypic evaluation in trials to identify the best lines based on their phenotypic expression as well. A timeline study for the FHB inoculation trials need to be done to determine the best time to harvest sample material for gene expression studies.

Lines with wheat rust and FHB resistance have been successfully developed in this study using different methods to validate resistance within these lines. Although the developed lines are not perfect yet, there are many possibilities to further improve them. Valuable informative information was obtained in this study that can be applied in future studies to improve their effectiveness and consequent outcomes. The developed lines can further be considered for use in pre-breeding programmes of breeding companies to improve the level of resistance to wheat rust and FHB, lowering the risk of future epidemic outbreaks. Resistant cultivars will also help wheat producers to produce wheat more cost effectively and ensure sustainable wheat production in a more environmentally friendly manner.

Appendix A

Molecular marker data generated for the rust resistant female lines

Line name	STSLr19$_{130}$ (*Lr19*)[1]	cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]
KSF_1-159	1	1	1	0.5	0.5
KSF_2-159.1	0	1	1	0.5	0.5
KSF_2-159.3	0	1	1	0	0
KSF_2-159.4	0	1	1	0.5	0
KSF_2-159.5	0	1	1	0.5	0.5
KSF_2-159.6	0	1	1	0.5	0.5
KSF_2-159.7	1	1	1	0	0.5
KSF_2-159.8	0	1	1	0	0.5
KSF_2-159.9	1	1	1	0.5	0.5
KSF_2-159.10	1	1	1	0.5	0
KSF_2-159.11	0	1	0	0	0
KSF_2-159.12	1	1	1	0.5	0
KSF_2-159.13	0	1	1	0.5	0
KSF_2-159.14	1	1	0	0.5	0
KSF_2-159.15	1	1	0	0	0.5
KSF_2-159.16	1	1	0	0.5	0.5
KSF_2-159.17	1	1	1	0.5	0.5
KSF_2-159.18	1	1	1	0.5	0.5
KSF_2-159.19	0	1	1	0.5	1
KSF_2-159.20	1	1	1	0.5	0
KSF_2-159.21	1	1	1	0.5	0.5
KSF_2-159.22	1	1	1	0.5	1
KSF_2-159.23	1	1	1	0	1
KSF_2-159.24	1	1	1	0	1
KSF_2-159.25	1	1	0	0	0
KSF_2-159.26	1	1	0	0.5	0.5
KSF_2-159.27	0	1	1	0	0.5
KSF_2-159.28	0	1	0	0	0.5
KSF_2-159.29	0	1	0	0.5	0.5
KSF_2-159.30	1	1	1	0.5	0
KSF_2-159.31	1	1	1	0.5	1
KSF_2-159.32	1	1	0	0.5	0.5
KSF_2-159.33	1	1	1	0	0.5
KSF_2-159.34	1	1	0	0	0.5
KSF_2-159.35	0	1	0	0.5	0.5
KSF_2-159.36	1	1	1	0.5	0.5
KSF_2-159.37	1	1	1	0	0
KSF_2-159.38	1	1	1	0.5	0
KSF_2-159.39	0	1	0	0	0

Molecular marker data generated for the rust resistant female lines (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]
KSF$_2$-159.40	1	1	0	0	0.5
KSF$_2$-159.41	0	1	1	0.5	0
KSF$_2$-159.42	0	1	1	0.5	0.5
KSF$_2$-159.43	0	1	0	0.5	0
KSF$_2$-159.44	0	1	1	0.5	0.5
KSF$_2$-159.45	0	1	0	0.5	0
KSF$_2$-159.46	1	1	1	0.5	0
KSF$_2$-159.47	0	1	1	0.5	0.5
KSF$_2$-159.48	1	1	1	0.5	0.5
KSF$_2$-159.49	1	1	1	0.5	0
KSF$_2$-159.50	0	1	0	0	0
KSF$_2$-159.51	1	1	0	0	0.5
KSF$_2$-159.52	0	1	1	0	0
KSF$_2$-159.53	0	1	1	0	0.5
KSF$_2$-159.54	1	1	1	1	0.5
KSF$_2$-159.55	1	1	1	0	1
KSF$_2$-159.56	1	1	1	0.5	0.5
KSF$_2$-159.57	0	1	1	0	0.5
KSF$_2$-159.58	0	1	1	0	0.5
KSF$_2$-159.59	0	1	0	0.5	0.5
KSF$_2$-159.60	1	1	0	0.5	0
KSF$_2$-159.61	0	1	1	0	0.5
KSF$_2$-159.62	1	1	1	0.5	0
KSF$_2$-159.63	1	1	0	0.5	1
KSF$_1$-182	1	1	1	0.5	0.5
KSF$_2$-182.1	1	1	1	0	0
KSF$_2$-182.2	1	1	1	0.5	0
KSF$_2$-182.3	1	1	0	0.5	1
KSF$_2$-182.4	1	1	0	0.5	1
KSF$_2$-182.5	1	1	1	0	0.5
KSF$_2$-182.6	1	1	1	0.5	0.5
KSF$_2$-182.7	1	1	1	0	0
KSF$_2$-182.8	1	1	0	0.5	0.5
KSF$_2$-182.9	1	1	1	0.5	0.5
KSF$_2$-182.10	0	1	1	0	0.5
KSF$_2$-182.11	1	1	1	0.5	1
KSF$_2$-182.12	1	1	1	0	0.5
KSF$_2$-182.13	1	1	1	0.5	0
KSF$_2$-182.14	1	1	1	0.5	0.5
KSF$_2$-182.15	1	1	1	0.5	0.5

Molecular marker data generated for the rust resistant female lines (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]
KSF$_2$-182.16	1	1	1	0.5	0.5
KSF$_2$-182.17	1	1	1	0.5	0
KSF$_2$-182.18	1	1	1	0.5	0
KSF$_2$-182.19	1	1	1	0.5	0.5
KSF$_2$-182.20	1	1	1	0.5	0.5
KSF$_2$-182.21	1	1	1	0.5	0.5
KSF$_2$-182.22	1	1	1	0	0.5
KSF$_2$-182.23	0	1	1	0	0.5
KSF$_2$-182.24	0	1	0	0	1
KSF$_2$-182.25	1	1	1	0.5	1
KSF$_2$-182.26	1	1	1	0.5	0.5
KSF$_2$-182.27	0	1	1	0.5	0.5
KSF$_2$-182.28	1	1	1	0.5	0
KSF$_2$-182.29	0	1	0	0.5	0
KSF$_2$-182.30	1	1	1	0.5	0
KSF$_2$-182.31	1	1	1	0	0.5
KSF$_2$-182.32	1	1	0	0	0.5
KSF$_2$-182.33	1	1	1	0	0.5
KSF$_2$-182.34	1	1	1	1	0.5
KSF$_2$-182.35	1	1	1	0	1
KSF2-182.36	1	1	1	0.5	0.5
KSF$_2$-182.37	1	1	0	0.5	1
KSF$_2$-182.38	1	1	0	0	0.5
KSF$_2$-182.39	1	1	0	0.5	1
KSF$_2$-182.40	1	1	1	0.5	1
KSF$_2$-182.41	1	1	1	0.5	0.5
KSF$_2$-182.42	0	1	0	0	0
KSF$_2$-182.43	1	1	1	0.5	0.5
KSF$_2$-182.44	1	1	1	0.5	0
KSF$_2$-182.45	1	1	1	0.5	0
KSF$_2$-182.46	1	1	1	0	0.5
KSF$_2$-182.47	1	1	1	0	0.5
KSF$_2$-182.48	1	1	1	0.5	1
KSF$_2$-182.49	0	1	0	0	0
KSF$_2$-182.50	1	1	0	0.5	0.5
KSF$_2$-182.51	0	1	1	0	0.5
KSF$_2$-182.52	0	1	1	0.5	0.5
KSF$_2$-182.53	1	1	1	0.5	1
KSF$_2$-182.54	0	1	0	0	0.5
KSF$_2$-182.55	0	1	1	0	0

Molecular marker data generated for the rust resistant female lines (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]
KSF$_2$-182.56	0	1	1	0.5	0.5
KSF$_2$-182.57	0	1	1	0	0
KSF$_2$-182.58	1	1	1	0.5	0.5
KSF$_2$-182.59	0	1	1	0.5	0.5
KSF$_2$-182.60	0	1	0	0.5	0.5
KSF$_1$-682	1	0.5	1	0.5	0.5
KSF$_2$-682.1	0	0.5	0	0.5	0
KSF$_2$-682.2	0	0	1	0	0
KSF$_2$-682.3	0	1	1	0	0.5
KSF$_2$-682.4	1	0.5	1	0	0
KSF$_2$-682.5	0	0.5	1	0	0.5
KSF$_2$-682.6	0	0.5	0	0.5	0
KSF$_2$-682.7	1	1	1	0	0.5
KSF$_2$-682.8	1	0.5	1	0.5	0.5
KSF$_2$-682.9	1	0	1	0.5	0.5
KSF$_2$-682.10	1	1	0	0	0.5
KSF$_2$-682.11	1	0	1	0.5	1
KSF$_2$-682.12	1	0.5	1	0.5	0.5
KSF$_2$-682.13	1	0.5	0	0.5	0.5
KSF$_2$-682.14	1	0.5	1	0	0.5
KSF$_2$-682.15	0	1	1	0	0.5
KSF$_2$-682.16	1	1	1	0.5	0.5
KSF$_2$-682.17	0	0.5	1	0.5	0.5
KSF$_2$-682.18	0	1	0	0.5	0
KSF$_2$-682.19	1	0	0	0.5	0.5
KSF$_2$-682.20	0	0	1	0.5	0.5
KSF$_2$-682.21	1	0.5	1	0.5	0.5
KSF$_2$-682.22	1	0.5	1	0	0.5
KSF$_2$-682.23	0	0	1	0.5	0
KSF$_2$-682.24	0	0.5	0	0.5	1
KSF$_2$-682.25	0	0	1	0	1
KSF$_2$-682.26	0	0.5	1	0.5	1
KSF$_2$-682.27	1	1	1	1	0.5
KSF$_2$-682.28	0	1	1	0.5	0.5
KSF$_2$-682.29	0	0.5	1	0.5	0.5
KSF$_2$-682.30	0	0	0	0	0
KSF$_2$-682.31	0	0.5	1	0.5	0.5
KSF$_2$-682.32	1	1	0	0	0.5
KSF$_2$-682.33	1	1	1	0.5	0.5
KSF$_2$-682.34	0	0.5	1	1	0.5

Molecular marker data generated for the rust resistant female lines (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2/Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]
KSF$_2$-682.35	0	0	1	0.5	0
KSF$_2$-682.36	1	1	1	0.5	0.5
KSF$_2$-682.37	0	1	1	0.5	0.5
KSF$_2$-682.38	1	0.5	1	0.5	0
KSF$_2$-682.39	1	1	1	0	0.5
KSF$_2$-682.40	1	0.5	1	0.5	0
KSF$_2$-682.41	0	0	0	0.5	0
KSF$_2$-682.42	1	0.5	1	0.5	0.5
KSF$_2$-682.43	0	1	0	0	0
KSF$_2$-682.44	0	0	1	0	0.5
KSF$_2$-682.45	1	1	0	0.5	0.5
KSF$_2$-682.46	1	1	1	0.5	0.5
KSF$_2$-682.47	1	1	1	0.5	0
KSF$_2$-682.48	1	0.5	1	0	0.5
KSF$_2$-682.49	1	0	0	0.5	0.5
KSF$_2$-682.50	1	1	0	0.5	0.5
KSF$_2$-682.51	1	0.5	1	0.5	0.5
KSF$_2$-682.52	1	0.5	1	0.5	0.5
KSF$_2$-682.53	1	0	1	0.5	1
KSF$_2$-682.54	0	1	0	0	0.5
KSF$_2$-682.55	1	1	1	0.5	0.5
KSF$_2$-682.56	0	1	1	0	0.5
KSF$_2$-682.57	1	1	0	0.5	0.5
KSF$_2$-682.58	0	1	0	0.5	0.5
KSF$_2$-682.59	0	1	0	0.5	0
KSF$_2$-682.60	1	1	0	0.5	0.5
KSF$_2$-682.61	1	1	0	0.5	0
KSF$_2$-682.62	0	1	0	0.5	0

Lines highlighted in grey are the three best selected KSF$_1$ lines used as the parental rust resistant lines in this study. [1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0).

Appendix B

Molecular marker data generated for the Fusarium head blight resistant male lines

Line name	Umn10 *Fhb1*	Gwm156 *Qfhs.ifa-5A-2*	Gwm293 *Qfhs.ifa-5A-1*	Gwm304 *Qfhs.ifa-5A-1*
BC_2F_2-7	0.5[1]	0[3]	0.5	0.5
BC_2F_3-7.1	1[2]	0	0	0
BC_2F_3-7.2	0.5	0	0	0
BC_2F_3-7.3	0.5	0	0.5	0.5
BC_2F_3-7.4	0.5	0	0.5	0.5
BC_2F_3-7.5	1	0	0.5	0.5
BC_2F_3-7.6	0.5	0	0	0
BC_2F_2-20	0.5	0	0.5	0.5
BC_2F_3-20.1	0	0	0	0
BC_2F_3-20.2	0	0	0.5	0.5
BC_2F_3-20.3	0	0	0	0
BC_2F_3-20.4	0.5	0	0	0
BC_2F_3-20.5	0.5	0	0	0
BC_2F_3-20.6	0.5	0	0	0
BC_2F_2-39	0.5	0.5	0.5	0.5
BC_2F_3-39.1	0.5	0	0.5	0.5
BC_2F_3-39.2	0.5	0.5	0.5	0.5
BC_2F_3-39.3	0	1	0	0
BC_2F_3-39.4	0	0	0	0
BC_2F_3-39.5	0.5	0	0	0
BC_2F_3-39.6	0.5	0.5	0.5	0.5
BC_2F_3-39.7	0.5	1	1	1
BC_2F_3-39.8	0.5	1	1	1
BC_2F_3-39.9	0.5	1	0	0
BC_2F_3-39.10	0	1	1	1
BC_2F_3-39.11	0.5	1	1	1
BC_2F_3-39.12	0	0.5	0.5	0.5
BC_2F_6-4.3	0	1	1	1
BC_2F_7-4.3.1	0	1	1	1
BC_2F_7-4.3.2	0	1	1	1
BC_2F_7-4.3.3	0	1	1	1
BC_2F_7-4.3.4	0	1	1	1
BC_2F_7-4.3.5	0	1	1	1
BC_2F_7-4.3.6	0	1	1	1
BC_2F_7-4.3.7	0	1	1	1
BC_2F_7-4.3.8	0	1	1	1
BC_2F_7-4.3.9	0	1	1	1
BC_2F_7-4.3.10	0	1	1	1
BC_2F_7-4.3.11	0	1	1	1
BC_2F_7-4.3.12	0	1	1	1
BC_2F_7-4.3.13	0	1	1	1
BC_2F_7-4.3.14	0	1	1	1

Molecular marker data generated for the Fusarium head blight resistant male lines (continued)

Line name	Umn10 *Fhb1*	Gwm156 *Qfhs.ifa-5A-2*	Gwm293 *Qfhs.ifa-5A-1*	Gwm304 *Qfhs.ifa-5A-1*
BC_2F_7-4.3.15	0	1	1	1
BC_2F_7-4.3.16	0	1	1	1
BC_2F_7-4.3.17	0	1	1	1
BC_2F_7-4.3.18	0	1	1	1
BC_2F_7-4.3.19	0	1	1	1
BC_2F_7-4.3.20	0	1	1	1
BC_2F_7-4.3.21	0	1	1	1
BC_2F_7-4.3.22	0	1	1	1
BC_2F_7-4.3.23	0	1	1	1
BC_2F_7-4.3.24	0	1	1	1
BC_2F_7-4.3.25	0	1	1	1
BC_2F_7-4.3.26	0	1	1	1
BC_2F_7-4.3.27	0	1	1	1
BC_2F_7-4.3.28	0	1	1	1
BC_2F_7-4.3.29	0	1	1	1
BC_2F_7-4.3.30	0	1	1	1
BC_2F_7-4.3.31	0	1	1	1
BC_2F_6-12.3	1	1	1	1
BC_2F_7-12.3.1	1	1	1	1
BC_2F_7-12.3.2	1	1	1	1
BC_2F_7-12.3.3	1	1	1	1
BC_2F_7-12.3.4	1	1	1	1
BC_2F_7-12.3.5	1	1	1	1
BC_2F_7-12.3.6	1	1	1	1
BC_2F_7-12.3.7	1	1	1	1
BC_2F_7-12.3.8	1	1	1	1
BC_2F_7-12.3.9	1	1	1	1
BC_2F_7-12.3.10	1	1	1	1
BC_2F_7-12.3.11	1	1	1	1
BC_2F_7-12.3.12	1	1	1	1
BC_2F_7-12.3.13	1	1	1	1
BC_2F_7-12.3.14	1	1	1	1
BC_2F_7-12.3.15	1	1	1	1
BC_2F_7-12.3.16	1	1	1	1
BC_2F_7-12.3.17	1	1	1	1
BC_2F_7-12.3.18	1	1	1	1
BC_2F_7-12.3.19	1	1	1	1
BC_2F_7-12.3.20	1	1	1	1
BC_2F_7-12.3.21	1	1	1	1
BC_2F_7-12.3.22	1	1	1	1
BC_2F_7-12.3.23	1	1	1	1
BC_2F_7-12.3.24	1	1	1	1
BC_2F_7-12.3.25	1	1	1	1
BC_2F_7-12.3.26	1	1	1	1

Molecular marker data generated for the Fusarium head blight resistant male lines (continued)

Line name	Umn10 *Fhb1*	Gwm156 *Qfhs.ifa-5A-2*	Gwm293 *Qfhs.ifa-5A-1*	Gwm304 *Qfhs.ifa-5A-1*
BC_2F_7-12.3.27	1	1	1	1
BC_2F_7-12.3.28	1	1	1	1
BC_2F_7-12.3.29	1	1	1	1
BC_2F_7-12.3.30	1	1	1	1
BC_2F_7-12.3.31	1	1	1	1
BC_2F_7-12.3.32	1	1	1	1
BC_2F_7-12.3.33	1	1	1	1
BC_2F_7-12.3.34	1	1	1	1
BC_2F_7-12.3.35	1	1	1	1
BC_2F_6-10.2	1	1	1	1
BC_2F_7-10.2.1	1	1	1	1
BC_2F_7-10.2.2	1	1	1	1
BC_2F_7-10.2.3	1	1	1	1
BC_2F_7-10.2.4	1	1	1	1
BC_2F_7-10.2.5	1	1	1	1
BC_2F_7-10.2.6	1	1	1	1
BC_2F_7-10.2.7	1	1	1	1
BC_2F_7-10.2.8	1	1	1	1
BC_2F_7-10.2.9	1	1	1	1
BC_2F_7-10.2.10	1	1	1	1
BC_2F_7-10.2.11	1	1	1	1
BC_2F_7-10.2.12	1	1	1	1
BC_2F_7-10.2.13	1	1	1	1
BC_2F_7-10.2.14	1	1	1	1
BC_2F_7-10.2.15	1	1	1	1
BC_2F_7-10.2.16	1	1	1	1
BC_2F_7-10.2.17	1	1	1	1
BC_2F_7-10.2.18	1	1	1	1
BC_2F_7-10.2.19	1	1	1	1
BC_2F_7-10.2.20	1	1	1	1
BC_2F_7-10.2.21	1	1	1	1
BC_2F_7-10.2.22	1	1	1	1
BC_2F_7-10.2.23	1	1	1	1
BC_2F_7-10.2.24	1	1	1	1
BC_2F_7-10.2.25	1	1	1	1
BC_2F_7-10.2.26	1	1	1	1
BC_2F_7-10.2.27	1	1	1	1
BC_2F_7-10.2.28	1	1	1	1
BC_2F_7-10.2.29	1	1	1	1
BC_2F_7-10.2.30	1	1	1	1
BC_2F_7-10.2.31	1	1	1	1
BC_2F_7-10.2.32	1	1	1	1
BC_2F_7-10.2.33	1	1	1	1
BC_2F_7-10.2.34	1	1	1	1

Molecular marker data generated for the Fusarium head blight resistant male lines (continued)

Line name	Umn10 *Fhb1*	Gwm156 *Qfhs.ifa-5A-2*	Gwm293 *Qfhs.ifa-5A-1*	Gwm304 *Qfhs.ifa-5A-1*
BC_2F_7-10.2.35	1	1	1	1
BC_2F_6-37.1	1	1	1	1
BC_2F_7-37.1.1	1	1	1	1
BC_2F_7-37.1.2	1	1	1	1
BC_2F_7-37.1.3	1	1	1	1
BC_2F_7-37.1.4	1	1	1	1
BC_2F_7-37.1.5	1	1	1	1
BC_2F_7-37.1.6	1	1	1	1
BC_2F_7-37.1.7	1	1	1	1
BC_2F_7-37.1.8	1	1	1	1
BC_2F_7-37.1.9	1	1	1	1
BC_2F_7-37.1.10	1	1	1	1
BC_2F_7-21.1	0	1	1	1
BC_2F_7-21.1.1	0	1	1	1
BC_2F_7-21.1.2	0	1	1	1
BC_2F_7-21.1.3	0	1	1	1
BC_2F_7-21.1.4	0	1	1	1
BC_2F_7-21.1.5	0	1	1	1
BC_2F_7-21.1.6	0	1	1	1
BC_2F_7-21.1.7	0	1	1	1
BC_2F_7-21.1.8	0	1	1	1
BC_2F_7-21.1.9	0	1	1	1
BC_2F_7-21.1.10	0	1	1	1
BC_2F_7-21.1.11	0	1	1	1
BC_2F_7-21.1.12	0	1	1	1
BC_2F_7-21.1.13	0	1	1	1
BC_2F_7-21.1.14	0	1	1	1
BC_2F_7-21.1.15	0	1	1	1
BC_2F_6-6.3	0	1	1	1
BC_2F_7-6.3.1	0	1	1	1
BC_2F_7-6.3.2	0	1	1	1
BC_2F_7-6.3.3	0	1	1	1
BC_2F_7-6.3.4	0	1	1	1
BC_2F_7-6.3.5	0	1	1	1
BC_2F_7-6.3.6	0	1	1	1
BC_2F_7-6.3.7	0	1	1	1
BC_2F_7-6.3.8	0	1	1	1
BC_2F_7-6.3.9	0	1	1	1
BC_2F_7-6.3.10	0	1	1	1
BC_2F_7-6.3.11	0	1	1	1
BC_2F_7-6.3.12	0	1	1	1
BC_2F_7-6.3.13	0	1	1	1
BC_2F_7-6.3.14	0	1	1	1
BC_2F_7-6.3.15	0	1	1	1

Molecular marker data generated for the Fusarium head blight resistant male lines (continued)

Line name	Umn10 *Fhb1*	Gwm156 *Qfhs.ifa-5A-2*	Gwm293 *Qfhs.ifa-5A-1*	Gwm304 *Qfhs.ifa-5A-1*
BC_2F_7-6.3.16	0	1	1	1
BC_2F_7-6.3.17	0	1	1	1
BC_2F_7-6.3.18	0	1	1	1
BC_2F_7-6.3.19	0	1	1	1
BC_2F_7-6.3.20	0	1	1	1
BC_2F_7-6.3.21	0	1	1	1
BC_2F_7-6.3.22	0	1	1	1
BC_2F_7-6.3.23	0	1	1	1
BC_2F_7-6.3.24	0	1	1	1
BC_2F_7-6.3.25	0	1	1	1
BC_2F_7-6.3.26	0	1	1	1
BC_2F_7-6.3.27	0	1	1	1
BC_2F_7-6.3.28	0	1	1	1
BC_2F_7-6.3.29	0	1	1	1
BC_2F_7-6.3.30	0	1	1	1
BC_2F_7-6.3.31	0	1	1	1
BC_2F_7-6.3.32	0	1	1	1
BC_2F_7-6.3.33	0	1	1	1
BC_2F_7-6.3.34	0	1	1	1
BC_2F_7-6.3.35	0	1	1	1

FHB-Fusarium head blight; Lines highlighted in grey are the three BC_2F_2 lines and seven BC_2F_6 lines selected as FHB resistant male plants; [1]Heterozygous gene/quantitative trait loci (QTL); [2]Homozygous resistant gene/QTL; [3]Homozygous susceptible gene/QTL.

Appendix C

Molecular marker data generated for the FMF_1 population

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
KSF_2-682.12 BC_2F_2-39.11	1	0.5	1	0.5	0.5	0.5	1	1	1
FMF_1-1.1	1	0	1	0	0	0	0.5	0.5	0.5
FMF_1-1.2	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-1.3	0	0	0	0	0	0.5	0.5	0.5	0.5
KSF_2-682.16 BC_2F_2-39.11	1	1	1	0.5	0.5	0.5	1	1	1
FMF_1-2.1	1	0.5	0	0	0	0	0.5	0.5	0.5
KSF_2-682.52 BC_2F_2-39.11	1	0.5	1	0.5	0.5	0.5	1	1	1
FMF_1-3.1	1	0	1	0	0	0	0.5	0.5	0.5
FMF_1-3.2	1	0	1	0	0	0.5	0.5	0.5	0.5
FMF_1-3.3	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-3.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-3.5	1	0.5	0	0.5	0	0	0.5	0.5	0.5
FMF_1-3.6	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-3.7	1	0	0	0	0	0	0.5	0.5	0.5
FMF_1-3.8	1	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-3.9	1	0	0	0.5	0	0	0.5	0.5	0.5
FMF_1-3.10	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-3.11	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-3.12	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-3.13	1	0	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-3.14	1	0	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-3.15	1	0	1	0	0	0	0.5	0.5	0.5
FMF_1-3.16	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-3.17	1	0	1	0	0	0	0.5	0.5	0.5
FMF_1-3.18	1	0	1	0	0	0	0.5	0.5	0.5
FMF_1-3.19	1	0.5	0	0	0.5	0	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-3.20	1	0	0	0	0	0.5	0.5	0.5	0.5
KSF_2-682.27 BC_2F_2-39.12	1	1	1	1	0.5	0.5	1	0.5	0.5
FMF_1-4.1	1	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_1-4.2	1	0.5	1	0.5	0.5	0	0.5	0	0
FMF_1-4.3	1	0.5	1	0.5	0.5	0	0.5	0	0
FMF_1-4.4	1	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_1-4.5	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-4.6	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
KSF_2-682.42 BC_2F_2-39.7	1	0.5	1	0.5	1	0.5	1	1	1
FMF_1-5.1	1	0.5	1	0	0.5	0	0.5	0.5	0.5
FMF_1-5.2	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-5.3	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-5.4	0	0	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-5.5	0	0	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-5.6	0	0.5	0	0	0.5	0	0.5	0.5	0.5
KSF_2-182.40 BC_2F_2-39.7	1	1	1	0.5	0.5	0.5	1	1	1
FMF_1-6.1	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-6.2	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-6.3	1	0.5	1	0	0.5	0	0.5	0.5	0.5
FMF_1-6.4	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-6.5	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-6.6	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-6.7	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-6.8	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-6.9	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-6.10	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-6.11	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-6.12	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-6.13	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-6.14	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-6.15	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-6.16	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-6.17	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-6.18	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-159.31	1	1	1	0.5	1				
BC_2F_2-39.7						0.5	1	1	1
FMF_1-7.1	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-7.2	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-7.3	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-7.4	0	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-7.5	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-7.6	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-7.7	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-7.8	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-7.9	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-7.10	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-7.11	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-7.12	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-7.13	0	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-7.14	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-7.15	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-7.16	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-7.17	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-7.18	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-7.19	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-682.46	1	1	1	0.5	0.5				
BC_2F_2-39.7						**0.5**	**1**	**1**	**1**
FMF_1-8.1	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-8.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-8.3	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-8.4	1	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-8.5	1	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-8.6	1	0.5	1	0	0	0	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-8.7	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-8.8	1	0.5	1	0	0	0.5	0.5	0.5	0.5
KSF_2-182.31	1	1	1	0	0.5				
BC_2F_2-39.10						**0**	**1**	**1**	**1**
FMF_1-9.1	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.2	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.3	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.4	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.5	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.6	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.7	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.8	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.9	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.10	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.11	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.12	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-9.13	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-9.14	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-9.15	1	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-9.16	1	0.5	0	0	0	0	0.5	0.5	0.5
KSF_2-182.34	1	1	1	1	0.5				
BC_2F_2-39.10						0	1	1	1
FMF_1-10.1	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-10.2	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-10.3	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-10.4	0	0.5	0	0.5	0.5	0	0.5	0.5	0.5
KSF_2-159.17	1	1	1	0.5	0.5				
BC_2F_2-39.10						0	1	1	1
FMF_1-11.1	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-11.2	0	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-11.3	0	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_1-11.5	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-11.6	0	0.5	0	0.5	0	0	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-11.7	0	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-11.8	0	0.5	0	0	0	0	0.5	0.5	0.5
KSF_2-682.32 BC_2F_2-39.8	1	1	0	0	0.5	0.5	1	1	1
FMF_1-12.1	1	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-12.2	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-12.3	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-682.42 BC_2F_2-39.6	1	0.5	1	0.5	1	0.5	1	0.5	0.5
FMF_1-13.1	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-13.2	0	0	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-13.3	0	0.5	1	0.5	0.5	0.5	0.5	0	0
FMF_1-13.4	0	0	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-13.5	0	0	0	0.5	0.5	0.5	0.5	0	0
FMF_1-13.6	0	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-13.7	0	0.5	0	0.5	0.5	0.5	0.5	0	0
FMF_1-13.8	0	0	0	0	0.5	0	0.5	0	0
FMF_1-13.9	0	0.5	0	0	0.5	0.5	0.5	0	0
FMF_1-13.10	0	0.5	1	0	0.5	0.5	0.5	0	0
FMF_1-13.11	0	0	0	0.5	0.5	0.5	0.5	0	0
FMF_1-13.12	0	0	0	0	0.5	0	0.5	0	0
FMF_1-13.13	0	0	0	0	0.5	0	0.5	0.5	0.5
KSF_2-682.32 BC_2F_2-39.6	1	1	0	0	0.5	0.5	1	0.5	0.5
FMF_1-14.1	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-14.2	0	0.5	0	0	0	0	0.5	0	0.5
FMF_1-14.3	0	0.5	1	0	0	0	0.5	0.5	0.5
KSF_2-159.18 BC_2F_2-39.11	1	1	1	0.5	0.5	0.5	1	1	1
FMF_1-15.1	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-15.2	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-15.3	0	0.5	1	0	0	0	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
KSF_2-159.48 BC_2F_2-39.11	1	1	1	0.5	0.5	0.5	1	1	1
FMF_1-16.1	0	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-16.2	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-16.3	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-16.4	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-16.5	0	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-16.6	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-16.7	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-16.8	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-16.9	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-16.10	1	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-16.11	1	0.5	1	0	0	0	0.5	0.5	0.5
KSF_2-182.48 BC_2F_2-39.11	1	1	1	0.5	1	0.5	1	1	1
FMF_1-17.1	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-17.2	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-17.3	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-17.4	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-17.5	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-17.6	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-17.7	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-17.8	0	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-17.9	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-17.10	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-17.11	0	0.5	1	0	0.5	0	0.5	0.5	0.5
FMF_1-17.12	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-17.13	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-17.14	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-17.15	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.26 BC_2F_2-39.9	1	1	1	0.5	0.5	0.5	1	0	0
FMF_1-20.1	0	0.5	0	0	0	0.5	0.5	0	0

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-20.2	0	0.5	1	0	0	0.5	0.5	0	0
FMF_1-20.3	0	0.5	0	0	0.5	0.5	0.5	0	0
FMF_1-20.4	0	0.5	0	0	0.5	0.5	0.5	0	0
FMF_1-20.5	0	0.5	0	0.5	0.5	0.5	0.5	0	0
FMF_1-20.6	0	0.5	1	0	0	0.5	0.5	0	0
FMF_1-20.7	0	0.5	1	0	0.5	0	0.5	0	0
FMF_1-20.8	1	0.5	0	0	0.5	0	0.5	0	0
FMF_1-20.9	1	0.5	0	0	0.5	0.5	0.5	0	0
FMF_1-20.10	1	0.5	1	0	0	0.5	0.5	0	0
FMF_1-20.11	1	0.5	0	0	0.5	0	0.5	0	0
FMF_1-20.12	0	0.5	0	0	0	0	0.5	0	0
FMF_1-20.13	0	0.5	0	0	0	0	0.5	0	0
FMF_1-20.14	0	0.5	0	0	0	0	0.5	0	0
KSF_2-159.37	1	1	1	0	0				
BC_2F_2-39.12						0.5	1	0.5	0.5
FMF_1-22.1	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-22.2	0	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-22.3	0	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-22.4	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-22.5	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-22.6	0	0.5	0	0	0	0	0.5	0	0
FMF_1-22.7	1	0.5	1	0	0	0	0.5	0	0
FMF_1-22.8	0	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-22.9	1	0.5	1	0	0	0.5	0.5	0	0
FMF_1-22.10	1	0.5	1	0	0	0.5	0.5	0.5	0.5
KSF_2-159.55	1	1	1	0	0.5				
BC_2F_2-39.12						0.5	1	0.5	0.5
FMF_1-23.1	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-23.2	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-23.3	0	0.5	0	0	0	0.5	0.5	0	0
FMF_1-23.4	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-23.5	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-23.6	0	0.5	1	0	0	0.5	0.5	0	0

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-23.7	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-23.8	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-23.9	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-23.10	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-23.11	1	0.5	1	0	0	0.5	0.5	0	0
FMF_1-23.12	1	0.5	0	0	0	0.5	0.5	0	0
FMF_1-23.13	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-23.14	0	0.5	0	0	0.5	0.5	0.5	0	0
FMF_1-23.15	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-23.16	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.58 BC_2F_2-39.12	1	1	1	0.5	0.5	0.5	1	0.5	0.5
FMF_1-24.1	1	0.5	1	0.5	0	0	0.5	0	0
FMF_1-24.2	1	0.5	1	0.5	0	0	0.5	0	0
FMF_1-24.3	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-24.4	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-24.5	1	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-24.6	1	0.5	0	0	0	0	0.5	0	0
FMF_1-24.7	1	0.5	1	0.5	0	0	0.5	0	0
KSF_2-159.21 BC_2F_6-37.1.2	1	1	1	0.5	0.5	1	1	1	1
FMF_1-27.1	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-27.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-27.3	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-27.5	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-27.6	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
KSF_2-159.47 BC_2F_2-39.9	0	1	1	0.5	0.5	0.5	1	0	0
FMF_1-29.1	0	0.5	1	0	0	0	0.5	0	0
FMF_1-29.2	0	0.5	1	0	0.5	0.5	0.5	0	0
FMF_1-29.3	0	0.5	1	0	0.5	0.5	0.5	0	0
FMF_1-29.4	0	0.5	0	0	0.5	0	0.5	0	0

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
KSF_2-682.39 BC_2F_6-37.1.2	1	1	1	0	0.5	1	1	1	1
FMF_1-30.1	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-30.2	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-30.3	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-30.4	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-30.5	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-30.6	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-30.7	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-30.8	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-30.9	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-30.10	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-30.11	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-30.12	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-30.13	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-30.14	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-30.15	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-30.16	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-30.17	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-30.18	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-30.19	1	0.5	1	0	0	0.5	0.5	0.5	0.5
KSF_2-682.39 BC_2F_6-37.1.2	1	1	1	0	0.5	1	1	1	1
FMF_1-35.1	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-35.2	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-35.3	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.21 BC_2F_6-37.1.3	1	1	1	0.5	0.5	1	1	1	1
FMF_1-36.1	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-36.2	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-36.3	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-36.4	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-36.5	1	0.5	1	0	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-36.6	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-36.7	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-36.8	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-36.9	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-36.10	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.43 BC_2F_2-39.10	1	1	1	0.5	0.5	0	1	1	1
FMF_1-37.1	1	0.5	1	0	0.5	0	0.5	0.5	0.5
FMF_1-37.2	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-37.3	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-37.4	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-37.5	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-37.6	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-37.7	1	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_1-37.8	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-37.9	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-37.10	1	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_1-37.11	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-37.12	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-37.13	0	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-37.14	0	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-37.15	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-37.16	1	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_1-37.17	1	0.5	1	0	0.5	0	0.5	0.5	0.5
KSF_2-159.36 BC_2F_6-37.1.3	1	1	1	0.5	0.5	1	1	1	1
FMF_1-39.1	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-39.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-39.3	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-159.36 BC_2F_6-12.3.5	1	1	1	0.5	0.5	1	1	1	1
FMF_1-40.1	1	0.5	1	0	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-40.2	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-40.3	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-40.4	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-40.5	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-40.6	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-40.7	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-40.8	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-40.9	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
KSF_2-682.42 BC_2F_6-37.1.8	1	0.5	1	0.5	1	1	1	1	1
FMF_1-41.1	0	0	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-41.2	0	0	1	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.20 BC_2F_6-37.1.8	1	1	1	0.5	0.5	1	1	1	1
FMF_1-42.1	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-42.2	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-42.3	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.15 BC_2F_6-37.1.4	1	1	1	0.5	1	1	1	1	1
FMF_1-44.1	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-44.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-44.3	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-44.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-682.36 BC_2F_6-37.1.4	1	1	1	0.5	0.5	1	1	1	1
FMF_1-45.1	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-45.2	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-45.3	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-45.4	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-45.5	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-45.6	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-45.7	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-45.8	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-45.9	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-45.10	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-45.11	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-45.12	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-45.13	1	0.5	1	0	0	0.5	0.5	0.5	0.5
KSF_2-182.31 BC_2F_6-37.1.4	1	1	1	0	0.5	1	1	1	1
FMF_1-46.1	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-46.2	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-46.3	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-46.4	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-46.5	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-46.6	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-46.7	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-46.8	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-46.9	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-46.10	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-46.11	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-682.26 BC_2F_2-39.8	0	0.5	1	0.5	1	0.5	1	1	1
FMF_1-49.2	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-49.3	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-49.4	0	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-49.5	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-49.6	0	0	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-159.26 BC_2F_2-39.8	1	1	0	0.5	0.5	0.5	1	1	1
FMF_1-51.1	1	0.5	0	0.5	0	0	0.5	0.5	0.5
FMF_1-51.2	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-51.3	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-51.4	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-51.5	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-51.6	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-51.7	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-51.8	0	0.5	0	0.5	0	0	0.5	0.5	0.5
FMF_1-51.9	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-51.10	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-51.11	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-51.12	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-51.13	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-51.14	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-51.15	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-51.16	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-51.17	1	0.5	0	0.5	0	0	0.5	0.5	0.5
FMF_1-51.18	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-51.19	1	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-51.20	1	0.5	0	0	0	0	0.5	0.5	0.5
KSF_2-159.30	1	1	1	0.5	0				
BC_2F_2-39.8						0.5	1	1	1
FMF_1-52.1	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-52.2	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-52.3	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-52.4	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_1-52.5	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-52.6	0	0.5	1	0.5	0	0	0.5	0.5	0.5
KSF_2-159.24	1	1	1	0	0.5				
BC_2F_6-37.1.7						1	1	1	1
FMF_1-54.1	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-54.2	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-54.3	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-54.4	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-159.22	1	1	1	0.5	1				
BC_2F_6-37.1.7						1	1	1	1
FMF_1-55.1	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-55.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
KSF_2-159.23	1	1	1	0	1				
BC_2F_2-39.11						0.5	1	1	1
FMF_1-56.1	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-56.2	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-56.3	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-56.4	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-56.5	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-56.6	1	0.5	1	0	0.5	0	0.5	0.5	0.5
FMF_1-56.7	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-56.8	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-56.9	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-56.10	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-56.11	1	0.5	0	0	0.5	0	0.5	0.5	0.5
KSF_2-159.54	1	1	1	1	0.5				
BC_2F_6-37.1.9						1	1	1	1
FMF_1-60.1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.2	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-60.3	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-60.4	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-60.5	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-60.6	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-60.7	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.8	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-60.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.10	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-60.12	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
KSF_2-682.36	1	1	1	0.5	0.5				
BC_2F_6-37.1.9						1	1	1	1
FMF_1-63.1	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-63.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-63.3	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-63.4	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-63.5	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-63.6	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-63.7	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-63.8	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-63.9	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
KSF_2-159.56 BC_2F_6-12.3.17	1	1	1	0.5	1	1	1	1	1
FMF_1-65.1	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.3	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.4	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.5	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-65.6	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.7	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.8	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-65.9	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.10	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.11	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.12	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-65.13	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.14	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.15	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-65.16	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-65.17	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.53 BC_2F_6-12.3.17	1	1	1	0.5	1	1	1	1	1
FMF_1-67.1	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-67.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-67.3	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-67.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-67.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-67.6	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-67.7	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-67.8	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-67.9	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-67.10	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-67.11	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-67.12	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-67.13	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-67.14	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-67.15	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-67.16	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.36 BC_2F_6-12.3.16	1	1	1	0.5	0.5	1	1	1	1
FMF_1-70.1	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-70.2	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-70.3	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-70.4	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-70.5	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-70.6	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-70.7	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-70.8	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-70.9	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-70.10	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-70.11	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-70.12	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-70.13	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-70.14	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-70.15	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-70.16	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-70.17	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-70.18	1	0.5	0	0	0	0.5	0.5	0.5	0.5
KSF_2-159.42 BC_2F_6-12.3.3	0	1	1	0.5	0.5	1	1	1	1
FMF_1-71.1	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-71.2	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-71.3	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-71.4	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-71.5	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-71.6	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-71.7	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-71.8	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-71.9	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-71.10	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-71.11	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-71.12	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-71.13	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.37	1	1	0	0.5	1				
BC_2F_6-12.3.16						1	1	1	1
FMF_1-72.1	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-72.2	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-72.3	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-72.4	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-72.5	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-72.6	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-72.7	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-72.8	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-72.9	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-72.10	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-72.11	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-72.12	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-682.34	0	0.5	1	1	0.5				
BC_2F_6-12.3.3						1	1	1	1
FMF_1-73.1	0	0	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-73.2	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-73.3	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-73.4	0	0	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-73.5	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-73.6	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-73.7	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-73.8	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-73.9	0	0	1	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.41 BC_2F_6-12.3.33	1	1	1	0.5	0.5	1	1	1	1
FMF_1-76.1	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-76.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-76.3	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-76.4	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-76.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-76.6	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-76.7	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-76.8	1	0.5	1	0	0	0.5	0.5	0.5	0.5
KSF_2-182.43 BC_2F_6-12.3.33	1	1	1	0.5	0.5	1	1	1	1
FMF_1-77.1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-77.2	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-77.3	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-77.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-77.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-77.6	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-77.7	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-77.8	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-77.9	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-77.10	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-77.11	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-77.12	1	0.5	1	0	0	0.5	0.5	0.5	0.5
KSF_2-682.51 BC_2F_6-12.3.33	1	0.5	1	0.5	0.5	1	1	1	1
FMF_1-78.1	1	0	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-78.2	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-78.3	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-78.4	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-78.5	1	0	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-78.6	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.26 BC_2F_6-12.3.34	1	1	1	0.5	0.5	1	1	1	1
FMF_1-79.1	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-79.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-79.3	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-79.4	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-79.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-79.6	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-79.7	0	0.5	1	0	0	0.5	0.5	0.5	0.5
KSF_2-682.37 BC_2F_6-12.3.4	0	1	1	0.5	0.5	1	1	1	1
FMF_1-80.1	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-80.2	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-80.3	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-80.4	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-80.5	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-80.6	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-80.7	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-80.8	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-80.9	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-80.10	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-80.11	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-80.12	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-80.13	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-80.14	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-682.46 BC_2F_6-12.3.4	1	1	1	0.5	0.5	1	1	1	1
FMF_1-81.1	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-81.2	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-81.3	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-81.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-81.5	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-81.6	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-81.7	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-81.8	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-81.9	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-81.10	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-81.11	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-81.12	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-81.13	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-81.14	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-81.15	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-81.16	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.32 BC_2F_6-12.3.24	1	1	0	0	0.5	1	1	1	1
FMF_1-83.1	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-83.2	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-83.3	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-83.4	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-83.5	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-83.6	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-83.7	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-83.8	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-83.9	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-83.10	1	0.5	0	0	0	0.5	0.5	0.5	0.5
KSF_2-159.55 BC_2F_6-12.3.35	1	1	1	0	0.5	1	1	1	1
FMF_1-86.1	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.2	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-86.3	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-86.4	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.5	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.6	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-86.8	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-86.9	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.10	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-86.11	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-86.12	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.13	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.14	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-86.15	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.16	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.17	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.18	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-86.19	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.40 BC_2F_6-12.3.35	1	1	1	0.5	0.5	1	1	1	1
FMF_1-87.1	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-87.2	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-87.3	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-87.4	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-87.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-87.6	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-87.7	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
KSF_2-182.39 BC_2F_6-12.3.13	1	1	0	0.5	1	1	1	1	1
FMF_1-88.1	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-88.2	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-88.3	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-88.4	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-88.5	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-88.6	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-88.7	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-88.8	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-88.9	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-88.10	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-88.11	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-88.12	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-88.13	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-88.14	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-88.15	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.52 BC_2F_6-12.3.13	0	1	1	0.5	0.5	1	1	1	1
FMF_1-89.1	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-89.2	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-89.3	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-89.4	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-89.5	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-89.6	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-89.7	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-89.8	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-89.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-89.10	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-89.11	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-89.12	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-89.13	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-89.14	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-89.15	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-89.16	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-89.17	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-89.18	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-89.19	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-89.20	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-89.21	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-89.22	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-89.23	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
KSF_2-182.39 BC_2F_6-12.3.14	1	1	0	0.5	1	1	1	1	1
FMF_1-92.1	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-92.2	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-92.3	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-92.4	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-92.5	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-159.23	1	1	1	0	1				
BC_2F_6-12.3.21						1	1	1	1
FMF_1-93.1	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.2	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.3	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.4	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.5	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.7	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.8	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.9	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.10	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.11	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.12	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.13	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.14	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.15	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-93.16	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.48	1	1	1	0.5	1				
BC_2F_6-12.3.30						1	1	1	1
FMF_1-97.1	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-97.2	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-97.3	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-97.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-97.5	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-97.7	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-97.8	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.36	1	1	1	0.5	0.5				
BC_2F_6-12.3.12						1	1	1	1
FMF_1-98.1	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-98.2	0	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-98.3	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-98.4	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-98.5	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-98.6	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-98.7	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-98.9	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-98.10	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-98.11	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-98.12	0	0.5	0	0	0	0.5	0.5	0.5	0.5
KSF_2-182.25 BC_2F_6-12.3.30	1	1	1	0.5	1	1	1	1	1
FMF_1-99.2	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-99.3	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-99.4	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-99.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-99.6	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.25 BC_2F_6-12.3.24	1	1	1	0.5	1	1	1	1	1
FMF_1-100.1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.3	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.4	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.6	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.7	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.8	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.10	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.11	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.12	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.14	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.15	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-100.16	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-100.17	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.18	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-100.19	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.39	1	1	0	0.5	1				
BC_2F_6-12.3.22						1	1	1	1
FMF_1-101.1	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-101.2	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-101.3	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-101.4	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-101.5	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-101.6	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-101.7	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-101.8	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-101.9	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-101.10	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-101.11	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-101.12	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.31	1	1	1	0	0.5				
BC_2F_6-12.3.21						1	1	1	1
FMF_1-102.1	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-102.2	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-102.3	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-102.4	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-102.5	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-102.6	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-102.7	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-102.9	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-102.10	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-102.11	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-102.12	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-102.13	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-102.14	0	0.5	1	0	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-102.15	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-102.16	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-159.63 BC_2F_6-37.1.10	1	1	0	0.5	1	1	1	1	1
FMF_1-105.1	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-105.2	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-105.3	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-105.4	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-105.5	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-105.6	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-182.34 BC_2F_6-12.3.23	1	1	1	1	0.5	1	1	1	1
FMF_1-106.1	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-106.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-106.3	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-106.4	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-106.5	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-106.6	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-106.7	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-106.8	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-106.9	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
KSF_2-182.35 BC_2F_6-12.3.32	1	1	1	0	1	1	1	1	1
FMF_1-109.1	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-109.2	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-109.3	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-109.4	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-109.5	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-682.21 BC_2F_6-12.3.19	1	0.5	1	0.5	0.5	1	1	1	1
FMF_1-110.1	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-110.2	0	0.5	1	0	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
KSF_2-159.48 BC_2F_6-12.3.11	1	1	1	0.5	0.5	1	1	1	1
FMF_1-111.1	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-111.2	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-111.3	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-111.4	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-682.51 BC_2F_6-12.3.27	1	0.5	1	0.5	0.5	1	1	1	1
FMF_1-112.1	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-112.2	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-112.3	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-112.4	1	0	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-112.5	1	0	1	0	0	0.5	0.5	0.5	0.5
FMF_1-112.6	1	0	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-112.7	1	0	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-112.8	1	0	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-112.9	0	0	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-112.10	1	0	1	0	0	0.5	0.5	0.5	0.5
KSF_2-682.37 BC_2F_6-6.3.31	0	0.5	1	0.5	0.5	0	1	1	1
FMF_1-113.1	0	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_1-113.2	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-113.3	0	0.5	0	0	0	0	0.5	0.5	0.5
FMF_1-113.4	0	0	0	0.5	0	0	0.5	0.5	0.5
FMF_1-113.5	0	0	1	0	0.5	0	0.5	0.5	0.5
FMF_1-113.6	0	0	0	0	0	0	0.5	0.5	0.5
FMF_1-113.8	0	0.5	0	0	0.5	0	0.5	0.5	0.5
FMF_1-113.9	0	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_1-113.10	0	0.5	0	0.5	0	0	0.5	0.5	0.5
FMF_1-113.11	0	0	0	0	0.5	0	0.5	0.5	0.5
FMF_1-113.12	0	0	0	0	0.5	0	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
KSF_2-159.54	1	1	1	0.5	0.5				
BC_2F_6-6.3.1						0	1	1	1
FMF_1-119.1	0	0.5	0	0	0.5	0	0.5	0.5	0.5
KSF_2-182.21	1	1	1	0.5	0.5				
BC_2F_6-12.3.1						1	1	1	1
FMF_1-120.1	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-120.2	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-120.3	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-120.5	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-120.6	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-120.8	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-120.9	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-120.10	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-682.16	1	1	1	0.5	0.5				
BC_2F_6-12.3.8						1	1	1	1
FMF_1-121.1	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-121.2	1	0.5	0	0	0	0.5	0.5	0.5	0.5
KSF_2-682.16	1	1	1	0.5	0.5				
BC_2F_6-12.3.8						1	1	1	1
FMF_1-122.1	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-159.31	1	1	1	0.5	1				
BC_2F_6-12.3.8						1	1	1	1
FMF_1-123.1	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-123.2	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
KSF_2-682.51	1	0.5	1	0.5	0.5				
BC_2F_6-12.3.28						1	1	1	1
FMF_1-124.1	1	0	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-124.2	1	0	1	0	0	0.5	0.5	0.5	0.5
FMF_1-124.3	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-124.4	1	0	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-124.5	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-124.6	1	0	1	0	0	0.5	0.5	0.5	0.5
FMF_1-124.7	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-124.8	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_1-124.9	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-124.10	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
KSF_2-682.16	1	1	1	0.5	0.5				
BC_2F_6-12.3.8						1	1	1	1
FMF_1-125.2	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-125.5	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_1-125.9	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
KSF_2-159.31	1	1	1	0.5	1				
BC_2F_6-12.3.8						1	1	1	1
FMF_1-126.1	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-126.2	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-126.3	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
KSF_2-182.16	1	1	1	0.5	0.5				
BC_2F_6-12.3.8						1	1	1	1
FMF_1-127.1	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-127.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-127.5	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-127.6	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5

FMF_1-First generation lines of the crosses made between the female (KSF_2 rust resistant) and male (BC_2F_2/BC_2F_6 FHB resistant) wheat lines highlighted in grey. [1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0).

Appendix D

Molecular marker data generated for the DCF_1 population

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-16.4	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-11.6	0	0.5	0	0.5	0	0	0.5	0.5	0.5
DCF_1-1.1	0	0	0	0.5	0	0	0.5	0.5	0.5
DCF_1-1.2	0	0.5	1	0	0	0	0.5	0.5	0.5
DCF_1-1.3	0	0.5	1	0	0	0	1	0.5	0.5
DCF_1-1.5	0	0.5	0	0.5	0.5	0	0.5	0.5	0.5
DCF_1-1.7	0	1	0	0	0.5	0	0.5	0.5	0.5
DCF_1-1.9	0	1	0	0	0.5	0	0.5	0	0
DCF_1-1.10	0	1	0	0	0	0	0.5	1	1
DCF_1-1.11	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-1.12	0	0	0	0	0	0.5	0.5	0	0
DCF_1-1.13	0	0.5	1	0.5	0	0	0.5	1	1
DCF_1-1.14	0	0.5	0	0	0.5	0	0.5	0.5	0.5
DCF_1-1.15	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
FMF_1-17.4	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-60.12	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-7.2	1	0.5	1	0	0	0	0.5	0.5	0.5
DCF_1-7.3	0	1	1	0.5	0.5	0	0.5	1	1
DCF_1-7.4	0	0	0	0	0	0	0.5	0	0
DCF_1-7.5	0	1	0	0.5	0	0	0.5	0.5	0.5
DCF_1-7.6	1	0.5	1	0	0	0	0.5	1	1
DCF_1-7.7	0	0.5	1	0.5	0	0	0.5	0.5	0.5
DCF_1-7.8	0	0	1	0.5	0	0	0.5	1	1
DCF_1-7.9	0	0	0	0	0	0	0.5	0.5	0.5
DCF_1-7.10	0	1	1	0	0	0	0.5	0.5	0.5
DCF_1-7.11	0	0	1	0.5	0	0	1	0	0
DCF_1-7.12	0	0.5	1	0	0	0.5	0.5	1	1
DCF_1-7.13	0	0.5	1	0	0	0	0.5	0	0
DCF_1-7.14	0	0	1	0.5	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-7.15	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-7.16	0	1	0	0.5	0	0	0.5	0.5	0.5
DCF_1-7.17	1	0.5	0	0	0	0	0.5	0.5	0.5
DCF_1-7.18	0	0.5	0	0	0	0	0.5	1	1
DCF_1-7.19	0	1	1	0	0	0	0.5	0.5	0.5
DCF_1-7.20	0	0.5	1	0	0	0.5	0.5	0	0
DCF_1-7.21	1	1	0	0	0	0.5	0.5	0	0
DCF_1-7.22	0	0	1	0	0.5	0	0.5	0.5	0.5
DCF_1-7.23	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-7.24	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
DCF_1-7.25	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-17.14	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-11.1	1	0	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-11.2	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-11.3	0	1	0	0.5	0	0	0.5	0	0
DCF_1-11.4	0	0.5	1	0.5	0.5	0	1	0.5	0.5
DCF_1-11.5	1	0	1	0	0	0.5	1	1	1
DCF_1-11.6	1	0.5	0	0	0	0	0.5	0.5	0.5
DCF_1-11.7	0	0.5	1	0	0	0	0.5	0	0
DCF_1-11.8	1	0.5	0	0	0	0	1	1	1
DCF_1-11.9	0	0.5	1	0	0	0	0.5	1	1
DCF_1-11.10	0	1	0	0.5	0	0	0.5	1	1
DCF_1-11.11	0	1	1	0	0.5	0	1	0.5	0.5
DCF_1-11.12	0	0.5	1	0.5	0	0	0.5	0.5	0.5
DCF_1-11.13	0	0.5	1	0	0	0	0.5	0	0
DCF_1-11.14	1	0.5	1	0.5	0	0	0.5	0	0
FMF_1-3.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-52.2	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-12.1	0	0.5	1	0.5	0	0.5	0.5	1	1
DCF_1-12.2	0	0.5	1	0	0	0.5	0.5	0.5	0.5
DCF_1-12.3	1	1	0	0.5	0	1	0.5	0	0
DCF_1-12.4	0	1	1	0	0	0	1	0.5	0.5

Molecular marker data generated for the DCF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-12.5	1	0.5	1	0.5	0	0	0.5	0	0
DCF_1-12.6	0	0.5	1	0.5	0	0.5	1	0	0
DCF_1-12.7	0	0.5	0	0.5	0	1	0.5	0.5	0.5
DCF_1-12.8	0	0	1	0.5	0	1	1	0.5	0.5
DCF_1-12.9	0	1	1	0	0	0	0.5	0.5	0.5
DCF_1-12.10	0	0.5	1	0	0	0	1	1	1
DCF_1-12.11	1	0.5	0	0.5	0	1	0.5	0	0
DCF_1-12.12	0	0.5	0	0	0	0.5	1	0.5	0.5
DCF_1-12.13	0	0.5	1	0.5	0	0	1	0	0
DCF_1-12.14	0	0.5	1	0.5	0	0.5	1	0	0
DCF_1-12.15	1	0.5	1	0	0	0.5	1	1	1
DCF_1-12.16	0	0.5	1	0.5	0	0	1	0.5	0.5
DCF_1-12.17	0	0	1	0	0	0	1	0.5	0.5
FMF_1-6.17	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-11.3	0	0.5	1	0.5	0	0	0.5	0.5	0.5
DCF_1-14.1	1	0	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-14.2	0	1	1	0.5	0	0	1	0.5	0.5
DCF_1-14.3	0	1	1	0	0.5	0	0.5	0.5	0.5
DCF_1-14.4	0	0.5	1	0	0	0	0.5	0.5	0.5
DCF_1-14.5	0	1	1	1	0	0	1	1	1
DCF_1-14.6	0	0	1	1	0	0.5	0.5	0	0
DCF_1-14.7	0	0	0	0.5	0	0.5	1	0	0
DCF_1-14.8	1	1	1	0.5	0.5	0	0.5	0	0
DCF_1-14.9	0	1	0	0.5	0	0	0.5	0	0
DCF_1-14.10	1	1	1	1	0	0	0.5	0.5	0.5
DCF_1-14.11	1	0.5	1	0.5	0.5	0	0.5	0	0
DCF_1-14.12	1	1	1	0	0.5	0	0.5	0	0
DCF_1-14.13	1	0	1	0.5	0.5	0	0.5	0	0
DCF_1-14.14	0	0	0	1	0.5	0	0.5	1	1
DCF_1-14.15	0	1	1	0	0	0	0.5	0	0
DCF_1-14.16	1	0	0	0	0	0	0.5	1	1
DCF_1-14.17	1	0.5	0	0.5	0.5	0	0.5	1	1
DCF_1-14.18	0	0.5	1	0.5	0.5	0	1	0.5	0.5

Molecular marker data generated for the DCF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-14.19	0	0.5	1	0	0.5	0	0.5	0.5	0.5
DCF_1-14.20	1	0.5	1	1	0.5	0	0.5	0.5	0.5
DCF_1-14.21	1	0.5	0	0	0	0	0.5	0.5	0.5
DCF_1-14.22	0	0.5	1	1	0	0	0.5	0.5	0.5
DCF_1-14.23	0	0.5	1	0	0	0	0.5	1	1
DCF_1-14.24	0	0.5	1	0.5	0	0	1	0	0
DCF_1-14.25	0	0	0	1	0.5	0	0.5	0	0
DCF_1-14.26	0	0	1	0.5	0	0	1	1	1
FMF_1-37.2	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-16.7	0	0.5	0	0	0	0.5	0.5	0.5	0.5
DCF_1-16.1	0	0.5	1	0	0.5	0.5	1	0.5	0.5
DCF_1-16.2	0	0	1	0	0.5	0.5	0.5	0	0
DCF_1-16.3	0	0.5	1	0.5	0.5	0	1	0.5	0.5
DCF_1-16.4	0	0	1	0.5	0	0.5	1	0	0
DCF_1-16.5	0	0	1	0	0.5	0.5	0.5	0.5	0.5
DCF_1-16.6	0	0.5	1	0	0	0.5	0.5	0	0
DCF_1-16.7	0	0.5	1	0	0.5	0.5	1	1	1
DCF_1-16.8	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-16.9	0	0	0	0.5	0	0.5	1	0.5	0.5
DCF_1-16.10	0	0	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-16.11	1	0	0	0	0.5	0.5	0.5	0.5	0.5
DCF_1-16.12	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-16.13	1	1	1	0	0.5	0	0.5	0.5	0.5
DCF_1-16.14	0	0	1	0.5	0.5	0	1	1	1
DCF_1-16.15	1	0.5	0	0	0	0.5	0.5	0	0
DCF_1-16.16	0	0.5	1	0.5	0.5	0	1	0	0
DCF_1-16.17	0	0.5	1	0.5	0.5	0	1	0	0
DCF_1-16.18	0	0	0	0.5	0.5	0.5	1	1	1
DCF_1-16.19	1	0	1	0	0.5	0	0.5	0.5	0.5
DCF_1-16.20	0	1	1	0.5	0	0.5	1	0.5	0.5
DCF_1-16.21	1	0.5	1	0.5	0	0	0.5	0.5	0.5
DCF_1-16.22	0	0.5	1	0.5	0.5	0	1	0.5	0.5
DCF_1-16.23	0	0.5	1	0	0	0	1	1	1

Molecular marker data generated for the DCF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-16.24	0	0.5	0	0	0	0.5	1	1	1
DCF_1-16.25	0	1	1	0.5	0.5	0	1	1	1
DCF_1-16.26	0	0.5	1	0.5	0	0.5	1	0.5	0.5
DCF_1-16.27	1	1	1	0.5	0	0	0.5	0.5	0.5
FMF_1-37.4	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-18.1	1	1	0	0.5	0	0.5	0.5	0.5	0.5
DCF_1-18.2	1	1	1	0.5	0	0.5	0.5	1	1
DCF_1-18.3	0	0.5	1	0.5	0.5	0	1	1	1
DCF_1-18.4	0	0	0	0.5	0.5	0.5	1	0.5	0.5
DCF_1-18.5	1	0	1	0.5	0.5	0	0.5	0	0
DCF_1-18.6	0	1	1	0.5	0	0	0.5	1	1
DCF_1-18.7	0	0.5	1	0	0.5	0	0.5	0.5	0.5
DCF_1-18.8	1	1	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-18.9	1	1	1	0	0.5	0.5	0.5	0.5	0.5
DCF_1-18.10	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-18.11	0	0.5	1	0.5	0.5	0.5	0.5	0	0
DCF_1-18.12	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-18.13	1	0	1	1	0	0	0.5	0	0
DCF_1-18.14	1	1	1	0	0	0.5	0.5	0.5	0.5
DCF_1-18.15	0	0	1	0	0.5	0.5	1	1	1
DCF_1-18.16	1	0.5	1	0	0	0.5	0.5	0	0
DCF_1-18.17	1	0.5	0	0	0.5	0.5	0.5	1	1
DCF_1-18.18	1	0.5	0	0.5	0	0	0.5	1	1
DCF_1-18.19	1	0	1	0.5	0.5	0	0.5	1	1
DCF_1-18.20	1	0	1	0.5	0	0.5	0.5	1	1
DCF_1-18.21	1	1	0	0.5	0.5	0.5	0.5	1	1
DCF_1-18.22	1	1	1	1	0	0.5	0.5	0.5	0.5
DCF_1-18.23	1	1	1	0.5	0.5	0.5	0.5	0	0
DCF_1-18.24	0	0.5	0	0	0.5	0.5	0.5	0.5	0.5
DCF_1-18.25	1	0.5	1	0	0.5	0	0.5	0.5	0.5
DCF_1-18.26	1	0.5	1	0.5	0	0	0.5	0.5	0.5
DCF_1-18.27	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5

Molecular marker data generated for the DCF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-18.28	1	0	1	0.5	0	0	0.5	0	0
DCF_1-18.29	1	0	1	0.5	0	0	0.5	0.5	0.5
DCF_1-18.30	1	1	1	0.5	0.5	0.5	0.5	1	1
DCF_1-18.31	1	0.5	1	0.5	0	0	0.5	0	0
DCF_1-18.32	1	0.5	1	0.5	0	0	0.5	1	1
FMF_1-36.8	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-19.1	1	0	1	1	0.5	0.5	0.5	0	0
DCF_1-19.2	0	1	1	0.5	0.5	0.5	1	1	1
DCF_1-19.3	1	0.5	1	0.5	0.5	1	0.5	0	0
DCF_1-19.4	0	0.5	1	0.5	0.5	0	1	1	1
DCF_1-19.5	1	0.5	1	0.5	0.5	0.5	0.5	0	0
DCF_1-19.6	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
DCF_1-19.7	0	1	1	0.5	0.5	0.5	1	0.5	0.5
DCF_1-19.8	1	0	1	0	0.5	0.5	0.5	0	0
DCF_1-19.9	0	1	0	0	0.5	0	0.5	0.5	0.5
DCF_1-19.10	1	0.5	0	0.5	0	0	0.5	0.5	0.5
DCF_1-19.11	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-19.12	1	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-19.13	1	0.5	1	0.5	0	0	0.5	0	0
DCF_1-19.14	0	0.5	1	0.5	0	0.5	1	1	1
DCF_1-19.15	1	0	1	1	1	0	0.5	1	1
DCF_1-19.16	0	0.5	1	0	0.5	0.5	1	1	1
FMF_1-3.11	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-60.1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-21.1	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-21.2	1	0.5	1	0.5	0	0.5	0.5	1	1
DCF_1-21.3	0	0.5	1	0.5	0	0.5	1	1	1
DCF_1-21.4	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
DCF_1-21.5	1	1	1	0	0.5	0	0.5	0	0
DCF_1-21.6	0	0.5	1	0.5	0	0	0.5	0	0
DCF_1-21.7	1	0.5	1	0.5	1	0	0.5	0.5	0.5
DCF_1-21.8	1	0.5	1	0.5	1	0	0.5	0.5	0.5

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-21.9	1	1	1	0	1	0.5	0.5	0.5	0.5
DCF_1-21.10	1	1	1	0.5	1	0	0.5	0.5	0.5
DCF_1-21.11	0	0.5	1	0	1	0.5	1	0.5	0.5
DCF_1-21.12	1	1	0	0	0.5	0.5	0.5	0.5	0.5
DCF_1-21.13	1	1	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-21.14	0	0.5	1	0	0.5	0	0.5	0.5	0.5
DCF_1-21.15	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-21.16	1	1	1	0.5	0.5	0	0.5	0	0
DCF_1-21.17	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-21.18	0	0.5	1	0.5	0.5	0	0.5	0	0
DCF_1-21.19	0	0.5	0	0.5	0.5	0.5	0.5	0	0
DCF_1-21.20	1	0.5	1	0.5	1	0	0.5	0.5	0.5
DCF_1-21.21	0	1	1	0.5	0.5	0.5	1	1	1
DCF_1-21.22	1	1	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-21.23	1	0	1	0.5	0.5	0.5	0	0	0
FMF_1-17.1	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-27.6	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
DCF_1-33.1	1	1	0	0	0.5	0	0	0	0
DCF_1-33.2	0	0.5	1	0	0	0.5	0	0	0
DCF_1-33.3	0	0.5	0	0.5	0.5	0	1	0	0
DCF_1-33.5	1	1	1	0	0	0.5	0.5	0.5	0.5
DCF_1-33.6	0	0.5	0	0	0	0.5	1	0	0
DCF_1-33.7	1	0	0	0.5	0	0.5	0.5	0.5	0.5
DCF_1-33.8	0	1	0	0	0	0	0.5	0	0
DCF_1-33.9	0	0.5	1	1	0.5	0.5	1	0.5	0.5
DCF_1-33.10	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
DCF_1-33.11	1	0.5	0	0.5	0.5	0.5	1	1	1
DCF_1-33.12	1	1	0	0	0	0.5	1	0.5	0.5
FMF_1-60.1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-34.1	0	0.5	1	0.5	0.5	0.5	0	0	0
DCF_1-34.2	1	0.5	0	0	0.5	1	1	1	1
DCF_1-34.3	1	1	1	0	0.5	0.5	1	0	0

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-34.4	1	1	1	1	0.5	0	1	1	1
DCF_1-34.5	0	0.5	0	0	0.5	0	1	0	0
DCF_1-34.7	0	1	0	1	0.5	1	0	0	0
DCF_1-34.8	1	0.5	0	0.5	0.5	0	1	0	0
DCF_1-34.9	1	0	0	0.5	0	1	0	0	0
DCF_1-34.10	1	0.5	0	0	0.5	0.5	1	0	0
DCF_1-34.11	1	0.5	1	0.5	0.5	0.5	1	0	0
DCF_1-34.12	0	0.5	1	0	1	0	1	1	1
FMF_1-37.11	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-60.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-35.2	1	0.5	1	0.5	0	0	0.5	0	0
DCF_1-35.3	1	0.5	1	1	0.5	0.5	0.5	0.5	0.5
DCF_1-35.4	1	1	0	0	0.5	0.5	1	0.5	0.5
FMF_1-36.10	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-36.1	0	0	0	1	0	0	1	0.5	0.5
DCF_1-36.2	0	1	1	0.5	0	0.5	1	0.5	0.5
DCF_1-36.3	0	1	1	0	0.5	0.5	1	1	1
DCF_1-36.4	1	0	1	0	0	0.5	1	0	0
DCF_1-36.5	1	0	1	0.5	0	0.5	0.5	0	0
DCF_1-36.6	0	1	0	0.5	0	1	1	1	1
DCF_1-36.7	0	1	1	0	0.5	0	0.5	0	0
DCF_1-36.8	0	0.5	1	1	0.5	0	1	0	0
DCF_1-36.9	0	0	1	1	0.5	1	1	0	0
DCF_1-36.10	0	0.5	1	0.5	0.5	0	1	0	0
DCF_1-36.11	1	0.5	1	0	0	0.5	0.5	0.5	0.5
DCF_1-36.12	1	0.5	1	0	0.5	0.5	1	0.5	0.5
DCF_1-36.13	0	0.5	0	1	0.5	0.5	1	0	0
DCF_1-36.14	0	0.5	1	0.5	0.5	0	1	0.5	0.5
DCF_1-36.15	0	0	1	0.5	0	0.5	1	1	1
DCF_1-36.16	0	1	1	1	0.5	0.5	1	0.5	0.5
DCF_1-36.17	1	1	1	0.5	0.5	0.5	0.5	1	1
DCF_1-36.18	0	0.5	1	0	0.5	0.5	1	0.5	0.5

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-36.19	1	1	1	0.5	0.5	0	0.5	1	1
DCF_1-36.20	1	0.5	1	1	0.5	0.5	1	1	1
DCF_1-36.21	1	0.5	1	0	0.5	0.5	0.5	0	0
DCF_1-36.22	1	0.5	1	0.5	0.5	0.5	1	0	0
FMF_1-4.6	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-37.1	1	0.5	1	1	0	0	0.5	0.5	0.5
DCF_1-37.2	0	0.5	0	1	0.5	0	1	0	0
DCF_1-37.3	1	0.5	1	0	0	0	0	0.5	0.5
DCF_1-37.4	0	0.5	0	1	0.5	0.5	0.5	0.5	0.5
DCF_1-37.5	1	0.5	1	0	0.5	0	1	0.5	0.5
DCF_1-37.6	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-37.7	1	1	1	0	0	0	1	1	1
DCF_1-37.8	1	1	1	0.5	0.5	0	1	1	1
DCF_1-37.9	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-87.4	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_1-52.2	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-38.1	0	0.5	0	0	0	1	1	1	1
DCF_1-38.2	1	0	1	1	0	0	1	0.5	0.5
DCF_1-38.3	1	0	0	0.5	0	1	1	0.5	0.5
DCF_1-38.4	1	0	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-38.5	1	0.5	1	1	0	1	1	0.5	0.5
DCF_1-38.6	1	0	0	0	0	0.5	1	1	1
DCF_1-38.7	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-38.8	0	0.5	1	1	0	0.5	0.5	0.5	0.5
DCF_1-38.9	1	0.5	1	1	0	0	1	0	0
DCF_1-38.11	0	1	0	0.5	0	0.5	0.5	0.5	0.5
DCF_1-38.12	1	1	0	0.5	0	0.5	0.5	0.5	0.5
DCF_1-38.13	0	1	1	0.5	0	0.5	1	1	1
FMF_1-37.8	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-52.2	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-39.1	0	0.5	1	0	0.5	0.5	1	0	0
DCF_1-39.2	1	0.5	1	0.5	0	0.5	1	0	0

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-39.3	0	0	0	0	0	0.5	1	0	0
DCF_1-39.5	0	1	1	1	0	0.5	1	0.5	0.5
DCF_1-39.6	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-39.7	0	0.5	1	0.5	0	0	0.5	0	0
DCF_1-39.8	0	0.5	1	0.5	1	0	1	0.5	0.5
DCF_1-39.9	0	0.5	1	0	0	0	0.5	0.5	0.5
DCF_1-39.10	0	0	0	1	1	0	0.5	0.5	0.5
DCF_1-39.11	0	0.5	0	0.5	0	0	1	1	1
DCF_1-39.12	0	1	1	0.5	0.5	0	0.5	0	0
FMF_1-51.10	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5
FMF_1-52.2	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-40.1	0	0.5	1	1	0.5	0	0.5	0.5	0.5
DCF_1-40.2	0	1	1	0.5	0	0	1	0.5	0.5
DCF_1-40.3	0	0.5	1	0.5	0	0	1	0.5	0.5
DCF_1-40.4	0	1	1	1	0.5	0.5	1	0	0
DCF_1-40.5	0	0	1	0	0	0.5	0.5	0	0
DCF_1-40.6	0	0	0	0	0.5	0	1	0	0
DCF_1-40.7	0	1	1	0.5	0.5	0.5	1	0.5	0.5
DCF_1-40.8	0	0.5	1	0	0.5	0.5	0.5	1	1
DCF_1-40.9	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-40.10	0	0	0	0.5	0.5	0.5	0.5	1	1
DCF_1-40.11	0	1	1	0.5	0	0.5	1	0.5	0.5
DCF_1-40.12	0	0	1	0	0	0.5	1	0.5	0.5
DCF_1-40.13	0	0.5	0	0.5	0	0	0.5	0.5	0.5
DCF_1-40.14	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-40.15	0	0.5	1	0.5	0	0.5	1	1	1
DCF_1-40.16	0	0.5	0	1	0	0.5	1	0	0
DCF_1-40.17	0	0.5	0	0.5	0.5	0.5	0.5	0	0
DCF_1-40.18	0	0	1	0	0	0	0.5	1	1
DCF_1-40.19	0	0.5	1	0	0.5	0	1	0.5	0.5
DCF_1-40.20	0	1	1	0	0.5	0	1	0.5	0.5
DCF_1-40.21	0	0	1	0	0	0.5	1	0.5	0.5
DCF_1-40.22	0	0	0	0.5	0	0.5	1	0	0

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-40.23	0	1	1	0.5	0.5	0	1	1	1
DCF_1-40.24	0	0	1	0	0	0	0.5	0	0
DCF_1-40.25	0	0.5	1	0	0	0.5	0.5	0	0
DCF_1-40.26	0	1	0	0.5	0.5	0.5	1	0.5	0.5
DCF_1-40.27	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-40.28	1	0.5	0	0.5	0.5	0.5	0.5	0	0
FMF_1-24.4	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-42.1	0	0	1	0.5	0	0.5	0.5	0	0
DCF_1-42.2	0	1	1	0	0.5	0	1	0.5	0.5
DCF_1-42.3	1	0	1	0	0	0	0.5	0.5	0.5
DCF_1-42.4	0	1	1	0	0.5	0	0.5	0	0
DCF_1-42.5	1	0.5	1	0	0	0	0.5	0.5	0.5
DCF_1-42.7	1	0.5	1	0.5	0	0	0.5	0	0
DCF_1-42.8	0	1	1	0	0.5	0.5	1	0.5	0.5
DCF_1-42.9	0	1	0	0.5	0	0.5	0.5	0	0
DCF_1-42.10	0	0	1	0.5	0.5	0.5	0.5	1	1
DCF_1-42.11	1	0.5	0	0.5	0.5	0.5	0	0	0
DCF_1-42.12	0	1	0	1	0	0	1	1	1
DCF_1-42.13	1	0.5	1	0.5	0	0	0.5	0.5	0.5
DCF_1-42.14	1	0.5	0	0.5	0	0	0.5	0.5	0.5
DCF_1-42.15	0	1	0	0.5	0	0.5	0	0.5	0.5
DCF_1-42.16	0	0.5	1	0.5	0.5	0.5	1	0.5	0.5
DCF_1-42.17	1	0.5	1	0	0	0.5	0.5	0.5	0.5
DCF_1-42.18	1	0	0	0	0.5	0.5	0.5	1	1
FMF_1-44.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-43.1	0	0.5	1	0	0	1	0.5	0.5	0.5
DCF_1-43.2	0	1	0	1	0	0.5	0.5	0	0
DCF_1-43.3	0	0	1	0.5	0.5	0.5	1	1	1
DCF_1-43.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-43.6	0	0.5	0	0.5	0.5	0.5	1	0.5	0.5
DCF_1-43.7	1	1	0	1	0	0.5	0.5	0	0

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-43.8	0	0.5	1	0.5	0.5	0.5	1	0.5	0.5
DCF_1-43.9	1	1	1	0	0	0.5	0.5	0.5	0.5
DCF_1-43.10	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-43.11	0	0	1	0	0.5	0.5	0.5	1	1
DCF_1-43.12	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-43.13	1	0	1	0	0.5	0.5	0.5	0.5	0.5
DCF_1-43.14	0	0.5	1	1	0.5	0.5	1	0.5	0.5
DCF_1-43.15	1	0.5	1	0.5	0	0	0.5	0.5	0.5
DCF_1-43.16	0	0.5	0	1	0.5	0.5	1	0.5	0.5
FMF_1-97.2	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-51.4	1	0.5	0	0	0.5	0.5	0.5	0.5	0.5
DCF_1-48.1	1	0.5	0	0.5	0	0.5	0.5	0	0
DCF_1-48.2	0	1	0	0	0.5	0.5	0.5	0.5	0.5
DCF_1-48.3	0	0.5	0	0	0.5	1	0.5	0.5	0.5
DCF_1-48.4	0	0.5	0	0	0.5	1	0.5	1	1
DCF_1-48.5	0	1	0	0	0	0.5	1	0.5	0.5
DCF_1-48.6	1	0.5	1	0	0	0	1	0.5	0.5
DCF_1-48.7	0	0.5	0	0	1	0.5	1	0.5	0.5
DCF_1-48.8	1	1	0	0	0.5	0.5	0.5	1	1
DCF_1-48.9	0	0	1	1	1	0	0.5	0.5	0.5
DCF_1-48.10	0	1	0	1	0.5	0.5	0.5	0.5	0.5
DCF_1-48.11	0	0.5	1	0.5	0.5	0	1	1	1
DCF_1-48.12	1	1	0	0.5	0.5	1	0	0	0
DCF_1-48.13	0	0.5	0	0.5	0	0.5	1	0	0
DCF_1-48.14	0	1	0	0	0.5	0.5	1	0	0
DCF_1-48.15	1	1	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-48.16	1	1	1	0.5	0	1	1	0	0
DCF_1-48.17	1	0.5	1	0	0.5	0.5	1	0	0
DCF_1-48.18	1	0.5	0	0.5	1	1	1	0	0
FMF_1-60.7	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-50.1	0	0.5	0	0.5	0	1	0.5	0.5	0.5
DCF_1-50.2	1	0.5	1	0.5	1	0.5	0.5	1	1

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-50.3	0	0.5	0	0.5	1	1	0.5	0.5	0.5
DCF_1-50.4	1	1	0	0.5	0.5	1	0.5	0.5	0.5
DCF_1-50.5	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-50.6	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-50.7	1	0	1	0	0	0.5	0.5	0.5	0.5
DCF_1-50.8	1	0.5	1	0.5	1	0	0	0	0
DCF_1-50.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-50.10	0	0	1	0	0.5	0	0	0	0
DCF_1-50.11	1	0.5	1	0.5	0	0	0.5	1	1
DCF_1-50.12	1	1	1	0.5	0.5	0	0	0.5	0.5
DCF_1-50.13	1	0.5	1	0	0	0	0.5	1	1
DCF_1-50.14	1	0.5	1	1	0	0	0.5	0.5	0.5
DCF_1-50.15	1	0	0	0	0	1	0.5	0.5	0.5
DCF_1-50.16	1	0	1	0.5	0.5	0	0.5	1	1
DCF_1-50.17	1	0.5	0	1	0.5	1	0.5	1	1
DCF_1-50.18	1	0.5	1	1	0	0	0	0	0
DCF_1-50.19	1	1	0	0.5	0.5	1	0	0	0
DCF_1-50.20	0	1	1	0	0.5	0	0	0	0
DCF_1-50.21	0	0.5	1	0	0	1	0.5	0	0
DCF_1-50.22	1	0	0	0.5	0.5	1	0.5	0	0
DCF_1-50.23	1	0	1	0.5	0.5	0.5	0.5	0	0
DCF_1-50.24	1	0	1	0.5	0	0	0.5	1	1
DCF_1-50.25	1	1	0	0.5	0.5	0.5	0.5	0	0
DCF_1-50.26	0	0.5	1	0.5	0.5	1	0.5	0	0
DCF_1-50.27	1	0.5	0	0.5	0.5	1	0.5	0.5	0.5
FMF_1-60.6	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_1-60.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-51.2	1	0.5	1	0.5	0	0	0.5	1	1
DCF_1-51.3	1	0	1	0.5	0.5	0.5	0.5	0	0
DCF_1-51.4	0	0	0	0.5	0.5	1	1	1	1
DCF_1-51.5	1	0.5	0	0.5	0.5	1	0.5	0.5	0.5
DCF_1-51.6	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-51.7	1	0.5	1	0	0	0	0.5	0	0

Molecular marker data generated for the DCF_1 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-51.8	1	0.5	1	0.5	0	0	0.5	0	0
DCF_1-51.9	1	0.5	1	1	0.5	0.5	0	0.5	0.5
DCF_1-51.10	1	1	1	1	0.5	1	0	0.5	0.5
DCF_1-51.11	1	0	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-51.12	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-51.13	1	1	1	0.5	0.5	0	0.5	1	1
DCF_1-51.14	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-51.15	1	0	0	0.5	0.5	1	0.5	0.5	0.5
DCF_1-51.16	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-51.18	1	0.5	0	0	0.5	0	0.5	0.5	0.5
DCF_1-51.19	1	1	0	0.5	0	0	0.5	0.5	0.5
DCF_1-51.20	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-51.21	1	0	0	0.5	0.5	1	0.5	0.5	0.5
DCF_1-51.22	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
DCF_1-51.23	0	0	0	1	0.5	1	0	0	0
DCF_1-51.24	1	1	0	0.5	0.5	0.5	0.5	1	1
DCF_1-51.25	0	1	1	0	0	0	0	0	0
FMF_1-39.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-51.3	1	0.5	0	0	0	0.5	0.5	0.5	0.5
DCF_1-52.1	0	0	0	0	0	0.5	1	0.5	0.5
DCF_1-52.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
DCF_1-52.3	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-52.4	0	0.5	0	0	0	0.5	0.5	0	0
DCF_1-52.5	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-52.7	0	0.5	0	0.5	0	0	0.5	0.5	0.5
DCF_1-52.8	0	0	0	0.5	0	1	0.5	0.5	0.5
DCF_1-52.9	0	0.5	1	0.5	0.5	0.5	1	0.5	0.5
DCF_1-52.10	0	1	1	0	0.5	0.5	0.5	0	0
DCF_1-52.11	0	1	0	0	0.5	0.5	0.5	0	0
DCF_1-52.12	0	0.5	0	0	0.5	1	0.5	0.5	0.5
DCF_1-52.13	0	1	0	0.5	0	0.5	0.5	1	1
DCF_1-52.14	0	1	1	0.5	0	0.5	1	0.5	0.5
DCF_1-52.15	0	0.5	1	0.5	0.5	0	1	1	1

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-44.2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-51.3	1	0.5	0	0	0	0.5	0.5	0.5	0.5
DCF_1-53.1	0	1	1	0.5	0	0.5	1	1	1
DCF_1-53.2	1	0.5	0	0	0	0.5	0.5	0.5	0.5
DCF_1-53.4	0	1	1	0	0	0.5	1	0	0
DCF_1-53.5	0	0.5	0	0.5	0	0.5	1	0.5	0.5
DCF_1-53.6	0	0.5	1	0	0.5	0	1	0.5	0.5
DCF_1-53.7	1	1	0	0	0	1	0.5	0	0
DCF_1-53.8	0	0	0	0.5	0.5	0.5	1	0.5	0.5
DCF_1-53.9	0	1	0	0	0.5	1	1	0.5	0.5
DCF_1-53.10	0	0.5	1	0	0.5	0	0.5	0.5	0.5
DCF_1-53.11	0	0	0	0	0	0.5	1	0.5	0.5
DCF_1-53.12	1	0	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-77.6	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-51.3	1	0.5	0	0	0	0.5	0.5	0.5	0.5
DCF_1-54.1	1	1	0	0.5	0.5	1	0.5	1	1
DCF_1-54.2	0	0	1	0.5	0.5	0	1	0.5	0.5
DCF_1-54.3	1	0.5	0	0.5	0	1	0.5	1	1
DCF_1-54.4	0	0.5	0	0.5	0.5	1	1	1	1
DCF_1-54.5	0	0	0	0.5	0	1	0.5	0.5	0.5
DCF_1-54.6	0	1	0	0.5	0.5	0.5	0.5	0	0
DCF_1-54.7	0	0	1	0	0.5	0	0.5	0	0
DCF_1-54.8	0	0	1	0	0.5	0	1	0.5	0.5
DCF_1-54.9	0	0.5	0	0.5	0	0.5	0.5	0.5	0.5
DCF_1-54.10	1	1	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-54.11	0	0.5	1	0.5	0	0.5	0.5	1	1
DCF_1-54.12	0	0.5	0	0	0.5	1	0.5	0	0
DCF_1-54.13	0	0	1	0.5	0	0	1	0	0
DCF_1-54.14	0	0.5	1	0	0	0	1	0.5	0.5
DCF_1-54.15	1	0	0	0.5	0.5	1	0.5	0.5	0.5
DCF_1-54.16	0	1	0	0.5	0.5	0.5	1	1	1
FMF_1-71.13	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-59.1	0	0	0	0	0.5	0	1	1	1
DCF_1-59.2	1	0	1	0.5	0.5	0.5	0.5	1	1
DCF_1-59.3	0	0.5	1	0.5	0	0.5	1	1	1
DCF_1-59.4	0	0.5	1	0.5	0	0.5	0.5	0	0
DCF_1-59.5	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
DCF_1-59.6	0	0	0	0.5	0.5	1	1	0.5	0.5
DCF_1-59.7	0	0.5	1	0.5	0	0	0.5	0.5	0.5
DCF_1-59.8	1	0	1	1	0.5	0.5	0.5	0.5	0.5
DCF_1-59.9	0	1	0	0.5	0	1	0.5	1	1
DCF_1-59.10	0	0	1	0.5	0.5	0	1	0.5	0.5
FMF_1-65.5	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-60.1	1	1	1	0.5	0.5	0	0.5	0.5	0.5
DCF_1-60.2	0	0.5	1	0.5	0	0.5	0.5	0	0
DCF_1-60.3	0	0.5	1	0.5	0.5	0.5	0.5	0	0
DCF_1-60.4	0	0	0	0.5	0.5	1	0.5	0.5	0.5
DCF_1-60.5	0	0.5	0	1	0.5	1	0.5	0	0
DCF_1-60.6	1	0.5	1	1	0	0.5	0.5	0.5	0.5
DCF_1-60.7	1	1	0	0.5	0	1	0.5	0.5	0.5
DCF_1-60.8	0	1	0	0.5	0.5	1	0.5	0	0
DCF_1-60.9	0	0.5	0	0	0	1	0.5	1	1
FMF_1-89.2	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-60.11	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-61.1	0	0.5	1	0	0	0.5	1	1	1
DCF_1-61.2	0	0	1	1	0	0.5	0.5	0	0
DCF_1-61.3	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF_1-61.4	0	0.5	1	0	0	0.5	0.5	1	1
DCF_1-61.5	1	0.5	1	0.5	0	0.5	0.5	0	0
DCF_1-61.6	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-97.4	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_1-51.9	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-86.1	0	0.5	1	1	1	0.5	1	1	1

Molecular marker data generated for the DCF_1 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF_1-86.2	0	1	0	0.5	0	1	1	0.5	0.5
DCF_1-86.3	1	0.5	1	1	0.5	0.5	0.5	0	0
DCF_1-86.4	1	0.5	1	0.5	1	0	0.5	1	1
DCF_1-86.5	0	1	1	1	0.5	0	1	1	1
DCF_1-86.6	0	0.5	1	0	0.5	0.5	1	0.5	0.5
DCF_1-86.7	0	0	1	0	0	0	0.5	0.5	0.5
DCF_1-86.8	0	0.5	0	0.5	1	1	1	0	0
DCF_1-86.9	0	0.5	0	0.5	0.5	0.5	0.5	1	1
DCF_1-86.10	0	0.5	1	0.5	0.5	0.5	1	1	1
DCF_1-86.11	1	0	1	0	0.5	0.5	1	0	0
DCF_1-86.12	0	0	0	0	1	0.5	0.5	0.5	0.5
DCF_1-86.13	0	1	1	0.5	0.5	1	1	0.5	0.5
DCF_1-86.14	0	0	1	0.5	1	0	1	0	0
DCF_1-86.15	1	0	1	1	1	0.5	0.5	0	0
FMF_1-100.14	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_1-51.9	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF_1-88.1	0	0.5	0	0	0.5	0.5	1	0.5	0.5
DCF_1-88.2	0	1	1	0.5	0.5	0	1	0.5	0.5
DCF_1-88.3	0	0.5	1	0	0.5	0.5	0.5	0	0
DCF_1-88.4	0	0.5	1	0	0	0.5	1	1	1
DCF_1-88.5	0	0	0	0	0	1	1	0	0
DCF_1-88.6	0	1	1	0	0.5	0	1	0	0
DCF_1-88.7	0	0	1	0.5	0.5	0.5	1	1	1
DCF_1-88.8	0	0	0	0.5	0.5	0.5	1	1	1
DCF_1-88.9	1	1	1	0.5	0.5	0	1	0.5	0.5
DCF_1-88.10	0	1	0	0	0	0.5	1	0.5	0.5
DCF_1-88.11	0	1	0	0.5	0.5	1	1	0.5	0.5
DCF_1-88.12	0	0.5	0	0.5	0.5	0	1	0.5	0.5
DCF_1-88.13	0	0.5	0	0	0	0	1	0	0
DCF_1-88.14	0	0.5	1	0	0.5	0	1	1	1
DCF_1-88.15	0	0.5	0	0	0	0	1	0	0

Lines highlighted in grey are the FMF_1 lines used to develop the cross. [1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0).

Appendix E

Molecular marker data generated for the FMF_2 population

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_1-60.2	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.2.1	0	1	1	0.5	0	0	0.5	1	1
FMF_2-60.2.2	0	0.5	0	0	0	0.5	0.5	1	1
FMF_2-60.2.3	1	0	0	0	0	0.5	0.5	1	1
FMF_2-60.2.4	0	0.5	1	0	0	0	0.5	1	1
FMF_2-60.2.5	1	0.5	1	0.5	0	0	0	0	0
FMF_2-60.2.6	0	0	1	0.5	0	0.5	0.5	1	1
FMF_2-60.2.7	0	1	1	0.5	0	0	0	0	0
FMF_2-60.2.8	0	0.5	1	0.5	0	1	0.5	0.5	0.5
FMF_2-60.2.9	0	0	1	0	0	0.5	0.5	1	1
FMF_2-60.2.10	0	0	1	0	0	0	1	0.5	0.5
FMF_2-60.2.11	0	0.5	1	0	0	0	0.5	0	0
FMF_2-60.2.12	0	1	1	0	0	0.5	0.5	0	0
FMF_2-60.2.13	0	0	1	0	0	0.5	0.5	0.5	0.5
FMF_2-60.2.14	0	1	0	0	0	1	0.5	0.5	0.5
FMF_2-60.2.15	0	0.5	0	1	0	1	0	0	0
FMF_2-60.2.16	0	1	1	0	0	0.5	0.5	0.5	0.5
FMF_2-60.2.17	0	1	1	0.5	0	0	0.5	1	1
FMF_2-60.2.18	0	0.5	1	0	0	0	0.5	0.5	0.5
FMF_2-60.2.19	0	0	0	0	0	0.5	0.5	0.5	0.5
FMF_2-60.2.20	1	1	1	0	0	0.5	0	1	1
FMF_2-60.2.21	0	0	1	1	0	0	0	0	0
FMF_2-60.2.22	0	0.5	0	0	0	0.5	0.5	0	0
FMF_2-60.2.23	0	0	1	0.5	0	0	0	0	0
FMF_2-60.2.25	1	0.5	1	0.5	0	0.5	0	0	0
FMF_2-60.2.26	0	0.5	0	0	0	0.5	0.5	0	0
FMF_2-60.2.27	0	0	0	0.5	0	1	1	1	1
FMF_2-60.2.28	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.2.29	1	0.5	1	0	0	0	0.5	0.5	0.5

Molecular marker data generated for the FMF_2 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_2-60.2.30	1	0.5	0	0	0	1	0.5	1	1
FMF_2-60.2.31	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_2-60.2.32	0	0.5	0	0.5	0	0	0.5	1	1
FMF_2-60.2.33	0	1	1	0.5	0	0.5	0.5	0	0
FMF_2-60.2.34	0	0.5	1	0.5	0	0.5	1	0	0
FMF_2-60.2.35	0	1	1	0	0	0	1	1	1
FMF_2-60.2.36	1	0.5	0	0	0	1	0.5	0	0
FMF_2-60.2.37	1	0	1	0.5	0	0.5	0	0	0
FMF_2-60.2.38	1	0.5	0	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.2.39	1	0	1	0.5	0	0	0.5	0.5	0.5
FMF_2-60.2.40	0	0.5	1	0.5	0	0.5	0.5	0	0
FMF_2-60.2.41	0	1	1	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.2.42	1	0.5	1	0.5	0	0.5	0.5	1	1
FMF_2-60.2.43	0	0	1	0.5	0	0	0.5	0.5	0.5
FMF_2-60.2.44	0	0.5	1	0	0	0.5	1	0.5	0.5
FMF_2-60.2.45	0	0	1	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.2.46	0	0.5	1	0.5	0	0	1	1	1
FMF_2-60.2.47	0	0	1	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.2.48	0	0.5	1	0.5	0	0.5	1	1	1
FMF_2-60.2.49	0	1	1	1	0	0.5	0	0.5	0.5
FMF_2-60.2.50	0	0	1	0.5	0	0.5	1	0.5	0.5
FMF_2-60.2.51	1	0.5	1	0	0	0.5	1	0.5	0.5
FMF_2-60.2.52	0	1	1	1	0	0	1	0.5	0.5
FMF_2-60.2.53	0	0.5	1	0	0	0.5	1	0	0
FMF_2-60.2.54	0	1	0	0	0	1	1	0.5	0.5
FMF_2-60.2.55	0	0.5	1	0.5	0	0	0.5	1	1
FMF_2-60.2.56	0	0.5	1	0.5	0	0	1	1	1
FMF_2-60.2.57	0	1	1	0.5	0	0	1	0.5	0.5
FMF_2-60.2.58	1	1	1	0.5	0	0	0.5	1	1
FMF_2-60.2.59	1	1	1	0.5	0	0.5	0.5	1	1
FMF_2-60.2.60	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.2.61	1	1	1	0.5	0	0	0.5	0	0
FMF_2-60.2.62	1	1	1	0.5	0	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_2 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_2-60.2.63	1	0.5	1	0	0	0.5	0.5	0	0
FMF_2-60.2.64	0	1	0	0	0	1	0.5	1	1
FMF_2-60.2.65	0	0.5	1	0.5	0	0.5	0.5	1	1
FMF_2-60.2.66	0	0.5	0	0	0	1	1	0	0
FMF_2-60.2.67	1	1	0	0	0	1	0.5	1	1
FMF_2-60.2.68	0	0	1	0.5	0	1	1	1	1
FMF_2-60.2.69	0	0.5	1	0	0	0	0.5	0	0
FMF_2-60.2.70	0	1	1	0	0	0.5	0.5	0	0
FMF_2-60.2.71	0	0.5	1	1	0	0.5	0.5	0	0
FMF_2-60.2.72	1	0.5	1	0.5	0	0.5	0.5	0	0
FMF_2-60.2.73	1	1	1	0	0	0.5	0.5	0	0
FMF_2-60.2.74	0	0	1	0.5	0	0	0.5	0	0
FMF_2-60.2.76	1	1	0	0.5	0	1	0.5	1	1
FMF_2-60.2.77	1	0.5	1	0.5	0	0	0.5	1	1
FMF_2-60.2.78	1	0.5	1	0.5	0	0	0.5	1	1
FMF_2-60.2.79	1	0	1	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.2.80	1	0.5	1	0.5	0	1	0.5	0.5	0.5
FMF_2-60.2.81	1	0.5	1	0.5	0	0	0.5	0	0
FMF_2-60.2.82	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_2-60.2.83	1	0.5	1	0	0	0	0.5	0.5	0.5
FMF_2-60.2.84	1	1	1	0	0	0	0.5	0.5	0.5
FMF_1-60.7	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.1	0	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_2-60.7.2	1	1	1	0	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.3	0	0	1	0	0	0.5	1	0.5	0.5
FMF_2-60.7.4	0	0	1	0	0	0.5	0	0	0
FMF_2-60.7.5	0	1	1	0	0	0.5	0.5	1	1
FMF_2-60.7.6	0	0.5	1	0	1	0.5	0.5	1	1
FMF_2-60.7.7	0	0	1	0	1	0.5	1	1	1
FMF_2-60.7.8	0	0.5	1	0	0	0	0.5	0.5	0.5
FMF_2-60.7.9	0	0.5	0	0	1	0	0.5	0.5	0.5
FMF_2-60.7.10	1	0.5	0	0	0	0.5	0.5	0.5	0.5
FMF_2-60.7.11	0	1	1	0	0.5	1	1	0	0

Molecular marker data generated for the FMF_2 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_2-60.7.12	0	0.5	1	0	0.5	0	0.5	1	1
FMF_2-60.7.13	0	0.5	1	0	0.5	0	1	0	0
FMF_2-60.7.14	0	1	0	0	0.5	0	0.5	0.5	0.5
FMF_2-60.7.15	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.16	0	1	0	0	0	0	0	0	0
FMF_2-60.7.17	0	1	0	0	0.5	0	1	0.5	0.5
FMF_2-60.7.18	0	0	1	0.5	0.5	0	1	0.5	0.5
FMF_2-60.7.19	0	0	0	0.5	0.5	0	0	0	0
FMF_2-60.7.20	0	0	1	1	0.5	0	1	1	1
FMF_2-60.7.21	0	0	1	0	0	0	0.5	0	0
FMF_2-60.7.22	1	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_2-60.7.23	1	0	1	0.5	0.5	0.5	0	0	0
FMF_2-60.7.24	0	1	1	0.5	1	0.5	0	0	0
FMF_2-60.7.25	0	1	1	0.5	1	1	0.5	0.5	0.5
FMF_2-60.7.26	0	1	1	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.7.27	1	0	1	0.5	1	0.5	0	0	0
FMF_2-60.7.28	1	0.5	1	0.5	1	0	0	0	0
FMF_2-60.7.29	1	0.5	1	0	0.5	0	0.5	0.5	0.5
FMF_2-60.7.30	1	0.5	1	0.5	1	0.5	0.5	0.5	0.5
FMF_2-60.7.31	0	0	1	0.5	1	0.5	0.5	0.5	0.5
FMF_2-60.7.32	0	0.5	1	0.5	1	1	0.5	0	0
FMF_2-60.7.33	1	0	1	0	1	0.5	0.5	0.5	0.5
FMF_2-60.7.34	1	0.5	1	0.5	0.5	1	0.5	0.5	0.5
FMF_2-60.7.35	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.36	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.38	1	0	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.39	1	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_2-60.7.40	0	0.5	1	0.5	0.5	0.5	0	0	0
FMF_2-60.7.41	1	0.5	1	0.5	1	0.5	0.5	0	0
FMF_2-60.7.42	1	0	1	0.5	0.5	0.5	0	0	0
FMF_2-60.7.43	0	0.5	1	1	0.5	0	1	0.5	0.5
FMF_2-60.7.44	1	0.5	1	1	0	0	0.5	0.5	0.5
FMF_2-60.7.45	0	0.5	1	0	0.5	0	0.5	0.5	0.5

Molecular marker data generated for the FMF_2 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_2-60.7.46	0	0	1	1	0.5	1	1	1	1
FMF_2-60.7.47	0	1	1	1	0.5	0	0.5	1	1
FMF_2-60.7.48	0	1	1	1	0.5	1	0.5	0.5	0.5
FMF_2-60.7.49	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.50	0	0.5	1	0.5	0.5	0.5	0	0	0
FMF_2-60.7.51	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5
FMF_2-60.7.52	0	1	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.53	0	0.5	1	0.5	0.5	0.5	0	0	0
FMF_2-60.7.54	0	0.5	1	0.5	0.5	0.5	1	0.5	0.5
FMF_2-60.7.55	1	0	1	0	0.5	0	0	0	0
FMF_2-60.7.56	1	0	1	0.5	0.5	0	0.5	0	0
FMF_2-60.7.57	0	0.5	1	0	0.5	0	0	0	0
FMF_2-60.7.58	0	1	1	0	0.5	0	0.5	0	0
FMF_2-60.7.59	0	0.5	1	1	0.5	0.5	0	0	0
FMF_2-60.7.60	0	0	1	0.5	0.5	0	0.5	0.5	0.5
FMF_2-60.7.61	0	0.5	1	0.5	0.5	0	0.5	1	1
FMF_2-60.7.62	0	1	1	0	0.5	1	1	0.5	0.5
FMF_2-60.7.63	0	0.5	1	0	0.5	0.5	1	0.5	0.5
FMF_2-60.7.65	1	0	1	0	0	0.5	0.5	0	0
FMF_2-60.7.66	0	0.5	0	0.5	0.5	1	0.5	0.5	0.5
FMF_2-60.7.67	1	0	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.68	0	0.5	1	0.5	0.5	1	0.5	0.5	0.5
FMF_2-60.7.69	1	0.5	0	0.5	0.5	1	0.5	0.5	0.5
FMF_2-60.7.70	1	1	0	0.5	0.5	0	0.5	0.5	0.5
FMF_2-60.7.71	1	0.5	1	0	0	0.5	0.5	0.5	0.5
FMF_2-60.7.72	0	1	1	0.5	0.5	0	0.5	0.5	0.5
FMF_2-60.7.73	1	0	1	0.5	0.5	0.5	0.5	1	1
FMF_2-60.7.74	1	0.5	1	0.5	0.5	0	0.5	0	0
FMF_2-60.7.75	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_2-60.7.76	1	0	0	0	0.5	1	0.5	0.5	0.5
FMF_2-60.7.77	0	0.5	0	0	0	0.5	1	1	1
FMF_2-60.7.78	1	1	1	0.5	1	0.5	0.5	0.5	0.5
FMF_2-60.7.79	1	0.5	0	0.5	0.5	1	0.5	0.5	0.5

Molecular marker data generated for the FMF_2 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_2-60.7.80	1	1	1	0.5	0	0.5	0.5	0	0
FMF_2-60.7.81	1	1	1	0.5	0	0	0.5	1	1
FMF_2-60.7.82	1	0.5	0	1	0	1	0.5	1	1
FMF_2-60.7.83	0	0	0	0.5	1	1	0.5	0	0
FMF_2-60.7.84	1	0.5	1	0.5	1	0.5	0.5	0	0
FMF_1-60.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.9.1	0	0	1	0.5	0.5	0	0.5	0.5	0.5
FMF_2-60.9.2	0	1	1	0	0.5	0.5	0	0	0
FMF_2-60.9.3	0	0.5	1	0.5	0.5	0.5	1	1	1
FMF_2-60.9.4	0	1	1	0	0.5	0.5	1	0.5	0.5
FMF_2-60.9.6	0	0	1	0.5	0.5	0.5	0.5	1	1
FMF_2-60.9.7	0	0	0	0	0	1	1	0.5	0.5
FMF_2-60.9.8	1	0	1	0	0.5	0.5	0	0	0
FMF_2-60.9.10	0	0.5	1	0.5	0	0	0.5	0.5	0.5
FMF_2-60.9.11	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.9.12	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.9.13	0	0.5	1	0	0.5	0.5	0	0	0
FMF_2-60.9.14	0	0.5	1	0.5	0.5	0.5	0	0	0
FMF_2-60.9.15	0	0.5	0	0	0.5	0	0.5	1	1
FMF_2-60.9.16	0	1	1	0	0	0.5	0.5	1	1
FMF_2-60.9.17	0	1	0	0	0.5	0.5	0.5	1	1
FMF_2-60.9.18	0	0.5	1	0.5	0.5	0.5	0	0.5	0.5
FMF_2-60.9.19	0	1	1	0	0	0.5	0.5	0.5	0.5
FMF_2-60.9.20	0	0.5	1	0	0.5	0	0	0	0
FMF_2-60.9.21	0	1	1	0	0	0.5	0.5	0.5	0.5
FMF_2-60.9.22	0	0.5	1	0.5	0	0.5	1	1	1
FMF_2-60.9.23	1	0	1	0	0.5	0.5	0.5	0	0
FMF_2-60.9.24	1	0	0	0	0.5	0	0	0	0
FMF_2-60.9.25	1	0.5	1	0.5	0.5	0	0.5	0	0
FMF_2-60.9.26	1	1	0	0.5	0.5	0.5	0	0	0
FMF_2-60.9.27	1	1	0	0.5	0.5	1	0.5	0	0
FMF_2-60.9.28	0	0.5	0	0	0	1	0.5	0	0
FMF_2-60.9.29	1	1	0	0	0	0.5	0	0	0

Molecular marker data generated for the FMF_2 population (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_2-60.9.30	0	0.5	0	0	0.5	1	1	1	1
FMF_2-60.9.31	1	0	1	0	0.5	0	0.5	0	0
FMF_2-60.9.32	1	0	0	0.5	0.5	1	0	0	0
FMF_2-60.9.33	1	0.5	0	0	0.5	1	0.5	0	0
FMF_2-60.9.34	1	0.5	0	0.5	0.5	0	0	0	0
FMF_2-60.9.35	1	0.5	1	0.5	0	0.5	0.5	0	0
FMF_2-60.9.36	1	1	1	0.5	0.5	1	0.5	1	1
FMF_2-60.9.37	1	0.5	1	0	0.5	0.5	0.5	0	0
FMF_2-60.9.38	1	0.5	0	0	0.5	1	0	0	0
FMF_2-60.9.39	1	1	1	0.5	0.5	0.5	0	0	0
FMF_2-60.9.40	0	0	0	0	0.5	0.5	0.5	0	0
FMF_2-60.9.41	0	0.5	1	0	0.5	0	0	0	0
FMF_2-60.9.42	1	0.5	0	0	0.5	1	0.5	0	0
FMF_2-60.9.43	1	1	1	0	0.5	0.5	0.5	0	0
FMF_2-60.9.44	1	0.5	1	0	0.5	0.5	0.5	0	0
FMF_2-60.9.45	0	0	1	0	0.5	0.5	1	1	1
FMF_2-60.9.46	0	0	1	0.5	0.5	0	1	0	0
FMF_2-60.9.47	0	0	1	0.5	0.5	0	1	0	0
FMF_2-60.9.48	1	1	1	0	0.5	0	1	0	0
FMF_2-60.9.49	1	0	1	0.5	0.5	0.5	0	0	0
FMF_2-60.9.50	0	0	1	0.5	0.5	0.5	0.5	1	1
FMF_2-60.9.51	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
FMF_2-60.9.52	1	1	1	0	0.5	0.5	1	1	1
FMF_2-60.9.53	0	1	1	0.5	0	0.5	0	0	0
FMF_2-60.9.55	1	0.5	1	0	0	0.5	1	1	1
FMF_2-60.9.56	0	0.5	1	0	0	0	0.5	0	0
FMF_2-60.9.57	1	0	1	0.5	0.5	0	0	0	0
FMF_2-60.9.58	0	1	1	0.5	0	0.5	0.5	0	0
FMF_2-60.9.59	0	0	1	0.5	0.5	0.5	1	0.5	0.5
FMF_2-60.9.60	1	0.5	1	0.5	0.5	0.5	1	0	0
FMF_2-60.9.61	0	0.5	1	1	0.5	0.5	1	0.5	0.5
FMF_2-60.9.62	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.9.63	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5

Molecular marker data generated for the FMF_2 population (continued)

Line name	$STSLr19_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF_2-60.9.64	1	0.5	1	0.5	0.5	0	0.5	1	1
FMF_2-60.9.65	0	0.5	0	0.5	0.5	0	1	0.5	0.5
FMF_2-60.9.66	0	0	0	0	0	1	1	1	1
FMF_2-60.9.67	0	0	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.9.68	1	0.5	1	1	0.5	0	1	1	1
FMF_2-60.9.69	1	0	1	0.5	0.5	0.5	1	1	1
FMF_2-60.9.70	0	0.5	1	0.5	0.5	0	1	0.5	0.5
FMF_2-60.9.71	0	0.5	1	0.5	0.5	0.5	1	1	1
FMF_2-60.9.72	0	0	0	0.5	0.5	1	0.5	0.5	0.5
FMF_2-60.9.73	0	0.5	1	0.5	0.5	0.5	1	1	1
FMF_2-60.9.74	0	0.5	1	0.5	0.5	1	1	1	1
FMF_2-60.9.75	0	0.5	0	0	0	0.5	0.5	0	0
FMF_2-60.9.76	0	0.5	1	0	0.5	0.5	0.5	0.5	0.5
FMF_2-60.9.77	0	1	1	0	0.5	0	0.5	0	0
FMF_2-60.9.78	1	0.5	1	0	0.5	0	0.5	1	1
FMF_2-60.9.79	0	0.5	1	0.5	0.5	0.5	1	1	1
FMF_2-60.9.81	0	0.5	1	0.5	0.5	0	0.5	0	0
FMF_2-60.9.82	0	0	1	1	0.5	0.5	1	1	1
FMF_2-60.9.83	0	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
FMF_2-60.9.84	0	0.5	0	1	0	1	1	1	1

Lines highlighted in grey are the FMF_1 lines selected for self-pollination. [1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible (0).

Appendix F

Rusts and Fusarium head blight resistance molecular marker data generated for the 2DCF$_1$, DCF$_2$ and FMF$_3$ populations

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF$_1$-50.27	1	0.5	0	0.5	0.5	1	0.5	0.5	0.5
DCF$_1$-51.11	1	0	1	0.5	0.5	0	0.5	0.5	0.5
2DCF$_1$-12.1	0	0	1	0.5	1	0.5	0	0	0
2DCF$_1$-12.2	0	0.5	1	1	1	0.5	0.5	0.5	0.5
2DCF$_1$-12.3	1	0.5	0	0.5	0.5	0.5	0	0	0
2DCF$_1$-12.4	1	0.5	1	1	1	0.5	0.5	0.5	0.5
2DCF$_1$-12.5	0	0	1	1	0.5	0.5	0	0	0
2DCF$_1$-12.6	0	0	1	1	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-12.7	0	0	0	1	0.5	0.5	0	0	0
2DCF$_1$-12.8	0	0.5	1	1	0.5	0.5	0	0	0
2DCF$_1$-12.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-12.10	0	0.5	1	0.5	1	0.5	0.5	0.5	0.5
2DCF$_1$-12.11	0	0	0	1	0	0.5	0.5	0.5	0.5
2DCF$_1$-12.12	1	0.5	1	1	1	0.5	0	0	0
2DCF$_1$-12.13	0	0.5	1	0.5	0	0.5	0	0	0
2DCF$_1$-12.14	0	0.5	1	1	1	0.5	0	0	0
2DCF$_1$-12.15	0	0.5	0	1	0.5	0.5	0	0	0
2DCF$_1$-12.16	0	0	1	1	0	0.5	0.5	0.5	0.5
2DCF$_1$-12.17	0	0.5	1	0.5	0.5	0.5	0	0	0
2DCF$_1$-12.18	0	0.5	1	1	1	0.5	0	0	0
2DCF$_1$-12.19	0	0	1	0.5	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-12.20	0	0	1	0.5	1	0.5	0	0.5	0.5
2DCF$_1$-12.21	0	0	1	1	0.5	0.5	0	0	0
DCF$_1$-51.16	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_1$-50.4	1	1	0	0.5	0.5	1	0.5	0.5	0.5
2DCF$_1$-20.1	1	1	0	0.5	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-20.2	0	1	0	0.5	0.5	0.5	0	0.5	0.5
2DCF$_1$-20.3	1	1	0	1	0.5	1	0.5	0.5	0.5
2DCF$_1$-20.4	1	1	1	0.5	0.5	1	0	0	0

Rusts and FHB resistance molecular marker data generated for the 2DCF$_1$, DCF$_2$ and FMF$_3$ populations (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF$_1$-50.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_1$-51.24	1	1	0	0.5	0.5	0.5	0.5	1	1
2DCF$_1$-28.1	1	1	0	0	0.5	0.5	0.5	1	1
2DCF$_1$-28.2	1	0.5	1	0	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-28.3	1	1	1	0.5	1	0.5	0.5	1	1
2DCF$_1$-28.4	0	0.5	1	0	1	0.5	0.5	1	1
2DCF$_1$-28.5	1	1	1	0	1	0.5	0.5	0.5	0.5
2DCF$_1$-28.6	0	0.5	1	1	0.5	0	0.5	0.5	0.5
2DCF$_1$-28.7	0	0.5	1	1	0.5	0	0.5	0.5	0.5
2DCF$_1$-28.8	1	1	1	0.5	0.5	0	0.5	1	1
2DCF$_1$-28.9	0	0.5	1	1	0.5	0.5	0.5	1	1
2DCF$_1$-28.10	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-28.11	0	0.5	1	1	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-28.12	1	1	1	0	0.5	1	0.5	1	1
2DCF$_1$-28.13	0	1	0	0	0.5	1	0.5	1	1
2DCF$_1$-28.14	0	0.5	1	0.5	1	1	0.5	1	1
2DCF$_1$-28.15	1	1	1	1	0	0	0.5	0.5	0.5
2DCF$_1$-28.16	0	1	1	0	0.5	0.5	0.5	1	1
2DCF$_1$-28.17	0	1	1	0	1	1	1	1	1
2DCF$_1$-28.18	0	1	1	1	1	0.5	0.5	1	1
2DCF$_1$-28.19	0	1	1	0	1	0.5	0.5	1	1
2DCF$_1$-28.20	0	0.5	1	0.5	0.5	1	0.5	0.5	0.5
DCF$_1$-18.30	1	1	1	0.5	0.5	0.5	0.5	1	1
DCF$_1$-40.10	0	0	0	0.5	0.5	0.5	0.5	1	1
2DCF$_1$-30.1	0	0.5	1	0	0.5	0	0.5	1	1
2DCF$_1$-30.2	0	0.5	0	0.5	0.5	0.5	0.5	1	1
2DCF$_1$-30.3	0	0.5	0	1	0.5	0.5	0.5	1	1
2DCF$_1$-30.4	1	0.5	0	1	0	1	0.5	1	1
2DCF$_1$-30.5	0	0.5	0	0.5	0.5	0.5	0.5	1	1
2DCF$_1$-30.6	0	0.5	1	0.5	0	0.5	1	1	1
2DCF$_1$-30.7	0	0.5	0	1	0.5	1	1	1	1
2DCF$_1$-30.8	0	0.5	1	0.5	0.5	0	0.5	1	1
2DCF$_1$-30.9	0	0.5	0	0.5	0.5	1	1	1	1

Rusts and Fusarium head blight resistance molecular marker data generated for the 2DCF$_1$, DCF$_2$ and FMF$_3$ populations (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF$_1$-18.30	1	1	1	0.5	0.5	0.5	0.5	1	1
DCF$_1$-54.10	1	1	1	0.5	0.5	0	0.5	0.5	0.5
2DCF$_1$-31.1	0	1	1	0	1	0	0.5	0.5	0.5
2DCF$_1$-31.2	0	1	1	0	0.5	0	0.5	0.5	0.5
2DCF$_1$-31.3	0	1	1	0.5	1	0	0.5	1	1
2DCF$_1$-31.4	1	1	1	1	1	0	0.5	0.5	0.5
2DCF$_1$-31.6	1	1	1	0	1	0	0.5	1	1
2DCF$_1$-31.7	1	1	1	0	1	0	0.5	1	1
2DCF$_1$-31.8	1	1	1	0	1	0	0.5	0.5	0.5
2DCF$_1$-31.9	0	1	1	0.5	1	0	0.5	0.5	0.5
2DCF$_1$-31.10	0	1	1	0	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-31.11	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
2DCF$_1$-31.12	1	1	1	0.5	0	0.5	1	1	1
2DCF$_1$-31.13	1	1	1	0	0.5	0	0.5	0.5	0.5
2DCF$_1$-31.14	1	1	1	1	0	0	0.5	0.5	0.5
2DCF$_1$-31.15	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_1$-59.6	0	0	0	0.5	0.5	1	1	0.5	0.5
FMF$_2$-60.7.78	1	1	1	0.5	1	0.5	0.5	0.5	0.5
2DCF$_1$-32.1	0	0.5	1	0	1	1	1	1	1
DCF$_1$-54.1	1	1	0	0.5	0.5	1	0.5	1	1
DCF$_1$-18.30	1	1	1	0.5	0.5	0.5	0.5	1	1
2DCF$_1$-34.2	0	1	1	1	0.5	1	0.5	1	1
2DCF$_1$-34.3	0	1	1	0.5	1	0.5	0.5	1	1
2DCF$_1$-34.4	1	1	1	0	0.5	1	0.5	1	1
2DCF$_1$-34.5	0	1	1	0.5	0	0.5	1	1	1
2DCF$_1$-34.6	0	1	1	1	1	1	1	1	1
2DCF$_1$-34.7	0	1	1	1	1	0.5	1	1	1
2DCF$_1$-34.8	0	1	1	1	0.5	0.5	0.5	1	1
2DCF$_1$-34.9	0	1	1	0.5	0.5	0.5	0.5	1	1
2DCF$_1$-34.10	1	1	0	0.5	0	1	0.5	1	1
DCF$_1$-18.30	1	1	1	0.5	0.5	0.5	0.5	1	1
DCF$_2$-18.30.1	1	1	1	0	0	0.5	0.5	1	1
DCF$_2$-18.30.2	0	1	1	0	1	0	1	1	1

Rusts and Fusarium head blight resistance molecular marker data generated for the 2DCF$_1$, DCF$_2$ and FMF$_3$ populations (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF$_2$-18.30.3	0	1	1	1	1	0	1	1	1
DCF$_2$-18.30.4	0	1	1	1	0.5	0.5	1	1	1
DCF$_2$-18.30.5	0	1	1	0.5	1	0	1	1	1
DCF$_2$-18.30.6	0	1	1	0.5	0.5	0.5	1	1	1
DCF$_2$-18.30.7	0	1	1	0.5	1	0	0.5	1	1
DCF$_2$-18.30.8	0	1	0	0	1	0.5	0.5	1	1
DCF$_2$-18.30.9	1	1	0	1	1	0.5	0.5	1	1
DCF$_2$-18.30.10	0	1	1	0.5	1	0	0.5	1	1
DCF$_2$-18.30.11	0	1	1	0.5	0.5	0	0.5	1	1
DCF$_2$-18.30.12	0	1	0	0.5	0.5	0.5	0.5	1	1
DCF$_2$-18.30.13	1	1	1	0.5	0	0.5	0.5	1	1
DCF$_2$-18.30.14	0	1	0	0	0.5	0.5	1	1	1
DCF$_2$-18.30.15	0	1	0	1	1	0.5	0.5	1	1
DCF$_2$-18.30.16	0	1	1	0.5	1	0.5	0.5	1	1
DCF$_2$-18.30.17	0	1	1	0	1	0	0.5	1	1
DCF$_2$-18.30.18	0	1	1	1	0.5	0.5	0.5	1	1
DCF$_2$-18.30.19	1	1	0	0.5	1	1	0.5	1	1
DCF$_2$-18.30.20	0	1	1	0.5	0	0.5	1	1	1
DCF$_2$-18.30.21	0	1	1	0	1	0.5	1	1	1
DCF$_2$-18.30.22	0	1	0	0	0.5	0.5	1	1	1
DCF$_2$-18.30.23	1	1	0	0.5	1	1	0.5	1	1
DCF$_2$-18.30.24	0	1	1	1	1	0.5	0.5	1	1
DCF$_2$-18.30.25	0	1	1	0	0.5	0.5	0.5	1	1
DCF$_1$-50.9	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_2$-50.9.1	1	0.5	1	0.5	0.5	1	0.5	1	1
DCF$_2$-50.9.2	1	1	1	0.5	1	0.5	0.5	0.5	0.5
DCF$_2$-50.9.3	0	0	1	0.5	0.5	0.5	0	0	0
DCF$_2$-50.9.4	0	0	1	0	1	0.5	1	1	1
DCF$_2$-50.9.5	0	0.5	1	0.5	0.5	0	0.5	0.5	0.5
DCF$_2$-50.9.6	0	0	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_2$-50.9.7	1	0.5	1	0	0	1	0.5	0.5	0.5
DCF$_2$-50.9.8	0	0.5	1	0.5	1	0.5	0.5	0.5	0.5
DCF$_2$-50.9.9	1	0.5	1	0.5	0.5	0.5	0	0	0

Rusts and Fusarium head blight resistance molecular marker data generated for the 2DCF$_1$, DCF$_2$ and FMF$_3$ populations (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
DCF$_2$-50.9.10	0	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_2$-50.9.11	0	0.5	1	0.5	0.5	0.5	0.5	1	1
DCF$_2$-50.9.12	0	0.5	1	0.5	0	0.5	0.5	0.5	0.5
DCF$_2$-50.9.13	1	1	1	0.5	1	0.5	0.5	0.5	0.5
DCF$_2$-50.9.14	1	0.5	1	0.5	1	0.5	0.5	0.5	0.5
DCF$_2$-50.9.15	1	0.5	1	0	0	0.5	0.5	0.5	0.5
DCF$_1$-51.16	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_2$-51.16.1	0	1	0	1	1	1	0.5	0.5	0.5
DCF$_2$-51.16.2	1	1	1	1	0.5	0	1	1	1
DCF$_2$-51.16.3	0	1	1	1	0	0.5	0.5	0.5	0.5
DCF$_2$-51.16.4	0	1	0	1	0.5	0.5	0	0	0
DCF$_2$-51.16.5	0	1	1	0.5	0.5	0.5	0	0	0
DCF$_2$-51.16.6	0	1	1	0.5	0.5	0.5	1	1	1
DCF$_2$-51.16.7	1	1	0	1	0.5	0.5	0.5	0.5	0.5
DCF$_2$-51.16.8	0	1	1	0.5	0.5	0	0.5	0.5	0.5
DCF$_2$-51.16.9	0	1	1	1	0.5	0	1	1	1
DCF$_2$-51.16.10	0	1	0	0.5	0.5	0.5	0.5	0.5	0.5
DCF$_2$-51.16.11	1	1	0	1	0.5	1	0.5	1	1
DCF$_2$-51.16.12	0	1	1	1	0.5	0	0.5	0.5	0.5
DCF$_2$-51.16.13	1	1	0	1	0	1	0.5	0.5	0.5
DCF$_2$-51.16.14	1	1	1	0.5	0.5	0	0	0	0
DCF$_2$-51.16.15	1	1	1	0.5	0	0.5	0	0	0
FMF$_2$-60.7.78	1	1	1	0.5	1	0.5	0.5	0.5	0.5
FMF$_3$-60.7.78.1	0	1	1	0.5	1	0.5	0.5	0.5	0.5
FMF$_3$-60.7.78.2	0	1	0	0	1	0.5	0.5	0	0
FMF$_3$-60.7.78.3	0	1	1	0.5	1	0	0.5	1	1
FMF$_3$-60.7.78.4	0	1	1	0.5	1	0.5	0.5	1	1
FMF$_3$-60.7.78.5	0	1	1	0	1	0	0.5	0.5	0.5
FMF$_3$-60.7.78.6	0	1	0	0.5	1	0.5	0.5	0.5	0.5
FMF$_3$-60.7.78.7	0	1	0	0.5	1	0.5	0.5	0.5	0.5
FMF$_3$-60.7.78.8	1	1	0	0	1	0.5	0	0	0
FMF$_3$-60.7.78.9	0	1	1	1	1	0	0	0	0
FMF$_3$-60.7.78.10	0	1	1	0.5	1	0	0.5	1	1

Rusts and Fusarium head blight resistance molecular marker data generated for the 2DCF$_1$, DCF$_2$ and FMF$_3$ populations (continued)

Line name	STSLr19$_{130}$ (*Lr19*)[1]	Cssfr5 (*Lr34/Yr 18/Sr57*)[2]	csSr2 (*Sr2*/*Yr30*)[1]	Sr26#43 and BE518379 (*Sr26*)[2]	Sr39 and BE500705 (*Sr39*)[2]	Umn10 (*Fhb1*)[2]	Gwm156 (*Qfhs.ifa-5A-2*)[2]	Gwm293 (*Qfhs.ifa-5A-1*)[2]	Gwm304 (*Qfhs.ifa-5A-1*)[2]
FMF$_3$-60.7.78.11	0	1	1	1	1	0.5	0	0	0
FMF$_3$-60.7.78.12	0	1	1	0.5	1	0.5	0.5	1	1
FMF$_3$-60.7.78.13	1	1	1	0.5	1	0	0.5	1	1
FMF$_3$-60.7.78.14	1	1	1	0.5	1	0.5	0	0	0
FMF$_3$-60.7.78.15	1	1	0	0.5	1	0.5	0.5	0	0

Lines highlighted in grey are the DCF$_1$ or the FMF$_2$ lines selected for developing crosses or self-pollination. [1]Markers linked to *Lr19* and *Sr2*/*Yr30* are dominant markers and were scored as present (1) and absent (0); [2]Co-dominant markers were scored as homozygous resistant (1), heterozygous resistant (0.5) and homozygous susceptible. (0).

Appendix G

Quality molecular marker data generated for the $2DCF_1$, DCF_2 and FMF_3 populations

Line name	Umn19[1]	MAR[1]	BxFp[1]	Umn25[1]	Umn26[1]	Glu-B3j[2]
DCF_1-50.27						
DCF_1-51.11						
$2DCF_1$-12.1	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.2	2*	7	7OE	2	12	0
$2DCF_1$-12.3	2*	7	7OE	2	12	0
$2DCF_1$-12.4	2*	7	7OE	2	12	0
$2DCF_1$-12.5	2*	7	7OE	2	12	0
$2DCF_1$-12.6	2*	7	7OE	2	12	0
$2DCF_1$-12.7	2*	7	7OE	2	12	0
$2DCF_1$-12.8	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.9	2*	7	≠	2	12	0
$2DCF_1$-12.10	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.11	2*	7	7OE	2	12	0
$2DCF_1$-12.12	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.13	2*	7	≠	2	12	0
$2DCF_1$-12.14	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.15	2*	7	7OE	2	12	0
$2DCF_1$-12.16	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.17	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.18	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.19	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.20	2*	7	≠/7OE	2	12	0
$2DCF_1$-12.21	2*	7	≠/7OE	2	12	0
DCF_1-51.16						
DCF_1-50.4						
$2DCF_1$-20.1	2*	7	≠	2/5	12/10	0
$2DCF_1$-20.2	2*	7	≠	2/5	12/10	0
$2DCF_1$-20.3	2*	7	≠	2/5	12/10	0
$2DCF_1$-20.4	2*	7	≠	2/5	12/10	0
DCF_1-50.9						
DCF_1-51.24						
$2DCF_1$-28.1	2*	7	≠/7OE	2	12	0
$2DCF_1$-28.2	2*	7	≠	2	12	0
$2DCF_1$-28.3	2*	7	7OE	2	12	0
$2DCF_1$-28.4	2*	7	≠	2	12	0
$2DCF_1$-28.5	2*	7	≠	2/5	12/10	0
$2DCF_1$-28.6	2*	7	7OE	2/5	12/10	0
$2DCF_1$-28.7	2*	7	≠/7OE	2	12	0
$2DCF_1$-28.8	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-28.9	2*	7	≠	2/5	12/10	0
$2DCF_1$-28.10	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-28.11	2*	7	≠	5	10	0
$2DCF_1$-28.12	2*	7	≠	2/5	12/10	0
$2DCF_1$-28.13	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-28.14	2*	7	≠/7OE	2	12	0
$2DCF_1$-28.15	2*	7	≠	5	10	0
$2DCF_1$-28.16	2*	7	≠	2/5	12/10	0
$2DCF_1$-28.17	2*	7	≠/7OE	5	10	0
$2DCF_1$-28.18	2*	7	≠/7OE	5	10	0
$2DCF_1$-28.19	2*	7	≠	2/5	12/10	0
$2DCF_1$-28.20	2*	7	7OE	2/5	12/10	0

Quality molecular marker data generated for the $2DCF_1$, DCF_2 and FMF_3 populations (continued)

Line name	Umn19[1]	MAR[1]	BxFp[1]	Umn25[1]	Umn26[1]	Glu-B3j[2]
DCF_1-18.30 DCF_1-40.10						
$2DCF_1$-30.1	2*	7	≠/7OE	5	10	0
$2DCF_1$-30.2	2*	7	≠/7OE	5	10	0
$2DCF_1$-30.3	2*	7	≠/7OE	5	10	0
$2DCF_1$-30.4	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-30.5	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-30.6	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-30.7	2*	7	≠/7OE	5	10	0
$2DCF_1$-30.8	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-30.9	2*	7	≠/7OE	5	10	0
DCF_1-18.30 DCF_1-54.10						
$2DCF_1$-31.1	2*	7/17	≠	5	10	0
$2DCF_1$-31.2	2*	7	≠/7OE	5	10	0
$2DCF_1$-31.3	2*	7/17	≠	2/5	12/10	0
$2DCF_1$-31.4	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-31.6	2*	7/17	≠	2/5	12/10	0
$2DCF_1$-31.7	2*	7	≠/7OE	5	10	0
$2DCF_1$-31.8	2*	7/17	≠	5	10	0
$2DCF_1$-31.9	2*	7	≠/7OE	5	10	0
$2DCF_1$-31.10	2*	7	≠/7OE	5	10	0
$2DCF_1$-31.11	2*	7/17	≠	2/5	12/10	0
$2DCF_1$-31.12	2*	7	≠/7OE	5	10	0
$2DCF_1$-31.13	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-31.14	2*	7	≠/7OE	5	10	0
$2DCF_1$-31.15	2*	7/17	≠	2/5	12/10	0
DCF_1-59.6 FMF_2-60.7.78						
$2DCF_1$-32.1	2*	7	≠/7OE	5	10	0
DCF_1-54.1 DCF_1-18.30						
$2DCF_1$-34.2	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-34.3	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-34.4	2*	7/17	≠	2/5	12/10	0
$2DCF_1$-34.5	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-34.6	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-34.7	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-34.8	2*	7	≠/7OE	2/5	12/10	0
$2DCF_1$-34.9	2*	7/17	≠	2/5	12/10	0
$2DCF_1$-34.10	2*	7/17	≠	2/5	12/10	0
DCF_1-18.30						
DCF_2-18.30.1	2*	7	≠	5	10	0
DCF_2-18.30.2	2*	7	≠	5	10	0
DCF_2-18.30.3	2*	7	≠	5	10	0
DCF_2-18.30.4	2*	7	≠	5	10	0
DCF_2-18.30.5	2*	7	≠	5	10	0
DCF_2-18.30.6	2*	7	≠	5	10	0
DCF_2-18.30.7	2*	7	≠	5	10	0
DCF_2-18.30.8	2*	7	≠	5	10	0
DCF_2-18.30.9	2*	7	≠	5	10	0
DCF_2-18.30.10	2*	7	≠	5	10	0
DCF_2-18.30.11	2*	7	≠	5	10	0
DCF_2-18.30.12	2*	7	≠	5	10	0
DCF_2-18.30.13	2*	7	≠	5	10	0
DCF_2-18.30.14	2*	7	≠	5	10	0

Quality molecular marker data generated for the $2DCF_1$, DCF_2 and FMF_3 populations (continued)

Line name	Umn19[1]	MAR[1]	BxFp[1]	Umn25[1]	Umn26[1]	Glu-B3j[2]
DCF_2-18.30.15	2*	7	≠	5	10	0
DCF_2-18.30.16	2*	7	≠	5	10	0
DCF_2-18.30.17	2*	7	≠	5	10	0
DCF_2-18.30.18	2*	7	≠	5	10	0
DCF_2-18.30.19	2*	7	≠	5	10	0
DCF_2-18.30.20	2*	7	≠	5	10	0
DCF_2-18.30.21	2*	7	≠	5	10	0
DCF_2-18.30.22	2*	7	≠	5	10	0
DCF_2-18.30.23	2*	7	≠	5	10	0
DCF_2-18.30.24	2*	7	≠	5	10	0
DCF_2-18.30.25	2*	7	≠	5	10	0
DCF_1-50.9						
DCF_2-50.9.1	2*	7	≠/7OE	2/5	12/10	0
DCF_2-50.9.2	2*	7	7OE	2	12	0
DCF_2-50.9.3	2*	7	≠	2	12	0
DCF_2-50.9.4	2*	7	7OE	2/5	12/10	0
DCF_2-50.9.5	2*	7	7OE	2/5	12/10	0
DCF_2-50.9.6	2*	7	≠/7OE	2/5	12/10	0
DCF_2-50.9.7	2*	7	≠	2	12	0
DCF_2-50.9.8	2*	7	7OE	2	12	0
DCF_2-50.9.9	2*	7	≠/7OE	2/5	12/10	0
DCF_2-50.9.10	2*	7	≠/7OE	2/5	12/10	0
DCF_2-50.9.11	2*	7	7OE	2/5	12/10	0
DCF_2-50.9.12	2*	7	≠	2/5	12/10	0
DCF_2-50.9.13	2*	7	7OE	2/5	12/10	0
DCF_2-50.9.14	2*	7	≠/7OE	5	10	0
DCF_2-50.9.15	2*	7	7OE	2/5	12/10	0
DCF_1-51.16						
DCF_2-51.16.1	2*	7	≠	5	10	0
DCF_2-51.16.2	2*	7	≠	5	10	0
DCF_2-51.16.3	2*	7	≠	5	10	0
DCF_2-51.16.4	2*	7	≠	5	10	0
DCF_2-51.16.5	2*	7	≠	5	10	0
DCF_2-51.16.6	2*	7	≠	5	10	0
DCF_2-51.16.7	2*	7	≠	5	10	0
DCF_2-51.16.8	2*	7	≠	5	10	0
DCF_2-51.16.9	2*	7	≠	5	10	0
DCF_2-51.16.10	2*	7	≠	5	10	0
DCF_2-51.16.11	2*	7	≠	5	10	0
DCF_2-51.16.12	2*	7	≠	5	10	0
DCF_2-51.16.13	2*	7	≠	5	10	0
DCF_2-51.16.14	2*	7	≠	5	10	0
DCF_2-51.16.15	2*	7	≠	5	10	0
FMF_2-60.7.78						
FMF_3-60.7.78.1	2*	7	7OE	2/5	12/10	0
FMF_3-60.7.78.2	2*	7	7OE	2	12	0
FMF_3-60.7.78.3	2*	7	7OE	2/5	12/10	0
FMF_3-60.7.78.4	2*	7	7OE	5	10	0
FMF_3-60.7.78.5	2*	7	7OE	5	10	0
FMF_3-60.7.78.6	2*	7	7OE	2/5	12/10	0
FMF_3-60.7.78.7	2*	7	7OE	2/5	12/10	0
FMF_3-60.7.78.8	2*	7	7OE	2	12	0
FMF_3-60.7.78.9	2*	7	7OE	2/5	12/10	0
FMF_3-60.7.78.10	2*	7	7OE	2	12	0
FMF_3-60.7.78.11	2*	7	7OE	2/5	12/10	0
FMF_3-60.7.78.12	2*	7	7OE	2/5	12/10	0

Quality molecular marker data generated for the $2DCF_1$, DCF_2 and FMF_3 populations (continued)

Line name	Umn19[1]	MAR[1]	BxFp[1]	Umn25[1]	Umn26[1]	Glu-B3j[2]
FMF_3-60.7.78.13	2*	7	7OE	2/5	12/10	0
FMF_3-60.7.78.14	2*	7	7OE	2/5	12/10	0
FMF_3-60.7.78.15	2*	7	7OE	5	10	0

Lines highlighted in grey are the DCF_1 or the FMF_2 lines selected for developing crosses or self-pollination. [1]Markers were scored based on the HMW-GS allele (2*-*Glu-Ax2**; 7-*Glu-Bx7*; 7OE-over-expressed *Glu-Bx7*; ≠-do not contain *Glu-Bx7+By8**; 2-*Glu-Dx2*; 5-*Glu-Dx5*; 12-*Glu-Dy12*; 10-*Glu-Dy10 present*; [2]Markers were scored as present (1) and absent (0).

Appendix H

Quantitative polymerase chain reaction data of the Fusarium head blight inoculated samples

Cultivar/line	Spray inoculation *β-tubilin:TEF* expression ratio							
	Chaff	SE	Rachis	SE	Seed	SE	Total	SE
Kariega	5.47	0.37	5.82	0.44	25.47	2.57	36.76	3.38
Avocet*YrSP*	2.17	0.35	1.31	0.12	3.70	0.72	7.18	1.19
Blade	2.74	0.35	23.02	2.96	8.85	0.53	34.61	3.84
CS*Lr19*-149-299	3.78	0.73	43.98	0.11	2.42	0.38	50.18	1.22
Kingbird	1.57	0.17	2.48	0.31	1.05	0.27	5.10	0.75
2S#/163	4.51	0.39	12.24	0.81	9.60	1.64	26.35	2.84
CM-82036	1.23	0.69	0.54	0.16	1.08	0.23	2.85	1.08
Krokodil	4.61	0.14	11.37	0.18	6.84	0.24	22.82	0.56
Sumai3	0.16	0.02	0.18	0.01	0.02	0.00	0.36	0.03
Frontana	1.30	0.38	1.39	0.96	2.12	1.64	4.81	2.98
SST-835	5.05	0.01	23.20	0.01	3.07	0.00	31.32	0.02
BC_2F_6-2.3	3.24	0.30	0.41	0.01	0.40	0.09	4.05	0.40
BC_2F_6-1.1	5.42	0.35	2.67	0.19	8.72	1.45	16.81	1.99
BC_2F_6-13.2	0.27	0.30	2.62	0.57	0.72	0.14	3.61	1.01
BC_2F_6-6.3	0.01	0.00	0.00	0.00	0.58	0.05	0.59	0.05
BC_2F_6-32.2	0.28	0.02	0.10	0.01	0.02	0.01	0.40	0.04
BC2F6-12.3	0.27	0.04	0.07	0.01	0.09	0.03	0.43	0.08
BC_2F_6-37.1	0.12	0.01	0.00	0.00	0.07	0.01	0.20	0.02
BC_2F_6-25.2	6.19	0.29	27.62	1.61	7.30	0.85	41.11	2.75

TEF-Translation elongation factor; SE-Standard error.

Quantitative polymerase chain reaction data of the Fusarium head blight inoculated samples (continued)

Cultivar/line	Point inoculation *β-tubilin:TEF* expression ratio							
	Chaff	SE	Rachis	SE	Seed	SE	Total	SE
Kariega	0.50	0.05	0.79	0.14	0.60	0.10	1.90	0.29
Avocet*YrSP*	1.20	0.13	1.18	0.16	3.13	0.78	5.51	1.07
Blade	1.61	0.11	0.78	0.12	0.20	0.02	2.59	0.25
CS*Lr19*-149-299	2.10	0.43	3.53	0.37	2.76	0.55	8.39	1.35
Kingbird	2.78	0.3	1.10	0.07	7.20	1.22	11.07	1.59
2S#/163	1.35	0.24	0.84	0.08	0.02	0.00	2.22	0.32
CM-82036	0.51	0.03	0.02	0.00	0.01	0.01	0.54	0.04
Krokodil	0.70	0.17	1.25	0.29	7.29	0.66	9.25	1.12
Sumai3	0.54	0.08	1.42	0.14	0.17	0.01	2.13	0.23
Frontana	0.22	0.03	0.11	0.02	0.93	0.19	1.27	0.24
SST-835	7.02	0.83	5.73	0.78	13.33	1.24	26.08	2.85
BC_2F_6-2.3	0.39	0.03	0.53	0.03	0.32	0.05	1.23	0.11
BC_2F_6-1.1	2.05	0.24	3.29	0.49	19.13	2.33	24.46	3.06
BC_2F_6-13.2	1.28	0.17	2.49	0.37	8.40	1.07	12.17	1.61
BC_2F_6-6.3	2.47	0.27	5.33	0.84	9.17	0.59	16.97	1.70
BC_2F_6-32.2	1.10	0.11	0.11	0.01	0.05	0.01	1.25	0.13
BC2F6-12.3	0.21	0.03	0.96	0.05	0.16	0.01	1.33	0.09
BC_2F_6-37.1	0.48	0.08	0.98	0.15	0.76	0.11	2.22	0.34
BC_2F_6-25.2	1.42	0.20	2.45	0.35	2.86	0.57	6.73	1.12

TEF-Translation elongation factor; SE-Standard error.

Quantitative polymerase chain reaction data of the Fusarium head blight inoculated samples (continued)

Cultivar/line	Spray inoculation *Tri5:TEF* expression ratio							
	Chaff	SE	Rachis	SE	Seed	SE	Total	SE
Kariega	1.02	0.05	0.81	0.12	2.91	0.29	4.74	0.46
Avocet*YrSP*	1.12	0.21	0.68	0.05	1.09	0.21	2.89	0.47
Blade	1.25	0.21	0.74	0.12	1.83	0.14	3.82	0.47
CS*Lr19*-149-299	1.36	0.24	1.50	0.21	1.23	0.08	4.09	0.53
Kingbird	0.92	0.10	1.13	0.16	1.64	0.12	3.69	0.38
2S#/163	0.91	0.14	1.49	0.14	1.64	0.32	4.04	0.60
CM-82036	0.94	0.13	0.75	0.04	1.12	0.08	2.81	0.25
Krokodil	0.70	0.08	0.75	0.09	0.58	0.05	2.03	0.22
Sumai3	0.81	0.10	1.25	0.21	0.77	0.09	2.83	0.40
Frontana	1.23	0.18	1.06	0.09	1.53	0.21	3.82	0.48
SST-835	1.04	0.18	1.83	0.05	0.87	0.21	3.74	0.44
BC_2F_6-2.3	0.97	0.05	0.58	0.04	0.92	0.10	2.47	0.19
BC_2F_6-1.1	0.93	0.09	0.80	0.09	1.32	0.22	3.05	0.40
BC_2F_6-13.2	0.84	0.11	1.53	0.31	0.50	0.11	2.87	0.53
BC_2F_6-6.3	0.87	0.16	0.97	0.18	0.66	0.09	2.50	0.43
BC_2F_6-32.2	0.94	0.06	1.02	0.06	0.26	0.02	2.22	0.14
BC2F6-12.3	0.77	0.14	0.82	0.14	0.44	0.05	2.03	0.33
BC_2F_6-37.1	0.86	0.08	1.56	0.17	1.07	0.17	3.49	0.42
BC_2F_6-25.2	1.65	0.23	1.13	0.15	0.93	0.07	3.71	0.45

TEF-Translation elongation factor; SE-Standard error.

Quantitative polymerase chain reaction data of the Fusarium head blight inoculated samples (continued)

Cultivar/line	Point inoculation *Tri5:TEF* expression ratio							
	Chaff	SE	Rachis	SE	Seed	SE	Total	SE
Kariega	1.10	0.09	1.09	0.24	1.03	0.03	3.22	0.36
Avocet*YrSP*	0.97	0.11	0.81	0.10	0.79	0.08	2.57	0.29
Blade	1.20	0.08	1.13	0.08	1.41	0.21	3.74	0.37
CS*Lr19*-149-299	0.99	0.10	1.31	0.12	1.19	0.32	3.49	0.54
Kingbird	1.53	0.13	1.11	0.08	2.11	0.47	4.75	0.68
2S#/163	0.74	0.09	0.82	0.14	1.41	0.12	2.97	0.35
CM-82036	1.06	0.13	1.38	0.11	0.81	0.07	3.25	0.31
Krokodil	1.11	0.28	0.56	0.05	1.16	0.27	2.83	0.60
Sumai3	1.08	0.03	1.18	0.09	0.83	0.07	3.09	0.19
Frontana	0.85	0.04	0.66	0.07	1.13	0.19	2.64	0.30
SST-835	1.16	0.20	1.46	0.19	1.05	0.11	3.67	0.50
BC_2F_6-2.3	0.58	0.11	0.75	0.02	0.77	0.03	2.10	0.16
BC_2F_6-1.1	1.24	0.08	0.88	0.08	1.23	0.18	3.35	0.34
BC_2F_6-13.2	0.90	0.05	0.77	0.06	1.71	0.13	3.38	0.24
BC_2F_6-6.3	0.97	0.08	1.72	0.39	1.66	0.16	4.35	0.63
BC_2F_6-32.2	0.96	0.08	0.97	0.08	0.62	0.08	2.55	0.24
BC2F6-12.3	1.14	0.13	0.84	0.11	0.45	0.08	2.43	0.32
BC_2F_6-37.1	0.91	0.09	0.98	0.17	0.60	0.05	2.49	0.31
BC_2F_6-25.2	0.94	0.12	0.94	0.14	0.62	0.07	2.50	0.33

TEF-Translation elongation factor; SE-Standard error.