Marker-assisted backcross breeding for Fusarium head blight resistance in South African wheat

by

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Thesis submitted in fulfilment of the requirements for the degree Philosophiae Doctor in Plant Breeding in the Department of Plant Sciences, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa

June 2014

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Declaration

"I, Scott Lloyd Sydenham, do hereby declare that the thesis hereby submitted by me for the degree Philosophiae Doctor in Plant Breeding at the University of the Free State represents my own independent work and has not previously been submitted by me at another University/faculty.

I furthermore cede copyright of this thesis in favour of the University of the Free State."

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Scott Lloyd Sydenham Date

Motivational poem

by

Nathan Watson

It Will Come

When life's largest pressures leave you struck dumb,
Just search for an answer; the solution will come.
When a tragedy occurs leaving you feeling numb,
Just wait for your health; the strength will come.

When everyone relies on you and there is no way you can see,
Trust your mind to think with time; patience is the key.
When you have made it where others always flee,
Just wait to gather courage; soon you'll be where you want to be.

When it is nearing the end and you're in need,
Muster up your courage; endurance will lead.

IT WILL COME

This is just an example of what life and a PhD can put you through; the amazing life lessons and skills you learn along the way and in the end only patience and endurance pay off.

Dedication

I would like to dedicate this PhD thesis to my family, without whom this would not be possible.

Thank for you for your unending love, support and belief in me.

And finally in honour of my late Dad, Stuart

(10th May 1941 - 29th December 2006),

this is for you Dad, we all miss you!

"Family is where life begins and love never ends."

- Unknown

Acknowledgements

To my wife and best friend, Cindy-Lee, thank you for being so supportive, patient, loving, caring and motivational. Thank you for your unique ability to be able to make me laugh when needed. I found the strength to see this through in the belief you had in me that it can be done as well as your listening ear, unconditional love, motivation and encouragement. Thank you for taking care of our son Spencer during the long nights and hard weekends.

To my son Spencer, thank you for your love and making me laugh and smile each day.

Thank you to my Mom for her unbelievable constant motivation and encouragement which kept me going when times got tough. Your continual love and moral support each day got me through all the difficulties. I know it has been hard without dad the last few years.

Mom and Dad Pool thank you for giving me the support, love and encouragement to finalise this study. Thank you for giving me your amazing daughter Cindy-Lee.

Margie and Harry thank you for your continual support and encouragement to me and my family. You both have always been like the grandparents that I never had.

Cassey thank you for your assistance and help with editing.

Adré thank you for your amazing friendship, support and encouragement throughout the study.

Sadie, thank you for your continual support and wiliness to listen at any time, always offering very sound advice on life.

A word of thanks goes to Ansori, Chrisna, Fred, Magriet and Rouxléne from Plant Breeding at the university.

A special thank you goes to the following people at ARC-SGI who offered their friendship, support and encouragement: Annelie, Barend, Chrissie, Cathy, Eben, Elri, Hestia, Nyiko, Robbie, Shunay and Vicki.

I would like to thank the Winter Cereal Trust for personal and project financial support.

I would like to thank the University of the Free State (UFS) for the use of facilities provided and the privilege to study.

I would like to thank the ARC-SGI for the personal financial support of my study the last two years.

An extended and very special thanks goes to both my promoters:

I thank Prof. Liezel Herselman whom I respect and admire for everything you have taught me over the years. We have travelled a long difficult path to get to this point. Thank you for your continual support, guidance and encouragement. A special thank you for your contributions and assistance in making this project a success. When I needed it, you were able to tell me the right words of motivation and encouragement to give me a needed confidence boost. Thank you for your professionalism and countless hours and hard work.

A thank you to Me Wilmarie Kriel for your plant pathology enthusiasm and advice and for my first introduction to FHB in a wheat field. Thank you for your valued contributions, continual support, belief in me and encouragement.

Lastly, I would like to thank the Lord God, for blessing me with the abilities to carry out this PhD study and especially for laying your supportive and protective hand over me and my family during trying times.

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15-ADON	15-Acetyl-deoxynivalenol
3-ADON	3-Acetyl-deoxynivalenol
AFLP	Amplified fragment length polymorphism
APS	Ammonium persulfate
ARC-SGI	Agricultural Research Council-Small Grain Institute
Barc	Beltsville Agricultural Research Center
BC	Backcross
bp	Base pair(s)
SCAR	Sequence characterised amplified region
CIMMYT	International Maize and Wheat Improvement Centre
cM	CentiMorgan
CTAB	Hexadecyltrimethylammonium bromide
DArT	Diversity arrays technology
DH	Double haploids
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DON	Deoxynivalenol
dpi	Days post-inoculation
EDTA	Ethylene-diaminetetraacetate
EtBr	Ethidium bromide
F_1	First generation
FDK	*Fusarium*-damaged kernels
Fg	*Fusarium graminearum*
FGSC	*Fusarium graminearum* species complex
FHB	Fusarium head blight
g	Gravitational force
GCPSR	Genealogical concordance phylogenetic species recognition
GGT	Geographical genotypes
Gwm	Gatersleben Wheat Microsatellite
HMW-GS	High molecular weight glutenin subunits
HDGP	Heterozygous donor genome percentage
HRGP	Homozygous recurrent genome percentage
LMW-GS	Low molecular weight glutenin subunits

LOD	Log-likelihood score
MAB	Marker-assisted breeding
MABC	Marker-assisted backcrossing
MAS	Marker-assisted selection
Mb	Millions of bases
MDP	Marker data points
$MgCl_2$	Magnesium chloride
NaCl	Sodium chloride
NGS	Next generation sequencing
NIV	Nivalenol
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
ppm	Parts per million
PSB	Potato sucrose broth
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RIP	Repeat induced point mutation
RPGP	Recurrent parent genome percentage
rpm	Revolutions per minute
r/s	Revolutions per second
SA	South Africa
SCAR	Sequenced-characterised amplified region
SDR	Segregation distortion regions
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
STS	Sequence tagged site
Taq	*Thermus aquaticus*
TBE	Tris-Cl/Boric acid/EDTA
TE	Tris-Cl/EDTA
TEMED	Tetramethylethylenediamine
Tris-Cl	Tris(hydroxymethyl) aminomethane
U	Unit(s)
UFS	University of the Free State
UNTAN	Tris-Cl/EDTA/Acetic acid buffer
USA	United States of America

UV	Ultraviolet
V	Volt(s)
v/v	Volume per volume
W	Watt(s)
Wmc	Wheat Microsatellite Consortium
w/v	Weight per volume

Chapter 1
General introduction

In our modern era there are growing concerns around the supply of enough food to an ever increasing global population, climate change, extreme weather phenomena and the unsustainable use of natural resources. All these factors will have a significant negative impact on food security, especially in vulnerable third-world countries (Brodt et al. 2011). Currently, there exists a growing need for global and national commitment to invest in crop improvement to ensure food security for the future. Without this needed commitment the negative impacts are likely to increase and may even lead to a global food shortfall which will lead to food price increases in the coming decades resulting in possible wide spread poverty and malnutrition (Shiferaw et al. 2013). A focus is needed on sustainable agriculture to increase crop production per land area by using natural resources more efficiently and responsibly to avoid losses in productive capacity. Given the time lag between research and development and widespread policy implementation, immediate action is required, both at national and international levels (Brodt et al. 2011).

Wheat (*Triticum aestivum* L.) can play a vital role in addressing some of these global concerns. Wheat is one of the most important cereals in the world and forms a critical part of human society. Over 220 million hectares of wheat are planted annually with a global production average of around 670 million tons, making it one of the most widely cultivated cereals in the world (Shiferaw et al. 2013). For the 2014-2015 season the global wheat production estimate is predicted to reach 703 million tons (FAO 2014). Importantly, more than 50% of the world's wheat is produced in developing countries. Stable and reliable wheat production at an affordable level is therefore paramount for regional political stability and global food security (Shiferaw et al. 2013).

Wheat in South Africa (SA) is the second most important crop grown across the country. The majority of the wheat planted in SA is dryland winter/intermediate type wheat and on average 20-25% is irrigated spring wheat. Wheat production has steadily been declining in SA over the last decade. Currently SA is a net wheat importer for the shortfall required to achieve the annual local demand of 2 million tons. This decline in production is primarily due to lower wheat prices, lack of government subsidy and interest, a decline in

the number of commercial farmers planting wheat, and in terms of climate change, risks related to diseases and pests.

There are a number of wheat diseases and pests that cause significant financial loss and result in increased input costs for SA farmers. Fusarium head blight (FHB), or head scab, is a destructive fungal disease of wheat (Foroud and Eudes 2009) and occurs throughout the world wherever wheat is grown. It was first noted in England in 1884 by WG Smith. FHB was first reported in SA in 1980, after which epidemic outbreaks of FHB were recorded in 1985, 1986 (Scott et al. 1988), 1994 (Scott and Smith 1995) and 2000 (Kriel and Pretorius 2008). Since 2000 the area planted to wheat has been declining steadily, reaching record lows in 2013-2014. However, FHB still remains a sporadic disease in SA that predominantly occurs on irrigated spring wheat. Control of this disease is further complicated as local farmers in SA tend to use maize-wheat crop rotations in combination with no-till practices that can harbour *Fusarium* inoculum and significantly increase the risk of FHB disease (Scott and Smith 1995; Kriel and Pretorius 2008).

FHB is caused by a number of trichothecene-producing species in the genus *Fusarium* (Foroud and Eudes 2009). The most common casual species around the world are members of the *Fusarium graminearum* (Schwabe) species complex (FGSC). *Fusarium graminearum* [anamorph*: Gibberella zeae* Schwein Petch] (Boshoff et al. 1998; Boutigny et al. 2011) from lineage 7 predominates in SA (Minnaar-Ontong 2011). FHB can cause significant yield losses of up to 70% under high inoculum pressure and favourable environmental conditions (Kriel and Pretorius 2008). A secondary problem is reduction in seed quality due to FHB disease infection and mycotoxin contamination. Wheat is susceptible to infection from early flowering until hard dough stage. The most favourable conditions for infection are prolonged periods of high humidity (48-72 h) and warm temperatures (20-25°C). FHB disease severity varies from year to year as it is depends on environmental conditions (McMullen et al. 1997; 2012).

Typical symptoms of the disease are the partial or full bleaching (whitening) of wheat heads. The *Fusarium* fungus can also cause infection in the stem (peduncle) directly under the infected wheat head. Often wheat kernels from infected heads are commonly called “tombstone” kernels as they can be shrivelled, lightweight and white to dull greyish in appearance (McMullen et al. 1997; 2012). These kernels can be contaminated with harmful mycotoxins such as deoxynivalenol (DON), also known as vomitoxin that is harmful to both humans and animals after consumption (Foroud and Eudes 2009). In the USA, and most of Europe, advisory levels for DON of 1 ppm in finished wheat products,

e.g. flour, bran and germ, have been put in place to protect humans and animals from mycotoxin exposure. However, in SA as well as Australia, New Zealand and Kenya there are no official advisory levels in place or enforced for DON levels (Kubo 2012). Importantly the presence or symptoms of FHB infection do not automatically mean that mycotoxins are present. Factors such as disease severity, species of *Fusarium*, aggressiveness and chemotype of an isolate and host plant resistance types can all influence mycotoxin levels (McMullen et al. 1997; 2012).

FHB is best managed by implementing/combining multiple management strategies such as host plant resistance, better tillage practices, crop rotations, staggered planting dates, fungicide treatment and biological control. Use of a single management strategy may not be effective or may even fail when environmental conditions favour severe disease development (McMullen et al. 2012). Control measures are limited especially in SA due to the fact that there are no official fungicides registered for the control of FHB on wheat. In general limited success has been achieved with chemical and biological control (Parry et al. 1995). Now in 2014, a fungicide resistant to a *F. graminearum* isolate was identified in the USA (Spolti et al. 2014). FHB host plant resistance is considered the most effective management strategy to be used in combination with better tillage practices, chemical or bio-control and crop rotation systems (Foroud and Eudes 2009).

Breeding for FHB host plant resistance has been one of the most focused areas of research and development in wheat for the last two decades worldwide (Parry et al. 1995). A number of initiatives have been undertaken in the USA, China and the European Union by large research groups to improve resistance levels in wheat cultivars. As a result, a number of FHB sources of resistance have been well documented, from different parts of the world, such as Brazil (Frontana; Steiner et al. 2004), China (Sumai 3; Anderson et al. 2001; Buerstmayr et al. 2002 and Wangshuibai; Ma et al. 2006), Europe and USA but these are often landraces or unadapted wheat varieties. Concerns surrounding breeding with exotic resistance sources such as these are the possible negative effects on yield, quality and other important agronomic traits that may be associated with FHB resistance. Mesterhazy (1995) was one of the first to describe two of the most commonly used types of FHB resistance, namely resistance to initial infection (Type I) and resistance to spread of disease symptoms within the wheat spike (Type II).

However, breeding for FHB resistance is labour intensive and difficult due to the complex nature of the trait. Since 2000, a number of important FHB resistance genes/quantitative

trait loci (QTL) present in non-adapted sources have been mapped and tagged with linked molecular markers (Buerstmayr et al. 2009). One of the most commonly used FHB sources of resistance in the world has been Sumai 3. In recent years especially molecular markers identified in Sumai 3 have been implemented successfully in breeding programmes to transfer targeted FHB resistance genes/QTL (Cuthbert et al. 2006). The most effective breeding method for FHB resistance has been the use of marker-assisted selection (MAS) in combination with strict phenotypic screening during required generations.

Currently in SA there are limited moderately-resistant or resistant commercial wheat cultivars available or marketed, but differences in tolerance levels have been previously noted (Scott et al. 1988; Minnaar-Ontong 2011). Local farmers will only consider the adoption of resistant varieties if yields are competitive with current cultivars as this will reduce the risk to the farmer. It is speculated if the area planted to wheat in SA increases once again to levels of those 15-20 years ago, in combination with a wet-humid season the potential for a wide-spread FHB epidemic is a real probability.

In SA there is a critical need for the development of wheat lines with improved FHB resistance levels while retaining high quality grain and maintaining market related competitive yields. The main objective of this study was to transfer targeted FHB resistance genes/QTL into the background of a SA spring wheat cultivar in a marker-assisted backcrossing (MABC) programme. A second and third objective were to observe and quantify the expression of FHB resistance genes/QTL in a SA cultivar background and to attempt to select for improved lines with the highest amount of recurrent parent genome percentage (RPGP) with the aim of retaining good bread making qualities.

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Chapter 2

Breeding for Fusarium head blight resistance

"An infamous and unrestrained cereal killer"

2.1 Sustainable agriculture for a food secure future

With the reality of an ever increasing global population, which is likely to plateau at nine billion people by 2050, the demand for food is increasing. Looking forward the world faces a complex challenge in matching the rapidly changing demand for food due to the increasing world population. Ways need to be found that are socially and environmentally sustainable and that ensure that people in developing countries are no longer malnourished and hungry (Godfray et al. 2010). Sustainable agriculture is defined as food production or development of products derived from plants or animals by using farming methods that safeguard the environment, the use of natural resources, public health, human communities and animal welfare. This enables the production of healthy food without compromising future generations' ability to do the same (Brodt et al. 2011). The modern challenge of food security requires the implementation of new methods for food production, storage, processing, distribution and accessibility (Godfray et al. 2010). All-encompassing these issues is the added difficulty the threat climate change and large climate shifts pose to food production (Godfray et al. 2010; Brodt et al. 2011). Currently crop improvement research is aimed at improving yields, disease resistance and quality in an attempt to increase world food production on the same area of arable land available or even less (Godfray et al. 2010). A multifaceted invested commitment, linked at both national and international levels, is required to successfully implement sustainable agriculture practices to prevent the loss in potential food production capacity (Godfray et al. 2010; Brodt et al. 2011). For important crops such as maize, rice and especially wheat, research is focused on more sustainable food production under biotic and abiotic environmental stresses for a more food secure future.

2.2 Wheat

Wheat is an economically important cereal among the 'big three' cereal crops of the world, with over 600-700 million tons being harvested every year (Shewry 2009; Brenchley et al. 2013). Wheat accounts for 20% of the calories consumed by mankind worldwide and is a good human dietary source of protein, vitamins, minerals, (Simons et al. 2006; Brenchley et al. 2013), essential amino acids and dietary fibre (Shewry 2009).

Wheat is a self-pollinating annual polyploid grass consisting of a number of *Triticum* species that originated from the fertile crescent of the Middle East (Bell 1987). Currently, about 95% of the wheat grown worldwide is common hexaploid bread wheat grown for human food end-use, with the majority of the remaining 5% being tetraploid durum wheat (Shewry 2009). The key feature that has given wheat an advantage over other crops is the unique dough properties formed from various wheat flours that are processed into a range of breads, pastas, Asian noodles, baked products (including cakes, pies, muffins, cupcakes and biscuits) and other processed foods (such as cereal bars and breakfast cereals; Peña 1999; Shewry 2009).

Wheat is not only an important nutritional crop playing a significant role to alleviate food safety concerns today; it also had a significant historical influence on the evolutionary development of mankind over time (Peña 2007; Shewry 2009). Wheat is unrivalled in its cultivation and diversity range compared to crops like maize and rice (Shewry 2009). The optimum growing temperature for wheat is about 25°C, with minimum and maximum growth temperatures of around 3-4°C and 32-36°C respectively. Wheat is adapted to a broad range of annual rainfall from 250-1 750 mm (Mergoum et al*.* 2009).

Today wheat is grown over approximately 210-230 million hectares annually, with a global yield average around 3.0 t/ha (USDA 2013). Global wheat production needs to increase by 2% each year in order to meet the increasing global demand for wheat since the global population increases exponentially each year. By the year 2020 the global wheat production needs to be 1 billion metric tons per annum to meet demand (Mergoum et al. 2009). Of major concern is the fact that during the last few years the world wheat production has been fluctuating between 650-700 million metric tons (USDA 2013).

2.2.1 Wheat genomics

Bread wheat or common wheat is an allohexaploid with three interrelated genomes (A, B and D) and each genome consists of seven chromosomes (Gupta et al*.* 2002; Gill et al. 2004; Dieguez et al. 2006; Zaharieva and Monneveux 2006). Wheat has a genome size of 16-17 x 10^9 base pairs (bp), which is considered large when compared to other crops (Gupta et al*.* 2002; Brenchley et al. 2013). The common bread wheat genome (AABBDD) is about eight times larger than the size of maize and 40 times larger than the rice genome (Gill et al*.* 2004). Identification of new molecular markers in a relatively young polyploid species such as wheat is difficult and further complicated by high levels of orthologous gene similarity and functions (Akhunov et al. 2010). The resulting

duplication and triplication of genes further complicate the analysis of segregation patterns, epistatic effects and effects of gene components, affecting breeding and crop improvement (Worland et al. 1987). The wheat genomes A, B and D are in order of increasing magnitude and complexity and decreasing order of diversity compared with other plant model genomes (Akhunov et al. 2010). Within each wheat genome there are gene-rich regions surrounded by gene-empty regions or non-coding regions with approximately 80% of the entire wheat genome being repeated regions (Gupta et al. 2002; Brenchley et al. 2013).

2.3 South African wheat production

From the 1960's onwards, agriculture in SA had a fast growth rate, with significant expansions in production, farms were modernised and mechanised and the adoption of scientific farming methods became common. From the 1960's there were extensive records kept on wheat production and it was from this point that wheat yields in SA started to increase and improve. In 1960, the SA wheat production was only 0.769 million tons (USDA 2013). In 1966, this declined to a record low of 0.548 million tons. Over the next few decades, the annual wheat production steadily increased with record highs in 1976 (2.239 million tons), 1981 (2.339 million tons), 1982 (2.420 million tons), 1984 (2.224 million tons) and 1986 (2.321 million tons), all well over the two million tons. This increase in production was mainly due to improved yields. The two best wheat production years ever recorded were in 1987 (3.135 million tons) and 1988 (3.535 million tons). However, since the record high in 1988, annual production for several years has been hovering just below or above the two million tons level. In 2010 wheat production was low (1.430 million tons) and in 2011 the targeted two million tons was achieved but since 2012-2014 only 1.8 million tons was achieved (USDA 2013).

SA wheat production can be sub-divided into three climatically and geographical distinct wheat production areas namely, dryland winter rainfall (Western Cape), dryland summer rainfall (Free State) and irrigated production areas (Northern Cape, North West and parts of KwaZulu-Natal, Eastern Cape and Limpopo provinces). The most significant wheat production areas of SA by province is the Western Cape (40%), Free State (25%), Northern Cape (16%), Limpopo (8%), North West (6%) and KwaZulu-Natal (2%), while the remaining provinces (Mpumalanga, Eastern Cape and Gauteng) make up the remaining 3% of the total wheat production in SA. Over the past decade there has been a significant change in hectares of wheat planted in irrigation production areas compared to dryland production in SA. The area of wheat planted in the Free State dryland production areas, in particular for 2012 and 2013, was the lowest for decades. However,

since 2007 the annual national wheat production has steadily been decreasing and the local production is not meeting local demand (FAO 2013).

The inability of SA to be self-sufficient for wheat production is further compounded by the lack of government support or subsidy for farmers, a decline in large scale commercial wheat farmers, uncompetitive wheat prices, higher disease (e.g. FHB and wheat rusts) and pest (e.g. Russian wheat aphid) risks compared to other crops, higher input costs, environmental extremes (droughts and floods) and competition from more favourable crops, such as maize, sunflower and soybean.

FHB is a fungal disease of wheat that is of importance worldwide and is a growing concern in SA as a sporadic disease that occurs on irrigated spring wheat. This disease and multiple factors that influence its development and control will be discussed in detail.

2.4 Fusarium head blight on wheat

FHB of wheat, alternatively named head scab or ear blight, is one of the most economically important diseases of wheat and other major cereals (barley, rye, oats and maize) worldwide (Li et al. 2005; Nicholson et al*.* 2005; Chen et al. 2006; Najaphy et al. 2006; Xue et al. 2010a; Zhang et al. 2011). A number of *Fusarium* species are widely associated with FHB of wheat and other important small grain crops. Considering the large number of *Fusarium* species isolated from blighted cereals infected with FHB, only a small number are in fact considered significant casual agents of FHB (Nicholson et al. 2004). *Fusarium graminearum* is the most common causal agent of FHB worldwide. FHB can cause severe yield losses of between 10-70% under high inoculum pressure during epidemic years (Nicholson et al*.* 2005; Zhang et al. 2011) with an overall reduction in grain quality (Nicholson et al. 2004; Chen et al*.* 2006; Xue et al. 2010a) resulting in large revenue losses on susceptible cultivars.

Fusarium species have the ability to produce various secondary metabolites commonly known as mycotoxins. Apart from yield loss, secondary losses include a danger to food safety caused by mycotoxin contamination of harvested infected grain (Browne 2007). Certain FHB causal *Fusarium* species produce trichothecene mycotoxins such as DON and nivalenol (NIV), which are dangerous and pose a serious health risk to humans and livestock if consumed (Nicholson et al. 2004; Li et al. 2005; Najaphy et al. 2006; Saharan and Naef 2008; Zhang et al. 2011). FHB infection prevents sufficient nutrient uptake by the plant during grain filling, resulting in reduced kernel number, reduced kernel weight

and finally severe disruption to the starch granules and storage proteins within the grain (Snijders 1990; Nicholson et al. 2005).

Xue et al. (2010a) stated that FHB resistance in wheat is controlled by the complex interaction of QTL that are significantly influenced by the environment. To date, there is no completely immune wheat genotype available or released (Ittu et al. 2000; Xue et al. 2010b), although historically a few highly resistant FHB sources have been identified across different continents of the world such as Asia, South and North America and Europe (Xue et al. 2010a; Zhang et al. 2011). Importantly, there is a limited number of moderately to highly tolerable FHB resistant wheat cultivars available or released to date in SA.

2.4.1 Disease symptoms

An abundance of natural inoculum, in combination with high humidity and high temperatures (22-25°C) during wheat flowering (a two week window) and early grain filling stages present favourable conditions for FHB infection (Zhou et al. 2002b). Recent reports stated that the perfect microclimate for FHB infection only exists for extremely short time intervals within a 24 h period (Boutingy et al. 2011). Under favourable environmental conditions and a surplus of initial inoculum, symptoms may start to develop within 3-5 days after infection (Kriel 2007). The presence of two compounds, choline and betaine, in high concentrations in anthers of flowering florets have been shown to stimulate the initial growth stage of *Fusarium* pathogens, in particular *F. graminearum*, directly after spore germination. Spread of disease can occur through various animal vectors, water (rain or irrigation) splash dispersal of conidia and/or by air-borne ascospores that are present on crop residues or on the base of the host plant (Nicholson et al. 2005).

The first signs of FHB infection are small brown water soaked markings on glumes within the spikelets, eventually expanding to affect the entire spikelet (glumes and florets) (Figure 2.1; Nicholson et al. 2005). Under optimal humidity conditions the affected spikelet can become covered with white or pink mycelium and at this point the disease will spread from spikelet to spikelet, slowly progressing through the entire wheat spike (Nicholson et al. 2005). Severe infection is characterised by ears/spikes that appear to be ripening early, resulting in a white-bleached appearance (Figure 2.2). There may even be an accumulation of saprophytic mould growth on the surface causing the wheat spike to become darker as time progresses. This white-bleached characteristic is a result of fungal infection that has spread into the central axis of the spike that prevents the

transfer of water and nutrients from other parts of the host plant to the spike under stress (Nicholson et al. 2005; OMAFRA 2009)

Figure 2.1 Infected florets with brown water markings on the glumes, showing the entire spikelet with Fusarium head blight infection

FHB infection of spikelets results in sterile flowers (Zhou et al. 2002b). Kernels infected with *Fusarium* are often light in weight and appear shrunken and shrivelled, also commonly known as “tombstone kernels” (Figure 2.3). This results in reduced seed and baking quality (Buerstmayr et al. 1999) in comparison with uninfected grain (Oettler and Wahle 2001) and in severe yield and revenue losses (Zhou et al. 2002b; Nicholson et al. 2005). The infected kernels are rough, scabby in appearance and range from light brown to pink to greyish white in colour (OMAFRA 2009). The germination rate and seedling vigour of infected seeds are drastically reduced (Nicholson et al. 2005; OMAFRA 2009). Planting of infected seed can often result in the development of seedling blight, which is an entirely different phase of the *Fusarium* disease. Seedlings infected at an early age, will appear reddish-brown and will possibly be covered in white or pink mould. They will develop slower, produce a reduced number of tillers and generally be smaller and stunted in size in comparison with uninfected seedlings (OMAFRA 2009).

Figure 2.2 A characteristic Fusarium head blight infected wheat spike showing bleached colouration and bent back awns, well before expected ripening (photo: Cathy de Villiers)

Figure 2.3 The characteristic "tombstone kernels" on the right compared to normal grain on the left (photo: Cathy de Villiers)

2.4.2 Historical Fusarium head blight epidemics

During the last two or three decades there have been severe outbreaks of FHB worldwide (Foroud and Eudes 2009). These outbreaks have been attributed to the following important causes: the widespread (large areas) cultivation of susceptible cultivars; increased presence of infected crop residues due to conservation tillage practices; use of maize in crop rotation systems with small grains such as wheat or barley and changes in regional weather patterns as a result of global warming (Nakajima 2005).

The first FHB-outbreak to be recorded occurred in England in 1884, where the disease got its common name “wheat scab”. Over the last few decades FHB disease outbreaks have been reported worldwide. In North America’s Midwest region epidemics occurred during 1993-1998, causing economic losses close to three billion dollars (Nganje et al*.* 2004; Jansen et al. 2005; Dill-Macky 2008; Foroud and Eudes 2009). On top of the yield loss, the majority of the harvested crop was rejected by industry, since the DON contamination levels present in flour was over the recommended 1 ppm tolerance level (Jansen et al*.* 2005). In the 2010 and 2011 seasons the USA had some of the most severe and wide-spread FHB epidemics in recent history. In China, FHB occurred on barley and wheat which was a particular problem for wheat breeders with a number of epidemics between the years 1951-1990. Since 1990, the release of moderately resistant varieties has reduced the number of severe FHB epidemics (Foroud and Eudes 2009). The very wet season of 2012 in China resulted in the worst FHB epidemic in decades. Due to the presence of such high inoculum levels susceptible varieties were destroyed and even moderately resistant varieties that covered more than 50% of total area of the wheat planted were heavily infected (personal communication: Dr Guihua Bai).

2.4.3 Fusarium head blight epidemics in South Africa

FHB was first recorded in the North West province of South Africa in 1980 (Scott et al. 1988; Kriel and Pretorius 2008). After the first report, epidemic outbreaks of FHB were recorded in 1985, 1986 (Scott et al. 1988), 1994 (Scott and Smith 1995) and 2000 from various localities across the country. An increased use of double cropping systems in SA of maize as a summer crop and wheat as a winter crop, in combination with conservation tillage practices, have increased the threat FHB poses under irrigation (Kriel and Pretorius 2008; Foroud and Eudes 2009). Some small localised FHB outbreaks, known as “hot spots” on a few commercial farms in the 2008, 2010, 2012 and 2013 seasons were communicated (personal communication: Mrs Cathy de Villiers). Worldwide

epidemics in recent years have been reported in new wheat production areas to a certain extent and were surprisingly severe. This can be related to climate change or shifts and some of these epidemics have occurred even in countries where host plant resistance is well established. These global epidemics should make SA FHB wheat researchers aware of the potential dangers future local epidemics may pose.

2.4.4 Fusarium graminearum infection cycle

Most FHB epidemics around the world are predominantly caused by *F. graminearum.* The *F. graminearum* infection cycle starts with the mycelium overwintering in the soil or on plant residues (Goswami and Kistler 2004; Kazan et al. 2012). FHB disease is initiated in the wheat field by the airborne ascospores (sexual stage) and microconidia (anamorph) landing on the flowering spikelets. These spores germinate and enter the plant through the lemma, palea and vulnerable degenerating anther tissues (Bushnell et al. 2003; Trail 2009). Over the years there has been a contentious issue about whether *F. graminearum* exhibits a biotroph lifestyle during the initial stages of infection (Trail 2009). Recently is has been concluded that *F. graminearum* displays a hemibiotrophic lifestyle (Brown et al. 2010; Kazan et al. 2012; Ma et al. 2013). In a recent report new penetration mechanisms unique to *F. graminearum* have been identified, namely ‘subcuticular hyphae’ and ‘bulbous infection hyphae’; the latter are specific structures designed for initial infection (Rittenour and Harris 2010).

The point of infection inside the plant tissue is commonly called the infection front, from which the *F. graminearum* fungus grows intercellular and asymptomatically (Bushnell et al. 2003; Jansen et al. 2005; Trail 2009), spreading (through central axis of the inflorescence) through the xylem and pith tissues of the host plant (Bushnell et al. 2003; Ma et al. 2013). Behind the infection front the fungus rapidly colonises the tissues by spreading radially and with the intracellular growth of the fungus, tissue necrosis starts. A characteristic symptom of this stage is water soaking of the collenchyma tissues after which infected colonised tissues appear bleached. The fungal spread from floret to floret and spikelet to spikelet is via the vascular bundles in the rachis and rachilla (Goswami and Kistler 2004) which occurs on average 4-7 days post-inoculation (dpi; Kazan et al. 2012). However, during the colonisation phase, the extensive expression of reporter genes by the *F. graminearum* pathogen, which control DON biosynthesis, have already been detected in rachis nodes (Ilgen et al. 2009). This indicated that rachis tissue elicits DON biosynthesis. This confirmed previous reports that the rachis present in wheat is a challenging physical barrier to FHB infection and DON production is required to break down this significant obstacle (Jansen et al. 2005; Kazan et al. 2012). The biosynthesis

of DON in *F. graminearum* is considerably higher during host plant infection than during culture growth (Voigt et al. 2005; Kazan et al. 2012). This suggests that signals generated by the host plant are recognised as important triggers by *F. graminearum* for the activation of mycotoxin biosynthesis (Kazan et al. 2012).

2.4.5 Life cycle of Fusarium graminearum

The fungus grows as a haploid colony (Ma et al. 2013) for most of its life cycle and the sexual developmental stage begins with the formation of hyphae with binucleate cells. *Fusarium graminearum* is homothallic, which means it does not need two sexually distinct partners; the two nuclei formed by the binucleate cells are genetically identical clones (Goswami and Kistler 2004; Ma et al. 2013). The homothallic state is a result of the presence of genes (*Mat1-1* and *Mat2-2)* linked to both mating types (Yun et al. 2000; Goswami and Kistler 2004). Small asexual spores, known as microconidia (Figure 2.4) are produced by the mycelium (Ma et al. 2013). The characteristic long “canoe” shaped septated spores, known as macrocondia (Figure 2.4) are produced in cushion shaped aggregations of condiophores called sporodochia (Trail 2009; Ma et al. 2013).

The airborne ascospores are the primary inoculum source of the FHB disease. Copious asexual spores otherwise known as conidia (macro- and micro-conidia), are produced on the surfaces of infected plants and on crop residues during moist humid periods. Conidia have a unique fusiform shape that form of slimy spore masses that have been associated with rain-splash dispersal of the FHB disease, from one neighbouring host wheat plant to another (Trail 2009). The predominant form of short-distance dispersal is considered to be as a result of conidia (Shaner 2003; Trail 2009). Guenther and Trail (2005) suggested that the most important activity for the pathogen during the vegetative growth phase within the host plant is to accumulate carbon resources. The carbon resources are initially gathered during the first colonisation of the host stalk and play a vital role in overwinter survival and sexual reproduction (Guenther and Trail 2005). Sexual development is a crucial part of the FHB disease life cycle. The perithecium initials (Figure 2.4), together with the binucleate hypae from which they arise, are the overwintering structures that survive on secondary hosts or wheat crop residues (Goswami and Kistler 2004; Trail 2009).

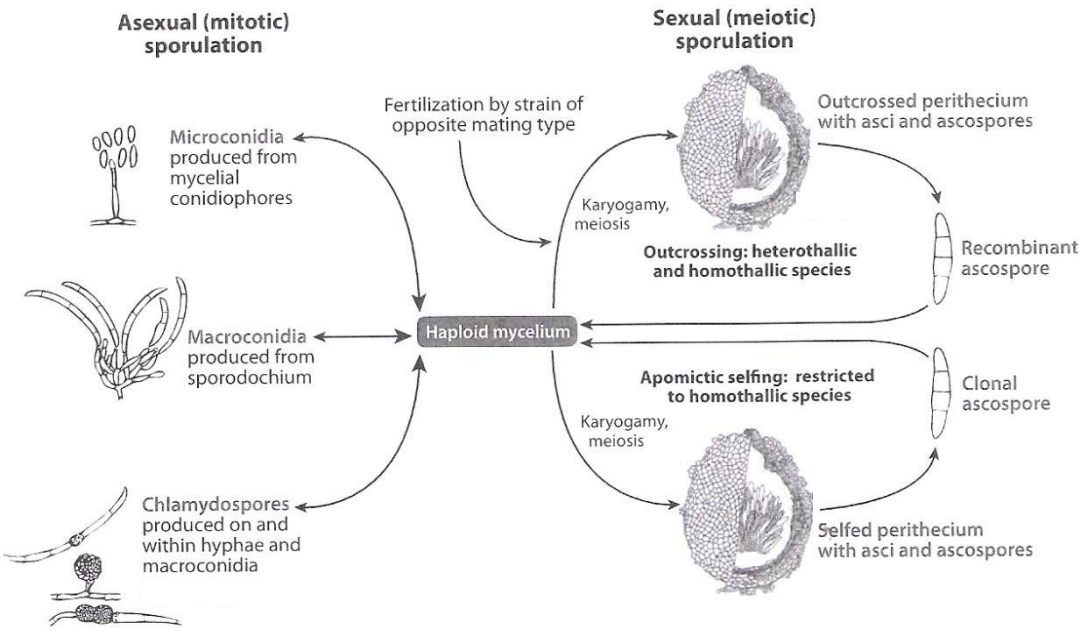

Figure 2.4 A graphical illustration of the general life cycle of *Fusarium* showing both asexual and sexual stages (Ma et al. 2013)

2.4.6 Fusarium graminearum taxonomy

Fungal species are often defined in reference to certain species concepts such as biology, ecology, morphology and phylogenetics (Taylor et al. 2000). Often when all four concepts are applied the same result will be obtained most of the time. *Fusarium graminearum* has been subjected to this scrutiny by all four of these concepts and often no consensus or conclusive conclusion can be drawn at species level (Leslie and Bowden 2008). Morphological differentiation of species of *Fusarium* is difficult if not impossible (O' Donnell et al. 2000; 2004; Wang et al. 2011). However, the most contention has been over biology versus phylogenetic species concepts of *F. graminearum* (Leslie and Bowden 2008).

Since 2000, the phylogenetic species concept has been applied extensively to *Fusarium* using neutral DNA sequences to trace potential lineages (O' Donnell et al. 2000; Leslie and Bowden 2008). *Fusarium graminearum* was thought to be a single panmictic species which spanned across all six continents of the world until a genealogical concordance phylogenetic species recognition (GCPSR; Taylor et al. 2000) approach was used to test the exact species limits (O' Donnell et al. 2000). Initially it was proposed that the ancestral origin of the *F. graminearum* (*Fg)* clade could be divided into seven or eight

different lineages based on geographical continental origins (Taylor et al. 2000; O' Donnell et al 2000). It was suggested that Africa was the point of origin of the *Fg* clade (O' Donnell et al. 2000). In 2004, O' Donnell et al. suggested that these eight proposed species lineages within the *Fg* clade were to be abandoned after an extensive look into using a GCPSR approach while comparing DNA sequences of certain mating type genes present in the *F. graminearum* B-trichothecene producing clade. From the O' Donnell et al. (2004) study nine lineages of the *Fg* clade and species were identified. More recently the *Fg* clade was preferably seen as the FGSC (O' Donnell et al. 2008). After the use of a high-throughput multi-locus genotyping assay combined with GCPSR, 13 phylogenetically distinct species were successfully identified as member species of the FGSC (O' Donnell et al. 2008; Yli-Mattila et al. 2009; Wang et al. 2011). In 2011 a review done by Cai et al. concluded that the use of GCPSR as an extension of the traditional phylogenetic species concept provided an improved method for species discrimination and demarcation. Now there might be as many as nineteen different member species of the FGSC that have been reported to cause FHB on wheat (Zhang et al. 2011).

2.4.7 Casual species of Fusarium head blight

FHB occurs primarily in warm and humid climatic conditions during flowering, predominantly due to *F. graminearum sensu latu* infections (Ittu et al. 2000; Zhang et al. 2011). These warm and humid climates are found in Australia, China, Canada, central Europe, parts of the USA (Zhang et al. 2011) and certain provinces of SA. Species such as *F. crookwellense* Burgess, Nelson and Tousson, *F. culmorum* (WG Smith), *F. avenaceum* (Fries) Saccardo, *F. sporotrichioides* Sherbakoff and *F. langsethiae* Torp and Nirenberg are associated with FHB disease in cool and wet/humid conditions, while *F. poae* (Peck) Wollenweber is important in warmer and dryer climates (Rossi et al. 2001; Xu and Nicholson 2009; Zhang et al. 2011). Two species, *F. graminearum* and *F. culmorum* are pathogenic to most cereals (wheat and maize) and grasses without displaying a specific crop specialisation, therefore these species are non host-specific (Van Eeuwijk et al. 1995). In a recent *Fusarium* survey report done in SA, *F. graminearum* was the most predominant lineage, followed by *F. boothii, F. meridionale, F. acacia-mearnsii, F. cortaderiae and F. brasilicum* in decreasing frequency (Boutingy et al. 2011). Environmental conditions, specific geographical regions and the favourability of the year dictate the predominance of a particular species (Van Eeuwijk et al. 1995; Nicholson et al. 2004). This suggests that environmental factors and geographical landscapes play a primary role in the distribution of FHB, besides the genetic diversity of the *Fusarium* pathogens (Xu and Nicholson 2009; Zhang et al. 2011).

2.4.8 Fusarium graminearum genomics

The genome size of *F. graminearum* is 36.1 million bases (Mb), which is characteristic for a filamentous fungus. The genome contains genes that encode approximately 13 937 different known proteins. These genes are evenly distributed across the four chromosomes (Goswami and Kistler 2004; Trail 2009) and 5 812 of these genes show homology to proteins of unknown function while 2 001 have no similarity to any other previously sequenced organisms (Trail 2009). The availability of the complete genome sequence of *F. graminearum* (Cuomo et al. 2007) has assisted in gaining significant new insights into this pathogen (Kazan et al. 2012). Based on previous studies, some fungi have the ability to create duplicated gene sequences in their genomes which are non-functional by a process called “repeat induced point mutation” (RIP). RIP randomly fills copies of duplicated genes with point mutations, rendering them non-functional (Galagan and Selker 2004). Cuomo et al. (2007) demonstrated that the lack of multi-copy sequences in the genome of *F. graminearum* is due to the presence of a RIPing mechanism.

2.4.9 Mycotoxins

Mycotoxins are secondary metabolites produced by various *Fusarium* species that play a significant role in isolate aggressiveness, host preference and virulence (Jansen et al. 2005). *Fusarium* species produce three major types of mycotoxins, namely trichothecenes, zearalenones and fumonisins (Hollins et al*.* 2003; Nicholson et al. 2004). Mycotoxins such as these groups mentioned are present in a large portion of the world’s food supply in processed end products and pose a potentially serious threat to global food safety (Nakajima 2005).

2.4.9.1 Trichothecenes

A major mycotoxin class is the trichothecenes that are toxic sesquiterpenoid compounds (Afshar et al*.* 2007; Moon et al*.* 2007; Foroud and Eudes 2009) and are implicated in FHB disease spread and aggressiveness (Foroud and Eudes 2009). Trichothecene toxins are potent inhibitors of eukaryotic protein biosynthesis (Afshar et al*.* 2007; Moon et al*.* 2007). There are four types of trichothecenes namely types A (T-2 toxin), B [(DON; 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON) and (NIV)], C and D, of which type A are considered highly toxic (Moss 2002; Foroud and Eudes 2009). These mycotoxins accumulate in the kernels of diseased spikelets (Foroud and Eudes 2009), making the grain unfit for human and animal consumption (Kimura et al*.* 2003; Afshar et al*.* 2007; Riungu et al*.* 2008; Foroud and Eudes 2009). *Fusarium graminearum* contaminates maize, barley and wheat grains with the trichothecenes NIV

and DON and its two derivatives 3-ADON and 15-ADON (Kimura et al. 2003; Goswami and Kistler 2004; Trail 2009).

2.4.9.1.1 Deoxynivalenol

DON is known as a virulence factor that helps the spread of infection that causes tissue necrosis (Proctor et al. 1995) and allows the fungus to spread into the rachis from the wheat florets (Jansen et al. 2005). However, DON is thus far the only mycotoxin revealed to be a virulence factor (Jansen et al. 2005; Trail 2009). The colonisation of the fungus within developing kernels coincides with DON mycotoxin accumulation in the grain. DON and its two derivatives, 3-ADON and 15-ADON are primarily produced by two *Fusarium* species namely *F. graminearum* and *F. culmorum* (Moss 2002; Nicholson et al. 2004; Moon et al. 2007; Foroud and Eudes 2009; Wegulo 2012). In plants, DON and 3-ADON have been shown to have a phytotoxic effect (Wegulo 2012). Guidance levels for DON toxicity were set in 1982 at 1 ppm for finished wheat products for human consumption and 4 ppm for wheat or milled by-products of wheat used in animal feed (Collins et al. 2006).

2.4.9.1.2 Nivalenol

NIV is considered more toxic than DON or its derivatives, which is of great concern for food safety (Nicholson et al. 2004). The occurrence of NIV in the field as well as food and feed products is far rarer than that of DON (Yazar and Omurtag 2008). NIV is similar in structure to that of DON, except it has an additional hydroxyl group at the C-4 position (Yoshizawa 2013). Acute NIV exposure like most trichothescene mycotoxins produce similar adverse effects on humans and animals as DON exposure does (Yazar and Omurtag 2008).

2.4.10 Fusarium head blight management strategies

Proper FHB management strategies are vital for reducing the economical short fall, damage and potential health risks associated with severe outbreaks of the disease. Important strategies have been established to target each of the following: inoculum source, susceptibility of the host and favourable environmental conditions that are necessary for an outbreak (Foroud and Eudes 2009). As with most plant diseases it should be possible to control FHB by using some general control strategies such as exclusion or quarantine, host resistance, chemical fungicide treatment, biological control and cultural practices (Yuen and Schoneweis 2007). However, it must be noted that exclusion or quarantine is not an option for FHB disease control since the FHB causal

pathogens have already formed resident populations in most wheat growing areas of the world (O' Donnell et al*.* 2000; Yuen and Schoneweis 2007).

Foroud and Eudes (2009) suggested that the primary inoculum source is usually in the form of ascospores in the soil which are produced during late spring and early summer from perithecia that have developed on crop residues. This problem is made worse when a maize crop rotation system is used in conjunction with wheat production (Dill-Macky 2008; Gilbert and Tekauz 2011). Kochler et al*.* (1924) demonstrated that both large amounts of maize residue remaining after harvest and the abundance of perithecia on maize residues are problematic for disease incidence on the next crop.

A second essential component is that the pathogen requires a suitable host for spores to germinate for disease establishment and development. The problem associated with susceptible wheat hosts can be addressed if farmers can be encouraged and convinced by breeders/seed companies to buy and plant resistant varieties (Buerstmayr et al. 2009; Foroud and Eudes 2009).

Finally the third essential component for epidemic outbreaks is favourable environmental conditions. FHB thrives in wet, humid and warm conditions with an optimal temperature of 25ºC during flowering and grain filling stages (Foroud and Eudes 2009). Weather cannot be controlled but tools such as disease forecasting models together with historical weather records and forecasts should be used to better plan and utilise planting times, an effective spraying schedule and post-harvest management methods (Gilbert and Tekauz 2011; Matarese et al. 2012).

A number of studies indicated that the correct crop rotation system, tillage practices, chemical or biological control methods and use of FHB resistant cultivars can all help reduce the amount of primary inoculum and inoculum remaining in the soil by reducing the amount of *Fusarium* contaminated crop debris (Foroud and Eudes 2009; Gilbert and Tekauz 2011). Farmers will experience the benefit from multiple practices that can be integrated as a single management strategy, instead of depending on a single control method (Gilbert and Tekauz 2011; Matarese et al. 2012; Kleczewski 2014).

2.4.10.1 Host plant resistance

Success in FHB resistance breeding has been elusive, as it is a multi-genic trait that is controlled by a number of QTL that generally contribute relative small amounts to its expression. FHB host plant resistance is significantly affected by genetic background in

which the resistance genes/QTL are placed in (Buerstmayr et al. 2009; Gilbert and Tekauz 2011). Resistance to FHB is not complete, meaning resistant cultivars will still show signs of FHB infection, but disease progress is significantly reduced (Gilbert and Tekauz 2011). However, according to Foroud and Eudes (2009), if all efforts, resources and money available should be spent on one strategy, it should be directed towards the development of FHB resistant lines. Resistant varieties still remain one of the most important management strategies as it is the first line of defence and the area of intensive research (Pirgozliev et al. 2003; Gilbert and Tekauz 2011). FHB resistant cultivars can positively improve all three concepts discussed. However, the use of moderately or tolerant/resistant cultivars containing a single FHB resistance gene/QTL only is prone to resistance breakdown under severe disease pressure (Buerstmayr et al. 2009; Foroud and Eudes 2009) and a combined control method is rather suggested to protect deployed resistance genes/QTL (Gilbert and Tekauz 2011).

2.4.10.2 Chemical control

Chemical control of FHB incidence using fungicide treatment depends on a complex set of different variables such as level of cultivar resistance, fungicide efficacy, fungicide coverage, method/instrument and nozzle used for spraying, timing and the aggressiveness of pathogen isolates. Often fungicides are applied in an attempt to control the severity and spread of FHB disease, but the effectiveness is a concern and often unsatisfactory (Mesterhazy et al. 2003; Oettler et al. 2004). Suppression of FHB disease can be achieved by the timeous application of recommended fungicides on wheat (Gilbert and Tekauz 2011). However, fungicide treatment as a preventive measure for FHB incidence is difficult to manage and only partly effective at best (Oettler et al. 2004). The most consistent performing fungicides that reduced both FHB infection and DON concentration by up to 50% are those that contain tebuconazole (Mesterhazy et al. 2003). In 2014, the first tebuconazole resistant *F. graminearum* isolate (isolate Gz448NY11) was identified on wheat from Steuben County, New York, USA. This resistant isolate showed a sensitivity rate to tebuconazole of EC_{50} = 8.09 mg/ℓ, the lowest reported to date for the species. Future studies are needed to monitor potential fungicide resistant isolates, to fully understand the risk fungicide resistance can pose to FHB disease management and food security (Spolti et al. 2014). An additional problem associated with the use of fungicides on wheat to control FHB is the accumulation of residues in harvested grain (Riungu et al. 2008). To date, there is no official registered fungicide in SA against FHB to be used on wheat.

2.4.10.3 Cultural practices

Crop residue management

Fusarium graminearum thrives on decaying plant residues, such as maize, wheat and barley (Kleczewski 2014). These crop residues allow the pathogen to overwinter producing ascospores in spring, which is the source of primary inoculum for the next crop (Gilbert and Tekauz 2011; Kazan et al. 2012). Originally, it was thought that inoculum dispersal was only a few metres, but since then is has become a well-known fact that wind assisted spore dispersal can occur over many kilometres (Maldonado-Ramirez et al. 2005; Schmale et al. 2012). Tillage can bury most of the crop residues under the soil surface, allowing for natural decay (Kleczewski 2014), but it is further recommended that remaining crop residue is chopped up finer and spread over the land, which should aid in decomposition and subsequently reduce the amount of inoculum available (Gilbert and Tekauz 2011). Reduced or conservation tillage has been used worldwide in agriculture over the last 25 years. This shift towards no-till or minimal tillage was suggested in an attempt to limit fertile soil erosion, caused by winds or flood water, when the soil surface is exposed for extended periods between seasonal crops (Dill-Macky 2008). It has also become apparent that the shift to no-till has resulted in more crop residue left on the surface after harvesting, which has caused a significant change for the worse regarding FHB incidence and epidemics on cereal crops (Dill-Macky 2008; Kazan et al. 2012; Kleczewski 2014). Some studies have shown that *F. graminearum* in or on the surface of buried seed can possibly survive up to 24 months (Inch and Gilbert 2003) but this can be overcome with a dry heat treatment at 70°C for five days (Gilbert et al. 2005).

Crop rotation

Fusarium graminearum is not host specific and have been isolated from many different cereal crops, especially maize (Gilbert and Tekauz 2011). Additionally, *F. graminearum* is not just a pathogen restricted to heads of cereals, but can also cause crown and root rot diseases which may be important in other environments (Kazan et al. 2012). It has become apparent that a rotation involving maize should be avoided if at all possible (Pirgozliev et al. 2003; Gilbert and Tekauz 2011). Some significant success in reducing FHB severity and DON content has been reported when planting spring wheat into soybean (Kleczewski 2014) or canola crop residues (Gilbert and Tekauz 2011).

Multiple cultivar plantings and varying planting dates

Planting several cultivars, that differ in growing periods (long, intermediate and short growers) and/or staggered planting have been proposed as a possible FHB control

method (Gilbert and Tekauz 2011). The principle behind this is that the farmer may still be able to have a good yield from one of the options even during a bad FHB season. However, as the exact weather during flowering or the length of time with optimal weather cannot accurately be predicted, this still remains risky (De Wolf et al. 2003; Gilbert and Tekauz 2011).

2.4.10.4 Biological control

Biological control of FHB is a relatively new management strategy and an ideal method to combine with host plant resistance to lower the impact of FHB disease incidence during high potential seasons. Biological control needs agents that are aggressive against, or antagonists of, the problematic pathogen. Biological control agents can also help prevent inoculum build-up on crop residues (Matarese et al. 2012; Musyimi et al. 2012). Additionally the correct biological antagonist can further help prevent FHB disease development on wheat heads (Musyimi et al. 2012). In a recent study, certain *Trichoderma* isolates that have the ability to grow in the presence of DON were identified as potential antagonists to inhibit the growth and DON production process of *F. graminearum* and *F. culmorum* (Matarese et al. 2012). Musyimi et al. (2012) indicated some species of *Alternaria, Epicoccum* and *Trichoderma* that are effective antagonists of *F. graminearum* and *F. poae* with up to 60% colony reduction. These studies proved that there is potential in the application of biological antagonistic agents to help control FHB disease.

2.5 Factors affecting durable resistance

Durable resistance, as defined by Michelmore (2003), is a form of resistance by a host plant that has remained effective during prolonged and widespread use in environments conducive to the disease. Increasing the durability of host plant resistance to plant pathogens such as *Fusarium* is one of the vital goals of virulence management (Lo Iacono et al. 2013). The concept of durable resistance and responsible resistance gene deployment have been considered in plant breeding for years (Michelmore 2003). Many different resistance genes have been identified and tagged with molecular markers, especially in wheat germplasm, but identifying the underlining factors and gene interactions that provide effective resistance remains a challenging task (Leach et al. 2001; Lo Iacono et al. 2013). The extensive deployment of strong resistance genes/QTL in resistant cultivars imposes a tough selection pressure on the pathogen population, which can lead to pathogen virulence shifts or mutations that should be considered (Quenouille et al. 2014).

McDonald and Linde (2002) suggested that the evolutionary potential of a pathogen population, in this case *Fusarium*, is reflected in its population structure. A host plant's genetic resistance is more easily overcome by pathogen populations with a high evolutionary potential than those populations with a lower evolutionary potential. Specific pathogens that pose the greatest risk of breaking down host plant resistance genes, display a mixed reproductive system, a high potential for genotype flow, large effective population sizes and high mutation rates (McDonald and Linde 2002).

The nature of the resistance deployed like polygenic, quantitative and/or horizontal resistance, as FHB resistance often is, should be more durable than single gene or vertical resistance. Selection pressure imposed by these different types of resistances are different. Single gene or vertical resistance is often pathogen specific and complete resistance, which leads to high pressure on the pathogen to change virulence; this is not the case for quantitative or horizontal resistance (Boyd 2006; Palloix et al. 2009; Lo Iacono et al. 2013). However, over the years there have been some exceptions to this norm with some polygenic resistance erosion occurring rapidly (Stuthman et al. 2007; Lo Iacono et al. 2012) and *vice versa*. More recently it has been suggested, based on experimental data, that in fact major gene resistance durability is directly dependent on the genetic background it is placed into, i.e. in a completely susceptible background the gene will have a shorter life span compared to being placed in a genetic background that already contains a form of quantitative resistance (Quenouille et al. 2014). The fact remains that any form of resistance needs to be deployed with a structured and responsible plan.

2.6 Breeding for Fusarium head blight resistance

Cultivation of FHB resistant lines/varieties will play a key role in the integrated control of *Fusarium* diseases and the prevention of accumulation of mycotoxins such as DON within infected grain. An important breeding goal of a number of cereal and small grain breeders in recent times is to improve FHB resistance in commercially released lines (Buerstmayr et al. 2009). Breeding for FHB resistance is rather complex due to the polygenic nature of the trait, the negative association of exotic resistant germplasm with undesired agronomical traits and environmental influences on disease expression (Mardi et al. 2006). Resistance breeding against FHB confers resistance against multiple *Fusarium* species and isolates although resistance expression are dependent on isolate aggressiveness (Hollins et al. 2003).

There exists a large level of genetic variation for FHB resistance in many different wild relatives and exotic sources. The taxing task for wheat breeders is to develop regionally adapted cultivars that perform well in terms of yield and quality but that also show improved resistance to local pests and diseases including FHB (Buerstmayr et al. 2009). Other factors that need to be considered when breeding and evaluating FHB resistant lines are the influence of high inoculum pressure and the effect of isolate/species cocktails and aggressiveness of isolates. These factors significantly contribute to more severe disease symptoms than would normally be expected, even on moderate to highly resistant varieties (Bai and Shaner 2004).

Over the years a number of foreign sources of resistance have been used worldwide in breeding programmes to develop cultivars with improved FHB resistance (Xue et al. 2010a). The improvement of FHB host plant resistance achieved through conventional and more recently MAS methods combined with phenotypic validation has been reasonable (Buerstmayr et al. 2009). However, Xue et al. (2010a) is of the opinion that genetic breeding gain with regard to FHB host plant resistance has been moderate, purely based on the tremendous amount of scientific effort, time and funding invested with limited actual reward. This can be attributed to a number of complicated factors that are at play within the FHB resistance complex.

2.6.1 Types and forms of resistance

FHB resistance is controlled by a number of polygenes, with small effects being significantly influenced by the environment (Hollins et al. 2003; Xue et al. 2010a). This gene-for-gene by environment interaction makes it difficult to select for stable FHB resistance expression when only conventional breeding strategies are being used and can result in low heritability (Xue et al. 2010b).

Schroeder and Christensen (1963) were the first to describe two types of resistance to FHB, namely Type I (initial infection) and Type II (spread of infection within the spike). In 1995, Mesterhazy described five different resistance types involved in FHB resistance: Type I is resistance or tolerance to initial infection within the host plant, Type II is resistance to the spread of the fungal pathogen within the wheat spike from the first infection site, Type III is resistance to kernel infection and additionally the reduction in DON mycotoxin accumulation (Mesterhazy 1995; McCartney et al. 2007; Xue et al. 2010b),Type IV is tolerance or limitation, to kernel damage (yield tolerance) and Type V is the resistance to mycotoxin accumulation in the grain (Mesterhazy 1995; Xue et al. 2010b). However, Hollins et al. (2003) believe that there are only three types of

resistance that can be analysed namely Type I and Type II as mentioned above and Type III, that is resistance against the accumulation of mycotoxins or the ability to degrade mycotoxins within the infected grain. Generally over the last few years most research has focused purely on Type II resistance due to the relative ease of the single floret injection method often used to test for a resistance response under controlled conditions (Klahr et al. 2007). In recent years it has come to light that the Type II single point inoculation test done in glasshouse experiments is not necessarily a true reflection of host plant resistance levels, since multiple infection sites within one spike are possible under field conditions with high natural inoculum pressure (Tamburic-Ilincic 2012). Type I resistance is important to all breeders for successful FHB resistance breeding as it is the host plant's first barrier to infection by *Fusarium* species (Lin et al. 2006).

2.6.2 Mode of Fusarium head blight inheritance

The FHB resistance response is said to be of oligo-genic as well as polygenic inheritance, which is significantly influenced by environmental factors (Buerstmayr et al. 2002; Jia et al. 2005; Klahr et al. 2007). Many reports have suggested that FHB resistance is a complex quantitative trait that is governed by a few major, as well as a number of minor genes (Bai and Shaner 2004; Jia et al. 2005). Oettler et al. (2004) concluded that FHB resistance is a quantitatively inherited trait and contains high levels of additive gene action. Bai and Shaner (2004) suggested that FHB resistance involves a complex and extensive interacting network of signalling molecules and pathways within the host plant. The introduction of FHB resistance in a number of studies has been correlated with two agronomical traits, namely an increase in plant height and a possible shift in heading date, both of which are not always desirable effects for a wheat breeder (Klahr et al. 2007).

Little is truly known about the dynamics and manner in which the FHB genes/QTL operate in the host defence response during disease development (Klahr et al. 2007; Buerstmayr et al. 2009; Gilbert and Tekuaz 2011). With the assistance of molecular markers genes/QTL involved in initial infection and resistance responses during different phases of disease development have been identified. This is a step towards combining genes/QTL from different sources that operate at specific stages in the host plant defence (Klahr et al. 2007; Gilbert and Tekuaz 2011).

2.7 Fusarium head blight resistance associated chromosomes

Over the years a number of mapping studies have been done to identify which chromosomes from various resistant cultivar sources contain promising FHB resistance genes/QTL. Buerstmayr et al*.* (1999) suggested that chromosomes 3B, 4D, 5A, 6B, 6D and 7A are continually associated with FHB resistance in a number of sources of resistance. However, more recently, some new prominent chromosomes have been identified to contain novel QTL which vary in resistance effectiveness namely 2D, 3A, 3B, 4B, 5A, 5B, 6B, 7A and 7B (McCartney et al. 2007). In 2009 a review by Buerstmayr et al. indicated that FHB resistance QTL have been mapped to almost every wheat chromosome.

2.7.1 Fusarium head blight resistance genes/QTL

One particular QTL is not necessarily restricted to a single form/type of FHB resistance (McCartney et al. 2007). The stability and heritability of QTL is of great importance when it comes to the effective transfer into new backgrounds and expression across different environments. A QTL identified from a similar genomic region across many different studies, using related resistance sources but entirely different susceptible parents, is considered stable, in terms of its consistent expression in different genetic backgrounds. If a QTL, for example, was found repeatedly significant from results of various phenotypic screening methods, especially when different inoculation techniques were used in totally independent biological experiments, then the QTL is said to be stable across environments (Buerstmayr et al. 2009).

A limited number of single genes responsible for FHB resistance, identified from previously well documented QTL mapping studies, have been fine mapped. Two single genes namely *Fhb1* on chromosome 3B and *Fhb2* on chromosome 6B (Anderson et al. 2001; Cuthbert et al*.* 2006; 2007) were identified in the resistant cultivar Sumai 3. Specific sequence tagged site (STS) markers within and around this *Fhb1* region have been successfully tested in a number of studies and proven to be useful in a marker-assisted breeding (MAB) programme (Cuthbert et al*.* 2006). In 2008 a third FHB resistance gene was mapped from the alien species *Leymus racemosus* (Lam.; common name mammoth wild rye)*,* called *Fhb3.* This single gene resides in the distal region of the short arm of chromosome 7Lr#1. A number of STS polymerase chain reaction (PCR) based markers have been developed for the future implementation of this translocation region in FHB resistance breeding programmes (Qi et al. 2008). The Chinese FHB resistant landrace Wangshuibai has been identified with major QTL on chromosomes 4B and 5A. The *Qfhi.nau-4B* QTL region was recently fine mapped by Xue et al. (2010b)

that was designated as *Fhb4*. This region offers Type I resistance in comparison to the predominantly Type II resistance of *Fhb1* and *Fhb2* from Sumai 3 (Xue et al. 2010b). In 2011 Xue et al. successfully fine mapped the *Fhb5* gene/locus on chromosome 5A which predominantly confers Type I and Type III resistance in Wangshuibai.

2.8 Resistant cultivars and landraces

Genetic variation for different types and forms of resistance to FHB is well documented in wheat, its close relatives and wild relatives (Mesterhazy 1995; Zhou et al. 2004; Buerstmayr et al. 2009). However, no completely resistant FHB germplasm has been identified to date, although high to moderate to partial levels of resistance to FHB can be found in a number of diverse germplasm sources across different continents and countries (Shen et al. 2003; Paillard et al. 2004; Browne 2007). These sources of resistance often differ in the chromosomal regions that govern the partial FHB resistance response (Shen et al. 2003; Paillard et al. 2004; Browne 2007). A characteristic observation of partial or quantitative resistance is a reduced rate of FHB epidemic development within a host wheat population. This can be attributed to a number of important components, including lower infection incidence and a longer latent period from inoculation to sporulation. Prime examples of FHB sources of resistance are the highly to moderately FHB resistant genotypes Sumai 3 and Wangshuibai (Table 2.1) from Asia (Ma et al. 2006b; Browne 2007) and Frontana (Steiner et al. 2004) from Brazil which have all been considered effective sources of FHB resistance (Wilde et al. 2007). Other exotic resistant germplasm and related FHB QTL are summarised in Table 2.1. The importance of using cultivar resistance as the best and most cost effective control method of FHB has been realised since the 1940's, especially in China (Zhou et al. 2004).

In recent years extensive mapping studies have been undertaken on a number of Chinese and Japanese FHB resistant landraces, namely Baishanyuehuang (Zhang et al. 2012b), Haiyanzhong (Li et al. 2011), Huangcandou (Cai and Bai 2012) and Huangfangzhu (Li et al. 2012; Table 2.1). These unadapted sources of resistance have disadvantages such as lower yield, kernel shattering, excessively tall, susceptibility to other diseases such as powdery mildew and rusts and poor quality traits which require a number of backcrosses to elite, well performing cultivars (Wilde et al. 2007).

2.8.1 Sumai 3

The Chinese cultivar Sumai 3 and its derivatives (Wilde et al. 2007) have been used in many breeding programmes across the world since the 1970's (Zhou et al. 2004).

Table 2.1 A summary of well-known Fusarium head blight sources of resistance, country of origin, pedigree, FHB resistance genes/QTL and relevant associated markers

Resistant source/cultivar	Country of origin and pedigree	QTL chromosome position	Gene/QTL	Markers/map interval	Reference
Sumai 3	China	2B[2]	***Qfhs.ndsu-***		Anderson et al. 2001;
	Funo/Taiwanxiaomei	3BS[2+3]	***3BS***	Gwm533-Barc133-Gwm493	Bai and Shaner 2004;
		3BS[2]	***Fhb1***	St-3B-66; St-3B-138; St-3B-142	Cuthbert et al. 2006;
		5A[1]	***Qfhs.ifa-5A***	Gwm293-Gwm304-Gwm156-Barc197-2	Cuthbert et al. 2007;
		6B[2]	***6B-QTL***	Bcd331-Cdo524	Wilde et al. 2007;
		6B[2]	***Fhb2***	Gwm133-Gwm644	Jayatilake et al. 2011
		7A[2+3]	*7A-QTL*	Gwm130-Gwm233	
		2B[1+2]	*2B-QTL*		
Frontana	Brazil	3A[1]	***3A-QTL***	Dupw227-Gwm720	Steiner et al. 2004;
	Fronteira/Mentana	4B[1+2]	*4B-QTL*		Yang et al. 2006
		5A[1]	***5A-QTL***	Gwm129-Barc197-2	
		6B[1+2]	*6B-QTL*		
		7A[1]	*7A-QTL*		
CM-82036		3B[1+2+3]	***Qfhs.ndsu-***	Gwm533-Barc133-Barc147-Gwm493	Buerstmayr et al.
	Sumai 3/Thornbird-S	5A[1+2]	***3BS***	Gwm293-Gwm304-Gwm156	2002, 2003, 2009;
		1B[2]	***Qfhs.ifa-5A***	Glub1	Lemmens et al. 2005
			1B-QTL		

Table 2.1 Continued

Resistant source/cultivar	Country of origin and pedigree	QTL chromosome position	Gene/QTL	Markers/map interval	Reference
Wangshuibai	China	1B[2]	*1B-QTL*	Gwm759	Zhou et al. 2004; Jia et al. 2005; Ma et al. 2006b; Mardi et al. 2006; Yang et al. 2006; Yu et al. 2008b
	Landrace	2A[2+3]	*2A-QTL*	Gwm425	
		2D1	*2D-QTL*	Gwm261-Gwm484	
		3AS[1]	*3A-QTL*		
		3BS[1+2]	***3B-QTL***	Gwm533-Barc147-Gwm133-Gwm493	
		3DL[2]	*3D-QTL*		
		4B[1+2]	***4B-QTL***	Gwm368-Gwm149	
		4B[1]	***Fhb4***	Hbg226-Gwm149	Xue et al. 2010b;
		5A[1+3]	***Fhb5***	Gwm304-Gwm415	Xue et al. 2011
		5A[1+3]	***5A-QTL***	Wmc96-Gwm304-Gwm156	
		5B[1+2]	*5B-QTL*	Gwm186-Gwm304-Gwm156	
		5DL	*5D-QTL*	Gwm292	
		6B[2]	***6B-QTL***	Gwm539-Barc024	
Arina		1B	***1B-QTL***		Paillard et al. 2004; Semagn et al. 2007; Draeger et al. 2007
		3B[1]	*3B-QTL*	Cfa2134b-Gwm131b	
		4A[1]	*4A-QTL*	Cdo545-Gwm160	
		4D[1]	***4D-QTL***	*Rht-D1*	
		5A[1]	*5A-QTL*	Gwm291-Glk348c	
		6D[1]	***6D-QTL***		

Table 2.1 Continued

Resistant source/cultivar	Country of origin and pedigree	QTL chromosome position	Gene/QTL	Markers/map interval	Reference
Ning7840	China	2AS[2]	*2BL-QTL*	Gwm614	Van Ginkel et al. 1996;
	Aurora/Anhhui11//Sumai 3	2BL[2]	*2AS-QTL*	Gwm120	Zhou et al. 2002b;
		3BS[2]	***Qfhs.ndsu-3BS***	Gwm389-Gwm533-Barc147-Gwm493	Yang et al. 2006
F201R		1B[2]	*1B-QTL*	Barc8	Shen et al. 2003
		3A[2]	*3A-QTL*	Barc76, Gwm674	
		5A[2]	*5A-QTL*	Gwm304	
Renan		2A[1]	*2A-QTL*	Gwm311-Gwm382	Gervais et al. 2003
	Mironovskaia 808/Maris	2BS[1]	***Qfhs.inra-2b***	Gwm388-Gwm257a; Gwm374	
	huntsman//VPM	3A	*Qfhs.inra-3a*	Bcd0372	
	moisson4/Courtot	3B	*Qfhs.inra-3b*	Tam61; Gwm383b	
		5A	***5A-QTL***	Gwm639b	
		5AL[1]	***Qfhs.inra-5a2***	Cbd0508-Gwm271b; Gwm639b	
		5AL[1]	***Qfhs.inra-5a3***	Gwm595-B1	
Ernie		2B[2]	*Qfhs.umc-2B*	Gwm276b	Liu et al. 2007
		3B[2]	***Qfhs.umc-3B***	Gwm285	
		4BL[2]	*Qfhs.umc-4B*	Gwm495	
		5A[2]	***Qfhs.umc-5A***	Barc65-Barc165	

Table 2.1 Continued

Resistant source/cultivar	Country of origin and pedigree	QTL chromosome position	Gene/QTL	Markers/map interval	Reference
W14		3BS[1+2]	***3BS-QTL***	Gwm533-Barc133-Gwm493	Chen et al. 2006
		5A[1]	***5A-QTL***	Barc117-Barc186	
Dream	Germany	2B[1]	*2B-QTL*		Schmolke et al. 2005
	Disponent/Kronjuwel//Monopol/3/Orestis	6AL[1]	***6A-QTL***	Gwm82-Barc107	
		7BS[1]	***7B-QTL***	Gwm46	
DH181	Canada	1D	***1D-QTL***	Gdm126	Yang et al. 2003, 2005, 2006
	Sumai 3/HY368	2DS[1+2]	***2D-QTL***	Wmc144-Gwm539	
		3BS[1+2]	*3B-QTL*	Gwm533	
		3BC[2]	*3BC-QTL*	Wmc612	
		4DL[1]	***4D-QTL***	Wmc331	
		5AS[1]	*5A-QTL*	Gwm293	
		6BS[1+2]	*6BS-QTL*	Wmc397	
		7BL[2]	*7BL-QTL*	Wmc526	
CJ9306		1AS[2+3]	*1AS-QTL*	Barc148	Jiang et al. 2007
		2DL[2+3]	***2DL-QTL***	Gwm157-Wmc041	
		3BS[2+3]	***3BS-QTL***	Gwm533-Gwm493	
		5AS[3]	*5AS-QTL*	Gwm425-Barc186	
		7BS[2]	*7BS-QTL*	Gwm400	

Table 2.1 Continued

Resistant source/cultivar	Country of origin and pedigree	QTL chromosome position	Gene/QTL	Markers/map interval	Reference
Haiyanzhong	Landrace	6BS	*6BS-QTL*	Wmc121, Cfd46 and Wmc702	Li et al. 2011
		5AS	*5AS-QTL*	Gwm493-Gwm389	
		1AS	*1AS-QTL*	Barc164-Wmc78	
		7DL	***7DL-QTL***	Barc95-Gwm26	
Huangcandou	Landrace	3BSc	*3BSc-QTL*	Umn10-Barc147	Cai and Bai 2012
		3BSd	***Fhb1***		
		2D	***2D-QTL***	Gwm276-Barc121	
Huangfangzhu	Landrace	3B	***Fhb1***		Yu et al. 2006; 2008a;
		7AL	***7A-QTL***		Li et al. 2012
Heyne	PI 612577	3AS	***3AS-QTL***	Gwm5-Wmc428	
		4DL	***4DL-QTL***	Wmc720	Zhang et al. 2012a
		4AL	***4AL-QTL***	Wmc219-Gwm160	
Baishanyuehuang	Landrace	3B	***Fhb1***	Umn10	Zhang et al. 2012b
		3Bsc	***3BSc-QTL***	Wmc307-Gwm566	
		3A	*3AS-QTL*	Wmc651-Barc356	
		5A	*5AS-QTL*	Gwm186-Barc141	

[1]Type I resistance; [2]Type II resistance; [3]Type III resistance; ***Major quantitative trait locus (QTL)*** *or minor QTL*

Breeding highly resistant cultivars has not been particularly effective in wheat, especially when the amount of time and money invested into FHB resistance breeding is considered. The moderate to high resistance that has been associated with the Sumai 3 cultivar has been extensively explored and used to develop new resistant cultivars (Mesterhazy et al. 2003). However, it is suggested that under high FHB disease pressure, like all cultivars under severe pressure, resistance in Sumai 3 tends to break down (Trail 2009). Most FHB QTL that have been reported and studied in greater detail to date are from Sumai 3 and its derivatives (Zhou et al. 2004). A study by Zhou et al. (2002a) detected a number of chromosomes associated with Type II resistance (2B, 3B, 6B and 7A), while 3B and 7A were additionally associated with reduced DON content. The same study, however identified three chromosomes (1B, 2D and 4D) highly associated with an increase in DON content and concentration (Zhou et al. 2002a).

Sumai 3 and its derivatives generally all show excellent Type II resistance and FHB disease symptoms do not tend to spread to uninfected spikelets (Bai and Shaner 2004). One important major FHB QTL that has been identified in Sumai 3 and its derivatives from many different research groups was located on the distal end of chromosome 3BS, named *Qfhs.ndsu-3BS* (Anderson et al. 2001; Shen et al. 2003; Zhou et al. 2004) and this QTL is mostly associated with Type II resistance (Wilde et al. 2007). This region has been confirmed in many different mapping and breeding populations over the years (Zhou et al. 2004). Since then this region has been fine mapped and the first single gene for FHB resistance known as *Fhb1* was mapped within this *Qfhs.ndsu-3BS* QTL region by Cuthbert et al. (2006). The specific STS and simple sequence repeat (SSR) markers developed for the *Fhb1* major gene and flanking the *Qfhs.ndsu-3BS* region, are indicated in Table 2.1.

Other important major QTL for different types of resistance have been identified in Sumai 3 or its derivatives on chromosomes 5A and 6B (Zhou et al. 2004). The QTL on chromosome 5A was named *Qfhs.ifa-5A*. This QTL confers predominantly a Type I resistance response (Anderson et al. 2001; Zhou et al. 2004). This QTL has been studied less in Sumai 3 compared to *Fhb1.* However *Qfhs.ifa-5A* QTL regions remain an important FHB resistance QTL for resistance breeding. In 2007, Cuthbert et al. fine mapped the 6B QTL region of Sumai 3 and identified another major single gene, later named *Fhb2*. Recently a fourth major QTL named *Fhb7AC,* was identified in Sumai 3 on chromosome 7A. After haplotype analysis, the original source of the *Fhb7AC* QTL was traced back to the Italian cultivar, Funo which was a parent of Sumai 3 (Jayatilake et al. 2011).

2.8.2 Wangshuibai

Wangshuibai is a FHB resistant landrace that originated from the Jiangsu province of China. However, it is not known to be related to Sumai 3 (Zhou et al. 2004). It contains a high level of Type II resistance and some level of Type I resistance and is reported to be a more stable resistant genotype than Sumai 3, possibly even under high disease pressure (Lu et al. 2001; Zhou et al. 2004; Lin et al. 2006). A study by Ma et al. (2006b) identified QTL on chromosomes 2A, 3B and 5A in Wangshuibai. Together with the identification of DNA markers associated with these QTL, this cultivar would be ideal for use in MAB programmes to develop new resistant cultivars. In 2010 Xue et al. (2010b) mapped *Fhb4* to chromosome 4B and *Fhb5* was later mapped to chromosome 5A (Xue et al. 2011) in Wangshuibai. However, the one great concern of the resistance of Wangshuibai is that in a number of studies the resistance was poorly transferred to developed lines and showed poor heritability. With the use of MAS lines containing targeted Wangshuibai alleles can be selected (Lin et al. 2006).

2.8.3 CM-82036

CM-82036 was developed from a cross between Sumai 3 and Thornbird, where the two major QTL from Sumai 3 on chromosomes 3B and 5A, were successfully transferred to the newly developed cultivar (Buerstmayr et al. 2002; 2003; 2009; Lemmens et al. 2005; Wilde et al. 2007). The QTL on chromosome 3B explained around 60% of the phenotypic variation for the Type II resistance reaction. A minor QTL was detected on chromosome 1B in association with the high molecular weight glutenin locus identified on this chromosome (Buerstmayr et al. 2002). SSR markers flanking these respective QTL are listed in Table 2.1. Lemmens et al. (2005) discovered from their study on CM-82036 that the *Qfhs.ndsu-3BS* QTL/*Fhb1* (now referred to as *Fhb1*) is involved with resistance to DON accumulation in the kernels and DON degradation. The *Fhb1* gene region conferring a high level of resistance towards DON accumulation assists in the prevention to the spread of FHB within spikes. DON that was injected into single florets in known concentrations was converted to DON-3-*O*-glucoside, the safe detoxified product, in a similar ratio as the initial DON concentrations. *Fhb1* plays an important role in the FHB resistance complex and is believed to either encode for DON-glucosyl-transferase or that genes present in this region regulate the expression of such an enzyme (Lemmens et al. 2005). Jointly the two QTL on chromosomes 3B and 5A can reduce the DON content by around 70% (Miedaner et al. 2006). The QTL on chromosome 3BS is mostly associated with Type II resistance, while the *Qfhs.ifa-5A* QTL on chromosome 5A is mostly associated with Type I resistance. Buerstmayr et al. (2002) indicated based on their previous QTL mapping studies on Sumai 3, that the QTL detected on chromosome 6B in

Sumai 3 was not detected in CM-82036, suggesting that the putative 6B QTL allele may not be present in CM-82036 or is masked by background (Buerstmayr et al. 2002).

2.8.4 Frontana

The Brazilian wheat cultivar Frontana was released in 1943 (Singh et al. 1995). Frontana has a significant FHB resistance QTL that was mapped by Steiner et al. (2004) to chromosome 3A (Wilde et al. 2007). The moderate resistance response of Frontana is thought to be controlled by the additive interaction of at least three known minor genes/QTL (Singh et al. 1995) which are listed in Table 2.1. A unique resistance mechanism of Frontana is its ability to degrade DON through the production of specific enzymes. When Frontana was inoculated using a single point inoculation method under glasshouse conditions, it did not display resistance to spread of the disease within the spike (Type II). However, in the field Frontana displayed high levels of resistance to initial infection (Type I; Bai and Shaner 2004).

2.9 Marker-assisted breeding

The term “marker-assisted selection” was first used in literature almost three decades ago (Beckman and Soller 1986). By improving the precision and efficiency in which targeted phenotypes of certain genotypes can be predicted, the development of improved cultivars with enhanced disease and pest resistances and higher agronomic performance can be significantly accelerated (Varshney et al. 2009; Randhawa et al. 2013). With modern developments in genomics, especially in a crop such as wheat, a number of tools for the discovery and tagging of novel alleles and genes are available. These tools can make public or private sector breeding programmes more efficient in the form of applied MAS (Mosse and Mumm 2008; Xu and Crouch 2008). MAS allows breeders the opportunity to gain an enhanced response from selection as molecular markers can be applied at early seedling stage, with accurate and precise selection, thus reducing costs (Miedaner and Korzun 2012). MAB or MAS can overcome certain cumbersome restraints of traditional breeding strategies and enables breeders to select superior genotypes that might not have been achievable or feasible using only conventional and traditional breeding methods (Somers et al. 2007; Xu and Crouch 2008; Randhawa et al. 2013).

2.9.1 Benefits of marker-assisted selection to plant breeding

MAS assists in the more accurate and efficient characterisation of all available germplasm and identifies potential genetic variation that is not visible (Xu and Crouch 2008; Randhawa et al. 2013). MAS is particularly useful for selecting traits with the

following characteristics: difficult or expensive and labour intensive to phenotype, a recessive trait/gene that needs to be in a homozygous state for expression and/or controlled by multiple QTL or small effect genes that combine to confer a targeted phenotype (Xu and Crouch 2008). One of the most frequent uses of MAS has been the construction of linkage maps for important crops to identify chromosomal regions that contain important genes of interest (Collard et al. 2005). DNA markers can then be used to successfully tag and clone, manipulate and stack multiple genes and QTL into new backgrounds and improve current germplasm (Xu and Crouch 2008; Randhawa et al. 2013). MAS can assist with the protection of plant varieties (plant breeder's rights) and intellectual property rights (Randhawa et al. 2013). MAS can be used to speed up the backcross breeding process and select for recurrent parent alleles to ensure faster recurrent parent recovery (Collard et al. 2005; Xu and Crouch 2008). The availability of makers closely linked to a targeted gene/QTL (with a relatively high phenotypic response), or, if possible, even markers developed from gene sequences, are most critical to the success of MAS and/or MABC (Miedaner and Korzun 2012).

2.9.2 Marker-assisted backcrossing

In plant breeding, a key objective as defined by Semagn et al. (2006), is the transfer of one or more targeted genes/traits from a donor source into the background of an elite variety or recurrent parent. This objective is generally achieved by the backcross breeding method. There are many modelling and computer simulation studies indicating the power of markers with regard to recovery rate and precision in a backcross breeding programme. For most crops is has been observed that 90% of the recurrent parent genotype can be recovered within two to three backcross generations when enough markers (one every 10 cM or less) and large enough populations are used (Xu and Crouch 2008). Molecular markers intended for the use in a MABC programme can be selected based on the following principles: genome distribution (previous maps); haplotype or polymorphic information on the selected lines and the close linkage with targeted genes or traits of interest (Xu and Crouch 2008; Miedaner and Korzun 2012).

2.9.2.1 Key principles of backcrossing

The process of backcrossing as stated by Hospital (2005) is a distinguished and recognised breeding scheme in which the progeny with a particular trait of interest are selected, which are then crossed back to the recurrent parent in a number of successive generations, ensuring that the portion of donor parent genome transferred is minimal and decreases after each generation, except on the targeted chromosome(s). It is necessary to reduce the targeted donor chromosome segments carrying the characteristic of

interest to the smallest possible fragment size (Hospital 2005). In a backcross breeding programme where selection is carried out for the trait of interest only, the reduction in donor genome per generation will be 50%, except for the targeted chromosome where the reduction rate will be much slower (Salina et al. 2003; Babu et al. 2004; Hospital 2005; Semagn et al. 2006), resulting in the phenomenon known as linkage drag on the targeted chromosome (Hospital 2005).

However, if selection could be done against the donor parent's genome or a distinction could be made between both parental alleles, recovery of the recurrent parent genome could be much faster. Selection based on molecular marker alleles of both parents was suggested to be the first potential application of MAS within breeding programmes (Beckman and Soller 1983; Tanksley et al. 1989; Hospital 2005). Molecular markers linked to economically important traits have been identified and used in MABC for several plant species, including wheat (Collard et al. 2005; Semagn et al. 2006; Xu and Crouch 2008; Miedaner and Korzun 2012). MABC using molecular markers with good, even coverage across the entire genome, can be implemented to select individuals with the highest percentage of recurrent parent genome, reducing the number of backcross generations required (Frisch and Melchinger 2001; Semagn et al. 2006). Application of markers to select for the recurrent parent genome is known as background selection (Frisch and Melchinger 2001). Babu et al. (2005) identified a number of maize plants with a recurrent parent percentage between 93-96% in the BC_2 generation with the aid of a two-generation MABC approach.

2.9.2.2 Factors for consideration

Several factors must be taken into account for the successful application of MABC. These include: The interval between markers across the entire genome (marker map and density), number of targeted genes/QTL to be introgressed, genetic architecture and nature of the trait of interest, number of individuals that can be screened per generation (generation size), specific recurrent parent genetic background, type and number of molecular markers to be used, availability of technologically equipped facilities, applied selection strategy and the crossing scheme to be used (Weeden et al. 1992; Semagn et al. 2006; Prigge et al. 2008). Other points for consideration are how many marker data points (MDP) are required to meet the desired objectives, influence of the selection strategy applied on the number of MDP required and how many MDP can be feasibly dealt with considering technologies and level of skills available (Frisch et al. 1999b). A co-dominant type of marker can distinguish between homozygous and heterozygous progeny which is an advantage and favoured in MABC (Holland 2004). There are many

markers available which are linked to specific traits which will always need validation before use. Potential genetic gain from MABC increases with an increase in marker linkage tightness, as shorter genetic distances between linked markers and the trait of interest reduce the possibility for recombination (Semagn et al. 2006).

Foreground selection

Foreground selection is used as a diagnostic tool for the precise selection of targeted marker allele(s) linked to specific traits of interest, being transferred from the donor line into the recipient line or recurrent parent thus indicating the presence of the trait in individual plants (Hospital 2001; Semagn et al. 2006).

Background selection

Background selection as defined in Semagn et al. (2006) is used for identifying individuals with a low proportion of donor genome which can prevent the transfer of undesirable traits to the recurrent parent. Individuals that are homozygous for alleles of the recurrent parent at all background markers across the entire genome are favoured for selection (Frisch et al. 1999b). An important objective of background selection is to reduce the donor segments on targeted chromosomes to the smallest possible size within the recurrent parent. This is just as important on non-targeted chromosomes. However, this is normally more efficiently applied from the second backcross generation onwards (Frisch et al. 1999a; Hospital 2001).

2.9.2.3 Different types of individuals observed in a marker-assisted breeding programme

Based on the genotypes observed at the targeted locus and considering the flanking and background markers that surround the targeted region of interest, selected individuals can be described as one of following types: Type 1 would be classed as a double recombinant individual (limited or minimal linkage drag) which is heterozygous for the donor allele at the targeted locus and homozygous for the recurrent parent alleles for both flanking markers that surround the targeted locus. Type 2 and Type 3 individuals are similar and are classed as single recombinants (expected levels of linkage drag) which are heterozygous for the donor allele at the targeted locus and are homozygous for the recurrent parent allele at one of the flanking markers that surround the trait of interest, irrespective of the genotypes at the other flanking marker. Type 4 is a non-preferred recombinant individual (with high amounts of linkage drag) which is heterozygous for the donor allele at the targeted locus and heterozygous at both flanking markers. Type 5 individuals do not have the trait of interest and is a non-recombinant at

the targeted locus, which is homozygous for the recurrent parent allele at the targeted locus (Frisch et al. 1999b; Semagn et al. 2006).

2.10 Concerns of QTL introgression

QTL detection and functional analysis remain completely separate from normal MAS applications as a discovery or basic phase of marker research. In modern times QTL detection has been far more accurate and applied at higher thresholds (Lv et al. 2014) However, concerns around QTL detection and introgression of about ten years ago are still mostly applicable today. Marker-assisted introgression of specific traits such as QTL is not always completely successful (Hospital 2005). In most cases QTL introgression in breeding programmes ends in failure in a MAS programme when errors were made during the initial QTL detection process (Semagn et al. 2006; Ma et al 2006a). The true success of trait/QTL introgression depends on the ability of the targeted genes/QTL to exhibit the expected and desired effects once introduced in the "new genetic background" (Hospital 2005). A new introduction into a genetic background can alter the epistatic interactions. Nevertheless, if a targeted QTL is not giving expected effects there are two possible reasons; it could be due to new epistatic effects in the new genetic background or that the QTL transferred had no effect at all in that background, meaning its effect is being masked (Hospital 2005). There are a number of possible reasons for unexpected effects of introgressed traits over and above the epistatic effects discussed already. It could possibly be as a result of QTL by environment interactions or another possible reason is that the chromosome segment containing the QTL region transferred in fact contains not one gene but a number of genes. It is vital for a MAS programme that targeted traits/QTL be verified in a number of genetic backgrounds, across environments before use (Semagn et al. 2006; Collard et al. 2005; Collard and Mackill et al. 2008). From a breeder's point of view when it comes to making selection decisions, it is risky to make decisions based only on the presence of DNA markers. In most cases it is much wiser for the breeder to support marker data selections with phenotypic selection. The choice of using phenotypic selection will depend on the targeted trait and on the crop or species used within the MAB programme (Hospital 2005; Collard and Mackill et al. 2008; Lv et al. 2014).

2.11 Application of molecular markers in wheat

The plant biotechnology era began in the early 1980's (Mosse and Mumm 2008) and since then molecular markers have been successfully applied in wheat (Bagge et al. 2007). Molecular markers that are extensively applied in wheat breeding, in the past and presently include SSR, sequenced-characterised amplified region (SCAR), or STS

markers and more recently single nucleotide polymorphisms (SNPs). SSR and SNP markers are still used to a great extent in marker-assisted research. In fact SNP markers have only really being fully exploited in wheat breeding programmes the last few years. In 2007 no more than 20 genes that control various important agronomical traits have been cloned in wheat (Bagge et al. 2007), but this is currently changing by the month. Less than ten years ago when molecular marker technologies were under pressure by a driven need for higher-throughput systems, a move was made from measuring DNA fragment polymorphisms to hybridisation-based technologies with high multiplexing capabilities (Akarbi et al. 2006; Wenzl et al. 2006). One such technology, diversity arrays technology (DArT) has been used extensively the last five to ten year period to conduct targeted wheat research (Wenzl et al. 2006; Akarbi et al. 2006). What makes DArT technology unique is its ability to type hundreds to thousands of genomic loci in parallel (Akarbi et al. 2006) and even performs well in the complicated genome of hexaploid wheat. A number of high density linkage maps of wheat have been generated using DArT markers in combination with SSR and SNP markers over the last five years (Marone et al. 2012). It has been suggested that the use of a combination of SNP and DArT markers are the way forward for whole-genome profiling. During this similar period the advances in next generation sequencing (NGS) technology has been rapid and causing a revolution in genetics and genomics (Varshney et al. 2009; Berkman et al. 2012). NGS technologies are capable of generating DNA sequence data cheaper and at a far faster rate than ever before. Now the advances in NGS technologies are driving the costs lower and increasing the sequence capacity exponentially almost on a monthly basis (Berkman et al. 2012). Genomics assisted breeding approaches are advancing significantly with the increasing availability of genome sequence data (Varshney et al. 2009), especially in wheat. An application of NGS has been for faster and more accurate discovery of SNPs (Berkman et al. 2012) which can then be used in a targeted MAS programmes.

2.11.1 Sequenced-tagged sites

A STS marker is a short, unique sequence that identifies a specific target locus and is amplified by PCR (Gupta et al. 2002; Hu et al. 2014). A STS is characterised by a set of PCR primers that are developed from sequencing of a restriction fragment length polymorphism (RFLP) or random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) fragment linked to a specific trait of interest. STS primers amplify specific, targeted DNA regions and therefore are locus specific in nature (Gupta et al. 2002). STS markers combine the simplicity of RAPD applications with the better specificity offered by SSRs (Perry and Bousquet 1998). These markers are useful

for the detection of specific desired genes/QTL (Gupta et al. 2002; Hu et al. 2014). In the last five years, a number of STS markers have been developed in wheat, including STS markers for the *Fhb1* gene namely St-3B markers by Cuthbert et al. (2006).

2.11.2 Microsatellites or simple sequence repeats

Microsatellites or SSRs are a unique class of repetitive DNA sequences that are highly polymorphic and abundant throughout the genomes of plants and animals (Powell et al. 1996; Fahima et al. 1998; Röder et al. 1998; Varshney et al. 2000; Hayden et al. 2001; Rakoczy-Trojanowska and Bolibok 2004). SSRs are known to be mutational hotspots present in genomes (Luo et al. 2012) and are recognised as powerful and informative markers (Peakall et al. 1998) even today, especially in wheat. A microsatellite region is generally a repeat motif of di- $(TA)_n$, tri- $(CAA)_n$, tetra- $(TAGA)_n$, or even penta and hexa-nucleotides (Powell et al. 1996; Peakall et al.1998; Luo et al. 2012) which are flanked by unique and conserved regions (Rakoczy-Trojanowska and Bolibok 2004; Xu et al. 2013). The flanking regions define the microsatellite region and primers are developed based on the sequence of these flanking regions (Powell et al. 1996; Xu et al. 2013). SSRs can be further classed into three different types namely compound, interrupted and pure SSRs. Compound SSRs consist of two or more repeat types e.g.$(CT)_n(GT)_m$ while interrupted SSRs contain a specific interruption within the repeat e.g.$(AT)_nCC(AT)_m$ and pure SSRs consist of uninterrupted repeats e.g. $(GA)_n$ (Peakall et al. 1998). The length of the microsatellite region depends on the number of motif repeats between the conserved flanking regions which is often highly polymorphic (Fahima et al. 1998). The nature of the SSR repeat units are naturally unstable and prone to mutation by DNA slippage or unequal crossing over (Luo et al. 2012). Microsatellite or SSR analysis is a PCR-based technique (Röder et al. 1998; Gupta et al. 2002; Somers et al. 2004) that still makes it a popular marker choice for rapid and reliable genotyping that can be used between laboratories and for high-throughput analysis (Röder et al. 1998; Somers et al. 2004; Lou et al. 2012; Xu et al. 2013).

Over the years there has been reported evidence that there are a large number of SSR regions located in transcribed regions, protein coding regions as well as in expressed sequence tags. It has been speculated that SSR regions play a significant role in chromatin organisation, gene activity regulation and are influential in recombination and DNA replication (Li et al. 2004). SSR markers are multi-allelic and mostly co-dominant in nature, which makes them ideal for use in evolutionary studies, genetic relationship studies, DNA fingerprinting and genetic mapping. Most SSRs are locus specific and often chromosome specific (Powell et al. 1996; Röder et al. 1998; Hayden et al. 2001;

Gupta et al. 2002; Xu et al. 2013) that is ideal for anchoring markers to specific chromosomes (Röder et al. 1998) which is useful during mapping (Somers et al. 2004). Once microsatellite markers have been developed, their application in breeding programmes for MAS is highly effective (Röder et al. 1998). Compared with SNP markers that are generally only bi-allelic, SSR markers are generally more informative due to their multi-allelic nature, which is one of the reasons SSR markers are still commonly used today (Xu et al. 2013).

2.11.3 SSR wheat maps

The first comprehensive SSR map of wheat was produced by Röder et al. (1998), where over 279 SSR markers were mapped to the 21 chromosomes of wheat. SSR markers used were designated the abbreviation Gwm, after the research group that identified them. In 2002 a study by Gupta et al. mapped a further 66 SSR markers that extended the microsatellite genetic map of wheat. These particular SSR markers from their study were given the abbreviation Wmc from the Wheat Microsatellite Consortium (Gupta et al. 2002). A number of high density marker maps have been generated in wheat which have used different targeting strategies and types markers (mostly SSR) and different mapping populations (Somers et al. 2004; Sourdille et al. 2004). Sourdille et al. (2004) created a microsatellite-based deletion map of wheat by making use of 725 SSR markers. A high-density microsatellite consensus map of bread wheat was constructed by Somers et al. (2004), where 1 235 different SSR markers were mapped in bread wheat at an average marker interval of 2.2 cM. Microsatellite markers from all different research groups were used to construct this consensus map (Wmc, Gwm, Gdm, Cfa, Cfd and Barc; Somers et al. 2004). Recently the A and B genome high density consensus map of wheat was developed and generated from DArT and SSR marker data (Marone et al. 2012). The construction of consensus maps is important and relies on the development of new genetic tools that provide an essential basis for further genomic research (Marone et al. 2012). These consensus maps and other SSR linkage maps mentioned above are like libraries and points of reference for SSR markers to be used and applied in many applications of MAS and MABC on wheat.

2.12 Conclusions

From this literature study it was determined that there exists a need to increase food production under stressed environments for more sustainable agriculture globally and locally. An economically important diverse crop such as wheat can assist in ensuring a more food secure future. In SA wheat production is declining, especially dryland wheat production, although irrigated wheat makes up approximately 27% of national wheat

production. FHB disease is a real sporadic threat and a risk to commercial irrigated wheat farmers in SA. The causal agents of FHB and mycotoxins they produce are diverse and complex. This disease causes significant yield losses and loss in revenue due to the negative impacts on grain quality. An integrated management strategy using stable FHB host plant resistance at its core, needs to be implemented for SA. Therefore, the FHB resistance levels in current SA irrigated spring wheat varieties need to be improved. There are a number of well documented exotic FHB sources of resistance that can be exploited for FHB resistance improvement. A number of FHB resistance genes/QTL have been mapped to different chromosomes and have been tagged with molecular markers in these sources of resistance. The use of MAS in combination with phenotypic validation in a targeted backcross pre-breeding programme can help to achieve the development of improved FHB resistance lines in the background of a SA wheat cultivar, while maintaining competitive yields and good grain quality.

2.13 References

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Chapter 3

Marker-assisted development of F_1 and BC_1F_1 lines containing Fusarium head blight resistance genes/quantitative trait loci

Abstract

The fundamental focus of plant breeding is the selection of specific individual plants with traits of interest. Breeders are continually using different methods to improve existing wheat varieties. There is a need in SA for the improvement of FHB resistance levels in irrigated spring wheat cultivars. The main objective of this study was to track the introgression of targeted FHB resistance genes/QTL. The crossing of the SA spring wheat cultivar Krokodil with FHB resistant source CM-80236 in a MABC pre-breeding programme for targeted FHB resistance gene/QTL introduction is discussed. Developed F_1 individuals were validated with SSR marker Gwm493, which is associated with FHB resistance gene *Fhb1.* A BC_1F_1 population was generated from identified true F_1 hybrids. A two stage MAS approach was employed with foreground selection done for targeted FHB resistance genes/QTL and background selection against unwanted donor alleles. A partial linkage map of targeted chromosomes 3B, 5A, 6B and 7A was generated on a BC_1F_1 population of 120 individuals. From this linkage map GGT profiles were developed for each individual. This allowed for the analysis and comparison of the following: single versus double recombinants, recurrent parent percentage recovered and segregation distortion. Families containing three and four targeted FHB resistance genes/QTL were identified. These lines will be used in the development of the BC_2F_1 generation.

"Your present circumstances don't determine where you can go;
they merely determine where you start."

by

Nido Qubein

3.1 Introduction

The fundamental foundation of plant breeding is the selection of specific individual plants with desirable traits (Collard and Mackill 2008). Breeders are continually using different methods to improve existing varieties for a number of targeted traits such as yield, grain quality and disease resistance. FHB has become a disease of increasing economic importance on wheat in SA. With the overall wheat production in SA on the decline, a production shift is occurring, from previously dominated dryland winter wheat to irrigated spring wheat. A potential higher profit margin from more consistent and reliable high yields will further encourage the production of irrigated spring wheat which can create potential FHB epidemic hot spots from season to season, placing a higher importance on the disease (Duveiller et al. 2007).

Host plant resistance is often improved by crossing existing wheat cultivars with either diverse foreign resistant donors or by doing wide crosses using wild relatives (Semagn et al. 2006). The use of unadapted foreign resistant donors can be problematic due to the negative association of the specific targeted FHB resistance gene/QTL with unwanted agronomic traits like yield penalties, an increase in plant height and a reduction in grain quality (Suzuki et al. 2012). Furthermore, introgression of a targeted trait/QTL from a donor line into a recipient line is often impeded by the phenomenon of linkage drag (Hospital 2005; Semagn et al. 2006).

For decades the backcross breeding method has been used to reduce linkage drag around a targeted locus. With every backcross generation, linkages between unwanted traits are broken and the size of the chromosome segments transferred from foreign donors are reduced in the resulting breeding material (Hospital 2005; Semagn et al. 2006; Randhawa et al. 2009; Wang et al. 2009). Theoretically, with each conventional backcross generation, the offspring should be 50% more similar or related to the original recurrent parent on non-targeted (non-carrier) chromosomes (BC_1=75.00%, BC_2=87.50%, BC_3=93.75%, BC_4=96.88%, BC_5=98.44% and BC_6=99.2%; Hospital 2005; Randhawa et al. 2009). On chromosomes containing the introgressed targeted trait (carrier chromosomes), the recovery of RPGP is slower, resulting in linkage drag. With this methodology and theory in mind, breeders are aware of “linkage drag” but are mostly selecting “blind” or cannot directly select against this phenomena. Before the application of MAS, breeders had gone well beyond the BC_6 generation in an attempt to reduce the amount of unwanted donor genome retained carrying negative traits (Semagn et al. 2006; Wang et al. 2009). In recent years breeding programmes aim to achieve targeted

objectives in BC_1 or BC_2 generations and it has been suggested that there is minimal to no advantage going to BC_3 or beyond (Randhawa et al. 2009; Wang et al. 2009).

In the past 10-20 years the application of MAS in crop improvement has helped breeders to introduce new resistance genes and different combinations thereof into breeding material. More recently, molecular markers have been used in MABC programmes. MABC efficiency depends on the following factors: experimental design, marker density, population size and selection strategy (Frisch and Melchinger 2005). A MABC approach often involves two forms of selection; foreground and background selection. Foreground selection is the first phase of selection in which markers associated with targeted genes/QTL are used to narrow down the targeted population. Foreground selection in each backcross generation is based on the presence of molecular markers linked to the trait of interest. Background selection on the other hand makes use of markers that are not linked to the targeted gene/QTL and are useful for selecting against the donor parent's genome (Collard and Mackill 2008). Background selection is the final step of selection in which individuals containing the targeted trait and with the highest amount of RPGP are preferred (Semagn et al. 2006; Collard and Mackill 2008; Randhawa et al. 2009). Often referred to as recurrent parent genome recovery, background selection is done with specifically chosen background markers that cover large genomic regions of either specific carrier chromosomes or the entire genome (Randhawa et al. 2009). The foreground and background marker data can be used to create genetic linkage maps.

A MABC approach has been used successfully on other cereal crops namely barley (Jefferies et al. 2003), rye (Falke et al. 2009), pearl millet (Serraj et al. 2005), maize (Babu et al. 2005; Ribaut and Ragot 2007) and rice (Khush 2005; Steele et al. 2006). In most of these studies a RPGP of 93-98% was achieved in just two generations of backcrossing (Babu et al. 2005; Randhawa et al. 2009). Wang et al. (2009) discussed the value of using a single backcrossing breeding strategy in the International Maize and Wheat Improvement Centre (CIMMYT) breeding programmes. They stated that two generations of backcrossing is enough to recapture most of the important agronomical qualities of adapted breeding material (Wang et al. 2009). Randhawa et al. (2009) successfully introgressed targeted genes into popular wheat varieties in just two backcross generations using a MAB approach. They identified a superior $BC_2F_{2:3}$ line that contained the targeted traits with an overall RPGP of 97%, compared to a BC_4F_7 line with a RPGP of 82% developed from traditional backcrossing and phenotypic selection. The largest difference between these two lines was for the targeted chromosomes as a

result of linkage drag. Results obtained by Randhawa et al. (2009) validated the efficacy and advantages of MABC over traditional backcross methods.

The objective of the current study was to use marker-assisted foreground and background selection to track the introgression of targeted FHB resistance genes/QTL within F_1 and BC_1F_1 generations developed from a cross between SA irrigated spring wheat cultivar Krokodil and CIMMYT FHB resistant line CM-82036. This chapter examines if MAS will assist with the successful tracking of the transfer of targeted FHB resistance genes/QTL combinations while retaining the highest amount of RPGP in the first backcross generation. Also discussed is whether MAS will assist in the efficient selection of lines with reduced linkage drag around targeted FHB resistance gene/QTL regions between backcross generations. Additionally, it is discussed whether MABC will significantly allow for the faster improvement of elite pre-breeding lines.

3.2 Materials and methods

3.2.1 Plant material

The following four SA spring wheat irrigation cultivars were considered as possible recurrent parents; Duzi, Krokodil (Agricultural Research Council-Small Grain Institute; ARC-SGI), PAN3434 (PANNAR) and SST886 (Sensako). Four FHB sources of resistance were considered as FHB donor lines; Frontana (FHB resistant spring wheat cultivar from Brazil; Steiner et al. 2004), Sumai 3 (a Chinese FHB resistant line; Anderson et al. 2001; Zhou et al. 2002; del Blanco et al. 2003), BMC (0YMC) and CM-82036 (CIMMYT line; Buerstmayr et al. 2002; 2003; 2009). CM-82036 was developed from a cross between Thornbird and Sumai 3. These lines were all initially considered as potential SA recurrent parents and FHB resistance donor parents respectively for this study.

3.2.2 Glasshouse trials

All glasshouse trials (2008-2010) for each generation's development were done at the University of the Free State (UFS). Glasshouse conditions were set at 20°C day and 15°C night temperatures using natural day/night lengths. Seeds were planted in 3 ℓ pots containing a clay-topsoil mixture to which 2 g of 3:2:1 (N:P:K) fertiliser was added. A solution of micronutrients [Chemicult® fertiliser (Chemicult Products, Pty Ltd., SA)] was applied weekly to growing seedlings once seedlings had reached two weeks of age. A macronutrient solution of 3:2:1 fertiliser (100 g/10 ℓ) was applied every second week when seedlings were four weeks old. Plants were watered twice daily by hand.

3.2.3 Screening of parental lines

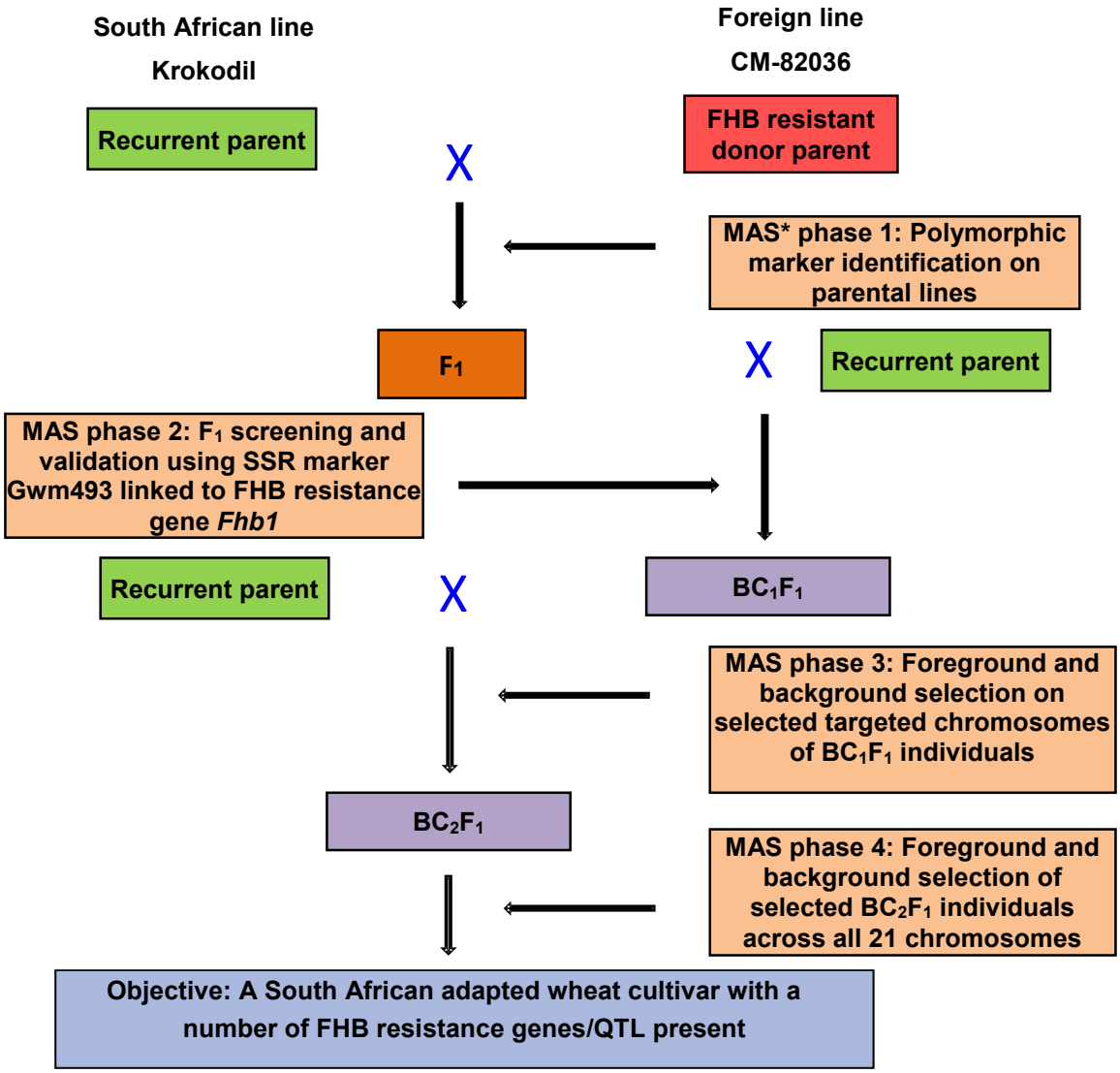
Eight seeds of each of the eight potential parental lines discussed in section 3.2.1 were planted. Two seeds were planted per pot; with leaf samples for DNA isolation collected from four week old seedlings. The emerging heads were covered with glycine bags to prevent cross pollination and were left to mature to maintain pure seed stocks.

These four potential recurrent parental lines as well as four potential FHB resistant donor lines were screened with 14 foreground markers linked to FHB resistance genes/QTL, plus 20 additional randomly selected (1-2 markers per chromosome) background markers. Individual plants of each parental line were screened to determine the line purity for the targeted FHB resistance genes/QTL. The two parental lines to be used in the MAB programme were selected using the following criteria: absence of interplant variation within the line; absence of similar FHB resistance marker alleles in the recurrent parent; presence of FHB resistance markers in the expected donor parent and large enough allelic variation for targeted FHB resistance markers between the recurrent and donor parent line combination. Based on the molecular data obtained from screening eight individuals of each potential parental line (SA cultivars: Duzi, Krokodil, PAN3434 and SST886 and FHB resistance donor lines: Sumai 3, CM-82036, Frontana and BMC) Krokodil was selected as the recurrent parent and CM-82036 as the FHB resistant donor parent. The selected parental lines were screened with 256 background SSR markers. DNA from ten individual plants per line was bulked to obtain a representative sample from each line. SSR markers which were polymorphic between the parental lines were used for screening of the BC_1F_1 and BC_2F_1 populations.

3.2.4 Marker-assisted backcross breeding scheme

The MABC programme used in this study was based on the work of Babu et al. (2005). Figure 3.1 indicates how the selected SA recurrent parent (Krokodil) was used throughout the breeding programme as the female parent. The selected FHB resistant donor line (CM-82036) was used as the male parent (pollinator) to develop the F_1 lines. Figure 3.1 furthermore indicates at what point and phase in the breeding programme specific types of MAS were applied.

MAS phase 1 involved screening the selected parental lines with SSR and STS markers linked to FHB resistance genes/QTL present in CM-82036. Additionally, around 250 background SSR markers, covering the entire wheat genome [21 chromosomes, 3 sub-genomes (A, B and D)], were used to screen the selected parental lines to identify polymorphic markers.



MAS*=marker-assisted selection

Figure 3.1 **A schematic representation of the four phases of marker-assisted backcrossing used for the introgression of targeted Fusarium head blight resistance genes/QTL into the South African spring wheat cultivar Krokodil**

These background markers were selected from the SSR consensus map of wheat (Somers et al. 2004), using an average 10 cM distance between markers as basis for selection. These polymorphic markers were then applied during phases 3 and 4 of MAS for RPGP selection.

During the second phase of MAS, F_1 individuals were screened using SSR marker Gwm493 linked to FHB resistance gene *Fhb1* in order to trace the successful transfer of FHB resistant donor male parent alleles. Only confirmed F_1 individuals were taken further in the backcross breeding programme.

The third phase of MAS involved screening of BC_1F_1 individuals with polymorphic foreground and background SSR markers linked to the targeted chromosomes. A partial linkage map of each targeted chromosome was created based on the molecular data generated from screening BC_1F_1 individuals.

The fourth phase of MAS involved the narrowing down of the BC_2F_1 population with foreground SSR markers. BC_2F_1 individuals were selected based on FHB resistance genes/QTL present. These selected individuals were screened with polymorphic background markers across the entire genome. A linkage map of all 21 chromosomes was created based on the molecular data generated from screening the selected BC_2F_1 individuals.

3.2.5 FHB resistant line development

3.2.5.1 F_1 generation

Three seeds of each parental line were planted per 3 ℓ pot. Three plantings spaced over a five week period with 10 pots per planting were planted of each parental line. A one week interval between plantings was used to allow for differences in flowering dates. The recurrent parent Krokodil was used as the female parent and the FHB resistant donor line CM-82036 as the male parent.

3.2.5.2 BC_1F_1 generation

Three plantings each consisting of 20 pots containing F_1 crosses and 30 pots containing recurrent parent Krokodil were planted. Within each planting, the core of each planting was ten pots of F_1 seed and then ten pots of the recurrent parent, planted three days apart. This was repeated once for each planting. Planting one was started with an additional ten pots of recurrent parent Krokodil, while plantings two and three were

followed by ten additional pots of recurrent parent Krokodil, for optimal anthesis synchronisation. Each planting sequence had a one week interval.

3.2.6 DNA isolation

Leaf material used for DNA isolation was sampled from four week old seedlings, using strict sterile conditions. Sample leaves were immediately placed on ice followed by freeze-drying for 3-5 days using a Viritis Advantage Freeze mobile II (New York, NY, USA) and stored at -70°C. Freeze-dried leaf material was homogenised using Qiagen's TissueLyser (Haan, Germany). Three to five freeze-dried leaf pieces of 1-2 cm in length were transferred into a 2 ml microcentrifuge tube together with two 5 mm stainless-steel ball bearings and homogenised for 1 min at 30 r/s.

A modified one-day CTAB (hexadecyltrimethylammonium bromide) extraction method was used for total genomic DNA isolation (Saghai-Maroof et al. 1984). Per 2.0 ml microcentrifuge tube, 750 µl CTAB buffer [100 mM Tris-Cl (tris(hydroxymethyl) aminomethane), pH 8.0, 20 mM EDTA (ethylene-diaminetetraacetate), pH 8.0, 1.4 M NaCl, 2% (w/v) CTAB, 0.2% (v/v) β- mercaptoethanol] was dispensed into 250 µl of fine leaf powder and incubated for 1 h at 65°C. The suspension was extracted with 500 µl chloroform:isoamylalcohol [24:1 (v/v)] and centrifuged at 12 000 *g* for 5 min at 4°C. DNA was precipitated with 0.66 volumes ice-cold 2-isopropanol at room temperature (20-25°C) for 20 min and centrifuged at 12 000 *g* for 5 min at 4°C. A DNA wash step was performed at room temperature for 20 min by adding 500 µl 70% (v/v) ethanol followed by centrifugation at 12 000 *g* for 10 min at 4°C. The DNA pellet was air-dried for 1 h at room temperature. The DNA was resuspened in 100-200 µl TE buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA, pH 8.0) and treated simultaneously with 100 µg/ml DNase-free RNase A enzyme followed by incubation at 37°C for 2 h. Samples were stored at 4°C till the next day. DNA quantity and quality were estimated from a 0.8% (w/v) agarose gel run at 80 V for 1 h in 1x UNTAN (40 mM Tris-Cl, 2 mM EDTA, pH adjusted to 7.4 with acetic acid) buffer containing ethidium bromide. A digital gel image was recorded after exposure to ultraviolet (UV) light. DNA concentrations were determined with a spectrophotometer and diluted to 50 ng/µl.

3.2.7 SSR analysis

PCR reactions were performed in a DNA Engine Dyad® Thermal Cycler (Bio-Rad Laboratories (Pty) Ltd, Johannesburg, SA). PCR reactions were set up in a final volume of 10 µl for all SSR and STS PCR primers used during this study. PCR reactions contained 200 ng genomic DNA, 2 mM $MgCl_2$, 1x GoTaq Flexi polymerase buffer

(Promega Corporation, Madison, WI, USA), 200 µM of each dNTP (deoxynucleotide triphosphate), 25 ng of each primer and 0.25 U GoTaq® Flexi DNA *Taq* polymerase (Promega). Primers were manufactured by Integrated DNA Technologies (Coralville, IA, USA).

All 14 markers linked to targeted FHB resistance genes/QTL used in foreground selection in this study had specific annealing temperatures (50-60°C) depending on the specific PCR primer pair used (Table 3.1). The following cycle conditions were used for all annealing temperatures: 1 cycle at 94°C for 3 min; 35 cycles at 94°C for 45 s, Tm °C for 45 s and 72°C for 75 s and final elongation step at 72°C for 10 min. PCR cycling conditions for all wheat SSR marker types were used as obtained from publications and the Grain Genes website (http://wheat.pw.usda.gov/GG2/index.shtml) namely, Gwm (Gatersleben Wheat Microsatellite; Röder et al. 1998), Gdm (Pestsova et al. 2000), Wmc (Wheat Microsatellite Consortium; Gupta et al. 2002), Barc (USDA-ARS Beltsville Agricultural Research Center; Song et al. 2002; 2005), Cfa (Sourdille et al. 2003), Cfd (Guyomarc'h et al. 2002; Somers et al. 2004) and St SST markers (Cuthbert et al. 2006). Background SSR markers used during polymorphic marker screening, marker names, polymorphic status and chromosome location are indicated in Appendix A.

All foreground and background SSR (Barc, Cfa, Cfd, Gdm, Gwm and Wmc) and SST (St) markers were visualised using denaturing polyacrylamide gel electrophoresis (PAGE). PCR products were mixed with 5 µl formamide loading dye [98% (v/v) de-ionised formamide, 10 mM EDTA, pH 8.0, 0.05% (w/v) bromophenol blue, 0.05% (w/v) xylene cyanol]. Reactions were denatured in a thermal cycler for 5 min at 95°C and immediately placed on ice prior to loading. PCR products (4.5 to 5.0 µl) were separated on a 5% (w/v) denaturing polyacrylamide gel [19:1 acrylamide:bis-acrylamide, 7 M urea, 1x TBE Buffer (89 mM Tris-Borate, 2.0 mM EDTA)]. A 25 bp size standard DNA ladder (Promega) was loaded with each sample set. Electrophoresis was performed at constant power of 80 W for 1-2 h depending on expected PCR product size. The Silver Sequence™ DNA Sequencing System protocol supplied by Promega was used to visualise the separated PCR products. Silver stained gels were left standing upright overnight to air-dry and to be photographed. Photographs were done by exposing photographic paper (Ilford Multigrade IV RC) directly under the gel to a dim white light for approximately 15-20 s. This produced a negative image of the gel of exactly the same dimensions. SSR allele sizes were determined by visual comparison with a 25 bp (25-300 bp) DNA ladder (Promega).

Table 3.1 General PCR annealing temperatures and targeted genes/QTL for 14 foreground SSR markers used during the study

Marker name	Targeted gene/QTL	Annealing temperature	References
Gwm533	*Qfhs.ndsu.3BS/Fhb1*	60°C	Anderson et al.
Barc133		50°C	2001; Buerstmayr
Gwm493		60°C	et al. 2002; 2003;
St-3B-66		55°C	2009; Cuthbert et
St-3B-138		60°C	al. 2006
St-3B-142		60°C	
Gwm304	*Qfhs.ifa.5A*	55°C	Buerstmayr et al.
Gwm293		55°C	2002; 2003; 2009
Gwm156		60°C	
Barc197-2		50°C	
Gwm133	*Fhb2*	60°C	Cuthbert et al.
Gwm644		60°C	2007
Gwm130	*7A* QTL	60°C	
Gwm233		50°C	

3.2.8 F_1 hybrid identification

The 180 F_1 plants planted out for backcrossing were validated as true F_1 hybrid plants using SSR marker Gwm493 linked to the *Fhb1* FHB resistance gene. The FHB donor line CM-82036 was used as male parent and alleles from this line were tracked to confirm a true F_1 individual. Only individuals identified as true F_1's were retained in the backcrossing programme.

3.2.9 BC_1F_1 screening

One-hundred-and-twenty BC_1F_1 individuals that were used in crosses were screened with 14 foreground and 20 background markers across chromosomes 3B, 5A, 6B and 7A. These individuals were genotyped and classed based on the targeted FHB resistance gene/QTL combinations present in each individual. Individuals were classed as either three or four gene/QTL containing families.

3.2.10 Data analysis

Genotypic SSR data was generated for each BC_1F_1 individual based on the presence/absence of the recurrent parent and/or donor parent allele(s). The following coding or scoring system was used to convert the genotypic data into mapping data: SA recurrent parent allele = A, FHB resistant donor parent allele = B and heterozygous for both parental alleles = H. Mapping data in the form of a partial linkage map of the targeted chromosomes (3B, 5A, 6B and 7A) was generated using Map Manger QTXb 2.0 software with an log-likelihood score (LOD) > 3.0 (Manly et al. 2001; Cheema and Dicks 2009; http://mapmgr.roswellpark.org/mmQTX.html;). The software programme Record (REcombination Counting and ORDering; Van Os et al. 2005; http://www.plantbreeding.wur.nl/UK/software_record.html) was used to validate and sequentially order the SSR marker positions within the specific linkage groups. MapChart 2.2 software (http://www.wageningenur.nl/en/show/Mapchart.htm; Voorrips 2002) was used to draw the partial linkage maps from the mapping data. A chi-squared goodness of fit test and analysis was carried out on the mapped individual polymorphic SSR markers to determine the segregation deviation of marker loci from the expected 1:1 segregation ratio ($P < 0.05$; Francki et al. 2009). Mapping data, marker positions and genotypic data was used in Geographical Genotypes-GGT 2.0 software (http://en.bio-soft.net/other/GGT.html) to draw each of the targeted chromosomes for each of the BC_1F_1 individuals (Van Berloo 2008). Based on the GGT analysis the specific crossover points between markers on targeted chromosomes of each individual in the BC_1F_1 population could be identified and double or single recombinants distinguished. Additionally, data obtained from the GGT analysis was used in the calculation of RPGP

present within each individual across the specific targeted chromosomes. The predicted RPGP was calculated as the homozygous recurrent genome percentage (HRGP) value plus half the heterozygous donor genome percentage (HDGP; RPGP = HRPG + ½ HDGP). Missing value percentages were excluded or ignored.

3.3 Results

3.3.1 Parental line selection

During the parental line screening process, one or two individuals from SST886 and Sumai 3 consistently showed additional individual allelic differences for a number of important SSR markers, indicating within line variation. SST886 and Sumai 3 were thus considered unfavourable for parental selection. Duzi and PAN3434 displayed limited allelic differences for some of the targeted SSR markers compared to the four FHB donor sources. As a result of these limited polymorphic differences between these two cultivars (Duzi and PAN3434) and the FHB resistance donors Frontana and BMC, these were all excluded. Krokodil showed no interplant variation and was polymorphic across all 34 markers compared to the four FHB donor cultivars, especially Sumai 3 and CM-82036. Krokodil was thus selected as the recurrent parent. CM-82036 (a Sumai 3 derived source) was selected as the FHB resistant donor line. The genotypic data for all SSR markers, linked to FHB resistance genes/QTL (*Qfhs.ndsu.3BS/Fhb1, Qfhs.ifa.5A, Fhb2* and *7A* QTL) screened on CM-82036 showed no within line variation and displayed the same allelic sizes compared to its parental cultivar Sumai 3. Additionally, CM-82036 is agronomically more advanced than Sumai 3 which further favoured its selection.

Krokodil was commercially released in 2004 as an irrigated spring bread wheat by the ARC-SGI and has excellent hectolitre mass, good straw strength, poor aluminium tolerance and reasonable sprouting tolerance. Krokodil has poor overall disease resistance. It is susceptible to all three wheat rusts [stem rust - *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and Henn., stripe rust – *P. striiformis* Westend f. sp. *tritici* Eriks. and Henn. and leaf rust - *P. triticina* Erikss.] and moderately susceptible to FHB. Krokodil is an above average yielding cultivar of around 6.4 t/ha for the warmer northern irrigation areas, 7.6 t/ha for the cooler central irrigation areas and 4.5 t/ha for the eastern Highveld irrigation areas.

3.3.2 Polymorphic foreground and background marker screening on selected parents (Krokodil and CM-82036)

All 14 foreground markers and 256 background SSR markers were screened on DNA bulks of Krokodil and CM-82036.

3.3.2.1 Foreground marker screening

All 14 markers linked to specific FHB resistance genes/QTL (Table 3.2) gave allelic differences between the two selected parental lines, Krokodil and CM-82036. The SSR marker allele sizes amplified in CM-82036 for *Qfhs.ndsu.3BS/Fhb1, Qfhs.ifa.5A, Fhb2* and *7A* QTL were the same size on the silver stained PAGE gels compared to the allele sizes in Sumai 3. Allele sizes in Table 3.2 were calculated after visual comparison with a 25 bp DNA ladder (Promega). A number of markers such as Barc197-2, Gwm644 and Gwm130 had a small 2-4 bp difference between the two parental lines, which were difficult to score in the segregating populations. However, these markers were reliable and diagnostic. Markers St-3B-138, St-3B-142 and Gwm133 were unreliable and difficult to score.

3.3.2.2 Background SSR marker screening

In total 256 background SSR markers were screened on bulked DNA of the two parental lines. Table 3.3 shows results represented per genome and specific chromosome number for markers polymorphic between the two parental lines. The 256 markers included an additional 80 SSR markers that were selected and screened after an initial screening to fill gaps on chromosomes with poor polymorphic marker coverage. The highest number of polymorphic markers between Krokodil and CM-82036 were detected for genome B (74%), followed by D (72%) and A (62%). Across the different chromosomes the number of polymorphic markers per chromosome was similar and relatively consistent within a range of 61-76% polymorphic markers per chromosome. The polymorphic marker percentages between Krokodil and CM-82036 on the four targeted chromosomes were as follows: 3B-87%, 5A-71%, 6B-69% and 7A-65%. The average number of polymorphic markers observed between the selected parents Krokodil and CM-82036 across all chromosomes and genomes was 69%.

During background marker screening it was observed that although markers may be polymorphic between the two parental lines, it may not be a reliable or informative marker due to the similar allele size (allele masking or blurring) of the two parental lines. When the recurrent and donor parent allele sizes are too similar, the PCR fragments of specific marker alleles could blur together as one broad fragment or mask each other on PAGE when the segregating BC_1 population is screened.

3.3.3 F_1 hybrid identification

A total of 43 crosses were made to create the F_1 population and 511 seeds harvested. The 180 F_1 individuals (180/511 F_1 seed) that were planted out for the generation of the

BC_1F_1 population were screened with a FHB resistance gene/QTL specific marker, Gwm493, linked to FHB resistance gene *Fhb1* to validate true F_1 plants. Marker Gwm493 was selected since it amplified a 202 bp fragment in CM-82036 that was used as the male parent and a 165 bp fragment in the recurrent (female) parent Krokodil. Of the 180 F_1 individuals screened, 173 individuals (96.1%) displayed two alleles, one allele (165 bp) from the recurrent parent Krokodil and the second allele (202 bp) from the FHB resistance donor parent CM-82036, which confirmed these individuals as true F_1 offspring. Seven individuals were identified as homozygous for the Krokodil allele, indicating that these were self-pollinated and not crosses or F_1 hybrids and were not selected for further use to produce BC_1F_1 off-spring.

Table 3.2 Allele sizes in base pairs (bp) of 14 foreground markers linked to specific FHB resistance genes/QTL screened on the selected parental lines, Krokodil and CM-82036 and the relevant targeted FHB resistance gene/QTL

Marker	Targeted gene/QTL	Krokodil allele size (bp)	CM-82036 allele size (bp)
Gwm533	*Qfhs.ndsu.3BS/Fhb1*	120	145
Barc133	*Qfhs.ndsu.3BS/Fhb1*	115	127
Gwm493	*Qfhs.ndsu.3BS/Fhb1*	165	202
St-3B-66	*Qfhs.ndsu.3BS/Fhb1*	310	300
St-3B-138	*Qfhs.ndsu.3BS/Fhb1*	360	350
St-3B-142	*Qfhs.ndsu.3BS/Fhb1*	125	127
Gwm293	*Qfhs.ifa.5A*	195	204
Gwm304	*Qfhs.ifa.5A*	200	215
Gwm156	*Qfhs.ifa.5A*	280	310
Barc197-2	*Qfhs.ifa.5A*	180	183
Gwm133	*Fhb2*	111	114
Gwm644	*Fhb2*	145	143
Gwm130	*7A* QTL	126	130
Gwm233	*7A* QTL	230	251

Table 3.3 Background SSR marker screening of the recurrent parent Krokodil and FHB resistance donor line CM-82036, indicating polymorphic markers expressed per chromosome of each genome

Wheat chromosome	Polymorphic/ screened	Polymorphic/ screened	Polymorphic/ screened	Polymorphic/ screened
	A	**B**	**D**	**TOTAL**
1	6/11	8/10	10/13	24/34
2	7/11	7/13	8/12	22/36
3	5/11	13/15	9/10	27/36
4	10/16	5/8	7/12	22/36
5	10/14	8/10	8/10	26/34
6	7/10	9/13	9/15	25/38
7	11/17	10/12	10/13	31/42
TOTAL	**56/90**	**60/81**	**61/85**	**177/256**

3.3.4 Screening of BC_1F_1 generation and partial linkage mapping

During BC_1F_1 development 131 crosses were made with the 173 available F_1 plants. A number of primary spikes of some F_1 plants flowered earlier than expected and could not be used in crosses due to elevated temperatures during this period. The 131 crosses yielded 1 637 seeds of which 600 BC_1F_1 seeds were planted out to create the BC_2F_1 generation, but only 120 BC_1F_1 plants could be successfully crossed to yield a BC_2F_1 generation. These 120 BC_1F_1 individuals were genotyped and screened with all markers on the four targeted chromosomes previously identified as polymorphic between the two parental lines. However, not all polymorphic markers were informative in the BC_1F_1 individuals when screened using PAGE and silver staining.

3.3.4.1 Partial linkage map analysis

A partial linkage map was developed based on the genotypic data generated from the informative polymorphic markers on the four targeted chromosomes namely 3B, 5A, 6B and 7A using 120 segregating BC_1 individuals (Figure 3.2).

Nine polymorphic markers were successfully mapped to chromosome 3B (Figure 3.2). Four markers expected to map to this chromosome could not be positioned on chromosome 3B possibly as a result of incorrect genotyping and poor marker reliability. A total distance of 183.7 cM was covered by markers for this chromosome. The

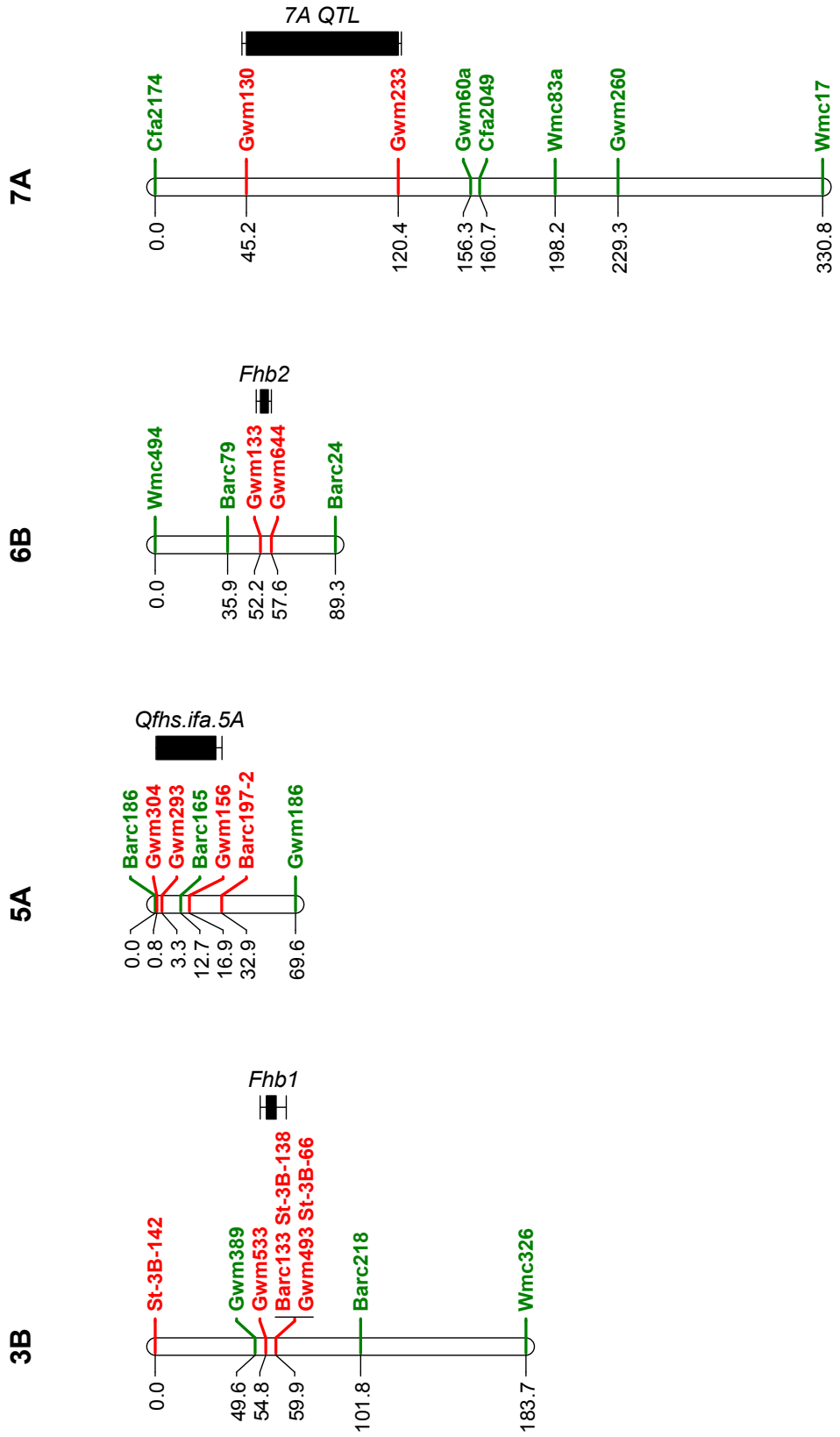


Figure 3.2 **Partial linkage map of targeted chromosomes 3B, 5A, 6B and 7A. Left: distance between markers given in centiMorgans (cM). Right: specific marker and QTL names: markers linked to FHB resistance genes/QTL are indicated in red, mapped polymorphic background markers in green and FHB resistance gene/QTL regions by black bars**

Qfhs.ndsu.3BS/Fhb1 region spanned a 5.1 cM distance. The background markers on chromosome 3B mapped far apart (>50 cM between markers). Marker St-3B-142 mapped 54.8 cM away from the nearest marker linked to FHB resistance, namely marker Gwm533. This surprising position of marker St-3B-142 away from other markers linked to FHB resistance on chromosome 3B could be as result of the small population size and genotyping error. Although marker St-3B-142 has previously been shown to be linked to FHB resistance it was treated as a background marker due to it being designated as the distal end of chromosome 3B. The *Qfhs.ndsu.3BS/Fhb1* QTL region mapped to the short arm of chromosome 3B and was flanked by markers Gwm533, Barc133, St-3B-138, Gwm493 and St-3B-66. The four FHB resistance markers, Barc133, St-3B-138, Gwm493 and St-3B-66, all mapped to the same position, primarily due to the small BC_1F_1 population size. Marker Gwm389 mapped 5.2 cM distal from marker Gwm533. Gwm389 has previously been reported to be associated with the *Qfhs.ndsu.3BS/Fhb1* gene/QTL region of Sumai 3 and other derived/related Sumai 3 lines.

Three of the ten polymorphic markers expected to map to chromosome 5A (Figure 3.2) did not map to this or any of the other targeted chromosomes. The remaining seven SSR markers mapped as expected and map positions were similar to the SSR wheat consensus map. Markers that mapped to chromosome 5A covered a total distance of 69.6 cM. Although there was a high marker density around the two QTL regions on chromosome 5A (*Qfhs.ifa.5A-1* and *Qfhs.ifa.5A-2*), only three additional background markers mapped to chromosome 5A. The *Qfhs.ifa.5A-1* QTL was flanked by markers Gwm304 and Gwm293, spanning a 2.5 cM region. The two QTL regions mapped 13.6 cM apart. Background marker Barc165 separated the two QTL regions, *Qfhs.ifa.5A-1* and *Qfhs.ifa.5A-2.* Barc165 mapped 9.4 cM distal from flanking marker Gwm293 (*Qfhs.ifa.5A-1*) and 4.2 cM proximal to flanking marker Gwm156 (*Qfhs.ifa.5A-2*). The total targeted *Qfhs.ifa.5A* QTL region spanned 32.1 cM and the five SSR markers mapped in the following order: Gwm304, Gwm293, Barc165, Gwm156 and Barc197-2.

Only five SSR markers were successfully and reliably mapped to chromosome 6B (Figure 3.2). Four of the tested polymorphic markers could not be mapped successfully. The total distance covered by mapped markers for chromosome 6B was 89.3 cM. The *Fhb2* gene region was flanked by markers Gwm133 and Gwm644 spanning 5.4 cM. Background SSR markers Barc79 distal (16.3 cM) and Barc24 proximal (31.7 cM) flanked the *Fhb2* gene region.

Eight SSR markers were successfully mapped to chromosome 7A (Figure 3.2). Two of the tested polymorphic markers did not map to this or any other of the targeted chromosomes. A total map distance of 330.8 cM was covered for chromosome 7A. The *7A* QTL region was poorly defined and was flanked by markers Gwm130 and Gwm233, spanning a 75.2 cM distance. There were bigger genetic distances between background markers on chromosome 7A than the other three chromosomes.

3.3.4.2 Segregation distortion

The chi-square fitness analysis (Table 3.4) was performed on the 29 markers used to construct the partial linkage map. Since the unmapped markers displayed significant segregation distortion they were removed from the data set. Twenty-six of the 29 markers displayed a good fit to the expected allelic ratio of 1:1 (homozygous recurrent parent allele: heterozygous parent alleles) for the BC_1F_1 population. Markers Gwm644 (χ^2=3.217), Barc218 (χ^2=6.667) and Gwm186 (χ^2=7.500) respectively all deviated significantly from the expected 1:1 allelic ratio. Results indicated that the BC_1F_1 population was slightly skewed towards heterozygous individuals at a number of marker loci that flanked the resistance genes/QTL, possibly due to high levels of linkage drag around these regions. In total six markers namely, St-3B-66 (chromosome 3B; χ^2=2.617), Barc218 (chromosome 3B; χ^2=6.667), Gwm186 (chromosome 5A; χ^2=7.500), Barc79 (chromosome 6B; χ^2=2.167), Gwm133 (chromosome 6B; χ^2=2.267) and Gwm644 (chromosome 6B; χ^2=3.217), displayed a possible level of segregation distortion. Limited segregation distortion regions (SDR) were observed across the four targeted chromosomes for a number of adjacent markers. Markers Barc79, Gwm133 and Gwm644 on chromosome 6B seemed to form a distorted cluster spanning 21.7 cM around the *Fhb2* gene region that is a gene rich region. Markers St-3B-66 (*Fhb1*) and background marker Barc218 on chromosome 3B, appeared to distort as a region spanning 41.9 cM in length.

3.3.4.3 GGT data of BC_1F_1 generation

GGT data generated for the BC_1F_1 generation for the targeted chromosomes 3B, 5A, 6B and 7A are shown in Figures 3.3, 3.4, 3.5 and 3.6, respectively. These figures indicate the theoretical crossover points (predicted to be the middle point between two adjacent markers) and possible parental chromosome segments of each BC_1F_1 individual exchanged and retained between the two parental lines used to create the BC_1F_1 population. A number of differences were observed across the different targeted

Table 3.4 Marker loci chi-square segregation fitness test of number of observed homozygous recurrent parent alleles (Krokodil) compared to number of observed heterozygous parental alleles regarding the expected ratio of 1:1 (*P < 0.05, df =1*) in the BC_1F_1 population

Marker	Ch	No. of plants	Observed hetero	Missing values	Observed homo	$\chi 2$
St-3B-142	3B	120	66	1	53	1.416
Gwm389	3B	120	56	3	61	0.283
Gwm533	3B	120	57	2	61	0.167
Barc133	3B	120	62	0	58	0.133
St-3B-138	3B	120	62	2	56	0.333
Gwm493	3B	120	61	2	57	0.167
St-3B-66	3B	120	66	5	49	2.617
Barc218	3B	120	72	4	44	6.667
Wmc326	3B	120	57	6	57	0.300
Barc186	5A	120	58	0	62	0.133
Gwm304	5A	120	59	0	61	0.033
Gwm293	5A	120	56	1	63	0.416
Barc165	5A	120	57	0	63	0.300
Gwm156	5A	120	51	2	67	2.167
Barc197-2	5A	120	57	8	55	0.567
Gwm186	5A	120	75	0	45	7.500
Wmc494	6B	120	59	1	60	0.017
Barc79	6B	120	67	2	51	2.167
Gwm133	6B	120	66	4	50	2.267
Gwm644	6B	120	67	5	48	3.217
Barc24	6B	120	66	1	53	1.417
Cfa2174	7A	120	62	1	57	0.217
Gwm130	7A	120	66	2	52	1.667
Gwm233	7A	120	61	1	58	0.083
Gwm60a	7A	120	53	3	65	1.233
Cfa2049	7A	120	58	3	59	0.083
Wmc83a	7A	120	60	2	58	0.067
Gwm260	7A	120	52	1	67	1.883
Wmc17	7A	120	56	2	62	0.333

Ch=chromosome; hetero=heterozygous for Krokodil and CM-82036 alleles; homo=homozygous for Krokodil alleles

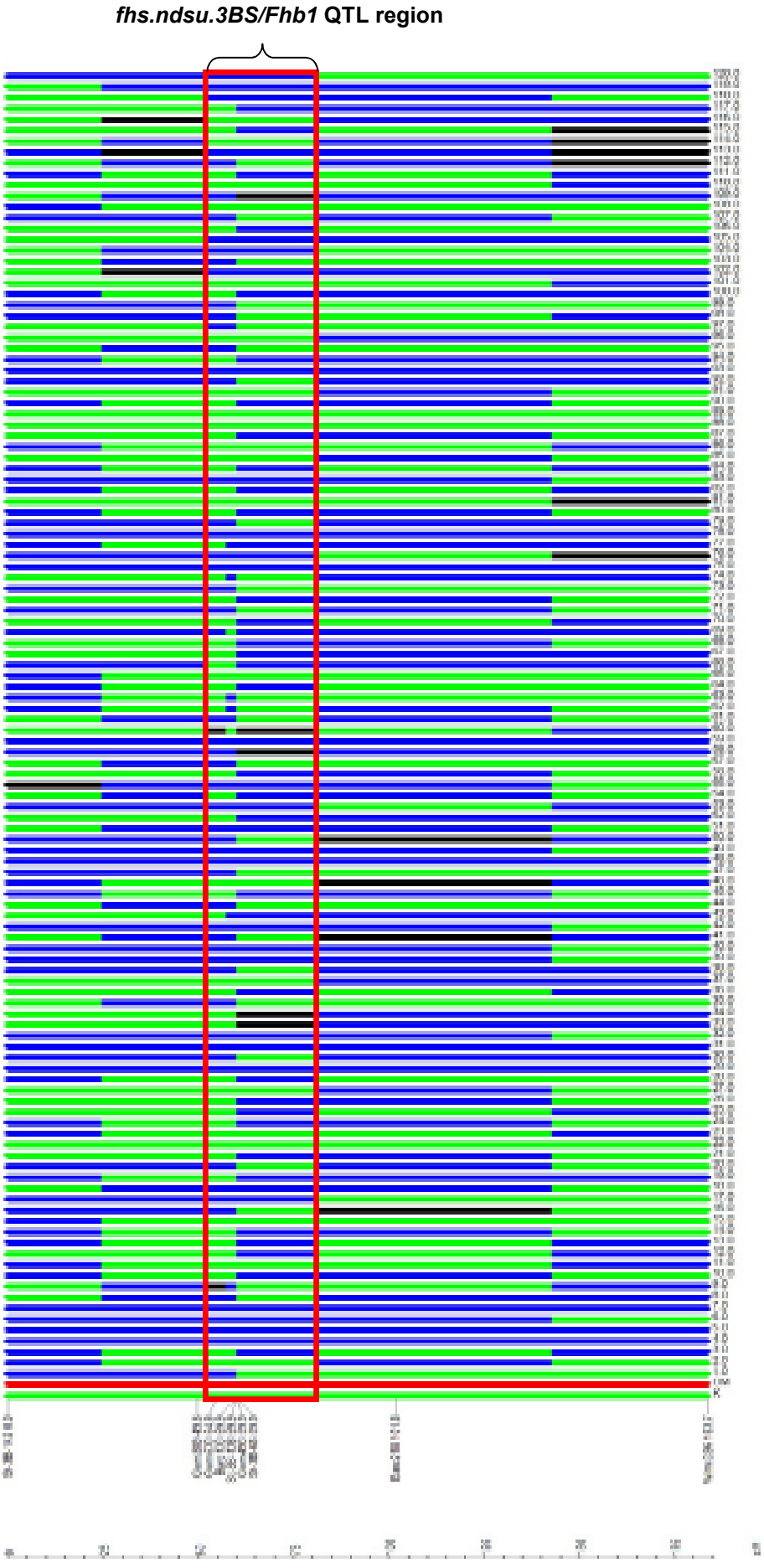


Figure 3.3 GGT data of targeted chromosome 3B, indicating theoretical marker crossover points and chromosome fragment transfer per BC_1F_1 individual (x-axis) against a centimorgen scale (cM; y-axis). Recurrent parent Krokodil (K) chromosome fragments are indicated by green bars, donor parent CM-82036 (CM) by red bars, heterozygous chromosome segments by blue bars, missing marker data by black bars while the red boxed area represents the targeted *Qfhs.ndsu.3BS/Fhb1 QTL* region

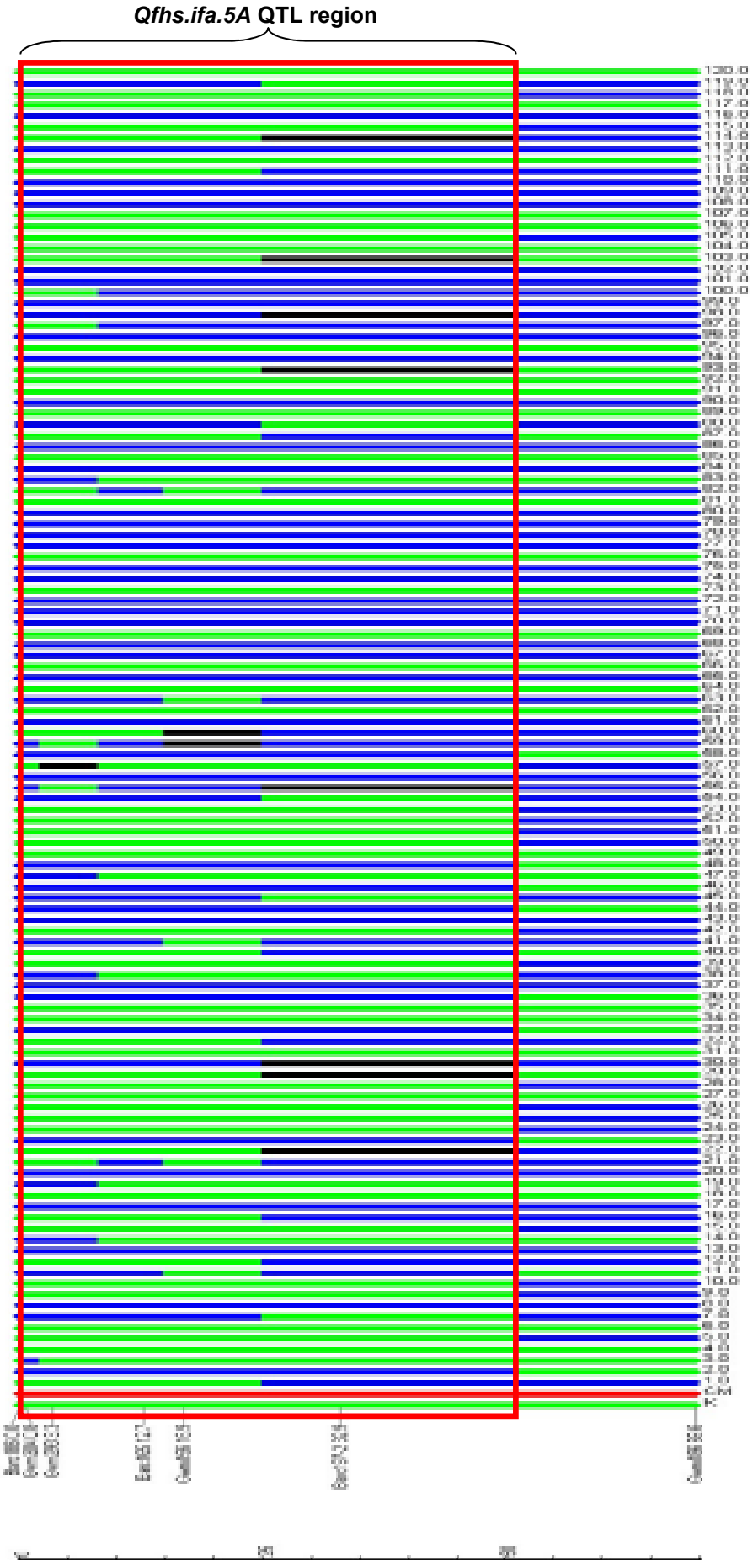


Figure 3.4 GGT data of targeted chromosome 5A, indicating theoretical marker crossover points and chromosome fragment transfer per BC_1F_1 individual (x-axis) against a centimorgan scale (cM; y-axis). Recurrent parent Krokodil (K) chromosome fragments are indicated by green bars, donor parent CM-82036 (CM) by red bars, heterozygous chromosome segments by blue bars, missing marker data by black bars, while the red boxed area represents the targeted *Qfhs.ifa.5A* QTL region

Figure 3.5 GGT data of targeted chromosome 6B, indicating theoretical marker crossover points and chromosome fragment transfer per BC_1F_1 individual (x-axis) against a centimorgan scale (cM; y-axis). Recurrent parent Krokodil (K) chromosome fragments are indicated by green bars, donor parent CM-82036 (CM) by red bars, heterozygous chromosome segments by blue bars, missing marker data by black bars while the red boxed area represents the targeted *Fhb2* gene/QTL region

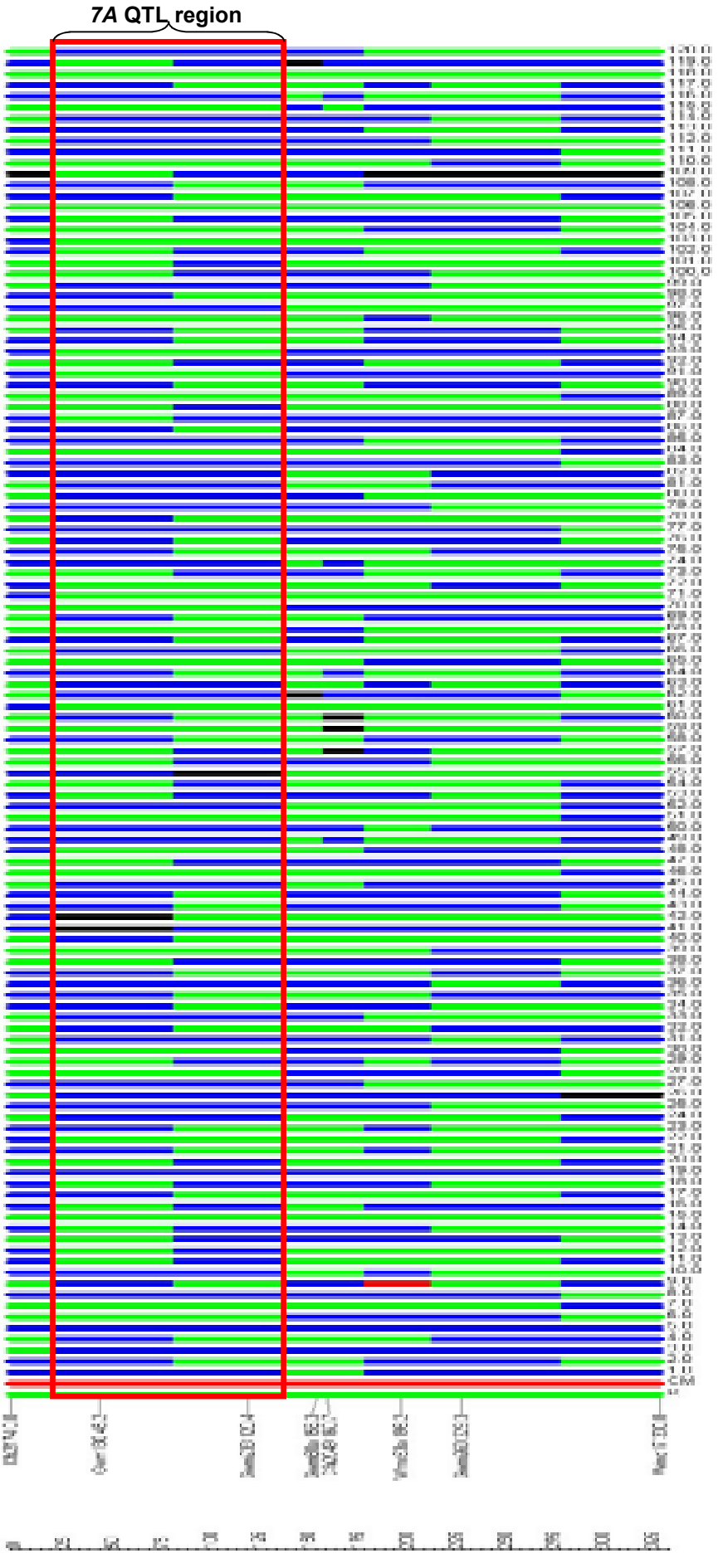


Figure 3.6 GGT data of targeted chromosome 7A, indicating theoretical marker crossover points and chromosome fragment transfer per BC_1F_1 individual (x-axis) against a centimorgan scale (cM; y-axis). Recurrent parent Krokodil (K) chromosome fragments are indicated by green bars, donor parent CM-82036 (CM) by red bars, heterozygous chromosome segments by blue bars, missing marker data by black bars while the red boxed area represents the targeted *7A* QTL region

chromosomes when single and double recombinants around respective targeted resistance gene/QTL regions were analysed and compared.

3.3.4.3.1 Chromosome 3B

The GGT data for chromosome 3B (Figure 3.3) indicated that BC_1F_1 individuals on average had three recombination events or crossover points within the mapped region. Individuals 3, 13, 54, 61 and 111 had four crossover points each while individual 62 had five crossover points. The position of the targeted FHB resistance gene/QTL region *Qfhs.ndsu.3BS/Fhb1* (5.1 cM) on chromosome 3B (middle of chromosome) allowed double recombination to occur around the targeted region. The theoretical length covered by mapped markers on chromosome 3B was 183.7 cM. The unwanted chromosome segments (everything except the targeted gene/QTL region) of donor CM-82036 retained ranged from 24.8 cM (14%) for individuals 65 and 108 to 178.6 cM (100%) for individuals 4, 5, 7, 29, 31, 48, 59, 75, 78 and 93. Individuals 97 and 106 with most of the targeted gene/QTL region (interval between markers Gwm533-Gwm493) present as a small heterozygous segment within an entire Krokodil region, indicating the smallest amount of linkage drag possible on chromosome 3B, should be selected. The calculated chromosome fragment sizes for each targeted chromosome are reflected in Appendix B. Individuals 1^1, 14^1, 19^1, 35^1, 44^1, 52^1, 57^1, 87^1 and 95^1 (type of recombinant: ^{1} indicates a single recombination around the QTL) are examples of some of the single recombinants around the targeted *Qfhs.ndsu.3BS/Fhb1* QTL region with varying amounts of linkage drag present in the tested population. As expected a high number of BC_1F_1 individuals still contained large chromosome 3B segments from FHB resistant donor CM-82036. However, individuals 22, 88 and 89 showed no recombination for chromosome 3B and were 100% similar to Krokodil. Overall the GGT data indicated a high degree of linkage drag around the *Qfhs.ndsu.3BS/Fhb1* (5.1 cM) QTL region (red shaded region in Figure 3.3), covered by markers Gwm533, Barc133, St-3B-138, Gwm493 and St-3B-66. Individuals 3^2, 12^2, 13^2, 25^2, 28^2, 36^2, 64^2, 70^2, 82^2, 84^2, 106^2, 111^2 and 115^2 (type of recombinant: ^{2} indicates a double recombination around the QTL) are double recombinants around the targeted *Qfhs.ndsu.3BS/Fhb1* QTL region.

These genotypes potentially have most of the 3B (*Qfhs.ndsu.3BS/Fhb1)* QTL region with a reduced level of linkage drag. If selection was based on targeted chromosome 3B only, individuals 97 and 106 containing only the *Qfhs.ndsu.3BS/Fhb1* QTL region would be considered the best lines with the highest amount of recurrent parent Krokodil genome present possible.

3.3.4.3.2 Chromosome 5A

Chromosome 5A (Figure 3.4) is covered by a smaller genetic distance (69.6 cM) compared to chromosomes 3B (183.7 cM) and 7A (330.7 cM). For chromosome 5A each BC_1F_1 individual on average had between one and two crossover points. Due to poor marker coverage and density, large parts of chromosome 5A were not covered in this study. Large donor chromosome segments were observed for chromosome 5A, indicating higher levels of linkage drag compared to chromosome 3B. The *Qfhs.ifa.5A* QTL region (Figures 3.2 and 3.4) spans from markers Gwm304 to Barc197-2. This *Qfhs.ifa.5A* QTL can be divided into two smaller QTL regions namely *Qfhs.ifa.5A-1* and *Qfhs.ifa.5A*-2 separated by background marker Barc165 (Figure 3.2). The *Qfhs.ifa.5A-1* QTL is flanked by markers Gwm304 and Gwm293, while *Qfhs.ifa.5A*-2 is flanked by markers Gwm156 and Barc197-2.

However, the GGT data indicated that the entire chromosome block represented by markers Barc186, Gwm304, Gwm293, Barc165, Gwm156 and Barc197-2 (Figure 3.4) was often inherited as one block, although a certain amount of recombination still occurred in this region. Perhaps background marker Barc165 in future could be associated with this QTL or would completely map outside the *Qfhs.ifa.5A* region in a larger population. The low levels of recombination observed around background marker Barc186 was due to the short 0.8 cM distance between this marker and Gwm304 (flanking the *Qfhs.ifa.5A* QTL region). Since the *Qfhs.ifa.5A* QTL region was inherited as a large chromosome block in the BC_1F_1 population and due to the close proximity to the distal end of chromosome 5A, the possibility of obtaining double recombinants around the targeted QTL was slim. Forty-nine of the 120 individual genotypes tested (Figure 3.4) still contained between 75% and 100% of the 5A chromosome region in a heterozygous state. Individuals 2^1, 23^1, 33^1, 36^1, 41^1, 44^1, 46^1, 48^1, 58^1 and 63^1 were single recombinants around the QTL region for chromosome 5A and contained most of the *Qfhs.ifa.5A* QTL region (Gwm304-Gwm293 and Gmw156-Barc197-2; Figure 3.4) with varying amounts of linkage drag present. Individuals $11^{1,2}$ and $45^{1,2}$ had a recombination event between the two QTL regions (*Qfhs.ifa.5A-1* and *Qfhs.ifa.5A*-2) between background marker Barc165 and Gwm165. These two lines are single recombinants around *Qfhs.ifa.5A-1* and double recombinants around *Qfhs.ifa.5A*-2 with the best possible levels of the genome of recurrent parent Krokodil. Lines 11 and 45 were the best lines based on GGT data for chromosome 5A which potentially contained the entire *Qfhs.ifa.5A* QTL with the lowest amount of linkage drag. No true double recombinants positive for the presence of the complete *Qfhs.ifa.5A* QTL region were identified.

3.3.4.3.3 Chromosome 6B

Chromosome 6B was represented by a small number of mapped markers (Figure 3.5) resulting in a small genetic distance of 89.3 cM. The *Fhb2* QTL region was well defined between flanking markers Gwm133 and Gwm644, spanning a distance of 5.4 cM. Since the QTL was positioned towards the middle of the chromosome, it allowed for double recombination to occur around the targeted QTL region. Individuals 10^1, 32^1, 39^1, 53^1, 73^1, 74^1, 82^1, 91^1, 98^1 and 106^1 were single recombinants around the *Fhb2* gene region with at least a recombination event between flanking marker Gwm644 and background marker Barc24 (Figures 3.2 and 3.5). These lines had large amounts of linkage drag around the QTL region on chromosome 6B. Selections made based on the GGT data for double recombinant individuals on chromosome 6B are: 3^2, 25^2, 27^2 and 64^2. The Gwm133/Gwm644 marker interval confirmed the presence of the FHB QTL region on chromosome 6B (Figure 3.5). A high number of individuals retained 75% to 100% of chromosome 6B in a heterozygous state. Four double recombinants were selected compared to chromosomes 5A and 7A where none were observed.

3.3.4.3.4 Chromosome 7A

For chromosome 7A (Figure 3.7), BC_1F_1 individuals generally had smaller and less donor chromosome segments in comparison to the other targeted chromosomes. Chromosome 7A was covered by a large 330.8 cM distance. However, the large genetic distances between adjacent markers allowed high levels of recombination. Since the *7A* QTL region was poorly defined, flanked by a distance of 75.2 cM, a number of recombination events occurred between markers Gwm130 and Gwm233. Fifty-four individuals of the 120 BC_1F_1 individuals showed a recombination event between the *7A* QTL flanking markers. Due to the large genetic distance between the flanking markers for the *7A* QTL region, the presence of the two flanking markers might indicate the presence of the full region but in fact a number of recombination events could have occurred between them. The following individuals still contained 75% or more of donor chromosome fragments: 3, 8, 26, 36, 41, 50, 77, 83, 86, 87, 111 and 119. There were double recombinant events around the *7A* QTL region (24^2, 45^2, 63^2, 66^2 and 81^2) and could be selected for the presence of the *7A* QTL. Of these lines individuals 24 and 66 contained higher amounts of Krokodil genome than the other double recombinant individuals and would be better for selection.

3.3.4.3.5 FHB resistance gene/QTL combinations

When only double or single recombinants around the gene/QTL regions of targeted chromosomes in GGT profiles were being considered, a majority of the selected individuals across the four targeted chromosomes contained a single FHB resistance gene/QTL. However, selected individuals 3, 25 and 64 contained both the *Qfhs.ndsu.3BS/Fhb1* and *Fhb2* regions. Selected individual 36 contained both *Qfhs.ndsu.3BS/Fhb1* and *Qfhs.ifa.5A* QTL regions. If single or double recombinations around the targeted QTL regions were ignored, a number of more promising QTL combinations were possible. Individuals 17, 30, 44 and 75 all contained the three major targeted resistance QTL, *Qfhs.ndsu.3BS/Fhb1*, *Qfhs.ifa.5A* and *Fhb2* but with a high percentage of linkage drag. All four targeted FHB resistance gene/QTL regions were positively identified within individuals 8 and 113, although these lines showed significant linkage drag and contained a low recurrent parent percentage. Results indicated that FHB resistance gene/QTL combinations across genomes A or B occurred far less frequently than within the same genome (A/A or B/B) combinations. However, this might have been as a result of different marker coverage levels for these targeted chromosomes and due to the relatively small BC_1F_1 population studied.

3.3.4.4 Frequency of genotypes observed

The most frequently observed genotype in the BC_1F_1 population screened contained *Fhb1* (Type II) in combination with *Fhb2* (Type II) genes/QTL and was present in 19 (15.8%) of the 120 BC_1F_1 individuals. The *Fhb2* gene in combination with the *7A* QTL and the *Qfhs.ifa.5A* QTL (Type I) in combination with the *Fhb2* gene were present in 10 (8.3%) individuals each. Nine individuals (7.5%) tested positive for the *Fhb1* gene in combination with *Qfhs.ifa.5A* QTL. Two (1.7%) individuals (8 and 113; Appendix C) tested positive for all four of the targeted FHB resistance genes/QTL (*Fhb1, Qfhs.ifa.5A*, *Fhb2* and *7A* QTL). Individual 83 tested positive for three-and-a-half of the targeted genes/QTL and contained the *Fhb1, ½ Qfhs.ifa.5A*, *Fhb2* and *7A* QTL genes/QTL (Appendix C). Additionally, four (3.3%) individuals (17, 30, 44 and 75) tested positive for three major genes/QTL of interest (*Fhb1, Qfhs.ifa.5A* and *Fhb2*). Nine individuals (5, 31, 50, 74, 77, 79, 99, 116 and 120; Appendix C), tested positive for a combination of three genes/QTL of which one QTL was always the minor *7A* QTL. The presence of only a single resistance gene/QTL (*Fhb1, Qfhs.ifa.5A, Fhb2* or *7A* QTL) was confirmed in 9, 8, 7 and 4 BC_1F_1 individuals respectively. Nine individuals, based on SSR marker data, were identified to contain none of the targeted FHB resistance genes/QTL. FHB resistance genes/QTL combinations across the three genomes (A, B or D) appeared to occur less frequently than within the same genome (A with A, B with B or D with D) combinations. A

preliminary observation was that FHB resistance genes/QTL that confer either Type I or Type II resistance combined better with the same type of resistance (Type I with Type I and Type II with Type II) than the two different types present in combination (Type I with Type II).

3.3.4.5 Recurrent and donor parent genome percentages

The average HRGP (Krokodil) for the BC_1F_1 population of 120 individuals was 46.5% and the average HDGP (CM-82036) was 51.5% across the four targeted chromosomes. On average 3-5% of the predicted RPGP value could not be accounted for due to missing values per BC_1F_1 individual. The HRGP values for the 120 BC_1F_1 individuals across the four targeted chromosomes ranged from 8.4% (individual 83) up to 94.7% (individual 89; Appendix C). The HDGP range observed across the four targeted chromosomes ranged from 5.3% up to 91.6%. The HDGP tended to increase as more FHB resistance genes/QTL were present per individual, reducing the HRGP present, which meant the overall predicted RPGP of that individual would be significantly lower. Ideally, a 50% HRGP or greater combined with all/most of the targeted FHB resistance genes/QTL in an individual is required for more favourable predicted RPGP.

3.3.4.6 Predicted recurrent parent genome percentage recovered

The predicted RPGP values are indicated in Appendix C. Theoretically, in a BC_1F_1 generation an associated linkage drag of around 50% can be expected on certain targeted chromosomes around the trait/QTL of interest. However, in the BC_1F_1 population it is expected that on non-targeted chromosomes the expected RPGP could be closer to 75% due to limited linkage drag. In the BC_1F_1 population predicted RPGP values ranged from 46.2% within individual 109 up to 97.4% for individual 89 (containing no FHB resistance QTL) at an overall population average of 72.2%. Each BC_1F_1 individual's RPGP value (green bars), the population average (red line) and expected theoretical BC_1F_1 average of 75% (blue line) are indicated in Figure 3.7. The average RPGP for the BC_1F_1 population tested was 2.8% lower than the expected 75% average of a theoretical BC_1F_1 population that is not experiencing any linkage drag.

The primary objective of this study was the introduction of the best FHB resistance gene/QTL combinations into the background of a SA cultivar and a secondary objective was to select for the highest RPGP.

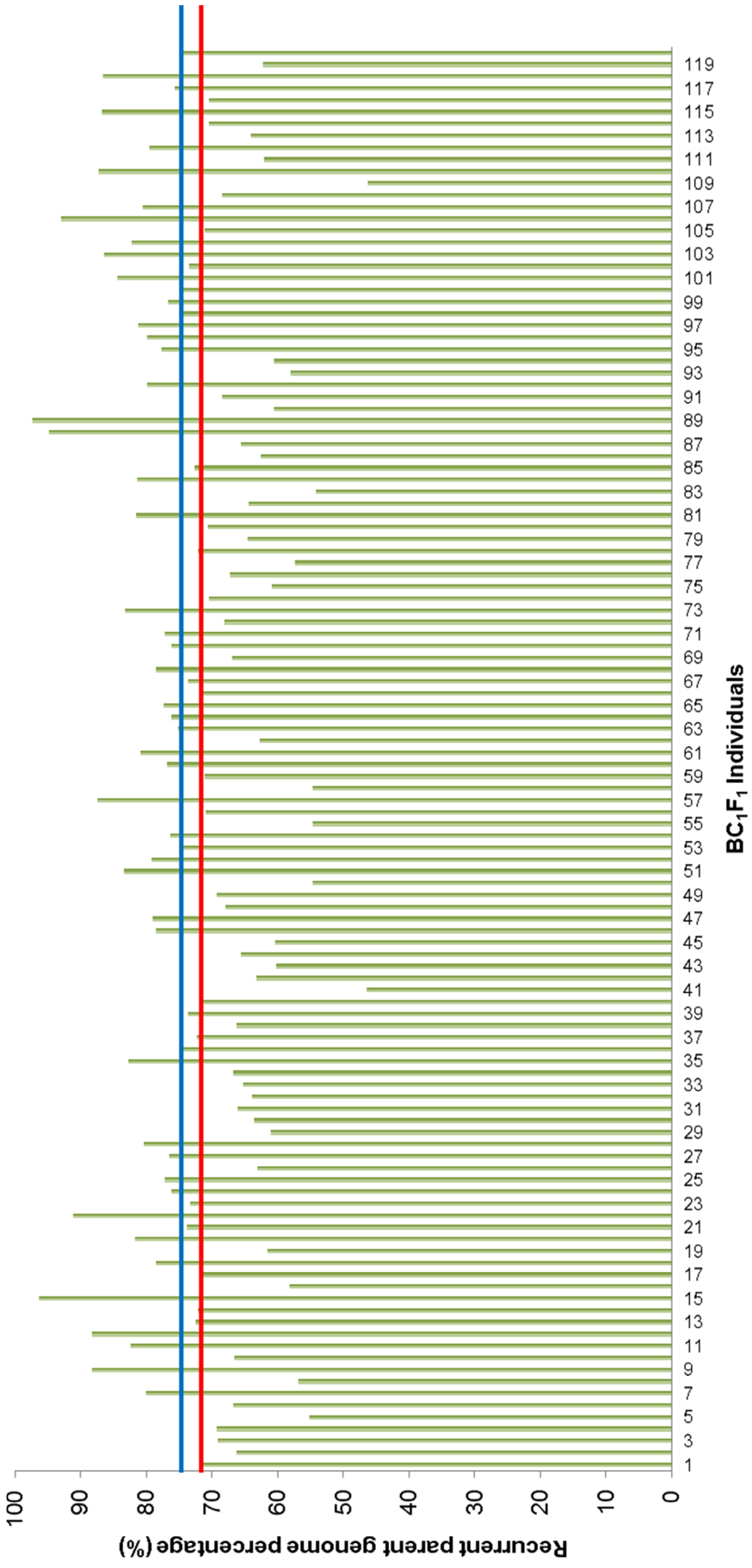


Figure 3.7 Each individual's predicted recurrent parent genome percentage (RPGP; green bars) across the four targeted chromosomes 3B, 5A, 6B and 7A. The red line indicates the population RPGP average of 72.2% and the blue line the expected theoretical RPGP average of 75% if there was no linkage drag

The red line in Table 3.5 indicates a potential 80% threshold (if RPGP values were used only as a selection criterion) for the predicted RPGP values which included the first 20 BC_1F_1 individuals. However, not all targeted FHB resistance gene regions were present in these 20 individuals identified. Lines listed below the red line in Table 3.5 that contained three or four FHB resistance genes/QTL were selected/classed as families for the use in BC_2 development. These lines were selected and ordered based on QTL importance (*Fhb1*, then *Qfhs.ifa.5A*, then *Fhb2;* the *7A* QTL was seen as a bonus and not a necessity) and highest RPGP values. This was done to ensure that the highest number of targeted FHB resistance genes/QTL should occur within a single genotype. Individuals shown above the threshold all had above 80% of the SA recurrent parent genome Krokodil but only the introgression of a single or two gene/QTL regions. Importantly, none of the selected double recombinant individuals mentioned in previous sections featured in these lines with higher than 80% RPGP. Table 3.5 indicates a significant trend that an increase in the number of targeted FHB resistance genes/QTL present in a genotype generally lowered the predicted RPGP, especially in genotypes with three or more FHB resistance genes/QTL present.

3.4 Discussion

3.4.1 Parental selection

The CIMMYT line CM-82036 was selected as the FHB resistant donor line since it did not show any within genotype variation. In a molecular marker-assisted study genotypes used in the breeding programme need to be stable and true breeding. CM-82036 displayed similar allelic sizes for all 14 markers linked to FHB resistance genes/QTL tested. These allele sizes compared to those present in Sumai 3 which CM-82036 was derived from.

CM-82036 tested positive at marker level for the three major FHB genes/QTL, *Fhb1* (Buerstmayr et al. 2003; Cuthbert et al. 2006), *Qfhs.ifa.5A* (Buerstmayr et al. 2003) and *Fhb2* (Cuthbert et al. 2007) and one minor QTL for FHB resistance on chromosome 7A. Since only the expression of *Fhb1* and *Qfhs.ifa.5A* QTL in CM-82036 have been confirmed by a number of studies (Miedaner et al. 2011; Tamburic-Ilincic 2012), the presence and expression of the QTL on chromosomes 6B and 7A has yet to be confirmed in the CM-82036 line background. Since CM-82036 tested positive for markers linked to the resistance genes/QTL on chromosomes 6B and 7A it was decided to continue following these markers in the current study.

Table 3.5 Selected BC_1F_1 individuals' genotype for all four of the targeted FHB resistance genes/QTL (*Fhb1, Qfhs.ifa.5A, Fhb2* and *7A* QTL) in decreasing order of recurrent parent genome percentage (RPGP)

Individual no.	Genotype	RPGP
88	½ *Qfhs.ifa.5A*	94.9%
106	*Fhb2*	92.9%
9	*Fhb1*	88.3%
57	*Fhb1*	87.4%
110	*Qfhs.ifa.5A*	87.3%
118	*Fhb1*	86.6%
103	*Fhb1* and *Fhb2*	86.4%
101	*Qfhs.ifa.5A* and *Fhb2*	84.4%
51	*Fhb1* and *Fhb2*	83.4%
73	*Fhb1* and *Fhb2*	83.2%
35	*Fhb1*	82.8%
11	*Fhb2*	82.4%
104	*Fhb1* and *Fhb2*	82.3%
20	*Fhb1 and Qfhs.ifa.5A*	81.7%
81	*7A* QTL	81.6%
84	*Qfhs.ifa.5A* and *Fhb2*	81.4%
97	*Fhb1,* ½ *Qfhs.ifa.5A* and *7A* QTL	81.2%
61	*Fhb1* and *Qfhs.ifa.5A*	80.8%
107	*Fhb1*	80.6%
7	*Fhb1,* ½ *Qfhs.ifa.5A* and *Fhb2*	80.0%
47	*Fhb1,* ½ *Qfhs.ifa.5A* and *7A* QTL	79.0%
99	*Fhb1, Qfhs.ifa.5A* and *7A* QTL	76.7%
98	*Fhb1,* ½ *Qfhs.ifa.5A* and *Fhb2*	74.8%
120	*Fhb1, Fhb2* and *7A* QTL	74.5%
17	*Fhb1, Qfhs.ifa.5A* and *Fhb2*	71.7%
74	*Qfhs.ifa.5A, Fhb2* and *7A* QTL	70.4%
116	*Qfhs.ifa.5A, Fhb2* and *7A* QTL	70.4%
44	*Fhb1, Qfhs.ifa.5A* and *Fhb2*	65.6%
79	*Fhb1, Qfhs.ifa.5A* and *7A* QTL	64.6%
113	*Fhb1, Qfhs.ifa.5A, Fhb2* and *7A* QTL	64.1%
30	*Fhb1, Qfhs.ifa.5A* and *Fhb2*	63.6%
75	*Fhb1, Qfhs.ifa.5A* and *Fhb2*	60.8%

Red line=80% threshold RPGP value

Krokodil was selected as the recurrent parent because no within cultivar variation was observed at molecular level for this cultivar. Furthermore, Krokodil is a reliable, high yielding irrigation cultivar that is phenotypically and genotypically distinct from the CM-82036 donor line. Large allelic variation was observed for all 14 foreground markers tested between Krokodil and CM-82036. This would allow for more effective foreground and background selections in segregating generations. Addition of the FHB resistance genes/QTL into Krokodil will notably improve the cultivar.

3.4.2 Background marker screening

In order to conduct successful background selection, markers that are polymorphic between the two parents are required. Background markers were selected from the SSR consensus map at a 10 cM distance, throughout the wheat genome for background screening. Background marker screening of the two selected parental lines revealed a 69% polymorphic rate. However, although background marker screening of the parental lines revealed a high number of polymorphic markers, after careful analysis not all of these markers were informative and useful when being applied in a segregating population. A similar observation was documented by Collard and Mackill (2008). This was due to small differences in allele sizes between the donor and recurrent parental lines. The relative small bp differences between the parental lines potentially mask one another on a PAGE gel, making scoring difficult or impossible. This problem can in future be overcome by using an automated gel documentation system using florescent detection for these markers.

When individual genomes (A, B and D) were compared, genome A showed the lowest level of polymorphism between the two selected parental lines. However, for genome A, chromosome 5A showed a higher level of polymorphism compared to other chromosomes. This was expected since chromosome 5A is one of the targeted chromosomes carrying the *Qfhs.ifa.5A* FHB resistance QTL, which primarily confers Type I (fungal penetration) resistance, present on the donor genome (CM-82036; Buerstmayr et al. 2002; 2003). Furthermore, since the relative short genetic distance (69.6 cM) covered by markers mapped to the 5A chromosome did not give a good coverage of the entire chromosome, it significantly influenced the level of recombination observed. Since the targeted QTL region on chromosome 5A was expected to be a gene rich region that should differ significantly between the two parental lines, it should be more polymorphic compared to other genomic regions. It has been well documented that recombination primarily occurs around gene rich regions (Erayman et al. 2004). However, the recombination rate is influenced by gene density, gene length or the

position of the gene rich region on the chromosome. Recombination across the full chromosome length is distributed unevenly. Recombination increases towards the telomeres (Gill et al. 1996) and is significantly suppressed around the centromeres (Gill et al. 1996; Erayman et al. 2004). Genes or gene rich regions are randomly and unevenly distributed throughout the wheat genome which could be associated with the variation in recombination observed (Erayman et al. 2004).

Targeted chromosome 3B had the highest number of polymorphic markers for genome B, as expected, due to the presence of the targeted *Qfhs.ndsu.3BS/Fhb1* gene/QTL (Buerstmayr et al. 2002; 2003) on this chromosome. Results indicated that marker St-3B-142 previously indicated to be associated with *Fhb1* resistance (Cuthbert et al. 2006), acted as a background marker in the current population and not as a foreground marker as expected. Importantly, marker St-3B-142 was difficult to genotype in this population, possibly resulting in a marker positioning error. This marker mapped to the theoretical distal end and starting point of the 3B chromosome in this study.

Gill et al. (1996) concluded that marker density is high within/around gene rich regions and can account for about 80-90% of markers present. Even though in this study partial linkage maps with limited marker densities were developed in comparison to other major high density mapping studies done recently, the principles still seemed to hold true. In this study better marker coverage was observed around the targeted FHB gene/QTL rich regions. Due to background markers mapping far apart on most of the targeted chromosomes, these distances allowed for high levels of recombination to be detected between markers. Better marker coverage is thus required to more accurately predict recombination events and RPGP for targeted chromosomes, especially for chromosomes 6B and 7A. However, the main objective of this study was achieved with a number of important BC_1F_1 lines containing three or four FHB resistance genes/QTL as well as high levels of RPGP that were selected.

A number of non-targeted chromosomes of the D genome displayed a high number of polymorphic markers between the two selected parental lines. This high polymorphic rate could be due to the presence of a number of agronomical, quality and bread baking traits on chromosomes of the D genome of wheat. Traits associated with general agronomic characteristics (plant height, photoperiod, grain yield and time to maturity) and traits associated with end-use bread baking quality (protein content, kernel hardness, dough strength and flour yield), present on chromosomes 1D, 2D, 4D, 5D, 6D and 7D have been identified in a number of mapping studies (Perretant et al. 2000; Budak et al. 2004;

McCartney et al. 2005; Huang et al. 2006; Nelson et al. 2006; Dodig et al. 2012). The two selected parental lines, Krokodil and CM-82036, would have been developed and selected for further use with entirely different agronomical and bread quality standards, target markets and end-use objectives in mind. Krokodil was selected for optimal protein content and good quality bread properties to comply with the demanding SA bread making standards and the targeted consumer market, compared to the CIMMYT line CM-82036 developed in Mexico.

3.4.3 F_1 identification

During F_1 hybrid identification, marker Gwm493 linked to the *Qfhs.ndsu.3BS* QTL/*Fhb1* resistance gene was successfully used to identify true F_1 hybrids. Gwm493 was selected since the marker displayed a large allelic difference between Krokodil and CM-82036, enabling easy and accurate scoring of data. Seven of the 180 F_1 individuals were identified to have originated from self-pollination and were therefore removed from the breeding programme. This ensured that further generated material was derived from true F_1 hybrids.

3.4.4 Partial linkage map analysis

A partial linkage map of the four targeted chromosomes was constructed in order to obtain the correct marker order and distances between markers for the Krokodil/CM-82036//*Krokodil (pedigree given as stated in Purdy et al. 1968) backcross population. The partial linkage map was needed to accurately and precisely perform segregation distortion and GGT analysis on the BC_1F_1 population. An accurate linkage map of a targeted population is an essential tool for the successful application of MAS by breeders in order to develop new targeted improved plant varieties (Cheema and Dicks 2009). Most markers mapped to the correct chromosomes and the order of the markers was not significantly different from what was observed for the wheat SSR consensus map (Somers et al. 2004). Markers on targeted chromosomes 3B, 5A and 6B respectively mapped in similar order and positions that were reported in the recent high density map of the A and B wheat genomes (Marone et al. 2012).

3.4.5 Segregation distortion regions

Segregation distortion analysis detected a limited number of SDR's which were non-randomly distributed across chromosomes 3B and 6B, especially around markers which deviated significantly from the expected 1:1 ratio (Francki et al. 2009). A similar observation was made by Paillard et al. (2003) in which it was stated that SDRs were non-random and some spanned as much as 58 cM. The maintenance or segregation of

the targeted FHB gene/QTL regions as large intact chromosome blocks between gene/QTL flanking markers and the adjacent surrounding background markers seemed to predefine a given SDR in this population. This might be a further indication of the degree of linkage drag within and around gene rich regions, specifically around the four targeted FHB resistance gene/QTL donor regions. It has been observed that segregation can be distorted around or near chromosome centromeres (negatively) and telomeres (positively) and therefore would affect SDRs observed. The recombination rate is higher at the distal ends of chromosomes than at the proximal ends (Erayman et al. 2004). This seemed to be the case for chromosomes 3B and 5A where the targeted gene/QTL regions were closer to the distal ends of the chromosome. In contrast, the gene/QTL region on chromosome 6B was situated closer to the middle of the mapped chromosome and did not show segregation distortion.

3.4.6 GGT analysis

The GGT data provided a visual aid for identifying the number of recombination hotspots on all targeted chromosomes. Recombination hotspots are small genomic regions along a DNA sequence where the recombination rate increases more significantly than an expected normal distribution of segregating alleles in a random population (de Lorio et al. 2005). These recombination hotspots were mostly visible around targeted FHB resistance genes/QTL on chromosomes 3B, 5A and 6B. This validated two findings namely that hotspots occur within small regions and recombination rates tend to increase around gene rich regions (Gill et al. 1996; Faris et al. 2000; de Lorio et al. 2005).

Based on the GGT data the linkage drag around each targeted gene/QTL was visually apparent for each genotype for each chromosome based on the large heterozygous (donor and recurrent) chromosome segments/portions that remained. GGT data helped to identify, per individual, per targeted chromosome, genotypes that had no recombination, single recombination or double recombination around the targeted gene/QTL region. Without the visualisation aid of the GGT data it would have been difficult to select and distinguish between single and double recombinants around the targeted gene/QTL regions. By selecting the BC_1F_1 individuals in this manner, there was a greater potential to significantly reduce the linkage drag that would be observed in the BC_2 generation. At chromosome level, certain chromosome segments that were transferred from the foreign donor parent remained large. This phenomenon of large donor chromosome segments retained has been well documented in a number of crops (Randhawa et al. 2009). Many individuals were still carrying a complete full heterozygous length of chromosomes 5A (69.6 cM) and 6B (89.3 cM). The number of unwanted and

non-targeted traits in the excess donor fragments cannot be predicted. This high level of linkage drag on chromosome 5A and 6B can be attributed to the relative short map distances and poor marker coverage. However, these large chromosome segments would otherwise normally have been retained without the use of MAB.

Based on GGT data for the BC_1F_1 population, chromosomes can be presented in the following descending order for the level of linkage drag visible; 5A, 6B, 3B and 7A. However, this order was directly correlated with chromosome length, since chromosomes represented by the shortest cM distance showed the highest level of linkage drag and *vice versa*. Therefore, the longer the chromosome length covered by good marker density the greater the potential to accurately detect recombination events, thus a higher potential to reduce linkage drag. However, a limiting factor in this study for accurate recombination analysis was the large genetic distances between adjacent background markers since most recombination events could have gone unnoticed once genetic distances started to exceed 25 cM, especially when approaching 50 cM or more. It is safe to assume that as a result of the large (75.2 cM) distance between flanking markers (Gwm130 and Gwm233) on chromosome 7A, recombination events between these markers were also not fully detected.

3.4.7 Frequency of genotypes observed

Genotypes with QTL on both chromosomes 3B *(Fhb1*) and 6B (*Fhb2)* were observed 19 times, double the frequency of any of the other two gene/QTL combinations. This suggested that these two gene/QTL regions on genome B were possibly easier to combine or transfer in combination. QTL combinations involving genes/QTL on chromosomes 3B (*Fhb1*) and 7A as well as 5A (*Qfhs.ifa.5A*) and 7A were not observed in the BC_1F_1 population. However, the *7A* QTL region was present in approximately a third of the BC_1F_1 individuals suggesting the *7A* QTL is not easily combined with certain FHB resistance genes/QTL. A high level of recombination was observed within the *7A* QTL region (Gwm130 and Gwm233), often resulting in a loss of the *7A* QTL region. The *7A* QTL region was observed 50% less frequently compared to other single QTL regions.

Results indicated that the *Qfhs.ifa.5A* QTL that confers Type I resistance on chromosome 5A was more difficult to transfer as indicated by a lower frequency of occurrence. The reduced frequency of *Qfhs.ifa.5A* containing genotypes implied a lower heritability than that of the *Fhb1* or *Fhb2* gene/QTL regions. However, other genetic factors play a role in combining ability and heritability. The *Qfhs.ifa.5A* region appeared less frequently in combination with other genes/QTL as well, further confirming the

difficulty of introgression of this QTL region in combination with other targeted FHB resistance genes/QTL. This observation is in line with a number of publications suggesting that QTL regions conferring Type I FHB resistance response, like the *Qfhs.ifa.5A* QTL region, show lower levels of heritability and are potentially more difficult to transfer/introgress into new backgrounds (Buerstmayr et al. 2002; 2003; 2009). The *Fhb1* gene/QTL, compared to the other FHB resistance genes/QTL, showed a higher level of heritability based on a higher observed combination frequency, especially with *Fhb2*. *Fhb1* and *Fhb2* are known to predominantly confer a Type II FHB resistance response (Cuthbert et al. 2006; 2007). Two individuals (8 and 113) with all four targeted FHB resistance genes/QTL, four individuals (17, 30, 44 and 75) with the three major gene/QTL regions (*Fhb1*, *Qfhs.ifa.5A* and *Fhb2*) and one individual (83) with three and a half gene/QTL regions (*Fhb1, ½ Qfhs.ifa.5A* with recombination in the Qfhs.ifa.5A QTL region*, Fhb2* and *7A QTL*) were observed in the BC_1F_1 population, further enforcing the difficulty in transferring and combining Type I resistance in combination with Type II resistance types. Considering the small population size of just 120 BC_1F_1 individuals the identification of these seven (8, 17, 30, 44, 75, 83 and 113) lines as well as others is a significant achievement.

3.4.8 Recurrent parent percentage recovery

Across the four targeted chromosomes (3B, 5A, 6B and 7A) a more limited level of linkage drag was observed than expected, when considering the overall population RPGP average. A BC_1F_1 population experiencing no linkage drag should theoretically be at a 75% RPGP expected population mean. The population mean observed was 72.2%, only 2.8% lower than a population without significant linkage drag. However, this is for the four targeted chromosomes only and not all 21 chromosomes of the wheat genome, which will be discussed in chapter 5. It is expected that RPGP recovery on non-targeted chromosomes should be better than that of the four targeted chromosomes. The top twenty BC_1F_1 individuals out of 120 screened, which contained different FHB resistance genes/QTL combinations present and with more than 80% (Krokodil) RPGP were identified. Some individuals tested positive for one resistance gene/QTL with more than 87% RPGP in the BC_1F_1 generation.

Targeted lines with three and four FHB resistance genes/QTL were identified and selected for use in the BC_2F_1 generation development. These targeted lines were selected based firstly with the main aim of FHB resistance in mind and not so much on RPGP values. As expected the presence of more than one FHB resistance gene/QTL within a genotype significantly reduced the RPGP recovered in that individual. However,

large genetic gain still remains possible with foreground and background marker selections made during this MAS phase, which can significantly reduce linkage drag and make selections of "extreme genotypes" in a population possible. Two individuals (88 and 106) with well over 90% RPGP and a single FHB gene/QTL were already identified in the BC_1F_1 generation. This further indicated that in just two MABC generations it should be possible to select an individual containing improved FHB resistance levels and 94-97% RPGP in the BC_2F_1 generation. The net genetic gain by selecting these extreme BC_1F_1 individuals with different FHB resistance gene/QTL combinations will hopefully show a significant improvement in the BC_2F_1 generation across all 21 chromosomes. Randhawa et al. (2009) with an optimised two generation MABC programme achieved development of a wheat line with 97% RPGP and the target trait of interest in the BC_2F_1 generation. So far results of this chapter suggested that improved lines with targeted FHB resistance gene/QTL combinations and similar levels of RPGP to Randhawa et al. (2009) may be achievable by the BC_2 generation of this study.

3.5 Conclusions

With the aid of marker-assisted foreground and background selection a number of promising BC_1F_1 lines with different FHB resistance gene/QTL combinations for the four targeted chromosomes (3B, 5A, 6B and 7A) were identified. Importantly, a number of BC_1F_1 lines (8, 17, 30, 44, 75, 83 and 113) with three/four FHB resistance gene/QTL combinations with limited linkage drag around the targeted QTL regions of chromosomes 3B, 5A, 6B and 7A were identified. As expected when mainly targeting four different FHB resistance genes/QTL, the recovery of RPGP will be limited, but linkage drag could be reduced. These elite lines have been grouped into three and four QTL families to be used in the development of the BC_2F_1 generation. With the aid of MAS it was possible to track the successful introgression of *Fhb1*, *Qfhs.ifa.5A*, *Fhb2* and *7A* QTL gene/QTL combinations into the background of the irrigated SA spring wheat cultivar Krokodil. This will help ensure the selection of improved BC_2F_1 pre-breeding lines containing the optimal FHB resistance gene/QTL combinations. The pre-set target of retaining the highest (94-97%) RPGP as possible in the BC_2F_1 generation across the entire wheat genome (all 21 chromosomes), aimed at retaining the most important quality traits seems achievable. This should speed up the development process of elite pre-breeding wheat lines to be used in private sector to develop new FHB resistant wheat cultivars.

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Chapter 4

Phenotypic validation of Fusarium head blight resistance gene/quantitative trait loci expression

Abstract

FHB is a sporadic disease that causes significant yield losses under irrigation in SA. FHB disease is best managed by integrated management practices involving the development of resistant cultivars. The aim of this study was to phenotypically validate the successful transfer and expression of targeted FHB resistance genes/QTL into the background of SA irrigated spring wheat cultivar Krokodil from FHB resistance source CM-80236. Spray and point inoculation methods were used in a glasshouse trial to evaluate Type I and Type II FHB resistance responses on six selected controls and 130 segregating BC_1F_1 genotypes. All BC_1F_1 individuals were genotyped with markers associated with targeted FHB resistance genes/QTL on chromosomes 3B, 5A, 6B and 7A. FHB disease was expressed as a percentage disease per spike and assessed at 4, 7, 10, 14, 18 and 21 dpi. The negative controls remained clear of FHB disease. BC_1F_1 genotypes containing the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL contributed additively to FHB resistance and the prevention of yield loss. Preliminary results indicated that the *Fhb1* gene reduced the presence of tombstone kernels (*Fusarium*-damaged kernels). The *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL was successfully transferred using MAS and validated phenotypically in the background of Krokodil displaying improved FHB resistance levels.

"Don't judge each day by the harvest you reap but by the seeds that you plant."

by

Robert Louis Stevenson

4.1 Introduction

FHB is a sporadic fungal disease that mainly occurs on irrigated spring wheat in SA. Currently there are a limited number of FHB resistant cultivars commercially available in SA. A situation compounded by the fact that there are no official fungicides registered for control of FHB on wheat. Large losses in revenue for farmers are related to yield loss, mycotoxin accumulation such as DON within contaminated grain (contamination of grain may lead to entire seed lots being rejected) and the presence of *Fusarium*-damaged kernels (FDK) affecting seed grading (Tamburic-Ilincic 2012).

FHB is often best managed using integrated management practices involving development of resistant germplasm, use of better crop rotations and optimal application of registered fungicides (Schaafsma et al. 2001; Mesterhazy et al. 2003). However, this could be complicated further in future with the recent identification of the first fungicide resistant *F. graminearum* isolate found in the state of New York, USA (Spolti et al. 2014). The best long term approach is the continual development of resistant cultivars. However, it is well documented that breeding for FHB resistance is complex (Tamburic-Ilincic 2012). The two main types of FHB resistance that have been reported include Type I resistance against initial infection by the pathogen and Type II resistance against fungal spread within the wheat spike and tolerance to the accumulation of mycotoxins (Shroeder and Christensen 1963; Mesterhazy 1995). Recently it has been accepted that spray inoculation methods evaluate both Type I and Type II resistance response, while the point inoculation method solely assess Type II resistance (Tamburic-Ilincic 2012).

MAS can aid breeders in making specific selections using markers linked to traits of interest and by introducing new novel FHB resistance genes/QTL. However, the use of markers alone is often not sufficient to confirm the presence and expression of the trait of interest. MAS introgression of targeted genes/QTL requires phenotypic confirmation of resistance, especially when being transferred into different backgrounds. Genotype selections based on marker data are only as strong as the phenotypic data which validates expression (Agostinelli et al. 2012).

A number of FHB sources of resistance have been identified over the last 20 years. Important resistance QTL have been characterised in a number of non-adapted sources, identified in different mapping populations and validated across diverse genetic backgrounds (Miedaner et al. 2011). Sumai 3, the most commonly used FHB resistance source around the world, contains four important genes/QTL, namely *Fhb1* (Anderson et al. 2001; Cuthbert et al. 2006), *Qfhs.ifa-5A* (Anderson et al. 2001), *Fhb2* (Cuthbert et al.

2007) and *Fhb7AC* QTL (Jayatilake et al. 2011). CM-82036, a derived line from Sumai 3 and developed at CIMMYT, contains at least *Fhb1* and *Qfhs.ifa-5A* that confer Type II and Type I resistance, respectively (Buerstmayr et al. 2002; 2003; Miedaner et al. 2011). Frontana is a moderately resistant Brazilian cultivar that contains a major QTL on chromosome 3A that confers a Type I resistance response (Steiner et al. 2004). Asian lines documented with a number of different resistance genes/QTL include Wangshuibai (*Fhb4* and *Fhb5*; Xue et al. 2010; 2011), CJ9036 (QTL on chromosomes 1A and 2D; Jiang et al. 2007) and more recently Chinese landraces Baishanyuehuang (*Fhb1* and QTL on chromosomes 3A and 5A; Zhang et al. 2012b), Haiyanzhong (QTL on chromosome 7D; Li et al. 2011), Huangcandou (*Fhb1* and a QTL on chromosome 3AS; Cai 2012) and Huangfangzhu (*Fhb1* and a QTL on chromosome 7A; Li et al. 2012). In 2012 a USA hard winter wheat named Heyne containing three novel major FHB resistance QTL on chromosomes 3A, 4D and 4AL was identified (Zhang et al. 2012a).

These sources of resistance all contain FHB resistance genes/QTL that have been reported to confer certain types of FHB resistance and are responsible for specific levels of phenotypic variation. Examples of specific phenotypic variation percentages are 25-50% for *Fhb1* (Anderson et al. 2001; Buerstmayr et al. 2002; Cuthbert et al. 2006), 5-12% for *Fhb2* (Cuthbert et al. 2007), 15-25% for *Qfhs.ifa-5A* (Buerstmayr et al. 2003), 5-10% for a QTL on chromosome 3BS near the centromere (Zhang et al. 2012b), 55% for *Fhb5* (Xue et al. 2011), 5-15% for a QTL on chromosome 3A (Steiner et al. 2004; Cai 2012; Zhang et al. 2012a; 2012b) and 22% for the *Fhb7AC* QTL (Jayatilake et al. 2011). Observed variations depended on the specific inoculation method used for phenotypic screening, FHB resistance type conferred by the targeted gene/QTL, selected susceptible genetic background and selection of acceptable susceptible and resistant checks. The observed phenotypic variation gives an indication of the improved resistance levels within a genotype containing the targeted resistance genes/QTL.

The main aim of this study was to phenotypically validate the introgression of FHB resistance genes/QTL into the background of the SA cultivar Krokodil. The aim was achieved through five objectives. Firstly, confirmation of SSR marker associations with specific FHB resistance genes/QTL. Secondly, determination whether different gene/QTL combinations confer varying levels of FHB resistance. Thirdly, identification of the optimal FHB resistance genes/QTL combinations in the studied population. Fourthly, confirmation of the predominant type of FHB resistance associated with specific genes/QTL. And lastly, assigning relative phenotypic variation percentages to specific

FHB resistance genes/QTL for FHB disease severity, reduction in potential yield loss and reduced FDK.

4.2 Materials and methods

4.2.1 Plant material

Six cultivars including the recurrent parent Krokodil and donor parent CM-82036 were included as a series of controls during the FHB resistance glasshouse screening trial. CRN826 and SST876 were included as susceptible checks (seed obtained from the Plant Breeding division's germplasm bank at the UFS), while Sumai 3 (containing Type I and Type II resistance) and Frontana (containing Type I resistance) were included as resistant checks (seed for the FHB resistant checks was obtained from Prof Buerstmayr from the University of Natural Resources and Applied Life Sciences Vienna, Austria).

4.2.2 Phenotypic glasshouse trial design and layout

Eight 3 ℓ pots per control cultivar (a total of 48 pots) containing three seeds per pot were planted. Seed from the BC_1F_1 population seed was used for phenotypic validation and three seeds per 3 ℓ pot were planted. One-hundred-and-sixty-eight BC_1F_1 seeds were planted in 56 pots. Each seed should have a unique genotype and represented different BC_1F_1 (Krokodil/CM-82036//*Krokodil) cross combinations. All material used in the phenotypic trials were planted simultaneously in the glasshouse. Plants were allowed to grow until 50-75% head emergence, followed by inoculation and incubation and then placed in a separate glasshouse cubicle.

The glasshouse conditions were set to 18°C/25°C night/day temperatures with natural night/day lengths. Pots were filled with a clay-topsoil mixture with 2 g of 3:2:1 fertiliser added per pot. A solution of Chemicult fertiliser (Chemicult Products, Pty Ltd., SA) for micronutrient supply was applied to growing seedlings after two weeks of growth. A solution of 3:2:1 fertiliser for the supply of macronutrients was applied to seedlings every second week once plants were four weeks old. Plants and evaporation pans were watered or filled twice daily.

A maximum of 52 pots were placed on each bench within the glasshouse cubicle. Separate benches were used for the spray and point inoculation methods (Figure 4.1). Four pots of each of the six checks, CRN826, SST876, Frontana, Sumai 3, CM-82036 and Krokodil, were placed on each bench. Twenty-eight pots containing different BC_1F_1 genotypes were randomly selected for each inoculation method. To increase the relative humidity in the cubicle two large 10 ℓ shallow plastic containers were placed on each

bench to serve as evaporation pans. Twelve heads from each of the six control cultivars per inoculation method were screened. In total 130 different BC_1F_1 plants were inoculated and evaluated for FHB resistance. The remaining BC_1F_1 plants were used as randomly selected negative controls that were "inoculated" with autoclaved double distilled water.

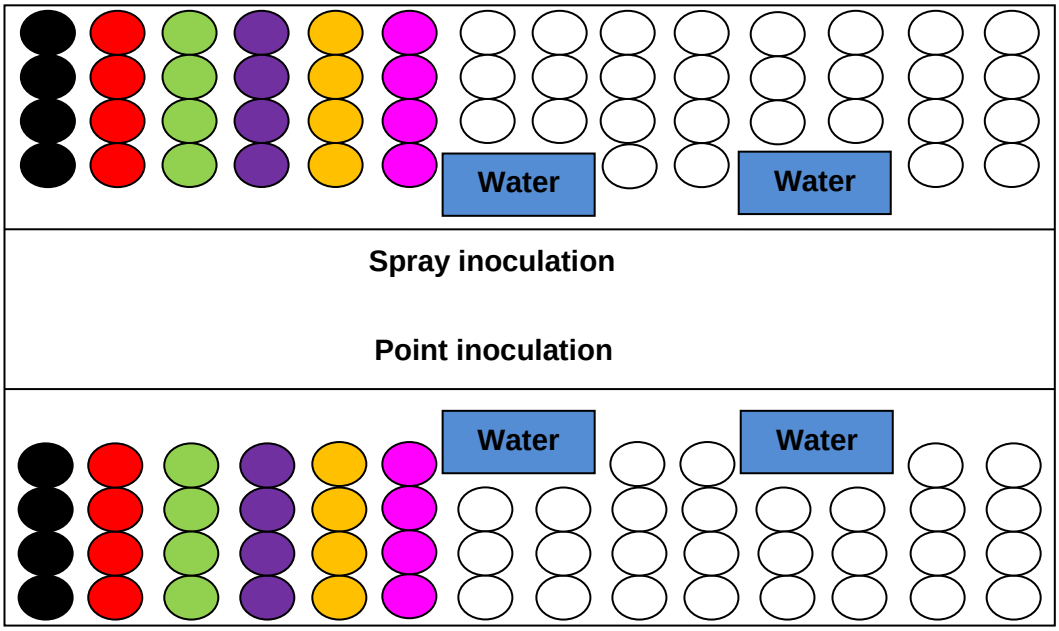


Figure 4.1 Schematic design of the pot layout used in the glasshouse for both spray and point inoculation methods to screen for Type I and Type II FHB resistance response. Each circle represents a 3 ℓ pot and the rectangle a 10 ℓ water tray. Controls are indicated in colour, namely black for CRN826, red for SST876, green for Frontana, purple for Sumai 3, gold for CM-82036 and pink for Krokodil. The white pots represent BC_1F_1 individuals

4.2.3 Inoculum preparation

The most pathogenic aggressive isolate (A841) of *F. graminearum* obtained from Dr Adré Minaar-Ontong that was identified during her PhD study (Minnaar-Ontong 2011) was used in the inoculum preparation. A potato sucrose broth (PSB) was made from freshly peeled potatoes. Diced potatoes (400 g/ℓ) were boiled in water for approximately 10 min until soft and then discarded (Waller et al. 2001). The remaining liquid was poured through a fine metal sieve and combined with 20 g/ℓ sucrose before autoclaving. The pure culture of A841 was added to 250 ml flasks containing the PSB and incubated

in an orbital shaker (250 rpm) at 25°C for spore production (Wegener 1992). After 5-7 days of incubation fungal cultures from each flask were bulked and stored. Fresh liquid spore suspension used during inoculation was prepared by filtering fungal mycelium through a double layer of cheesecloth.

4.2.4 Inoculation methods

Two different inoculation methods were used; a hand held spray method and a cotton wool point inoculation method. The spray method can be unreliable and hard to optimise due to the number of environmental factors that can play a role. However, the spray method is the preferred method to simulate a natural field infection and it can be used to simultaneously screen for Type I and Type II resistance response (Buerstmayr et al. 2012; Tamburic-Ilincic 2012). The point inoculation method is easier to perform in glasshouse screenings and is used to solely evaluate a Type II resistance response.

4.2.4.1 Spray inoculation (Type I and Type II resistance)

The spray inoculation method was used for Type I and Type II FHB resistance response screening. A 750 ml hand spray bottle containing 100 ml fresh spore suspension (Buerstmayr et al. 2012) of *F. graminearum* isolate A841, 400 ml autoclaved double distilled water and 20 µl Tween, was used during each inoculation. Inoculation was done at an early head emergence stage (50-75%) for Type I resistance response. The targeted head was sprayed from a distance of approximately 15 cm with a fine mist until small droplets were visible on the head (Figure 4.2A). The inoculated head was allowed to dry for 30 min after which it was covered with a 15x10 cm clear plastic zip lock bag (Mesterhazy 1978). Pots were moved to the glasshouse cubicle for a 60 h incubation period (Figure 4.2B). After the 60 h incubation period the zip lock bags were removed and disposed of by incineration. Additional plant heads were selected as negative controls and sprayed with autoclaved double distilled water containing Tween solution, during each inoculation session. Each inoculated head's percentage of FHB symptoms was determined at 4, 7, 10, 14, 18 and 21 dpi, recorded and captured by digital photographs.

4.2.4.2 Cotton wool point inoculation (Type II resistance)

The second inoculation method used to screen solely for Type II FHB resistance response, made use of fresh spore suspension soaked cotton wool balls. Small cotton wool balls, 2-3 mm in diameter, were rolled under sterile conditions. The cotton wool balls were placed in a glass petri dish, autoclaved and dried in an oven at 40°C. Each cotton wool ball was autoclaved three times in total. Inoculation was done during late

spike emergence (75-100%) for optimal infections. A cotton wool ball was soaked in fresh spore suspension (prepared as for the spray inoculation method) for 30 s and placed within a floret situated near the middle of each targeted head using tweezers (Wang and Miller, 1988; Bai and Shaner, 1996; Buerstmayr et al. 2012). Each inoculated head was immediately covered with a 15x10 cm clear zip lock bag and moved to the glasshouse cubicle and incubated for 60 h (Mesterhazy 1978). After the 60 h incubation period the bag was removed and disposed of by incineration. As with the spray inoculation method additional plant heads were selected as negative controls and a floret situated towards the middle of the spike was “infected” with a cotton wool ball soaked in distilled water containing Tween solution. Each inoculated head’s percentage FHB symptoms was determined at 4, 7, 10, 14, 18 and 21 dpi, recorded and captured by digital photographs.

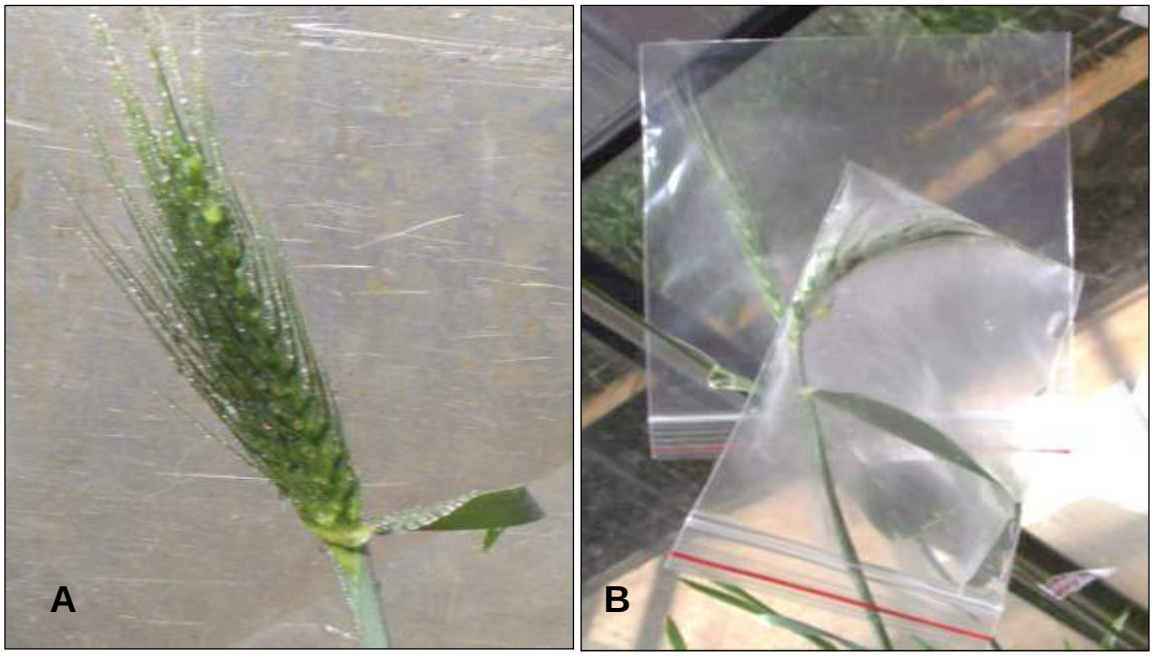


Figure 4.2 **Spray inoculation of wheat heads for Type I and Type II FHB resistance screening. A: Moisture droplets containing the water spore suspension visible on the targeted head. B: A 15x10 cm clear zip lock bag covering the targeted head after inoculation for an incubation period of 60 h**

4.2.4.3 Data analysis

The percentage bleached spikelets per inoculated head were scored at 4, 7, 10, 14, 18 and 21 dpi for both inoculation methods. FHB disease percentage scores were

calculated by determining the number of individual florets showing FHB symptoms out of the total number of florets per wheat spike. FHB disease percentages of each inoculated head were used to calculate representative FHB disease averages for each day of measurement per line/cultivar/specific BC_1F_1 genotype for both inoculation methods. A digital photo documenting stages/symptoms of infection of each specific inoculated head for susceptible checks (CRN826 and SST876), resistant checks (Frontana and Sumai 3), parental lines (Krokodil and CM-82036) and each individual BC_1F_1 plant at each day of measurement after inoculation was taken for both inoculation methods.

4.2.5 Resistance gene/QTL genotyping

4.2.5.1 DNA isolation

DNA isolation was performed as described in Chapter 3 (section 3.2.6). Leaf samples were collected from 168 BC_1F_1 individuals for foreground marker screening.

4.2.5.2 SSR analysis

Marker analysis was performed as described in Chapter 3 (section 3.2.7). The 14 foreground markers (Table 3.2) linked to various FHB resistance genes/QTL were used to screen each of the BC_1F_1 individuals used in the phenotypic trial. PCR products were screened using the Gel Scan 3000 Real-Time DNA Fragment Analysis System (Corbett Research, Sydney, Australia). Each one of the 5% non-denaturing gels was made up to an end volume of 25 ml consisting of 1x TBE buffer, 5% (w/v) acrylamide:bis-acrylamide (19:1), 0.12% (v/v) tetramethylethylenediamine (TEMED) and 0.08% (v/v) ammonium persulfate (APS). Gels were poured and left to polymerise for 3-4 hours or overnight. The upper buffer chamber (negative electrode) of the system contained 0.5x TBE buffer made up in deionised water. The bottom buffer chamber (positive electrode) contained 0.5x TBE buffer and 1% (v/v) ethidium bromide (EtBr) in deionised water. PCR products were mixed with deionised formamide loading dye. A pre-run step at 800 V for 45 min at 37°C was done prior to sample loading. A 1-2 µl volume of diluted sample was loaded on the gel and a pulse-run initiated for 10 s then excess product was rinsed out. The run was at 1 200-1 500 V for an average of 30-45 min at 37°C until all targeted fragments were visible and detected. A 25 bp ladder was loaded flanking each gel run and used to determine relative amplified fragment sizes. The targeted marker alleles were scored as present or absent.

4.2.6 Phenotypic variation analysis of observed Fusarium head blight symptoms

Using Microsoft Excel, the relative disease progress averages for each dpi measurement (averages at 4, 7 dpi etc.) were calculated for all susceptible checks, resistant checks

and both parental lines for comparison between the two inoculation methods used. The average disease scores at each dpi for the recurrent parent Krokodil were compared to BC_1F_1 genotypes that contained no FHB resistance genes/QTL. This was done to see the similarity of disease score averages between pure Krokodil individuals and developed BC_1F_1 individuals with no known FHB resistance gene/QTL. Then averages at each dpi were calculated per BC_1F_1 genotype class (presence of the *Fhb1* on its own; the *Qfhs.ifa-5A* QTL on its own and *Fhb1* in combination with the *Qfhs.ifa-5A* QTL) for both inoculation methods used. The averages for the no FHB BC_1F_1 genotypes and each BC_1F_1 genotype class at the same dpi were used for statistical T-test analysis (two tail, unequal variance analysis) and graphical comparisons across both inoculation methods. The disease score averages at 21 dpi of Krokodil/no FHB BC_1F_1 were then subtracted from each of BC_1F_1 genotype class averages (at 21 dpi) to calculate the observed phenotypic variation percentages. This observed phenotypic variation percentage calculation was done the same for both the spray and point inoculation methods.

4.2.7 Seed analysis

Each individual infected primary wheat spike (per check, per parental line, per BC_1F_1 individual and per negative control) was harvested for yield and FDK analysis. Each individual head was thrashed by hand, seed counted manually and the percentage FDK versus normal gradable seed percentages were calculated based on the total seed harvested for that specific head. This method was modified from the principles of Jones and Mirocha (1999) which used visual matching of a series of 100 g seed standards containing certain FDK percentages. This was not possible during the current study due to low amounts of seed that was harvested from single heads.

Yield of the primary infected spikes was compared to the average yield of negative controls (primary spikes) and non-targeted primary spikes which had already flowered, of each of the respective lines, which gave a representative average yield loss/gain percentage specific to that cultivar/line. This was done for both the spray and point inoculation methods used. The secondary spikes of the different entries were not used as this would affect the yield percentages. Yield averages were determined from a representative number of infected wheat spikes per check, parental line and BC_1F_1 line with the same genotype class (presence of the *Fhb1* gene on its own; the *Qfhs.ifa-5A* QTL on its own and the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL) were calculated, using Microsoft Excel, for both inoculation methods. Genotype yield averages and FDK percentages were calculated from a representative pool of segregating BC_1F_1 individuals for each of the above mentioned FHB resistance gene/QTL combinations per

inoculation method. These genotype class yield and FDK averages were subtracted from the same averages of BC_1F_1 individuals containing no FHB resistance genes/QTL. This gave the relative phenotypic contribution per FHB gene/QTL combination for the traits of prevention of FDK and potential yield loss. A digital photo of the FDK versus normal seed was taken per infected wheat spike for both inoculation methods.

4.3 Results

4.3.1 Phenotypic screening results: checks and parental lines

4.3.1.1 Spray inoculation (Type I and Type II resistance screening)

The assigned negative controls inoculated with sterile water remained clear of FHB symptoms. The susceptible checks, resistant checks and parental lines performed differently for the spray inoculation method compared to that of the point inoculation method. After 21 dpi SST876 (99.2%) was the most susceptible line, followed by recurrent parent Krokodil (80.0%) and resistant check Frontana (45.2%) conferring Type I resistance (Figure 4.3). Susceptible check CRN826 (35% at 21 dpi) performed better than expected displaying a moderate to tolerant resistance response. Resistant check Sumai 3 (15.0%) and FHB resistance donor parent CM-82036 (19.57%) showed high levels of resistance with limited spread of infection through the wheat spike.

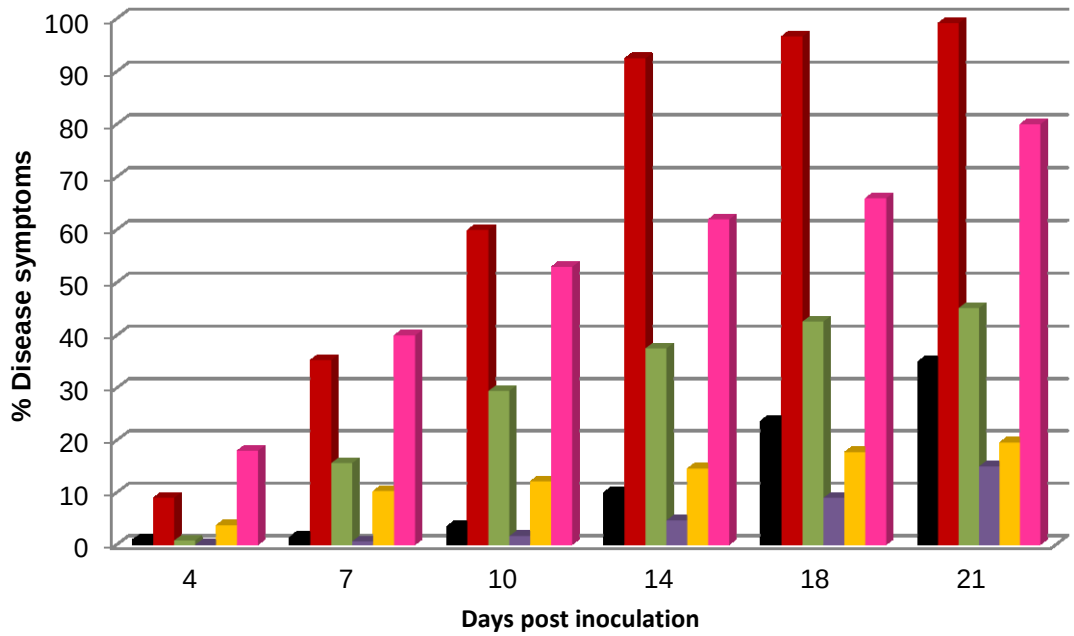
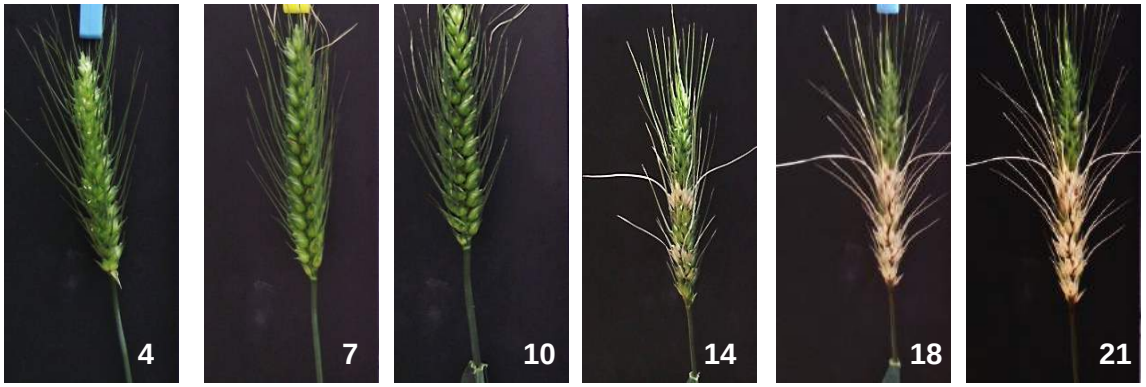


Figure 4.3 Average FHB resistance response scores reflected as percentages of infected spikelets at 4, 7, 10, 14, 18 and 21 days post spray inoculation for susceptible checks [CRN826 (black) and SST876 (red)], resistant checks [Frontana (green) and Sumai 3 (purple)] and parental lines [CM-82036 (gold) and Krokodil (pink)]

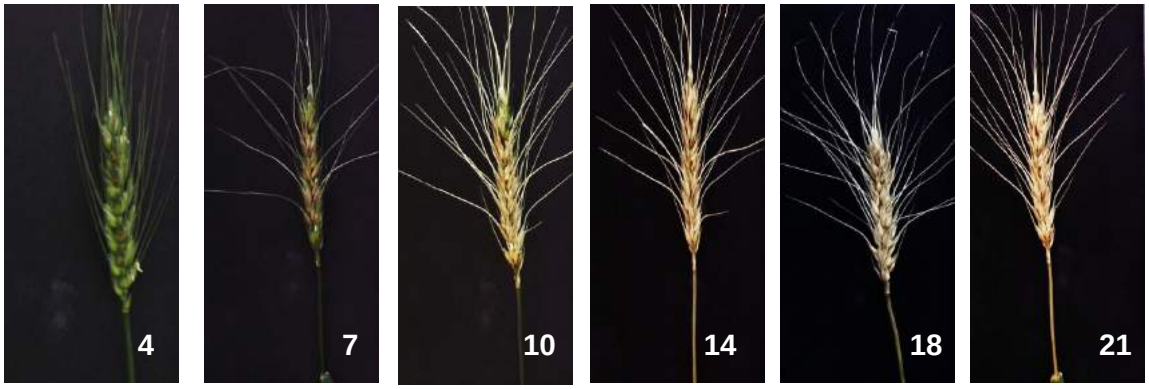
When looking at the 4-10 dpi interval considering only the Type I resistance action or tolerance to infection, susceptible check SST876 and recurrent parent Krokodil performed the worse. However, the rate of disease initiation was initially faster in Krokodil compared to SST876. At 10 dpi SST876 displayed on average higher levels of disease symptoms than Krokodil. It appeared that somewhere between 7-10 dpi Krokodil displayed some level of tolerance to the spread of FHB disease by slowing the rate of disease progress, compared to an accelerated disease rate displayed by SST876. If the same dpi interval was considered for CRN826, Frontana, CM-82036 and Sumai 3, CRN826 performed as well as the resistant checks and donor lines well up to 10 dpi. CM-82036 and Frontana performed worse than CRN826 during the initial phase of infection, indicating a physical barrier or high tolerance to initial infection in CRN826. After 14 dpi (Figure 4.3), CRN826 began to show progressively more FHB symptoms suggesting a lack of tolerance or resistance to spread of the disease. Resistant check Sumai 3 and FHB resistant donor CM-82036, expected to contain Type II resistance, displayed slower rates of disease progress from 10 dpi onwards. This suppression of FHB disease symptoms indicated the action of Type II resistance 10-21 dpi. When Sumai 3 and CM-82036 were compared across the measured time intervals, Sumai 3 performed better during the disease establishment phase. However, if the slower rate of disease progress was considered, especially across time intervals 14-21 dpi, CM-82036 appeared to outperform Sumai 3 for prevention of disease spread. With the spray inoculation method there appeared to be two important phases of resistance response; an initial establishment of infection from 0-7 dpi and the spread of infection from 7-21 dpi. It appeared that there was an overlapping period around 7-10 dpi, where disease establishment transitioned into disease spread through the wheat spike where perhaps both modes of resistance could be acting simultaneously. This validated the fact that a spray method can be used to screen for both Type I and Type II resistance.

Characteristic differences in FHB resistance response levels were displayed by visualisation of the disease progress (Figures 4.4 and 4.5) shown for the respective susceptible checks, resistant checks and two parental lines inoculated using the spray method. Susceptible check CRN826, displayed limited disease symptoms between 0-10 dpi visually confirming a tolerance to initial infection, thereafter disease symptoms spread rapidly throughout the wheat spike from 14 dpi onwards. Frontana performed well and showed limited signs of infection from 0-10 dpi, after which disease symptoms spread (Figure 4.5A). The initial sites of infection in Sumai 3 and resistant donor parent CM-82036 were suppressed from spreading further by what would be classed as Type II

A: CRN826



B: SST876



C: Krokodil

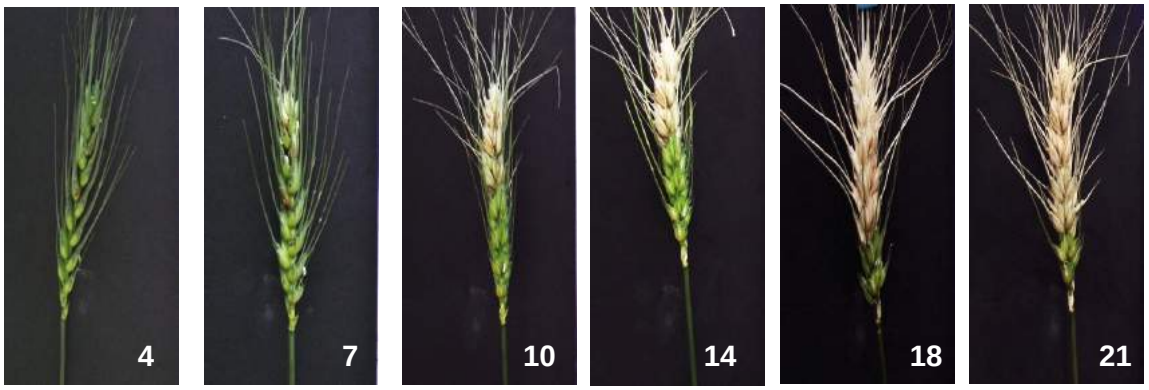
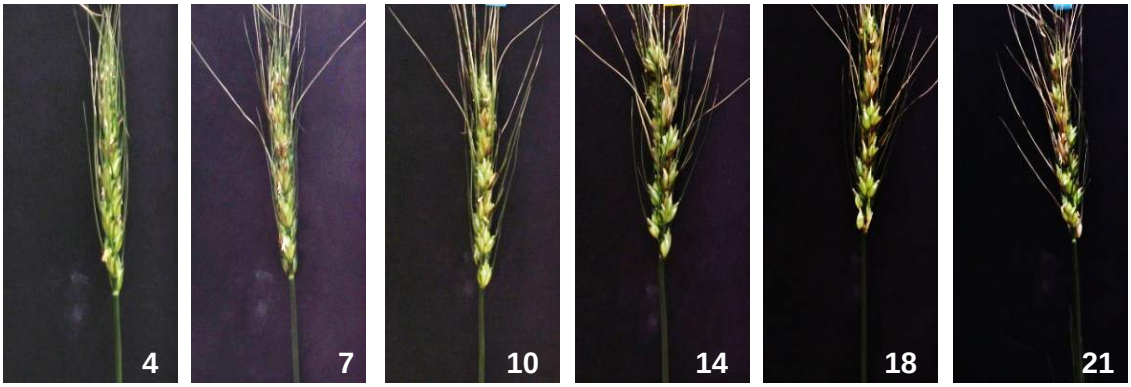


Figure 4.4 **Digital photos of FHB disease symptoms per infected wheat spike at 4, 7, 10, 14, 18 and 21 days post inoculation using the spray inoculation method for the susceptible checks CRN826 (A) and ST876 (B) and recurrent parent Krokodil (C)**

A: Frontana

B: Sumai 3



C: CM-82036

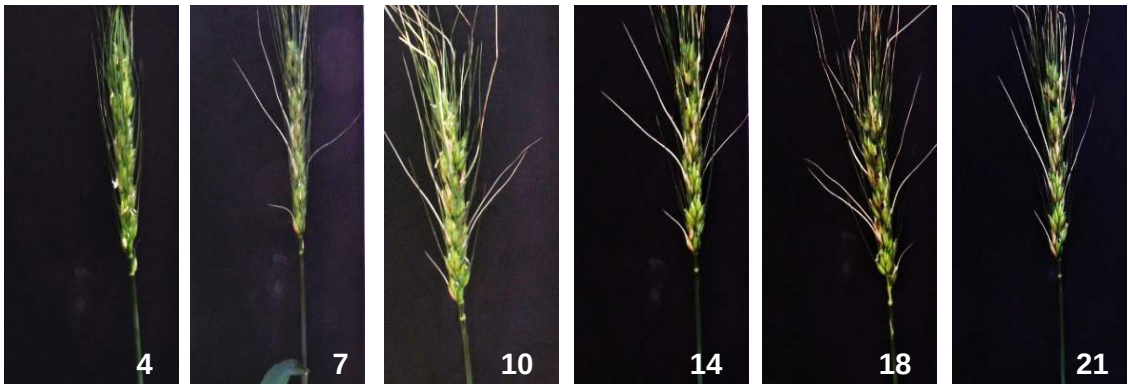


Figure 4.5 **Digital photos of FHB disease symptoms per infected wheat spike at 4, 7, 10, 14, 18 and 21 days post inoculation using the spray inoculation method for the FHB resistant checks Frontana (A) and Sumai 3 (B) and FHB resistant donor parent CM-82036 (C)**

resistance response (Figures 4.5B and C), after 7-10 dpi. Generally the rate of disease progress across the susceptible checks, resistant checks and parental lines for the spray inoculation method was slower, showing disease symptoms a few days later compared to the point inoculation method. However, the disease rate and spread was faster using the spray inoculation method once the *Fusarium* fungus was established as a result of multiple sites of infection. In the susceptible check, SST876, and recurrent parent Krokodil (Figures 4.4A and C) the visible white bleaching of the florets occurred rapidly after 7 dpi compared to the resistant check Sumai 3 and FHB resistant donor CM-82036. The visual disease symptoms of Sumai 3 and CM-82036 were worse compared to the point inoculation method. Sumai 3 and CM-82036 resistance responses were stable and showed durable resistance for the spray inoculation method even under high inoculum pressure.

4.3.1.2 Point inoculation (Type II resistance screening)

The assigned negative controls were clear of infection. The average percentage of FHB disease symptoms per inoculated head using the point inoculation method for each susceptible check, resistant check and parental lines (Figure 4.6) were as expected.

The measurement at 21 dpi was selected for comparison of overall disease levels of all lines for the point inoculation method. SST876 was the most susceptible (97.5% at 21 dpi), followed by CRN826 (92.5% at 21 dpi). CRN826 performed significantly worse with the point inoculation method compared to the spray inoculation method. A 57.5% difference in FHB disease rating for CRN826 at 21 dpi was observed between the two inoculation methods. CRN826 performed competitively with all lines at the 4 dpi reading but showed an accelerated rate of disease spread from 7 dpi onwards. The point inoculation method confirmed a number of observations seen with the spray inoculation method. CRN826 showed tolerance to initial infection which was significantly negated using the point inoculation method and Krokodil showed some tolerance to disease spread as the rates were slower compared to that of SST876 and CRN826 (Figure 4.6).

Genotypes showing a Type II FHB resistance response suppressed the spread of disease symptoms. This action was significant from around 7-10 dpi, similar to that of the spray method. This validated the need to use two different types of inoculation methods to accurately screen and identify resistant germplasm. The overlapping time interval where both Type I and Type II resistance are activated could be from as early as 4-10 dpi. The Type I FHB resistance present in Frontana notably reduced the initial infection rate. However, after 7-10 dpi, once infection was established, disease symptoms rapidly

spread through the infected head. The 70% FHB average infection rating at 21 dpi of Frontana further validated the absence of Type II resistance response in Frontana. Resistant check Sumai 3 and resistant donor parent CM-82036 were stable and performed similarly with 11.3% and 10.0% infection at 21 dpi respectively. This result provided strong evidence for Type II resistance expression in these two lines based on the reduced rate of disease progress within the infected wheat spike. Recurrent parent Krokodil with 89.1% infection at 21 dpi was considered susceptible showing low levels of tolerance to initial infection and disease spread.

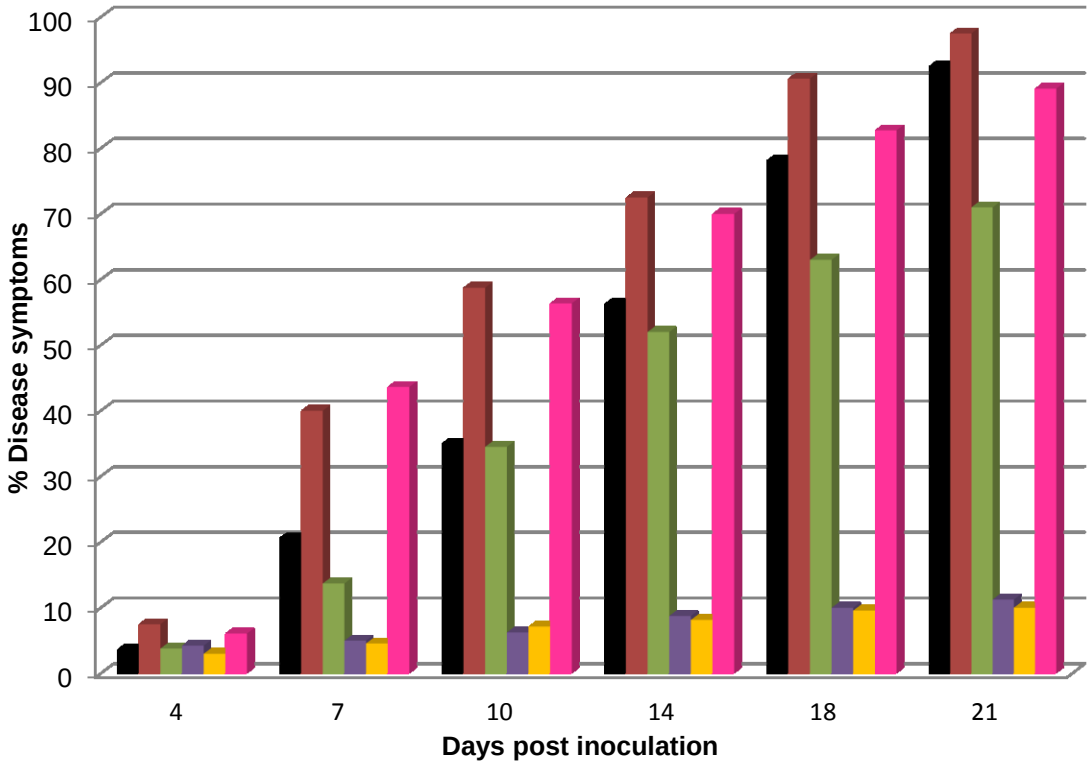
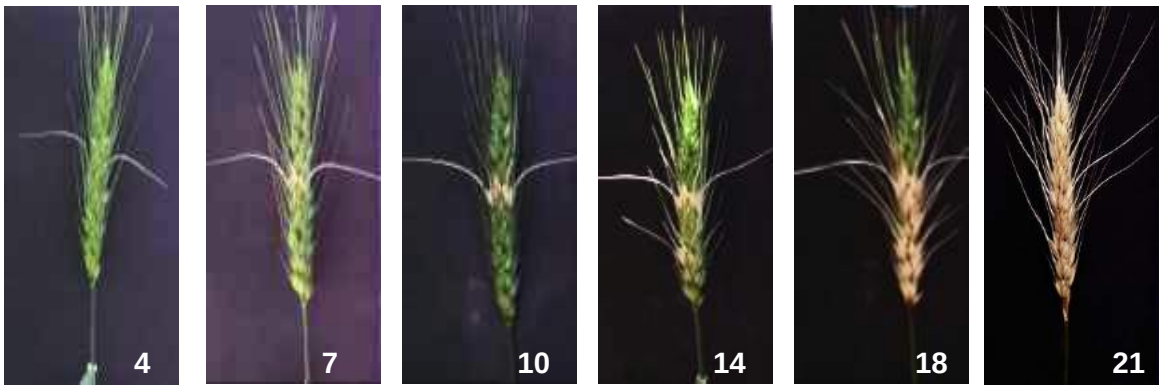


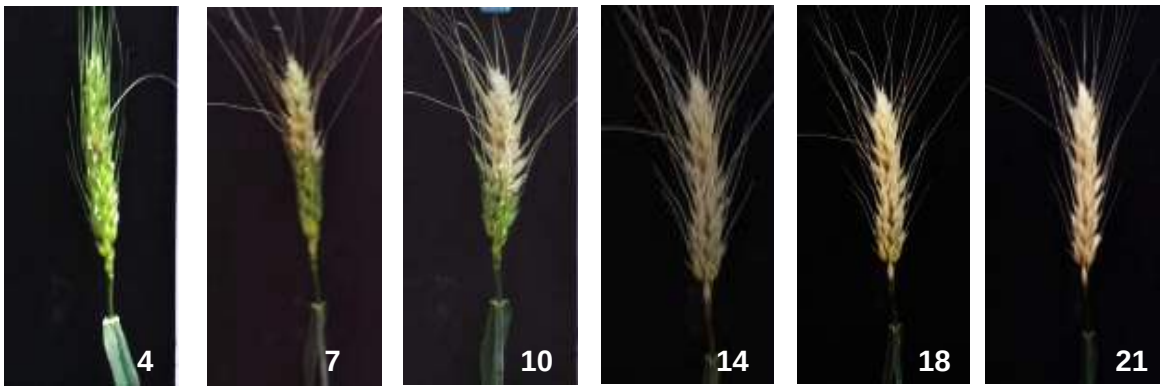
Figure 4.6 Average FHB resistance response scores reflected as percentages of infected spikelets at 4, 7, 10, 14, 18 and 21 days post point inoculation for susceptible checks [CRN826 (black) and SST876 (red)], resistant checks [Frontana (green) and Sumai 3 (purple)] and parental lines [CM-82036 (gold) and Krokodil (pink)]

The visual FHB disease symptoms observed per susceptible check (Figures 4.7A and B), resistant check (Figures 4.8B and C) and parental lines (Figures 4.7C and 4.8C) using the point inoculation method 4, 7, 10, 14, 18 and 21 dpi can clearly distinguish between resistant (Sumai 3 and CM-82036), moderately resistant/tolerant (Frontana) and susceptible material with low levels of tolerance (CRN826, SST876 and Krokodil).

A: CRN826



B: SST876



C: Krokodil

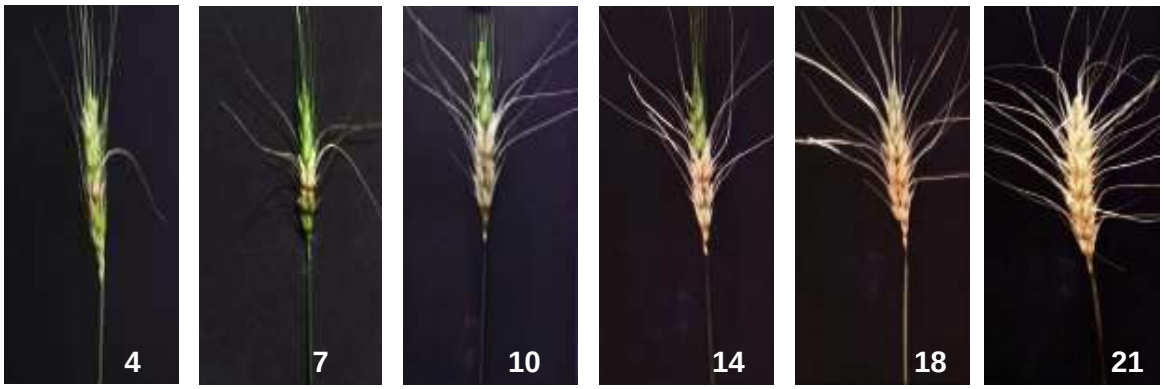
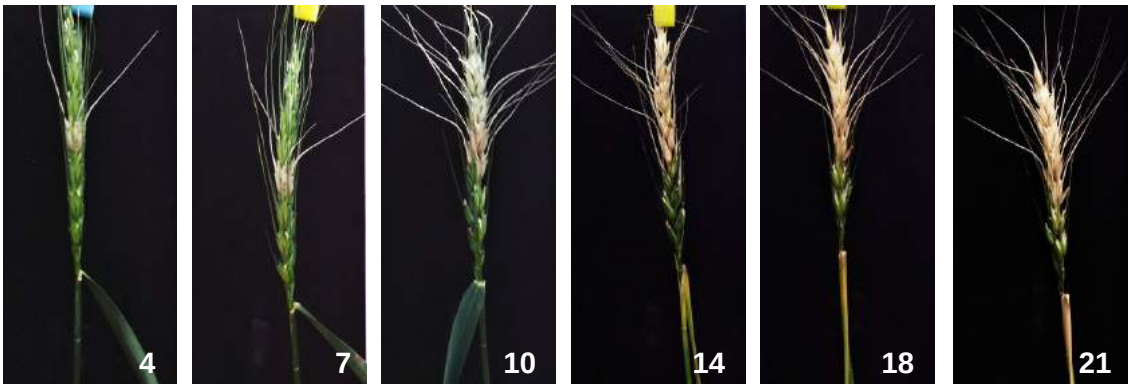
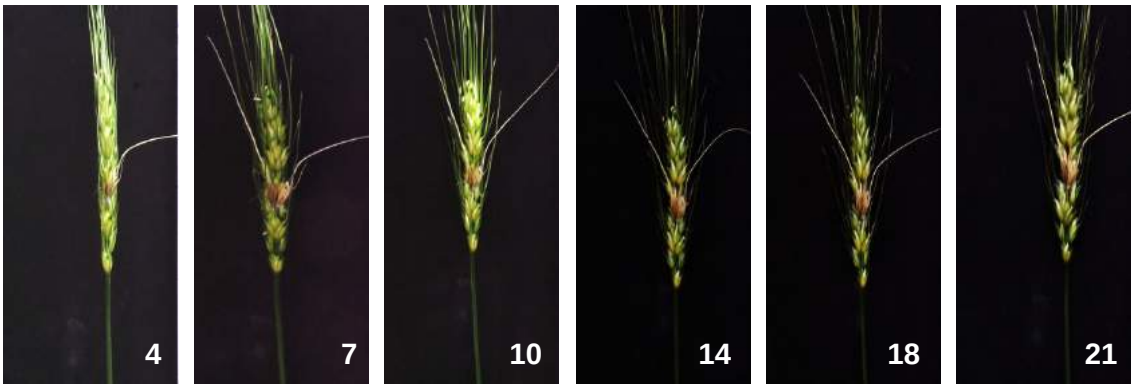


Figure 4.7 **Digital photos of FHB disease symptoms per infected wheat spike at 4, 7, 10, 14, 18 and 21 days post inoculation using the point inoculation method for the susceptible checks CRN826 (A) and ST876 (B) and recurrent parent Krokodil (C)**

A: Frontana



B: Sumai 3



C: CM-82036

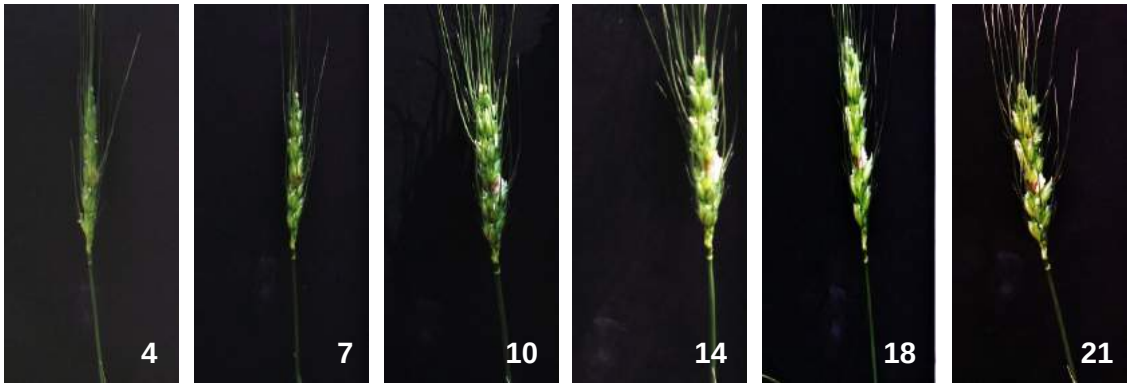


Figure 4.8 **Digital photos of FHB disease symptoms per infected wheat spike at 4, 7, 10, 14, 18 and 21 days post inoculation using the point inoculation method for the FHB resistant checks Frontana (A) and Sumai 3 (B) and FHB resistant donor parent CM-82036 (C)**

CRN826 showed high levels of FHB bleaching from 14 dpi. SST876 and Krokodil already showed high percentages of FHB disease symptoms at 10-14 dpi (Figures 4.7B and C). This indicated a lack of a Type II resistance response but low levels of tolerance to spread of the disease in cultivars CRN826, SST876 and Krokodil. In Figure 4.8A the number of bleached florets in Frontana rapidly progressed after the first 7 dpi, further validating the lack of Type II resistance in Frontana. Both Sumai 3 and CM-82036 responded similarly completely suppressing the FHB disease to a single spikelet. Sumai 3 and CM-82036 displayed a high level of Type II resistance and visually indicated the synergistic advantages of lines containing both Type I and Type II resistance.

4.3.2 Genotyping of BC_1F_1 individuals

All possible combinations of the different FHB resistance genes/QTL per individual were not observed in the random BC_1F_1 population. The phenotypic disease ratings, inoculation method tested and genotype assigned to each of the 128 BC_1F_1 individuals tested are indicated in Appendix D. The following number of BC_1F_1 individuals contained a specific single FHB resistance gene/QTL; eight tested positive for the *Fhb1* gene only, four had the *Qfhs.ifa-5A* QTL, six the *Fhb2* gene and two the *7A* QTL. Different combinations of two genes/QTL were observed in the BC_1F_1 individuals; five lines had the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL; ten lines the *Fhb1* gene in combination with the *Fhb2* gene; four lines the *Fhb1* gene in combination with *7A* QTL; ten lines the *Qfhs.ifa-5A* QTL in combination with the *Fhb2* gene and five lines the *Fhb2* gene in combination with the *7A* QTL. The *Qfhs.ifa-5A* QTL in combination with the *7A* QTL was not observed within the tested population. The following three gene/QTL combinations were observed: the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL and the *Fhb2* gene were present in seven individuals; the *Fhb1* gene in combination with the *Fhb2* gene and the *7A* QTL were present in six lines; the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL and the *7A* QTL were present in three lines while the *Qfhs.ifa-5A* QTL in combination with the *Fhb2* gene and the *7A* QTL were present in six BC_1F_1 individuals. A combination of all targeted FHB resistance genes/QTL was identified in nine lines.

Comparison of different genotypes (where a genotypes for the purpose of this study is being defined as those individuals containing the same FHB resistance gene/QTL combinations) with the average phenotypic scores for each genotype, indicated that the *Fhb2* gene and *7A* QTL on their own provided no or a limited resistance response. Measurement of any phenotypic variation observed for these two genes/QTL was difficult. Individuals that, based on molecular data tested positive for the presence of

either one of these two genes/QTL, performed phenotypically the same as individuals containing no FHB resistance genes/QTL as well as to the recurrent parent Krokodil. Additionally, it seemed that there was no additive contribution of either *Fhb2* or *7A* QTL to resistance in the various gene/QTL combinations. However, for the spray inoculation method the four genes/QTL combination seemed to perform slightly better compared to other genotype combinations. However, the limited number of plants detected with this genotype across both inoculation methods, especially the spray inoculation method, made the accurate calculation of averages difficult. Additionally, since the BC_1F_1 lines were still segregating every genotype was genetically diverse. Due to the small amount of seed available from segregating material leading to the small nature of the trial that was done without replications, it was impossible to validate all possible small additive gene/QTL effects. Based on available data, the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL was considered the best combination for a high level of stable FHB resistance disease expression in the Krokodil background. It was decided that only these two genes/QTL (*Fhb1* gene and *Qfhs.ifa-5A* QTL) present on their own and in combination would be used to explain the observed phenotypic variation percentages at 21 dpi per genotype across both inoculation methods.

4.3.2.1 Spray inoculation method: phenotypic response of specific genotypes

The *Fhb1* gene contributed significantly to FHB disease resistance levels during the initial phase of infection but especially in prevention of disease spread over the full 21 dpi period (Figure 4.9). The *Qfhs.ifa-5A* QTL performed better than the *Fhb1* gene in the initial phase of infection at 4 dpi and contributed individually more to the phenotypic variation observed. The effectiveness of the presence of the *Qfhs.ifa-5A* QTL dropped after 7 dpi compared to individuals containing the *Fhb1* gene. This is an expected response for the *Qfhs.ifa-5A* QTL which confers a Type I resistance response. The *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL performed better and additively compared to being present as a single gene/QTL. Compared to genotypes containing no FHB resistance genes/QTL as well as Krokodil (Figure 4.9), the *Fhb1* gene contributed on average 34.5%, the *Qfhs.ifa-5A* QTL 12.5% and the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL 42.5% to the observed phenotypic variation for the spray inoculation method.

4.3.2.2 Point inoculation method: phenotypic response of specific genotypes

The performance trends of genotypes observed with the spray inoculation method were similar for the point inoculation method. *Qfhs.ifa-5A* genotypes contributed on average more to the phenotypic variation observed at 21 dpi for the point inoculation method compared to the spray inoculation method. The *Fhb1* genotypes (Figure 4.10) performed better in limiting (single point of infection) disease symptoms from 0-7 dpi compared to *Qfhs.ifa-5A* genotypes. However, the *Qfhs.ifa-5A* QTL did still play a limited role in the resistance response during the window period when Type I and Type II resistance seem to be acting simultaneously at 4-10 dpi. The *Fhb1* gene in combination with *Qfhs.ifa-5A* QTL performed better than the *Fhb1* gene or *Qfhs.ifa-5A* QTL on their own, especially from 7-21 dpi (Figure 4.10). The phenotypic variation observed for the point inoculation further validated that the *Qfhs.ifa-5A* QTL is predominantly involved with Type I resistance while the *Fhb1* gene is a dominant resistant gene that confers Type II resistance.

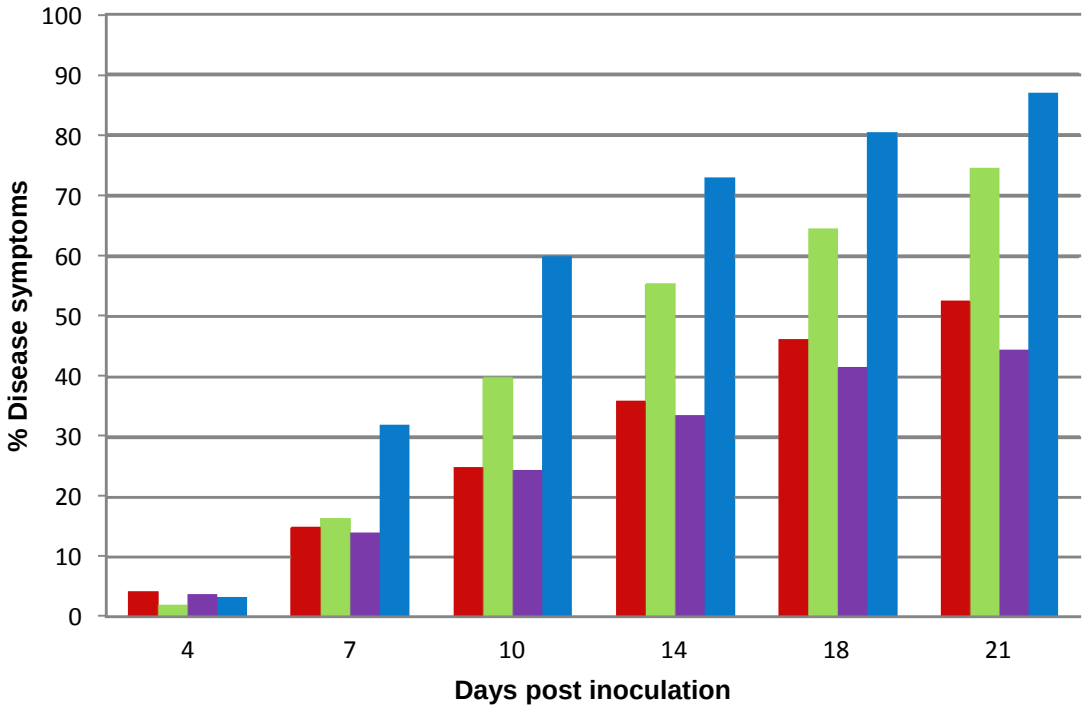


Figure 4.9 **Average FHB severity scores reflected as percentages of infected spikelets at 4, 7, 10, 14, 18 and 21 days post spray inoculation for genotypes containing the *Fhb1* gene (red), *Qfhs.ifa-5A* QTL (green), *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL (purple) and no FHB resistance genes/QTL (blue)**

Compared to genotypes containing no FHB resistance genes/QTL as well as Krokodil, the *Fhb1* gene contributed on average 40.4%, the *Qfhs.ifa-5A* QTL 27.1% and the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL on average 56.4% to the phenotypic variation observed at 21 dpi for the point inoculation method. These phenotypic variations were significantly higher compared to the spray inoculation method which was expected. From this data it was evident that *Fhb1* was predominantly involved with a Type II resistance active from 7 dpi and the *Qfhs.ifa-5A* QTL conferred a Type I resistance response active from 0-4 dpi. These two genes/QTL in combination, conferred more stable and durable FHB resistance, therefore are the optimal FHB resistance gene/QTL combination in the population under study.

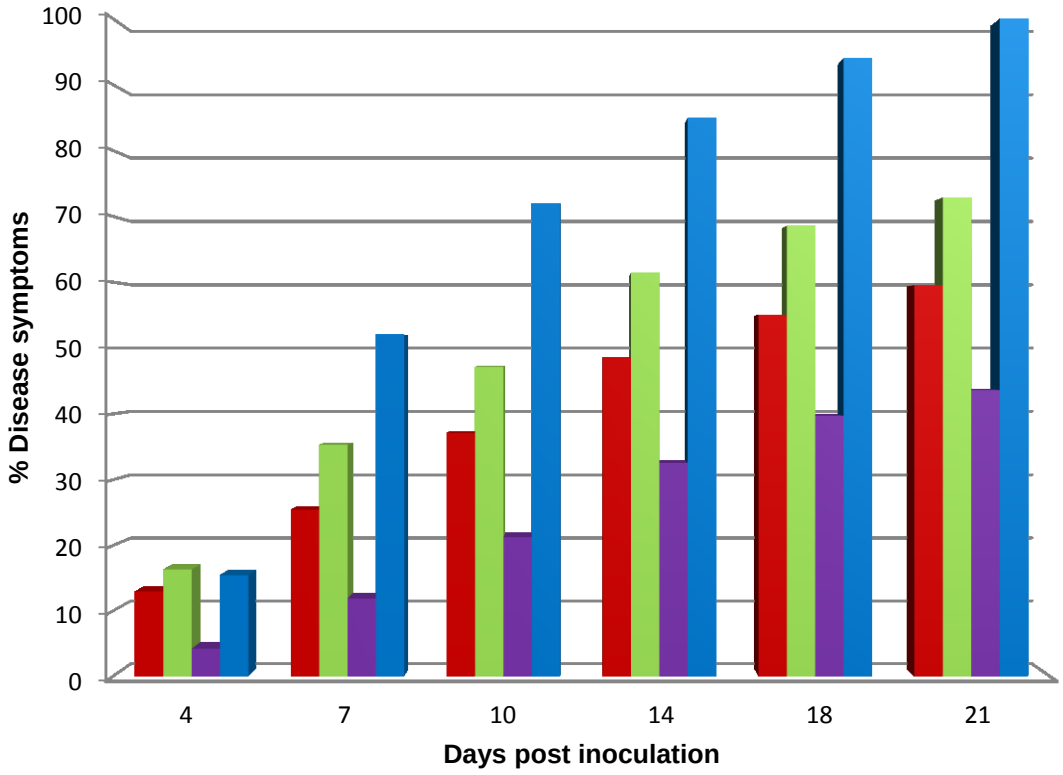


Figure 4.10 **Average FHB severity scores reflected as percentages of infected spikelets at 4, 7, 10, 14, 18 and 21 days post point inoculation for genotypes containing the *Fhb1* gene (red), *Qfhs.ifa-5A* QTL (green), *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL (purple) and no FHB resistance genes/QTL (blue)**

The *Fhb1* gene, *Qfhs.ifa-5A* QTL and the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL genotypes all performed similarly when compared across both the spray and point inoculation methods. If both inoculation methods (Figures 4.9 and 4.10) are considered, the FHB resistance genes/QTL contributed the following to the observed phenotypic

variation: 34.5-40.5% for the *Fhb1* gene, 12.5-27.1% for the *Qfhs.ifa-5A* QTL and 42.5-56.4% for the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL. The *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL present in the Krokodil background, maintained an average FHB disease infection rating at 21 dpi of about 43% for both inoculation methods. This is an overall resistance improvement of about 45-50% which is significant and further validates the successful transfer of stable FHB resistance into the background of Krokodil.

4.3.2.3 T-test comparison of the observed phenotypic response across genotypes

No significant differences were observed when the same genotype class series of disease progress means were compared across the two different inoculation methods used. Table 4.1 indicates the observed P values for the different genotypes class disease progress means compared per inoculation method used.

Table 4.1 T-test statistical comparison of different BC_1F_1 genotype classes across both spray and point inoculation methods used

Genotype	Spray	Point
Fhb1 vs *Qfhs.ifa-5A*	0.387	0.366
Fhb1 vs *Fhb1 and Qfhs.ifa-5A*	0.512	0.700
Qfhs.ifa-5A vs *Fhb1 and Qfhs.ifa-5A*	0.121	0.234
Fhb1 vs *No QTL*	0.009*	0.134
Qfhs.ifa-5A vs *No QTL*	0.024*	0.537
Fhb1 and Qfhs.ifa-5A vs *No QTL*	0.004*	0.082

*P ≤ 0.05

The T-test probabilities validated the phenotypic response data observed in the previous section. From the P values it can be seen that the *Fhb1* gene confers resistance under both inoculation methods used. The difference in the P values of 0.512 and 0.700 (*Fhb1* vs *Fhb1 and Qfhs.ifa-5A*) for the spray and point inoculation methods respectively substantiates the influence the *Qfhs.ifa-5A* QTL has on the prevention of initial infection. The P value of 0.537 for *Qfhs.ifa-5A* QTL vs No QTL for the point inoculation method validated the lack of a Type II resistance response conferred by this QTL. This is in contrast to the significant 0.024 value observed for *Qfhs.ifa-5A* QTL genotypes under the spray inoculation method indicating a strong Type I resistance response. Significant differences were furthermore observed for the spray inoculation method for genotypes

with the *Fhb1* gene present (0.009) and the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL (0.004) when compared with genotypes containing No FHB resistance QTL. The T-test values observed in Table 4.1 suggest that the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL is the optimal gene combination for durable FHB resistance response.

4.3.3 Seed analysis

4.3.3.1 Yield loss and Fusarium-damaged kernel evaluation for susceptible checks, resistant checks and parental lines

Spray inoculation

The spray inoculation method used to evaluate Type I and II resistance is often the closest artificial inoculation method to simulate *Fusarium* inoculum pressure under natural field conditions. Significant differences were observed when the two susceptible checks, two resistant checks and two parental lines were compared for FDK percentage, normal kernel percentage and overall potential yield loss percentage (Figure 4.11). Recurrent parent Krokodil had the largest average potential yield loss of -34.2% compared to the other susceptible lines. Although Krokodil showed the biggest potential yield loss, the loss in potential revenue for a farmer should be less since a higher percentage of seed harvested could be classed as higher marketable grade (77.8%). FDK percentage exceeded the normal seed percentage observed in susceptible checks CRN826 (52.8%) and SST876 (60.7%) with yield losses of -15.6% and -26.0%, respectively (Figure 4.11). The FDK percentages were 37.8% for Frontana, 9.7% for Sumai 3 and 6.6% for the FHB resistant donor parent CM-82036. These three resistant lines all showed similar limited yield losses of -14.67%, -18.10% and -16.30% for Frontana, Sumai 3 and CM-82036 respectively.

Point inoculation

The point inoculation method used to evaluate Type II resistance significantly increased the potential yield loss and the amount of FDK observed in the susceptible checks CRN826, SST876 and susceptible recurrent parent Krokodil (Figure 4.12). CRN826, SST876, Frontana and Krokodil had higher percentages FDK than normal gradable kernels present. The predominant Type I resistance present in Frontana did not reduce the amount of FDK observed using the spray inoculation method.

The yield loss percentage of Frontana was however comparable with the resistant check Sumai 3 and lower than the FHB resistant donor parent CM-82036. Resistant lines Sumai 3 and CM-82036, containing both Type I and Type II FHB resistance, showed low

FDK percentages (2.2% and 16.7%), high percentage normal seed (97.8% and 83.3%), and low yield loss percentages (-17.14% and -26.80%; Figure 4.12).

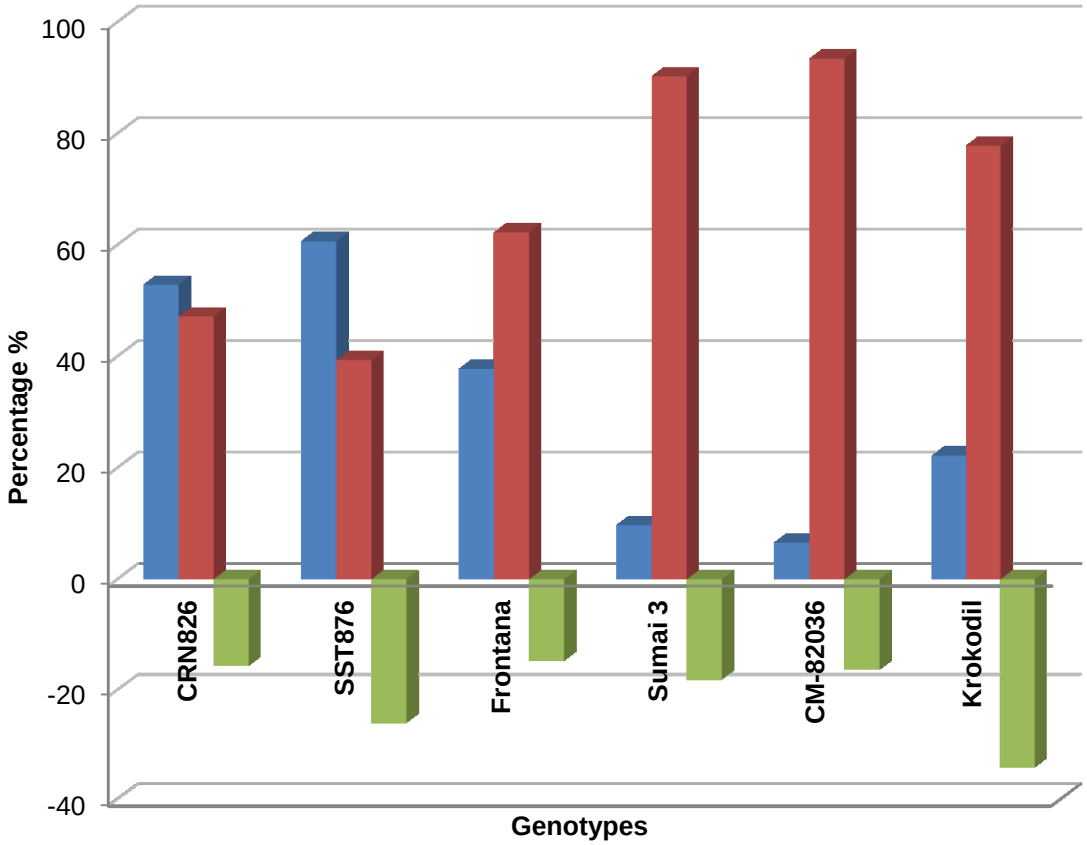


Figure 4.11 Analysis of seed harvested after spray inoculation (evaluation of Type I and II resistance) for the susceptible checks (CRN826 and SST876), resistant checks (Frontana and Sumai 3) and parental lines [CM-82036 (FHB resistant donor) and Krokodil (recurrent parent)]. Blue bars represent average Fusarium-damaged kernels percentage, the red bars average percentage normal graded kernels and the green bars average respective yield loss per genotype

4.3.3.2 Yield loss and Fusarium-damaged kernel evaluation for BC_1F_1 genotypes containing different FHB resistance genes/QTL

Spray inoculation

Seed analysis for genotypes inoculated using the spray inoculation method containing either the *Fhb1* gene, the *Qfhs.ifa-5A* QTL, the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL or no FHB resistance genes/QTL showed significant variation for the

average percentage of FDK present, percentage normal gradable seed and relative yield loss (Figure 4.13). The *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL resulted in fewer FDK, higher number of normal gradable seed and the lowest potential yield loss, followed by individuals containing the *Fhb1* gene or *Qfhs.ifa-5A* QTL. The presence of the *Fhb1* gene explained 9.69% and the *Fhb1* gene in combination with *the Qfhs.ifa-5A* QTL 19.80% of the observed phenotypic variation. The *Qfhs.ifa-5A* QTL on its own explained limited levels of phenotypic variation for FDK after comparison with genotypes that contained no FHB resistance genes/QTL. *Fhb1* contributed -34.91%, *Qfhs.ifa-5A* QTL -28.29% and *Fhb1* in combination with the *Qfhs.ifa-5A* QTL -50.67% of the phenotypic variation towards yield loss reduction (Figure 4.13).

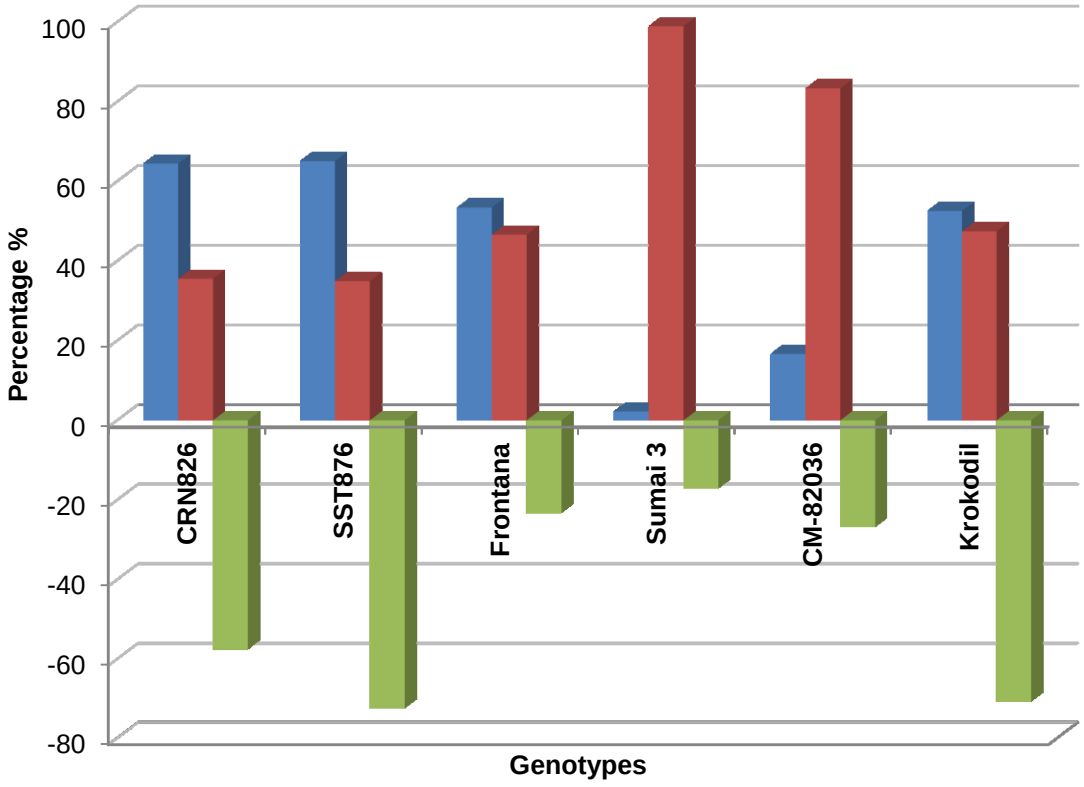


Figure 4.12 Analysis of seed harvested after point inoculation (evaluation of Type II resistance) for the susceptible checks (CRN826 and SST876), resistant checks (Frontana and Sumai 3) and parental lines [CM-82036 (FHB resistant donor) and Krokodil (recurrent parent)]. Blue bars represent average Fusarium-damaged kernels percentage, the red bars average percentage normal graded kernels and the green bars average respective yield loss per genotype

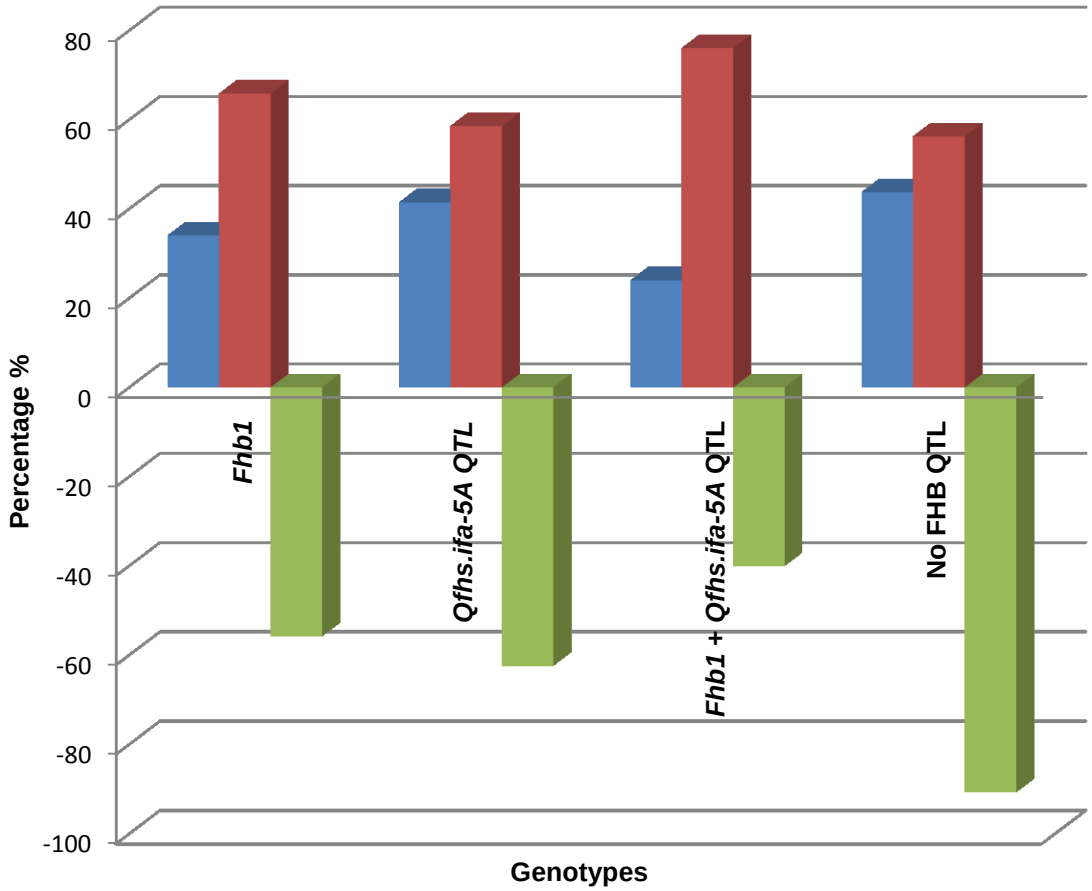


Figure 4.13 Analysis of seed harvested after the spray inoculation (evaluation for Type I and Type II resistance) method for the BC_1F_1 genotypes containing different FHB resistance genes/QTL namely *Fhb1*, *Qfhs.ifa-5A* QTL, *Fhb1* in combination with the *Qfhs.ifa-5A* QTL and no QTL. Blue bars represent average Fusarium-damaged kernels percentage, red bars average percentage normal graded kernels and green bars average respective yield loss per genotype

The FHB resistance gene *Fhb1* conferred resistance towards the reduction of both FDK and potential yield loss. The *Qfhs.ifa-5A* QTL only contributed towards the reduction or prevention in yield loss. The observed phenotypic variation for the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL was additive for reduced FDK and yield loss reduction that resulted in an improved performance compared to the single FHB resistance gene/QTL genotypes.

Point inoculation

A similar trend in genotype performance was observed for the FDK percentage and potential yield loss between the spray and point inoculation methods. The *Fhb1* gene (as expected since it confers Type II resistance) resulted in a larger decrease in FDK compared to genotypes containing no FHB resistance genes/QTL or only the *Qfhs.ifa-5A* QTL. A similar trend for the *Fhb1* gene was observed for yield reduction. The relative order of genotype importance for the different traits for better reduction of FDK, an increase in normal gradable seed levels and reduction in potential yield loss was as follows; the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL, the *Fhb1* gene on its own and the *Qfhs.ifa-5A* QTL on its own (Figure 4.14).

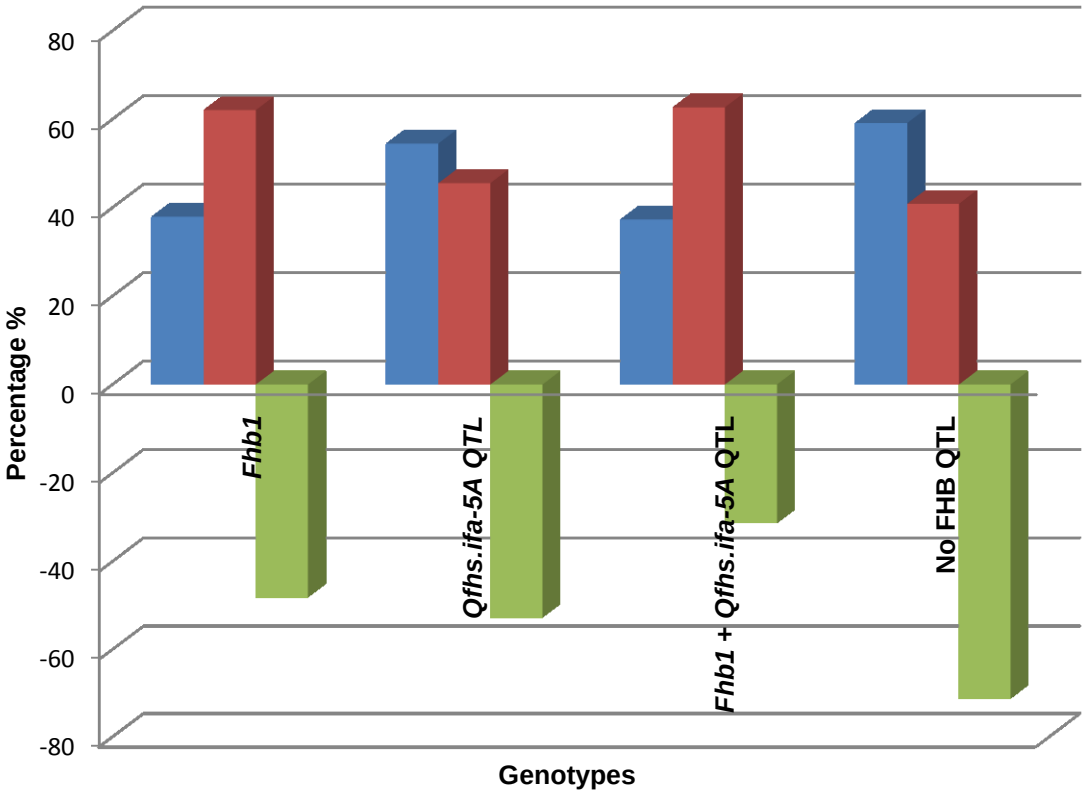


Figure 4.14 **Analysis of seed harvested after the point inoculation (evaluation for Type II resistance) method for the BC_1F_1 genotypes containing the different FHB resistance genes/QTL namely *Fhb1*, *Qfhs.ifa-5A* QTL, *Fhb1* in combination with the *Qfhs.ifa-5A* QTL and no QTL. Blue bars represent average Fusarium-damaged kernels percentage, red bars average percentage normal graded kernels and green bars average respective yield loss per genotype**

Presence of the *Fhb1* gene accounted for a 21.2% reduction in observed FDK and a 23.1% reduction in potential yield loss, while the presence of the *Qfhs.ifa-5A* QTL showed no significant reduction in FDK (compared to no FHB resistance QTL genotypes) and a 18.6% reduction in potential yield loss. The *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL resulted in a similar reduction in FDK percentage (38.0% for *Fhb1* compared to 37.3% for the *Fhb1* in combination with the *Qfhs.ifa-5A* QTL) and normal seed percentage (62.1% for *Fhb1* compared to 62.7% for the *Fhb1* in combination with the *Qfhs.ifa-5A* QTL) as the *Fhb1* gene. However, the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL resulted in an additive effect for reduction in potential yield loss contributing up to 39.9% compared to the no FHB resistance gene/QTL genotypes. This is an additive improvement of 16.8-21.4% for potential yield loss for this FHB resistance gene/QTL combination compared to the *Fhb1* gene or *Qfhs.ifa-5A* QTL acting individually. Results indicated that the *Fhb1* gene conferred more towards prevention of FDK compared to the *Qfhs.ifa-5A* QTL. However, the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL acted additively to reduce the potential yield loss from FHB disease.

The phenotypic variation observed across the two inoculation methods for reduction in FDK ranged from 9.7-21.2% for genotypes containing the *Fhb1* gene, the presence of the *Qfhs.ifa-5A* QTL had no noticeable influence while the presence of the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL indicated an additive effect using the spray inoculation method (19.80%), but no obvious difference was observed for the point inoculation method. All genotypes had a significant phenotypic variation for yield loss reduction across both screenings. *Fhb1* explained 23.1-34.9% of the variation, the *Qfhs.ifa-5A* QTL 18.5-28.3%, while *Fhb1* in combination with the *Qfhs.ifa-5A* QTL showed additive effects and explained 39.9-50.7% of the variation. Lower phenotypic variation was observed overall for all traits using the point inoculation method. Results indicated that the FHB resistance gene *Fhb1* conferred resistance to FDK and potential yield loss, whereas the *Qfhs.ifa-5A* QTL only contributed to reduction in yield loss. The *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL performed better for both resistance to FDK and reduction in yield loss, compared to when the respective gene/QTL was present on its own.

4.4 Discussion

This phenotypic screening trial made use of two inoculation methods namely the spray method to simulate field infections and to screen for Type I and Type II resistance and the point inoculation method to evaluate Type II resistance on its own. One-hundred-

and-twenty-eight BC_1F_1 plants were genotyped and screened randomly using the two inoculation methods. During the phenotypic evaluation trial none of the negative controls (using sterile water) for the two inoculation methods displayed any FHB disease symptoms. The two inoculation methods indicated significant differences between the six lines tested (CRN826, SST876, Frontana, Sumai 3, CM-82036 and Krokodil) and different BC_1F_1 individuals containing different combinations of FHB resistance genes/QTL.

4.4.1 Phenotypic screening

Susceptible checks

Susceptible line CRN826 unexpectedly showed tolerance to initial infection under spray inoculation. No foreground markers were run on any of the check cultivars used in the phenotypic trial so it is unknown whether CRN826 contains similar alleles to the targeted FHB resistance genes/QTL. There is no published evidence of any studies that have been done to investigate or document the tolerance detected in CRN826. This tolerance could be as result of CRN826 flowering at a far later stage of head emergence compared to the other lines. The flowering period and corresponding relative humidity at flowering are crucial in FHB disease development. Disease development was potentially hampered for CRN826 as inoculation and flowering may not have been optimal. Secondly, the floret structure of CRN826 was compact; this may have been a significant physical barrier in preventing/delaying initial *Fusarium* infection. A physical barrier is often the first defence mechanism of a host plant; various morphological and agronomic traits may affect the development of FHB disease symptoms (Steiner et al. 2004). Designated as passive resistance mechanisms by Mesterhazy (1995) these traits can interfere with the measurements of FHB resistance, resulting in inaccurate or higher resistance levels.

Resistant checks and parental lines

The moderately resistant cultivar Frontana that contains a major resistance QTL on chromosome 3A that confers Type I resistance (Steiner et al. 2004) was previously reported to explain on average 16% towards the observed phenotypic variation for FHB severity. Frontana performed moderately resistant, with average disease FHB disease rating of 45.2% at 21 dpi, for the spray inoculation method. This was expected and compared well with the 52% average FHB disease rating documented by Steiner et al. (2004). However, Frontana performed notably worse using the point inoculation method with an average of 71% disease rating at 21 dpi, primarily due to a lack of a known Type II disease resistance response. This result further confirmed the previously documented

reports that the FHB resistance QTL on chromosome 3A in Frontana mainly confers Type I FHB resistance (Steiner et al. 2004). This furthermore validated that under high inoculum pressure and multiple sites of infection Frontana should display moderate to high levels of FHB disease symptoms. The presence of only Type I resistance in a genotype will not provide adequate control under severe inoculum pressure and optimal environmental conditions as was the case for both the spray and point inoculation evaluations of Frontana.

Resistant check Sumai 3 and resistant donor parent CM-82036 performed similarly for both the spray and point inoculation screening methods. The presence of both Type I and Type II resistance in these two lines enabled the identification of the time intervals at which the different resistance responses were activated. Results indicated that the Type I resistance response was activated from 0-7 dpi while the Type II resistance response was activated from 4-7 dpi. These two types of resistances appeared to work successively and synergistically within a small overlapping window of 4-10 dpi. Results indicated that the combined Type I and Type II resistance in genotypes such as Sumai 3, CM-82036 and certain BC_1F_1 lines complemented each other and were essential in effective, stable and durable reduction of FHB disease symptoms. As expected the recurrent parental line Krokodil was highly susceptible for both inoculation methods tested. The point inoculation method did however indicate limited tolerance to spread of infection in Krokodil since the rate of disease progression was slower compared to that of SST876 and CRN826. This tolerance to disease spread exhibited by Krokodil has been noted previously (personal communication, Ms Wilmarie Kriel).

4.4.2 BC_1F_1 genotyping

All possible FHB resistance gene/QTL combinations were not observed in the BC_1F_1 population due to the relative small population size of 128 individuals. Since BC_1F_1 individuals were randomly selected for each of the two inoculation methods, the different FHB resistance gene/QTL combinations were also randomly distributed throughout individuals used in the two methods. However, each of the observed genotypes were present in at least two individuals of each inoculation method.

The presence of the FHB resistance gene/QTL *Fhb1*, *Qfhs.ifa-5A* and the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL were confirmed using specific foreground SSR markers. These genotypes expressed varying levels of FHB disease resistance within the Krokodil background. The additive FHB resistance effect expected to be conferred by the *Fhb2* gene and *7A* QTL, as indicated by the presence of linked markers, were not

detected phenotypically. The FHB disease score ratings for individuals containing either the *Fhb2* gene or the *7A* QTL were not significantly different compared to the susceptible recurrent parent Krokodil or genotypes confirmed genotypically to contain no FHB resistance genes/QTL. A number of previous mapping studies have identified only the *Fhb1* gene and the *Qfhs.ifa-5A* QTL as major contributing genes conferring stable FHB resistance across multiple backgrounds developed from the FHB resistant donor parent CM-82036 (Buerstmayr et al. 2002; 2003; 2009), although it was a source derived from Sumai 3. It is possible that the *Fhb2* gene and *7A* QTL regions have minor non-dectable effects on FHB resistance in CM-82036 and Krokodil backgrounds. Resistance QTL identified in one background will often not be as effective in other genetic backgrounds (Collard and Mackill 2008). Secondly, although similar marker allele sizes were present in CM-82036 compared to Sumai 3 for the *Fhb2* gene and the *7A* QTL, suggesting common backgrounds, these genetic regions might not have been transferred from Sumai 3 to CM-82036.

Phenotypic results confirmed that *Fhb1* predominantly confers Type II resistance with a limited influence on Type I resistance (Anderson et al. 2001; Buerstmayr et al. 2002; 2003; 2009) and explained larger phenotypic variation in the resistance response compared to that of the *Qfhs.ifa-5A* QTL region. The phenotypic data and visual disease symptoms indicated that the *Qfhs.ifa-5A* QTL region mainly contributed to Type I resistance and minimally to Type II resistance (Buerstmayr et al. 2002; 2003; 2009).

4.4.3 Analysis of variation in observed phenotypic resistance

The FHB resistance gene *Fhb1* accounted for 34.5-40.5% of the phenotypic variation observed. This phenotypic range is in line with previous publications for different sources of resistance containing *Fhb1* (Anderson et al. 2001; Buerstmayr et al. 2002; 2003; 2009; Shen et al. 2003; Yang et al. 2003; Zhang et al. 2004; Cuthbert et al. 2006). The *Qfhs.ifa-5A* QTL conferred 12.5-27.1% and the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL 42.5-56.4% of the phenotypic variation observed. These values are similar to phenotypic ranges reported previously (Buerstmayr et al. 2002; 2003; 2008; 2009; Miedaner et al. 2006). The statistical T-test analysis validated the resistance types and level of resistance conferred by each gene/QTL and the combination there of for both inoculation methods used. Significant differences were observed when different genotypes were compared to genotypes containing No FHB resistance QTL especially for the spray inoculation method. The *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL displayed significant additive gene effects and was validated as the most effective in reducing FHB disease severity within the Krokodil background. This gene/QTL

combination has been suggested and documented as the optimal FHB resistance gene/QTL combination to be used in a MAS programme (Buerstmayr et al. 2002; Miedaner et al. 2006; 2011). Although the performance of the BC_1F_1 genotypes was better than that of the recurrent parent Krokodil, they did not show the same resistance levels as the Sumai 3 resistant check and/or the resistance donor CM-82036. This reduced level of FHB resistance in developed lines has been well documented (Miedaner et al. 2011; Tamburic-Ilincic 2012) and can be attributed to background effects and to other minor resistance genes/QTL that may have an additive resistance response when in combination with the major genes but these could not be selected for directly. The presence of the major *Fhb2* gene (Cuthbert et al. 2007) and *Fhb7AC* QTL (Jayatilake et al. 2011) in Sumai 3 will have an influence on FHB resistance. Likewise for CM-82036 there could have been other minor FHB resistance QTL with total additive effects that were not targeted during selection. Additionally, the introduction of resistance genes/QTL into new genetic backgrounds can have a diluting or weakening effect on resistance expression (Buerstmayr et al. 2012).

4.4.4 Seed analysis

During seed analysis it was observed that the *Fhb1* resistance gene played a major role in the reduction of the number of FDK observed as well as in reducing potential yield loss. Reduction in yield loss due to the presence of the *Fhb1* gene region has been reported previously in other resistant cultivars such as CJ9306, in which it accounted for 8-15% of the phenotypic variation for reducing yield loss (Jiang et al. 2007). The presence of the *Qfhs.ifa-5A* QTL on its own did not seem to have any significant effect on reducing the number of FDK since the percentage observed was similar to genotypes containing no FHB resistance QTL. The *Qfhs.ifa-5A* QTL did however contribute to the reduction in potential yield loss. The phenotypic variation due to the presence of both Type I and Type II resistance tended to be additive in limiting the potential yield loss and in reducing FDK in the presence of both the *Fhb1* gene and *Qfhs.ifa-5A* QTL. This gene/QTL combination reduced FDK by 19.8% and lowered yield loss between 39.9-50.7%. Both resistance genes/QTL had a larger influence on the reduction in yield loss than FDK. Results for specific BC_1F_1 genotypes indicated that lower disease incidence did not imply improved yield or less observed FDK. Some genotypes with moderately to high levels of FHB symptoms had a limited number of FDK with relatively high yields. This has been recorded before in some FHB resistant genotypes before, for example Frontana, where additional components of resistance, such as the ability to degrade or tolerate higher mycotoxin (DON) levels appeared to be involved (Wang and Miller 1988; Steiner et al. 2004). Although there was not a direct correlation due to high variation in

expression, it was observed in general that the higher the resistance to FHB incidence, the higher the probability of having a higher percentage of plumb marketable seed with limited yield loss.

These findings are important when making selections and advising breeders. FHB resistant genotypes that show little disease symptoms, low incidence of FDK and slight yield reductions should be selected for and not just genotypes that show high levels of resistance to FHB disease incidence. A reduction in tombstone seed should imply improved control and reduction of mycotoxin contaminated grain (Agostinelli et al. 2012), although there remains the possibility of mycotoxin presence in “healthy” looking kernels. Perhaps in future resistance to mycotoxin accumulation, such as DON, should be used as an additional selection criterion for breeders when selecting the best possible FHB resistant genotypes. The ideal situation to limit the potential economic loss to farmers is to obtain the lowest yield loss possible with the highest amount of high quality market gradable seed.

4.5 Conclusions

The phenotypic and molecular results confirmed the successful transfer and expression of the major FHB genes/QTL, *Fhb1* and *Qhs.ifa-5A*, conferring a combination of Type I and Type II FHB resistance. Both inoculation methods should be used for the accurate determination of specific resistant genotypes since they were able to distinguish the specific time intervals post inoculation at which Type I resistance is activated and Type II resistance takes over. As expected there was a time interval where the action of Type I resistance appeared to overlap with the initiation of a Type II resistance response. The *Fhb1* gene was confirmed as the dominant gene explaining the highest level (35-40%) of observed phenotypic variation and predominantly conferred Type II resistance. The *Qfhs.ifa-5A* QTL region conferred only Type I resistance and explained less of the observed phenotypic variation (12-27%). The observed phenotypic variation data was further validated by significant difference values obtained with the T-test analysis especially under the spray inoculation method. The data indicated that the introgression of the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL was the optimal FHB gene/QTL combination to provide a significant improvement in overall FHB resistance levels in the background of the irrigated spring wheat cultivar Krokodil.

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Chapter 5

Partial BC_2F_1 linkage map construction and recurrent parent genome percentage analysis

Abstract

The genetic improvement of wheat has placed emphasis on certain research areas, namely yield, resistance to both biotic and abiotic stresses, adaptability and end product quality. Traditional backcross breeding was often employed to address these focus areas. Since the development of gene technologies using molecular markers, the application of MABC has allowed for more accurate recurrent parent selection. In this study the use of markers for foreground and background selection in a BC_2F_1 population derived from FHB resistance QTL families is described. The primary objective of this study was to select BC_2F_1 individuals that contain the FHB resistance gene *Fhb1* in combination with the *Qfhs.ifa-5A* QTL. A secondary objective was to select for the highest RPGP (from Krokodil) values across the 21 wheat chromosomes in an attempt to retain competitive yields and good bread making quality traits. From the BC_2F_1 population consisting of 238 individuals, 44 lines were selected for further marker-assisted background and recurrent parent genome analysis. In total 120 polymorphic markers were mapped across the 21 wheat chromosomes. These 44 lines were ranked based on the importance of FHB resistance genes/QTL genotype present as well as the highest RPGP values. Sixteen high value lines that contained *Fhb1* and/or the *Qfhs.ifa-5A* QTL with a minimum RPGP value of 80% were identified and selected. This strategy was a highly successful selection process for lines containing targeted FHB resistance genes/QTL. However, identified lines with high RPGP values still contained a high level of recombination on important quality related chromosomes. An RPGP value can only be used as a guide for improved line selection and not on its own. Lines identified in this pre-breeding programme can be used to initiate the further development of FHB resistant wheat cultivars in SA.

"Only those who have learned the power of sincere and selfless contribution experience life's deepest joy: true fulfilment."

by

Tony Robbins

5.1 Introduction

The genetic improvement/engineering of wheat has certain general focus areas of research namely yield improvement, overcoming both biotic and abiotic stresses, adaptation and enhanced end product quality (Mann et al. 2009). Traditional backcross breeding was often employed to address these focus areas by using recurrent parent selection in an attempt to retain as much of the market related traits of an elite cultivar as possible, while avoiding the negative alleles of the donor parent (Tanksley and Nelson 1996). Since the development of gene/QTL tagging using molecular markers, application of MABC has allowed for more accurate recurrent parent selection. This is often combined with simultaneous foreground selection for targeted traits from the donor. Randhawa et al. (2009) successfully employed a two stage MABC selection approach, by selecting a $BC_2F_{2:3}$ line that contained 97% of the recurrent parent genome as well as the targeted introgressed trait. This was achieved within two MABC generations. The MAS phase four of this study will be discussed in this chapter. Targeted FHB resistance gene/QTL containing chromosomes as well as relevant chromosomes important for end-use product quality were analysed, compared and considered.

Many wheat chromosomes carrying important gene/QTL regions for protein content, protein composition, bread making qualities and dough characteristics, yield and other important agronomic traits have been identified. Genes that control the expression of proteins such as high molecular weight glutenin subunits (HMW-GS) are encoded by the *Glu-1* loci present on the long arms of chromosomes 1A, 1B and 1D (Payne 1987). HMW-GS can be divided into two types, namely x-type and y-type. Loci *Glu-B1* and *Glu-D1* each encode for both types, while the *Glu-A1* locus codes for only the x-type or no subunit at all (Payne and Lawrence 1983). The short arms of these chromosomes (1A, 1B and 1D) also carry the loci that control the low molecular weight glutenin subunits (LMW-GS), namely *Glu-A3, Glu-B3* and *Glu-D3*. The short arms of chromosomes 1A, 1B, 1D, 6A, 6B and 6D have been associated with the presence of genes that code for gliadin proteins (Gao et al. 2007). Although the identification of the glutenin and gliadin alleles present should provide an idea of the potential overall quality of a bread wheat cultivar, it is not a conclusive predictor. These genes that code for different protein fractions can be differentially expressed and are influenced by various environmental factors that interact to determine the end bread quality. Other traits such as grain yield and grain protein QTL have been identified in certain mapping populations on chromosomes 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 5A, 5B, 5D, 6A, 6B, 6D, 7A and 7B (Kato et al. 2000; Perretant et al. 2000; Prasad et al. 2003; Carter et al. 2011; Heidari et al. 2011; Bennett et al. 2012; Ma et al. 2012; Mengistu et al. 2012; Wang et al. 2012; Plessis

et al. 2013; Rustgi et al. 2013). The interaction of many QTL from different chromosomes is essential for good yield, relevant protein content and required end-use quality (Mengistu et al. 2012). However, when breeding for disease resistance such as FHB, there are often associated negative effects on potential yield and end-use quality traits.

Over the last 15 years genetic mapping has led to the identification of two important FHB resistance genes/QTL namely the *Fhb1* gene (on chromosome 3B; Anderson et al. 2001; Lemmens et al. 2005) and the *Qfhs.ifa-5A* QTL (on chromosome 5A; Buerstmayr et al. 2002; 2003; 2009). The *Fhb1* gene and *Qfhs.ifa-5A* QTL originated from the resistant cultivar Sumai 3 and confer different resistance expression mechanisms which have been transferred successfully into elite germplasm around the world (Buerstmayr et al. 2009). Since the fine mapping of the *Fhb1* gene there has been a demand for the development of more closely linked markers with the aim to clone the *Fhb1* gene (Cuthbert et al. 2006). In 2008, Lui et al. developed the closely linked STS marker known as *Umn-10* to be used in MAS for *Fhb1*. In recent times a number of SNP markers have been identified within the *Fhb1* gene region that can be used for MAS (Bernardo et al. 2012). These SNP markers can distinguish between different *Fhb1* coding sequences and even link certain haplotypes to resistant source clusters from different parts of the world. In 2013, transcriptomic characterisation identified some potential novel candidate genes for FHB resistance conferred by *Fhb1* and *Qfhs.ifa-5A* (Schweiger et al. 2013).

It has been well documented that due to the position of the *Fhb1* gene on chromosome 3B and the *Qfhs.ifa-5A* QTL on chromosome 5A, there are small but significant adverse effects on grain yield, important quality traits and other agronomic traits (Kato et al. 2000; Carter et al. 2011; Miedaner et al. 2011; Suzuki et al. 2012). However, Sumai 3 is still being studied around the world to identify other QTL that have not been documented yet. In 2012, a unique FHB susceptibility QTL was identified on chromosome 2DS of Sumai 3. Selection done against this QTL resulted in an improvement of overall FHB resistance levels in Sumai 3 derived lines (Basnet et al. 2012).

The associated undesirable effects as well as effects on FHB disease resistance conferred by *Fhb1* and *Qfhs.ifa-5A* are significantly influenced by the genetic background and winter or spring wheat type introductions (Miedaner et al. 2011; Tamburic-IIlincic 2012). It has also been considered feasible to select lines with improved FHB resistance levels while retaining the high yield of the desired recurrent parent (Miedaner et al. 2011; Tamburic-IIlincic 2012).

It was the aim of this chapter to identify high value BC_2F_1 lines which contain the major FHB resistance genes/QTL *Fhb1* and/or *Qfhs.ifa-5A* on their own or in combination (foreground selection); as well as select for the highest percentage of recurrent parent genome (Krokodil; background selection across all 21 wheat chromosomes) in an attempt to retain competitive yields and good bread making quality traits. These pre-breeding lines were selected with the vision to be used in future resistant cultivar development. However, it was not clear if selection based solely on RPGP of BC_2F_1 lines containing targeted FHB resistance QTL would retain important quality traits and have potential high yields.

5.2 Materials and methods

5.2.1 Plant material

The best BC_1F_1 lines identified in Chapter 3 were used to create the BC_2F_1 population. The breeding scheme as well as planting regime for development of the BC_1F_1 population, as described in Chapter 3, was used. A total of 238 BC_2F_1 individuals which were developed from selected BC_1F_1 mother plants were planted in the glasshouse. These 238 plants were grouped in two families, namely three QTL and four QTL families, containing three or four FHB resistance QTL respectively. Groupings were done based on foreground FHB resistance marker data of each BC_1F_1 mother plant used during BC_2F_1 generation development. The four FHB resistance QTL family consisted of 84 individuals and the three FHB resistance QTL family consisted of 154 individuals. All plants were allowed to self-pollinate to produce BC_2F_2 seed which was harvested and stored for future selection of the best lines.

5.2.2 Glasshouse conditions

All BC_2F_1 individuals of the two QTL families were cultivated in the glasshouse. Three seeds were planted per 3 ℓ pot. The glasshouse conditions, soil type used, fertiliser schedule and watering frequency were as described in Chapter 3 (section 3.2.2).

5.2.3 DNA extraction

Genomic DNA extractions were performed as described in Chapter 3 (section 3.2.6) using freeze-dried leaf material sampled from all 238 BC_2F_1 individuals. DNA concentrations and purity were determined using a spectrophotometer. Each sample was diluted to an end concentration of 50 ng/µl. DNA quality was validated on a 0.8% (w/v) standard agarose gel run at 80 V for 2 h.

5.2.4 PCR reactions

All PCR reactions were performed as described in Chapter 3 (section 3.2.7) and Table 3.1. A complete list of background markers used is given in Appendix A. Wheat SSR markers developed and published by different scientific groups as described in section 3.2.7 were used.

5.2.5 PCR product visualisation

PCR products were visualised and analysed using the Gel Scan 3000 Real-Time DNA Fragment Analysis System (Corbett Research, Sydney, Australia) as described in Chapter 4 (section 4.2.5.2).

5.2.6 Linkage map construction and recurrent parent analysis

Map Manager QTX 3.0 (Manly et al. 2001) with an LOD > 3.0 was used for linkage map construction across all 21 wheat chromosomes for the selected BC_2F_1 individuals. The recurrent parent Krokodil's alleles were coded as A, heterozygous individuals as H and alleles of the FHB resistance donor as B. Best fit marker orders were determined using Record as described in section 3.2.10 (within each linkage group shortest chromosome lengths with the lowest number of crossovers were preferred). Additionally, marker orders were compared to that of the SSR wheat consensus maps (Somers et al. 2004; Marone et al. 2012) to confirm linkage groups. After marker orders and positions were determined, the map was drawn using MapChart 2.2 (Voorrips 2002). Some of the theoretical starting points of certain chromosomes were offset after comparison with the SSR consensus map. Expected theoretical starting positions of the targeted FHB resistance genes/QTL were adjusted and indicated on the relevant chromosomes of the linkage map. Geographical genotypes (GGT) 2.0 software (Van Berloo 2008) was used to calculate the predicted RPGP (HRPG + ½ HDPG = RPGP) recovered per BC_2F_1 individual, linkage drag analyses (percentage of chromosome length still heterozygous) and to observe each individuals haplotype across all 21 chromosomes.

5.3 Results

5.3.1 Foreground selection

Based on the strong phenotypic expression of FHB resistance shown by the *Fhb1* gene and *Qfhs.ifa-5A* QTL region individually and in combination and detected during the BC_1F_1 phenotypic trial (Chapter 4), these two regions were prioritised for foreground selection in the BC_2F_1 families. In total 238 individual BC_2F_1 plants were screened with five *Fhb1* resistance gene associated markers (Gwm533, Barc133, St-3B-138, Gwm493 and St-3B-66) and four *Qfhs.ifa-5A* QTL region markers (Gwm293, Gwm304, Gwm156

and Barc197-2). Out of the 238 lines screened, 44 lines were selected (Table 5.1) based on the presence of the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL, or individually in some lines.

Table 5.1 A list of the 44 BC_2F_1 lines selected for whole genome background screening. The line numbers as well as the BC_2F_1 plant identification numbers are indicated

Line number	BC_2F_1 plant ID	Line number	BC_2F_1 plant ID
1	S10 (4)*	23	S154 (3)*
2	S11 (4)	24	S134 (3)
3	S15 (4)	25	S16 (4)
4	S20 (4)	26	S21 (4)
5	S38 (4)	27	S28 (4)
6	S1 (3)	28	S33 (4)
7	S6 (3)	29	S37 (4)
8	S39 (4)	30	S19 (4)
9	S47 (4)	31	S67 (4)
10	S62 (4)	32	S83 (4)
11	S54 (4)	33	S73 (4)
12	S82 (3)	34	S40 (4)
13	S95 (3)	35	S11 (3)
14	S124 (3)	36	S26 (3)
15	S151 (3)	37	S27 (3)
16	S3 (4)	38	S47 (3)
17	S31 (4)	39	S54 (3)
18	S7 (4)	40	S15 (3)
19	S13 (3)	41	S63 (3)
20	S34 (3)	42	S65 (3)
21	S131 (3)	43	S87 (3)
22	S147 (3)	44	S16 (3)

* (3) or (4) indicates the three or four FHB resistance genes/QTL family origin of the line

The 44 lines were used further for background screening with previously identified polymorphic SSR markers (Chapter 3) for linkage map construction and recurrent parent genome percentage analysis.

5.3.2 Partial linkage map construction

A total of 120 polymorphic markers were mapped across the 21 wheat chromosomes and covered a total genetic distance of 3 026 cM at an average distance of 144 cM per chromosome. The 120 markers comprised of 22 (18.3%) Barc, four (3.3%) Cfa, 12 (10.0%) Cfd, two (1.6%) Gdm, 53 (44.1%) Gwm, 22 (18.3%) Wmc SSRs and five (4.2%) other markers. The average genetic distances covered per chromosome across the three genomes were 162 cM for the A genome, 126 cM for the B genome and 144 cM for the D genome. The average marker distance between markers on targeted chromosomes was 21.7 cM compared to 25.0 cM distance between markers for non-targeted chromosomes. The total number of markers mapped was 44, 42 and 34 respectively for genomes A, B and D. Both genomes A and B had an average of six markers per chromosome while the D genome had five markers on average.

5.3.2.1 Chromosome group 1

A total of 12 SSR markers mapped across the three chromosomes in this group, with five markers on chromosome 1A, three on 1B and four on 1D, with total covered genetic distances of 143 cM, 79 cM and 105 cM for the respective chromosomes (Figure 5.1). Chromosome 1D started at 48 cM with marker Gwm337 the most distal marker on this chromosome based on comparison with the position of this marker on the SSR consensus map. No other tested markers that are closer to the physical starting point of chromosome 1D on the consensus map could be mapped successfully in this BC_2F_1 mapping population. The average genetic distances between mapped adjacent markers were 35.8 cM, 39.5 cM and 35.0 cM for chromosomes 1A, 1B and 1D respectively.

5.3.2.2 Chromosome group 2

A total of 17 markers mapped across the three chromosomes in this group, with six markers on chromosome 2A, six on 2B and five on 2D, with total covered genetic distances of 105 cM, 174 cM and 108 cM respectively (Figure 5.1). Average distances between mapped adjacent SSR markers of 21.0 cM, 34.8 cM and 27.0 cM were observed for chromosomes 2A, 2B and 2D respectively. The starting points of chromosomes 2B and 2D were adjusted to 12 cM and 41 cM respectively as no markers near the respective physical chromosome starting points, after comparison with the wheat consensus map could be mapped.

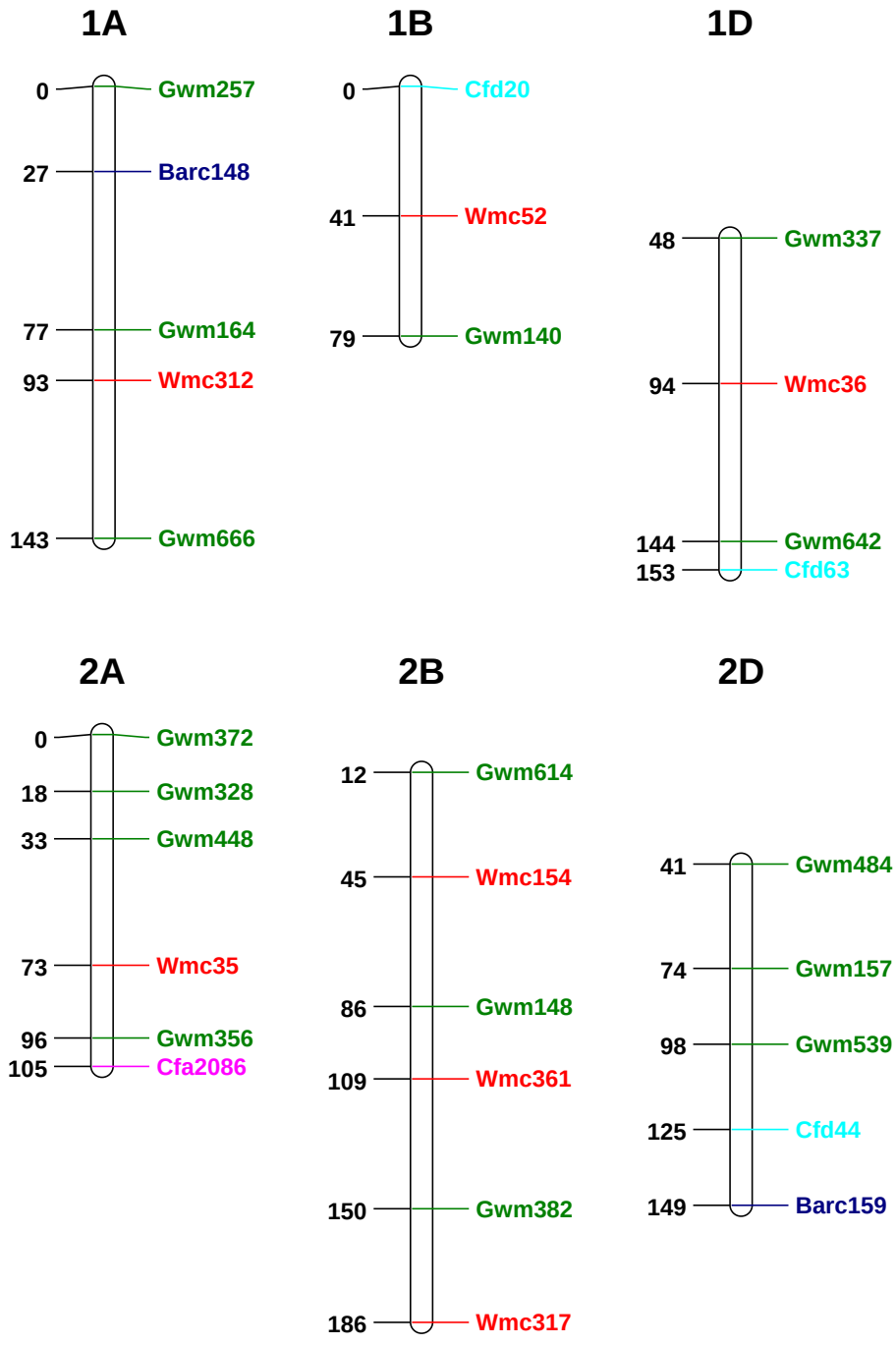

Figure 5.1 **A partial linkage map of wheat chromosomes 1A, 1B, 1D, 2A, 2B and 2D constructed using 44 BC_2F_1 individuals and data of 120 polymorphic SSR marker loci. All marker loci were colour coded as follows: Barc (dark blue), Cfa (pink), Cfd (light blue), Gwm (green) and Wmc (red) representing the different research groups which developed the SSRs. Mapped marker positions are indicated in cM to the left of the chromosome while marker names and order are indicated to the right**

5.3.2.3 Chromosome group 3

Good marker coverage was observed for chromosomes 3A (five markers), 3B (13 markers) and 3D (seven markers) as indicated in Figure 5.2. In total 25 foreground and background SSR markers were mapped successfully across these three chromosomes. The average genetic distance between adjacent markers was 31.0 cM, 19.3 cM and 45.5 cM for chromosomes 3A, 3B and 3D respectively.

Targeted chromosome 3B contained the *Fhb1* gene region (Figure 5.2) which covered the area between markers Gwm533 and Gwm493. Marker Stm559 is a STS marker used extensively in the past to indicate the presence of the slow rusting stem rust gene *Sr2* which originally was closely associated with the Gwm533 locus. As Stm559 was polymorphic between Krokodil and CM-82036 it was used as background marker for chromosome 3B in this study. Stm559 mapped within the *Fhb1* gene region, 10 cM away from flanking marker Gwm533. This confirmed previous reports that *Sr2* is present in a similar chromosome region on chromosome 3B as the *Fhb1* gene region close to the Gwm533 locus.

5.3.2.4 Chromosome group 4

A total of 12 polymorphic SSR markers mapped across chromosomes 4A (six markers), 4B (two markers) and 4D (four markers), representing poor marker coverage/density (Figure 5.3) for these three respective chromosomes. Total genetic distance covered for each chromosome is relatively short at 120 cM, 19 cM and 100 cM respectively for chromosomes 4A, 4B and 4D (Figure 5.3). The average genetic distance between adjacent markers was 24.0 cM, 19.0 cM and 33.3 cM for chromosomes 4A, 4B and 4D respectively. These average distances compared favourably with other linkage groups. Marker Gwm495 was positioned at 16 cM and Cfd193 at 32 cM, the theoretical starting points of chromosome 4B and 4D respectively, after positional comparison with other consensus maps. The short total distance of 19 cM covered by two SSR markers of chromosome 4B was due to low levels of polymorphisms between Krokodil and CM-82036 for this chromosome.

5.3.2.5 Chromosome group 5

A total of 19 SSR markers mapped across chromosomes 5A (eight markers), 5B (seven markers) and 5D (four markers; Figure 5.4). The major FHB resistance QTL *Qfhs.ifa-5A* was present on chromosome 5A and mapped between markers Gwm293 and Barc197-2.

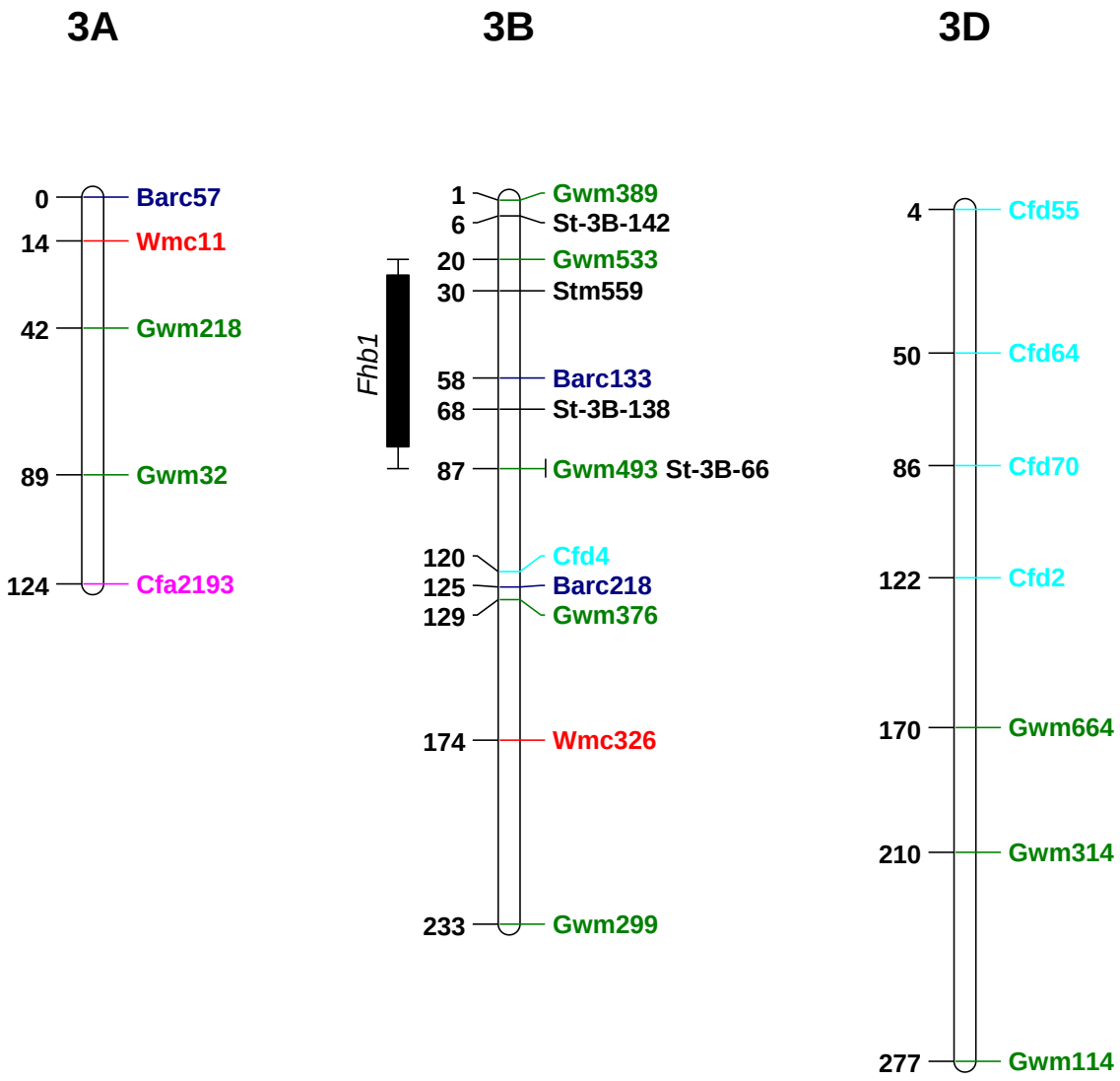

Figure 5.2 **A partial linkage map of wheat chromosomes 3A, 3B (*Fhb1* gene position is indicated to the entire left of the chromosome) and 3D constructed using 44 BC_2F_1 individuals and data of 120 polymorphic SSR marker loci. All marker loci were colour coded as follows: Barc (dark blue), Cfa (pink), Cfd (light blue), Gwm (green) and Wmc (red) representing the different research groups which developed the SSRs and STS markers (black). Mapped marker positions are indicated in cM on the left of the chromosome while marker names and order are indicated to the right**

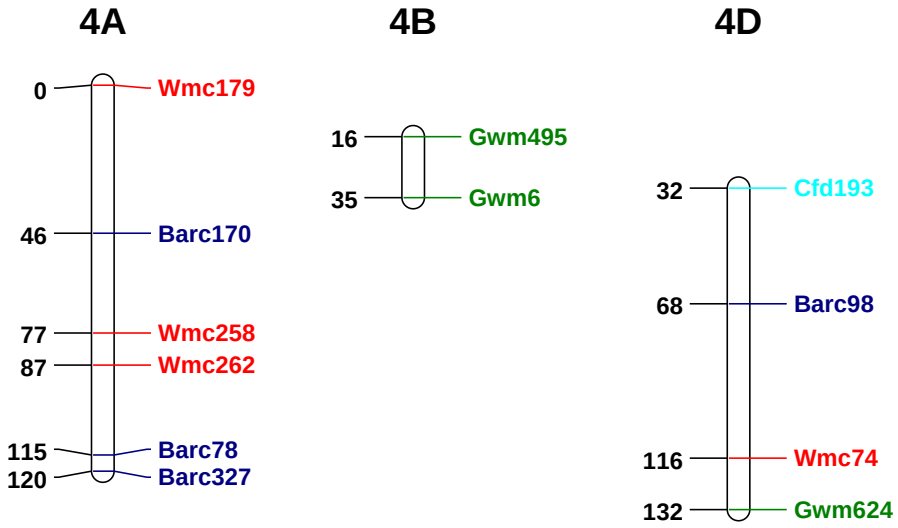

Figure 5.3 **A partial linkage map of wheat chromosomes 4A, 4B and 4D constructed using 44 BC_2F_1 individuals and data of 120 polymorphic SSR marker loci. All marker loci were colour coded as follows: Barc (dark blue), Cfd (light blue), Gwm (green) and Wmc (red) representing the different research groups which developed the SSRs. Mapped marker positions are indicated in cM to the left of the chromosome while marker names and order are indicated to the right**

These markers have been previously identified to be associated with this same Type I FHB resistance QTL present on chromosome 5A of the FHB resistant cultivar CM-82036. Mapping results indicated that there were most likely two important regions, designated *Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2* on chromosome 5A. The *Qfhs.ifa-5A-1* QTL covered a 5 cM region between markers Gwm293 and Gwm304 (Figure 5.4), while the *Qfhs.ifa-5A-2* QTL region covered a 8 cM region flanked by markers Gwm156 and Barc197-2. These two regions were separated by background marker Barc165. This entire chromosome segment between markers Gwm293 and Barc197-2, including background marker Barc165, was targeted for transfer as an entire block. The average genetic distance between adjacent markers was 35.0 cM, 22.4 cM and 40.3 cM for chromosomes 5A, 5B and 5D respectively.

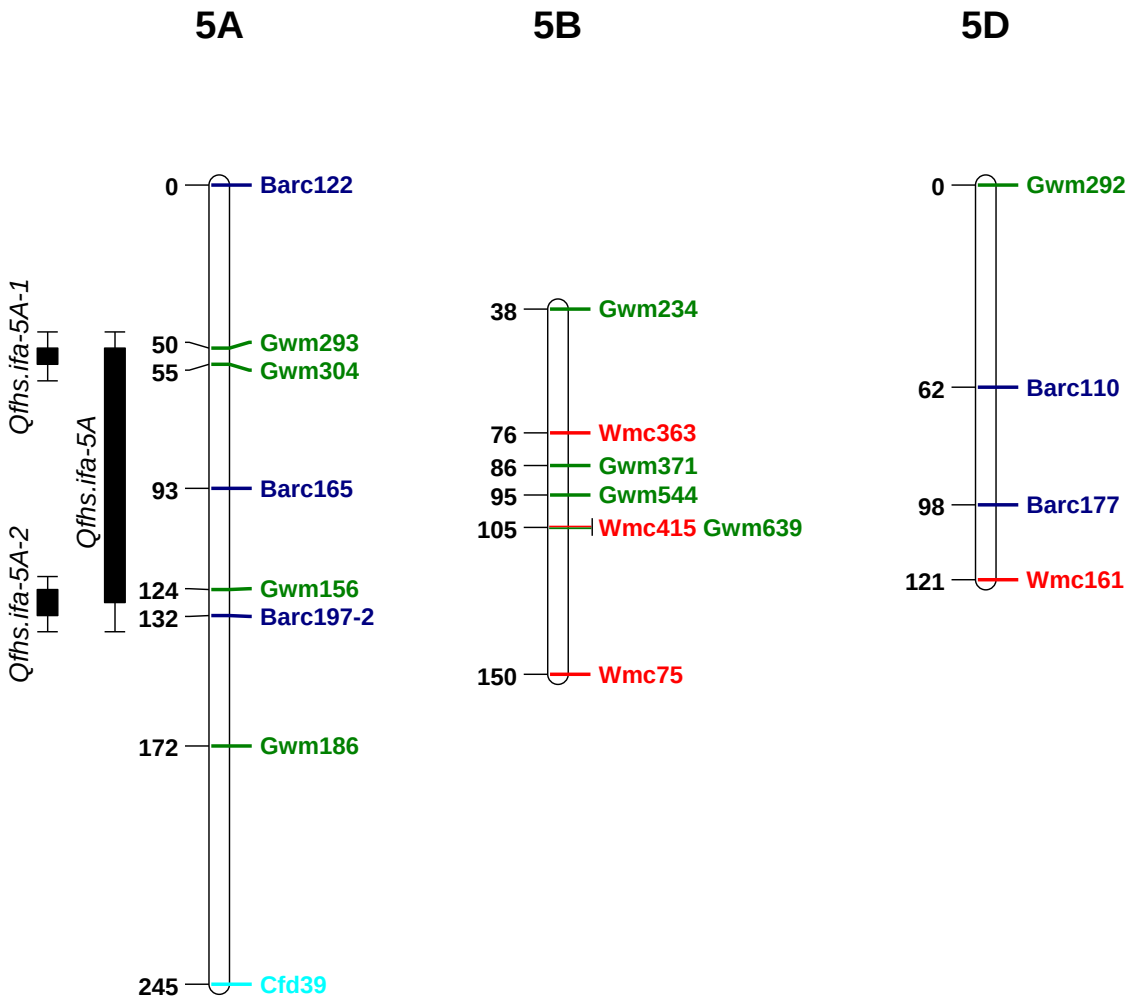

Figure 5.4 **A partial linkage map of wheat chromosomes 5A (*Qfhs.ifa-5A-1* and *Qfhs.ifa-5A-2* QTL regions' positions are indicated to the entire left of the chromosome), 5B and 5D constructed using 44 BC$_2$F$_1$ individuals and data of 120 polymorphic SSR marker loci. All marker loci were colour coded as follows: Barc (dark blue), Cfd (light blue), Gwm (green) and Wmc (red) representing the different research groups which developed the SSRs. Mapped marker positions are indicated in cM to the left of the chromosome while marker names and order are indicated to the right**

5.3.2.6 Chromosome group 6

A total of 18 markers mapped across chromosomes 6A (six markers), 6B (eight markers) and 6D (four markers). The total genetic distance covered was 178 cM, 219 cM and 120 cM respectively for chromosomes 6A, 6B and 6D. The average genetic distance between adjacent markers was 35.6 cM, 32.7 cM and 40.0 cM for chromosomes 6A, 6B and 6D respectively (Figure 5.5). The starting points of chromosomes 6B and 6D were adjusted to 14 cM (Gwm132) and 25 cM (Gwm469) respectively after comparison with the wheat consensus map. No other markers closer to the physical starting position could be mapped in this study.

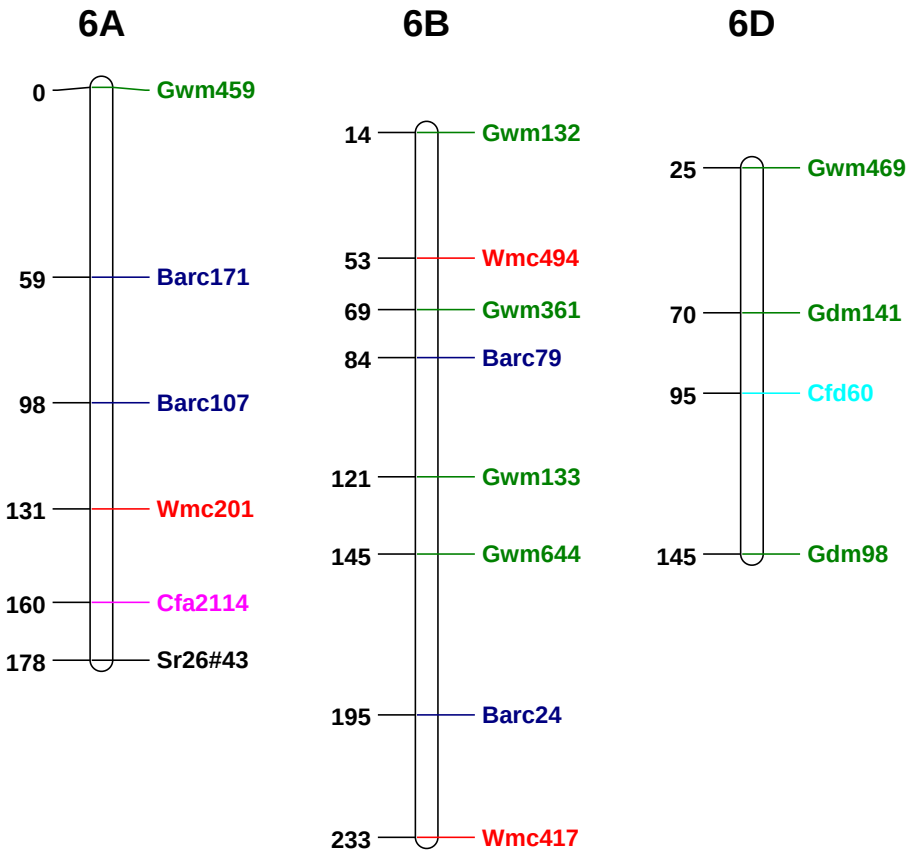

Figure 5.5 **A partial linkage map of wheat chromosomes 6A, 6B and 6D constructed using 44 BC_2F_1 individuals and data of 120 polymorphic SSR marker loci. All marker loci were colour coded as follows: Barc (dark blue), Cfa (pink), Cfd (light blue), Gdm and Gwm (green) and Wmc (red) representing the different research groups which developed the SSRs and other markers (black). Mapped marker positions are indicated in cM to the left of the chromosome while marker names and order are indicated to the right**

5.3.2.7 Chromosome group 7

A total of 17 polymorphic markers mapped on the three chromosomes 7A (eight markers), 7B (three markers) and 7D (six markers) with genetic distances of 219 cM, 57 cM and 183 cM covered respectively (Figure 5.6). The average genetic distance between adjacent markers was 31.3 cM, 28.5 cM and 36.6 cM for chromosomes 7A, 7B and 7D respectively. The starting positions of chromosome 7A, 7B and 7D were adjusted according to the SSR consensus map as follows: marker Gwm635 at 8 cM for chromosome 7A, marker Gwm400 at 41 cM for chromosome 7B and marker Cfd51 for chromosome 7D respectively.

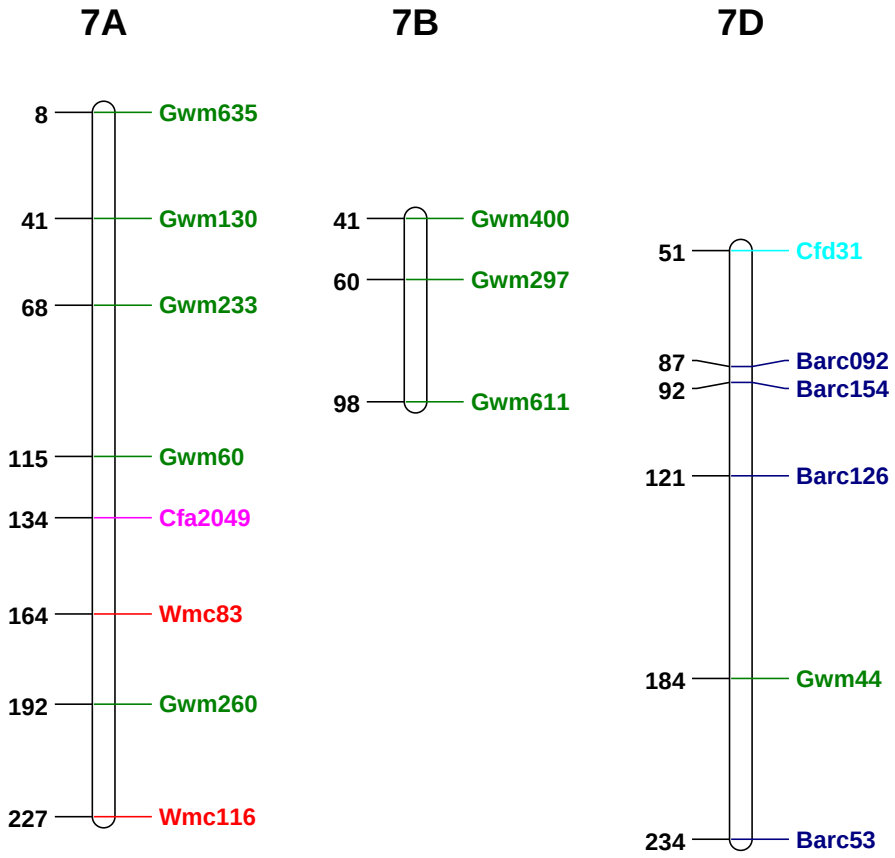

Figure 5.6 **A partial linkage map of wheat chromosomes 7A, 7B and 7D constructed using 44 BC_2F_1 individuals and data of 120 polymorphic SSR marker loci. All marker loci were colour coded as follows: Barc (dark blue), Cfa (pink), Cfd (light blue), Gwm (green) and Wmc (red) representing the different research groups which developed the SSRs. Mapped marker positions are indicated in cM to the left of the chromosome while marker names and order are indicated to the right**

5.3.3 Recovered recurrent parent percentage analysis

The linkage map data was used to order and determine optimal marker positions based on the genotypic data of the polymorphic mapped markers on each of the 44 BC_2F_1 individuals. From the linkage map marker order, GGT analysis was used to visualise each genotype and monitor the inheritance (A - Krokodil, B - CM-82036 and H - heterozygous) of each marker per BC_2F_1 individual. The GGT profiles for targeted chromosomes 3B and 5A containing the *Fhb1* gene and the *Qfhs.ifa-5A* QTL respectively for each of the 44 BC_2F_1 individuals are displayed in Figures 5.7 and 5.8 respectively.

GGT profiles for some of the non-targeted (1A, 1B, 1D, 6A, 6B and 6D) chromosomes are given in Appendix E. A high number of heterozygous segments (blue bars) were present in the 44 selected lines after foreground marker selection across the two targeted chromosomes, 3B and 5A. Visualisation using GGT profiles assisted with grouping of selected lines into genotype groups/classes (*Fhb1* on its own; the *Qfhs.ifa-5A* QTL present on its own and the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL). With the aid of GGT the predicted RPGP values per BC_2F_1 individual were calculated. The HRGP ranged from 37.1-77.1% and the HDGP from 22.9-62.3%. The total RPGP for the entire genome recovered per BC_2F_1 individual selected based on the presence of the *Fhb1* gene and/or *Qfhs.ifa-5A* QTL ranged between 68.3-88.6%. Lines were grouped in decreasing order (the *Fhb1* gene in combination with *Qfhs.ifa-5A* QTL; then the *Fhb1* gene on its own; then the *Qfhs.ifa-5A QTL* on its own) of FHB resistance genes/QTL importance based on the phenotypic results described in Chapter 4. Within each group, lines were further ordered based on decreasing RPGP. A line was given a ranking based on the importance of the FHB resistance gene/QTL combination present as well as overall RPGP (Table 5.2). Seventeen of the 44 lines designated as group 1 contained the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL region with RPGP ranging from 82.9-72.7%. Twelve lines (group 2) had the major resistance gene *Fhb1* in combination with only a portion of the *Qfhs.ifa-5A* QTL due to a recombination event in the *Qfhs.ifa-5A* QTL region, with RPGP values ranging between 86.5-71.1%. The best five lines of group 2 [S1 (3), S47 (4), S87 (3), S63 (3) and S124 (3)] had higher RPGP values compared to any line in group 1. The two lines in group 3 consisted of BC_2F_1 lines that showed recombination between some markers for the *Fhb1* gene but still contained the entire *Qfhs.ifa-5A* QTL region. Nine lines of group 4 contained only the major *Fhb1* gene and two lines of group 5 contained only the *Qfhs.ifa-5A* QTL. Two lines of group 6 tested positive for a portion of *Fhb1* gene region but with recombination between some markers with RPGP values of 88.6% and 81.4%.

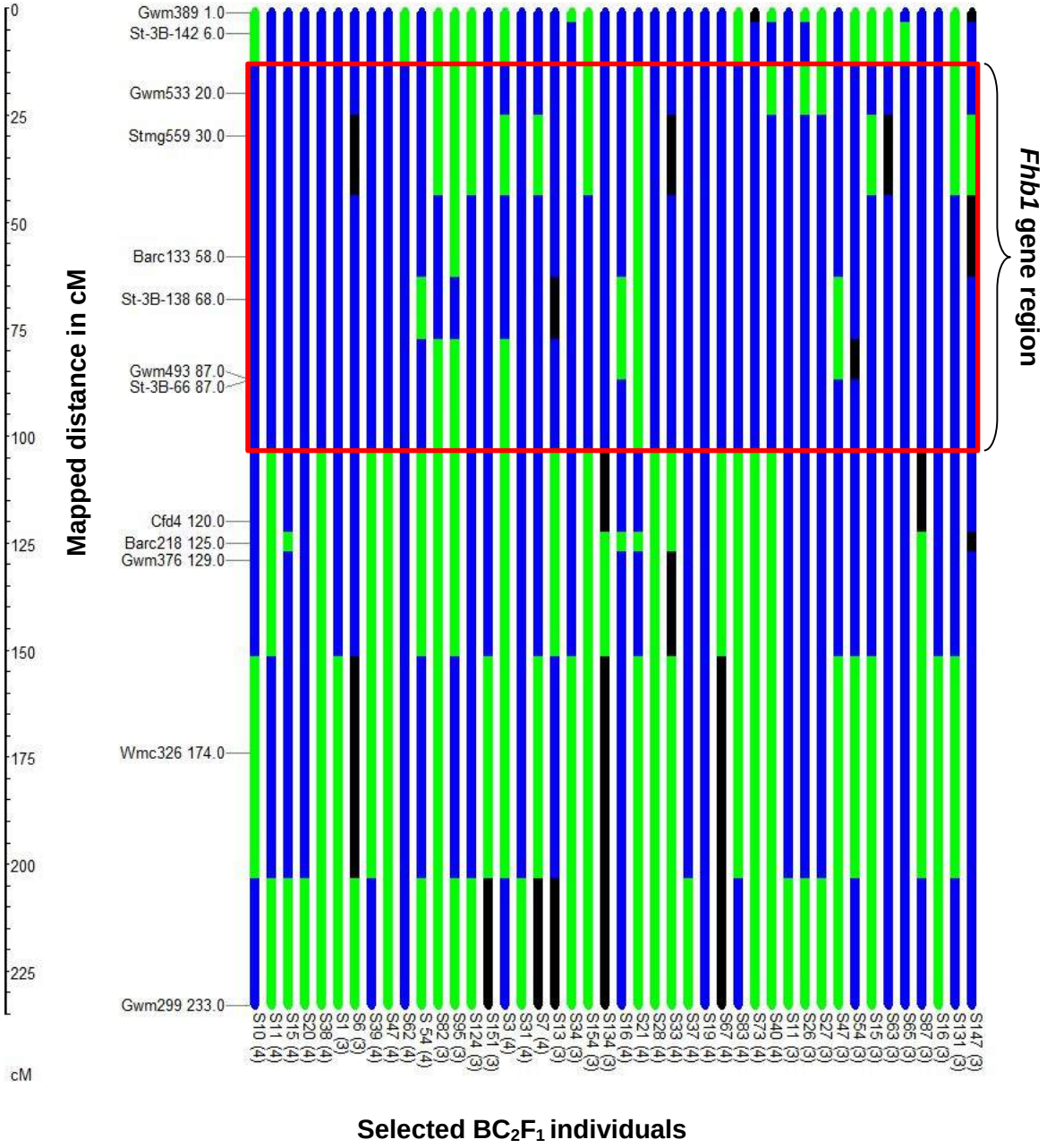

Figure 5.7 **GGT profiles of each of the 44 selected BC_2F_1 individuals for targeted chromosome 3B containing the *Fhb1* gene. Green bars represent homozygous genome sections of Krokodil, blue bars heterozygous genome segments and black bars segments with missing marker data. The red blocked area indicates the targeted *Fhb1* gene region. Mapped marker positions and marker names and order are indicated on the left, along with a cM scale**

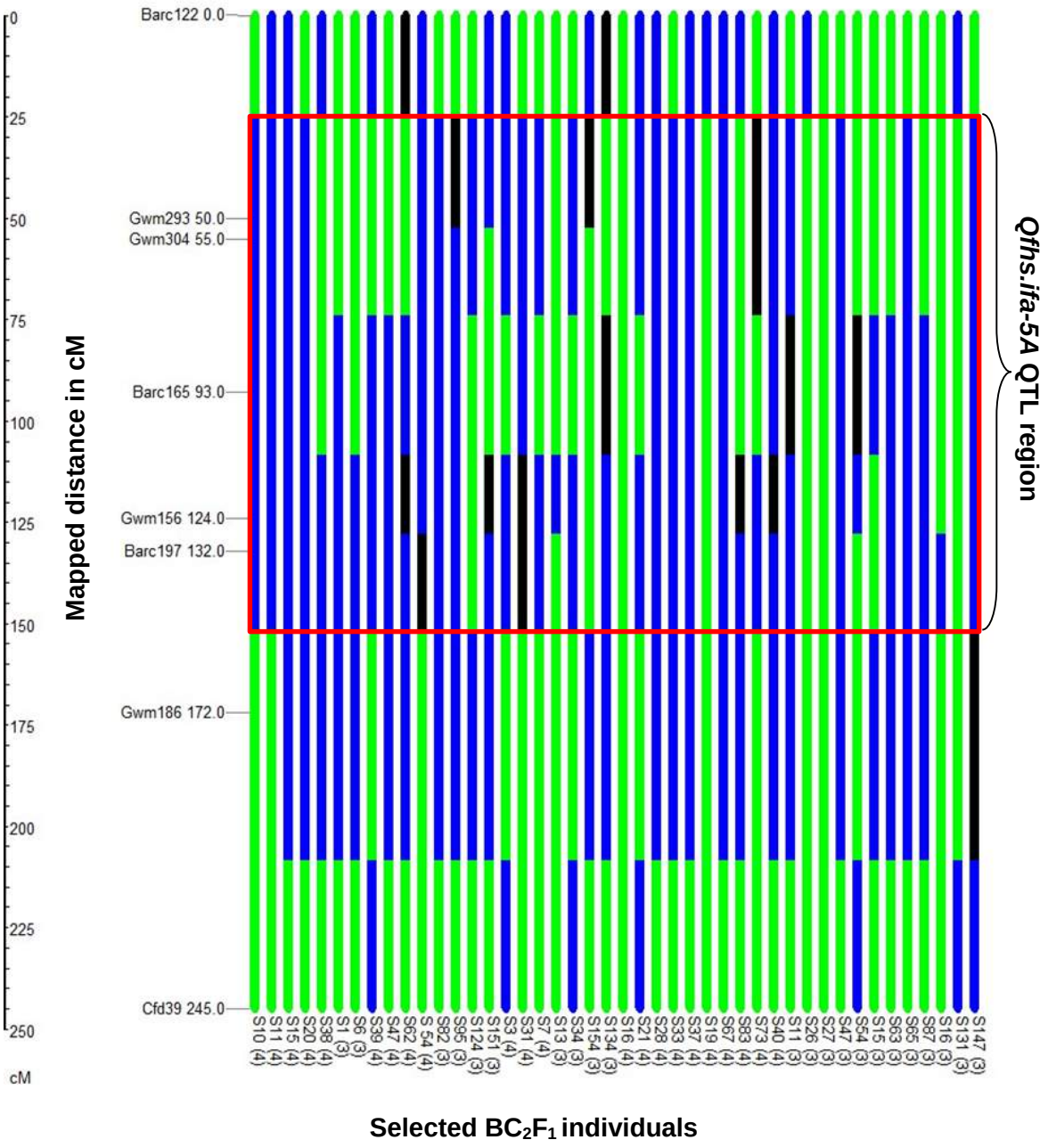

Figure 5.8 **GGT profiles of each of the 44 selected BC_2F_1 individuals for targeted chromosome 5A containing the *Qfhs.ifa-5A* QTL. Green bars represent homozygous genome sections of Krokodil, blue bars heterozygous genome segments and black bars segments with missing marker data. The red blocked area indicates the targeted *Qfhs.ifa-5A* QTL region. Mapped marker positions and marker names and order are indicated on the left, along with a cM scale**

Table 5.2 Line ranking, sample name, homozygous recurrent (Krokodil) genome percentage (HRGP), heterozygous donor genome percentage (HDGP), calculated recurrent parent genome percentage (RPGP = HRGP + ½ HDGP) and FHB resistance gene/QTL genotype of 44 selected BC_2F_1 individuals

Line ranking	Sample	HRGP (%)	HDGP (%)	RPGP (%)	Genotype
	Group 1				
1	S65 (3)	65.7	34.3	82.9	*Fhb1 + Qfhs.ifa-5A*
2	S7 (4)	65.0	35.0	82.5	*Fhb1 + Qfhs.ifa-5A*
3	S34 (3)	64.1	35.9	82.1	*Fhb1 + Qfhs.ifa-5A*
4	S151 (3)	62.8	37.2	81.4	*Fhb1 + Qfhs.ifa-5A*
5	S11 (3)	61.8	38.2	80.9	*Fhb1 + Qfhs.ifa-5A*
6	S20 (4)	58.7	41.3	79.4	*Fhb1 + Qfhs.ifa-5A*
7	S31 (4)	58.5	41.5	79.3	*Fhb1 + Qfhs.ifa-5A*
8	S40 (4)	58.2	41.8	79.1	*Fhb1 + Qfhs.ifa-5A*
9	S33 (4)	55.9	44.1	78.0	*Fhb1 + Qfhs.ifa-5A*
10	S67 (4)	53.2	46.8	76.6	*Fhb1 + Qfhs.ifa-5A*
11	S28 (4)	52.6	47.3	76.3	*Fhb1 + Qfhs.ifa-5A*
12	S37 (4)	52.2	47.8	76.1	*Fhb1 + Qfhs.ifa-5A*
13	S15 (4)	51.7	48.3	75.9	*Fhb1 + Qfhs.ifa-5A*
14	S11 (4)	50.9	49.1	75.5	*Fhb1 + Qfhs.ifa-5A*
15	S54 (4)	50.7	49.3	75.4	*Fhb1 + Qfhs.ifa-5A*
16	S10 (4)	49.2	50.8	74.6	*Fhb1 + Qfhs.ifa-5A*
17	S147 (3)	45.4	54.6	72.7	*Fhb1 + Qfhs.ifa-5A*
	Group 2				
18	S1 (3)	72.9	27.1	86.5	*Fhb1 + ½ Qfhs.ifa-5A*
19	S47 (4)	70.1	29.9	85.1	*Fhb1 + ½ Qfhs.ifa-5A*
20	S87 (3)	68.6	31.4	84.3	*Fhb1 + ½ Qfhs.ifa-5A*
21	S63 (3)	66.7	33.3	83.4	*Fhb1 + ½ Qfhs.ifa-5A*
22	S124 (3)	64.9	35.1	82.5	*Fhb1 + ½ Qfhs.ifa-5A*
23	S134 (3)	62.5	37.5	81.3	*Fhb1 + ½ Qfhs.ifa-5A*
24	S73 (4)	61.3	38.7	80.7	*Fhb1 + ½ Qfhs.ifa-5A*
25	S39 (4)	58.6	41.4	79.3	*Fhb1 + ½ Qfhs.ifa-5A*
26	S3 (4)	58.4	41.6	79.2	*Fhb1 + ½ Qfhs.ifa-5A*
27	S38 (4)	56.4	43.6	78.2	*Fhb1 + ½ Qfhs.ifa-5A*
28	S6 (3)	55.0	45.0	77.5	*Fhb1 + ½ Qfhs.ifa-5A*
29	S62 (4)	42.1	57.9	71.1	*Fhb1 + ½ Qfhs.ifa-5A*
	Group 3				
30	S47 (3)	62.5	37.5	81.3	*½ Fhb1 + Qfhs.ifa-5A*
31	S82 (3)	61.5	38.5	80.8	*½ Fhb1 + Qfhs.ifa-5A*
	Group 4				
32	S13 (3)	70.1	29.9	85.1	*Fhb1*
33	S27 (3)	63.0	37.0	81.5	*Fhb1*
34	S26 (3)	62.2	37.8	81.1	*Fhb1*
35	S154 (3)	61.7	38.3	80.9	*Fhb1*
36	S16 (3)	60.3	39.7	80.2	*Fhb1*
37	S15 (3)	59.0	41.0	79.5	*Fhb1*
38	S19 (4)	57.6	42.4	78.8	*Fhb1*
39	S54 (3)	53.3	46.7	76.7	*Fhb1*
40	S83 (4)	37.1	62.3	68.3	*Fhb1*
	Group 5				
41	S21 (4)	73.1	26.9	86.6	*Qfhs.ifa-5A*
42	S95 (3)	61.4	38.6	80.7	*Qfhs.ifa-5A*
	Group 6				
43	S131 (3)	77.1	22.9	88.6	*½ Fhb1*
44	S16 (4)	62.8	37.2	81.4	*½ Fhb1*

The best five ranked BC_2F_1 individuals of group 1 [S65 (3), S7 (4), S34 (3), S151 (3) and S11 (3)] tested positive for both the *Fhb1* gene and *Qfhs.ifa-5A* QTL with higher than 80.0% RPGP.

The average RPGP in a theoretical BC_2F_1 population without any linkage drag is expected to be 87.25% (blue line, Figure 5.9). The average RPGP for this population of 44 BC_2F_1 individuals was 79.7% (red line, Figure 5.9). The RPGP average was 7.55% below the expected 87.25%. However, due to the segregating nature of the BC_2F_1 genotypes which contained at least one major FHB resistance gene/QTL, varying amounts of linkage drag were expected. All selected BC_2F_1 lines were below the expected RGPG of 87.25% with the exception of selection 43 [sample S147 (3)] of group 6, with a RPGP of 88.6%.

5.3.4 Recommended line selections for further development

Line selections from Table 5.2 can be further narrowed down with a threshold RPGP value of 80% set across groups. Selections were done and ranked based on the presence of the *Fhb1* gene on chromosome 3B in combination with the *Qfhs.ifa-5A* QTL, with a RPGP threshold of 80%. Additionally favourable GGT profiles of specific quality related chromosomes (1A, 1B, 1D, 6A, 6B and 6D) were considered (Appendix E). The following lines were selected from the different genotype groups with RPGP equal or above 80%: group 1: S65 (3), S7 (4), S34 (3), S151 (3) and S11 (3); group 2: S1 (3), S47 (4), S87 (3), S63 (3), S124 (3), S134 (3) and S73 (4); group 3: S47 (3) and S82 (3). Due to the relatively large phenotypic response percentage conferred by the two single genes/QTL (*Fhb1* and *Qfhs.ifa-5A*) studied when present, especially by the *Fhb1* gene, lines from groups 4, 5 and 6 with high RPGP should not be over looked for further development. Lines S13 (3), S21 (4) and S131 (3) with the highest RPGP values from groups 4, 5 and 6 respectively, as well as being in the top five lines of all lines screened based on RPGP values, should possibly be considered for further crossing or inter-line crossing to reduce the amount of foreign donor genome retained in the developed or improved material. Quality related chromosome groups 1 (1A, 1B and 1D) and 6 (6A, 6B, 6D) which are essential for good baking quality were additionally targeted for evaluation based on the GGT profiles (Appendix E) of each of these chromosomes per selected individual. The GGT profiles of these six chromosomes were compared to recurrent parent Krokodil for each of the selected individuals (Table 5.3).

Figure 5.9 **A graphical representation of the predicted recurrent parent genome percentages (RPGP) of each of the 44 selected BC_2F_1 individuals. The blue line indicates the expected theoretical BC_2 RPGP of 87.25% without any linkage drag while the red line indicates the average observed RPGP of 79.7%. Lines were placed in ranking order from left to right based on the ranking given within each genotype group (group 1; group 2; group 3; group 4; group 5 and group 6)**

Table 5.3 The sixteen elite FHB resistance BC_2F_1 line selections ranked by genotype group and recurrent parent genome percentage (RPGP). GGT profiles of chromosome groups 1 and 6 that are important for protein composition were compared against recurrent parent Krokodil

Selections	Chromosome						RPGP	Genotype
	1A	1B	1D	6A	6B	6D	(%)	group
S65 (3)	✓	×	×	×	*✓	✓	82.9	1
S7 (4)	*✓	×	✓	×	×	×	82.5	1
S34 (3)	✓	✓	×	*✓	×	×	82.1	1
S151 (3)	✓	×	×	✓	*✓	*✓	81.4	1
S11 (3)	*✓	×	*✓	×	✓	✓	80.9	1
S1 (3)	✓	×	✓	*✓	*✓	*✓	86.5	2
S47 (4)	✓	×	✓	×	✓	*✓	85.1	2
S87 (3)	×	✓	✓	×	×	✓	84.3	2
S63 (3)	✓	×	×	✓	*✓	*✓	83.4	2
S124 (3)	✓	✓	×	×	✓	✓	82.5	2
S134 (3)	✓	✓	×	✓	✓	✓	81.3	2
S73 (4)	✓	✓	✓	×	×	×	80.7	2
S47 (3)	✓	×	×	×	*✓	✓	81.3	3
S13 (3)	*✓	*✓	×	×	×	✓	85.1	4
S21 (4)	×	×	*✓	✓	×	*✓	86.6	5
S131 (3)	×	✓	✓	*✓	*✓	✓	88.6	6

✓=80% or more similar to Krokodil; *✓=100% similar to Krokodil; ×=less than 80% similar to Krokodil

Based on the data presented in Table 5.3, variation was observed between the sixteen selected lines. The retention of important quality traits such HMW-GS, LWM-GS, gliadin proteins and grain yield may be significantly affected by recombination events on chromosomes 1A, 1B, 1D, 6A, 6B and 6D. Selections where haplotypes were not 80% similar to Krokodil on at least four of the targeted quality chromosomes were considered not suitable. The following selections were identified to potentially have poorer quality traits and need to be analysed further in future before exclusion: S65 (3), S7 (4), S34 (3), S87 (3), S73 (4), S47 (3), S13 (3) and S21 (4).

The following eight lines may potentially be better for quality traits and thus recommended for future development after further quality analysis, yield assessments and phenotypic screening; S151 (3) (group 1, 81.4% RPGP), S11 (3) (group 1, 80.9% RPGP), S1 (3) (group 2, 86.5% RPGP), S47 (4) (group 2, 85.1% RPGP), S63 (3) (group 2, 83.4% RPGP), S124 (3) (group 2, 82.5% RPGP), S134 (3) (group 2, 81.3% RPGP) and S131 (3) (group 6, 88.6% RPGP). The majority of these selected lines came from genotype group 2. It is thus essential that these lines should undergo further quality and phenotypic validations to monitor the FHB resistance levels of these genotypes because recombination was present in the *Qfhs.ifa-5A* QTL region. However, the focus should be on the two selected lines, S151 (3) and S11 (3) of group 1, with high levels of Krokodil on important quality related chromosomes, specifically for baking quality and yield determination to fast track the development of these elite lines.

5.4 Discussion

5.4.1 Marker-assisted selection

Marker-assisted foreground selection for two targeted FHB resistance genes/QTL, *Fhb1* and *Qfhs.ifa-5A* was successfully applied in the current study. A total of 44 lines from 238 BC_2F_1 (developed from three and four FHB resistance gene/QTL containing BC_1F_1 mother plants) individuals were selected using marker-assisted foreground selection. This approach ensured that a larger number of high value BC_2F_1 lines containing the targeted FHB resistance alleles from the donor parent CM-82036 could be selected that would not have been possible using conventional breeding. With this holistic approach, improved genetic gain and stricter selection criteria was applied to the selected individuals. The identification of a set of high value lines such as these containing different combinations of the targeted FHB resistance genes/QTL allowed for further evaluation and selection to be done. This set of 44 lines were narrowed down further after extensive application of background MAS; firstly based on RPGP in the background of each line and secondly the potential retention of other important traits needed for good bread baking and end-use quality in mind.

5.4.2 Partial linkage map construction

A number of polymorphic markers identified on silver stained PAGE during parental screening earlier in the study (Chapter 3) that were difficult to score during background screening of the segregating BC_2F_1 lines, gave difficulties when scoring even with the more sensitive capabilities of the Gel Scan 3000. This was mainly attributed to the masking effect (due to small differences in allele sizes) of certain alleles in the heterozygous state present in the segregating BC_2F_1 individuals which made scoring

more complex than anticipated. Co-dominant marker loci which were not easily distinguishable as either heterozygous or homozygous for the marker alleles were not included during the mapping analysis. Most of the marker positions and orders compared auspiciously with the SSR wheat consensus map (Somers et al. 2004).

Construction of the partial linkage map was negatively influenced by the small population size and limited number of polymorphic markers. However, the mapping population size was determined based on results obtained from foreground selection for the targeted FHB resistance gene/QTL. This resulted in a significantly limited number of recombination events being detected. The small population size and relative small number of markers tested, most likely played a significant role in some of the larger genetic distances observed between some adjacent markers on certain chromosomes (Frisch and Melchinger 2000). This can be seen when the partial linkage maps of the targeted chromosomes of the BC_1F_1 population (120 individuals; Chapter 3) were compared with the partial linkage maps of the BC_2F_1 population (44 selected BC_2F_1 individuals) from this chapter. Targeted chromosome maps for the larger BC_1F_1 mapping population displayed smaller genetic distances between markers compared to maps of the much smaller BC_2F_1 mapping population. The relatively small BC_2F_1 population size and lack of polymorphic markers negatively influenced the accurate statistical prediction of recombination rates and events between adjacent markers (Hospital 2005; 2009). Computer simulations indicated that populations can remain small and even constant, but marker density and marker data points needed to be high in order to reduce the genetic distances between markers (Frisch et al. 1999; Collard and Mackill 2008). When MABC was first considered it was recognised that an average marker distance of 10-20 cM would be required for successful recovery of the recurrent parent's genome (Visscher et al. 1996). The question arises as to whether these principles should still be applied today, 15-20 years later, with the advent and availability of high density linkage maps and high throughput marker systems such as DArTs and SNPs. The BC_2F_1 linkage map in the current study displayed an average genetic distance of 25 cM between markers. A number of marker linkage clusters were observed on certain chromosomes which signified the presence of different gene rich regions.

Narrowing down of the BC_2F_1 population during foreground selection to contain individuals with the targeted FHB resistance genes/QTL could have skewed the general population background towards a smaller RPGP since large levels of linkage drag are known to be associated with targeted resistance traits such as FHB resistance genes/QTL. However, it is expected that when the number of targeted FHB resistance

genes/QTL present in an individual is increased the potential recovery of RPGP could be hampered significantly. Additionally the small population size would not have allowed for the maximum number of recombination events to occur to further increase the level of RPGP recovered. However, the differences between Krokodil and CM-82036 for other non-targeted traits that could not be accounted for, may have played a significant role in segregation distortion.

Mapping results on the BC_2F_1 population furthermore confirmed previous reports that the number of polymorphic markers mapped usually decreases from the A to B to D genomes. A number of comparisons have been made between different wheat linkage and deletion maps after which it was concluded that wheat chromosomes can be subdivided into regions differing in relative gene density to the point of gene-rich and gene-poor regions (Akhunov et al. 2003; Erayman et al. 2004; Sidhu and Gill 2004). Recombination in wheat genomes occurs preferentially in distal chromosome regions and recombination events are not uniform along the length of a chromosome (Akhunov et al. 2003; Kumar et al. 2012), resulting in inaccurate estimates of genetic to physical map distances (Kumar et al. 2012). In addition a positive gradient of gene density from the chromosome centromere to the telomeres has been suggested (Akhunov et al. 2003; Rustenholz et al. 2011). In fact, recombination hot spots are known to be interspersed with recombination cold spots (Kumar et al. 2012). In addition these gene rich regions (gene islands) are groups of co-expressed genes that share similar functions (Rustenholz et al. 2011). It has been reported that 90% of the crossover events that occur on chromosome 3B (one of the targeted chromosomes of this study) take place at the distal-telomeric chromosome region and this represents just 40% of the entire 3B chromosome (Kumar et al. 2012). Therefore certain genomes and chromosomes will naturally have lower recombination rates and it will be more difficult to map markers on them as a result.

Larger chromosome segments were observed for the targeted chromosomes 3B (containing the *Fhb1* gene) and 5A (containing the *Qfhs.ifa-5A* QTL), particularly when compared to non-targeted chromosomes. This phenomenon of linkage drag for targeted traits especially from exotic donors has been well documented in wheat and other crops. However, chromosome segments retained on these two chromosomes (visually displayed by the GGT profiles) were, as expected (Hospital 2001; 2005; Collard and Mackill 2008), significantly smaller than in the BC_1F_1 generation.

5.4.3 Targeted chromosomes

The targeted FHB resistance gene *Fhb1* and the *Qfhs.ifa-5A* QTL have been well documented in many mapping studies and in different backgrounds to confer varying levels of resistance to the observed phenotypic variation for FHB disease incidence, disease severity and DON mycotoxin content (Anderson et al. 2001; Buerstmayr et al. 2002; 2003; Lemmens et al. 2005). In 2006, it was reported that jointly these two QTL can reduce DON content by 78% and FHB disease rating by 55% (Miedaner et al. 2006). However, in general it is accepted that these two QTL will jointly provide on average 20-45% reduction in FHB disease depending on the genetic background in which they are deployed (Buerstmayr et al. 2009; Miedaner et al. 2011). This was confirmed in the current study as described in the phenotypic validation trial in Chapter 4. Both these FHB resistance genes/QTL do have limited, but significant effects on other agronomic characteristics such as stem and spike length, plant height and delayed heading (Suzuki et al. 2012). In the current study measurements regarding plant height, stem and spike length and heading dates were not taken for the segregating BC_2F_1 material, so no conclusions could be made regarding the influence that these targeted FHB resistance genes/QTL introductions may have had on these traits. However, it was observed that plants containing FHB resistance appeared significantly taller than the original Krokodil cultivar. However, this observed segregation in plant height was to be expected since the original FHB donor parent CM-82036 was much taller than the recurrent parent Krokodil. In a number of different backgrounds these same targeted chromosomes (3B and 5A) have also been associated with yield QTL and other baking quality traits. These traits may be negatively affected by the introgression of FHB resistance genes/QTL into these same chromosome regions (Kato et al. 2000; Carter et al. 2011; Bennett et al. 2012).

5.4.4 Retained recurrent parent genome analysis

The retained recurrent parent genome analysis revealed a population RPGP average of 79.7% that was significantly lower than the expected 87.25%. The lower percentage can be attributed to high levels of linkage drag around targeted donor segments and the small population size studied. Significantly, 16 elite lines containing different FHB resistance gene/QTL combinations with more than 80% RPGP were already identified in the BC_2F_1 generation out of the 44 lines initially selected. Based on this RPGP value, 63.6% of the 44 lines initially selected were not selected for further quality related chromosome analysis. S131 (3) with a RPGP of 88.6% was identified as the best line regarding the genome percentage of recurrent parent Krokodil present. This was significantly lower than the best line (97%) identified by Randhawa et al. (2009). However, Randhawa et al. (2009) targeted only one trait and worked with much larger

populations. In the current study at least two FHB resistance genes/QTL were targeted. It is however possible that these selected lines with high Krokodil RPGP values could still have inferior yield and baking quality when compared to other lines if unwanted recombination had occurred on certain important quality related chromosomes as discussed above.

5.4.5 Quality and yield related chromosomes

Since wheat is produced with the end-user in mind, baking quality and yield should also be considered when advanced lines with FHB resistance and high RPGP values are selected. Visual analysis of the recombination events present on chromosomes 1A, 1B and 1D (Payne and Lawrence 1983) as well as chromosomes 6A, 6B and 6D (Gao et al. 2007; Heidari et al. 2011) was included when the best BC_2F_1 lines from the study were selected. If recombination on these chromosomes can be kept to a minimum, lines with potentially better quality and yield can be identified for later assessment and further cultivar development. Krokodil was originally selected as a good recurrent parent based on its market accepted bread making quality and competitive yields. Therefore, the retention of as much of Krokodil's genome on chromosome groups 1 and 6 may have a significant additive effect on selecting better performing FHB resistant lines. The next point of selection of these elite lines will be the determination of the HMW-GS subunits that are present and should give a good indication of the potential baking quality of these lines before further line development (Goutam et al. 2013). It is vital to remember however, that the interactions of multiple genes/QTL with one another and the environmental conditions in the field, especially during grain filling, play a significant role in determining the grain protein composition which will affect end-use quality and potentially the yield obtained (Mengistu et al. 2012).

5.4.6 The selection path followed towards elite FHB resistant line recommendations

It is valuable to understand and recap the selection path these eight elite BC_2F_1 lines, now harvested as BC_2F_2 material, have followed to be ready for further assessment, development or use as crossing parents. The process was started with developing a BC_1F_1 generation of 120 individuals from 173 true F_1 hybrids. These individuals were genotyped across four targeted chromosomes (3B, 5A, 6B and 7A) thought to be containing different FHB resistance genes/QTL at the time of screening. These lines, based on foreground marker selection, were grouped based on the presence of either three or four FHB resistance genes/QTL. Background markers were screened and RPGP values were determined across the four targeted chromosomes (3B, 5A, 6B and

7A) on these individuals although RPGP values were not used as a selection criterion at this point in time since the main objective of this study was firstly to introgress the best possible FHB resistance gene/QTL combinations. These BC_1F_1 families containing either three or four FHB resistance genes/QTL when crossed back to Krokodil, yielded 238 BC_2F_1 individuals which needed to be screened firstly for the presence of FHB resistance genes/QTL. Subsequently it was determined in a phenotypic trial on additional BC_1F_1 individuals of the population that the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL was the optimal FHB resistance gene/QTL combination. This gene/QTL combination conferred stable and durable resistance levels accounting for on average 45-50% reduction in observed FHB disease symptoms at 21 dpi in the background of irrigated spring wheat cultivar Krokodil. These 238 BC_2F_1 individuals were narrowed down to 44 individuals that contained the two targeted FHB resistance genes/QTL (*Fhb1* and *Qfhs.ifa-5A* QTL) in different combinations. This meant that only 18.5% of the 238 BC_2F_1 individuals were selected for further background screening. Marker-assisted background screening was carried out on these 44 lines across all 21 chromosomes of the wheat genome. These lines were grouped according to the importance of the FHB resistance gene/QTL combination present in that line and ordered using RPGP. Based on an 80% RPGP threshold value 16 high value BC_2F_1 lines were selected, implying that only 36.4% of the 44 lines selected for background screening passed this additional phase of selection. After haplotype comparison of these 16 high value BC_2F_1 lines across targeted baking quality related chromosomes 1A, 1B, 1D, 6A, 6B and 6D, two sets of lines consisting of eight lines each, were identified. One set potentially would have inferior baking quality and/or yields due to unwanted recombination occurring on the targeted quality related chromosomes and the other set was identified as possible better performers. Therefore, eight elite lines [S151 (3), S11 (3), S1 (3), 47 (4), S63 (3), S124 (3), S134 (3) and S131 (3)] containing various combinations of the targeted FHB resistance genes/QTL (*Fhb1* and *Qfhs.ifa-5A* QTL) as well as a RPGP value higher than 80% and favourable levels of recurrent parent Krokodil genome present on key quality related chromosomes were selected. Thus only 18.2% of 44 BC_2F_1 lines were selected or only 3.4% of the original 238 BC_2F_1 lines screened. If the two best elite lines, S151 (3) and S11 (3), positive for the *Fhb1* gene and *Qfhs.ifa-5A* QTL are given first priority and recommended for further development then only 0.84% (2 out of 238 BC_2F_1 individuals/lines) of the screened lines were selected. This is a significant achievement and validates the power of MAS. It is highly unlikely that such strict and accurate selection could be applied during development of similar lines using conventional and phenotypic selection only. The successful identification of this set of elite lines in a relatively short development time with high levels if genetic gain being made would not

have been possible without the different phases of MAS. Conventional breeding and phenotypic screening methods, may have taken a minimum of 5-7 years to identify some promising lines. The true value, genetic gain and selection power of MAS and MABC was revealed in this study.

5.5 Conclusions and recommendations

The main aim of this study was to develop and select backcross lines with improved FHB resistance levels in the background of a SA cultivar, which was successfully achieved. Additionally, these high potential BC_2F_1 FHB resistant lines were developed by the aid of four successive phases of applied MAS over a three year period. Based on what is publicly available, this is one of the first public documentation of the successful application of MAS to develop FHB resistant irrigated spring wheat lines in SA.

Furthermore, it was concluded that the RPGP value should only be used as a selection guide for improved line selection and not on its own. Recombination events around important quality and yield related chromosome regions as seen in this study could significantly affect the performance of selected lines even with high RPGP values. However, there exists a small possibility that some lines with lower RPGP values that were not selected, could possibly have less recombination on the targeted chromosomes for quality related traits, but this is unlikely. The application of MAS is aimed at complementing conventional breeding approaches and phenotypic screening to make the process more efficient (Goutam et al. 2013). It is recommended when selecting advanced lines that the RPGP values need to be used in combination with other measurable quality tests that require only small amounts of seed, to make selections in early generations which would allow even better potential for genetic gain. It will be of great interest to compare the quality related profiles of the 16 high value BC_2F_1 line selections (eight poorer quality versus eight elite better quality line selections) in future. This will give needed insight and may gain a better understanding into the merits of this form of recurrent parent genome selection on targeted quality related chromosomes.

The advantages displayed by applying MABC to select simultaneously for multiple targeted FHB resistance traits while attempting to retain a high level of the RPGP in this study can also be attributed to the relative stable nature of the FHB resistance expression of the *Fhb1* gene and the *Qfhs.ifa-5A* QTL. It is recommended that only resistance genes/QTL or traits that confer 10-20% of the observed phenotypic variation should be targeted to successfully apply MAS. The stability of the trait being transferred ultimately is the key factor in determining whether a MAS or MABC programme will be

successful (Hospital 2009). However, the application of MABC using manual marker systems as in this case, raises the question as to whether it will truly be practical for application in large commercial breeding programmes. This study formed part of a pre-breeding programme which is of a much smaller scale compared to a full commercial breeding programme. This made the use of manual marker systems such as silver stained PAGE and the Gel Scan 3000 in this case a practical choice. Modern technologies have moved on to fully automated marker systems and technologies that has become practically more accepted and used in commercial breeding programmes (Helguera et al. 2003; Ashikari et al. 2005; Nocente et al. 2007; Goutam et al. 2013). More recently rapid developments in NGS technologies has opened the possibilities of genotype by sequence selections. This has been successfully applied in recent years to different wheat populations and is proving to be a valuable tool, especially for identifying SNP markers closely linked to traits of interest that can be used by commercial breeding companies that require even more precise selection (Varshney et al. 2014).

Development of double haploids (DH) of these elite lines should also be considered to fast track the homozygosity level and potential use as crossing parents. However, DH development can be labour intensive, has a relative low success rate and is time consuming with additional costs and is more often applied in early generations of development. It is recommended that at least the eight elite BC_2F_1 lines which were allowed to self to form BC_2F_2 lines, should be planted out once again in a glasshouse as families and evaluated for plant height, flowering time, growth period and the presence of quality traits detected using MAS linked to protein alleles. It is recommended that the eight elite line selections should be multiplied (seed increased) followed by evaluations in small protected field trial strips/yield plots to further confirm FHB resistance gene/QTL marker presence (homozygous or heterozygous status of the targeted FHB resistance genes/QTL), phenotypic validation and extensive quality tests (bread making characteristics, hectolitre mass, falling number, yield, etc.) which requires larger amounts of seed. This will facilitate that more informed decisions regarding the potential field performance of these selections can be done. Comparison of quality profiles together with FHB resistance expression should lead to recommendation of a set of improved lines with variation in plant height, straw strength, yield and growth period that can be used in cultivar development.

Once enough seed has been generated per family in subsequent generations, these lines should be released by the UFS to the different SA wheat industry role players to be exploited as crossing parents or to be fast tracked into their various breeding

programmes as a potential future commercial FHB resistant spring wheat cultivar. Once these elite FHB resistant spring wheat lines are advanced and validated a potential international germplasm registration may be possible, making these selected lines eventually available to the international wheat research community.

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Chapter 6

General conclusions and future perspectives

Risks of climate change/shifts, extreme weather phenomena, crop yield gaps and the potential thresholds in crop yields reached a reality and these areas of concern will all have a significant negative impact on global food security. There thus exists a growing need for global and national commitment towards investment in crop improvement. Addressing crop production limitations under climate change, breeding crops with better water use efficacy and durable resistance to diseases and pests will assist the drive for better yields to ensure a better food secure future. However, research and development progress will remain slow unless extensive national and international multi-disciplinary collaborations are initiated and maintained to tackle needed crop research areas.

In regards to FHB, such an initiative was undertaken by the USA with the SCAB initiative which has been in place since 1998. This initiative coordinates national research and funding across multiple national role players and disciplines to tackle FHB control. An annual forum is hosted to report on important research findings and project progress. A similar FHB workshop or forum is needed in SA to coordinate research activities by all role players to make faster progress and to prevent duplication of work.

6.1 South African focus

In SA, during the 2013/2014 season the smallest area was planted to wheat in recent history. This was primarily due to the increased risk of common fungal diseases and pests on wheat, high input costs in relation to expected income from average yields of around 2.2 t/ha and the low wheat price which is making dryland winter wheat production in particular less profitable. As a result there has been a steady increase in the area planted to irrigated spring wheat in SA over the last decade. This is due to the more favourable profit margins that can be achieved under irrigation by producers, with higher and more stable yields of 8-10 t/ha. However, FHB disease on commercial irrigated spring wheat still remains an important sporadic threat in SA.

In SA there exists a unique diversity and a set of complex interactions between member species of the FGSC, which makes breeding and development of FHB resistant material a vital pillar for the development of an optimal management strategy for this disease. An

immediate and future concern is the adoption of FHB resistant cultivars by producers. Unless resistant cultivars can compete with yields of current market leading susceptible cultivars, it will remain a difficult task to motivate producers to make the switch. Farmers continually weigh up the advantages and disadvantages of the risk taken when planting a susceptible cultivar with a potential higher yield. A higher yielding FHB susceptible cultivar can still produce enough lower grade seed to cover the offset in potential income compared to planting a lower yielding FHB resistant cultivar with a higher grade seed. However, in years/seasons that SA has an extensive FHB epidemic, like recently experienced in the USA and China, farmers could lose almost everything. Currently, since the FHB disease only occurs sporadically and in isolated cases it is not of the utmost priority for irrigated wheat producers in SA.

6.2 General conclusions

This study successfully transferred the FHB resistance gene *Fhb1* (Type II resistance) and the *Qfhs.ifa-5A* QTL (Type I resistance) from the FHB resistant donor line CM-82036 into the background of the SA irrigated spring wheat cultivar Krokodil. Krokodil was selected as a well-adapted irrigated cultivar that in the past was used across multiple production areas, with a competitive yield and good bread making qualities. However, the downfall of Krokodil like most SA irrigation cultivars is the high level of FHB susceptibility that is risky. This is the first publicly documented improvement of FHB resistance levels for SA in a spring wheat cultivar assisted by MAS. The improved FHB resistance levels of these high value backcross Krokodil lines were phenotypically validated in a small glasshouse trial with both spray and point inoculation methods. The FHB resistance gene *Fhb1* and the *Qfhs.ifa-5A* QTL conferred observed phenotypic variation ranges of 34.5-40.5% and 12.5-27.1% respectively. These observed phenotypic variation ranges are in line with other international publications which targeted the transfer of these FHB resistance genes/QTL. However, the expression of resistance to FHB was not at the same levels as that of FHB resistant check Sumai 3 or FHB resistant donor parent CM-82036, which was expected, but was at least a 45-50% improvement in FHB resistance levels compared to the original cultivar Krokodil. It is thus imperative to only target well characterised genes/QTL for a MAS programme. These genes/QTL should confer a minimum of 15-20% of the observed phenotypic response which should be stable across environments leading to significant genetic gain.

The MAS phases used for the duration of the study supported the selection of the best FHB resistant genotypes already in the BC_1F_1 population while simultaneously accelerating potential retention of the Krokodil background across the four targeted

chromosomes. This process assisted in limiting the potential linkage drag around the targeted genes/QTL successfully.

It is important to remember that genotypic data and the interpretation thereof is only truly useful when accompanied by good reliable phenotypic data. Application of two different inoculation or screening methods for FHB disease resistance employed in this study was vital to accurately phenotype resistance levels of tested germplasm. This would prevent the inaccurate identification of a degree of tolerance and/or resistance that some lines may naturally express. During the phenotypic validation a clear overlapping window was observed around 7-10 dpi when Type I resistance was still activated and Type II resistance was initiated. Genotypes that contained both Type I and Type II resistance conferred more stable FHB resistance expression. This study confirmed previous reports that the *Fhb1* gene confers predominantly Type II FHB resistance and is responsible for the best levels of observed phenotypic variation for FHB disease resistance. The *Qfhs.ifa-5A* QTL confers Type I resistance, but less of the phenotypic variation compared to the *Fhb1* gene. Based on preliminary seed analysis data, the *Fhb1* gene had a significant impact on reducing the number of tombstone or FDK kernels observed from infected heads. Such an observation for the *Fhb1* gene was reported a number of years ago. The *Qfhs.ifa-5A* QTL did not appear to influence or reduce the number of observed FDK during seed analysis. During this study no DON concentration levels present in infected grain were determined as this did not fall in the scope of this study. However, it is suggested that this aspect should be investigated in future as these two targeted FHB resistance genes/QTL may play a significant role in the prevention of DON accumulation or even DON degradation. Importantly, the *Fhb1* gene in combination with *Qfhs.ifa-5A* QTL acted additively to reduce the potential yield loss as a result of FHB infection. Therefore the optimal FHB resistance gene/QTL combination identified in this study was the *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL, with an additive and more stable resistance response.

The foreground selection of FHB resistance genes/QTL in combination with whole genome background selection was invaluable in identifying the 16 high value BC_2F_1 lines containing the targeted FHB resistance genes/QTL and above 80% RPGP. However, it was concluded that background selection and high RPGP values cannot solely be used to ensure that lines with good bread making qualities are selected. Recombination present on the six targeted quality related chromosomes (1A, 1B, 1D, 6A, 6B and 6D), important for HMW-GS, LMW-GS and glutin composition should also be taken into consideration. It is recommended that in future studies simultaneous MAS for targeted

FHB resistance genes/QTL together with certain important quality traits should rather be done in early backcross generations to truly accelerate the potential genetic gain. However, larger backcross populations will be needed to ensure selection of individuals with all desired traits. This simultaneous foreground selection of many different targeted traits (FHB resistance genes/QTL and quality traits) in early generations may be more beneficial in combination with MABC. This might in the long run be a more practical and cost effective use of MAS in large commercial breeding programmes. Modern trends are moving towards new marker technologies that are making use of NGS applications for SNP marker discovery associated with multiple traits of interest to be applied in high-thoughtput marker systems and even the application of genotype by sequence selection to allow for whole genome based selection to rapidly improve cultivar development.

The sixteen selected high value BC_2F_1 lines identified in this study with different combinations of the *Fhb1* gene and *Qfhs.ifa-5A* QTL, were finally narrowed down to eight elite BC_2F_1 lines which were recommended for further development. Lines developed in this study was achieved with in a short three year period and could be the needed catalyst for the continual improvement of FHB resistance levels in SA irrigated spring wheat cultivars. Each line will differ slightly in potential yield, flowering time, growth period, height and straw strength, giving breeders some needed variation when making line or plant selections for their specific breeding programme. Lines identified from this work will have to undergo a series of seed increases, quality traits have to be evaluated as suggested and FHB resistance levels need to be validated extensively in field trials. Once this is done successfully these lines can be made publicly available to be used as potential crossing parents by the different breeding companies in SA, and possibly other parts of the world for the further development of FHB resistant cultivars.

6.3 South African Fusarium head blight control implications

Now with improved FHB resistance levels identified in lines developed in this study a focus needs to be placed on a complete FHB control package for SA. From what has been extensively reported, the best method for FHB control is an integrated control strategy that makes use of host plant resistance as its backbone in combination with improved cultural practices, fungicide use and/or bio-control. No fungicide has yet been officially registered for the control of FHB on wheat in SA. The moment a fungicide is registered, awareness will need to be raised around the correct, timeous and responsible application of fungicides, especially after the reported identification of the first fungicide resistant *Fusarium* isolate in the state of New York in 2014. In the coming years upon registration of fungicides in SA, it will be worth conducting *in vitro* fungicide trials to

evaluate any potential resistance/tolerance levels SA *Fusarium* isolates might have towards these fungicides.

The advantages and disadvantages of no-till or limited tillage practices need to be assessed and researched in terms of potential increase in FHB disease incidence in SA. Perhaps better recommendations in regards to tillage practices need to be made specifically to irrigation wheat farmers. Better crop rotation is often suggested as a method for the improved control of FHB disease. However, in SA certain production areas are limited to specific crops, due to unsuitability of specific climatic conditions, varying amounts of rainfall and soil types. The management of crop rotation as a FHB disease control strategy is not always possible for certain wheat farmers. Producers must not overlook the value in terms of soil nutrition and needed cash turnover which a wheat crop offers a SA crop farmer even when wheat production is challenging.

Bio-control agents to control FHB disease on wheat is an area that can and should also be explored in SA in future. In the last year or two there have been reports from different countries on the successful identification of some bio-control agents for FHB control. Perhaps bio-control should be used in combination with the planting of resistant cultivars as supportive method to offer a better and more environmentally friendly control package for the future.

Regulation of mycotoxin levels in contaminated grain, especially DON content in SA grain needs to be monitored more strictly. Guidelines and regulations need to be in place and enforced to protect consumers from unnecessary exposure to mycotoxin contaminated wheat and other grains. This has become an area of great concern across the world with recent reports of the association of *Fusarium* species with human related infections.

6.4 Food for thought

A number of focus areas need the attention of the general SA *Fusarium* research community. It is a well-documented fact that spores of *Fusarium* species can travel long distances by wind and general air turbulence. Recently, it was suggested by Minnaar-Ontong that the river and water systems of SA that supply water directly to irrigated wheat farms may play an important role as a primary source of *Fusarium* inoculum. The river systems and water sources that supply the irrigated wheat production areas in SA are diverse and cover large distances that run through many different provinces. An investigation into the natural water sources needs to be conducted looking at aspects

such as the period of time, if at all, that *F. graminearum* spores will remain viable in different natural water sources. This could answer some valuable questions before progressing further. In the last five years a number of reports have identified colonies of certain *Fusarium* species present in the old water pipes of hospitals in Europe. These colonies were diverse and in high enough concentrations to be linked to illnesses of patients. It is thus a possibility that *Fusarium* species which cause FHB disease on wheat could colonise irrigation systems and general water supply piping.

Additionally, *F. graminearum* spores could be artificially carried from one infected location to another by adhering to general farm implements such as contract harvesters which travel around the country as the wheat ripens. If these aspects and questions could be verified then better management practices of FHB disease can be used in conjunction with planting of stable FHB resistant varieties in future.

As previously mentioned, there are limited FHB resistant cultivars currently commercially available in SA or the private development of such has not been publicly documented as yet. The need for the targeted development of FHB resistant germplasm by a pre-breeding wheat programme to be used by all parties of the SA wheat industry was identified. However, to enable commercial release the following factors need to be considered in more detail in future: Pest and diseases for specific wheat production areas need to be considered by disease resistance breeding programmes, enabling the development of “tailor made” multi-pest and disease resistant wheat varieties for a specific production area. These resistant cultivars used in combination with better crop rotation will ensure the durability and versatility of the cultivar and lengthen its potential commercial market life span. On top of these considerations, special attention needs to go into developing a resistant cultivar that has market competitive yields and meets the strict bread baking quality standards of the SA wheat industry. The combination of all these traits into one cultivar package in the shortest time possible is a complex and difficult task, which normally takes up to 10 years to develop. Currently the demand for the use of molecular tools, such as MAS or MABC to reduce this development time of improved wheat cultivars from SA breeding companies is on the raise. In the years to come it is believed that the true benefits and impact of MAS applications, such as these achieved in the development of these FHB resistant lines of this study, will hopefully be seen in the more regular release of improved SA wheat cultivars.

Summary

In our modern era with the global population soon to reach the nine billion mark, there are rising concerns about food security. There is a driven need for more sustainable food production. Wheat is considered one of the most important cereal crops grown worldwide. However, wheat production in SA has been declining steadily over the last decade. A shift in SA wheat production has occurred from winter dryland to irrigated spring wheat being planted in a maize-wheat crop rotation system. Wheat production is vulnerable globally and locally due to yield losses and grain damage caused by sporadic FHB disease outbreaks, which are predominantly caused by *F. graminearum.* Currently the number of FHB resistant wheat cultivars available in SA is limited and not adequate. The aim of this study was to develop FHB resistant lines through backcross breeding and MAS to track the introgression of targeted FHB resistance genes/QTL (*Fhb1, Fhb2, Qfhs.ifa-5A* QTL and *7A* QTL) into the SA wheat cultivar Krokodil. The use of a MABC pre-breeding programme to transfer targeted FHB resistance genes/QTL from FHB resistant donor CM-82036 into the background of SA spring wheat cultivar Krokodil is discussed. F_1 individuals from which a BC_1F_1 generation was developed were validated using SSR marker Gwm493, which is linked to the *Fhb1* gene. Marker-assisted foreground selection was done for targeted FHB resistance genes/QTL combined with background selection against unwanted donor alleles. A partial linkage map of targeted chromosomes 3B, 5A, 6B and 7A was generated from a BC_1F_1 population consisting of 120 individuals. Based on this linkage map GGT profiles were developed for each individual which allowed analysis and comparison of recombination events on these chromosomes. Families containing three and four targeted FHB resistance genes/QTL were identified. These lines were backcrossed to Krokodil to yield a BC_2F_1 population of 238 individuals. Simultaneously, a phenotypic validation glasshouse trial was done on additional individuals of the BC_1F_1 population to confirm expression of the targeted FHB resistance genes/QTL. Spray and point inoculation methods were used to evaluate Type I and Type II FHB resistance responses on six selected controls and 130 segregating BC_1F_1 genotypes. These BC_1F_1 individuals were genotyped using markers associated with targeted FHB resistance genes/QTL. FHB disease symptoms were expressed as a percentage infection per spike and assessed 4, 7, 10, 14, 18 and 21 dpi. BC_1F_1 genotypes containing the targeted *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL contributed additively to overall FHB resistance and the prevention of expected yield loss. Preliminary results indicated that the *Fhb1* gene reduced the presence of

tombstone kernels. The *Fhb1* gene in combination with the *Qfhs.ifa-5A* QTL were successfully transferred using MAS and validated phenotypically in the background of Krokodil displaying improved FHB resistance levels. Since the primary objective was improvement of FHB resistance, this study successfully identified 44 BC_2F_1 individuals that contained the FHB resistance gene *Fhb1* and/or the *Qfhs.ifa-5A* QTL. A partial linkage map across all 21 wheat chromosomes was generated by testing 120 polymorphic SSR markers on these 44 individuals. With the aid of GGT profiles the RPGP analysis was done for each BC_2F_1 individual. Sixteen high value lines that contained *Fhb1* and/or the *Qfhs.ifa-5A* QTL with a minimum RPGP value of 80% were identified and selected for further analysis. Eight elite lines that contained the targeted FHB resistance genes/QTL as well as the potential for good baking quality were selected for further development. Lines identified and recommended from this study can be used to initiate the further development of FHB resistant wheat cultivars in SA.

Keywords: CM-82036, *Fhb1*, *Fusarium graminearum,* marker-assisted selection (MAS), *Qfhs.ifa-5A* QTL, recurrent parent genome percentage (RPGP), resistance breeding, simple sequence repeats (SSR), two generation backcrossing programme, wheat scab

Opsomming

Daar is ‘n groeiende besorgdheid oor voedselsekuriteit in ons moderne era waar die wêreldpopulasie binnekort die nege miljard merk gaan bereik. Daar is ‘n dringende behoefte aan verbeterde volhoubare voedselproduksie. Koring word as een van die belangrikste graangewasse wat wêreldwyd verbou word, beskou. Koringproduksie in Suid-Afrika (SA) het egter gedurende die laaste dekade geleidelik begin afneem. SA koringproduksie het vanaf droëland winterverbouing na somerkoring onder besproeiing gekoppel aan ‘n mielie-koring gewasrotasie-sisteem verander. Koringproduksie is wêreldwyd asook plaaslik kwesbaar weens opbrengsverliese en skade aan graan wat deur sporadiese Fusarium aarskroei (FHB) uitbrake, hoofsaaklik weens *Fusarium graminearum,* veroorsaak word. Die aantal FHB weerstandbiedende kultivars wat tans in SA beskikbaar is, is beperk en nie voldoende nie. Die doel van die studie was om FHB weerstandbiedende lyne te ontwikkel deur van terugkruisteling en merker-ondersteunde seleksie (MAS) om die inbou van die geteikende FHB weerstandsgene/kwantitatiewe eienskapslokusse (QTL; *Fhb1, Fhb2, Qfhs.ifa-5A* QTL en 7A QTL) in die SA koringkultivar Krokodil te volg, gebruik te maak. Die gebruik van ‘n merker-ondersteunde terugkruisteling vooraf-teelprogram om die geteikende FHB weerstandsgene/QTL vanaf die FHB weerstandsbron CM-82036 na die agtergrond van die SA koringkultivar Krokodil oor te dra, word bespreek. F_1 individue wat gebruik is om die BC_1F_1 generasie te ontwikkel se identiteit is bevestig deur die mikrosatellietmerker Gwm493, gekoppel aan die *Fhb1* geen, te gebruik. Merker-ondersteunde voorgrond-seleksie vir die geteikende FHB weerstandsgene/QTL gekombineer met agtergrond-seleksie teen ongewensde skenker allele is gedoen. ‘n Gedeeltelike koppelingskaart van die geteikende 3B, 5A, 6B en 7A chromosome is opgestel deur van 120 individue van die BC_1F_1 populasie gebruik te maak. Gebaseer op hierdie koppelingskaart is GGT profiele vir elke individu ontwikkel wat die analise en vergelyking van rekombinasie gebeurtenisse op hierdie chromosome moontlik gemaak het. Families wat drie en vier geteikende FHB weerstandsgene/QTL bevat het, is geïdentifiseer. Hierdie lyne is na Krokodil teruggekruis om ‘n BC_2F_1 populasie bestaande uit 238 individue te ontwikkel. Gelyktydig hiermee is ‘n fenotipiese glashuisproef op addisionele individue van die BC_1F_1 populasie gedoen om die uitdrukking van die geteikende FHB weerstandsgene/QTL te bevestig. Spuit- en punt-inokulasie metodes is gebruik om Tipe I en Tipe II FHB weerstandsreaksies op ses geselekteerde kontroles en 130 segregerende BC_1F_1 genotipes te evalueer. Hierdie BC_1F_1 individue is met merkers wat aan die geteikende FHB weerstandsgene/QTL

gekoppel is, gegenotipeer. FHB siekte simptome is as ‘n persentasie infeksie per aar uitgedruk en 4, 7, 10, 14, 18 en 21 dae na inokulasie geëvalueer. BC_1F_1 genotipes wat die geteikende *Fhb1* geen in kombinasie met die *Qfhs.ifa-5A* QTL bevat het, het additief tot die algehele FHB weerstand en in die voorkoming van die verwagte opbrengsverlies, bygedra. Voorlopige resultate het aangetoon dat die *Fhb1* geen die teenwoordigheid van *Fusarium*-beskadigde pitte verminder het. Die *Fhb1* geen in kombinasie met die *Qfhs.ifa-5A* QTL is suksesvol met behulp van MAS oorgedra en fenotipies in die Krokodil-agtergrond wat verbeterde FHB weerstandsvlakke vertoon het, bevestig. Aangesien die hoofdoel die verbetering van FHB weerstand was, was hierdie studie suksesvol in die identifikasie van 44 BC_2F_1 individue wat die FHB weerstandsgeen *Fhb1* en/of die *Qfhs.ifa-5A* QTL bevat het. ‘n Gedeeltelike koppelingskaart van al 21 koring chromosome is opgestel deur 120 polimorfiese mikrosatelliet merkers op hierdie 44 individue te toets. Die herhalende ouer se genoom persentasie analise is met behulp van GGT vir elke BC_2F_1 individu gedoen. Sestien hoë gehalte lyne wat *Fhb1* en/of *Qfhs.ifa-5A* QTL tesame met ‘n minimum herhalende ouer genoom persentasie van 80% bevat het, is geïdentifiseer en geselekteer vir verdere analises. Agt lyne wat die geteikende FHB weerstandsgene/QTL asook die potensiaal vir goeie bakkwaliteit bevat het, is vir verdere ontwikkeling geselekteer. Geïdentifiseerde en aanbevole lyne van hierdie studie kan vir die verdere ontwikkeling van FHB weerstandbiedende koringkultivars in SA gebruik word.

Sleutelwoorde: Aarskroei, CM-82036, *Fhb1*, *Fusarium graminearum*, herhalende ouer genoom persentasie, merker-ondersteunde seleksie (MAS), mikrosatelliete (SSR), *Qfhs.ifa-5A* QTL, twee-generasie terugkruisingsprogram, weerstandsteling

Appendix A All background molecular markers used during this study listed by number, marker names, polymorphic status of each marker and the chromosome(s) on which each marker is located

No.	Marker name	Polymorphic status	Chromosome(s) located on
1	Barc092	Yes	7D
3	Barc101	No	2B
4	Barc105	No	7D
5	Barc107	No	6A
6	Barc110	Yes	5D
7	Barc119	Yes	1A; 1B; 1D
8	Barc122	Yes	5A
9	Barc123	No	6D
10	Barc126	Yes	7D
11	Barc130	Yes	5D
12	Barc138	Yes	6B
13	Barc148	No	1A
14	Barc153	Yes	4A
15	Barc154	Yes	7A; 7D
16	Barc159	Yes	2D
17	Barc169	No	1D
18	Barc170	No	4A
19	Barc171	Yes	6A
20	Barc177	Yes	5D
21	Barc179	No	3A
22	Barc183	Yes	6D
23	Barc184	No	4A
24	Barc193	Yes	4B
25	Barc196	No	6D
26	Barc202	No	6D
27	Barc205	No	5D
28	Barc206	No	4A
29	Barc21	Yes	6D
30	Barc218	Yes	3B
31	Barc225	No	4D
32	Barc235	Yes	7D
33	Barc24	Yes	6B
34	Barc258	No	7B
35	Barc26	Yes	7D
36	Barc263	Yes	1A
37	Barc270	No	3D
38	Barc278	No	7B
39	Barc279	Yes	2A
40	Barc286	No	5D
41	Barc297	Yes	2D
42	Barc3	No	6A
43	Barc302	Yes	1B
44	Barc314	No	3A

No.	Marker name	Polymorphic status	Chromosome(s) located on
45	Barc315	Yes	4A
46	Barc344	No	3B
47	Barc349	No	2B
48	Barc354	Yes	6B
49	Barc40	No	5A
50	Barc44	No	5D
51	Barc45	Yes	3A
52	Barc48.2	No	6B
53	Barc5	No	2A
54	Barc52	Yes	4A
55	Barc53	No	7D
56	Barc57	Yes	3A
57	Barc59	No	5B
58	Barc65	Yes	7B
59	Barc69	No	5B
60	Barc7	No	2B; 3B
61	Barc76	No	7D
62	Barc78	Yes	4A
63	Barc79	Yes	6B
64	Barc84	Yes	3B
65	Barc98	Yes	4D
66	Barc99	No	1D
67	Cfa2028	Yes	7A
68	Cfa2049	Yes	7A
69	Cfa2086	Yes	2A
70	Cfa2104	No	5A
71	Cfa2114	Yes	6A
72	Cfa2129	Yes	1A; 1B; 1D
73	Cfa2134	Yes	3A; 3B
74	Cfa2141	Yes	5A
75	Cfa2147	No	1A
76	Cfa2174a	Yes	7A; 3B; 7D
77	Cfa2193	Yes	3A
78	Cfa2219	Yes	1A
79	Cfd12	Yes	5D
80	Cfd13	Yes	6B; 6D
81	Cfd15	No	1A; 1B
82	Cfd161	Yes	2D
83	Cfd17	No	2D
84	Cfd19	Yes	1D; 5D; 6D
85	Cfd193	Yes	4D
86	Cfd20	Yes	1B
87	Cfd211	Yes	3D
88	Cfd219	No	3D; 5B
89	Cfd238	No	2B
90	Cfd25	Yes	2B; 5A; 7D
91	Cfd257	Yes	4A
92	Cfd27	Yes	1D

No.	Marker name	Polymorphic status	Chromosome(s) located on
93	Cfd283a	No	4B
94	Cfd287	Yes	6D
95	Cfd2d	Yes	3D
96	Cfd30	No	6A
97	Cfd31	Yes	7D
98	Cfd32	Yes	1D
99	Cfd33	No	6D
100	Cfd35	Yes	3D
101	Cfd37	Yes	6D
102	Cfd38	Yes	6D
103	Cfd39b	Yes	4D; 5A
104	Cfd4	Yes	3B
105	Cfd41	Yes	7D
106	Cfd44	Yes	2D
107	Cfd5	No	6D
108	Cfd55	Yes	3D
109	Cfd56	Yes	2D
110	Cfd58	Yes	1D
111	Cfd60	No	6D
112	Cfd63	Yes	1D
113	Cfd64	No	3D
114	Cfd70	No	3D
115	Cfd73	Yes	2B; 2D
116	Cfd84	Yes	4D
117	Gdm141	Yes	6D
118	Gwm106	Yes	1A;1B;1D
119	Gwm107.3	No	3A
120	Gwm108	No	3B
121	Gwm114b	Yes	3D
122	Gwm121	No	5D; 7D
123	Gwm126	Yes	3B
124	Gwm132	Yes	6B; 6D
125	Gwm136	No	1A
126	Gwm146	Yes	7B
127	Gwm155	Yes	3A
128	Gwm157	Yes	2D
129	Gwm160	Yes	4A
130	Gwm164	Yes	1A
131	Gwm18	Yes	1B
132	Gwm181	Yes	3B
133	Gwm186	Yes	5A
134	Gwm194	No	4D
135	Gwm2	Yes	3A
136	Gwm218	Yes	3A
137	Gwm234	Yes	5B
138	Gwm24	No	1B; 2D
139	Gwm251	Yes	4B; 4D
140	Gwm257	Yes	1A

No.	Marker name	Polymorphic status	Chromosome(s) located on
141	Gwm259	Yes	1B
142	Gwm260	Yes	7A
143	Gwm261	No	2D
144	Gwm264b	No	4D
145	Gwm268	Yes	1B
146	Gwm271a	No	5B
147	Gwm273	Yes	1B
148	Gwm276	Yes	7A
149	Gwm284	No	3B
150	Gwm291	Yes	5A
151	Gwm292	No	5D
152	Gwm297	Yes	7B
153	Gwm299	Yes	3B
154	Gwm302	No	7B
155	Gwm314	Yes	3D
156	Gwm319	Yes	2B
157	Gwm32	No	3A
158	Gwm328	No	2A
159	Gwm33	No	1A; 1B; 1D
160	Gwm332	No	7A
161	Gwm337	Yes	1B
162	Gwm359	No	2A
163	Gwm361	No	6B
164	Gwm368	No	4B
165	Gwm371	Yes	5B
166	Gwm372b	No	2A
167	Gwm376	Yes	3B
168	Gwm391	No	3A
169	Gwm400	Yes	7B
170	Gwm44	Yes	7D
171	Gwm448	Yes	2A
172	Gwm459	Yes	6A
173	Gwm471	Yes	7A
174	Gwm484	Yes	2D
175	Gwm4b	No	4A
176	Gwm508	No	6B
177	Gwm512	No	2A
178	Gwm515	No	2D
179	Gwm538	Yes	4B; 4D
180	Gwm539	No	2D
181	Gwm550	Yes	1B
182	Gwm554	No	5B
183	Gwm55b	Yes	2B; 6D
184	Gwm604	Yes	1B; 5B
185	Gwm60a	Yes	7A
186	Gwm610	No	4A
187	Gwm611	Yes	7B
188	Gwm614	Yes	2B

No.	Marker name	Polymorphic status	Chromosome(s) located on
189	Gwm617	Yes	5A; 6A
190	Gwm639	Yes	5B
191	Gwm642	Yes	1D
192	Gwm674	No	3A
193	Sr26#43	No	6A
194	Stm559	Yes	3B
195	Wmc050	No	3A
196	Wmc11	Yes	3A
197	Wmc116	Yes	7A
198	Wmc121	No	7D
199	Wmc125	No	4B
200	Wmc143	No	7A
201	Wmc145	No	6A
202	Wmc147	No	1D; 3A
203	Wmc149	Yes	2B; 2D; 5B
204	Wmc153	No	3A
205	Wmc154	Yes	2B
206	Wmc158	No	7A
207	Wmc161b	Yes	5D
208	Wmc163	No	6A
209	Wmc17	Yes	7A
210	Wmc173d	No	4A
211	Wmc179.3	Yes	4A
212	Wmc190	Yes	2D
213	Wmc201	Yes	6A
214	Wmc206	Yes	3B
215	Wmc237	No	1A
216	Wmc243	Yes	2B; 2D
217	Wmc254	No	1A; 4B
218	Wmc258	Yes	4A
219	Wmc261e	No	7B
220	Wmc262	Yes	4A
221	Wmc27	No	5B
222	Wmc285	No	4D
223	Wmc286.4	No	7A
224	Wmc312	No	1A
225	Wmc317	Yes	2B
226	Wmc326	No	3B
227	Wmc35	Yes	2B
228	Wmc360	No	2B
229	Wmc361	Yes	2B
230	Wmc363	Yes	5B
231	Wmc387	Yes	6B
232	Wmc388	Yes	7A
233	Wmc397	Yes	6B
234	Wmc399	Yes	4D
235	Wmc415	Yes	5A; 5B
236	Wmc417b	Yes	6B

No.	Marker name	Polymorphic status	Chromosome(s) located on
237	Wmc418	Yes	3B; 3D
238	Wmc42	No	7D
239	Wmc422	No	2B; 7A
240	Wmc426b	No	7A
241	Wmc431	No	2D
242	Wmc486	Yes	6B
243	Wmc494	Yes	6B
244	Wmc500a	No	4A
245	Wmc52	No	1B; 4D
246	Wmc540	Yes	3B; 7B
247	Wmc553	Yes	6A
248	Wmc617	Yes	4A; 4B; 4D
249	Wmc69	Yes	2A; 3B
250	Wmc70	No	7B
251	Wmc74b	Yes	4D
252	Wmc75	Yes	5B
253	Wmc83a	Yes	7A
255	Wmc95	Yes	1A; 5D; 6B
256	Wmc96e	No	5A

Appendix B The exact theoretical chromosome fragment sizes of donor (CM-82036) parent remaining in each of the 120 BC_1F_1 individuals for the targeted chromosomes 3B, 5A, 6B and 7A

Individual	3B donor chromosome segments (cM)	5A donor chromosome segments (cM)	6B donor chromosome segments (cM)	7A donor chromosome segments (cM)
1	1 = 59.90	1 = 44.7	0	1 = 138.35; 2 = 151.35
2	1 = 24.8; 2 = 61.9	1 = 51.25	0	1 = 82.8; 2 = 100.6
3	1 = 24.8; 2 = 20.95; 3 = 40.95	1 = 1.65	1 = 29.40	1 = 308.2
4	1 = 183.7	0	1 = 71.35	1 = 60.2; 2 = 117.05
5	1 = 183.7	1 = 18.35	1 = 89.30	1 = 330.8
6	1 = 142.75	0	1 = 89.30	1 = 141.7
7	1 = 183.7	1 = 24.9; 2 = 18.35	1 = 71.35	1 = 50.75
8	1 = 35.1; 2 = 102.85	1 = 69.60	1 = 89.30	1 = 280.05
9	1 = 35.1; 2 = 40.95	1 = 18.35	0	1 = 60.2; 2 = 75.40; 3 = 50.75
10	1 = 24.8; 2 = 82.85	1 = 18.35	1 = 73.45	1 = 138.35; 2 = 34.3
11	1 = 24.8; 2 = 40.95	1 = 14.8; 2 = 26.35	1 = 71.35	1 = 22.6; 2 = 55.55; 3 = 50.75
12	1 = 20.95; 2 = 40.95	1 = 44.7	0	1 = 22.6; 2 = 55.55
13	1 = 24.8; 2 = 20.95; 3 = 40.95	1 = 69.60	0	1 = 197.25
14	1 = 24.8; 2 = 82.85	1 = 8.0	1 = 17.95	1 = 22.6; 2 = 130.95
15	1 = 24.8	1 = 18.35	0	0

Individual	3B donor chromosome segments (cM)	5A donor chromosome segments (cM)	6B donor chromosome segments (cM)	7A donor chromosome segments (cM)
16	1 = 59.9	1 = 44.70	1 = 89.30	1 = 22.6; 2 = 55.55; 3 = 151.35
17	1 = 80.85	1 = 69.60	1 = 89.30	1 = 82.8; 2 = 50.75
18	1 = 117.95	0	0	1 = 22.6; 2 = 130.95
19	1 = 24.8; 2 = 123.8	1 = 8.0	0	1 = 330.8
20	1 = 59.90; 2 = 61.9	1 = 69.60	1 = 15.85	1 = 55.55; 2 = 50.75
21	1 = 82.85	1 = 6.8; 2 = 44.7	1 = 17.95; 2 = 15.85	1 = 82.8; 2 = 34.3
22	0	1 = 18.35	0	1 = 22.6; 2 = 50.75
23	1 = 24.8; 2 = 40.95	1 = 51.25	1 = 89.3	1 = 82.8; 2 = 34.30
24	1 = 24.8; 2 = 61.9	1 = 18.35	1 = 17.95	1 = 115.75; 2 = 50.75
25	1 = 20.95; 2 = 40.95	1 = 18.35	1 = 29.4	1 = 213.75
26	1 = 82.85	1 = 18.35	1 = 89.3	1 = 257.45
27	1 = 61.9	0	1 = 29.4	1 = 179.45
28	1 = 24.8; 2 = 20.95	1 = 18.35	0	1 = 141.7
29	1 = 183.7	0	1 = 89.3	1 = 96.65; 2 = 66.3
30	1 = 59.9; 2 = 102.85	1 = 69.60	1 = 10.85	1 = 141.7
31	1 = 183.7	0	1 =89.3	1 = 191.15
32	1 = 142.75	1 = 44.7	1 = 73.45	1 = 60.2; 2 = 66.3
33	1 = 61.9	1 = 51.25	1 = 17.95; 2 = 15.85	1 = 156.85

Individual	3B donor chromosome segments (cM)	5A donor chromosome segments (cM)	6B donor chromosome segments (cM)	7A donor chromosome segments (cM)
34	1 = 102.85	0	1 = 89.3	1 = 82.8; 2 = 75.4
35	1 = 35.1	0	0	1 = 82.8; 2 = 117.05
36	1 = 20.95; 2 = 40.95	1 = 51.25	0	1 = 213.75; 2 = 50.75
37	1 = 102.85	1 = 69.60	0	1 = 82.8; 2 = 66.3
38	1 = 59.90; 2 = 102.85	1 = 8.0; 2 = 18.35	1 = 89.30	1 = 197.25
39	1 = 142.75	1 = 18.35	1 = 29.4	1 = 117.05
40	1 = 142.75	1 = 26.35	1 = 89.30	1 = 60.2
41	1 = 35.1; 2 = 40.95	1 = 14.80; 2 = 44.7	1 = 89.30	1 = 330.80
42	1 = 142.75	1 = 18.35	1 = 44.05; 2 = 15.85	1 = 22.60
43	1 = 126.35	1 = 69.60	1 = 26.10; 2 = 34.4	1 = 82.80; 2 = 141.7
44	1 = 35.1	1 = 51.25	1 = 89.30	1 = 82.80; 2 = 141.7
45	1 = 24.8; 2 = 82.85	1 = 24.9; 2 = 18.35	1 = 44.05; 2 = 34.4	1 = 115.75; 2 = 151.35
46	1 = 24.8; 2 = 40.95	1 = 51.25	1 = 10.85	1 = 50.75
47	1 = 59.90	1 = 8.0	0	1 = 197.25
48	1 = 183.70	1 = 51.25	0	1 = 22.6; 2 = 151.35
49	1 = 142.75	0	1 = 17.95	1 = 138.35; 2 = 20.95; 3 = 50.75
50	1 = 59.90; 2 = 40.95	1 = 18.35	1 = 89.30	1 = 179.45; 2 = 117.05
51	1 = 117.95	1 = 18.35	1 = 89.30	1 = 50.75

Individual	3B donor chromosome segments (cM)	5A donor chromosome segments (cM)	6B donor chromosome segments (cM)	7A donor chromosome segments (cM)
52	1 = 123.80	1 = 18.35	0	1 = 138.35; 2 = 50.75
53	1 = 80.85; 2 = 40.95	1 = 18.35	1 = 73.45	1 = 22.6; 2 = 130.95
54	1 = 27.4; 2 = 82.85	1 = 24.90; 2 = 18.35	1 = 54.90; 2 = 15.85	1 = 55.55; 2 = 50.75
55	1 = 117.95	1 = 2.05; 2 = 16.9; 3 = 18.35	1 = 89.30	1 = 82.80
56	1 = 82.85	1 = 69.60	1 = 89.30	1 = 130.95
57	1 = 35.1	1 = 18.35	0	1 = 55.55; 2 = 34.3
58	1 = 183.70	1 = 51.25	0	1 = 82.80; 2 = 100.6
59	1 = 183.70	1 = 2.05; 2 = 6.8; 3 = 44.7	1 = 71.35	1 = 330.8
60	1 = 40.95	1 = 44.7	1 = 15.85	1 = 60.2; 2 = 50.75
61	1 = 35.1; 2 = 61.90	1 = 69.60	1 = 44.05; 2 = 15.85	1 = 22.60
62	1 = 24.80; 2 = 2.55; 3 = 61.90	0	1 = 89.30	1 = 257.45
63	1 = 24.80; 2 = 2.55	1 = 14.8; 2 = 44.7	1 = 89.30	1 = 115.75; 2 = 34.3; 3 = 50.75
64	1 = 24.80; 2 = 20.95	0	1 = 17.95; 2 = 29.4	1 = 82.80; 2 = 20.95; 3 = 50.75
65	1 = 24.80	1 = 69.60	1 = 71.35	1 = 100.6
66	1 = 52.2; 2 = 123.80	0	1 = 89.30	1 = 115.75; 2 = 50.75
67	1 = 123.80	1 = 69.60	0	1 = 82.8; 2 = 41.1; 3 = 50.75
68	1 = 82.85	1 = 69.60	1 = 71.35	1 = 41.1
69	1 = 57.35; 2 = 123.80	0	1 = 71.35	1 = 60.2; 2 = 151.35

Individual	3B donor chromosome segments (cM)	5A donor chromosome segments (cM)	6B donor chromosome segments (cM)	7A donor chromosome segments (cM)
70	1 = 20.95; 2 = 40.95	1 = 69.60	1 = 17.95	1 = 192.45
71	1 = 59.90; 2 = 82.85	1 = 69.60	1 = 44.05; 2 = 15.85	1 = 22.6
72	1 = 82.85	1 = 69.60	1 = 89.30	1 = 22.6; 2 = 66.3
73	1 = 59.90	0	1 = 73.45	1 = 96.65; 2 = 50.75
74	1 = 2.55; 2 = 102.85	1 = 69.60	1 = 55.5	1 = 138.35; 2 = 20.95
75	1 = 183.70	1 = 69.60	1 = 71.35	1 = 82.80; 2 = 117.05
76	1 = 80.85	0	1 = 89.30	1 = 60.20; 2 = 141.7
77	1 = 24.80; 2 = 126.35	1 = 69.60	1 = 15.85	1 = 280.05
78	1 = 183.70	1 = 69.60	1 = 26.1; 2 = 18.55	1 = 60.20
79	1 = 59.90; 2 = 102.85	1 = 69.60	1 = 17.95; 2 = 15.85	1 = 213.75
80	1 = 24.80; 2 = 82.85	1 = 69.60	0	1 = 156.85
81	0	0	1= 17.95	1= 115.75 2 = 117.05
82	1 = 24.8; 2 = 20.95; 3 = 40.95	1 = 6.8; 2 = 44.7	1 = 73.45	1 = 138.35; 2 = 117.05
83	1 = 142.75	1 = 8.0	1 = 89.30	1 = 280.05
84	1 = 24.8; 2 = 20.95; 3 = 40.95	1 = 69.60	1 = 89.30	1 = 50.75
85	1 = 61.90	0	1 = 89.30	1 = 179.45; 2 = 50.75
86	1 = 24.8; 2 = 40.95	1 = 69.60	1 = 89.30	1 = 82.8; 2 = 192.45
87	1 = 82.85	1 = 26.35	1 = 71.35	1 = 22.6; 2 = 248.00

Individual	3B donor chromosome segments (cM)	5A donor chromosome segments (cM)	6B donor chromosome segments (cM)	7A donor chromosome segments (cM)
88	0	1 = 24.90; 2 = 18.35	1 = 15.85	1 = 55.55
89	0	0	1 = 17.95	1 = 50.75
90	1 = 24.8; 2 = 82.85	1 = 69.60	1 = 17.95	1 = 82.80; 2 = 100.6
91	1 = 61.90	0	1 = 73.45	1 = 22.6; 2 = 192.45
92	1 = 59.90; 2 = 102.85	0	1 = 71.35	1 = 96.65; 2 = 50.75
93	1= 183.70	0	1= 89.30	1= 22.6 2 = 192.45
94	1 = 24.8; 2 = 123.80	1 = 69.60	1 = 17.95; 2 = 15.85	1 = 82.8; 2 = 100.6
95	1 = 35.1	0	1 = 44.05; 2 = 18.55	1 = 60.2; 2 = 100.6
96	1 = 102.85	1 = 69.60	1 = 89.30	1 = 34.30
97	1 = 7.7	1 = 64.90	1 = 17.95; 2 = 15.85	1 = 138.35
98	1 = 59.90; 2 = 40.95	1 = 69.60	1 = 55.5	1 = 82.80
99	1 = 59.90	1 = 69.60	1 = 15.85	1 = 191.15
100	1 = 24.80; 2 = 123.80	1 = 64.90	0	1 = 130.95
101	1 = 40.95	1 = 69.60	1 = 73.45	1 = 55.55
102	1 = 131.5	1 = 69.60	1 = 17.95; 2 = 15.85	1 = 22.6; 2 = 96.65; 3 = 50.75
103	1 = 35.1	0	1 = 55.50	1 = 22.6
104	1 = 56.05	0	1 = 71.35	1 = 100.6
105	1 = 131.5	1 = 18.35	1 = 10.85; 2 = 15.85	1 = 22.6; 2 = 197.25

Individual	3B donor chromosome segments (cM)	5A donor chromosome segments (cM)	6B donor chromosome segments (cM)	7A donor chromosome segments (cM)
106	1 = 20.95	0	1 = 55.50	0
107	1 = 59.90; 2 = 61.9	0	1 = 15.85	1 = 82.8; 2 = 50.75
108	1 = 24.8	1 = 69.60	1 = 71.35	1 = 82.8; 2 = 151.35
109	1 = 158.9	1 = 69.60	1 = 17.95	1 = 96.65
110	1 = 40.95	1 = 69.60	0	1 = 66.3
111	1 = 24.8; 2 = 20.95; 3 = 40.95	1 = 44.7	1 = 71.35	1 = 280.05
112	1 = 35.1; 2 = 61.9	0	0	1 = 191.15
113	1 = 183.70	1 = 69.60	1 = 45.25	1 = 179.45; 2 = 50.75
114	1 = 27.4; 2 = 61.90	1 = 18.35	1 = 44.05	1 = 191.15; 2 = 50.75
115	1 = 20.95	1 = 18.35	0	1 = 20.15; 2 = 151.35
116	1 = 102.85	1 = 69.60	1 = 71.35	1 = 138.35; 2 = 20.95; 3 = 50.75
117	1 = 123.8	0	1 = 71.35	1 = 82.8; 2 = 34.30; 3 = 50.75
118	1 = 90.55	1 = 18.35	1 = 44.05	0
119	1 = 158.9	1 = 24.9; 2 = 18.35	1 = 89.30	1 = 22.6; 2 = 55.55; 3 = 172.3
120	1 = 80.85	0	1 = 89.30	1 = 156.85

Appendix C A list of the 120 BC_1F_1 individuals' specific FHB gene/QTL combination genotype, homozygous recurrent genome percentage (HRGP), heterozygous donor genome percentage (HDGP) and overall predicted recurrent parent genome percentage (RPGP) across the four targeted chromosomes 3B, 5A, 6B and 7A

BC_1F_1 individual	FHB gene/QTL present	HRGP (%)	HDGP (%)	Total cM homozygous recurrent genome	Total cM heterozygous genome	Predicted RPGP (%)
1	*Fhb1, ½ Qfhs.ifa.5A* and *7A* QTL	42.8	57.2	287.9	385.5	71.4
2	*Qfhs.ifa.5A*	32.6	67.4	219.3	454.1	66.3
3	*Fhb2* and *7A* QTL	38.1	61.9	256.7	416.7	69.1
4	*Fhb1* and *Fhb2*	38.6	61.4	259.6	413.8	69.3
5	*Fhb1, Fhb2* and *7A* QTL	10.3	89.7	69.6	603.8	55.2
6	*Fhb1* and *Fhb2*	33.5	66.5	225.9	447.5	66.8
7	*Fhb1, ½ Qfhs.ifa.5A* and *Fhb2*	59.9	40.1	403.4	270.0	80.0
8	*Fhb1, Qfhs.ifa.5A, Fhb2* and *7A* QTL	13.6	86.4	91.5	581.9	56.8
9	*Fhb1*	76.5	23.5	514.9	158.5	88.3
10	*Fhb2* and *7A* QTL	33.2	66.8	223.3	450.1	66.6
11	*½ Qfhs.ifa.5A* and *Fhb2*	64.7	35.3	435.7	237.7	82.4
12	No FHB QTL regions	76.3	23.7	513.7	159.7	88.2
13	*Qfhs.ifa.5A*	44.8	55.2	301.9	371.5	72.4
14	No FHB QTL regions	44.1	55.9	297.3	376.1	72.1

BC_1F_1 individual	FHB gene/QTL present	HRGP (%)	HDGP (%)	Total cM homozygous recurrent genome	Total cM heterozygous genome	Predicted RPGP (%)
15	No FHB QTL regions	92.6	7.4	623.8	49.6	96.3
16	*Fhb1* and *Fhb2*	28.5	59.3	191.9	399.6	58.2
17	*Fhb1, Qfhs.ifa.5A* and *Fhb2*	43.4	56.6	292.3	381.1	71.7
18	*Fhb1*	57.2	42.8	385.2	288.2	78.6
19	½ *Qfhs.ifa.5A* and *7A* QTL	23.2	76.8	156.5	516.9	61.6
20	*Fhb1* and *Qfhs.ifa.5A*	63.3	36.7	426.1	247.3	81.7
21	No FHB QTL regions	47.7	52.3	321.3	352.1	73.9
22	No FHB QTL regions	87.8	6.7	591.5	45.2	91.2
23	*Qfhs.ifa.5A* and *Fhb2*	46.5	53.5	313.4	360.0	73.3
24	*7A* QTL	52.4	47.6	353.0	320.4	76.2
25	*Fhb2* and *7A* QTL	54.2	45.8	365.1	308.3	77.1
26	*Fhb2* and *7A* QTL	25.9	74.1	174.7	498.7	63.0
27	*Fhb2* and *7A* QTL	52.9	47.1	356.2	317.2	76.5
28	No FHB QTL regions	60.5	39.5	407.4	266.0	80.3
29	*Fhb1* and *Fhb2*	27.4	67.2	184.4	452.3	61.0
30	*Fhb1, Qfhs.ifa.5A* and *Fhb2*	37.2	52.7	250.4	354.6	63.6
31	*Fhb1, Fhb2* and *7A* QTL	32.1	67.9	216.3	457.1	66.1
32	*Fhb1* and *Fhb2*	27.8	72.2	187.0	486.4	63.9
33	*Qfhs.ifa.5A* and *7A* QTL	41.4	47.6	279.0	320.8	65.2

BC_1F_1 individual	FHB gene/QTL present	HRGP (%)	HDGP (%)	Total cM homozygous recurrent genome	Total cM heterozygous genome	Predicted RPGP (%)
34	*Fhb2*	39.6	54.1	266.9	364.6	66.7
35	*Fhb1*	65.5	34.5	441.2	232.2	82.8
36	*Qfhs.ifa.5A* and *7A* QTL	49.4	50.6	332.6	340.8	74.7
37	*Fhb2*	44.6	55.4	300.0	373.4	72.3
38	*Fhb1* and *Fhb2*	32.6	67.4	219.2	454.2	66.3
39	*Fhb1* and *Fhb2*	49.7	47.9	334.8	322.3	73.7
40	*Fhb1* and *Fhb2*	42.8	57.2	288.5	384.9	71.4
41	*Fhb1, ½ Qfhs.ifa.5A* and *Fhb2*	16.0	60.7	107.5	408.8	46.4
42	*Fhb1*	42.4	41.7	287.1	280.6	63.3
43	*Fhb1* and *Qfhs.ifa.5A*	20.4	79.6	137.1	536.5	60.2
44	*Fhb1, Qfhs.ifa.5A* and *Fhb2*	31.1	68.9	209.3	464.1	65.6
45	*½ Qfhs.ifa.5A* and *7A* QTL	20.7	79.3	139.5	533.9	60.4
46	*Qfhs.ifa.5A*	69.3	18.5	466.9	124.6	78.6
47	*Fhb1, ½ Qfhs.ifa.5A* and *7A* QTL	58.0	42.0	390.4	283.0	79.0
48	*Fhb1* and *Qfhs.ifa.5A*	36.0	64.0	242.3	431.1	68.0
49	*Fhb1* and *7A* QTL	38.6	61.4	260.0	413.4	69.3
50	*Fhb1, Fhb2* and *7A* QTL	21.2	66.7	142.6	448.9	54.6
51	*Fhb1* and *Fhb2*	66.8	33.2	450.0	223.4	83.4
52	*7A* QTL	58.4	41.6	393.3	280.1	79.2

BC_1F_1 individual	FHB gene/QTL present	HRGP (%)	HDGP (%)	Total cM homozygous recurrent genome	Total cM heterozygous genome	Predicted RPGP (%)
53	*Fhb1* and *Fhb2*	48.7	51.3	328.2	345.2	74.4
54	*½ Qfhs.ifa.5A*	57.4	37.9	386.3	255.4	76.4
55	*Fhb1* and *Fhb2*	27.3	54.5	183.9	367.	54.6
56	*Qfhs.ifa.5A* and *Fhb2*	41.8	58.2	281.8	391.6	70.9
57	*Fhb1*	81.6	11.5	549.2	77.3	87.4
58	*Fhb1* and *Qfhs.ifa.5A*	20.1	69.0	135.4	464.4	54.6
59	*Fhb1* and *Fhb2*	50.3	41.8	338.6	281.3	71.2
60	*No FHB QTL regions*	68.5	16.6	461.0	111.9	76.8
61	*Fhb1* and *Qfhs.ifa.5A*	61.5	38.5	414.2	259.2	80.8
62	*Fhb2* and *7A* QTL	25.5	74.5	167.0	502.0	62.8
63	*½ Qfhs.ifa.5A, Fhb2* and *7A* QTL	50.3	49.7	338.7	334.7	75.2
64	*Fhb2*	52.1	47.9	351.0	322.4	76.1
65	*Qfhs.ifa.5A* and *Fhb2*	54.7	45.3	368.2	305.2	77.4
66	*Fhb2* and *7A* QTL	43.7	56.3	294.4	379.0	71.9
67	*Qfhs.ifa.5A*	47.2	52.8	317.7	355.7	73.6
68	*Qfhs.ifa.5A* and *Fhb2*	57.1	42.9	384.7	288.7	78.6
69	*Fhb1* and *Fhb2*	33.9	66.1	228.5	444.9	67.0
70	*Qfhs.ifa.5A*	52.2	47.8	351.5	321.9	76.1
71	*Fhb1* and *Qfhs.ifa.5A*	54.1	45.9	364.6	308.8	77.1

BC_1F_1 individual	FHB gene/QTL present	HRGP (%)	HDGP (%)	Total cM homozygous recurrent genome	Total cM heterozygous genome	Predicted RPGP (%)
72	*Qfhs.ifa.5A* and *Fhb2*	36.2	63.8	244.0	429.4	68.1
73	*Fhb1* and *Fhb2*	66.3	33.7	446.4	227.0	83.2
74	*Qfhs.ifa.5A, Fhb2* and *7A* QTL	40.8	59.2	274.7	398.7	70.4
75	*Fhb1, Qfhs.ifa.5A* and *Fhb2*	21.5	78.5	144.8	528.6	60.8
76	*Fhb1* and *Fhb2*	34.5	65.5	232.6	440.8	67.3
77	*Fhb1, Qfhs.ifa.5A* and *7A* QTL	14.8	85.2	99.6	573.6	57.4
78	*Fhb1* and *Qfhs.ifa.5A*	44.1	55.9	296.9	376.5	72.1
79	*Fhb1, Qfhs.ifa.5A* and *7A* QTL	29.2	70.8	196.8	476.6	64.6
80	*Qfhs.ifa.5A* and *7A* QTL	41.2	58.8	277.4	396.0	70.6
81	*7A* QTL	63.1	36.9	424.9	248.5	81.6
82	*Fhb2* and *7A* QTL	28.8	71.2	193.9	479.5	64.4
83	*Fhb1*, *½ Qfhs.ifa.5A, Fhb2* and *7A*	8.4	91.6	56.9	616.5	54.2
84	*Qfhs.ifa.5A* and *Fhb2*	62.8	37.2	423.0	250.4	81.4
85	*Fhb2* and *7A* QTL	45.1	54.9	304.0	369.4	72.6
86	*Qfhs.ifa.5A* and *Fhb2*	25.2	74.8	170.0	503.4	62.6
87	*Fhb2*	31.1	68.9	209.3	464.1	65.6
88	*½ Qfhs.ifa.5A*	89.8	10.2	604.6	68.8	94.9
89	No FHB QTL regions	94.7	5.3	637.5	35.9	97.4
90	*Qfhs.ifa.5A*	21.0	79.0	141.5	531.9	60.5

BC_1F_1 individual	FHB gene/QTL present	HRGP (%)	HDGP (%)	Total cM homozygous recurrent genome	Total cM heterozygous genome	Predicted RPGP (%)
91	*Fhb2*	42.0	52.7	282.5	355.0	68.4
92	*Fhb1* and *Fhb2*	59.5	40.5	400.4	273.0	79.8
93	*Fhb1* and *Fhb2*	21.4	73.2	144.0	492.7	58.0
94	*Qfhs.ifa.5A*	21.0	79.0	141.5	531.9	60.5
95	*Fhb1*	55.2	44.8	371.4	302.0	77.6
96	*Qfhs.ifa.5A* and *Fhb2*	59.6	40.4	401.5	271.9	79.8
97	*Fhb1, ½ Qfhs.ifa.5A* and *7A* QTL	62.3	37.7	419.2	254.2	81.2
98	*Fhb1, ½ Qfhs.ifa.5A* and *Fhb2*	55.0	39.6	370.1	266.6	74.8
99	*Fhb1, Qfhs.ifa.5A* and *7A* QTL	53.4	46.6	359.8	313.6	76.7
100	*½ Qfhs.ifa.5A*	49.6	50.4	334.2	339.2	74.8
101	*Qfhs.ifa.5A* and *Fhb2*	71.1	26.5	478.6	178.5	84.4
102	*Fhb1* and *Qfhs.ifa.5A*	46.9	53.1	316.2	357.4	73.5
103	*Fhb1* and *Fhb2*	78.4	16.2	527.8	108.9	86.5
104	*Fhb1* and *Fhb2*	64.6	35.4	435.2	238.2	82.3
105	*Fhb1*	42.1	57.9	283.5	389.5	71.1
106	*Fhb2*	85.8	14.2	578.1	95.3	92.9
107	*Fhb1*	61.1	38.9	411.2	262.2	80.6
108	*Qfhs.ifa.5A* and *Fhb2*	36.8	63.2	247.8	425.6	68.4
109	*Fhb1* and *Qfhs.ifa.5A*	25.7	40.9	172.8	275.5	46.2

BC_1F_1 individual	FHB gene/QTL present	HRGP (%)	HDGP (%)	Total cM homozygous recurrent genome	Total cM heterozygous genome	Predicted RPGP (%)
110	*Qfhs.ifa.5A*	74.6	25.4	502.3	171.1	87.3
111	*Fhb2* and *7A* QTL	23.9	76.1	161.0	512.4	62.0
112	*Fhb1* and *7A* QTL	59.0	41.0	397.1	276.3	79.5
113	*Fhb1, Qfhs.ifa.5A, Fhb2* and *7A* QTL	28.2	71.8	190.0	483.4	64.1
114	*7A* QTL	46.4	48.0	313.3	323.4	70.4
115	No FHB QTL regions	73.4	26.6	494.5	178.9	86.7
116	*Qfhs.ifa.5A, Fhb2* and *7A* QTL	40.8	59.2	274.7	398.7	70.4
117	*Fhb2*	51.2	48.8	344.7	328.7	75.6
118	*Fhb1*	73.1	26.9	492.3	181.1	86.6
119	*Fhb1, ½ Qfhs.ifa.5A* and *Fhb2*	24.6	75.4	165.9	507.5	62.3
120	*Fhb1, Fhb2* and *7A* QTL	48.9	51.1	329.3	344.1	74.5

Appendix D BC_1F_1 FHB resistance genes/QTL genotypes and corresponding phenotypic scores

			FHB disease symptoms scores as % for days post inoculation						Genes/QTL present/absent			
Sample	**Plant no**	**Inoculation method tested**	**4**	**7**	**10**	**14**	**18**	**21**	***Fhb1***	***Qfhs.ifa-5A QTL***	***Fhb2***	***7A* QTL**
1	BC_1F_1 1.1	Point	5	10-15	10-15	20	20	30	-	+/-	+	+/-
2	BC_1F_1 1.2	Point	5	20	30	40	50	50	+	-	+	-
3	BC_1F_1 1.3	Point	0-2	5-10	10-20	50	50-60	60	+	-	+	+/-
4	BC_1F_1 2.1	Negative check							-	+	+	-
5	BC_1F_1 2.2	Spray	5-10	10	20	30	30-40	30-40	+	-	-	+/-
6	BC_1F_1 2.3	Spray	10-20	20-30	30-40	50	60	60	+	-	+	+/-
7	BC_1F_1 3.1	Spray	2	30	60	70	70	70	+	-	+	-
8	BC_1F_1 3.2	Spray	0	10-20	20-30	20-30	30-40	40	+	+	+	+
9	BC_1F_1 3.3	Spray	0-2	40	60-70	70-80	90-100	95-100	+	+/-	-	-
10	BC_1F_1 4.1	Negative check							+	+	+/-	-
11	BC_1F_1 4.2	Point	2-5	30	40	50-60	70-80	80-90	-	-	-	.
12	BC_1F_1 5.1	Point	2-5	5-10	5-10	5-10	5-10	5-10	+	-	-	-
13	BC_1F_1 5.2	Point	0-2	10	20	20-30	40	50	-	-	.	.
14	BC_1F_1 5.3	Point	5-10	10	10-20	20-30	40	50	+	-	+	.
15	BC_1F_1 6.1	Negative check							+	-	+	-
16	BC_1F_1 6.2	Spray	0-2	0-2	0-2	2-5	2-5	2-5	+	+	-	+
17	BC_1F_1 6.3	Negative check							+	+	+	+
18	BC_1F_1 7.1	Point	0-2	10-20	50	60-70	100	100	+	-	-	-
19	BC_1F_1 7.2	Point	0	0	0-2	0-2	0-2	0-2	+	+	-	-
20	BC_1F_1 7.3	Negative check							-	+	+	+

Sample	Plant no	Inoculation method tested	FHB disease symptoms scores as % for days post inoculation						Genes/QTL present/absent			
			4	7	10	14	18	21	*Fhb1*	*Qfhs.ifa-5A* QTL	*Fhb2*	*7A* QTL
21	BC_1F_1 8.1	Point	10	10-20	10-20	10-20	10-20	20	+	.	+	+
22	BC_1F_1 8.2	Point	2-5	40	50	60	60-70	70-80	-	-	+	+
23	BC_1F_1 8.3	Point	5-10	10	10	10	10-15	10-20	+	+	+	-
24	BC_1F_1 9.3	Point	2-5	30	50	70	80	80-90	+	+	-	-
25	BC_1F_1 10.1	Negative check							-	-	-	-
26	BC_1F_1 10.2	Spray	0-2	2	5-10	30-40	50	60-70	+	-	-	+/-
27	BC_1F_1 10.3	Negative check							-	-	-	+
28	BC_1F_1 11.1	Point	5	20-30	40-50	70-80	80	80-90	-	-	+	-
29	BC_1F_1 11.2	Negative check							+	-	+	-
30	BC_1F_1 11.3	Point	10	10-20	10-20	50-60	70	80	+	-	+	+
31	BC_1F_1 12.1	Point	2-5	10	10-20	20	40	50	+	+	+	+
32	BC_1F_1 12.2	Point	0-2	2-5	40	60	70-80	80	-	+	+	-
33	BC_1F_1 12.3	Point	5	10	10-15	10-15	10-15	10-15	+	-	+	-
34	BC_1F_1 13.1	Spray	10	10-20	10-20	20	20-30	20-30	-	+	+	+
35	BC_1F_1 13.2	Spray	0	0	0	0-2	0-2	2-5	-	-	+	+/-
36	BC_1F_1 13.3	Negative check							+	+	-	+
37	BC_1F_1 14.1	Spray	0	2-5	10-20	40	50	50	-	+/-	-	-
38	BC_1F_1 15.1	Missed							+	-	-	-
39	BC_1F_1 15.2	Negative check							+	+	+	+/-
40	BC_1F_1 15.3	Missed							-	+/-	-	+
41	BC_1F_1 16.1	Point	0-2	0-2	0-2	0-2	0-2	0-2	+	+/-	-	-
42	BC_1F_1 16.2	Point	2-5	10	10-20	30-40	50	60	+	-	-	-

			FHB disease symptoms scores as % for days post inoculation						Genes/QTL present/absent			
Sample	**Plant no**	**Inoculation method tested**	**4**	**7**	**10**	**14**	**18**	**21**	***Fhb1***	***Qfhs.ifa-5A QTL***	***Fhb2***	***7A QTL***
43	BC_1F_1 16.3	Point	10-20	50	60	60-70	70	70-80	-	-	+	-
44	BC_1F_1 17.1	Point	0-2	10-20	40	60	70	70-80	-	+/-	+	+
45	BC_1F_1 17.2	Point	2	5-10	20	40	40-50	40-50	-	+/-	+	-
46	BC_1F_1 19.1	Point	2-5	20-30	30-40	50	60-70	80-90	-	+	+	-
47	BC_1F_1 19.2	Point	5	40	80	90	95-100	95-100	+	+	+	+
48	BC_1F_1 19.3	Point	5	90	100	100	100	100	-	-	-	-
49	BC_1F_1 20.1	Missed							+	+	-	+
50	BC_1F_1 20.2	Spray	2-5	60	70-80	70-80	80	80	-	+	+	+/-
51	BC_1F_1 20.3	Spray	30-40	80	90	95-100	95-100	100	-	-	+	+/-
52	BC_1F_1 21.3	Point	0-2	2-5	20-30	50	60	60	-	+	+	+
53	BC_1F_1 22.1	Spray	20	20	30-40	40-50	60	60-70	+	-	-	-
54	BC_1F_1 22.2	Negative check							+	-	+	+
55	BC_1F_1 22.3	Spray	5	40	60	90-100	95-100	100	-	-	-	-
56	BC_1F_1 23.1	Missed							+	-	+	-
57	BC_1F_1 23.3	Spray	20-30	50	80	90-100	100		-	-	+	+/-
58	BC_1F_1 24.1	Point	0-2	10	10-20	10-20	10-20	10-20	+	-	+	+
59	BC_1F_1 24.2	Point	2-5	10-20	20	20	20	20	-	+	+	+/-
60	BC_1F_1 24.3	Point	2-5	20-30	50	60-70	70-80	80	-	+	+	+/-
61	BC_1F_1 25.2	Spray	5-10	30-40	50-60	60	70	70-80	+	+/-	-	-
62	BC_1F_1 25.3	Spray	50	80	90-100	95-100	95-100	100	+	+/-	+/-	-
63	BC_1F_1 26.1	Spray	0-2	0-2	0-2	0-2	2-5	2-5	+	+	+/-	+
64	BC_1F_1 26.2	Negative check							+/-	+/-	+	+

			FHB disease symptoms scores as % for days post inoculation						Genes/QTL present/absent			
Sample	**Plant no**	**Inoculation method tested**	**4**	**7**	**10**	**14**	**18**	**21**	***Fhb1***	***Qfhs.ifa-5A QTL***	***Fhb2***	***7A QTL***
65	BC_1F_1 26.3	Spray	0-2	0-2	0-2	2-5	5	5	+	+	+	+
66	BC_1F_1 27.1	Spray	40	50	50	70	70	70	+	+	+/-	+/-
67	BC_1F_1 27.2	Spray	0-2	2-5	2-5	2-5	2-5	5-10	+	+	+	+
68	BC_1F_1 27.3	Spray	0-2	10	10-15	10-20	10-20		+	+	+	+
69	BC_1F_1 28.1	Point	10-15	10-20	10-20	10-20	10-20	10-20	+	-	+	+/-
70	BC_1F_1 28.2	Point	0-2	20-30	50	70-80	80-90	100	-	+	+	+/-
71	BC_1F_1 28.3	Point	5-10	20	30-40	30-40	40	60	+	-	+	+/-
72	BC_1F_1 29.2	Spray	0	0	0	0-2	0-2	0-2	+	-	+	+
73	BC_1F_1 29.3	Spray	2-5	10-20	30-40	70-80	90-100	100	+	+	+	-
74	BC_1F_1 30.2	Point	2-5	20-30	60	80	90-100	100	-	-	+	+/-
75	BC_1F_1 30.3	Point	2-5	20	40	60-70	80	80	+	+	-	+
76	BC_1F_1 31.1	Spray	0-2	40-50	80-90	90-100	100	100	-	-	-	+/-
77	BC_1F_1 31.2	Spray	2-5	5	5-10	5-10	10	10-20	+	-	+	-
78	BC_1F_1 32.1	Negative check							-	-	-	+
79	BC_1F_1 32.2	Missed							+	-	-	-
80	BC_1F_1 32.3	Spray	2-5	20-30	50-60	80-90	100	100	+	+/-	-	+
81	BC_1F_1 33.1	Point	5-10	20-30	40	60	60-70	60-70	+	+	-	-
82	BC_1F_1 33.2	Point	2-5	5-10	10	10-20	20	30	+	-	-	+
83	BC_1F_1 33.3	Negative check							-	+	+	+
84	BC_1F_1 34.1	Point	2-5	10	40-50	60	70	70	-	-	-	+
85	BC_1F_1 34.2	Negative check							-	+	+	+
86	BC_1F_1 34.3	Point	2-5	5	20	30-40	40	40	+	+	+	-

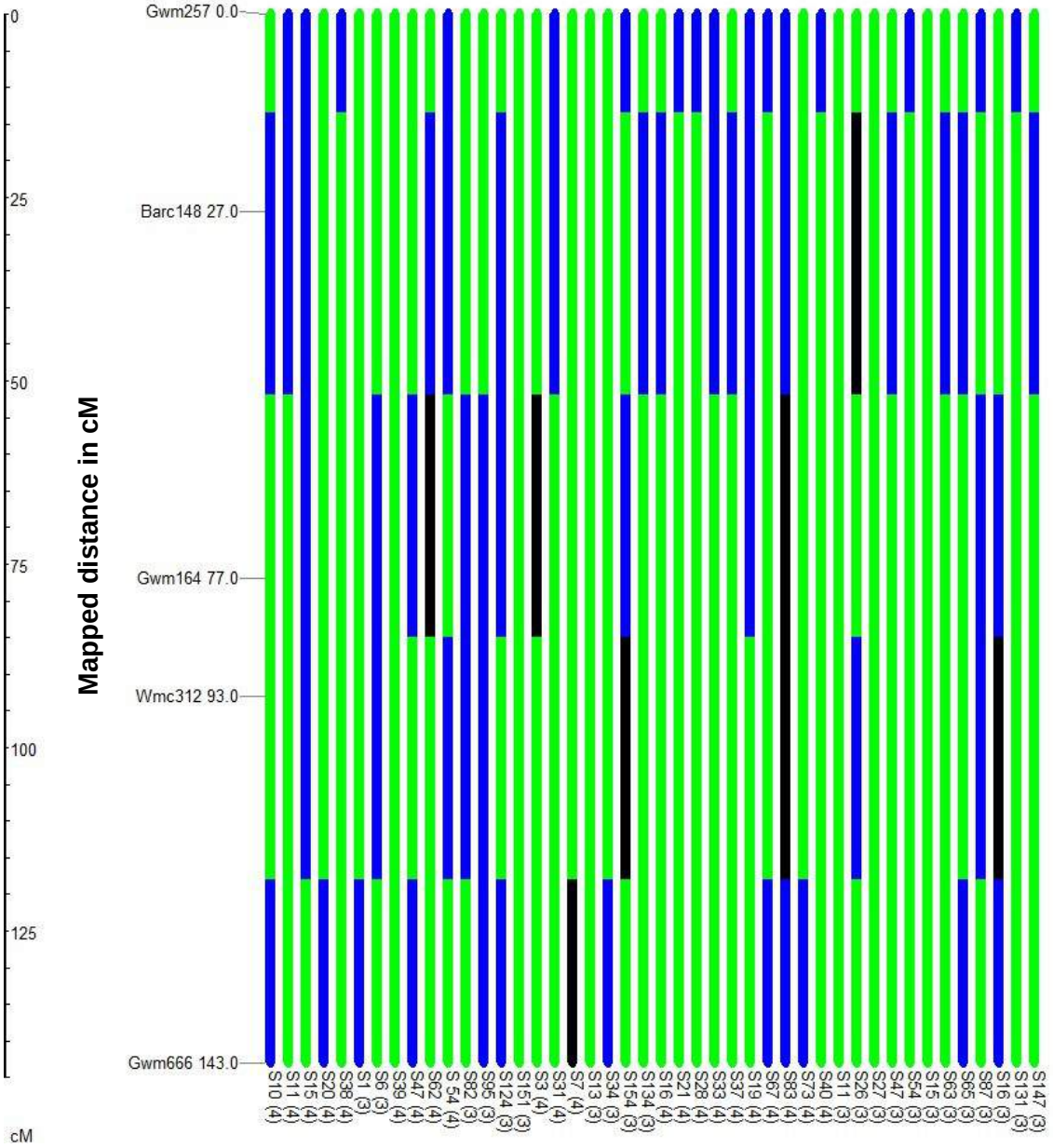
Sample	Plant no	Inoculation method tested	FHB disease symptoms scores as % for days post inoculation						Genes/QTL present/absent			
			4	7	10	14	18	21	*Fhb1*	*Qfhs.ifa-5A* QTL	*Fhb2*	*7A* QTL
87	BC_1F_1 36.3	Missed							.	+	-	.
88	BC_1F_1 37.2	Spray	0	10-20	20-30	40	80	90-100	.	-	-	.
89	BC_1F_1 38.2	Spray	0-2	10	30-40	50	60	60	.	-	-	+
90	BC_1F_1 38.3	Negative check							+	+	+	+/-
91	BC_1F_1 39.1	Spray	0	0	0	2	5	5-10	+	+/-	-	+
92	BC_1F_1 39.2	Spray	60	80-90	90-100	100	100	100	+	-	+	+
93	BC_1F_1 39.3	Spray	0-2	5-10	10-15	50	50	50	-	+	+	+
94	BC_1F_1 40.1	Point	2-5	20	40	50	60	60	+	-	.	+
95	BC_1F_1 40.2	Point	5-10	20	30	40	60-70	80	+	-	+	+
96	BC_1F_1 41.1	Negative check							+	+	+	+
97	BC_1F_1 42.1	Point	2-5	5-10	10	10-15	10-15	10-15	+	-	-	-
98	BC_1F_1 42.2	Point	5-10	10-20	30-40	40-50	50	50	-	+	+/-	+/-
99	BC_1F_1 42.3	Missed							+	+	+/-	-
100	BC_1F_1 43.1	Spray	0	0-2	0-2	2-5	10	10	-	+	+/-	+/-
101	BC_1F_1 43.2	Spray	2	2-5	10-20	20	30-40	30-40	+	-	-	+/-
102	BC_1F_1 43.3	Negative check							+	+	+	-
103	BC_1F_1 44.1	Point	5-10	30-40	60	70	70-80	80	-	+	-	-
104	BC_1F_1 44.2	Missed							-	+	+/-	+/-
105	BC_1F_1 44.3	Point	0-2	30-40	90-100	100	100	100	-	-	+	+/-
106	BC_1F_1 45.1	Spray	10-20	30-40	50	60	70	70-80	-	-	-	+/-
107	BC_1F_1 45.2	Spray	0-2	2-5	2-5	2-5	2-5	2-5	+	+	+/-	+/-
108	BC_1F_1 45.3	Negative check							+	+	+/-	+/-

Sample	Plant no	Inoculation method tested	FHB disease symptoms scores as % for days post inoculation						Genes/QTL present/absent			
			4	7	10	14	18	21	*Fhb1*	*Qfhs.ifa-5A* QTL	*Fhb2*	*7A* QTL
109	BC_1F_1 47.1	Negative check							+	+	+/-	-
110	BC_1F_1 47.2	Spray	5	20-30	60	70	80	90	+	+/-	+/-	+/-
111	BC_1F_1 47.3	Spray	80-90	90-100	100	100	100	100	-	+/-	+/-	-
112	BC_1F_1 48.1	Spray	0-2	0-2	2-5	30-40	50	50-60	+	+	+	+/-
113	BC_1F_1 48.2	Spray	0	0-2	0-2	0-2	0-2	0-2	-	-	-	-
114	BC_1F_1 48.3	Spray	20	20-30	60	80-90	100	100	-	-	+/-	-
115	BC_1F_1 50.1	Negative check							-	-	+/-	+
116	BC_1F_1 50.2	Point	5-10	30-40	50	60	70	80	-	+	-	+/-
117	BC_1F_1 50.3	Point	2-5	10	40	50	50	50	-	-	-	+/-
118	BC_1F_1 51.1	Spray	20	40	50	60	80	100	+/-	-	+	+/-
119	BC_1F_1 51.2	Spray	0-2	60-70	70-80	80-90	90	100	-	+/-	-	+/-
120	BC_1F_1 51.3	Spray	0	0-2	2	2	2	2	-	+	+/-	+
121	BC_1F_1 52.1	Point	2-5	20-30	50-60	70-80	80-90	90-100	-	+/-	+/-	+/-
122	BC_1F_1 52.2	Point	2-5	50-60	70-80	80-90	90	90	+	-	+/-	+/-
123	BC_1F_1 52.3	Point	2-5	20	70-80	90-100	95-100	100	-	-	+	+
124	BC_1F_1 53.1	Missed							+	-	+	+
125	BC_1F_1 53.2	Negative check							+	+	+/-	+/-
126	BC_1F_1 53.3	Point	2-5	2-5	30-40	50	60	60	+/-	-	+/-	+/-
127	BC_1F_1 54.1	Missed							+	+	+	-
128	BC_1F_1 54.2	Point	2	20	50	60	90-100	100	+/-	+/-	+	-

+ Presence of FHB gene/QTL; - Absence of FHB Gene/QTL; +/- Recombination within the targeted FHB gene/QTL region; Missed =The plant had already flowered

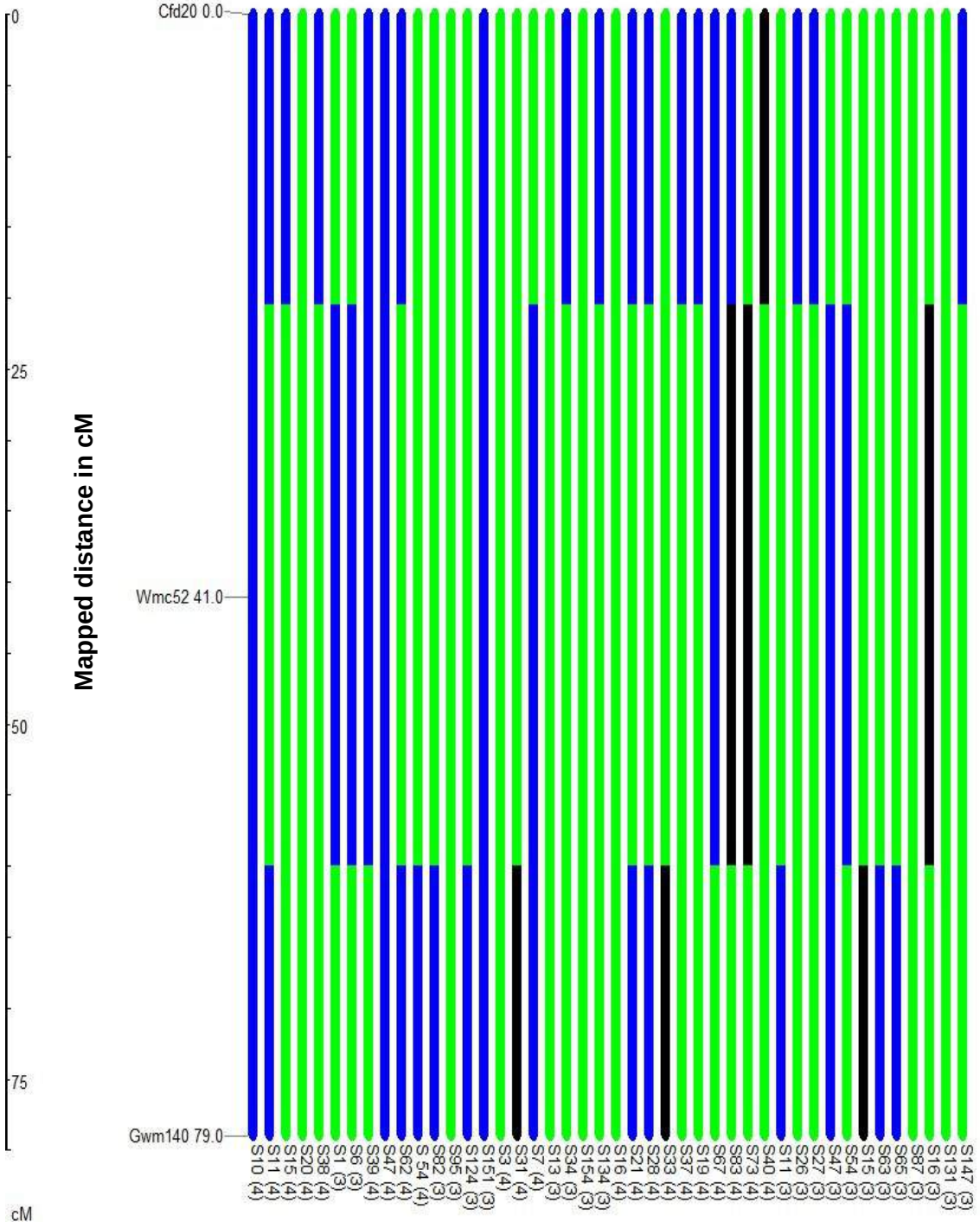
Appendix E **GGT profiles of each of the 44 selected BC_2F_1individuals for quality related chromosomes 1A, 1B, 1D, 6A, 6B and 6D. Green bars represent homozygous genome section of Krokodil, blue bars heterozygous genome segments and black bars segments with missing marker data. Mapped marker positions and marker names and order are indicated on the left, along with a cM scale**

Chromosome 1A



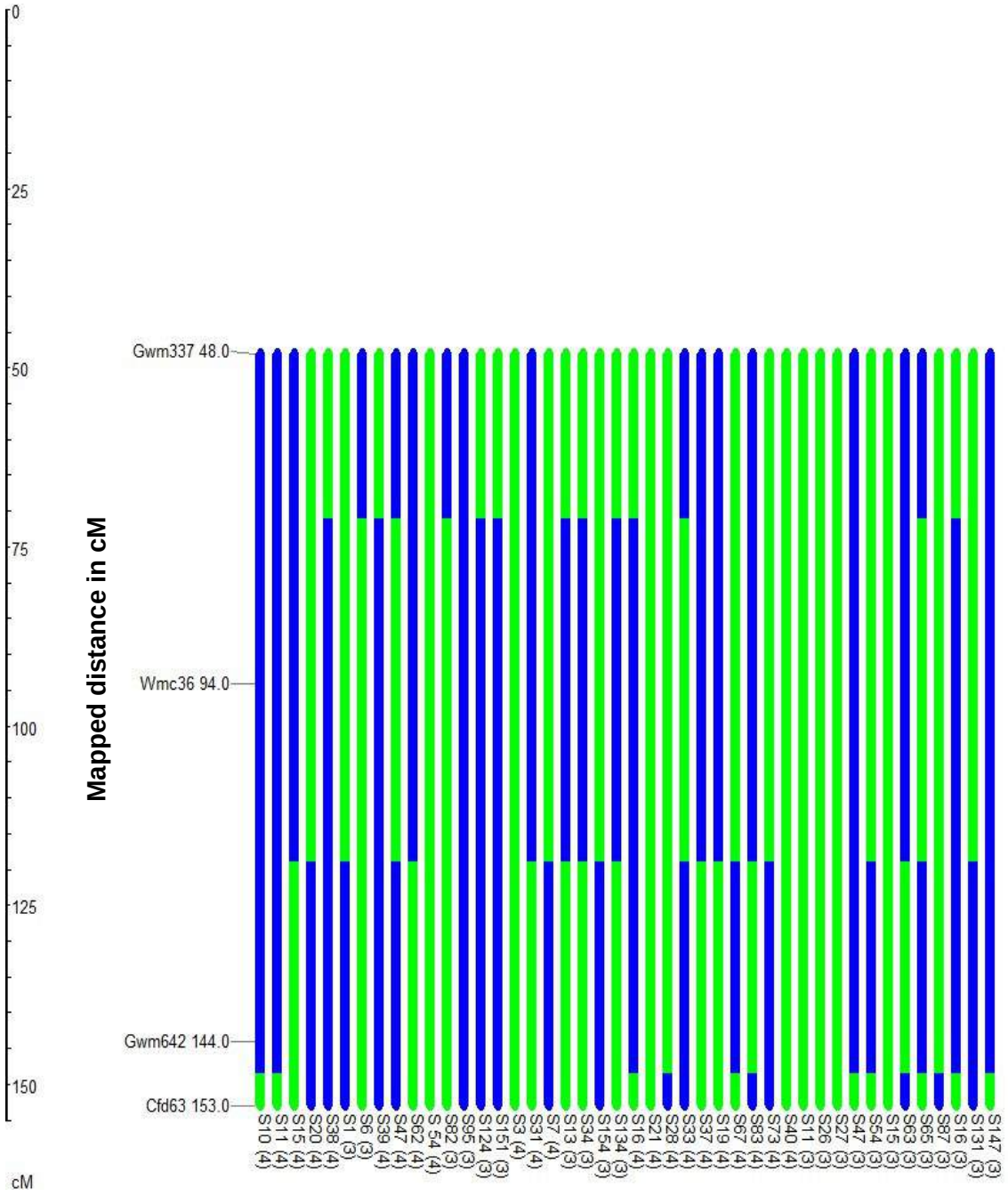
Selected BC_2F_1 individuals

Chromosome 1B



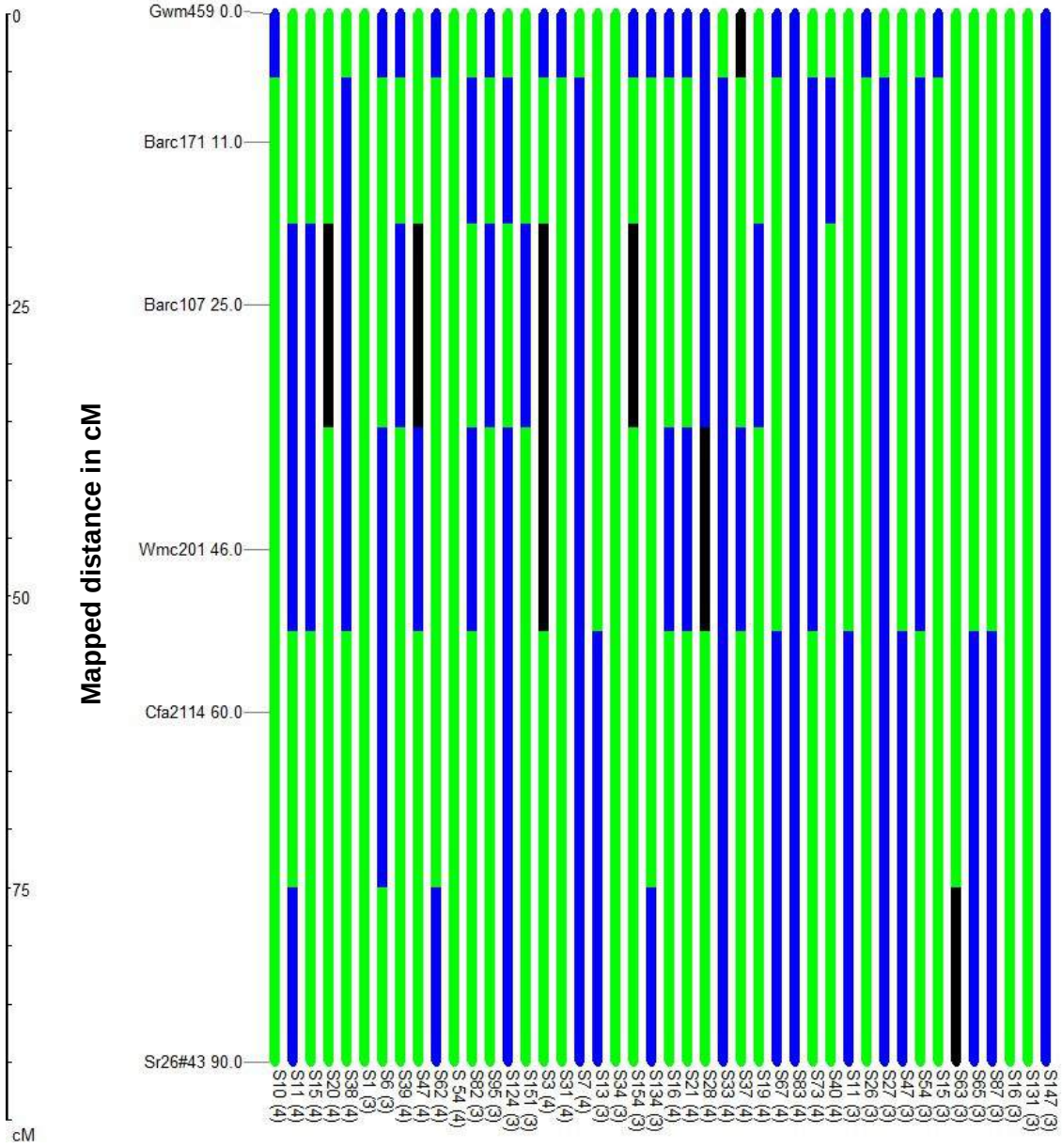
Selected BC_2F_1 individuals

Chromosome 1D



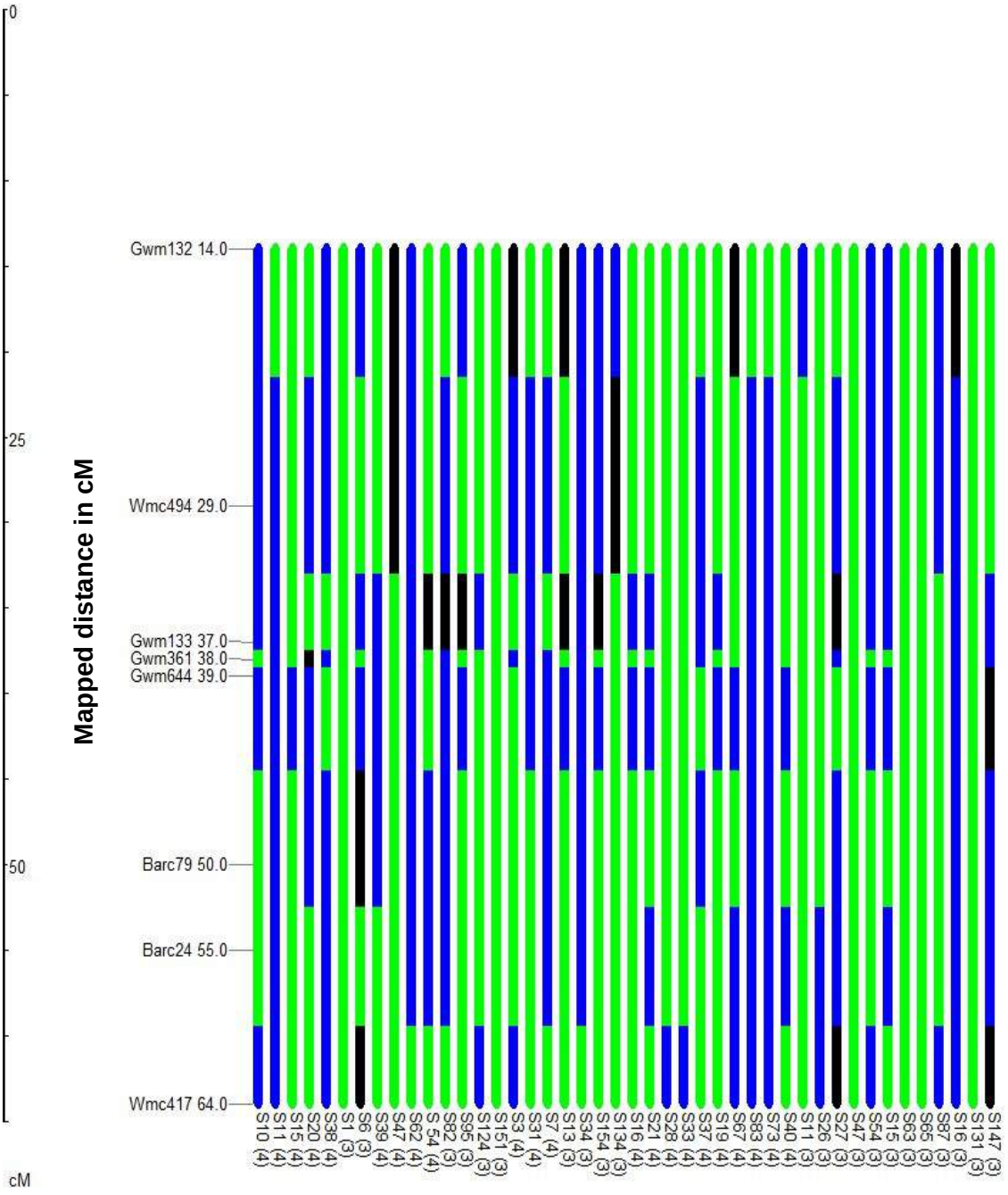
Selected BC_2F_1 individuals

Chromosome 6A



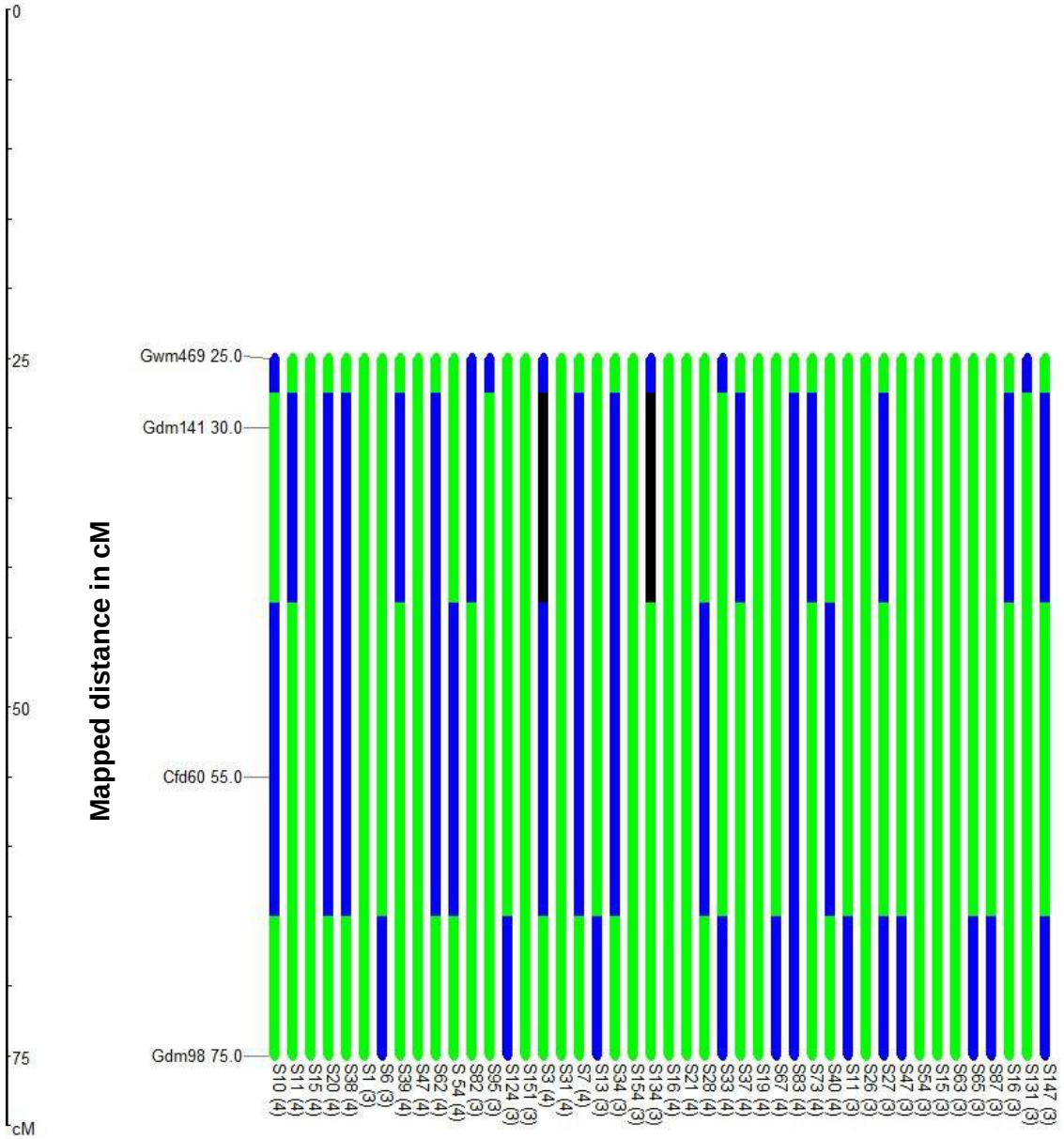
Selected BC_2F_1 individuals

Chromosome 6B



Selected BC_2F_1 individuals

Chromosome 6D



Selected BC_2F_1 individuals