Universität für Bodenkultur Wien

TULLN

Department for Agrobiotechnology - Tulln

Institute for Plant Production Biotechnology

Advisor
Univ. Prof. Dr. Hermann BÜRSTMAYR

MOLECULAR GENETIC ANALYSIS OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN A BACK – CROSS DERIVED POPULATION OF *TRITICUM DICOCCOIDES* WITH *TRITICUM DURUM*

Dissertation
for obtaining a doctorate degree at the University of Natural Resources and Applied Life Sciences, Vienna

Submitted by
Abdallah Alimari

Vienna – October 2009

Acknowledgements

This research would not have been successfully conducted without the help of many people whom I am thanking here. Firstly, it is an honor to me to present my appreciation to my supervisor Prof. Dr. Hermann Buerstmayr for his openness and his endless help to conduct this research in its all stages. I would like also to express my thanks and respects to Prof. Marc Lemmens and his lab. team for their help in inoculum preparation and for their constructive suggestions in the phytopathology. I offer my appreciation to Prof. Tamas Lelley for his consistent encouragement. My sincere thanks go to Dr. Barbra Steiner, Maria Buerstmayr, Karin, Kathrin, Matthias Fidesser, Gertraud Stift, Martin Pachner and all other colleagues in the Plant Production Biotechnology Institute for their technical help and for the construction of the nice and pleasant working atmosphere. Many thanks for my friends in Austria Hassan, Ammar, Abbas, Mohamed, Bashar and all friends in Palestine. I acknowledge the financial support by the Austrian Exchange Service (ÖAD). I will never be able to express the amount of appreciation I feel for my parents whom out of pure love have always allowed me to pursue my wildest dreams. Finally, my wife Abeer is the last but not least who I would like to thank for her priceless help and endless patience and support, and I will never forget our kids Talah and Essam who, along with their mother, gave me additional meaning for the life during this long journey.

Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum* is a serious disease problem on durum wheat (*T. durum*). Durum wheat is generally considered highly susceptible to FHB. Attempts to transfer resistance from hexaploid wheat to durum wheat have met with limited success. However, one potential source of resistance for durum wheat is the tetraploid wild emmer wheat (*T. dicoccoides*). A population of 105 BC_1F_6 lines from a backcross of *T. dicoccoides* (line 'Mt. Gerizim#36', resistant) with *T. durum* (cultivar 'Helidur', susceptible) has been evaluated for FHB resistance in five experiments (one in the field and four in the green house). At anthesis, individual heads were artificially inoculated with Fusarium spores (500 conidia/ head) using a single floret inoculation technique. A continuous variation was observed among genotypes studied with respect to all FHB-related traits and the variation was significant ($\alpha < 0.05$) for all FHB-related traits. The heritability results are ranging from 0.63 to 0.89. The population was genotyped with 522 DNA markers (142 SSR loci and 380 AFLP polymorphic fragments). Total map coverage equates to 2641 cM, an average distance between the markers of 5.1 cM. The major QTL effects associated with resistance to fungal spread (type II resistance) mapped to chromosome 3A and 6B. Two separate QTL were detected on chromosome 3A. The first QTL on chromosome 3A mapped to the flanking markers *Xgwm1121 - Xgwm720* and the second QTL on chromosome 3A mapped to the flanking markers *Xgwm2 - Xgwm779*. The most likely positions of these two QTL appeared at a distance of 39.6 cM. One QTL was detected on chromosome 6B. The position for the 6B QTL is between the flanking markers *XS23M17_5 - Xgwm626*. The two QTL on chromosome 3A explained 15 – 21% of the phenotypic variation for resistance to fungal spread and the QTL on chromosome 6B explained 17 – 23% of phenotypic variation. One QTL for FHB incidence was detected on chromosome 6B explaining 15 % of the phenotypic variation. The resistance for FHB incidence was conferred by alleles of *T. diccocoides* between flanking markers *XS13M25_3 - XS17M25_3*. One QTL for percent of wilted spikes was detected on chromosome 3A explaining 15 % of the phenotypic variation. The resistance for percent of wilted spikes was conferred by alleles of *T. diccocoides* between the flanking markers *Xgwm2 - Xgwm779* in the same region as the QTL for FHB spread on chromosome 3A. The plants were also assessed for developmental and morphological traits. QTL were detected for waxiness (1B and 2B), spike length (unknown group X6), plant height (4B), number of spikelets (6A and unknown group X3), brittle rachis (5A), date of anthesis (unknown group X3), colour of the spikes (5A) and powdery mildew resistance (6B).

Zusammenfassung

Ährenfusariose, verursacht durch Pilze der Gattung *Fusarium* ist eine bedeutende Pflanzenkrankheit an Weizen einschließlich Durumweizen (*Triticum durum*). Durumweizen gilt generell als hoch anfällig für Ährenfusariose. Versuche zur Übertragung von Ährenfusarioseresistenz aus hexaploidem Brotweizen in den tetraploiden Durumweizen waren bisher nur mäßig erfolgreich. Eine mögliche zusätzliche genetische Ressource für die Durumzüchtung stellt der wilde Emmerweizen (*T. dicoccoides*) dar.

Eine Population von 105 BC_1F_6 Linien abgeleitet von einer Rückkreuzung aus *T. dicoccoides* (Linie 'Mt. Gerizim#36', Donor, resistent) mit *T. durum* (Sorte 'Helidur', Rezipient, anfällig) wurde in fünf unabhängigen Experimenten (ein Feldversuch und vier Glashausversuche) auf Reaktion gegenüber Ährenfusariose überprüft. Dafür wurde eine Einzelähren-Inokulationsmethode benutzt. An blühenden Ähren wurde jeweils ein Ährchen in der Mitte der Ähre mit 10 µl einer Sporensuspension (50000 Konidien ml^{-1}) von *Fusarium graminearum* inokuliert. Das Ausmaß der Krankheitssymptome wurde zu mehreren Zeitpunkten nach der Inokulation bonitiert. Mehrere Parameter zur Quantifizierung des Fusariumbefalls wurden aus den Boniturwerten berechnet. Die Population zeigte eine quantitative Variation zwischen den untersuchten Linien für Fusariumbefall. Die Heritabilität für Fusariumbefall lag zwischen 0.63 bis 0.89, abhängig vom jeweiligen Parameter zur Quantifizierung des Fusariumbefalls. Die BC_1F_6 Linien wurden mit insgesamt 522 DNA Markern (142 polymorphe Mikrosatelliten (SSR) Marker and 380 polymorphe AFLP Marker) genetisch analysiert, relativ zu den beiden Elternlinien. Die Markerdaten erlaubten die Berechnung einer Kopplungskarte bestehend aus 36 Kopplungsgruppen mit einer Gesamtlänge von 2641 cM, und einem mittleren Abstand zwischen zwei Markern von 5.1 cM. Die gemeinsame Analyse der Resistenzdaten und der Markerdaten (QTL Analyse) ergab, dass die wichtigsten QTL (‚quantitative trait loci') für Ausbreitungsresistenz auf den Chromsomen 3A und 6B detektiert wurden. Auf Chromosom 3A wurden zwei separate QTL gefunden. Der erste QTL auf Chromosom 3A kartierte zwischen den beiden SSR Markern *Xgwm1121 - Xgwm720* und der zweite QTL auf diesem Chromosom zwischen den Markern *Xgwm2 - Xgwm779*. Die beiden QTL auf Chromosom 3A waren 39.6 cM voneinander entfernt. Ein dritter QTL für Resistenz gegenüber Ausbreitung von Ährenfusariose lag auf Chromosom 6B, flankiert von den Markern *XS23M17_5 - Xgwm626*. Die beiden QTL auf Chromosom 3A erklärten 15 – 21% der phänotypischen Variation für Ausbreitungsresistenz, und der QTL auf Chromosom 6B erklärte 17 – 23% der phänotypischen Variation. Für Resistenz gegenüber Eindringung von Ährenfusariose war nur ein QTL auf dem Chromosom 6B signifikant. In der Population wurden darüber hinaus morphologische- und

Entwicklungsmerkmale erhoben. QTL wurden detektiert für Bereifung der Ähren (Chromosomen 1B und 2B), Ährenlänge (nicht identifizierte Gruppe X6), Wuchshöhe (Chromosom 4B), Anzahl Ährchen je Ähre (Chromosom 6A und nicht identifizierte Gruppe X3), Spindelbrüchigkeit (Chromosom 5A), Blühdatum (nicht identifizierte Gruppe X3), Ährenfarbe (Chromosom 5A) und Befall mit Mehltau (Chromosom 6B). Die in dieser Arbeit kartierten QTL und die beschriebenen molekularen Marker können in der Züchtung von Durumweizen zur Verbesserung des Merkmales Ährenfusarioseresistenz eingesetzt werden.

Abbreviations

AFLP – amplified fragment length polymorphism

AUDPC – area under the disease progress curve

CIM – composite interval mapping

CTAB – mixed alkyltrimethyl-ammonium bromide

dai – days after inoculation

dNTPs – deoxynucleoside 5'-triphosphates

DON – deoxynivalenol

EDTA – ethylenediaminetetraacetate

FHB – Fusarium head blight

H^2 – broad sense heritability

LOD – logarithm of odds

LSD – least significant difference

MAS – marker assisted selection

n.DS - absolute number of infected spikelets

%DS - percent of infected spikelets

PM - powdery mildew

QTL – quantitative trait locus

SFS - speed of FHB spreading

SIM – simple interval mapping

SNP – single nucleotide polymorphism

SSR – simple sequence repeat

T.dic - T. dicoccoides

Table of contents

Introduction

Over the past decade, poverty, hunger and the related issue of inequality have moved to the top of the international development agenda. Growth in the agricultural sector has a crucial role to play in reducing poverty and increasing food supply to prevent famines (IFAD 2001).

The most important sources of food, both for direct human consumption and, indirectly, for livestock production, are cereals. What happens in the cereal sector is therefore crucial to world food supplies (FAO 2008). The world's major cereal crop is wheat, which accounts for 31 percent of the global cereal consumption in 1997-99. Over the coming years, wheat consumption is expected to increase in all regions, including the transition countries as their consumption intensifies (FAO 2008). In Austria, wheat production (2005/06) was 1,390,369 metric tons of common wheat (*Triticum aestivum*) and 62,704 metric tons of durum wheat (*T. durum*), whereas the use of common wheat and durum wheat was 1,047,168 and 82,768 metric tons, respectively (www.statistik.at). Consequently, there is a need to increase durum supply. Since land remains invariable, improving durum varieties through breeding is the only way to achieve this.

Yield loss due to diseases is among the main challenges for durum wheat breeders. Fusarium head blight (FHB) disease caused by *Fusarium* species is an economically devastating disease that affects wheat and other small grain cereal crops worldwide (Qu et al. 2008). FHB affects the developing heads of small grains directly, and yield losses that exceed 45% are common during years when disease is severe. FHB also negatively affects grain quality, often resulting in lower test weights and mycotoxin contamination (De Wolf et al. 2003). Durum wheat is generally considered highly susceptible to FHB. Attempts to transfer resistance from hexaploid wheat to durum wheat have met with limited success. However, one potential source of resistance for durum wheat is the tetraploid wild emmer wheat (*T. dicoccoides*) (Kumar et al. 2007). Tetraploid relatives like *T. dicoccoides* that have the same genome (AABB) as durum wheat, represent an important gene pool for durum improvement (Buerstmayr et al. 2003; Oliver et al. 2007). The progress in improving FHB resistance of wheat cultivars has been slow. This is in part due to the complexity of the disease evaluation procedures and the interaction between genotypes and environmental factors. Also, the most resistant germplasm is of exotic origin and has poor agronomic traits (Buerstmayr et al. 2002; Guo et al. 2003). The application of the molecular marker technology offers a wide range of novel approaches to improve the efficiency of selection for resistance to FHB. The techniques are based around the detection of sequence variation between accessions of wheat and the construction of a quantitative trait loci (QTL) map, where the sequence variant occupies a region of the genome that is closely linked to FHB resistance (Langridge et al. 2001; Buerstmayr et al. 2002).

Aims

The objectives of this study are

- to identify loci involved in resistance to fungal spread of FHB in tetrapoid wheat through molecular mapping,
- to develop durum wheat lines with QTL for FHB resistance derived from *Triticum dicoccoides*,
- to study the relationship between FHB resistance traits and some developmental and morphological traits, such as plant height, spike length, ear type, waxiness, number of spikelets per spike, date of anthesis, brittle riches, powdery mildew and colour of the spike,

In order to address these objectives, a population of 105 BC_1F_6 lines from a back cross of *T. dicoccoides* (line 'Mt. Gerizim#36', resistant) with *T. durum* (cultivar 'Helidur', susceptible) has been evaluated for FHB resistance. Multi seasonal block design was used in the field and greenhouse at the IFA-Tulln in spring 2007, autumn 2007, spring 2008, autumn 2008 and spring 2009. At anthesis, individual heads were artificially inoculated with Fusarium spores using a single floret inoculation technique. The development of Fusarium head blight was observed and recorded 14, 21 and 28 days after inoculation. FHB spread and FHB incidence were assessed. In addition, the BC_1F_6 lines were evaluated for plant height, spike length, date of anthesis, number of spikelets, waxiness, ear type, spike colour and powdery mildew severity. The FHB resistance data were biometrically analysed using analysis of variance. The same BC_1F_6 population was genotyped with SSR and AFLP markers, using standard protocols to construct a genetic linkage map. The combined analysis of the marker data and the phenotypic data allowed the detection of QTL that contribute to FHB resistance and to investigate their association with other plant characters.

1. State of the art

1.1. Wheat

Wheat (*Triticum ssp.)* is the world's most important cereal crop. The total harvested area of wheat amounts to 217,432,668 ha. This gives an average yield of 2791,8 kg/ha. In Austria, the total harvested area of wheat is 292,976 ha with an average yield 4776.3 kg/ha (Table 1).

Table 1: Production and yield of cereals and wheat worldwide and Austria in 2007*.

crop	Country	Area harvested (hectar)	Production Quantity (tons)	Yield per hectar (kg/ha)
cereals	world	699,813,132	2,342,426,995	3347.2
	Austria	811,173	4,594,730	5664.3
wheat	world	217,432,668	607,045,683	2791.8
	Austria	292,976	1,399,341	4776.3

*Source: www.faostat.fao.org.

Various plant diseases are the biggest biotic stress factors, reducing yield of wheat in most cultivated wheat areas. Fusarium head blight (FHB) or scab caused by *Fusarium spp*. is among the most destructive wheat diseases in many parts of the world. Several *Fusarium spp.* can cause root, stem and ear rot, resulting in severe reductions in crop yield and quality (Mesterhazy 1995; Stack et al. 2002).

1.1.1. History and use of durum wheat

Figure 1: Middle East, World Atlas.2008. Source: http://en.wikipedia.org/wiki/Fertile_Crescent

Wheat was domesticated in the Middle East (Figure 1) about 10,000 years ago. Durum wheat (*T. durum* wheat with the genome formula AABB, 2n = 4x = 28), was the most successful in expanding largely around the Mediterranean Sea. Most durum wheats are derived from wild emmer (*T. dicoccoides*), which could have arisen independently from crosses between two distinct diploid species and *T. urartu* (Bonjean and Angus 2001). Wild emmer is distributed throughout the 'Fertile Crescent' and was discovered by A. Aaronsohn in 1906 (Saranga et al. 2008). Durum wheat is used for semoules in the cous-cous, and it is also used for Levantine

dishes such as tabbula, kishk, kibba. When ground, its fine flour can be used for macaroni, pasta and bread.

1.1.2. Breeding of durum wheat

According to Pakendorf et al. (2008), the strategy of plant breeding is relatively simple. The basic elements comprise the following:

1) Recognise morphological traits and physiological and pathologic responses of plant species that are important for adaptation, yield and quality of crop plant species,

2) Design techniques that will evaluate the genetic potential for these traits in strains of the appropriate species,

3) Search out sources of genes for the desired traits that may be utilised in a breeding programme,

4) Devise means for combining the genetic potential for these traits into improved germplasm, varieties or cultivars.

Durum wheat, like bread wheat, is a self- fertilizing species. Therefore the durum wheat breeding methods are based on line selection. Breeders may use pedigree selection, bulk selection or combinations of these two methods for durum wheat breeding. In Figure 2 a breeding scheme for durum wheat breeding is shown. This scheme is used by the Austrian breeding company Saatzucht Donau as an example of one Austrian durum breeding program, which starts with crossing followed by selection several times until registration as a new cultivar.

The productivity of durum is generally accepted as an inherited factor with hundreds of genes being involved, so does the influence by the environment. Selecting for yield is considered one of the difficult jobs for the breeders because of the great heterozygosity and the genotype by environment interaction (Josephides 2000). In Italy and Spain the improvements in durum wheat varieties have increased through local breeding programmes. The increase in the number of grains per spike has been found to explain more than 50 percent of genetic gains in the number of grains per unit area (Royo et al. 2007). However, dwarfing genes and spike sterility are significant factors in yielding potential variation in durum wheat varieties (lvaro et al. 2008). In Turkey, Topala et al. (2004) found a significant variation in combining ability of thousand kernel weights and some other traits of local durum varieties. Breeding for yellow pigment content is one of the most important issues relating to durum wheat quality because consumers worldwide prefer pasta made from durum wheat of bright yellow colour. The bright yellow colour is the result of a higher concentration of carotenoid pigments in durum wheat endosperm (Patil et al. 2008). Durum was identified as salt-sensitive crop. Durum cultivars are relatively intolerant of

saline and sodic soils compared with bread or other hexaploid wheat cultivars. The selection by measuring the Na^+ and K^+ accumulated in the blade of a given leaf have the potential for improving salt tolerance in durum wheat breeding programs (Munns et al. 2000).

Breeding durum wheat for disease resistance is one of the most important issues regarding to durum breeding. The development of molecular markers will enhance breeding for disease resistance but the established techniques of plant breeding will remain relevant and important (Johnson 1992). To assist farmers, plant breeders work to develop cultivars with genetic resistance against most pathogens that may affect a particular region (Kelly and Miklas 1998). Since the rediscovery of Mendelian genetics, there have been technological breakthroughs that have been exploited in conventional plant breeding, such as hybrid vigor, polyploidy, biometry, chromosomal translocations and, recently, biotechnology (Rajaram 2005). Fusarium head blight (different *Fusarium spp*.), powdery mildew (*Blumeria graminis*), yellow rust (*Puccinia striiformis*), stem (black) rust (*Puccinia graminis*), leaf rust (*Puccinia triticina*) and tan spot (*Pyrenophora tritici-repentis*) are among the most important diseases of durum wheat in the world (Buerstmayr et al. 2009; Tomás and Solís 2000; Spielmeyer et al. 2005; Tadesse et al. 2007).

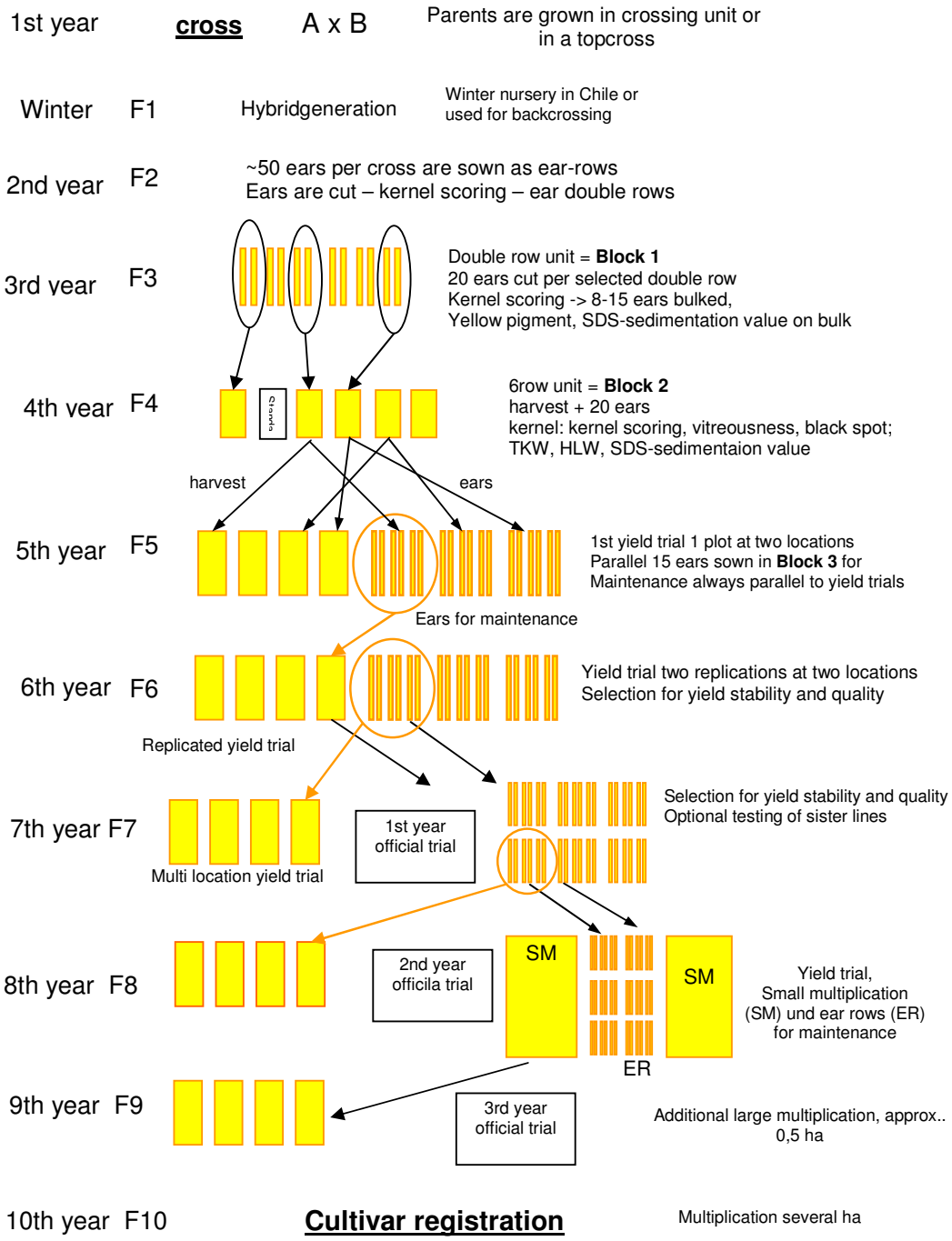


Figure 2: The breeding scheme for durum wheat practiced by the Austrian breeding company Saatzucht Donau (Lafferty 2008).

1.1.3. Genetic resources for durum wheat improvement

Apart from cultivated durum wheat (*T. durum*) other tetraploid wheat species like cultivated emmer wheat (*T. dicoccum*) or wild emmer wheat (*T. dicoccoides*) have potential as genetic recourses for durum wheat improvement (Table 2). Wild emmer is considered one of the best hopes for crop improvement because of their adaptive complexes to abiotic and biotic stresses (Oliver et al. 2007).

Agronomic traits in wild emmer include:

- Abiotic stress tolerance (salt, drought and heat)
- Biotic stress tolerances (powdery mildew, rusts, and Fusarium head blight)
- Grain protein quality and quantity and micronutrient concentrations (Zn, Fe and Mn) (Xie and Nevo 2008).

T. turaniacum is a tetraploid wheat and was grown in the past in the Mediterranean region and central Asia but now is almost disappeared. *T. carthlicum* is a tetraploid wheat with the Q factor and is considered a derivative of hexaploid wheat (Boniean and Angus 2001). *Agropyron* (Gaertn), which is a genus of the *Triticeae*, includes the crested wheatgrass complex as a representative species containing the P genome. This species is an important source for increasing the genetic variability of both durum and bread wheat. The P genome of *Agropyron* is a potential source of novel genes for traits, such as disease resistance, tolerance to drought, cold and moderate salinity (Soliman et al. 2001).

Table 2: The nomenclature of durum wheat* and their immediate wild relatives+.

Species	Common name
T. dicoccoides	Wild emmer
T. dicoccum	Cultivated emmer
T. paleocolchicum	Gorgian wheat
T. parvicoccum	None
T. durum	Macaroni or hard wheat
T. turgum	Rivet, cone or pollard wheat
T. polonicum	Polish wheat
T. turanicum	Khorassan wheat
T. carthlicum	Persian wheat

+Bonjean and Angus (2001), The nomenclature of durum wheat is according to Dorofeev *et al.* (1979).

1.2. Fusarium head blight

1.2.1. The genus *Fusarium*

The genus *Fusarium* was named 1809 by Link (Leslie and Summerell 2006). Fusarium is a large and common group of fungi. Because the spores are big and form readily on many substrate, many early mycologists described them (Booth 1971). Some *Fusarium* species lack a known sexual state (e.g. *F. culmorum*) and others have the perfect stage like for example *F. graminearum* (*Gibberella zeae*) (Bai and Shaner 1994). Fusarium is widely distributed on humans, plants and animals. Many plants have at least one Fusarium associated disease and it is found in normal mycoflora of commodities, such as rice, wheat, bean, soybean, and other crops. While most species are more common at tropical and subtropical areas, some inhabit in soil in cold climates (Leonard and Bushnell 2003).

1.2.2. Fusarium head blight species of cereals

The species found associated most frequently with head blight of wheat and other small-grain cereals are *F. graminearum, F. culmorum and F. avenaceum*. Among the other less frequently isolated species are *F. poae, F. cerealis, F. equiseti, F. sporotrichioides* and *F. tricinctum* (Bottalico and Perrone 2002; Šrobárová1 et al. 2008).

1.2.3. Host plants

The genus *Fusarium* can attack a wide range of host plants including: wheat, barley, oats, many other grains and plant species.

1.2.4. Biology of Fusarium on cereals

Figure 3: Macroconidia (top arrow), phialides (middle arrow), and sporodochium (bottom arrow) of *Fusarium graminearum*. Department of Agriculture and Agri-Food, Government of Canada. 2003)

Fusarium fungi are saprophytes and facultative parasites, the fungus can over winter and survives on crop debris such as grass stubble, chaff, cornstalk residues left on the soil surface and infected grain (Nelson et al. 1994). In most cases, inoculum may take the form of conidia (Figure 3), chlamydospores or hyphal fragments. In case where sexual spores are formed as for example *Gibberella zeae* (*F. graminearum*) and *Monographella nivalis* (*Fusarium nivale*), ascospores also represent an important form of inoculum. Spores are produced first on stem at the base of the plant or on crop debris. These spores are spread by rain or wind to the wheat ears.

Fusarium becomes a pathogen on wheat ears when it infects the flowers and causes Fusarium head blight (Parry et al. 1995).

1.2.5. Symptoms and damage

In humid and warm weather, mycelium and conidia of Fusarium develop abundantly in the infected spikelets, and the infection spreads via the rachis to adjacent spikelets or through the entire head. Partly white and partly green heads are diagnostic of FHB. The fungus also may infect the stem (peduncle) immediately below the head, causing a brown/purplish discoloration of the stem tissue. Additional indications of FHB infection are pink to salmon-orange spore masses of the fungus often seen on the infected spikelet and glumes during prolonged wet weather and many infected wheat kernels are shrivelled, lightweight, and are dull grayish or pinkish in colour (McMullen and Stack 1999). Infection with *Gibberella zeae* may lead to fruit bodies called perithecia on infected tissues, which contain ascospores (Guenther 2005). The estimation of yield reduction due to FHB may reach up to 80 percent (Bottalico et al. 2002, Windels 2000). In the United States and Canada, the re-emergence of Fusarium head blight in the 1990s has caused epidemics of varying severity on barley and on all classes of wheat (Windels 2000). The cumulative loss since the 1990's to the direct farm-gate value of Canadian wheat exceeds well over $1.5 billion. The USA has reported losses over $3.2 billion (Pandeya 2005). In China, more than seven million hectares of wheat are affected by FHB. It is estimated that up to 2.5 million metric tons of grain are lost to FHB epidemics in the Yangtze River Valley of China (Wang et al. 1987).

1.2.6. Fusarium mycotoxins

FHB infected grain may contain toxic substances called mycotoxins. The most frequently observed Fusarium mycotoxins are deoxynivalenol (DON) and zearalenone (ZON). These toxins are produced by several *Fusarium* species on a variety of cereal grains (Seeling et al. 2006). Zearalenone (ZON) with two metabolites (α-zearalenol and β-zearalenol), has a powerful estrogenic effect and causes fertility problems in pigs (D'Mello et al. 1999). Deoxynivalenol or DON (vomitoxin) is a mycotoxin that may cause vomiting and feed refusal in non-ruminant animals. Grain with DON would have to be ingested in very high amounts to pose a health risk to humans (McMullen and Stack 1999). The plasma elimination half-life of DON in pigs is about 4 hours. After oral administration of DON to rats, the major metabolic route is de-epoxidation to the corresponding methylene derivative. DON and its metabolites are primarily excreted via faeces but occur also in the urine (Schlatter 2004). Field fungi of the genus *Fusarium* have hardly an influence on both the sulfur speciation of wheat gluten proteins and the baking

properties (Prange et al. 2005). Since mycotoxins are considered to be unavoidable, the European Union has regulated the contamination levels of specific mycotoxins in food. Recently, the Scientific Committee on Food of the European Union set maximum limits of mycotoxins in cereals (Table 3), raw cereal grains , products derived from cereals and other manufactured foods (Gallo et al. 2008).

Table 3*: Maximum levels of mycotoxins, in the European Union.

Mycotoxin	Food product	Maximum Levels (μg/kg or ppb)
Deoxynivalenol	unprocessed cereals (other than durum wheat and maize)	1000 ppb
	unprocessed durum wheat	1500 ppb
	in durum wheat flour, maize flour and semolina,	750 ppb
	Product for direct human consumption containing cereals	400 – 500 ppb
	dried pasta	750 ppb
	processed food for infants and young children	100 – 200 ppb
Zearalenone		
	maize meal, maize flour, maize grits and maize oils	200 ppb
	cereal derived foodstuffs and breakfast cereals	75 ppb
	cereals based infant foods	20 ppb

*COMMISSION REGULATION (EC) No 856/2005 of 6 June 2005 amending Regulation (EC) No 466/2001 as regards Fusarium toxins.

1.3. Plant responses to FHB infection

In principle, there are three reasons for pathogen failure and a low level of disease. Either (1) the plant is unable to support the niche requirements of a potential pathogen; or (2) the plant possesses preformed structural barriers or toxic compounds that confine successful infections to specialized pathogen species; or (3) upon recognition of the attacking pathogen, defence mechanisms are elaborated and the invasion remains localised (Kosack and Jones 1996).

Information about the type of resistance is useful in choosing the most appropriate breeding and selection methodology. Resistance is broadly categorised into two groups:

1) race-specific type, 2) race-non specific type (Singh and Rajaram 2008).

The race-specific type of resistance is controlled by genes with major to intermediate effects, whereas the race-non-specific resistance is mostly controlled by genes with minor to intermediate and additive effects (Schippers et al. 1994). FHB resistance is considered non-race-specific, quantitatively inherited and involves several loci on different chromosomes (Kolb et al. 2001). In general, H_20_2, superoxide radicals (0_2^-) and hydroxyl radicals (OH^-) are thought to play key roles in defence responses in plants. Following infection, plants resistant to the invading pathogen develop a sustained increase in reactive oxygen species (Durner et al. 1997).

The mechanisms of the host-pathogen interaction between wheat and Fusarium are not fully understood. Some studies on *Fusarium graminearum* have shown that the ascospores develop on soil borne crop residues and can infect wheat florets during anthesis. Visible lesions are produced within 3±4 days under conditions favourable for disease development. From an infected floret, the fungus can spread up or down the spike from one spikelet to another. This spread is an important component in the overall damage caused by the disease (Pritsch et al. 2001). Some studies on *G. zeae* have shown that the hyphae of the fungus can directly penetrate ovaries, glumes and inner walls of the palea and lemma. Furthermore, symptoms progress down the stem from head infections (Guenther 2005).

Plants delay pathogen growth or resist pathogen attack by mobilizing a variety of biochemical and molecular defences. An incompatible interaction between the host and the pathogen results in the triggering of defence responses through signalling pathways (Geddes et al. 2008). Differences in activities of superoxide dismutase, catalase, phenylalanine ammonialyase, and ascorbic acid oxidase have been reported between resistant and susceptible varieties of wheat to FHB infection (Bernardo et al. 2007). Li et al. (2001) reported induction of several classes of chitinases in wheat spikes infected by *F. graminearum*. Kruger et al. (2002) identified a set of 29 different sequences in *F. graminearum* infected wheat. Some of these sequences encode proteins that may act in plant response to FHB infection. Geddes et al. (2008) identified 43 expressed

proteins due to FHB in barley 3 days after inoculation with *F. graminearum* such as malate dehydrogenase and peroxidases, and pathogenesis-related protein. These proteins could play a role in wheat defence to FHB. Fusarium mycotoxins like for example trichothecenes, play a role in the pathogenesis of Fusarium on plants and the importance of trichothecenes in disease may differ from one plant species to another (Desjardins et al. 1993).

With the immunogold labelling technique, Kang and Buchenauer (1999) found in *F. culmorum* infected wheat spikes and kernels a very close relationship of toxin accumulation with pathogenic changes in host cells, symptom appearance and colonization of host tissues by hyphae, suggesting that the toxins might play an important role in the disease development. Cell wall degrading enzymes are produced to assist infection during the infection of wheat spikes by *F. culmorum*, which lead to reduce cell wall components including cellulose, pectin and xylan (Kruger et al. 2002).

1.4. Mapping quantitative trait loci (QTL)

Many of the important traits for crops, such as yield potential and disease resistance, are controlled by multiple genes acting together to produce the desired trait. These quantitative traits are characterised by continuous variation. This is in contrast to qualitative traits, which show discrete variation and are controlled by one or few major genes. Quantitative trait loci (QTL) are regions of the DNA that are associated with a particular phenotypic trait. QTL analysis is considered a powerful approach to discover such agronomical useful genes.

The principle of genetic mapping is based on genes (loci) segregating via chromosome recombination during meiosis, thus allowing their analysis in the progeny. During meiosis, chromosomes assort randomly into gametes, such that the segregation of alleles of one gene is independent of alleles of another gene. When two genes are close together on the same chromosome, they do not assort independently and are said to be linked. Genes that are closer together or tightly-linked will be transmitted together from parent to progeny more frequently than those genes located far apart (Semagn et al. 2006). With the recent development of an arsenal of molecular markers that uncover population level polymorphisms, mapping genes that affect quantitative variation and associated with a particular phenotypic trait has now become feasible for natural populations (Mauricio 2001; Mueller and Wolfenbarger 1999).

The basic ideas behind QTL mapping are actually very simple, although the implementation of those ideas can be quite complex. In broad, the approach (Figure 4) according to Holsinger (1998) and Semagn et al. (2006) is:

1) producing a set of progeny of known parentage, such as recombinant inbred lines or backcross lines

2) constructing a linkage map for the molecular markers (ideally, a large enough number of markers to cover virtually every part of the genome)

3) measuring the phenotype and scoring the genotype at every marker locus of every individual in the progeny

4) collating the data and analysing these biometrically to identify the position and effects of QTL associated with variation to the phenotypic trait. The results also can be quite informative because they allow us to say more about the genetic influences on the expression of the trait in the study. QTL mapping programmes often estimate the effects at each locus individually.

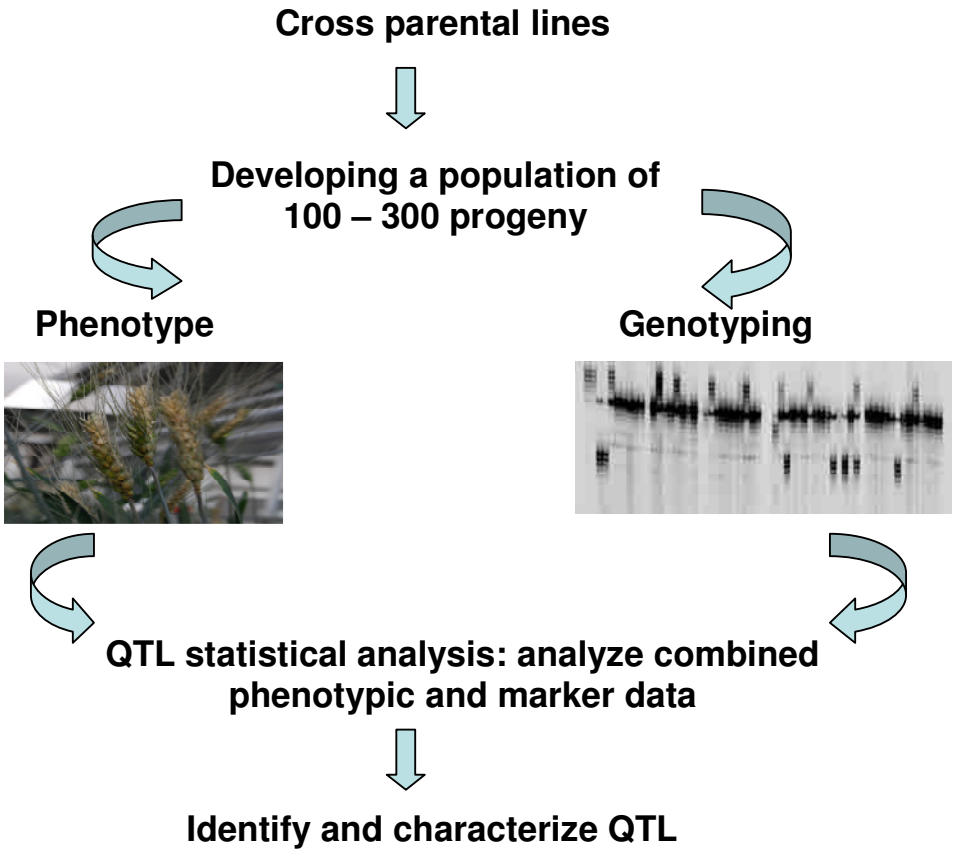


Figure 4: Major components of a QTL study

1.4.1. DNA markers for mapping

DNA markers (molecular markers) are tools that help to locate and identify parts of DNA that are located near a gene or genes of interest. DNA markers identify locations where the sequences differ among varieties. These can be locations within genes or in the DNA between genes, so long as they are unique sequences and differ between the plants of interest. Differences of this type are called polymorphisms, and there are a variety of ways to detect and use these signposts within the chromosomes (Suslow et al. 2002).

In plant breeding, molecular markers can be used as chromosome landmarks to facilitate the selection of chromosome segments including useful agronomic traits during the breeding process. These markers are particularly useful for incorporating genes for resistance to diseases that cannot be easily screened for and to accumulate multiple genes for resistance to specific pathogens and pests within the same cultivar (Dubcovsky 2004). Through the process of genetic linkage mapping, molecular markers that are linked to disease resistance genes can be identified, and these can then be applied in plant breeding programmes to assist in resistance gene

introgression (Vaughan et al. 1997). The use of markers in plant breeding is cost effective, highly reliable, accurate and many independent resistance genes can be diagnosed simultaneously (Gold et al. 1999). The marker assisted selection (MAS) approach took benefits from a combined product of traditional genetics and molecular markers. MAS is an efficient tool to speed up plant breeding. It also helps in the pyramiding of resistance genes (Slikova et al. 2003). Major DNA marker used in plant breeding and genetic research can be grouped into: RFLP-markers and PCR-based markers (e.g. RAPD, AFLP, SSR, SNP and DART) (Ovesna et al. 2002; Kassa et al. 2006).

1.4.2. Microsatellite markers

Microsatellites, alternatively known as simple sequence repeats (SSRs), short tandem repeats (STRs) or simple sequence length polymorphisms (SSLPs), are tandem repeats of sequence units generally less than 5 bp in length, e.g. $(TG)_n$ or $(AAT)_n$. The analysis of microsatellites is based on the polymerase chain reaction (PCR), which is much easier to perform than RFLP analysis and is highly amenable to automation. In plants, it has been demonstrated that microsatellites are highly informative, locus-specific markers in many species. The first microsatellite map in wheat possessed 279 microsatellites (Röder et al. 1998). There are several sources of wheat microsatellite primer sequences available in the public domain, which are coded by GWM (Röder et al. 1998), BARC (http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi), WMC (Somers et al. 2004), CFA (Sourdille et al. 2001) and CFD (Guyomarch et al. 2002).

1.4.3. Amplified fragment length polymorphism markers (AFLP)

The AFLP technique is based on selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases (Vos et al. 1995). Polymorphisms are detected from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis. The AFLP method is considered a powerful tool for the detection and evaluation of genetic variation in germplasm collections and in the screening of biodiversity as well as for fingerprinting studies (Ovesna et al. 2002). Tyrka (2002) confirms the usefulness of the AFLP technique for diversity studies and identification of common wheat cultivars. Lotti et al. (2000) suggested that AFLP can readily be applied for mapping durum wheat.

1.5. QTL mapping for FHB resistance in durum wheat

The main steps in QTL mapping of FHB resistance are: constructing a population, a linkage map, measuring the phenotype, scoring genotype and QTL analysis.

1.5.1. Population types for QTL mapping

Constructing a population is the first step in mapping FHB resistance. Populations are made by crossing two genetically divergent parents, which show clear genetic differences for FHB resistance. Progenies from the artificial population of recombinant inbred lines (RILs), backcross lines (BC) or doubled haploids (DHs) can be used for genetic mapping in self-pollinating species (He et al. 2001).

1.5.1.1. Recombinant inbred lines (RILs)

A population of recombinant inbred lines can be a powerful tool for genetic mapping. The RILs is formed by crossing two inbred strains followed by repeated selfing or sibling mating to create new inbred lines whose genome is a mosaic of the parental genomes (Broman 2005). RILs can be scored for the traits of interest and the data can then be compiled to develop a QTL map.

1.5.1.2. Back-cross populations (BC)

The backcross breeding method has been used by plant breeders for decades to incorporate specific traits into elite lines. Backcrossing requires a donor parent (has a gene of interest) and a recurrent parent (an elite line that could be improved by adding the gene of interest). This method works by crossing the F_1 hybrid (donor parent X recurrent parent) to the recurrent parent genotypes. The offspring, called the BC_1 generation for backcross 1, has 75% of the recurrent parent alleles and 25% of the donor parent alleles. Not all of the BC_1 offspring will have the desired alleles, so the plant breeder must have a way to determine which plants have the desired alleles and which do not (Poehlman 1990). To further reduce the number and size of the donor portion, backcross is repeated. With each round of backcrossing, the proportion of donor genome is reduced by 50%. The advanced backcross quantitative trait locus (AB-QTL) strategy, developed by Tanksley and Nelson (1996), has been proposed for introgression of exotic QTL alleles from a donor accession into elite germplasm. The method proved effective in detecting additive, dominant, partially dominant and over dominant QTL. Repeated backcrossing with the elite parent decreases the number and size of the exotic introgressions, which in turn reduces the burden of linkage drag from deleterious exotic QTL alleles.

This method uses molecular markers to identify beneficial alleles from unadapted germplasm with the potential to improve the agronomic performance of elite cultivated lines (Bernacchi et al. 1998).

1.5.1.3. Doubled haploid (DH) populations

Doubled haploids (DH) are increasingly used in plant breeding, and derivation of DH can fundamentally change the procedures of plant breeding. The terms haploid refers to a plant containing the gametic chromosome number (1n) or half of the somatic number of chromosomes (2n). By doubling the haploid complement the normal number of chromosomes is restored (Ahmad 2004). DH lines can be derived by anther or microspore culture or by interspecific crosses. The use of DH saves time, makes better use of genetic variation and increases selection efficiency by increasing genetic variance between families and by decreasing residual variance (Bordes et al. 2007).

1.5.2. Constructing a genetic map based on molecular markers

In recent years, wheat genomics research has increased the use of genetic maps to position a gene of interest between close flanking markers (Haley and Knott 1992). The application of molecular markers (RFLP, AFLP, SSR….*etc*.) in plant systems increases the efficiency of conventional plant breeding by carrying out indirect selection through molecular markers linked to the traits of interest (Gupta et al. 1999). A linkage map gives information on the position of markers within a linkage group. The map positions are inferred from estimates of recombination frequencies between markers. The distance between these markers is expressed in centimorgan (cM) which represents the recombination rates between them (Jones et al. 1997).

Based on polymorphic markers for parental lines, a segregating population as F_2, backcross, recombinant inbred and doubled haploids (DH) lines, can be scored and analysed. Then linkage analysis using computer programs will determine the distance between markers. For example CarthaGène (De Givry et al. 2005), JoinMap (Stam 1993) or Mapmaker (Lander et al. 1987). Programmes can be used for constructing a linkage map. Normally markers organized into linkage groups at the minimum LOD=3, and the maximum distance between two loci is 30 cM (Li et al. 2005). The mapping functions of Haldane (1919) or Kosambi (1944) used to calculate map units in centimorgan.

1.5.3. QTL analysis

Once the data are collected on each individual, statistical association between the markers and the quantitative trait are established through statistical approaches, such as analysis of variance (ANOVA) and simple linear regression statistics. These approaches tend to be methods of QTL detection that assess differences in the phenotypic means for single-marker genotypic classes (Doerge 2001). Lander and Botstein (1989) suggested interval mapping to test whether a QTL is likely to be present at the location within a specific markers interval or not. It actually tests a single QTL at each increment across the ordered markers in the genome. The results of the tests are expressed as LOD (logarithm of the odds) scores, which compare the evaluation of the likelihood function under the null hypothesis (no QTL) with the alternative hypothesis (QTL at the testing position) for the purpose of locating probable QTL. Zeng (1993) combines interval mapping technique with multiple regression analysis (composite interval mapping). This statistic test can be constructed by using a pair of markers to locate the testing position and at the same time using other markers to control the genetic background through a multiple regression analysis. A range of computer programs are available for conducting QTL analysis. Single marker regression can be done in any statistics package like SAS/STAT (Welch 2005) or Genestat (Payne et al. 2007) Software for interval mapping are included in specific QTL mapping programs like QTL Cartographer (Zeng et al. 1999), Plabqtl (Utz and Melchinger 1996) or QGene (Joehanes and Nelson 2008).

1.6. Breeding durum wheat for resistance of FHB

Resistance to FHB and its genetics has been the subject of many research projects in the world. As far back as the 1920s, plant pathologists and breeders observed that wheat genotypes seemed to differ in susceptibility to FHB, although it was difficult to clearly separate this from disease escape due to differences in maturity (Stack 2008). When FHB resistance in wheat is discussed today, the topic of “types of resistance” is sure to arise. In 1963, Schroeder and Christensen introduced this concept (Ambroz et al. 2006). Resistance to FHB is separated into five categories: Type I, resistance to initial infection; Type II, resistance to spread of infection; Type III, resistance to kernel infection; Type IV, tolerance; and Type V, resistance to the mycotoxin deoxynivalenol (DON) (Lemmens et al. 2005; Mesterhazy 2002).

Many sources of resistance to FHB have been reported and numerous genetic studies have been performed in hexaploid wheat (Buerstmayr et al. 2009). The spring wheat cultivar Sumai 3, including derived lines such as ‘Ning 7840’, is possibly the most widely used source of resistance to FHB in the world in hexabloid wheat (Rudd et al. 2002). On the other hand,

information in tetrapliod wheat is rather limited. However, a source of effective resistance to FHB has not been found in durum wheat so far. Wild emmer, with the genome AABB, represents an important gene pool for resistance to FHB in tetrapliod wheat (Buerstmayr et al. 2003; Oliver et al. 2007). Buerstmayr et al. (2003) evaluated 151 *T. dicoccoides* genotypes originating from Israel and Turkey for FHB spread. Most of the tetraploid accessions were highly susceptible, only a few showed moderate resistance. Among the eight *T. dicoccoides* lines with the lowest relative infection rates, five originated from the *Mt. Gerizim* population, and three from the *Mt. Hermon* population. A study by Oliver et al. (2007) using 416 accessions of wild emmer wheat tested for FHB spread, indicated a wide variation in response to FHB, ranging from highly resistant to highly susceptible. Several accessions showed minimal disease development across two or more seasons. Oliver et al. (2008) evaluated 376 accessions of five cultivated subspecies of *T. turgidum*. Evaluation data showed that 16 *T. turgidum* subsp. *carthlicum* and 4 *T. turgidum* subsp. *dicoccum* accessions consistently exhibited resistance or moderate resistance to FHB. These accessions likely carry genetic resistance to FHB. Otto et al. (2002) identified a major FHB resistance QTL on chromosome 3A, which explained 37% of the phenotypic variation. A linkage map around the QTL was constructed using a population of 83 RICL individuals for chromosome 3A of *T. dicoccoides* in a 'Langdon-16' durum background. Somers et al. (2006) found 2 QTL on chromosomes 2BL and 6BS associated with FHB resistance in a doubled-haploid (DH) population of a tetraploid cross of durum wheat ('Strongfield') with *Triticum carthlicum* ('Blackbird').

The review of Buerstmayr et al. (2009) shows that the most important QTL for FHB in common and durum wheat are located on chromosomes 1B (2 regions), 1D, 2A (2), 2B (2), 2D (2), 3A, 3B (2), 3D, 4B, 4D, 5A, 5B, 6A, 6B, 7A, and 7B (2).

2. Material and methods

2.1. Plant material

A population of 105 backcross lines (BC_1F_6) derived from the cross of the wild emmer (*Triticum dicoccoides),* line 'Mt. Gerizim#36' with *T. durum* (cultivar 'Helidur') was used in the project.

2.1.1. The donor parent

The wild emmer line *T. dicoccoides: Mt. Gerizim#36* (Figure 5) is a tetraploid wheat from Middle East. Altitude average 800 (m), average temperature (◦C) 17, average rainfall (mm) 700. Mt. Gerizim#36 was among the most Fusarium resistance wild emmer lines in a set of 150 wild emmer lines evaluated by Buerstmayr et al. (2003) for Fusarium resistance.

2.1.2. The recurrent parent

T. durum: cultivar Helidur is an Austrian durum wheat cultivar (registered in 1993) and it has well-adapted agronomic characters for cultivation in Europe. It is however highly susceptible to FHB (Buerstmayr et al. 1996).

Figure 5: A: Mt. Gerizim#36 , B: Helidur

2.1.3. Construction of mapping population

F_1 plants were produced in 2002 by crossing Mt. Gerizim#36 (donor parent) with Helidur (recurrent parent) in the greenhouse of IFA in Tulln. In the following several F_1 plants were backcrossed to the recurrent parent. 105 BC_1F_1 seeds were used to produce BC_1F_6 plants using single seed descent to achieve higher homozygousity (Figure 6). Seeds from individuals BC_1F_6 plants were harvested and used for the experiments.

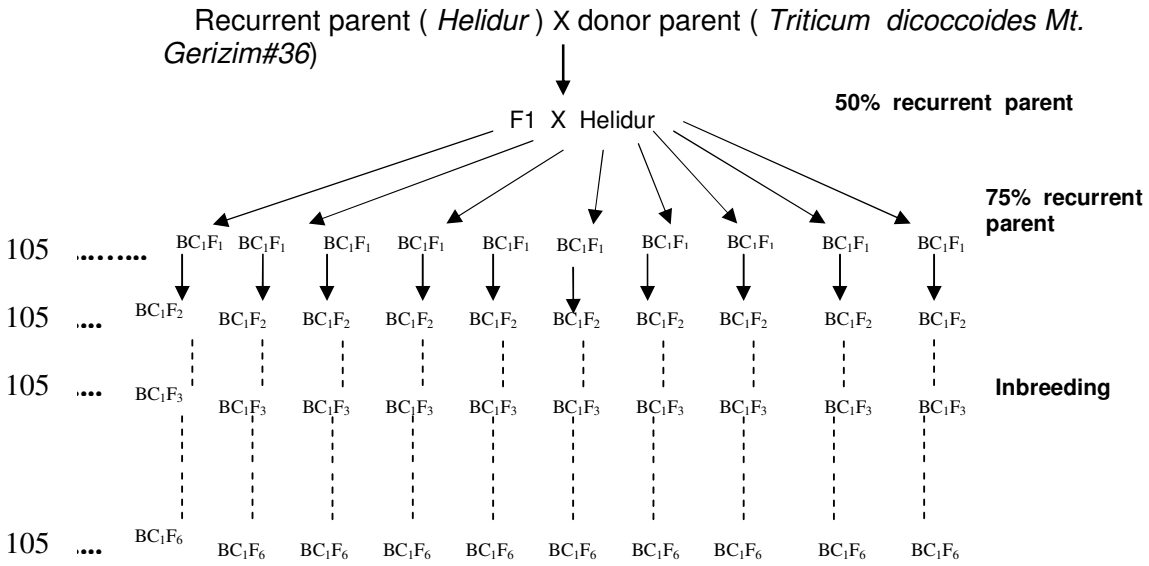


Figure 6: Schematic diagram illustrating the development of BC_1F_6 lines from the cross of Helidur with Mt. Gerizim#36.

2.2. Field and greenhouse experiments

The experiments (Table 4) were conducted in the field and the greenhouse of IFA in Tulln, 30 km west of Vienna, at 180 m above sea level. 105 B_1F_6 derived lines together with parents and controls were planted each season using multi seasonal block design.

Table 4: Summary of the experiments.

Exp.	code	year	sowing time	place	blocks	inoculation	seeds
1	Field	2007	Mid February	field	3	*F. culmorum*	$BC_1F_{6:7}$
2	GH1	2007	Mid July	greenhouse	2	*F. graminearum*	$BC_1F_{6:8}$
3	GH2	2008	20th January	greenhouse	3	*F. graminearum*	$BC_1F_{6:9}$
4	GH3	2008	Mid July	greenhouse	3	*F. graminearum*	$BC_1F_{6:10}$
5	GH4	2009	10th February	greenhouse	2	*F. graminearum*	$BC_1F_{6:11}$

2.2.1. Field experiment:

In total 40 seeds per genotype were sown in multi-trays. After germination the seedlings were vernalized at 4 ^{0}C for 4-6 weeks. 8 -10 seedlings were planted in each plot. Plots were 50 cm long, with 20 cm row spacing. The soil type is a meadow-czernosem. The average temperature and annual precipitation were 16°C and 320 mm. The experiment was twice fertilized with N-P-K (15-5-20) at a total rate of 20 g /m^2.

2. 2.2. Greenhouse experiments

In total 30 seeds for each genotype were sown in multi-trays, after germination the seedlings were vernalized at 4 ^{0}C for 4-6 weeks. After vernalization, 6-8 seedlings were planted in 15 cm pots for each genotype. Pots were filled with mixed soil of 70% compost, 20% peat and 10% sand. The blocks were planted several days apart, resulting in a few days difference in anthesis date between the blocks. During the first 30 days the temperature in the greenhouse was 14 °C during the day and 10 °C during the night with 12 h photoperiod. At anthesis the temperature was increased to 20-22 °C during the day and 18 °C during the night with 16 h photoperiod. Plants were fertilized two times with N-P-K (15-5-20) 1,5-2 g per pot, fumigation with sulphur was done twice per the week (Figure 7). For all experiments, fumigation and fertilization was stopped at anthesis to avoid effect on the Fusarium resistance tests.

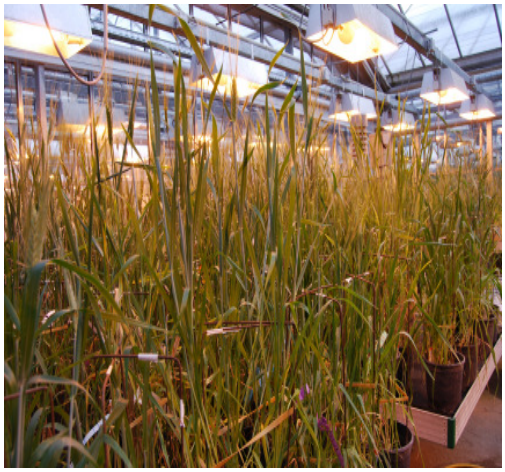


Figure 7: green house IFA Tulln

2.3. Inoculation experiments

The inoculation was done with two *Fusarium* species: *F. culmorum* (isolate - IFA 66) for the field experiment and *F. graminearum* (isolate - IFA 104) for green house experiments.

2.3.1. The preparation of inocula

A mixture of wheat and oat kernels (1 part oat, 2 parts wheat, v/v) was soaked overnight in water in baby food jars (20 g seeds/jar). Excess water was decanted and the jars were autoclaved at 121°C for 20 minutes. Then the kernels were seeded with *F. culmorum* conidia. Jars were left two weeks in diffuse daylight at room temperature and thereafter incubated for 4 weeks in the refrigerator at 5°C. Macroconidia were washed off the seeds with deionised water (Snijders and Eeuwijk 1990).

F. graminearum macroconidia were produced in liquid mung bean medium. Dry mung bean (*Vigna radiata L.*) seeds (20 g l^{-1}) were boiled in distilled water for 20 minutes. The liquid phase was transferred into glass bottles and autoclaved at 121°C for 20 minutes. After inoculation of the mung bean medium, continuous aeration with sterile air at room temperature caused macroconidia development within 5 days. Bottles of mung bean medium with conidia were stored over night in cold room (4 °C) to precipitate the conidia (Bürstmayr et al. 2002).

Conidial concentrations were determined for *F. culmorum* and *F. graminearum* using a Bürker-Türk counting chamber and adjusted to 50,000 conidia per ml with deionised water. Aliquots with the desired concentration were stored in 15 ml tubes at -80°C until use.

2.3.2. Inoculation technique

At anthesis plants were inoculated artificially with Fusarium macroconidia using two different variants of the single floret inoculation technique:

1) inoculation with injury using a micropipette injection (500 macroconidia) in a single spikelet (for the experiments field, GH2, GH3 and GH4) (Figure 8). A micropipette equipped with an injection needle was punched through the outer glumes and 10 µl inoculum of a 50,000 conidia per ml suspension was injected into one spikelet per spike (500 conidia / spike),

2) in the experiment GH1 (experiment 2, see Table 4) a droplet (10 µl) with 500 conidia was placed directly into two florets.

On each inoculation day we always used freshly prepared conidial suspensions using the frozen aliquots (one frozen tube of conidia was diluted in one liter water to reach 50,000 conidia per ml of suspension). On average 8 spikes were inoculated in each replication. After inoculation,

individual spikes were covered overnight with plastic bags to keep high relative air humidity in order to improve infection success.

Figure 8: A: Flowering ears were labelled, B: the inoculation of the plants with Fusarium spores using injection with a micropipette, C: spikes were covered with plastic bags to keep high humidity, D: disease development was scored.

2.4. Disease assessment

2.4.1. Spread of Fusarium symptoms (Type II resistance)

The BC_1F_6 lines together with the parents were evaluated for FHB spread within the spike. The number of visually infected spikelets was recorded 14, 21 and 28 days after inoculation (dai) (Table 5) as a measure for disease spread within the ear.

Table 5: Experimental layout for scoring infected plants.

Exp.	code	year	Season	place	scores taken at		
					14 dai *	21 dai*	28 dai*
1	field	2007	Spring	field		x	
2	GH1	2007	Fall	greenhouse		x	x
3	GH2	2008	Spring	greenhouse	x	x	x
4	GH3	2008	Fall	greenhouse	x	x	x
5	GH4	2009	spring	greenhouse	x	x	x

*dai: days after inoculation

Different methods were used for the calculation of FHB spread/severity: 1) as percent of infected spikelets (%DS) at 14, 21 and 28 dai, 2) absolute number of infected spikelets (n.DS) at 14, 21 and 28 dai, 3) the increase in numbers of infected spikelets per day as a measure for the speed of FHB spread (SFS) for the periods: a) 14 – 21 dai b) 21 – 28 dai and c) 14 – 28 dai and 4) the area under the disease progress curve (AUDPC) using percent of infected spikelets (%DS) parameter (Steiner et al. 2004).

2.4.2. Wilting

A head was classified as wilted when the upper part of the spike bleached at once due to a block of the supply of water and/or nutrients to the upper part of the ear. The number of wilted spikes was counted at the same time when FHB spread was recorded.

2.4.3. FHB incidence

An ear was classified as diseased when at least one spikelet was bleached. A small proportion of inoculated heads remained without symptoms until 28 dai. The percentage of symptomatic ears among all inoculated spikes was calculated as a measure for disease incidence after wounding.

2.4.4. Other traits and powdery mildew

Plant height was measured in the plots as the distance in cm from the soil surface to the top of the heads excluding awns. The number of spikelets per head was recorded for each plot of inoculated heads and the length of the heads in cm 'Spike length' for non-inoculated heads from the same plots. The plants were evaluated for spike colour in the non-inoculated heads for each plot. At ripening, ears of the genotypes were scored as green, greenish-black and black on a 1-3 scale. Plants heads were scored for ear type and brittle rachis on a 1 (*T. dicoccoides* type) - 3 (Helidur type) scale. Brittle rachis, which causes spontaneous shattering of spikes is considered an undesirable trait in domesticated species. After harvesting, the heads were separated in wild (*T. dicoccoides*) to cultivated (Helidur) heads. For waxiness, plants were scored in a 1 (*T. dicoccoides* type) to 5 (Helidur type) scale during the time of FHB assessment (Figure 9). Date of anthesis and spikelets number was also recorded for each spike (Table 6). The disease severity of powdery mildew (*Blumeria graminis*) was assessed visually (Figure 10) as the percentage of the disease on leaves and spikes on a 1-5 scoring scale.

Table 6: Assessment of developmental and morphological traits as well as powdery mildew severity evaluated on the BC_1F_6 lines of the mapping population.

Trait		**Experiments**				
		Field	**GH1**	**GH2**	**GH3**	**GH4**
	scale	**Spring 07**	**Fall 07**	**Spring 08**	**Fall 08**	**Spring 09**
Plant height	cm	x		x	x	x
Waxiness	1- 5			x	x	x
Ear type	1- 5			x	x	x
Spike length	cm			x	x	x
Brittle rachis	1- 3			x	x	x
Number of spikelets	no	x	x	x	x	x
Spike colour	1-3			x	x	x
Date of anthesis	-	x	x	x	x	x
Powdery mildew severity	1- 5	x		x		

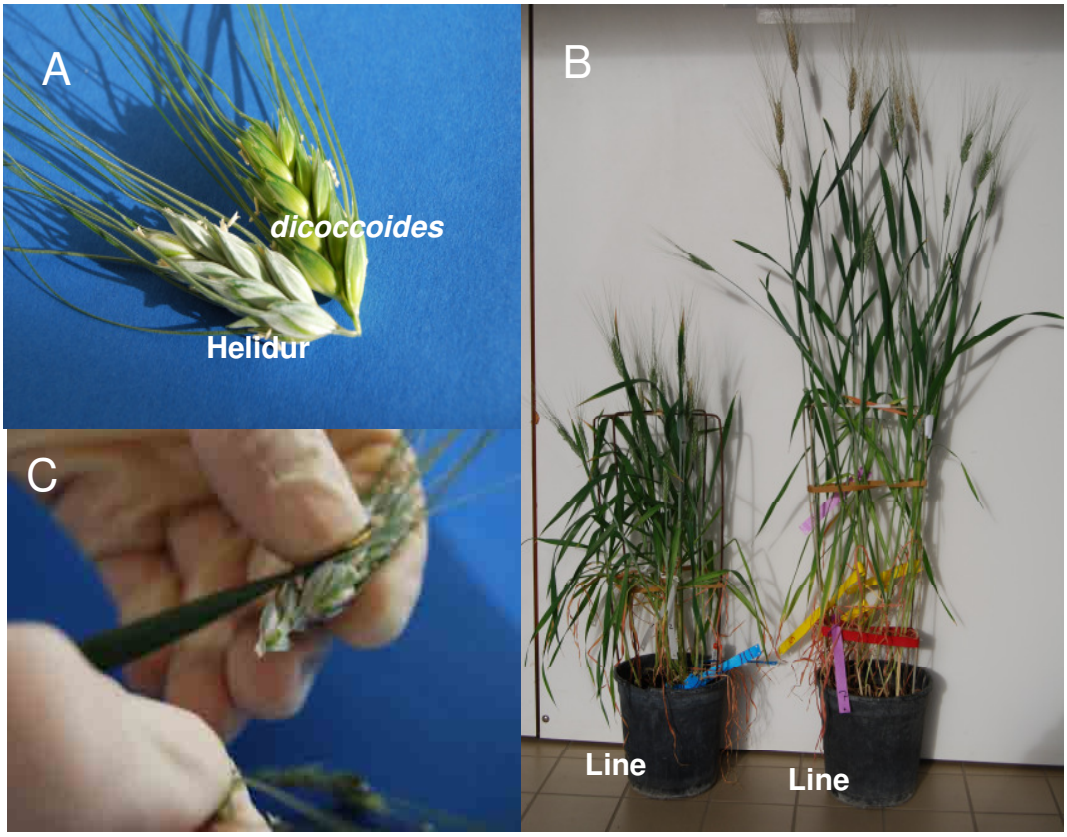


Figure 9: Developmental and morphological traits.
A: the differences between two lines for waxiness , B : plant hight and C: brittle rachis ,

Figure 10: Differences between lines for powdery mildew severity.

2.5. Genotyping

2.5.1. DNA extraction

The DNA was isolated from young leaves using the CTAB extraction method based on method of Saghai-Maroof et al. (1984).

After grinding of 300-400 mg of lyophilized tissue from each genotype in 15 ml polypropylene tubes, tubes were incubated at 65°C for 60-90 min with 9 ml of warm CTAB extraction buffer (appendix 1). Samples were left to cool down for 5 min and then 4.5 ml chloroform/isoamyl alcohol (24/1) was added to each sample. Samples were gently mixed for 5-10 min and then centrifuged for 10 min at 1300-1500 x g at room temperature (RT). The aqueous layer was transferred to new 15 ml tubes, 4.5 ml chloroform/isoamyl alcohol (24/1) was added, and samples were mixed and centrifuged again. Once more, the top aqueous layer was transferred into new 15 ml tubes containing 25 µl of 10 mg/ml RNase A (pre-boiled) mixed by gentle inversion and incubated for 30 min at RT. 6 ml of isopropanol (2-propanol) was added, gently mixed again. Then precipitated DNA was removed with a glass hook and placed in a 5 ml plastic tube containing 1 ml of TE-8 (10 mM Tris - 8.0, 1 mM EDTA - pH 8.0). Caped tubes were gently shaken overnight at RT to dissolve DNA. The next morning DNA was again precipitated by adding 50 µl of 5 M NaCl and then 2.5 ml absolute EtOH, removed with a glass hook and placed in a tube containing 3-4 ml of WASH 1 (76% EtOH, 0.2 M NaOAc) for about 20 min. DNA on hooks was briefly rinsed in 1-2 ml of WASH 2 (76% EtOH, 10 mM NH_4OAc) and transferred to 5 ml plastic tubes containing 0.5 ml TE buffer to dissolve DNA. The tubes were shaken gently overnight at room temperature to dissolve DNA.

DNA concentration was quantified on Pharmacia Gene Quant photometer. DNA samples were diluted to a concentration of 100 ng/µl and stored at –20°C.

2.5.2. Molecular marker analysis

Two types of molecular markers were used: 1) microsatellites (SSR) and 2) amplified fragment-length polymorphism (AFLP) markers.

2.5.2.1. Microsatellite markers (SSR)

Microsatellites were the first markers we used. 200 SSR primer pairs were tested on both parents and the polymorphic markers were applied to the population. The primers were synthesized according to the primer sequences published in the GrainGenes database (http:// graingenes.org). The analysis of microsatellites was performed using fluorescent fragment detection on a LI-COR 4200 DNA dual-dye sequencing system. For this method either one of the SSR primers was directly labelled with a fluorochrome (IRD700 or IRD800) or had a M13 tail. In the latter case a third primer a fluorochrome labelled M13-30 oligo (5' CCC AGT CAC GAC GTT G 3') was added to the PCR reaction.

PCR components of the M13 primers and direct labelling primers differ mainly in the addition of the M13-30 primer and the concentration of F-primer. The PCR reaction for direct labelling primers were performed using different programmes (depending on the annealing temperature of the primer). For M13 primers, touch down programmes were used (appendix 2).

PCR products were loaded on 0.7 % polyacrylamide gels. Samples were diluted before loading with 1:5 or 1:10 5µl of formamide tracking dye (95% formamide deionised, 0.5 mM EDTA, 0.1 mg/ml pink fuchsin) and denatured for 5 min at 95°C. Electrophoresis was performed at constant power of 40 W and a constant temperature of 48°C on a Li-COR 4200 dual dye sequencing system. Gels images were loaded on computer and scored visually using image processing programs.

2.5.2.2. Amplified fragment-length polymorphism markers (AFLP)

The AFLP marker method is based on Vos et al. (1995). Four steps were made starting with preparation of DNA and adapters. 250 ng of DNA was prepared for each genotype. Adapters were made from mixed single stranded oligos. The second step was restriction and ligation of the DNA. The next step is incubation at 37°C for 3 hours and then over night at room temperature. The samples were diluted four fold with ddH2O.

The third step was pre-selective PCR amplification of restricted - ligated DNA fragments (no selective nucleotide primers were used). The fourth step was selective amplification after dilution of pre-selective PCR amplification. This step was done using preselective dilution PCR product (appendix 3). PCR products were loaded on 0.7 % polyacrylamide gel after denaturion at 95°C for 5 min with 5 µl formamide tracking dye, using 1XTBE buffer in a C.B.S chamber.

Electrophoresis conditions were set at a constant current of 65 W and 40-50°C during 1 to 3 hours. Using the three fluorescent-labeled primers, FAM, C3 and C5 it was possible to load three different PCR products on the same gel. After electrophoresis, the glass plates containing the gels were placed on the laser scanner Typhoon (Typhoon Trio., Variable Mode Imager, Amersham Biosciences 2004), and were scanned with different emission filters for FAM, C3 and C5 . Images were scored visually using image processing programs (appendix 4).

2.6. Data analysis

2.6.1. Field data analysis

Field data were analysed using Genestat version 7, SPSS version 10.0, SAS/STAT (Version 8.02). Analysis of variance (ANOVA) was done to estimate the effects of experiments (Exp) and lines (Line) according to the following model (see 1):

(1) $Y_{ij} = \mu + Exp_i + e_i + Line_j + (Exp*Line)_{ij} + e_{ij}$

Pearson's correlation coefficient was used to calculate the correlation between experiments and traits. Broad sense heritability (H^2) was calculated according to Nyquist (1991).

$H^2 = 1- (MS_{GxE}/ MS_G)$

MS_{GxE}: mean square genotype x experiments

MS_G: mean square genotype

2.6.2. Marker and QTL analysis

A linkage map was constructed using CarthaGène (De Givry et al. 2005). This CarthaGène (www.inra.fr/mia/T/CarthaGene.) version was modified by C. Nelson for mapping advanced back cross population and running under LINUX assuming the Kosombi mapping function. A logarithm of odds (LOD) threshold of 3 and distance threshold equal 30 cM was set for grouping. The most-likely marker orders were determined using the build, annealing, flips and bestprintd commands for determining the best distance and order.

QTL analysis was done applying simple interval mapping (SIM) and composite interval mapping (CIM) (Zeng 1993). The program QGene (www.qgene.org) version-4.2.3 (Nelson 1997) was used for the analysis. Permutation test (Doerge and Churchill 1996) for the traits was performed at significance level 0.05 and 0.01. Graphical maps of chromosomes were generated using MapChart version 2.1 software (Voorrips 2001).

3. Results

3.1. The variation in population to Fusarium head blight resistance

The BC_1F_6 lines showed continuous variation to FHB spread at 14, 21, 28 days after inoculation (dai) as shown in Figure 11. The average increase in FHB symptom severity was larger in the interval 14 dai to 21 dai compared to 21 dai to 28 dai for percent of infected spikelets (%DS) and absolute number of infected spikelets (n.DS).

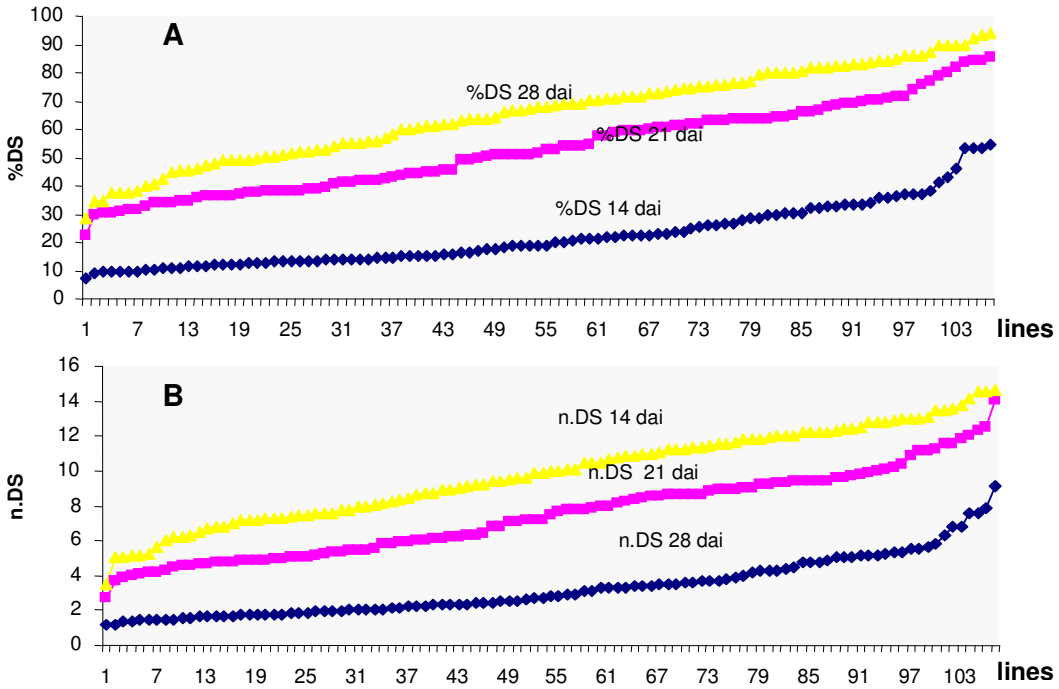


Figure 11: Variation among 105 BC_1F_6 lines for mean values of (**A**) %DS and (**B**) n.DS 14, 21 and 28 dai.

3.2. FHB spreading

The BC_1F_6 lines together with parents showed significant variation ($\alpha = 0.05$) for FHB spread within spikes measured as percent of infected spikelets (%DS) and absolute number of infected spikelets (n.DS) 14 dai for the experiments GH2, GH3 and GH4, 21 dai for all experiments and 28 dai for the experiments GH1, GH2, GH3 and GH4. The frequency distribution for %DS and n.DS is illustrated in Figure 12. The *T. dicoccoides* parental line showed significantly lower FHB spread in all experiments compared to the durum wheat parent Helidur (Table 7). For example *T. dicoccoides* had an average 35% for %DS and 3.8 for n.DS 21 dai, while Helidur had an average 48.3% for %DS and 6.9 for n.DS 21 dai. Population mean had low average (21.2 % for %DS as example) at 14 dai, whereas the average was higher (50.5% and 63.6%) at 21 and 28 dai. The

development of the disease on the parental lines is shown in Figure 13. The data in the Figure 13 were obtained from three times scored experiments (GH2, GH3 and GH4) at 14, 21 and 28 dai.

Table 7: Population mean values, parental mean values for % DS and n.DS obtained in each single experiment and mean values over all experiments, as well as broad sense heritability (H^2) for all mean values.

Trait	Days after inoculation	Experiments	Population mean	Population range	*T.dic**	Helidur	H^2
%DS	14	GH2	27.9	10.3-75.6	18.8	22.7	
		GH3	21.1	5-58.8	7.7	15.7	
		GH4	14.4	3.2-45.9	8.4	16.2	
		means	21.2	6.7-53	11.6	18.2	0.89
	21	Field	78.9	41.8-100	92.2	68.1	
		GH1	25.3	5.7-76.6	14.7	30.5	
		GH2	58.5	17.1-100	34	52.3	
		GH3	45.5	6.8-96.3	12.2	39.8	
		GH4	43.5	10-89	21.6	50.8	
		means	50.5	21-85	35	48.3	0.83
	28	GH1	37.9	6.1-100	32.6	50.9	
		GH2	83.9	36.8-100	91.3	88.9	
		GH3	64.4	19.4-100	47.3	68.9	
		GH4	67.4	19.4-100	30.3	66.2	
		means	63.6	26.7-92	50.4	68.7	0.83
n.DS	14	GH2	3.9	1.3-11.7	2	3.2	
		GH3	3.3	0.8-10.7	0.9	2.6	
		GH4	2.1	0.5-6.6	1	2.6	
		means	3.2	1.1-8.4	1.3	2.8	0.89
	21	Field	9.8	5.3-14.8	10	7.4	
		GH1	4.1	1-13.2	1.5	5.2	
		GH2	8.3	2-14	3.9	7.3	
		GH3	7.2	1-16.4	1.4	6.8	
		GH4	6.6	1.5-13	2.3	8	0.84
		means	7.2	2.6-13.8	3.8	6.9	
	28	GH1	6.2	1-16.7	3.1	8.1	
		GH2	11.8	4-17	10.1	12.6	
		GH3	10.2	1.2-17.4	5.4	11.5	
		GH4	10.1	2.2-18	3.3	10.4	
		means	9.6	3.3-14.3	5.5	10.7	0.84

**T. dic = T. dicoccoides Mt Gerizim # 36*

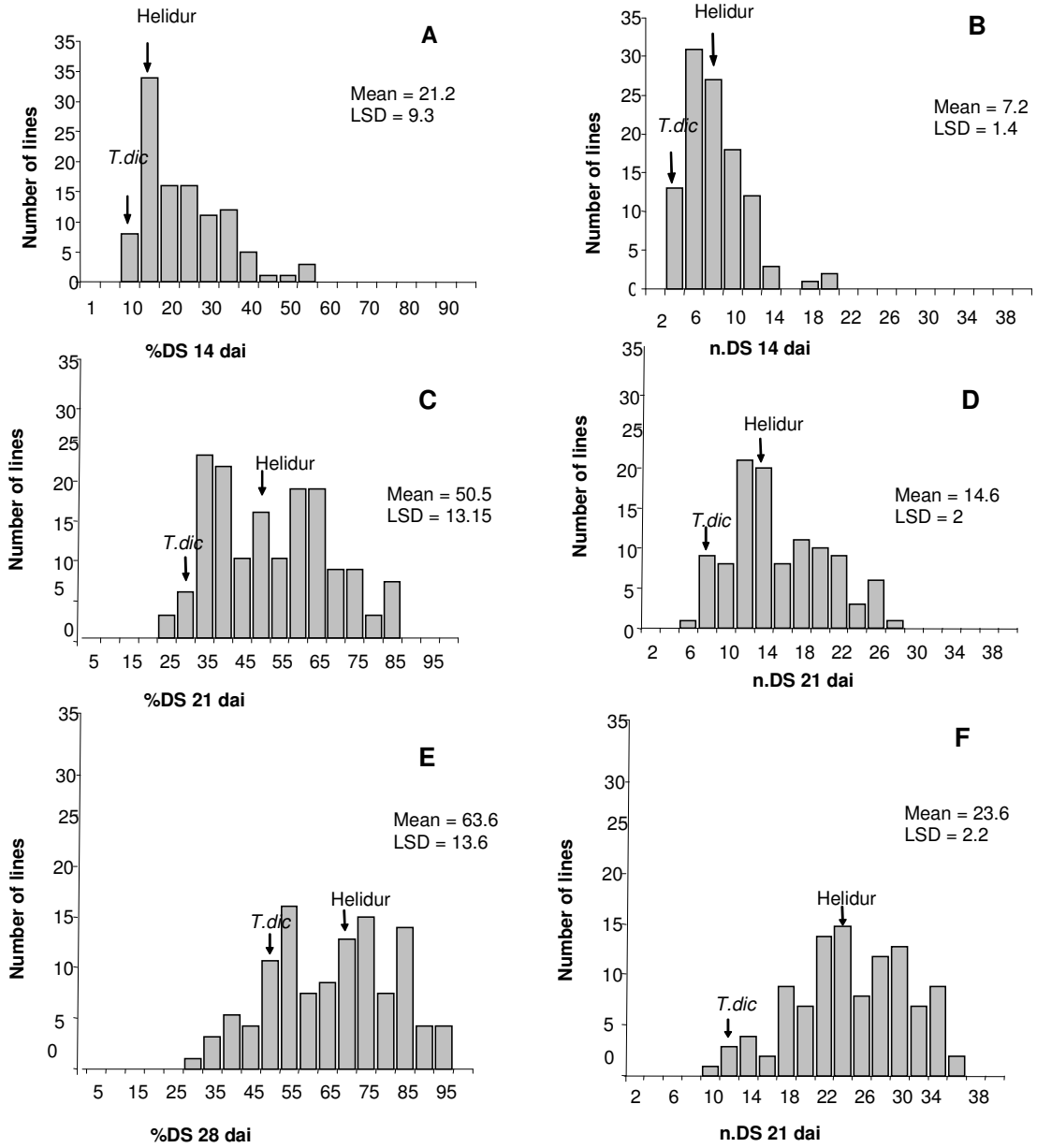


Figure 12: Histogram of 105 BC_1F_6 lines for mean values for FHB spread 14 dai (GH2,GH3 and GH4) (**A** and **B**), 21 dai (field ,GH1,GH2,GH3 and GH4) (**C** and **D**) and 28 dai (GH1,GH2,GH3 and GH4) (**E** and **F**) measured by percent of infected spikelets (%DS) and absolute number of infected spikelets (n.DS). The least significant difference (LSD) for comparison of lines means (α = 0.05) are given. Values of the parental lines are indicated by arrows. (T. dic: *T. dicoccoides*).

Analysis of variance (ANOVA) was calculated for %DS and n.DS assessed at each observation date (Table 8). The broad sense heritability of FHB spread ranged from $H^2 = 0.83$ to $H^2 = 0.89$ for %DS and n.DS 14, 21 and 28 dai (Table 6).

Table 8: Analysis of variance for FHB spread as %DS and n.DS (14 dai for GH2, GH3 and GH4. 21 dai for field, GH1, GH2, GH3 and GH4. 28 dai for GH1, GH2, GH3 and GH4).

	Scoring date	Source of variation	df	MS	F-value	P
%DS	14 dai	Exp	2	11997	16.96	0.006
		Exp*block (error 1)	5	707.3	4.89	
		Lines	106	1561.7	10.8	<.001
		Exp*Line	211	167.9	1.16	0.093
		Residual (error 2)	517	144.6		
	21 dai	Exp	4	106723.7	87.07	<.001
		Exp*block (error 1)	8	1225.7	4.20	
		lines	106	3021.4	10.34	<.001
		Exp*Lines	420	509.8	1.75	<.001
		Residual (error 2)	804	292.1		
	28 dai	Exp	3	91397.6	52.93	<.001
		Exp*block (error 1)	6	1726.7	5.53	
		Lines	106	3276	10.49	<.001
		Exp*Lines	315	541.3	1.73	<.001
		Residual (error 2)	579	312.2		
n.DS	14 dai	Exp	2	205.885	11.13	0.014
		Exp*block (error 1)	5	18.497	5.52	
		Line	106	36.843	10.99	<.001
		Exp.Line	211	4.117	1.23	0.035
		Residual (error 2)	516	3.353	2.92	
	21 dai	Exp	4	1165.96	42.86	<.001
		Exp*block (error 1)	8	27.203	3.73	
		Line	106	75.976	10.41	<.001
		Exp*Line	420	12.48	1.71	<.001
		Residual (error 2)	803	7.299	0.83	
	28 dai	Exp	3	1541.23	35.48	<.001
		Exp*block (error 1)	6	43.443	5.46	
		Line	106	87.138	10.95	<.001
		Exp*Line	315	13.954	1.75	<.001
		Residual (error 2)	579	7.955	6.35	

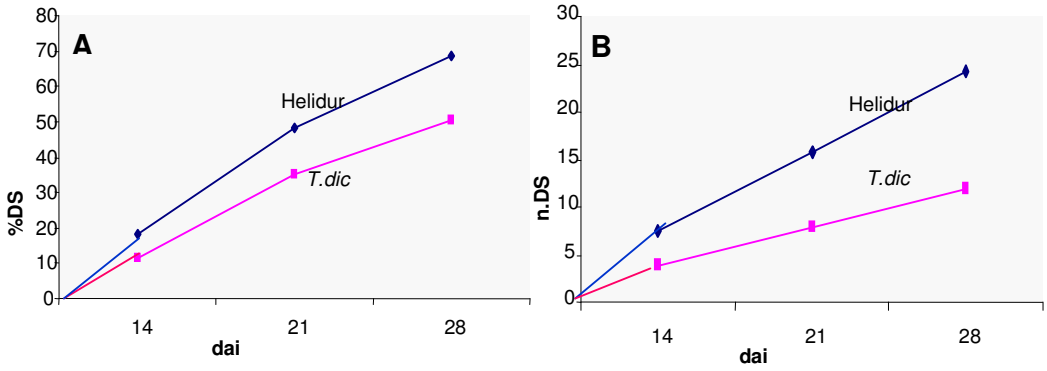


Figure 13: Spread of FHB on the parental lines for **(A)** %DS and **(B)** n.DS 14, 21 and 28 dai for mean values obtained from the experiments GH2, GH3 and GH4. (T. dic : *T. dicoccoides*).

Correlations between experiments for FHB spread (%DS) were significant for single experiments and means over experiments (Table 9). A high coefficient of correlation was revealed for GH2 with GH3 at 21 dai (r = 0.71).

Table 9: Pearson correlation coefficient for mean values of FHB spreading measured by %DS 21 dai among field, GH1, GH2, GH3 and GH4.

Traits	**GH1**	**GH2**	**GH3**	**GH4**
Field	0.20 *	0.41 **	0.36 **	0.32 **
GH1		0.48 **	0.64 **	0.45 **
GH2			0.71 **	0.58 **
GH3				0.60 **

** p < 0.01; * p < 0.05;

Correlations between experiments for FHB spread measured as n.DS were highly significant for single experiments and means over experiments (Table 10). A high coefficient of correlation was revealed for GH2 with GH3 at 21 dai (r = 0.71).

Table 10: Pearson correlation coefficient for mean values of FHB spreading measured by n.DS 21 dai among field, GH1, GH2, GH3 and GH4.

Traits	**GH1**	**GH2**	**GH3**	**GH4**
Field	0.28**	0.43**	0.41**	0.34**
GH1		0.47**	0.62**	0.43**
GH2			0.71**	0.59**
GH3				0.56**

** p < 0.01; * p < 0.05;

3.3. Speed of FHB spreading (SFS)

The average increase in numbers of infected spikelets per day as a measure for the speed of FHB spread was calculated for the periods: 1) 14 – 21 dai 2) 21 – 28 dai and 3) 14 – 28 dai. The speed of FHB spreading for both parents is illustrated in Figure 14. The results showed significant difference ($\alpha < 0.05$) for SFS 1 (14 – 21 dai) with on average 0.16 and 0.63 spikelets per day and 0.54 and 0.59 spikelets per day for SFS 2 (21 – 28 dai) for *T. dicoccoides* and Helidur, respectively. Table 11 shows that the average of FHB speed for the population during the period 14 – 21 dai (0.59) was faster than the speed for of FHB spread during the period 21 – 28 dai (0.44) indicative by the increase in mean population for both parental lines.

Table 11: Means and broad sense heritability (H^2) estimated for speed of FHB spreading in GH2, GH3, and GH4 during the periods 14 – 21 dai, 21 – 28 dai and 14 – 28 dai for the BC_1F_6 lines.

Trait	Days after inoculation	Population Mean	Population Range	*T.dic*	Helidur	H^2
SFS	14-21	0.59	0.14-1.19	0.16	0.63	0.77
	21-28	0.44	0.12-0.9	0.54	0.59	0.30
	14-28	0.53	0.6-0.8	0.38	0.63	0.63

T. dic. : *T. dicoccoides*

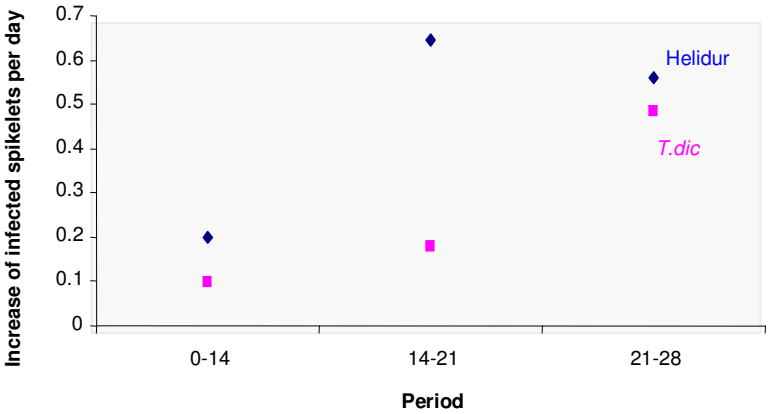


Figure 14: SFS (spikelets per day) during 0 to 14, 14 to 21 and 21 to 28 dai of the parental lines *T. dicoccoides* and Helidur for means across GH1, GH2, GH3 and GH4. (T. dic : *T. dicoccoides*).

ANOVA revealed significant variation ($\alpha = 0.05$) for SFS of spread of symptoms within the spike among the BC_1F_6 lines (Table 12). Broad sense heritability was high (0.77) during the period of 14-21dai. Correlations between SFS were highly significant for single experiments and means over experiments (Table 13). A high coefficient of correlation was revealed between GH2 and GH3 as well as GH3 and GH4 during the time period 14 to 21 dai ($r = 0.47$).

Table 12: Analysis of variance for SFS during 14 to 21, 21 to 28 and 14 to 28 dai for GH1, GH2, GH3 and GH4.

period	Source of variation	d.f	MS	F-value	P
21-14 dai	Exp	2	0.79026		
	Exp*block (error 1)	211	0.00068		
	Lines	106	0.53412	6.38	<.001
	Exp*Lines	211	0.12426	1.49	<.001
	Residual (error 2)	509	0.08368		
28-21 dai	Exp	3	2.93363		
	Exp*block (error 1)	315	0.00032		
	Lines	106	0.1723	2.3	<.001
	Exp*lines	315	0.12698	1.69	<.001
	Residual (error 2)	573	0.07493		
28-14 dai	Exp	2	0.676751		
	Exp*block (error 1)	211	0.000004		
	Lines	106	0.139	4.39	<.001
	Exp*Lines	211	0.0512	1.62	<.001
	Residual (error 2)	475	0.031659		

Table 13: Pearson correlation for mean values of SFS among GH2, GH3 and GH4 within 14 to 21 dai.

Traits		
	GH3	**GH4**
GH2	0.47**	0.38**
GH3		0.47**

** $p < 0.01$; * $p < 0.05$;

3.4. Area under the disease progress curve (AUDPC)

The AUDPC was used as an additional measure to calculate FHB severity using percent of infected spikelets (%DS) parameter. The BC_1F_6 lines showed continuous variation ($\alpha < 0.05$) for FHB severity measured by the AUDPC as shown in Figure 15. The resistant *T. dicoccoides* exhibited an average AUDPC of 418.7 units while Helidur had an AUDPC mean of 703.3 units (Table 14).

Table 14: means and abroad sense heritability (H^2) estimates for GH1, GH2, GH3 and GH4 as FHB severity (AUDPC) 14, 21 and 28 dai for the BC_1F_6 lines with parents

Trait	experiments	Population mean	population range	T.dic*	Helidur	H^2
	GH1	387.9	0-1295.8	300.5	560.9	
	GH2	972.4	396-1785	694.1	884	
FHB (AUDPC)	GH3	712.3	117-1566	344.8	610.3	
	GH4	692.6	216-1442	335.3	758.2	
	mean	674.7	260-1339	418.7	703.3	0.60

**T.dic:* *T. dicoccoides*

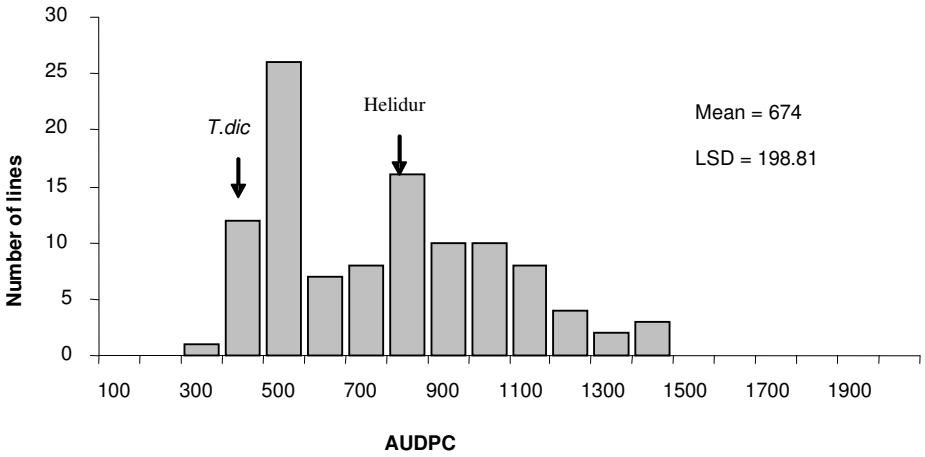


Figure 15: Histogram of BC_1F_6 lines for mean values of FHB severity measured by AUDPC (GH1, GH2, GH3 and GH4). (T. dic : *T. dicoccoides*).

Analysis of variance for FHB severity measured by AUDPC was significant ($\alpha < 0.05$) for the population (Table 15). Broad sense heritability was 0.60. Correlations between FHB severities were highly significant for the mean over GH1, GH2, GH3 and GH4 (Table 16). A high coefficient of correlation was revealed between GH2 and GH3 (r = 0.73).

Table 15: Analysis of variance for GH1, GH2, GH3 and GH4 mean values of FHB severity measured by AUDPC.

Source of variation	d.f	MS	F-value	P
Exp	3	5.58E+07	42.86	<.001
Exp*block (error 1)	6	1.302E+06	2.54	
Line	106	1.82E+06	3.55	<.001
Exp*Line	315	6.84E+05	1.33	0.001
Residual (error 2)	603	5.12E+05	2.8	

Table 16: Pearson correlation for mean values of FHB severity over GH1, GH2, GH3 and GH4 measured by AUDPC.

Traits	**GH 2**	**GH 3**	**GH 4**
GH1	0.56**	0.68**	0.39**
GH2		0.73**	0.61**
GH3			0.55**

** $p < 0.01$; * $p < 0.05$;

3.5. Wilting

The BC_1F_6 lines showed continuous variation ($\alpha < 0.05$) for percentage of wilted spikes 14, 21 and 28 dai. The parents showed (Figure 16) significant difference (LSD = 18) at 21 dai while the differences was not significant at 14 and 28 dai. Population mean was very high (68 %) at 28 dai (Table 17).

Table 17: means and broad sense heritability (H^2) estimates for wilted spikes at 14, 21 and 28 dai for the BC_1F_6 lines with parents (**T.dic: T. dicoccoides*).

Trait	Days after inoculation	Experiments	Population mean	Population range	T.dic*	Helidur	H^2
% Wilt	14	GH2	32	0-82	33	23	
		GH3	27	0-83	0	13	
		GH4	13	0-78	0	0	
		mean	24	0-83	11	12	0.83
	21	Field	88	33-100	100	82	
		GH1	25	0-83	13	14	
		GH2	68	12-100	57	52	
		GH3	53	0-100	7	51	
		GH4	48	0-100	0	58	
		mean	56	0-100	31	52	0.65
	28	GH1	38	0-100	40	43	
		GH2	91	38-100	100	86	
		GH3	69	14-100	67	72	
		GH4	72	17-100	100	58	
		mean	68	14-100	77	65	0.74

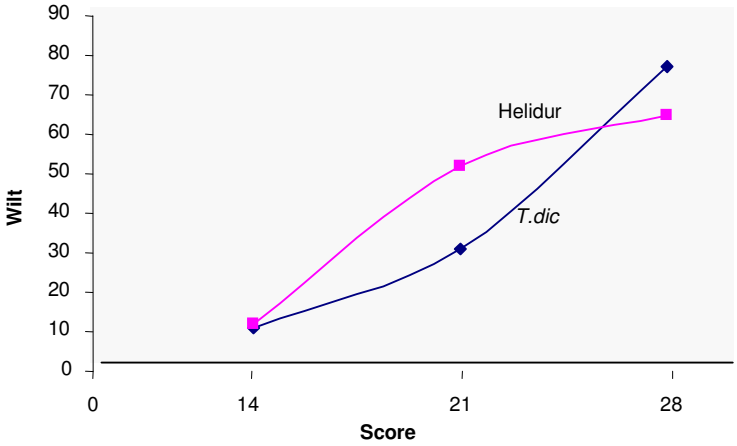


Figure 16: Disease progress curve of the parents for the mean percentage of wilted spikes 14, 21 and 28 dai for the experiments GH2, GH3 and GH4. (*T. dic*: *T. dicoccoides*).

Analysis of variance for percentage of wilted spikes was significant ($\alpha < 0.05$) for the population (Table 18). Broad sense heritability was high (0.83) at 14 dai. Correlations between experiments were significant for the mean over GH1, GH2, GH3 and GH4 experiments at 21 dai (Table 19). A high coefficient of correlation was found between GH1 and GH3 (r =0.59), whereas a low coefficient of correlation was found between field and GH4 (r =0.03).

Table 18: Analysis of variance at 14, 21 and 28 dai for all experiments.

Scoring FHB	Source of variation	d.f	MS	F-value	P
14 dai	Exp	2	2.21964	8.84	0.023
	Exp*block (error 1)	5	0.25115	5.09	
	Line	106	0.32532	6.59	<.001
	Exp*Line	212	0.05496	1.11	0.17
	Residual (error 2)	5	0.25115	5.09	
21 dai	Exp	4	15.5041	37.81	<.001
	Exp*block (error 1)	8	0.4101	2.91	
	Line	106	0.4194	2.98	<.001
	Exp*Line	421	0.1461	1.04	0.329
	Residual (error 2)	8	0.4101	2.91	
28 dai	Exp	4	15.5041	37.81	<.001
	Exp*block (error 1)	6	0.17523	3.44	
	Line	106	0.33534	6.57	<.001
	Exp*Line	316	0.08616	1.69	<.001
	Residual (error 2)	8	0.4101	2.91	

Table 19: Pearson correlation for percentage of wilted spikes (mean values) over all experiments at 21 dai.

Traits	GH1	GH2	GH3	GH4
Field	0.074	0.14	0.14	0.03
GH1		0.40 **	0.59**	0.38**
GH2			0.57**	0.40 **
GH3				0.41**

** $p < 0.01$; * $p < 0.05$;

3.6. FHB incidence

The distribution for percent of diseased spikes over all experiments at 21 dai is presented in Figure 17. This was calculated as percentage of diseased spikes with a mean of 91.8, a minimum of 73 and a maximum of 100.

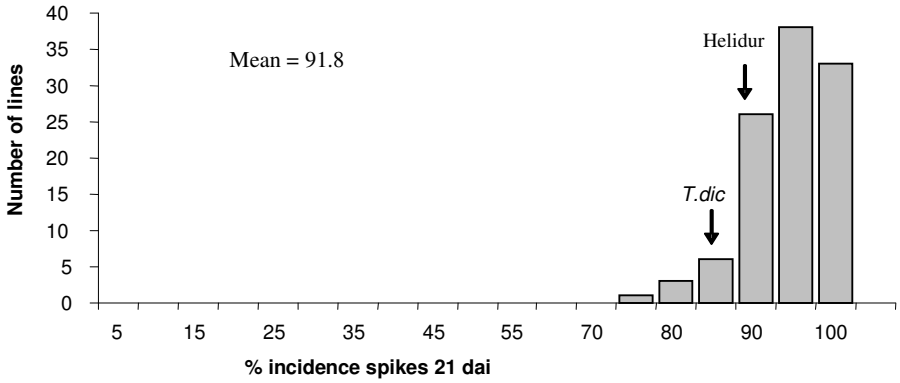


Figure 17: Histogram of BC_1F_6 lines for FHB incidence 21 dai over all mean values across 4 experiments (GH1, GH2, GH3 and GH4). (T. dic : *T. dicoccoides*).

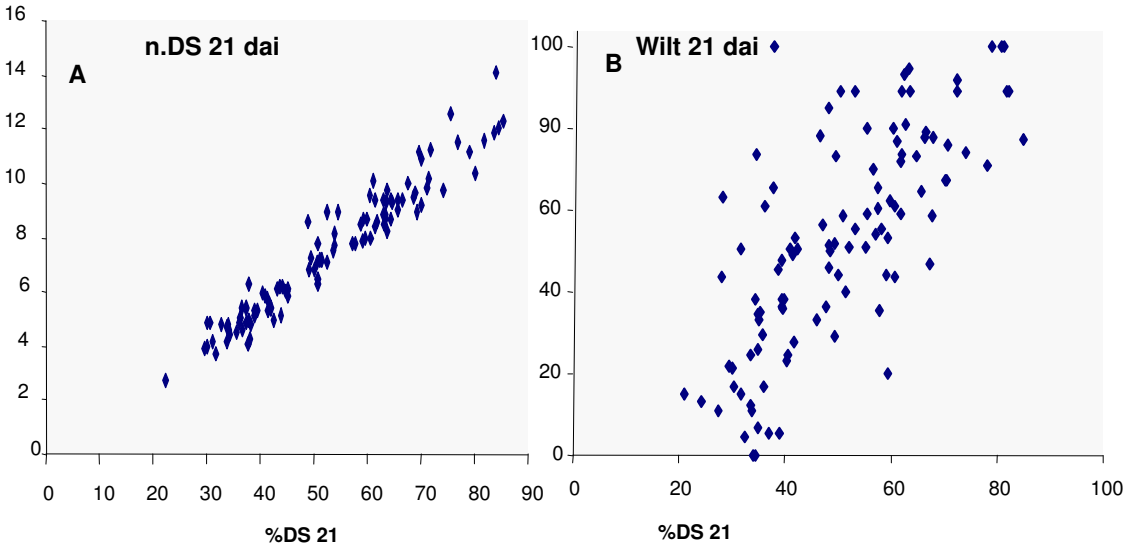
3.7. Correlation between FHB related traits

Correlations between %DS, n.DS, FHB speed of spread (SFS), AUDPC, FHB incidence and percent of wilted spikes were significant for single experiments and means over all experiments (Table 20). A high coefficient of correlation (Figure 18) was revealed for %DS and n.DS (r = 0.96). The lowest correlation coefficient was for percent of wilted spikes with FHB incidence (r = 0.14).

Table 20: Pearson correlation for mean values of %DS and n.DS21 dai, AUDPC, FHB incidence and percent of wilted spikes 21 dai over all experiments and the period 14-21 dai for SFS (GH2, GH3 and GH4).

Traits	n.DS 21	SFS14-21	Wilt 21 dai	AUDPC	incidence
%DS 21	0.96**	0.83**	0.82 **	0.96**	0.27**
n.DS 21		0.82**	0.83**	0.95**	0.27**
SFS14-21 dai			0.68**	0.73**	0.28**
Wilt 21 dai				0.80**	0.14
AUDPC					0.29**

** p < 0.01; * p < 0.05;



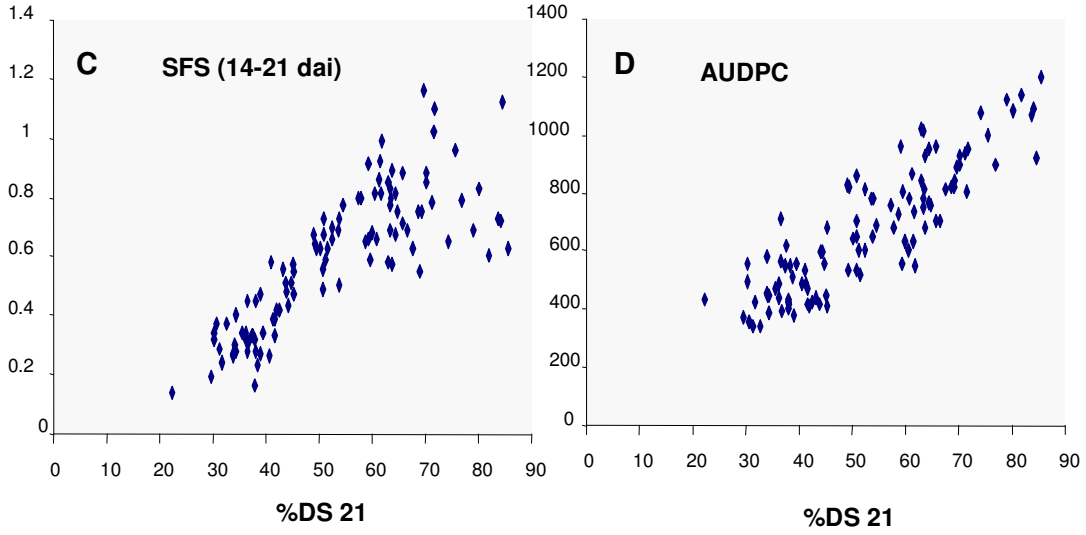


Figure 18: Scatter plot of mean values for FHB **(A)** spread as %DS and n.DS, **(B)** %DS and percent of wilted spikes, **(C)** %DS and FHB speed, **(D)** %DS abd AUDPC over all experiments 21 dai.

3.8. Other traits and powdery mildew

Genetic variation among the lines was significant ($\alpha < 0.05$) for plant height, spike length, ear type, waxiness, spikelets number, powdery mildew severity (PM), date of anthesis, colour of the spikes and brittle rachis (Table 21).

Table 21: Analysis of variance for developmental and morphological traits as well as powdery mildew.

Traits	scale	Source of variation	d.f	MS	F-value	P
Plant height	cm	Exp	3	16123.43	9.62	9.96
4 experiments		Exp*block (error 1)	2	1675.87	25.58	
		Lines	104	831.63	12.70	<.001
		Exp*lines	307	169.40	2.59	<.001
		Residual (error 2)	187	65.51		
Date of anthesis		Exp	4	57688	23.08	0.001
5 experiments		Exp*block (error 1)	8	2499.13	30.19	
		Lines	104	541.3	6.54	<.001
		Exp*lines	411	101.1	1.22	0.010
		Residual (error 2)	756	82.77	15.26	
Spikelets no		Exp	3	1524.22	12.52	0.005
5 experiments		Exp*block (error 1)	6	121.7	15.17	
		Lines	106	138.6	17.28	.001
		Exp*lines	315	14.7	1.83	.001
		Residual (error 2)	622	8.02	6.55	
PM	1-5	Exp.	1	27.72	0.63	0.573
2 experiments		Exp*block (error 1)	1	44.03	20.79	
		Lines	106	4.448	2.10	0.001
		Exp*lines	106	1.657	0.78	0.895
		Residual (error 2)	105	2.117	12.74	

Table 21: continue…

Traits	scale	Source of variation	d.f	MS	F-value	P
Waxiness	1-5	lines	106	3.7587	10	<.001
3 experiments		Residual	207	0.3759		
Ear type	1-5	lines	106	1.5131	3.25	<.001
3 experiments		Residual	204	0.4655		
Spike length	cm	lines	106	0.5893	2.12	<.001
3 experiments		Residual	206	0.2773		
Spikes colour	1-3	lines	106	0.4527	2.8	<.001
3 experiments		Residual	206	0.1619		
Brittle rachis	1-3	lines	106	1.0799	3.61	<.001
3 experiments		Residual	205	0.2994		

Correlation coefficients between the data for %DS, n.DS, SFS during 14-21 dai, AUDPC, percent of wilted spikes, FHB incidence and plant height, date of anthesis, spike length, ear type, waxiness, number of spikelets, brittle riches, spike colour and powdery mildew (PM) are shown in Table 22.

Table 22: The correlation coefficients between FHB related traits and developmental and morphological traits as well as powdery mildew (mean values).

Traits	%DS21	n.DS21	SFS14-21	wilt21	AUDPC	Incidence
Waxiness	-0.03	-0.09	0.04	-0.04	-0.03	-0.06
Plant height	0.25**	0.28**	0.14	0.18	0.37**	-0.14
Date of anthesis	-0.07	0.04	-0.01	-0.01	-0.10	0.13
Ear type	-0.09	0.00	0.01	0.01	-0.04	-0.11
Brittle rachis	-0.01	0.09	0.07	0.04	-0.05	0.04
Spikes length	-0.18	-0.13	-0.08	0.09	-0.15	-0.16
Number of spikelets	0.19*	0.41**	0.36**	0.24*	0.15	0.09
Spikes colour	-0.10	-0.13	-0.13	-0.09	-0.09	0.04
Pm	-0.12	-0.16	-0.10	-0.14	-0.13	0.13

** $p < 0.01$; * $p < 0.05$;

Significant correlation coefficients were found between %DS21, n.DS21, AUDPC and plant height. In addition, number of spikelets was significantly correlated with %DS21, n.DS21, SFS 14-21 dai and percent of wilted spikes, but not with AUDPC and diseases incidence (Figure 19).

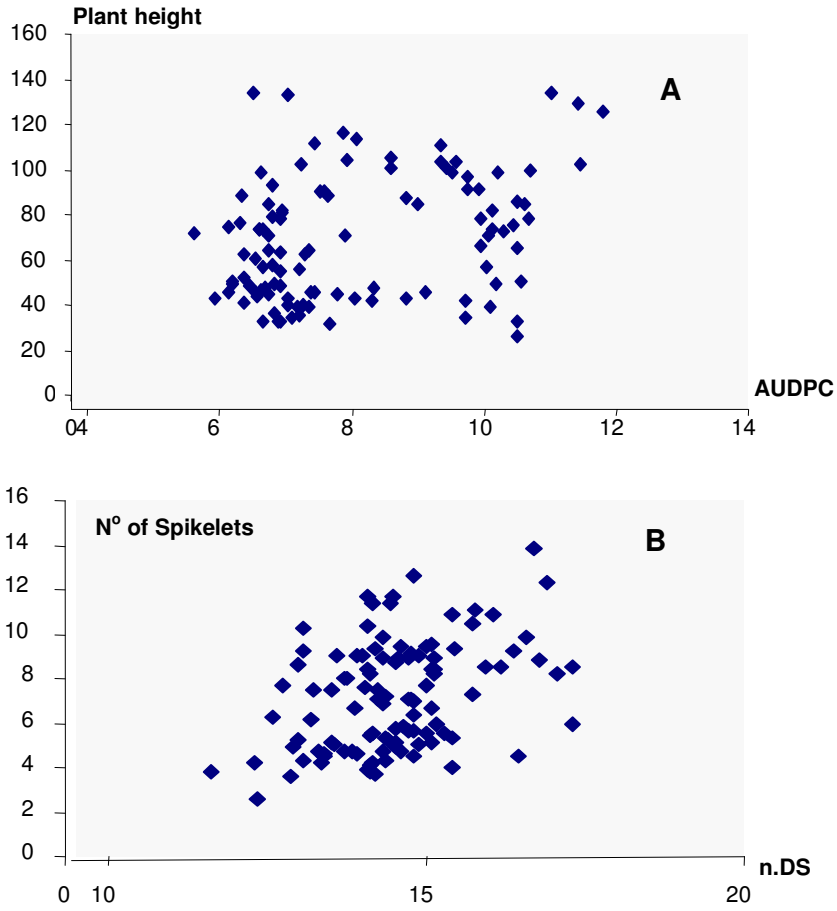


Figure 19: Scatter plot of mean value for **(A)** AUDPC and plant height, **(B)** n.DS and spikelets number over all experiments 21 dai.

Table 23: The Phenotypic correlation coefficients among developmental and morphological traits as well as powdery mildew.

Traits	Number of experiments	Plant Height	Date of anthesis	Ear type	Brittle rachis	Spike Length	PM	Spikes Colour
Waxiness	3	-0.17	0.03	-0.05	-0.000	0.015	0.22*	-0.45 **
Plant height	4		-0.14	0.01	-0.25**	-0.08	-0.49 (**)	0.28**
Date of anthesis	5			-0.04	0.05	0.02	0.01	-0.11
Ear type	3				0.54**	0.05	0.113	-0.17
Brittle rachis	3					-0.13	0.14	-0.28*
Spike length	3						-0.10	-0.02
Number of spikelets	5							-0.29**
PM	2							-0.10

** $p < 0.01$; * $p < 0.05$;

The results of the correlation analyses between the data of the morphological data and powdery mildew are summarized in Table 23. The colour of the spikes was correlated with four other morphological parameters: a) with waxiness (r =-0.45), b) with plant height (r =0.28), c) with the parameter "brittle rachis" (r =-0.28) and d) with number of spikes (r = -0.29). Significant correlation coefficients were also found between the data for powdery mildew and waxiness (r =0.22) and between powdery mildew and plant height (r =-0.49)(Figure 20). The data for the parameter "brittle rachis" were also related with the data for plant height (r =-0.25) and especially with the ear type(r =0.54). Spike length did not relate to any of the other investigated parameters.

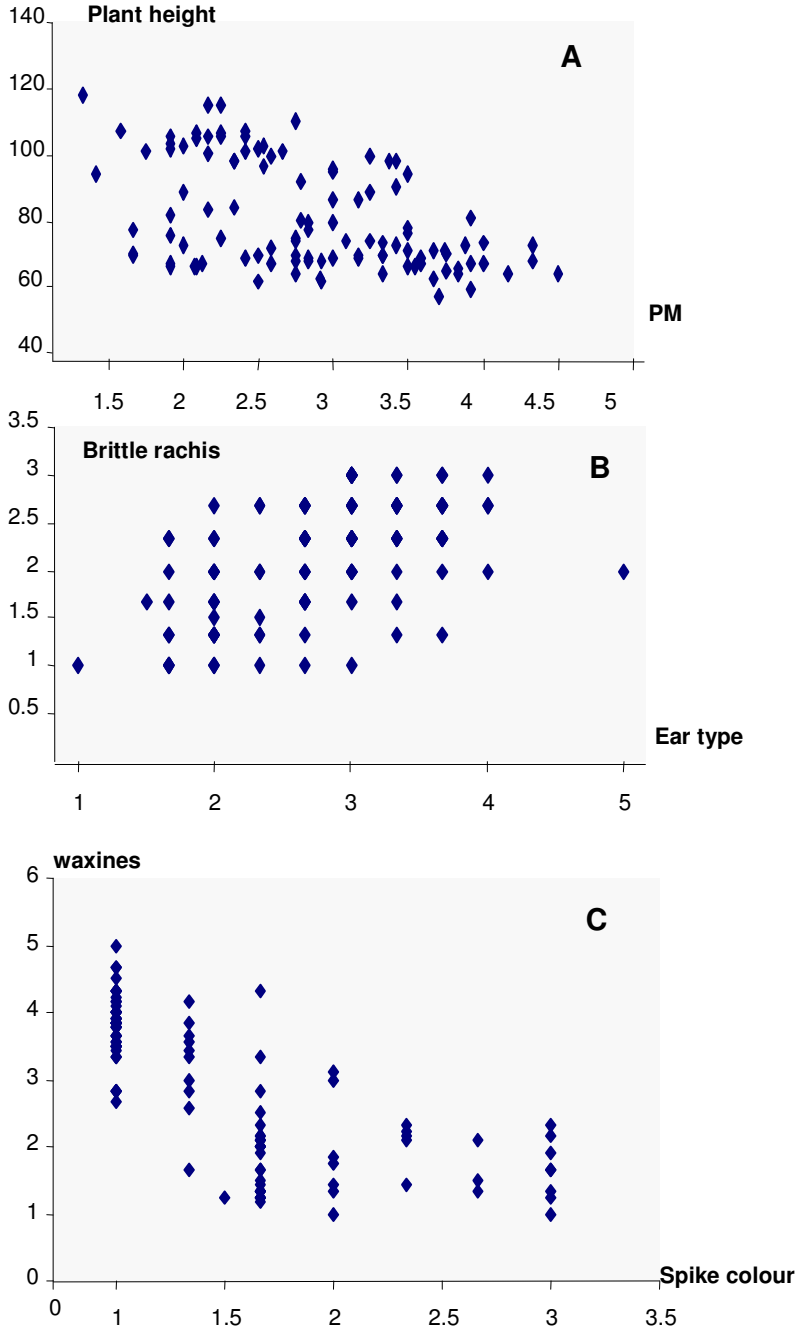


Figure 20: Scatter plot of over all mean values for **(A)** PM and plant height, **(B)** brittle rachis and ear type, **(C)** spikes colour and waxiness.

3.9. Linkage map

3.9.1. SSR markers

More than 200 SSR primers with known map position were tested on the two parents. 121 revealed polymorphisms between Helidur and *T. dicoccoides* and were applied to the mapping population. In total 142 SSR loci were obtained in the BC_1F_6 population.

3.9.2. AFLP markers

The 59 AFLP primer combinations generated 380 polymorphic fragments (mean 6.5 per combination). Table 24 gives the complete list of the numbers of polymorphic bands per primer combination.

Table 24: List of AFLP primer combinations applied for genotyping the BC_1F_6 population *T. diccocoides* x Helidur. Primers combination are indicated by the selective bases used for the respective Sse8387I and MseI primers, as well as the standard name for AFLP primer combination (www.keygene.com). The number of clearly polymorphic bands for each primer combination are presented.

no	Selective bases		Standard name	Polymorphic bands
	Sse8387I	*MseI*		
1	AA	AC	S11M12	5
2	AG	AC	S13M12	4
3	AT	AC	S14M12	7
4	CG	AC	S17M12	5
5	GC	AC	S20M12	5
6	GT	AC	S22M12	4
7	AA	AG	S11M13	10
8	AC	AG	S12M13	5
9	AG	AG	S13M13	9
10	AT	AG	S14M13	5
11	CG	AG	S17M13	6
12	GA	AG	S19M13	8
13	GC	AG	S20M13	6
14	GT	AG	S22M13	7
15	AA	CA	S11M15	7
16	AG	CA	S13M15	6
17	AT	CA	S14M15	8
18	CG	CA	S17M15	5
19	GC	CA	S20M15	7
20	GT	CA	S22M15	7
21	AA	CG	S11M17	3

no	Selective bases		Standard name	Polymorphic bands
	Sse8387I	*MseI*		
22	AG	CG	S13M17	3
23	AT	CG	S14M17	4
24	CG	CG	S17M17	4
25	GA	CG	S19M17	6
26	GC	CG	S20M17	3
27	GT	CG	S22M17	8
28	TA	CG	S23M17	11
29	AA	CT	S11M18	10
30	AG	CT	S13M18	5
31	AT	CT	S14M18	10
32	AA	GA	S11M19	4
33	AG	GA	S13M19	3
34	AT	GA	S14M19	4
35	CC	GA	S16M19	3
36	GT	GA	S22M19	5
37	CG	GG	S17M21	10
38	AA	GT	S11M22	4
39	AG	GT	S13M22	5
40	AT	GT	S14M22	6
41	GC	GT	S20M22	7
42	GT	GT	S22M22	3
43	AA	TC	S11M24	9
44	AG	TC	S13M24	16
45	AT	TC	S14M24	7
46	GC	TC	S20M24	4
47	GT	TC	S22M24	8
48	AA	TG	S11M24	13
49	AG	TG	S13M24	6
50	AT	TG	S14M24	6
51	CC	TG	S16M24	7
52	CG	TG	S17M24	9
53	GA	TG	S19M24	14
54	GC	TG	S20M24	4
55	GT	TG	S22M24	7
56	TA	TG	S23M24	9
57	AA	TT	S11M26	3
58	AG	TT	S13M26	3
59	AT	TT	S14M26	8
	Total			380

3.9.3. Genetic map

The molecular linkage map (Figure 23) consists of 495 loci of SSR and AFLP markers evident in 36 linkage groups. Total map coverage, resulting from 522 SSR and AFLP markers (495 linked and 27 unlinked markers), equates to 2641 cM, an average distance between the markers of 5.1 cM. The map length, divided among the two genomes, is 1142.5 cM, 1281.2 cM for the A- and B- genome, respectively, and 218 cM for unknown linkage groups (Figure 21). In average 82% of the markers segregated as expected and the distribution of Helidur alleles (A) on the chromosomes is a large fraction (Figure 22).

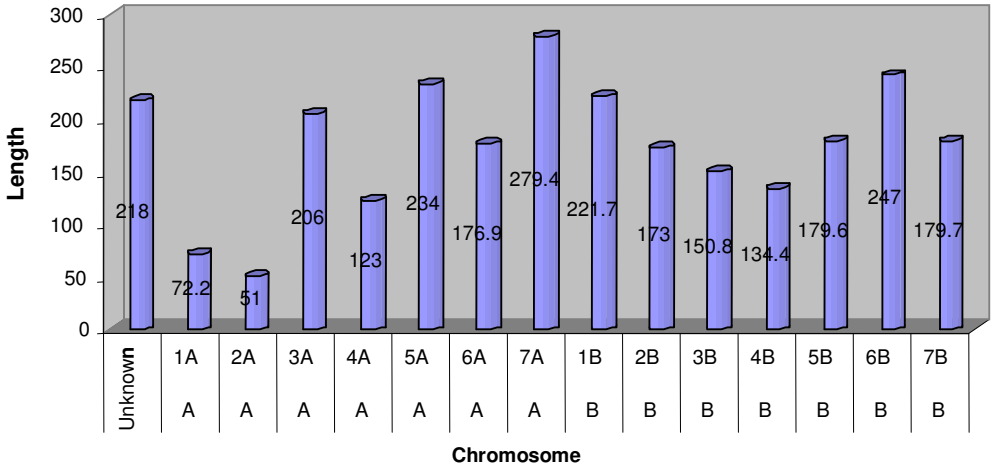


Figure 21: SSR and AFLP marker loci distribution in the genome of the BC_1F_6 population.

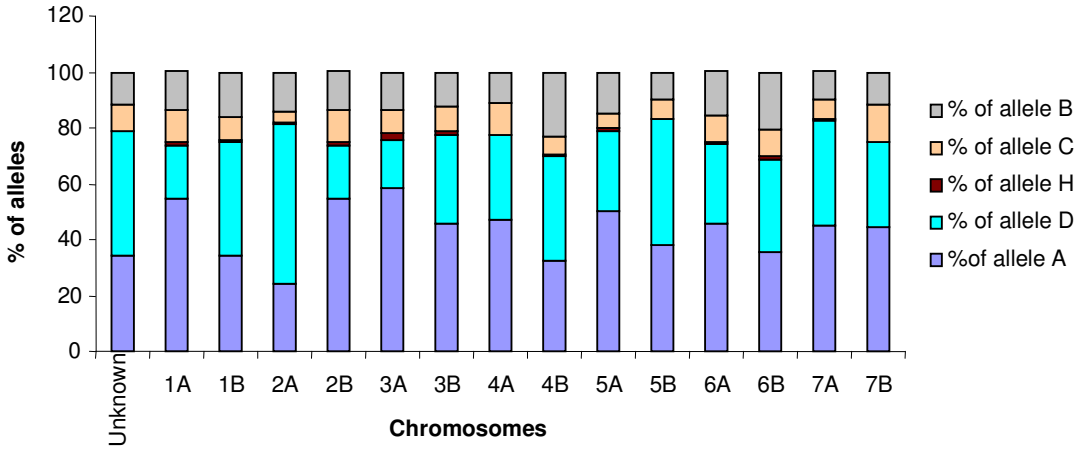
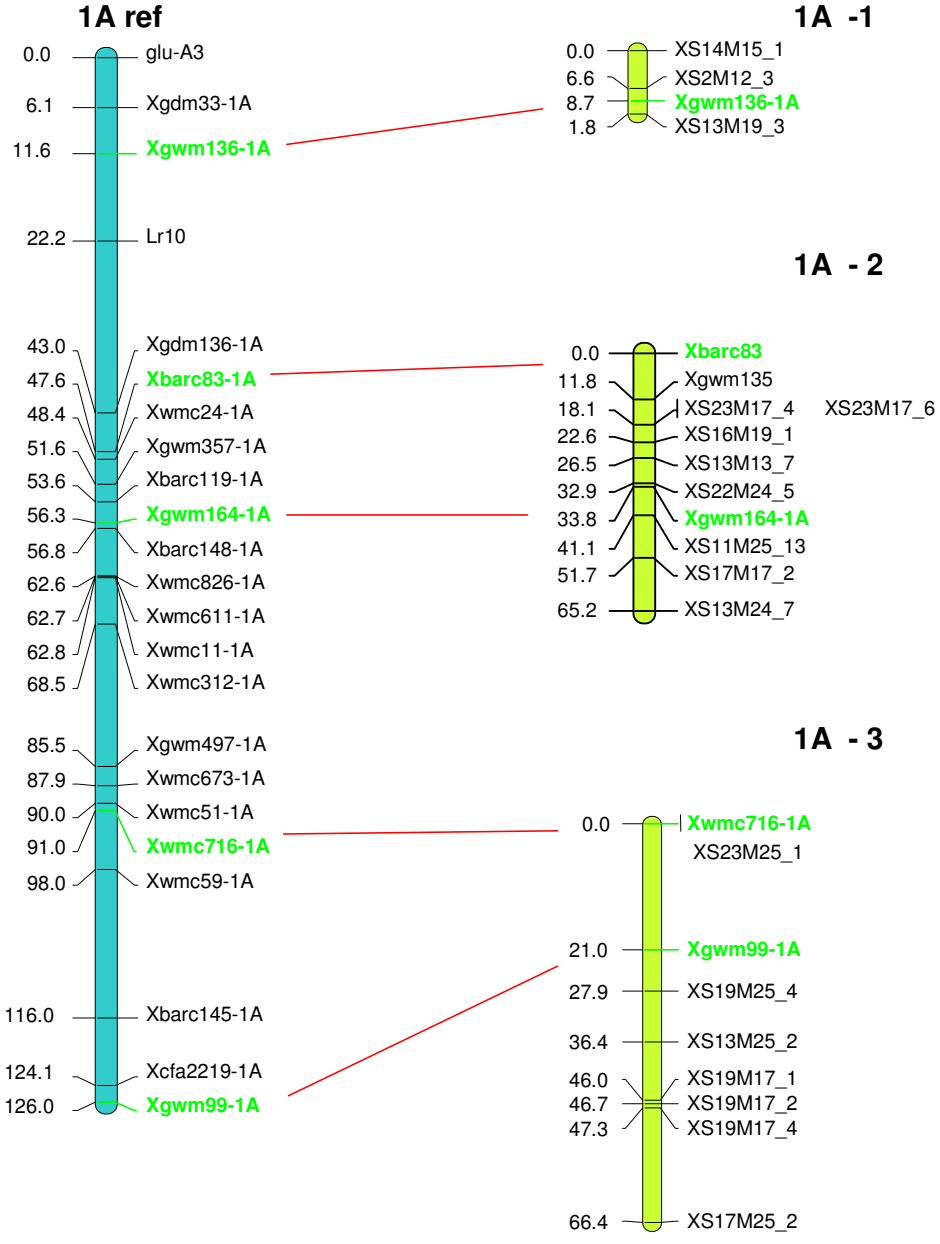
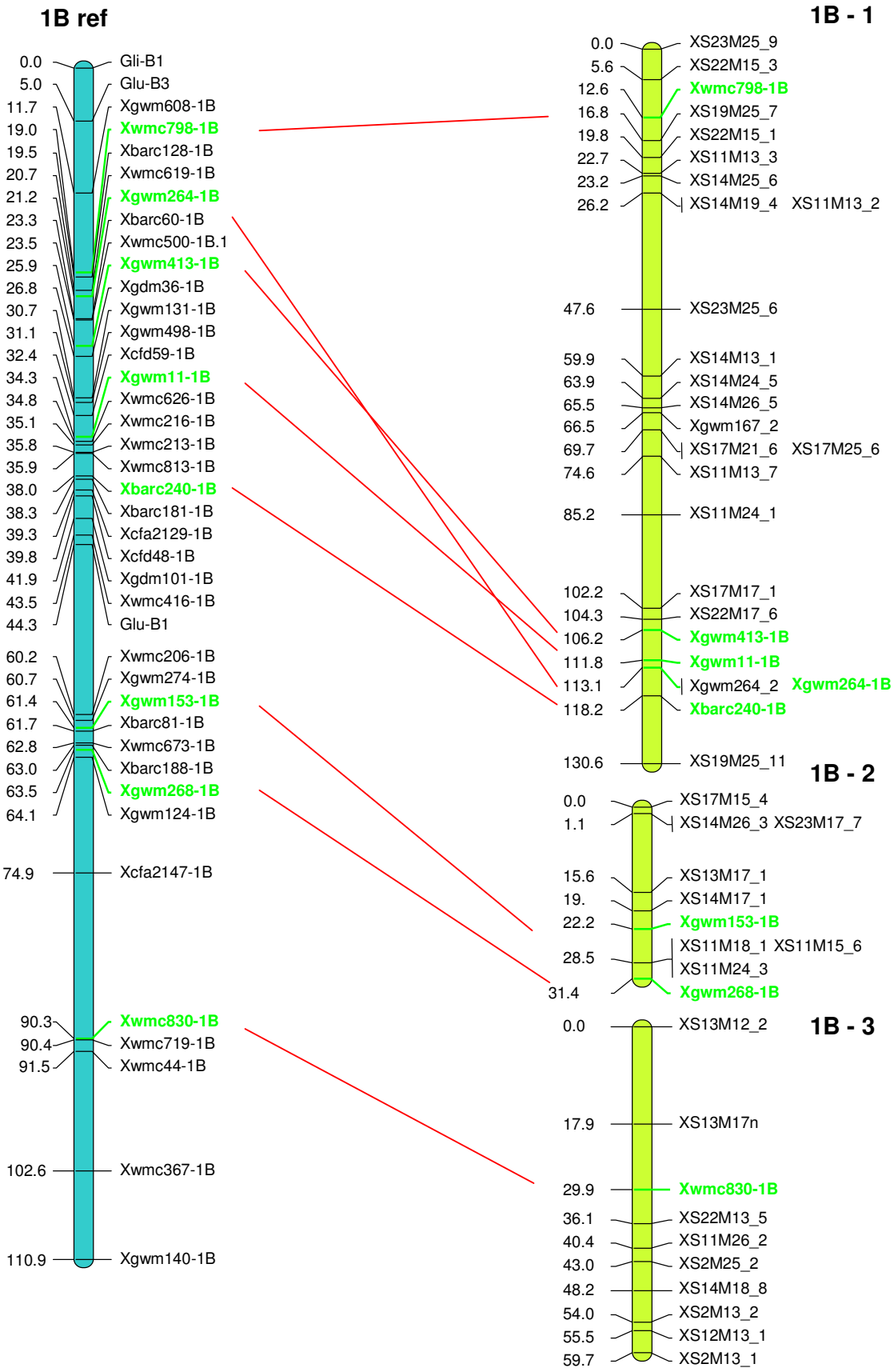
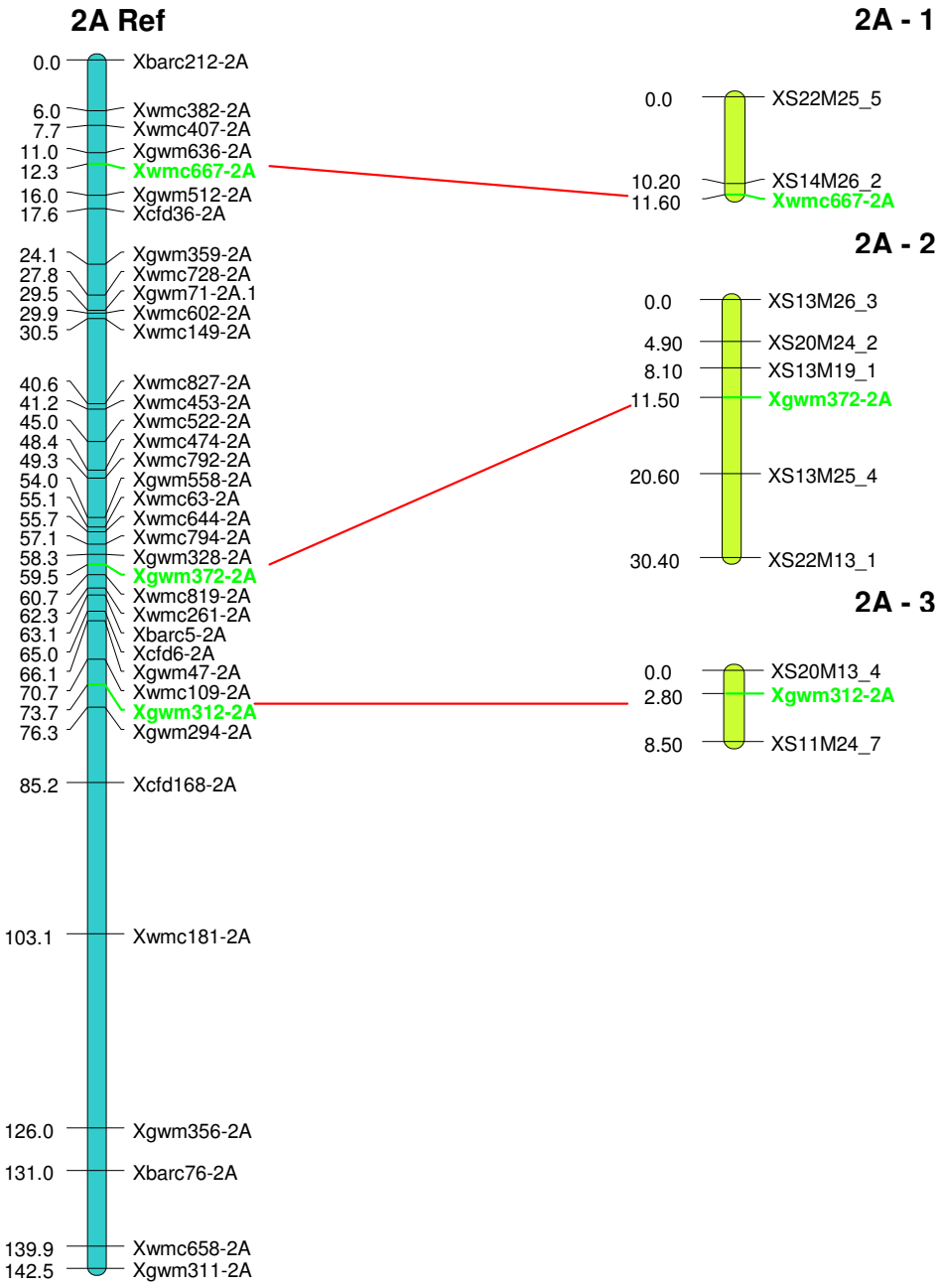


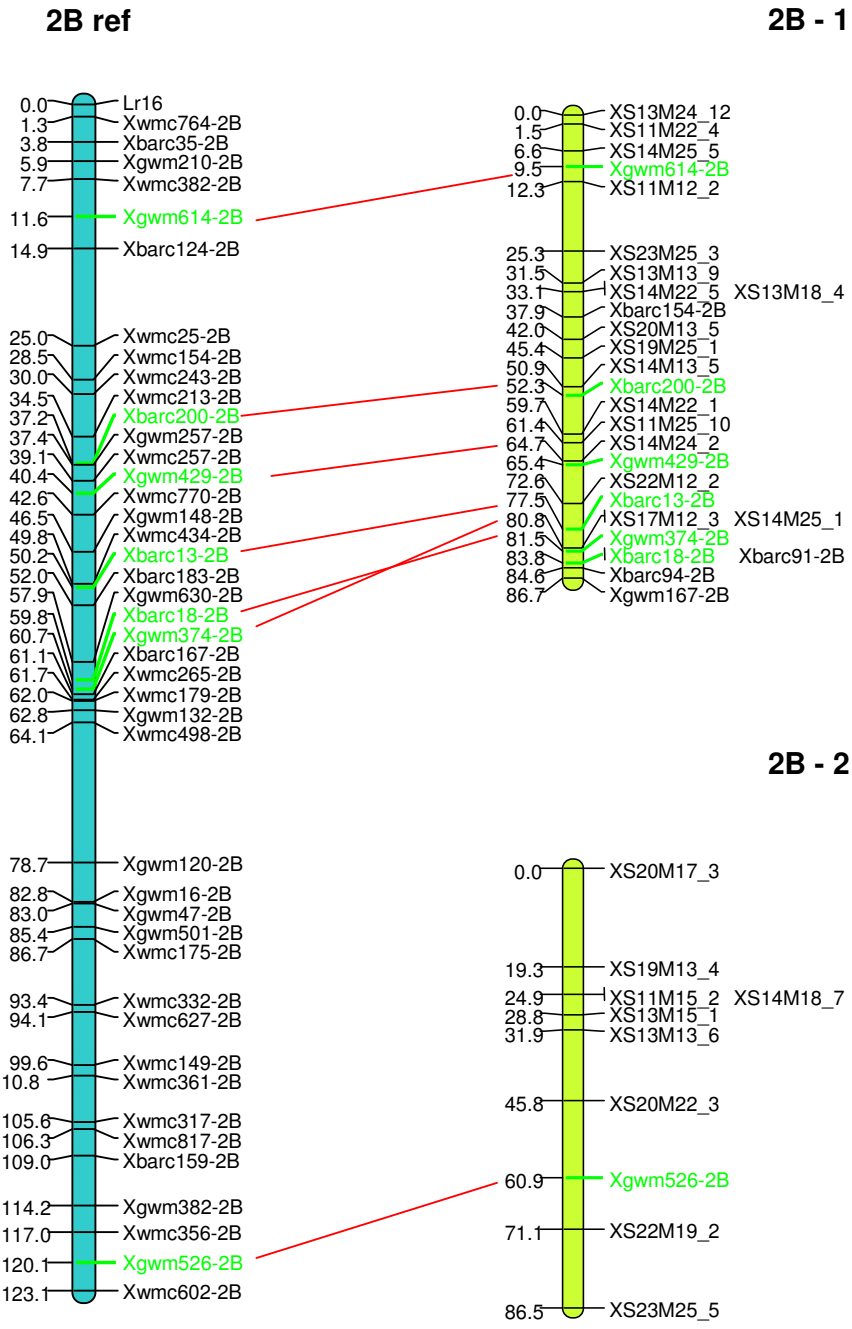
Figure 22: Fraction of alleles in each chromosome (A = Homozygous Helidur, B = Homozygous *T. dicoccoides*, H = heterozygous, D = not B, C = not A).

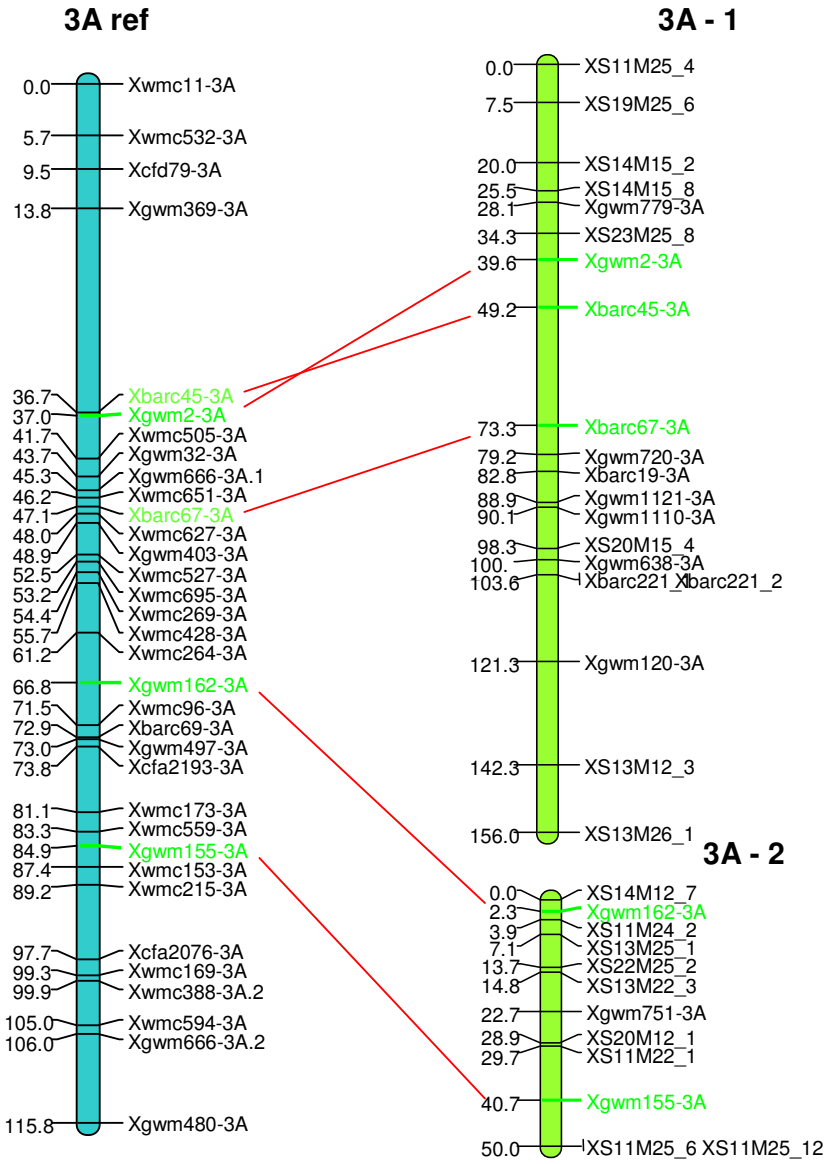
Figure 23: The obtained genetic linkage map (right) compared to the reference map (wheat consensus 2004 map (Somers et al. 2004) (left)). Linkage groups assigned to chromosomes are presented at the top of each group, markers used for map construction are shown on the right-hand side (**SSRs – green** colour is the homologues loci in both maps), genetic distance in cM is indicated on the left-hand side.

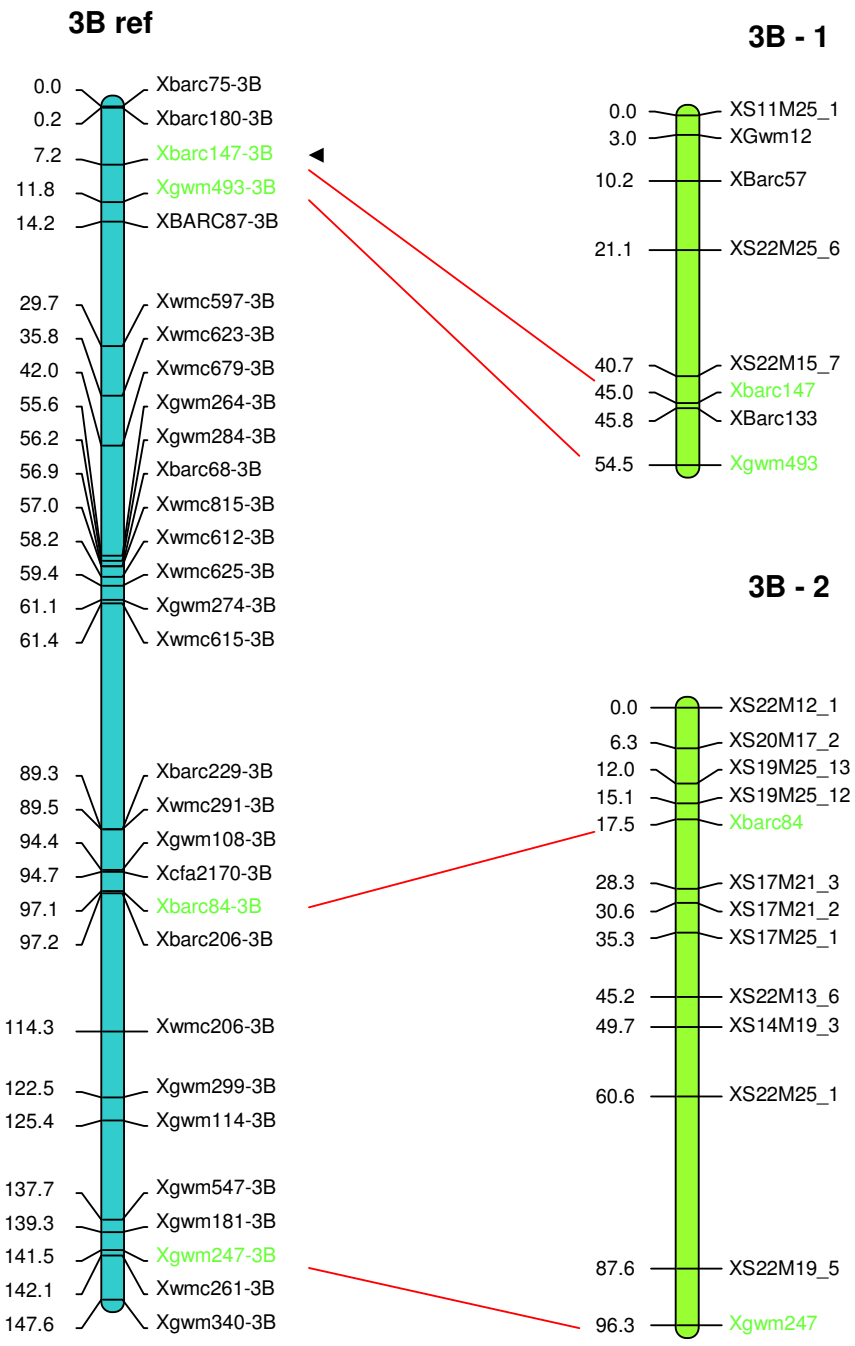


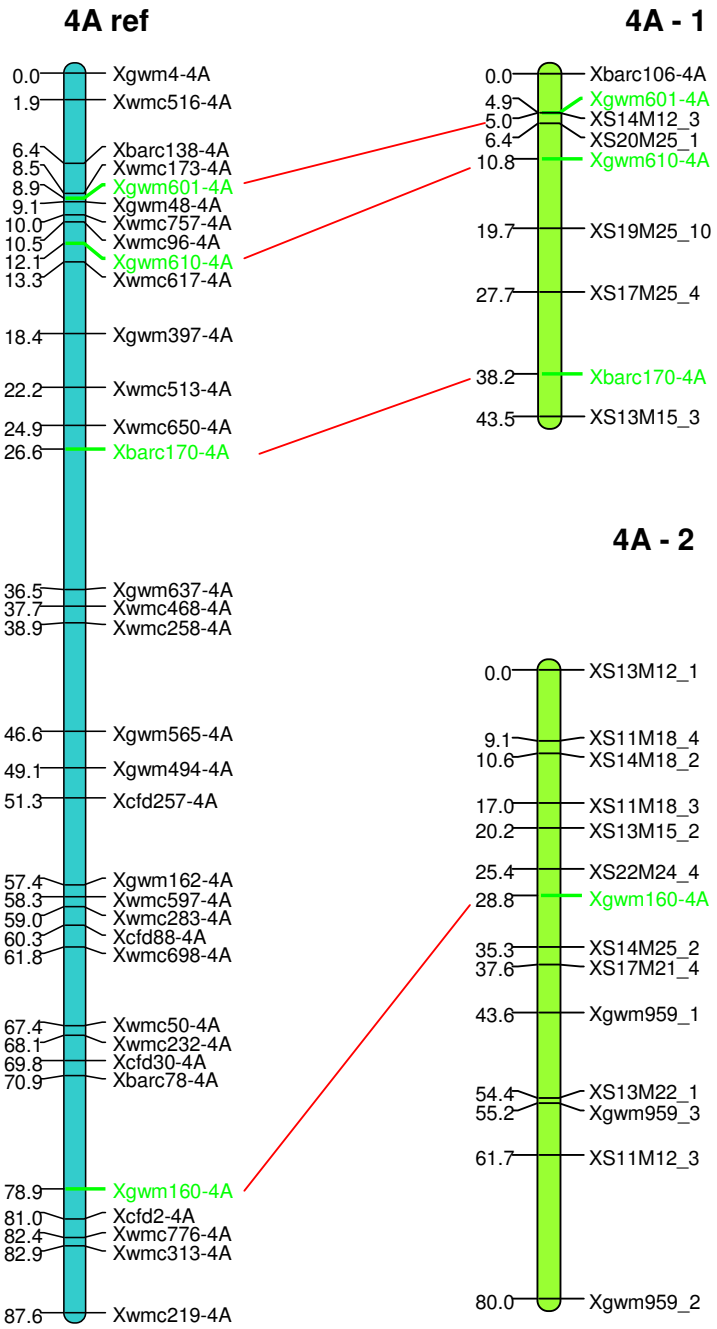

1B ref
0.0 Gli-B1
5.0 Glu-B3
11.7 Xgwm608-1B
19.0 Xwmc798-1B
19.5 Xbarc128-1B
20.7 Xwmc619-1B
21.2 Xgwm264-1B
23.3 Xbarc60-1B
23.5 Xwmc500-1B.1
25.9 Xgwm413-1B
26.8 Xgdm36-1B
30.7 Xgwm131-1B
31.1 Xgwm498-1B
32.4 Xcfd59-1B
34.3 Xgwm11-1B
34.8 Xwmc626-1B
35.1 Xwmc216-1B
35.8 Xwmc213-1B
35.9 Xwmc813-1B
38.0 Xbarc240-1B
38.3 Xbarc181-1B
39.3 Xcfa2129-1B
39.8 Xcfd48-1B
41.9 Xgdm101-1B
43.5 Xwmc416-1B
44.3 Glu-B1
60.2 Xwmc206-1B
60.7 Xgwm274-1B
61.4 Xgwm153-1B
61.7 Xbarc81-1B
62.8 Xwmc673-1B
63.0 Xbarc188-1B
63.5 Xgwm268-1B
64.1 Xgwm124-1B
74.9 Xcfa2147-1B
90.3 Xwmc830-1B
90.4 Xwmc719-1B
91.5 Xwmc44-1B
102.6 Xwmc367-1B
110.9 Xgwm140-1B
1B - 1
0.0 XS23M25_9
5.6 XS22M15_3
12.6 Xwmc798-1B
16.8 XS19M25_7
19.8 XS22M15_1
22.7 XS11M13_3
23.2 XS14M25_6
26.2 XS14M19_4 XS11M13_2
47.6 XS23M25_6
59.9 XS14M13_1
63.9 XS14M24_5
65.5 XS14M26_5
66.5 Xgwm167_2
69.7 XS17M21_6 XS17M25_6
74.6 XS11M13_7
85.2 XS11M24_1
102.2 XS17M17_1
104.3 XS22M17_6
106.2 Xgwm413-1B
111.8 Xgwm11-1B
113.1 Xgwm264_2 Xgwm264-1B
118.2 Xbarc240-1B
130.6 XS19M25_11
1B - 2
0.0 XS17M15_4
1.1 XS14M26_3 XS23M17_7
15.6 XS13M17_1
19. XS14M17_1
22.2 Xgwm153-1B
28.5 XS11M18_1 XS11M15_6
XS11M24_3
31.4 Xgwm268-1B
1B - 3
0.0 XS13M12_2
17.9 XS13M17n
29.9 Xwmc830-1B
36.1 XS22M13_5
40.4 XS11M26_2
43.0 XS2M25_2
48.2 XS14M18_8
54.0 XS2M13_2
55.5 XS12M13_1
59.7 XS2M13_1

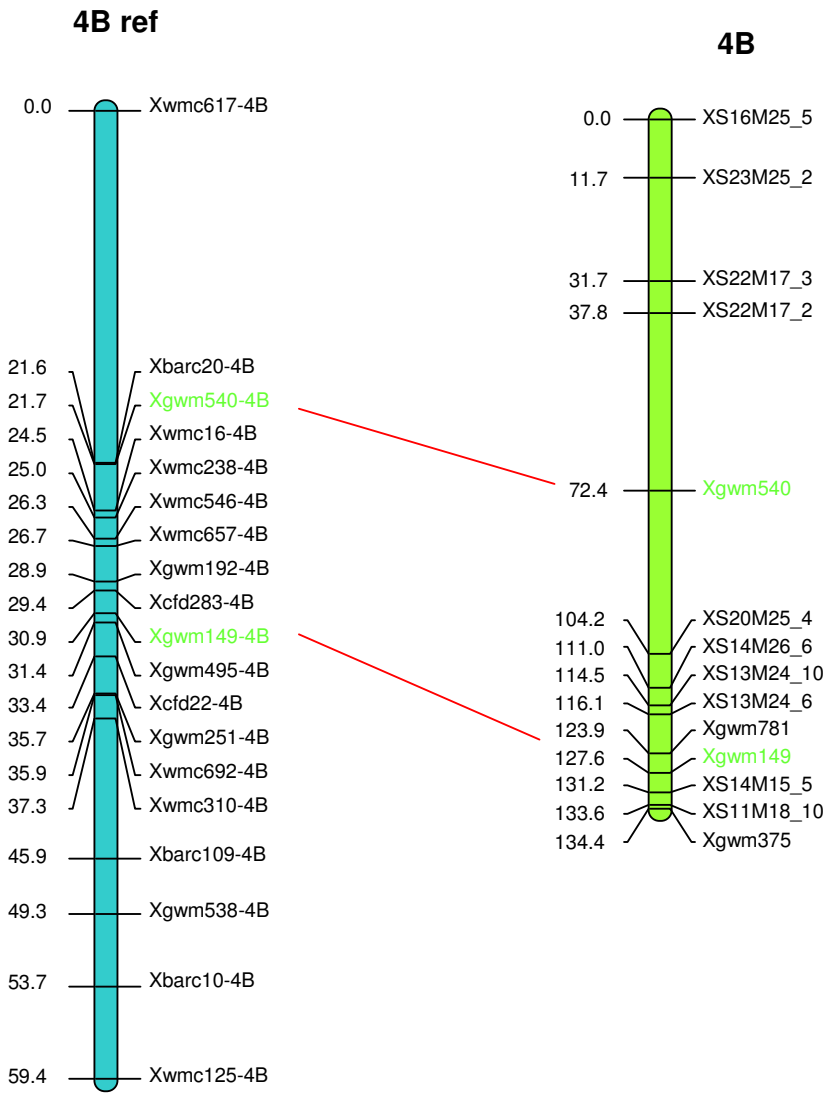

2A Ref
0.0 Xbarc212-2A
6.0 Xwmc382-2A
7.7 Xwmc407-2A
11.0 Xgwm636-2A
12.3 Xwmc667-2A
16.0 Xgwm512-2A
17.6 Xcfd36-2A
24.1 Xgwm359-2A
27.8 Xwmc728-2A
29.5 Xgwm71-2A.1
29.9 Xwmc602-2A
30.5 Xwmc149-2A
40.6 Xwmc827-2A
41.2 Xwmc453-2A
45.0 Xwmc522-2A
48.4 Xwmc474-2A
49.3 Xwmc792-2A
54.0 Xgwm558-2A
55.1 Xwmc63-2A
55.7 Xwmc644-2A
57.1 Xwmc794-2A
58.3 Xgwm328-2A
59.5 Xgwm372-2A
60.7 Xwmc819-2A
62.3 Xwmc261-2A
63.1 Xbarc5-2A
65.0 Xcfd6-2A
66.1 Xgwm47-2A
70.7 Xwmc109-2A
73.7 Xgwm312-2A
76.3 Xgwm294-2A
85.2 Xcfd168-2A
103.1 Xwmc181-2A
126.0 Xgwm356-2A
131.0 Xbarc76-2A
139.9 Xwmc658-2A
142.5 Xgwm311-2A
2A - 1
0.0 XS22M25_5
10.20 XS14M26_2
11.60 Xwmc667-2A
2A - 2
0.0 XS13M26_3
4.90 XS20M24_2
8.10 XS13M19_1
11.50 Xgwm372-2A
20.60 XS13M25_4
30.40 XS22M13_1
2A - 3
0.0 XS20M13_4
2.80 Xgwm312-2A
8.50 XS11M24_7

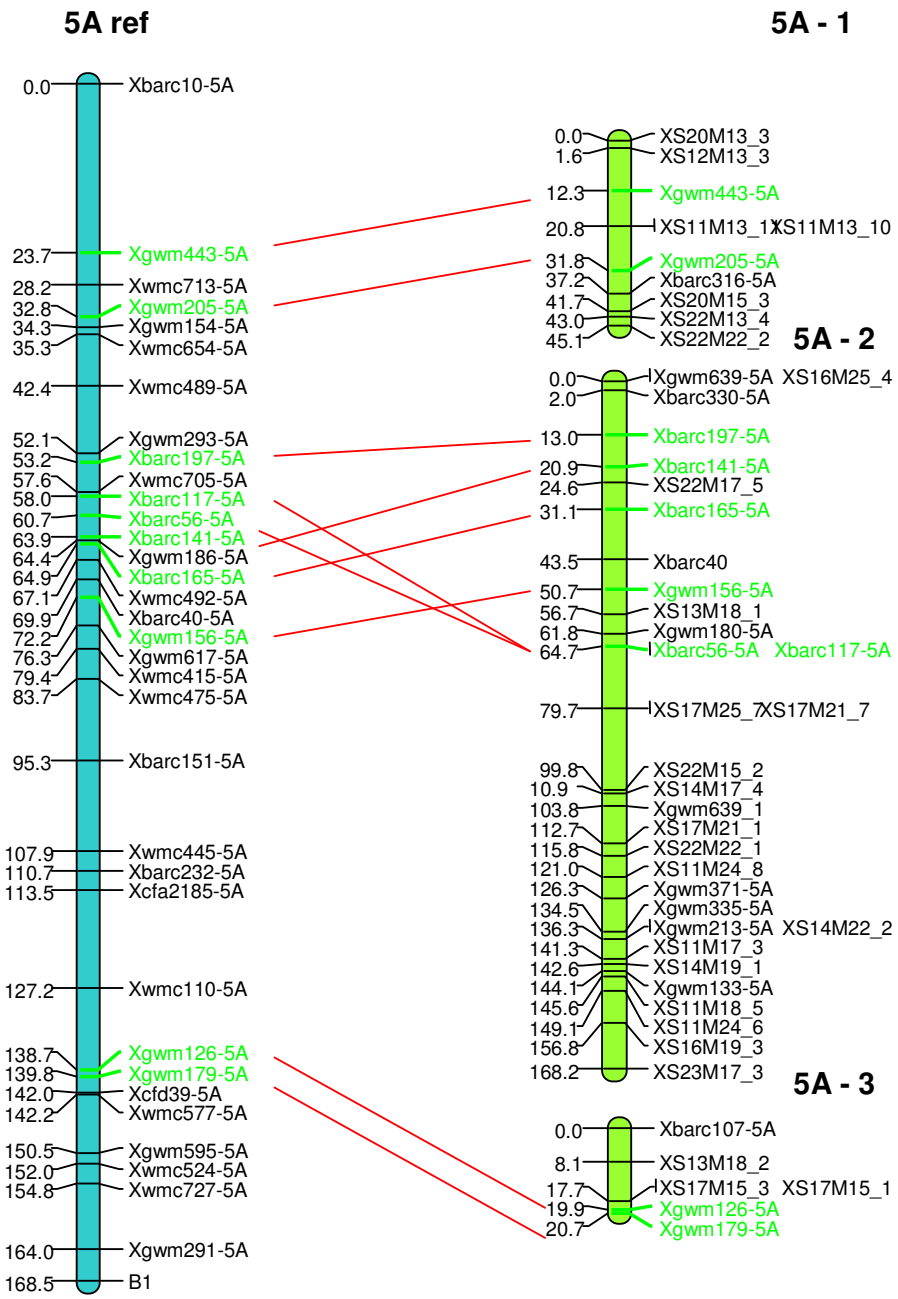

2B ref
2B - 1
2B - 2
0.0 Lr16
1.3 Xwmc764-2B
3.8 Xbarc35-2B
5.9 Xgwm210-2B
7.7 Xwmc382-2B
11.6 Xgwm614-2B
14.9 Xbarc124-2B
25.0 Xwmc25-2B
28.5 Xwmc154-2B
30.0 Xwmc243-2B
34.5 Xwmc213-2B
37.2 Xbarc200-2B
37.4 Xgwm257-2B
39.1 Xwmc257-2B
40.4 Xgwm429-2B
42.6 Xwmc770-2B
46.5 Xgwm148-2B
49.8 Xwmc434-2B
50.2 Xbarc13-2B
52.0 Xbarc183-2B
57.9 Xgwm630-2B
59.8 Xbarc18-2B
60.7 Xgwm374-2B
61.1 Xbarc167-2B
61.7 Xwmc265-2B
62.0 Xwmc179-2B
62.8 Xgwm132-2B
64.1 Xwmc498-2B
78.7 Xgwm120-2B
82.8 Xgwm16-2B
83.0 Xgwm47-2B
85.4 Xgwm501-2B
86.7 Xwmc175-2B
93.4 Xwmc332-2B
94.1 Xwmc627-2B
99.6 Xwmc149-2B
10.8 Xwmc361-2B
105.6 Xwmc317-2B
106.3 Xwmc817-2B
109.0 Xbarc159-2B
114.2 Xgwm382-2B
117.0 Xwmc356-2B
120.1 Xgwm526-2B
123.1 Xwmc602-2B
0.0 XS13M24_12
1.5 XS11M22_4
6.6 XS14M25_5
9.5 Xgwm614-2B
12.3 XS11M12_2
25.3 XS23M25_3
31.5 XS13M13_9
33.1 XS14M22_5 XS13M18_4
37.9 Xbarc154-2B
42.0 XS20M13_5
45.4 XS19M25_1
50.9 XS14M13_5
52.3 Xbarc200-2B
59.7 XS14M22_1
61.4 XS11M25_10
64.7 XS14M24_2
65.4 Xgwm429-2B
72.6 XS22M12_2
77.5 Xbarc13-2B
80.8 XS17M12_3 XS14M25_1
81.5 Xgwm374-2B
83.8 Xbarc18-2B Xbarc91-2B
84.6 Xbarc94-2B
86.7 Xgwm167-2B
0.0 XS20M17_3
19.3 XS19M13_4
24.9 XS11M15_2 XS14M18_7
28.8 XS13M15_1
31.9 XS13M13_6
45.8 XS20M22_3
60.9 Xgwm526-2B
71.1 XS22M19_2
86.5 XS23M25_5

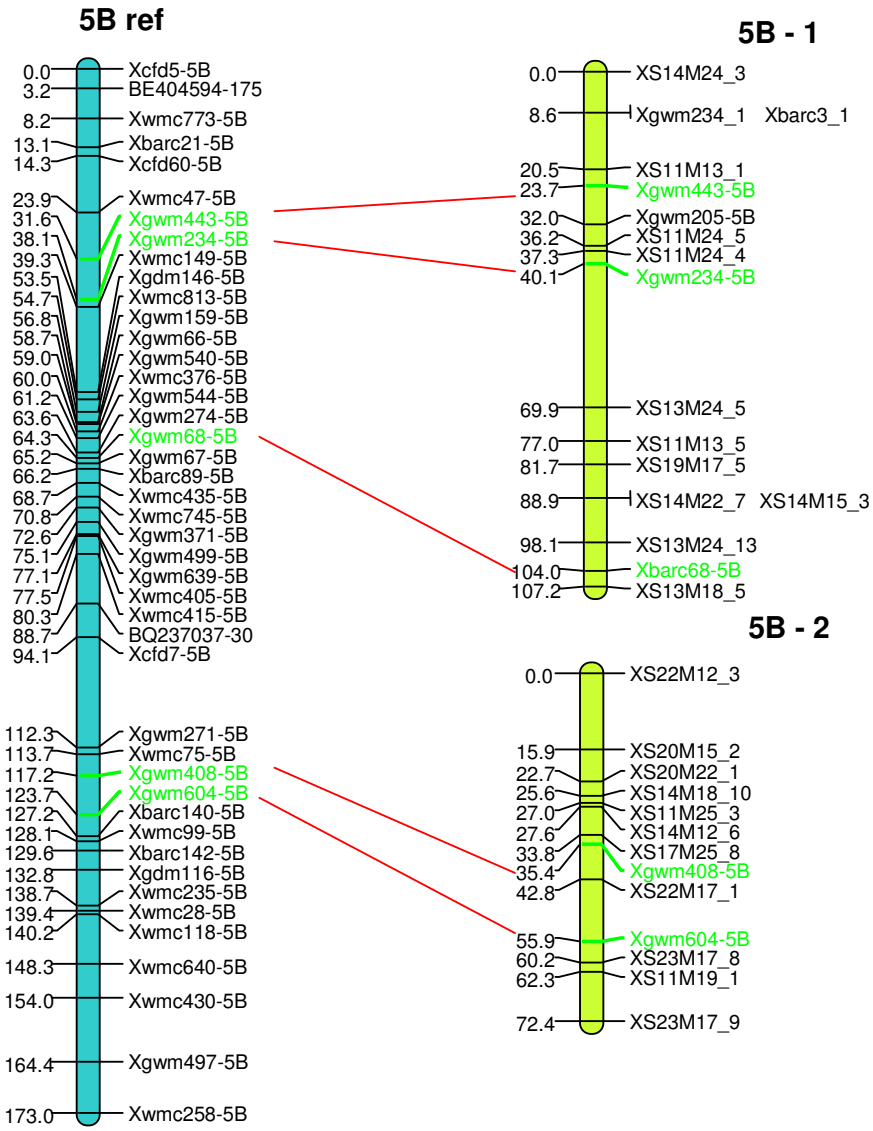

3A ref
0.0 Xwmc11-3A
5.7 Xwmc532-3A
9.5 Xcfd79-3A
13.8 Xgwm369-3A
36.7 Xbarc45-3A
37.0 Xgwm2-3A
41.7 Xwmc505-3A
43.7 Xgwm32-3A
45.3 Xgwm666-3A.1
46.2 Xwmc651-3A
47.1 Xbarc67-3A
48.0 Xwmc627-3A
48.9 Xgwm403-3A
52.5 Xwmc527-3A
53.2 Xwmc695-3A
54.4 Xwmc269-3A
55.7 Xwmc428-3A
61.2 Xwmc264-3A
66.8 Xgwm162-3A
71.5 Xwmc96-3A
72.9 Xbarc69-3A
73.0 Xgwm497-3A
73.8 Xcfa2193-3A
81.1 Xwmc173-3A
83.3 Xwmc559-3A
84.9 Xgwm155-3A
87.4 Xwmc153-3A
89.2 Xwmc215-3A
97.7 Xcfa2076-3A
99.3 Xwmc169-3A
99.9 Xwmc388-3A.2
105.0 Xwmc594-3A
106.0 Xgwm666-3A.2
115.8 Xgwm480-3A
3A - 1
0.0 XS11M25_4
7.5 XS19M25_6
20.0 XS14M15_2
25.5 XS14M15_8
28.1 Xgwm779-3A
34.3 XS23M25_8
39.6 Xgwm2-3A
49.2 Xbarc45-3A
73.3 Xbarc67-3A
79.2 Xgwm720-3A
82.8 Xbarc19-3A
88.9 Xgwm1121-3A
90.1 Xgwm1110-3A
98.3 XS20M15_4
100. Xgwm638-3A
103.6 Xbarc221_Xbarc221_2
121.3 Xgwm120-3A
142.3 XS13M12_3
156.0 XS13M26_1
3A - 2
0.0 XS14M12_7
2.3 Xgwm162-3A
3.9 XS11M24_2
7.1 XS13M25_1
13.7 XS22M25_2
14.8 XS13M22_3
22.7 Xgwm751-3A
28.9 XS20M12_1
29.7 XS11M22_1
40.7 Xgwm155-3A
50.0 XS11M25_6 XS11M25_12

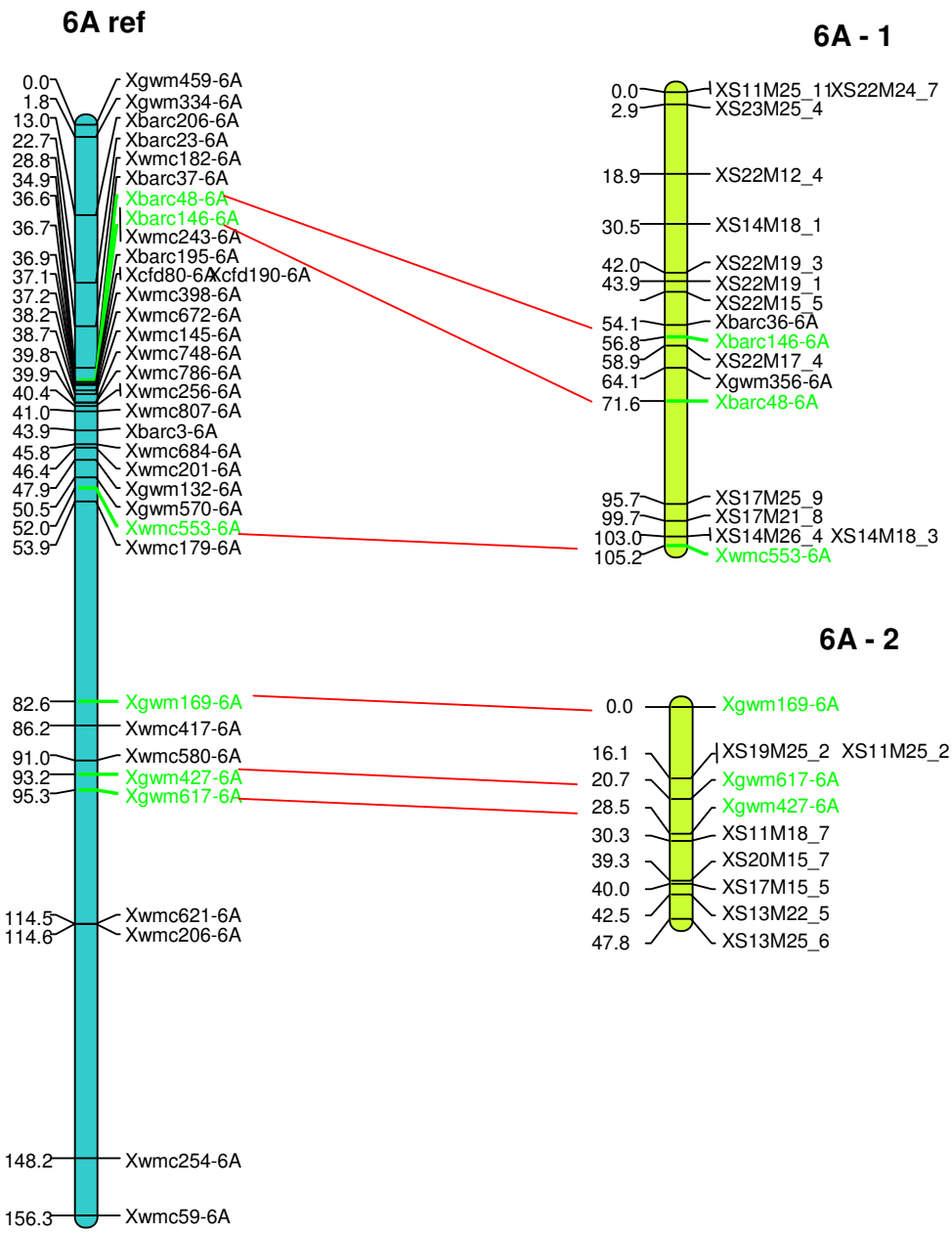

3B ref
0.0 Xbarc75-3B
0.2 Xbarc180-3B
7.2 Xbarc147-3B
11.8 Xgwm493-3B
14.2 XBARC87-3B
29.7 Xwmc597-3B
35.8 Xwmc623-3B
42.0 Xwmc679-3B
55.6 Xgwm264-3B
56.2 Xgwm284-3B
56.9 Xbarc68-3B
57.0 Xwmc815-3B
58.2 Xwmc612-3B
59.4 Xwmc625-3B
61.1 Xgwm274-3B
61.4 Xwmc615-3B
89.3 Xbarc229-3B
89.5 Xwmc291-3B
94.4 Xgwm108-3B
94.7 Xcfa2170-3B
97.1 Xbarc84-3B
97.2 Xbarc206-3B
114.3 Xwmc206-3B
122.5 Xgwm299-3B
125.4 Xgwm114-3B
137.7 Xgwm547-3B
139.3 Xgwm181-3B
141.5 Xgwm247-3B
142.1 Xwmc261-3B
147.6 Xgwm340-3B
3B - 1
0.0 XS11M25_1
3.0 XGwm12
10.2 XBarc57
21.1 XS22M25_6
40.7 XS22M15_7
45.0 Xbarc147
45.8 XBarc133
54.5 Xgwm493
3B - 2
0.0 XS22M12_1
6.3 XS20M17_2
12.0 XS19M25_13
15.1 XS19M25_12
17.5 Xbarc84
28.3 XS17M21_3
30.6 XS17M21_2
35.3 XS17M25_1
45.2 XS22M13_6
49.7 XS14M19_3
60.6 XS22M25_1
87.6 XS22M19_5
96.3 Xgwm247

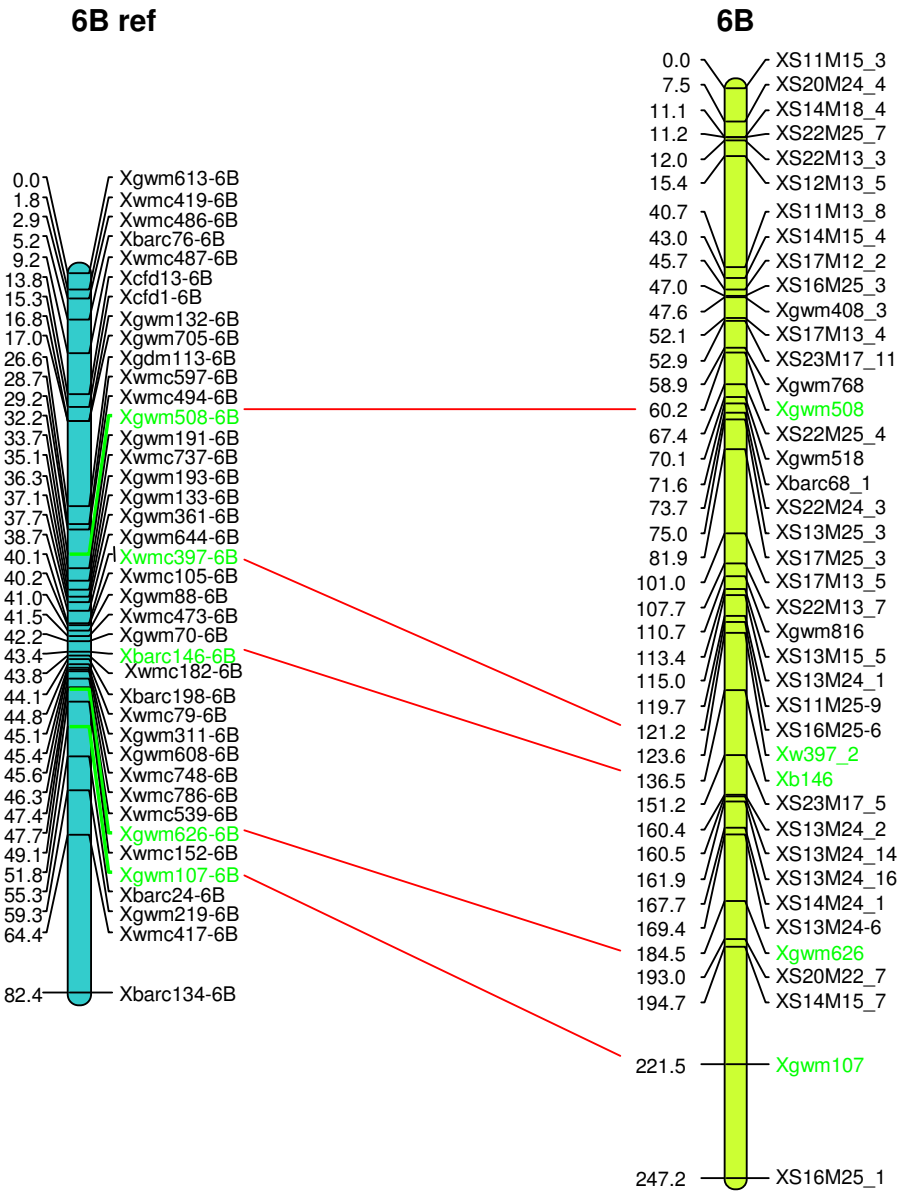

4A ref
0.0 Xgwm4-4A
1.9 Xwmc516-4A
6.4 Xbarc138-4A
8.5 Xwmc173-4A
8.9 Xgwm601-4A
9.1 Xgwm48-4A
10.0 Xwmc757-4A
10.5 Xwmc96-4A
12.1 Xgwm610-4A
13.3 Xwmc617-4A
18.4 Xgwm397-4A
22.2 Xwmc513-4A
24.9 Xwmc650-4A
26.6 Xbarc170-4A
36.5 Xgwm637-4A
37.7 Xwmc468-4A
38.9 Xwmc258-4A
46.6 Xgwm565-4A
49.1 Xgwm494-4A
51.3 Xcfd257-4A
57.4 Xgwm162-4A
58.3 Xwmc597-4A
59.0 Xwmc283-4A
60.3 Xcfd88-4A
61.8 Xwmc698-4A
67.4 Xwmc50-4A
68.1 Xwmc232-4A
69.8 Xcfd30-4A
70.9 Xbarc78-4A
78.9 Xgwm160-4A
81.0 Xcfd2-4A
82.4 Xwmc776-4A
82.9 Xwmc313-4A
87.6 Xwmc219-4A
4A - 1
0.0 Xbarc106-4A
4.9 Xgwm601-4A
5.0 XS14M12_3
6.4 XS20M25_1
10.8 Xgwm610-4A
19.7 XS19M25_10
27.7 XS17M25_4
38.2 Xbarc170-4A
43.5 XS13M15_3
4A - 2
0.0 XS13M12_1
9.1 XS11M18_4
10.6 XS14M18_2
17.0 XS11M18_3
20.2 XS13M15_2
25.4 XS22M24_4
28.8 Xgwm160-4A
35.3 XS14M25_2
37.6 XS17M21_4
43.6 Xgwm959_1
54.4 XS13M22_1
55.2 Xgwm959_3
61.7 XS11M12_3
80.0 Xgwm959_2

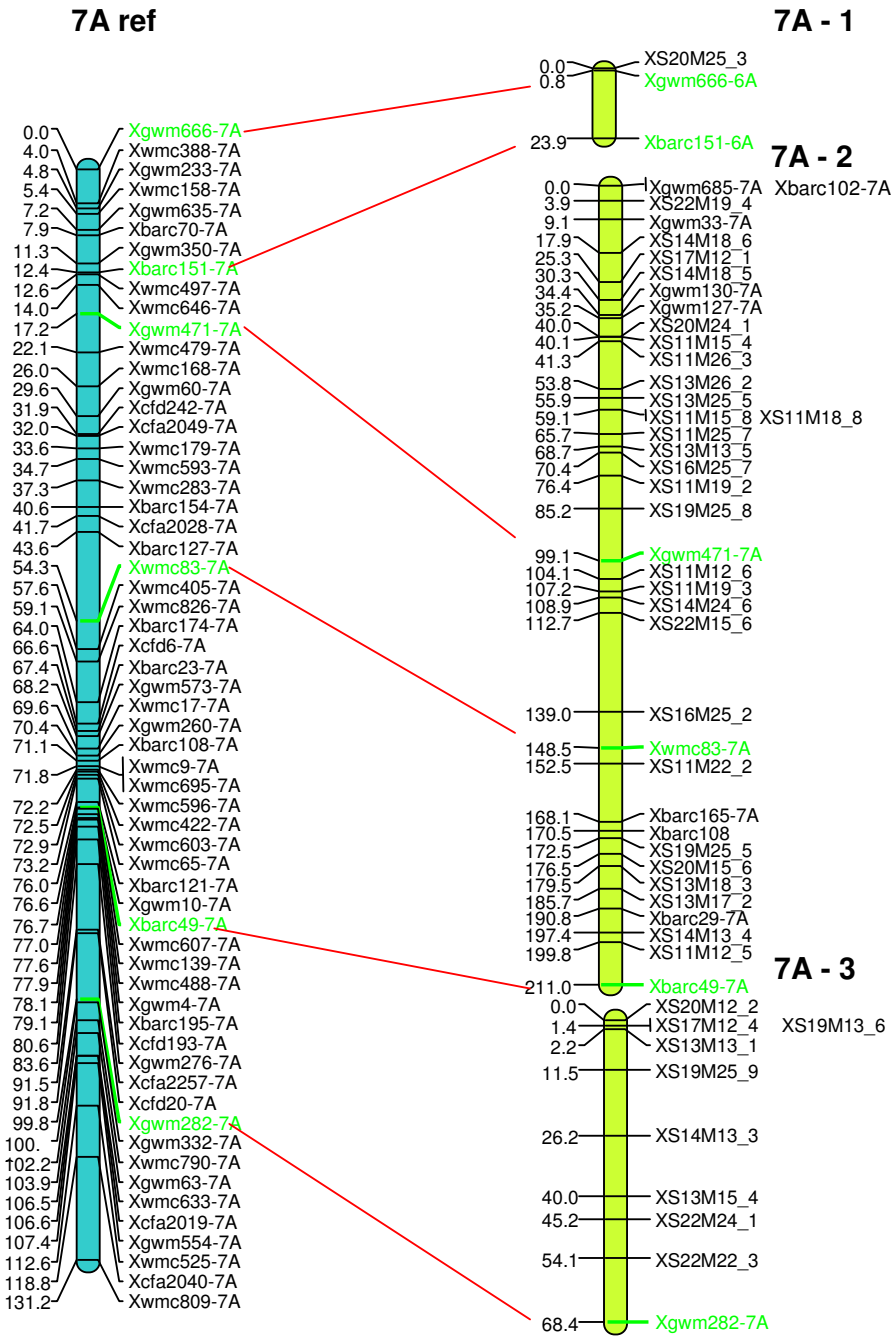

4B ref
0.0
Xwmc617-4B
21.6
Xbarc20-4B
21.7
Xgwm540-4B
24.5
Xwmc16-4B
25.0
Xwmc238-4B
26.3
Xwmc546-4B
26.7
Xwmc657-4B
28.9
Xgwm192-4B
29.4
Xcfd283-4B
30.9
Xgwm149-4B
31.4
Xgwm495-4B
33.4
Xcfd22-4B
35.7
Xgwm251-4B
35.9
Xwmc692-4B
37.3
Xwmc310-4B
45.9
Xbarc109-4B
49.3
Xgwm538-4B
53.7
Xbarc10-4B
59.4
Xwmc125-4B
4B
0.0
XS16M25_5
11.7
XS23M25_2
31.7
XS22M17_3
37.8
XS22M17_2
72.4
Xgwm540
104.2
XS20M25_4
111.0
XS14M26_6
114.5
XS13M24_10
116.1
XS13M24_6
123.9
Xgwm781
127.6
Xgwm149
131.2
XS14M15_5
133.6
XS11M18_10
134.4
Xgwm375

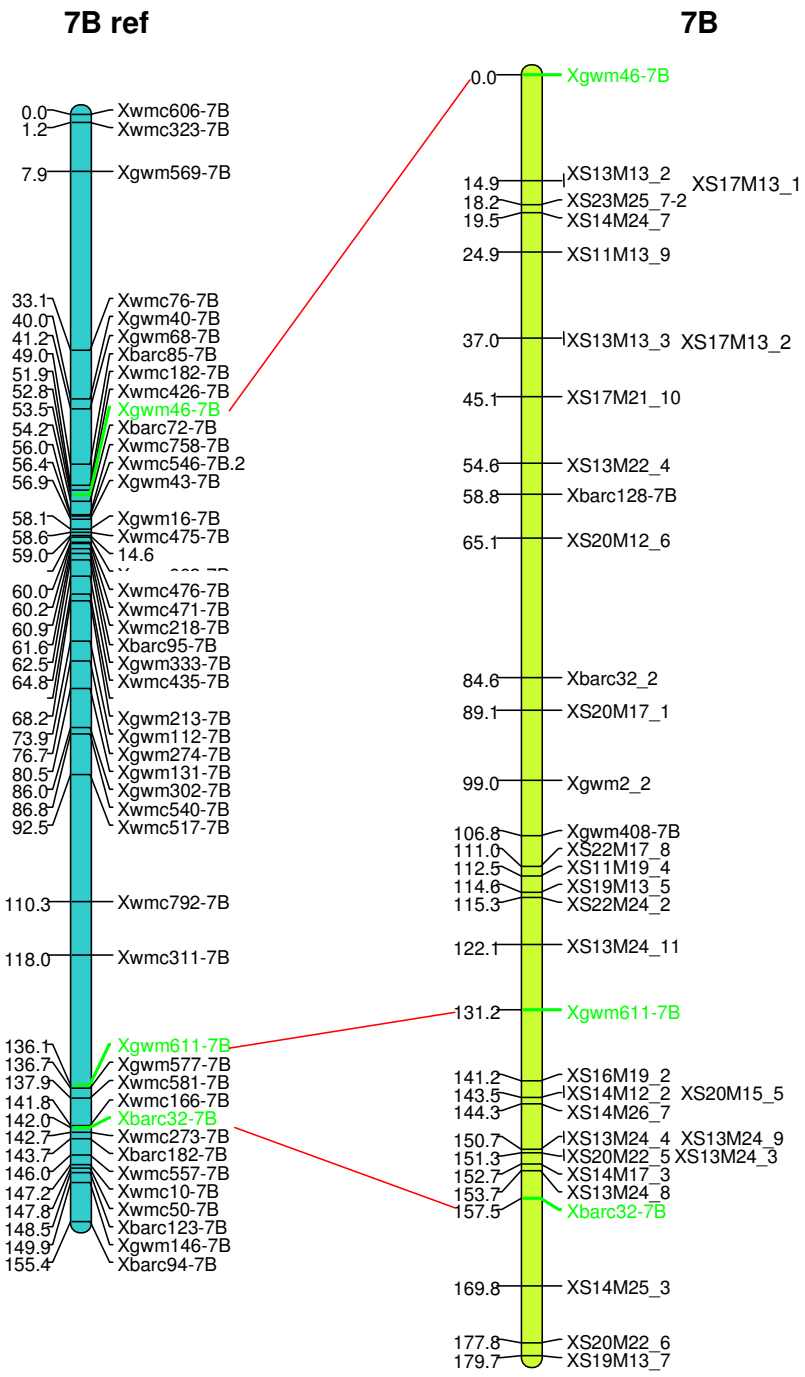

5A ref
5A - 1
5A - 2
5A - 3
0.0 Xbarc10-5A
23.7 Xgwm443-5A
28.2 Xwmc713-5A
32.8 Xgwm205-5A
34.3 Xgwm154-5A
35.3 Xwmc654-5A
42.4 Xwmc489-5A
52.1 Xgwm293-5A
53.2 Xbarc197-5A
57.6 Xwmc705-5A
58.0 Xbarc117-5A
60.7 Xbarc56-5A
63.9 Xbarc141-5A
64.4 Xgwm186-5A
64.9 Xbarc165-5A
67.1 Xwmc492-5A
69.9 Xbarc40-5A
72.2 Xgwm156-5A
76.3 Xgwm617-5A
79.4 Xwmc415-5A
83.7 Xwmc475-5A
95.3 Xbarc151-5A
107.9 Xwmc445-5A
110.7 Xbarc232-5A
113.5 Xcfa2185-5A
127.2 Xwmc110-5A
138.7 Xgwm126-5A
139.8 Xgwm179-5A
142.0 Xcfd39-5A
142.2 Xwmc577-5A
150.5 Xgwm595-5A
152.0 Xwmc524-5A
154.8 Xwmc727-5A
164.0 Xgwm291-5A
168.5 B1
0.0 XS20M13_3
1.6 XS12M13_3
12.3 Xgwm443-5A
20.8 XS11M13_1 XS11M13_10
31.8 Xgwm205-5A
37.2 Xbarc316-5A
41.7 XS20M15_3
43.0 XS22M13_4
45.1 XS22M22_2
0.0 Xgwm639-5A XS16M25_4
2.0 Xbarc330-5A
13.0 Xbarc197-5A
20.9 Xbarc141-5A
24.6 XS22M17_5
31.1 Xbarc165-5A
43.5 Xbarc40
50.7 Xgwm156-5A
56.7 XS13M18_1
61.8 Xgwm180-5A
64.7 Xbarc56-5A Xbarc117-5A
79.7 XS17M25_7 XS17M21_7
99.8 XS22M15_2
10.9 XS14M17_4
103.8 Xgwm639_1
112.7 XS17M21_1
115.8 XS22M22_1
121.0 XS11M24_8
126.3 Xgwm371-5A
134.5 Xgwm335-5A
136.3 Xgwm213-5A XS14M22_2
141.3 XS11M17_3
142.6 XS14M19_1
144.1 Xgwm133-5A
145.6 XS11M18_5
149.1 XS11M24_6
156.8 XS16M19_3
168.2 XS23M17_3
0.0 Xbarc107-5A
8.1 XS13M18_2
17.7 XS17M15_3 XS17M15_1
19.9 Xgwm126-5A
20.7 Xgwm179-5A

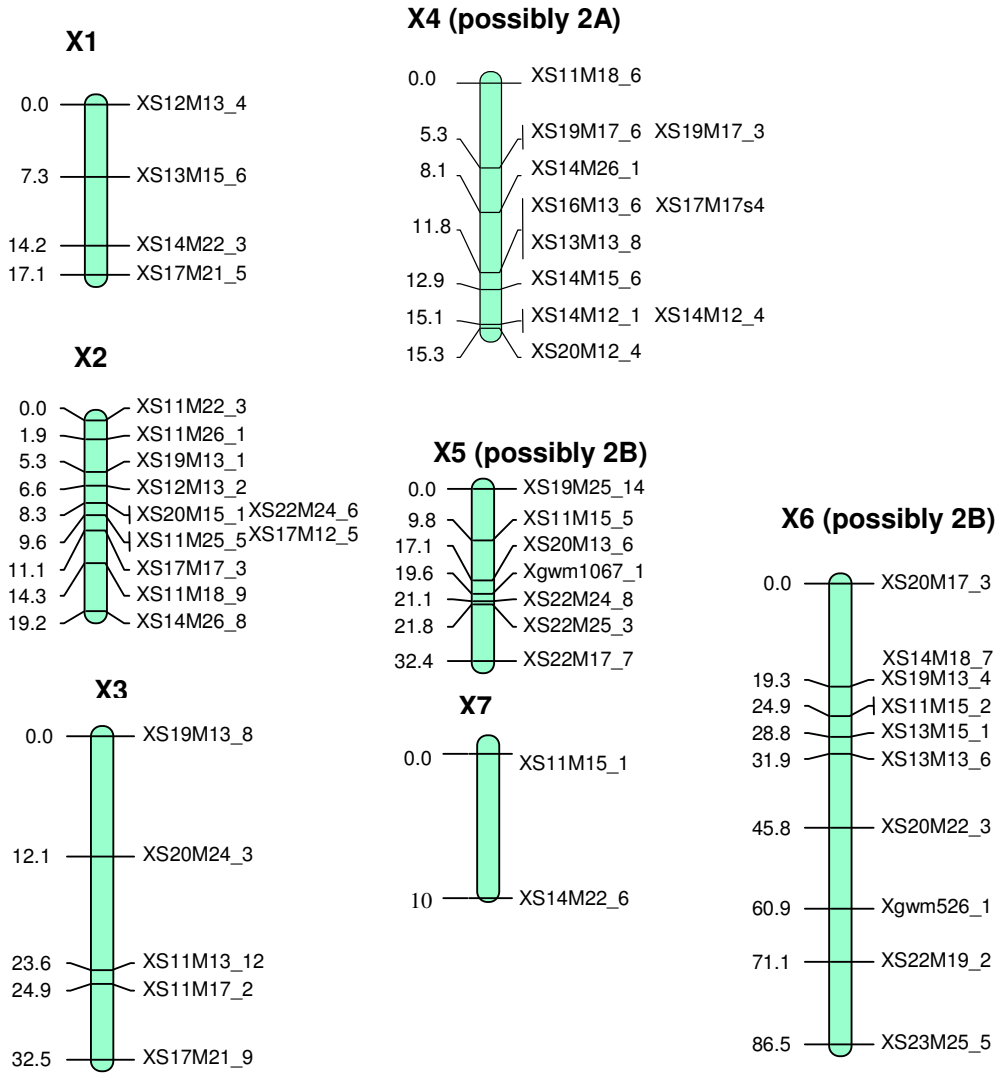

5B ref
0.0 Xcfd5-5B
3.2 BE404594-175
8.2 Xwmc773-5B
13.1 Xbarc21-5B
14.3 Xcfd60-5B
23.9 Xwmc47-5B
31.6 Xgwm443-5B
38.1 Xgwm234-5B
39.3 Xwmc149-5B
53.5 Xgdm146-5B
54.7 Xwmc813-5B
56.8 Xgwm159-5B
58.7 Xgwm66-5B
59.0 Xgwm540-5B
60.0 Xwmc376-5B
61.2 Xgwm544-5B
63.6 Xgwm274-5B
64.3 Xgwm68-5B
65.2 Xgwm67-5B
66.2 Xbarc89-5B
68.7 Xwmc435-5B
70.8 Xwmc745-5B
72.6 Xgwm371-5B
75.1 Xgwm499-5B
77.1 Xgwm639-5B
77.5 Xwmc405-5B
80.3 Xwmc415-5B
88.7 BQ237037-30
94.1 Xcfd7-5B
112.3 Xgwm271-5B
113.7 Xwmc75-5B
117.2 Xgwm408-5B
123.7 Xgwm604-5B
127.2 Xbarc140-5B
128.1 Xwmc99-5B
129.6 Xbarc142-5B
132.8 Xgdm116-5B
138.7 Xwmc235-5B
139.4 Xwmc28-5B
140.2 Xwmc118-5B
148.3 Xwmc640-5B
154.0 Xwmc430-5B
164.4 Xgwm497-5B
173.0 Xwmc258-5B
5B - 1
0.0 XS14M24_3
8.6 Xgwm234_1 Xbarc3_1
20.5 XS11M13_1
23.7 Xgwm443-5B
32.0 Xgwm205-5B
36.2 XS11M24_5
37.3 XS11M24_4
40.1 Xgwm234-5B
69.9 XS13M24_5
77.0 XS11M13_5
81.7 XS19M17_5
88.9 XS14M22_7 XS14M15_3
98.1 XS13M24_13
104.0 Xbarc68-5B
107.2 XS13M18_5
5B - 2
0.0 XS22M12_3
15.9 XS20M15_2
22.7 XS20M22_1
25.6 XS14M18_10
27.0 XS11M25_3
27.6 XS14M12_6
33.8 XS17M25_8
35.4 Xgwm408-5B
42.8 XS22M17_1
55.9 Xgwm604-5B
60.2 XS23M17_8
62.3 XS11M19_1
72.4 XS23M17_9


6A ref
0.0 Xgwm459-6A
1.8 Xgwm334-6A
13.0 Xbarc206-6A
22.7 Xbarc23-6A
28.8 Xwmc182-6A
34.9 Xbarc37-6A
36.6 Xbarc48-6A
36.7 Xbarc146-6A
Xwmc243-6A
36.9 Xbarc195-6A
37.1 Xcfd80-6A Xcfd190-6A
37.2 Xwmc398-6A
38.2 Xwmc672-6A
38.7 Xwmc145-6A
39.8 Xwmc748-6A
39.9 Xwmc786-6A
40.4 Xwmc256-6A
41.0 Xwmc807-6A
43.9 Xbarc3-6A
45.8 Xwmc684-6A
46.4 Xwmc201-6A
47.9 Xgwm132-6A
50.5 Xgwm570-6A
52.0 Xwmc553-6A
53.9 Xwmc179-6A
82.6 Xgwm169-6A
86.2 Xwmc417-6A
91.0 Xwmc580-6A
93.2 Xgwm427-6A
95.3 Xgwm617-6A
114.5 Xwmc621-6A
114.6 Xwmc206-6A
148.2 Xwmc254-6A
156.3 Xwmc59-6A
6A - 1
0.0 XS11M25_11 XS22M24_7
2.9 XS23M25_4
18.9 XS22M12_4
30.5 XS14M18_1
42.0 XS22M19_3
43.9 XS22M19_1
XS22M15_5
54.1 Xbarc36-6A
56.8 Xbarc146-6A
58.9 XS22M17_4
64.1 Xgwm356-6A
71.6 Xbarc48-6A
95.7 XS17M25_9
99.7 XS17M21_8
103.0 XS14M26_4 XS14M18_3
105.2 Xwmc553-6A
6A - 2
0.0 Xgwm169-6A
16.1 XS19M25_2 XS11M25_2
20.7 Xgwm617-6A
28.5 Xgwm427-6A
30.3 XS11M18_7
39.3 XS20M15_7
40.0 XS17M15_5
42.5 XS13M22_5
47.8 XS13M25_6


6B ref
6B
0.0 Xgwm613-6B
1.8 Xwmc419-6B
2.9 Xwmc486-6B
5.2 Xbarc76-6B
9.2 Xwmc487-6B
13.8 Xcfd13-6B
15.3 Xcfd1-6B
16.8 Xgwm132-6B
17.0 Xgwm705-6B
26.6 Xgdm113-6B
28.7 Xwmc597-6B
29.2 Xwmc494-6B
32.2 Xgwm508-6B
33.7 Xgwm191-6B
35.1 Xwmc737-6B
36.3 Xgwm193-6B
37.1 Xgwm133-6B
37.7 Xgwm361-6B
38.7 Xgwm644-6B
40.1 Xwmc397-6B
40.2 Xwmc105-6B
41.0 Xgwm88-6B
41.5 Xwmc473-6B
42.2 Xgwm70-6B
43.4 Xbarc146-6B
43.8 Xwmc182-6B
44.1 Xbarc198-6B
44.8 Xwmc79-6B
45.1 Xgwm311-6B
45.4 Xgwm608-6B
45.6 Xwmc748-6B
46.3 Xwmc786-6B
47.4 Xwmc539-6B
47.7 Xgwm626-6B
49.1 Xwmc152-6B
51.8 Xgwm107-6B
55.3 Xbarc24-6B
59.3 Xgwm219-6B
64.4 Xwmc417-6B
82.4 Xbarc134-6B
0.0 XS11M15_3
7.5 XS20M24_4
11.1 XS14M18_4
11.2 XS22M25_7
12.0 XS22M13_3
15.4 XS12M13_5
40.7 XS11M13_8
43.0 XS14M15_4
45.7 XS17M12_2
47.0 XS16M25_3
47.6 Xgwm408_3
52.1 XS17M13_4
52.9 XS23M17_11
58.9 Xgwm768
60.2 Xgwm508
67.4 XS22M25_4
70.1 Xgwm518
71.6 Xbarc68_1
73.7 XS22M24_3
75.0 XS13M25_3
81.9 XS17M25_3
101.0 XS17M13_5
107.7 XS22M13_7
110.7 Xgwm816
113.4 XS13M15_5
115.0 XS13M24_1
119.7 XS11M25-9
121.2 XS16M25-6
123.6 Xw397_2
136.5 Xb146
151.2 XS23M17_5
160.4 XS13M24_2
160.5 XS13M24_14
161.9 XS13M24_16
167.7 XS14M24_1
169.4 XS13M24-6
184.5 Xgwm626
193.0 XS20M22_7
194.7 XS14M15_7
221.5 Xgwm107
247.2 XS16M25_1


7A ref
7A - 1
7A - 2
7A - 3
0.0 Xgwm666-7A
4.0 Xwmc388-7A
4.8 Xgwm233-7A
5.4 Xwmc158-7A
7.2 Xgwm635-7A
7.9 Xbarc70-7A
11.3 Xgwm350-7A
12.4 Xbarc151-7A
12.6 Xwmc497-7A
14.0 Xwmc646-7A
17.2 Xgwm471-7A
22.1 Xwmc479-7A
26.0 Xwmc168-7A
29.6 Xgwm60-7A
31.9 Xcfd242-7A
32.0 Xcfa2049-7A
33.6 Xwmc179-7A
34.7 Xwmc593-7A
37.3 Xwmc283-7A
40.6 Xbarc154-7A
41.7 Xcfa2028-7A
43.6 Xbarc127-7A
54.3 Xwmc83-7A
57.6 Xwmc405-7A
59.1 Xwmc826-7A
64.0 Xbarc174-7A
66.6 Xcfd6-7A
67.4 Xbarc23-7A
68.2 Xgwm573-7A
69.6 Xwmc17-7A
70.4 Xgwm260-7A
71.1 Xbarc108-7A
71.8 Xwmc9-7A
Xwmc695-7A
72.2 Xwmc596-7A
72.5 Xwmc422-7A
72.9 Xwmc603-7A
73.2 Xwmc65-7A
76.0 Xbarc121-7A
76.6 Xgwm10-7A
76.7 Xbarc49-7A
77.0 Xwmc607-7A
77.6 Xwmc139-7A
77.9 Xwmc488-7A
78.1 Xgwm4-7A
79.1 Xbarc195-7A
80.6 Xcfd193-7A
83.6 Xgwm276-7A
91.5 Xcfa2257-7A
91.8 Xcfd20-7A
99.8 Xgwm282-7A
100. Xgwm332-7A
102.2 Xwmc790-7A
103.9 Xgwm63-7A
106.5 Xwmc633-7A
106.6 Xcfa2019-7A
107.4 Xgwm554-7A
112.6 Xwmc525-7A
118.8 Xcfa2040-7A
131.2 Xwmc809-7A
0.0 XS20M25_3
0.8 Xgwm666-6A
23.9 Xbarc151-6A
0.0 Xgwm685-7A Xbarc102-7A
3.9 XS22M19_4
9.1 Xgwm33-7A
17.9 XS14M18_6
25.3 XS17M12_1
30.3 XS14M18_5
34.4 Xgwm130-7A
35.2 Xgwm127-7A
40.0 XS20M24_1
40.1 XS11M15_4
41.3 XS11M26_3
53.8 XS13M26_2
55.9 XS13M25_5
59.1 XS11M15_8 XS11M18_8
65.7 XS11M25_7
68.7 XS13M13_5
70.4 XS16M25_7
76.4 XS11M19_2
85.2 XS19M25_8
99.1 Xgwm471-7A
104.1 XS11M12_6
107.2 XS11M19_3
108.9 XS14M24_6
112.7 XS22M15_6
139.0 XS16M25_2
148.5 Xwmc83-7A
152.5 XS11M22_2
168.1 Xbarc165-7A
170.5 Xbarc108
172.5 XS19M25_5
176.5 XS20M15_6
179.5 XS13M18_3
185.7 XS13M17_2
190.8 Xbarc29-7A
197.4 XS14M13_4
199.8 XS11M12_5
211.0 Xbarc49-7A
0.0 XS20M12_2
1.4 XS17M12_4 XS19M13_6
2.2 XS13M13_1
11.5 XS19M25_9
26.2 XS14M13_3
40.0 XS13M15_4
45.2 XS22M24_1
54.1 XS22M22_3
68.4 Xgwm282-7A


7B ref
7B
0.0 Xwmc606-7B
1.2 Xwmc323-7B
7.9 Xgwm569-7B
33.1 Xwmc76-7B
40.0 Xgwm40-7B
41.2 Xgwm68-7B
49.0 Xbarc85-7B
51.9 Xwmc182-7B
52.8 Xwmc426-7B
53.5 Xgwm46-7B
54.2 Xbarc72-7B
56.0 Xwmc758-7B
56.4 Xwmc546-7B.2
56.9 Xgwm43-7B
58.1 Xgwm16-7B
58.6 Xwmc475-7B
59.0 14.6
60.0 Xwmc476-7B
60.2 Xwmc471-7B
60.9 Xwmc218-7B
61.6 Xbarc95-7B
62.5 Xgwm333-7B
64.8 Xwmc435-7B
68.2 Xgwm213-7B
73.9 Xgwm112-7B
76.7 Xgwm274-7B
80.5 Xgwm131-7B
86.0 Xgwm302-7B
86.8 Xwmc540-7B
92.5 Xwmc517-7B
110.3 Xwmc792-7B
118.0 Xwmc311-7B
136.1 Xgwm611-7B
136.7 Xgwm577-7B
137.9 Xwmc581-7B
141.8 Xwmc166-7B
142.0 Xbarc32-7B
142.7 Xwmc273-7B
143.7 Xbarc182-7B
146.0 Xwmc557-7B
147.2 Xwmc10-7B
147.8 Xwmc50-7B
148.5 Xbarc123-7B
149.9 Xgwm146-7B
155.4 Xbarc94-7B
0.0 Xgwm46-7B
14.9 XS13M13_2 XS17M13_1
18.2 XS23M25_7-2
19.5 XS14M24_7
24.9 XS11M13_9
37.0 XS13M13_3 XS17M13_2
45.1 XS17M21_10
54.6 XS13M22_4
58.8 Xbarc128-7B
65.1 XS20M12_6
84.6 Xbarc32_2
89.1 XS20M17_1
99.0 Xgwm2_2
106.8 Xgwm408-7B
111.0 XS22M17_8
112.5 XS11M19_4
114.6 XS19M13_5
115.3 XS22M24_2
122.1 XS13M24_11
131.2 Xgwm611-7B
141.2 XS16M19_2
143.5 XS14M12_2 XS20M15_5
144.3 XS14M26_7
150.7 XS13M24_4 XS13M24_9
151.3 XS20M22_5 XS13M24_3
152.7 XS14M17_3
153.7 XS13M24_8
157.5 Xbarc32-7B
169.8 XS14M25_3
177.8 XS20M22_6
179.7 XS19M13_7


X1
0.0 XS12M13_4
7.3 XS13M15_6
14.2 XS14M22_3
17.1 XS17M21_5
X2
0.0 XS11M22_3
1.9 XS11M26_1
5.3 XS19M13_1
6.6 XS12M13_2
8.3 XS20M15_1 XS22M24_6
9.6 XS11M25_5 XS17M12_5
11.1 XS17M17_3
14.3 XS11M18_9
19.2 XS14M26_8
X3
0.0 XS19M13_8
12.1 XS20M24_3
23.6 XS11M13_12
24.9 XS11M17_2
32.5 XS17M21_9
X4 (possibly 2A)
0.0 XS11M18_6
5.3 XS19M17_6 XS19M17_3
8.1 XS14M26_1
11.8 XS16M13_6 XS17M17s4
XS13M13_8
12.9 XS14M15_6
15.1 XS14M12_1 XS14M12_4
15.3 XS20M12_4
X5 (possibly 2B)
0.0 XS19M25_14
9.8 XS11M15_5
17.1 XS20M13_6
19.6 Xgwm1067_1
21.1 XS22M24_8
21.8 XS22M25_3
32.4 XS22M17_7
X7
0.0 XS11M15_1
10 XS14M22_6
X6 (possibly 2B)
0.0 XS20M17_3
XS14M18_7
19.3 XS19M13_4
24.9 XS11M15_2
28.8 XS13M15_1
31.9 XS13M13_6
45.8 XS20M22_3
60.9 Xgwm526_1
71.1 XS22M19_2
86.5 XS23M25_5

3.10. QTL and Markers analysis

The QTL mapping was performed using the Qgene program (version-4.2.3) (Nelson 1997). QTL analysis revealed associations of several genomic regions with FHB traits. QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM). The reproducible QTL were defined based on the significance level of permutation test.

3.10.1. QTL analysis for FHB spread

QTL for %DS and n.DS were detected on chromosomes 3A and 6B. Two separate QTL were detected on chromosome 3A (significant at LOD = 3.3, $\alpha < 0.05$). The first QTL on 3A mapped to the flanking markers *Xgwm1121 - Xgwm720* and the second QTL on 3A mapped to the flanking markers *Xgwm2 - Xgwm779*. The most likely positions of these two QTL appeared at a distance of 50 cM (Figure 24). The effect of the first and second QTL was found significant ($\alpha < 0.05$) for FHB spread (%DS and n.DS) overall mean value and in four single experiments (field (n.DS), GH1, GH2 and GH3) for the first QTL and in two single experiments (GH2 and GH3) for the second QTL. One QTL was detected on chromosomes 6B (significant at LOD 3.2, $\alpha < 0.05$). The effect of the QTL was found significant ($\alpha < 0.05$) for FHB spread (%DS and n.DS) for the overall mean values and in three single experiments (GH2, GH3 and GH4). The position for 6B QTL is in the flanking markers *XS23M17_5 - Xgwm626* (Figure 25). The resistance was conferred by alleles of *T. diccocoides* in both chromosomes. The effects associated with FHB spread mapping to chromosome 3A, explained 20% and 15% of the phenotypic variation for the first and second QTL, respectively measured by %DS. The percent of explained phenotypic variation was 23% and 18% for the first and second 3A measured by n.DS. The effect associated with FHB spread mapping to chromosome 6B, explained 26% and 21% of the phenotypic variation for %DS and n.DS, respectively (Table 25).

Table 25: QTL detected for %DS and n.DS 21 dai. Chromosomal location, logarithm of odds (LOD), percentage of explained phenotypic variance (R^2) for single experiments and means over all experiments. QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM).

Chromosome	Flanking markers	Field	GH1	GH2	GH3	GH4	mean	mean
%DS		SIM	SIM	SIM	SIM	SIM	SIM	CIM
LOD								
3A -1	*Xgwm1121 - Xgwm720*	2.5	3.2	3.5	3.8	2.2	4.8**	4.3
3A -1	*Xgwm2 - Xgwm779*	1.4	2.3	4.1	3.7	1.2	4.5**	4.5
6B	*XS23M17_5 - Xgwm626*	2.8	1.4	5.7	3.8	4.8	6.4**	6.4
R^2								
3A -1	*Xgwm1121 - Xgwm720*	0.13	0.14	0.15	0.09	0.10	0.20	0.17
3A -1	*Xgwm2 - Xgwm779*	0.10	0.11	0.14	0.06	0.04	0.15	0.18
6B	*XS23M17_5 - Xgwm626*	0.07	0.24	0.17	0.20	0.12	0.26	0.24
n.DS								
LOD								
3A -1	*Xgwm1121 - Xgwm720*	3.1	3.8	4.4	4.1	2.4	6.0**	5.5
3A -1	*Xgwm2 - Xgwm779*	2.1	2.9	3.4	4.9	1.1	5.0**	4.8
6B	*XS23M17_5 - Xgwm626*	1.0	1.3	5.8	4.6	4.7	5.5**	4.4
R^2								
3A -1	*Xgwm1121 - Xgwm720*	0.12	0.16	0.18	0.17	0.10	0.23	0.21
3A -1	*Xgwm2 - Xgwm779*	0.09	0.12	0.14	0.19	0.05	0.18	0.19
6B	*XS23M17_5 - Xgwm626*	0.04	0.06	0.22	0.18	0.19	0.21	0.17

** $p < 0.01$; * $p < 0.05$;

The phenotypic effect of the three detected QTL for n.DS and %DS 21 dai. is illustrated in Table 26. Lines with the *T. diccocoides* allele of the QTL on chromosome 3A near *Xgwm720* showed an average 2.5 less infected spikelets compared to lines with the Helidure allele. Similarly lines with the *T. diccocoides* allele of the QTL on 3A near *Xgwm2* showed an average 2.8 less infected spikelets compared to lines with the Helidur allele. Lines with the *T. diccocoides* allele of the QTL on chromosome 6B near *XS13M24-6* showed an average 2.1 less infected spikelets compared to lines with the Helidur allele.

The Box plot in Figure 26 is an example of %DS peak markers mapping to chromosomes 3A and 6B.

Table 26: Numbers and mean values of lines alternative alleles of three QTL for % DS and n.DS 21 dai. (*T. dic: *T. diccocoides*, N: Number of lines).

				*T.dic		Helidur		Hetero.	
Traits	**chromosome**	**closest marker**	**Prob.***	**N**	**Mean**	**N**	**Mean**	**N**	**Mean**
	3A -1	*Xgwm720*	<.0001	20	42.3	69	57.2	7	40.9
	3A -1	*Xgwm2*	.0001	16	39.2	69	56	11	49.8
%DS	**6B**	*XS13M24-6*	<.0001	32	42.9	57	58		
	3A -1	*Xgwm720*	<.0001	20	5.4	69	7.9	7	5.3
	3A -1	*Xgwm2*	<.0001	16	4.9	69	7.7	11	6.8
n.DS	**6B**	*XS13M24-6*	<.0001	32	5.8	57	7.9		

* P- value from analysis of variance for mean differences between alternative alleles.

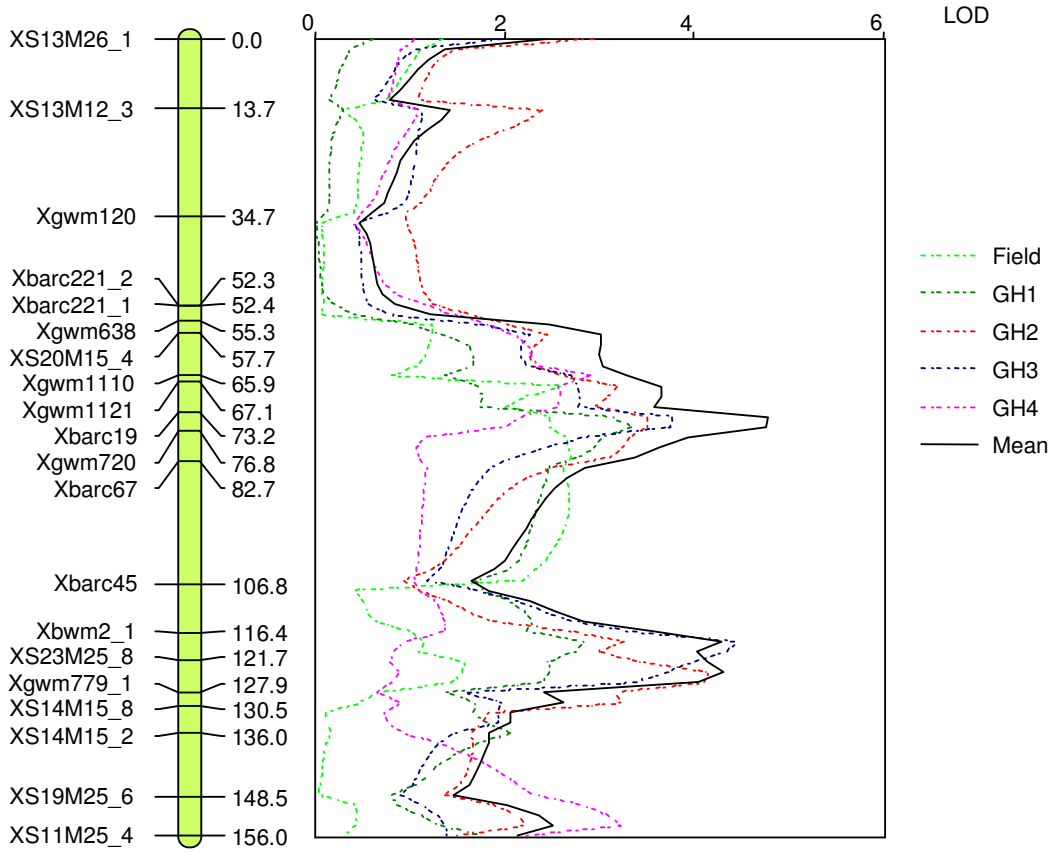


Figure 24: Interval analysis of a QTL 21 dai for %DS in field, GH1, GH2, GH3, GH4 and mean over all experiments is depicted. Analysis was performed by simple interval mapping (SIM). The QTL is on a linkage group corresponding to a part of chromosome 3A.

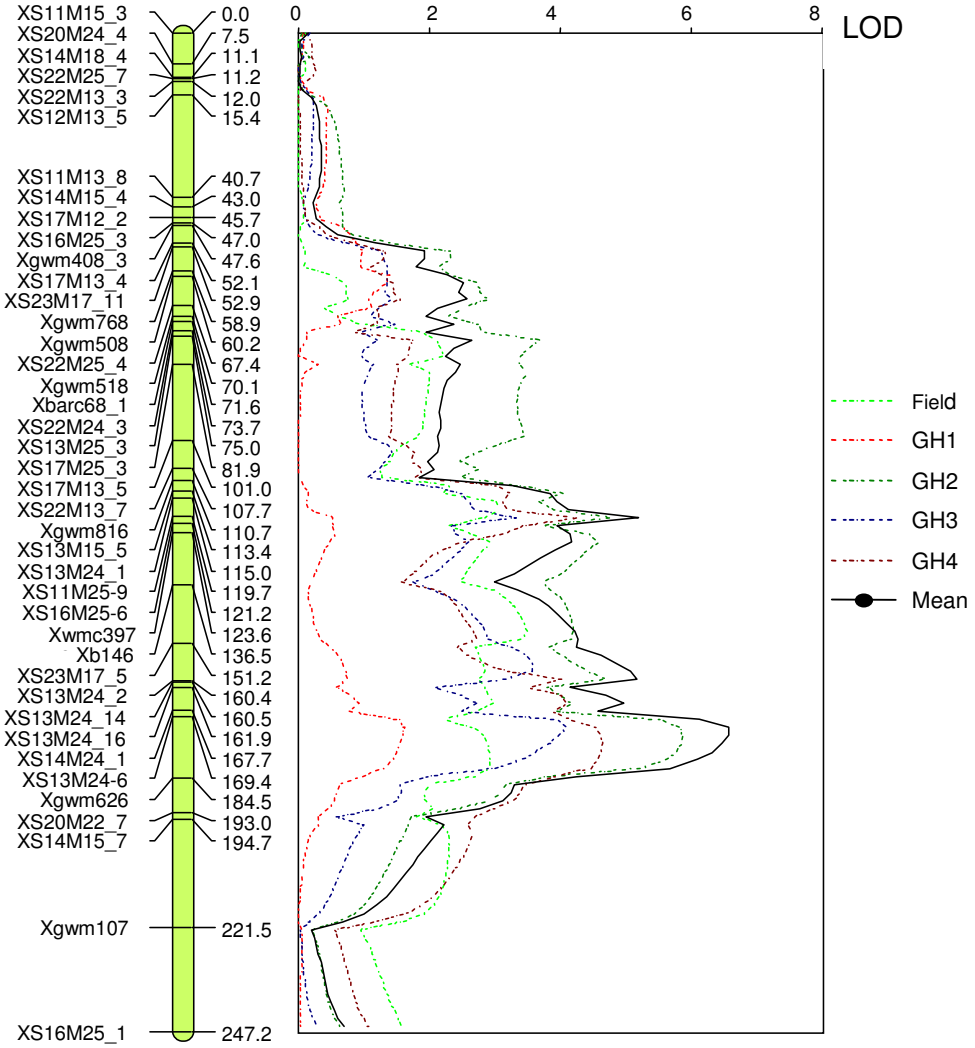


Figure 25: Interval analysis of a QTL for %DS 21 dai. The LOD curves for all experiments (Field, GH1, GH2, GH3, GH4) and for the mean over all experiments is depicted. Analysis was performed by simple interval mapping (SIM). The QTL is on a linkage group corresponding to a part of chromosome 6B.

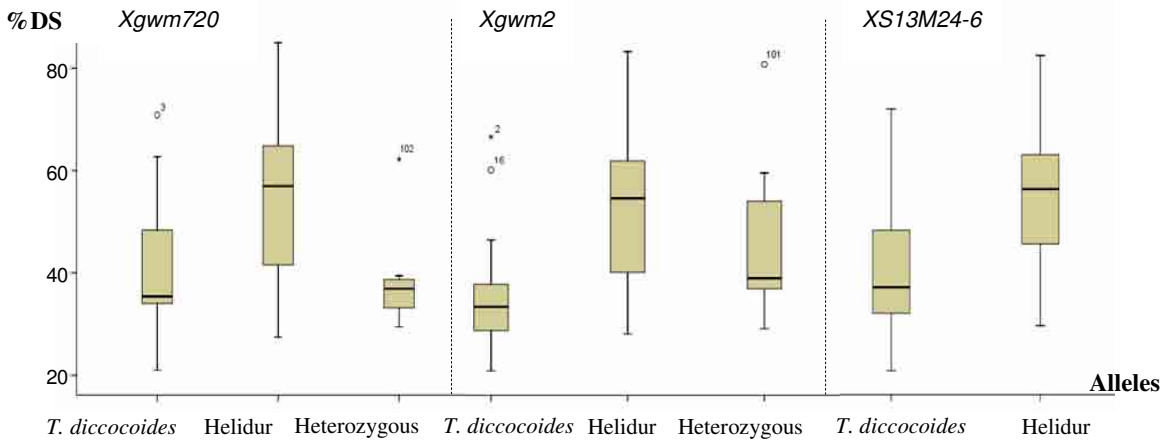


Figure 26: Box plot of %DS 21 dai. Line means for alternative alleles at the markers *Xgwm720* (3A), *Xgwm2* (3A) and *XS13M24-6* (6B).

3.10.2. Speed of FHB spreading (SFS)

QTL for FHB speed were detected on chromosomes 3A and 6B (Table 27) during the period 14 to 21 and 21 to 28 dai. The effects on chromosome 6B were significant ($\alpha < 0.05$) for overall mean values of the experiments GH2, GH3 and GH4 for the flanking makers *XS13M25_3 - Xgwm626*. Effects on chromosome 3A were significant ($\alpha < 0.05$) for overall mean values of the experiments GH2, GH3 and GH4 and in the GH3 experiment for flanking markers *Xgwm1121 - Xgwm720* and the effect was significant ($\alpha < 0.05$) for overall mean value of the experiments GH2, GH3 and GH4 and in the experiments GH2 and GH3 for flanking markers *Xgwm2 - Xgwm779*. The resistance was conferred by alleles of *T. diccocoides*. The effect associated with SFS mapping to chromosome 6B (Figure 27), explained 23% of the phenotypic variation for FHB speed. The effect associated with SFS mapping to chromosome 3A (Figure 28), explained 18% of the phenotypic variation for SFS. The QTL for 3A was significant at LOD = 3.3 ($\alpha <$ 0.05) and 3.2 LOD for 6B ($\alpha = 0.05$).

The phenotypic effect of the three detected QTL during the period 14 - 21 dai associated with the speed of FHB spread is illustrated in Table 28. Lines with the *T. diccocoides* allele on the QTL on chromosome 6B near *XS13M24-6* showed an average 0.2 spikelets per day slower infection compared to lines with the Helidur allele. Lines with the *T. diccocoides* allele of the QTL on chromosome 3A near *Xgwm720* showed an average 0.2 spikelets per day slower speed of spread of symptoms compared to lines with the Helidur allele. Similarly, lines with the *T. diccocoides* allele of the QTL on 3A near *Xgwm2* showed an average 0.1 spikelets per day slower infection compared to lines with the Helidur allele.

Table 27: flanking markers linked to the QTL estimated for FHB speed during the period 14 to 21 dai. Chromosomal location, logarithm of odds (LOD), the percentage of explained phenotypic variance (R^2) for GH2, GH3 and GH4 and mean across 3 experiments. QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM).

Chromosome	Flanking markers	GH2 SIM	GH3 SIM	GH4 SIM	mean SIM	Mean CIM
LOD						
3A -1	*Xgwm1121 - Xgwm720*	2.1	3.1	2.8	4.5**	4.5
3A -1	*Xgwm2 - Xgwm779*	3.2	3.8	1.39	4.5**	4.4
6B	*XS13M25_3 - Xgwm626*	2.1	3.9	4.6	6.1**	6.1
R^2						
3A - 1	*Xgwm1121 - Xgwm720*	0.09	0.13	0.12	0.18	0.18
3A - 1	*Xgwm2 - Xgwm779*	0.13	0.16	0.06	0.18	0.18
6B	*XS13M25_3 - Xgwm626*	0.09	0.16	0.18	0.23	0.23

** $p < 0.01$; * $p < 0.05$;

Table 28: Numbers and mean values of the lines for alternative alleles at three QTL for SFS during the time 14 -21 dai.

				T.dic#		Helidur		Heterozygous	
Traits	**chromosome**	**closest marker**	**Prob.***	**N**	**Mean**	**N**	**Mean**	**N**	**Mean**
SFS	**3A -1**	*Xgwm720*	0.0031	20	0.5	69	0.7	7	0.5
	3A -1	*Xgwm2*	0.0068	16	0.6	69	0.7	11	0.6
	6B	*XS13M24-6*	0.003	32	0.5	57	0.7	-	-

*P- value from analysis of variance for differences between alternative alleles.

#T.dic: *T. diccocoides*

The box plot in Figure 29 is an example of SFS during the period 14 -21 dai peak markers mapping to chromosomes 3A and 6B.

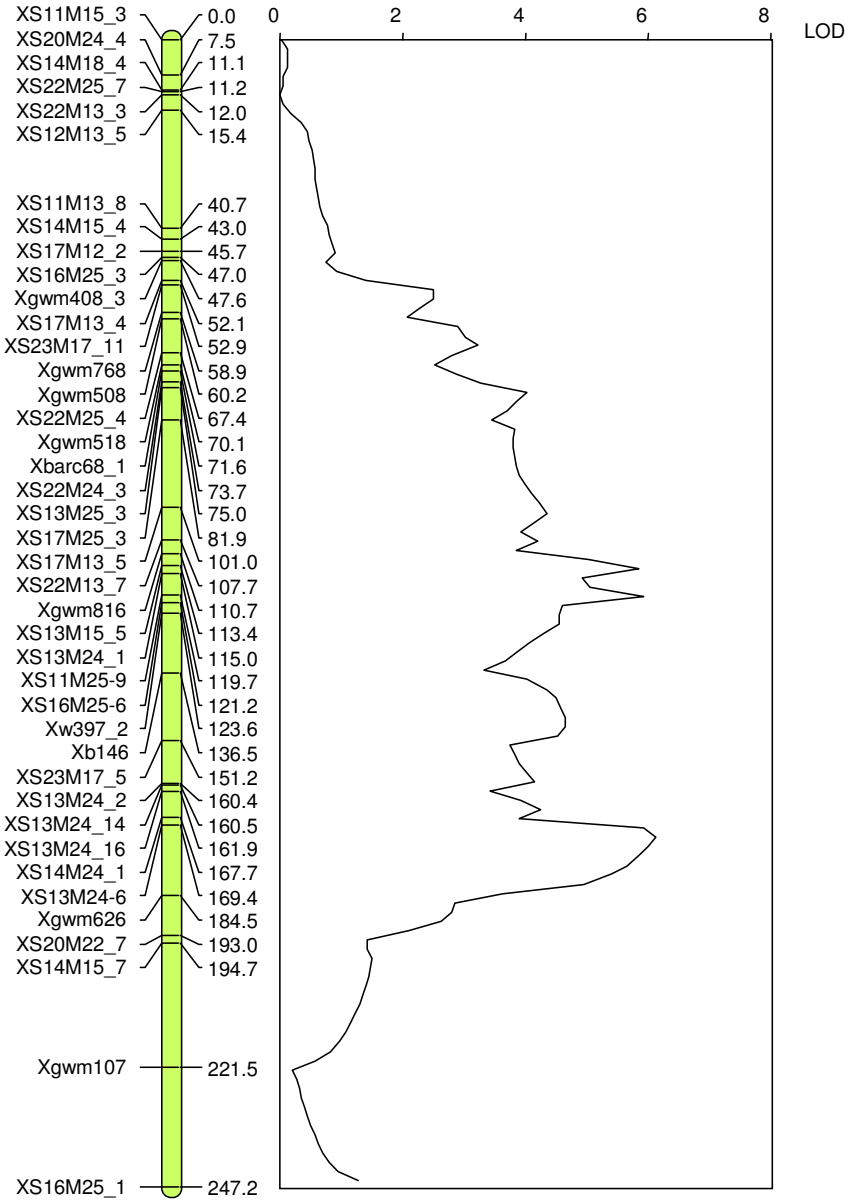


Figure 27: Interval analysis of a QTL of the mean of experiments GH2, GH3 and GH4 during the period 14 to 21 dai for FHB speed by simple interval mapping (SIM). The QTL is on a linkage group corresponding to a part of chromosome 6B.

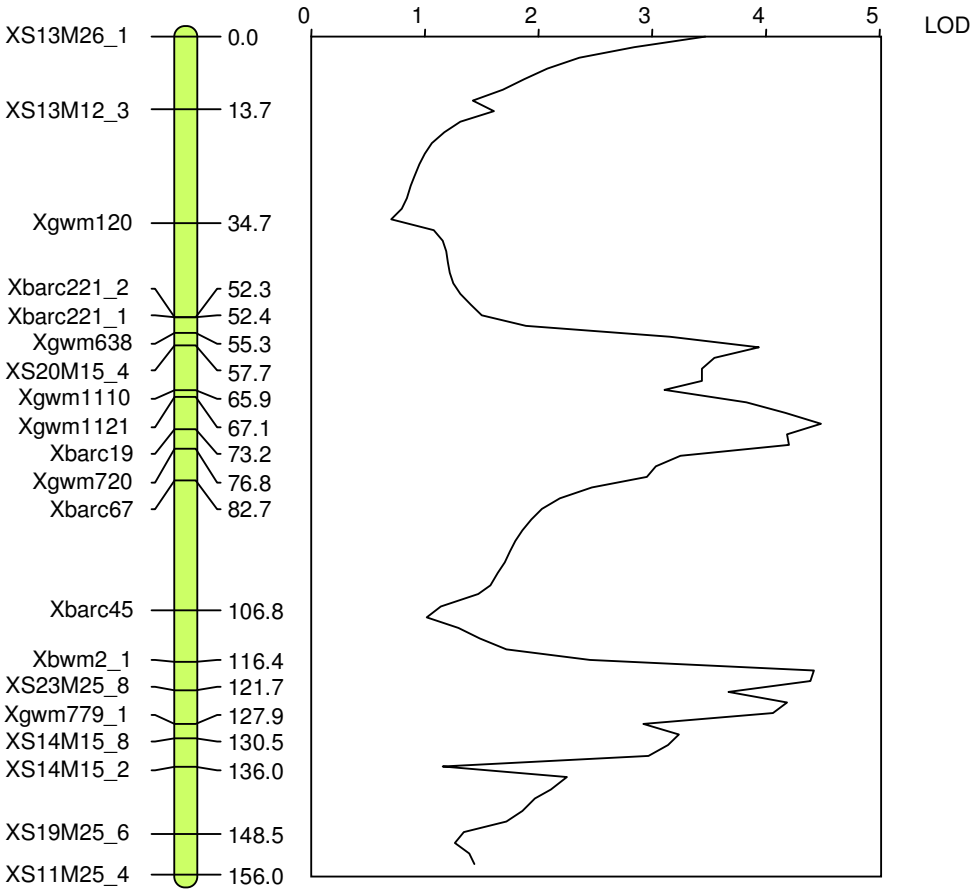


Figure 28: Interval analysis of a QTL of the Mean of experiments GH2, GH3 and GH4 during the period 14 to 21 dai for FHB speed by simple interval mapping (SIM). The QTL is on a linkage group corresponding to a part of chromosome 3A.

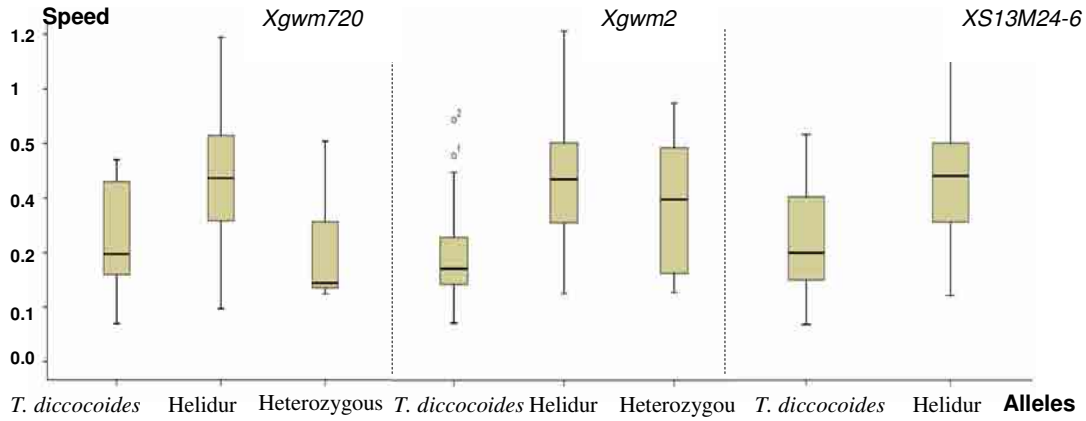


Figure 29: Box plot of speed of FHB spreading at the period 14 to 21 dai line means for alternative alleles at the markers *Xgwm720* (3A), *Xgwm2* (3A) and *XS13M24-6* (6B).

3.10.3. Area under disease progress curve (AUDPC)

QTL for FHB severity measured by AUDPC were detected on chromosomes 3A and 6B (Table 29). Effects on chromosomes 3A were significant ($\alpha < 0.05$) in the experiments GH2 and GH3 for flanking markers *Xgwm1121- Xgwm720* and *Xgwm2- Xgwm779*. Effects on chromosomes 6B was significant ($\alpha < 0.05$) in the experiments GH2, GH3 and GH4 for flanking makers *XS23M17_5 - Xgwm626*. The resistance was conferred by alleles of *T. diccocoides*. The effect associated with FHB severity mapping to chromosome 3A, explained 17% and 15% of the phenotypic variation for FHB severity, respectively. The effect on chromosome 6B (Figure 30), explained 19% of the phenotypic variation for FHB severity. The QTL for 3A and 6B was significant at LOD = 3.2 ($\alpha < 0.05$).

Table 29: Flanking markers linked to the QTL estimated for FHB severity measured by AUDPC. Chromosomal location, logarithm of odds (LOD), the percentage of explained phenotypic variance (R^2) for experiment means in GH1, GH2, GH3 and GH4 are out lined here. QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM).

Chromosome LOD	Flanking markers	GH1 SIM	GH2 SIM	GH3 SIM	GH4 SIM	mean SIM	mean CIM
3A - 1	*Xgwm1121 - Xgwm720*	2.2	3.7	3.0	2.5	4.2**	4.2
3A - 1	*Xgwm2 - Xgwm779*	2.3	3.4	3.4	1.0	3.7*	3.7
6B	*XS23M17_5 - Xgwm626*	1.2	5.5	3.6	4.3	4.8**	4.8
R^2							
3A - 1	*Xgwm1121 - Xgwm720*	0.10	0.15	0.12	0.10	0.17	0.17
3A - 1	*Xgwm2 - Xgwm779*	0.10	0.14	0.14	0.04	0.15	0.15
6B	*XS23M17_5 - Xgwm626*	0.05	0.22	0.15	0.17	0.19	0.19

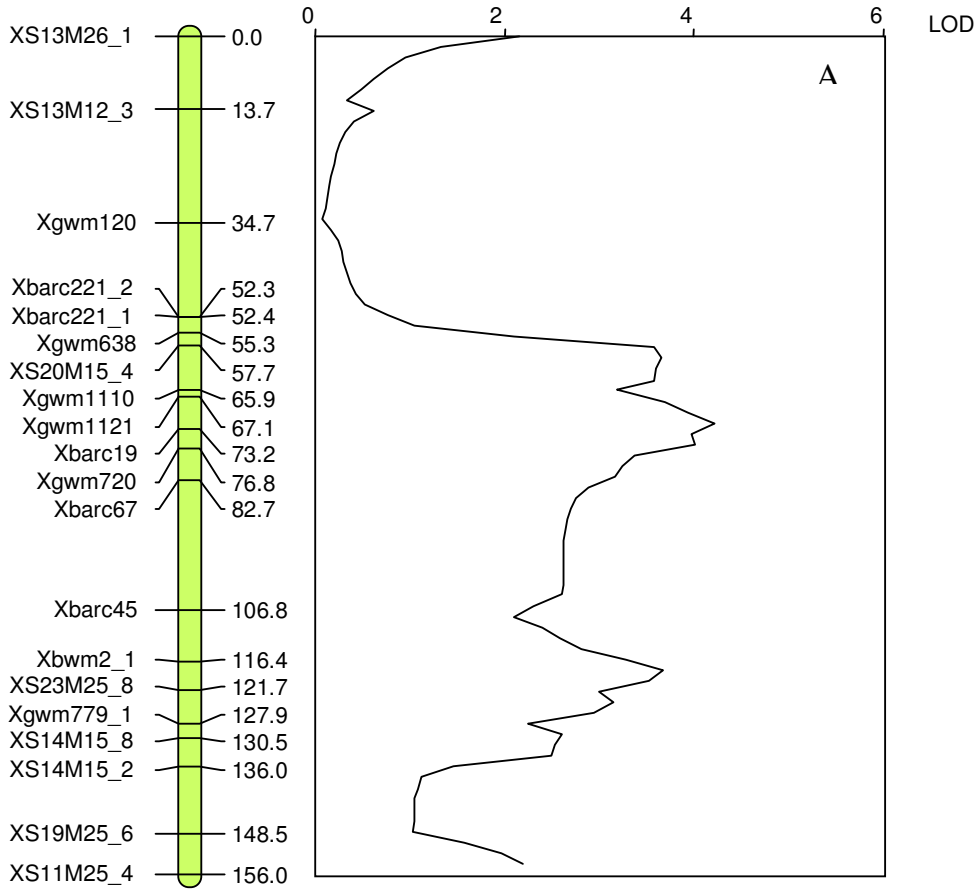
** $p < 0.01$; * $p < 0.05$;

The phenotypic effect of the three detected QTL with the severity of FHB symptoms is illustrated in Table 30. Lines with the *T. diccocoides* allele of the QTL on chromosome 3A near *Xgwm720* showed an average 249.3 less AUDPC units compared to lines with the Helidur allele. Similarly lines with the *T. diccocoides* allele of the QTL on 3A near *Xgwm2* showed an average 273.1 less AUDPC units compared to lines with the Helidur allele. Lines with the *T. diccocoides* allele of the QTL on chromosome 6B near *XS13M24-6* showed an average 240.5 less AUDPC units compared to lines with the Helidur allele.

Table 30: Numbers and mean values of the lines for alternative alleles of three QTL for AUDPC. (*T.dic: *T. diccocoides,* N: Number of lines).

Traits	Chromosome	Closest marker	Prob.*	*T.dic N	*T.dic Mean	Helidur N	Helidur Mean	Heterozygous N	Heterozygous Mean
	3A - 1	*Xgwm720*	<.0001	20	516.2	69	765.5	7	480.5
	3A – 1	*Xgwm2*	0.0006	16	472.6	69	745.7	11	619.6
AUDPC	**6B**	*XS13M24-6*	0.0001	32	538.5	57	779.0		

*P- value from analysis of variance for differences between alternative alleles.



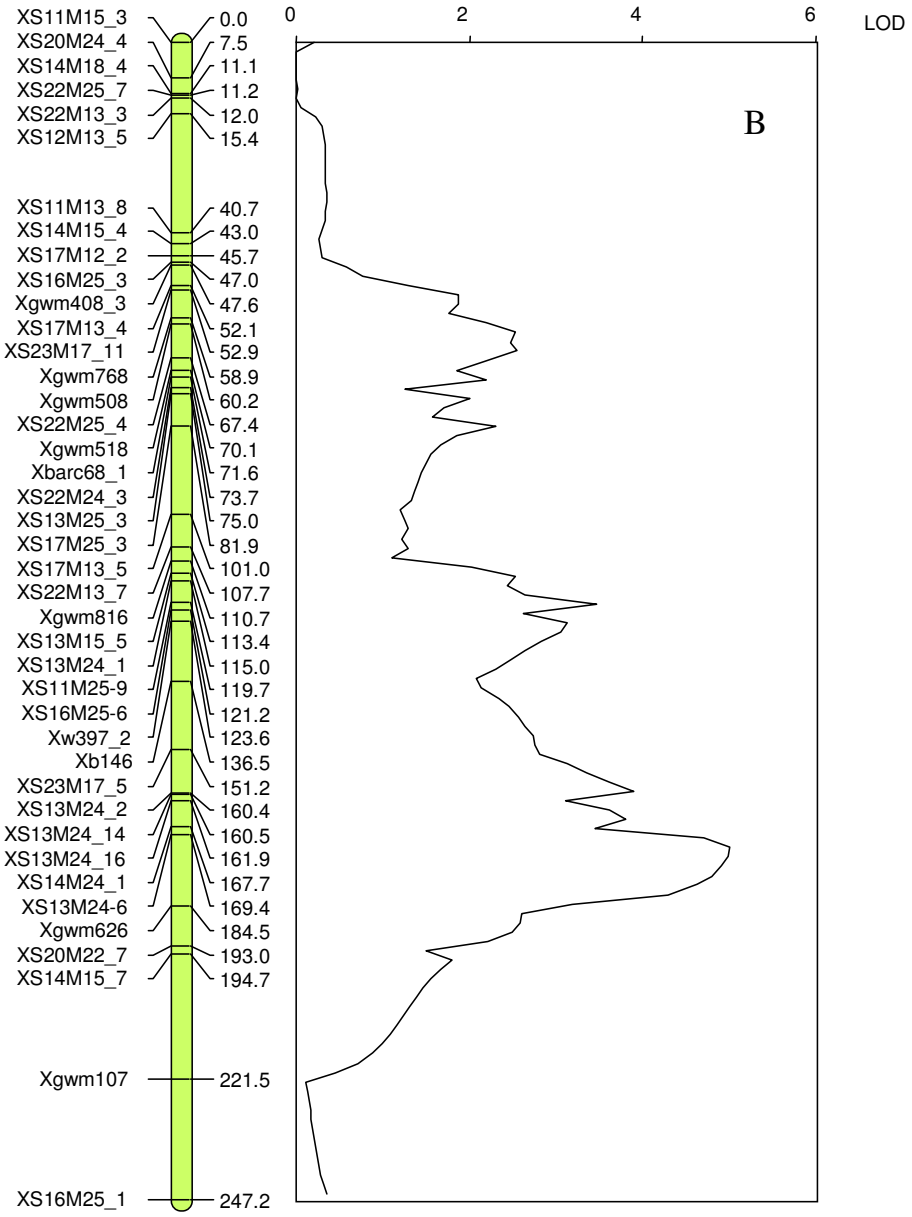


Figure 30: Interval analysis of a QTL of the mean for FHB severity in GH1, GH2, GH3 and GH4 by simple interval mapping (SIM). **(A)** The QTL is on a linkage group corresponding to a part of chromosome 3A. **(B)** The QTL is on a linkage group corresponding to a part of chromosome 6B (AUDPC).

3.10.4. FHB incidence

QTL analysis of FHB incidence detected one QTL on chromosome 6B (Table 31). This QTL (Figure 31) explained 15 % of the phenotypic variance. QTL analysis for FHB incidence was based mean values over all experiments. The most-likely position for 6B is in the flanking markers *XS13M25_3 - XS17M25_3*. The QTL was significant at LOD = 3.3 (α <0.05).

Table 31: Flanking markers linked to the QTL estimated for FHB incidence. Chromosomal location, logarithm of odds (LOD), the percentage of explained phenotypic variance (R^2). QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM).(mean values over all experiments)

Chromosome	Flanking markers	mean SIM	mean CIM
LOD			
6B	*XS13M25_3 - XS17M25_3*	3.6*	3.5
R^2			
6B	*XS13M25_3 - XS17M25_3*	0.15	0.15

** p < 0.01; * p < 0.05;

The phenotypic effect of the detected QTL associated with FHB incidence is illustrated in Table 32. Lines with the *T. diccocoides* allele of the QTL on chromosome 6B near *XS13M25_3* showed on average 3% less infected spikes compared to lines with the Helidur allele.

Table 32: Numbers and mean values of the lines for alternative alleles of QTL for FHB incidence. (*T. dic. *T. diccocoides*: N: Number of lines).

				*T.dic		Helidur		Hetero.	
Traits	**chromosome**	**Closest marker**	**Prob.**	**N**	**Mean**	**N**	**Mean**	**N**	**Mean**
incidence	6B	*XS13M25_3*	0.0396	15	89.5	65	92.5	-	-

*P- value from analysis of variance for differences between alternative alleles.

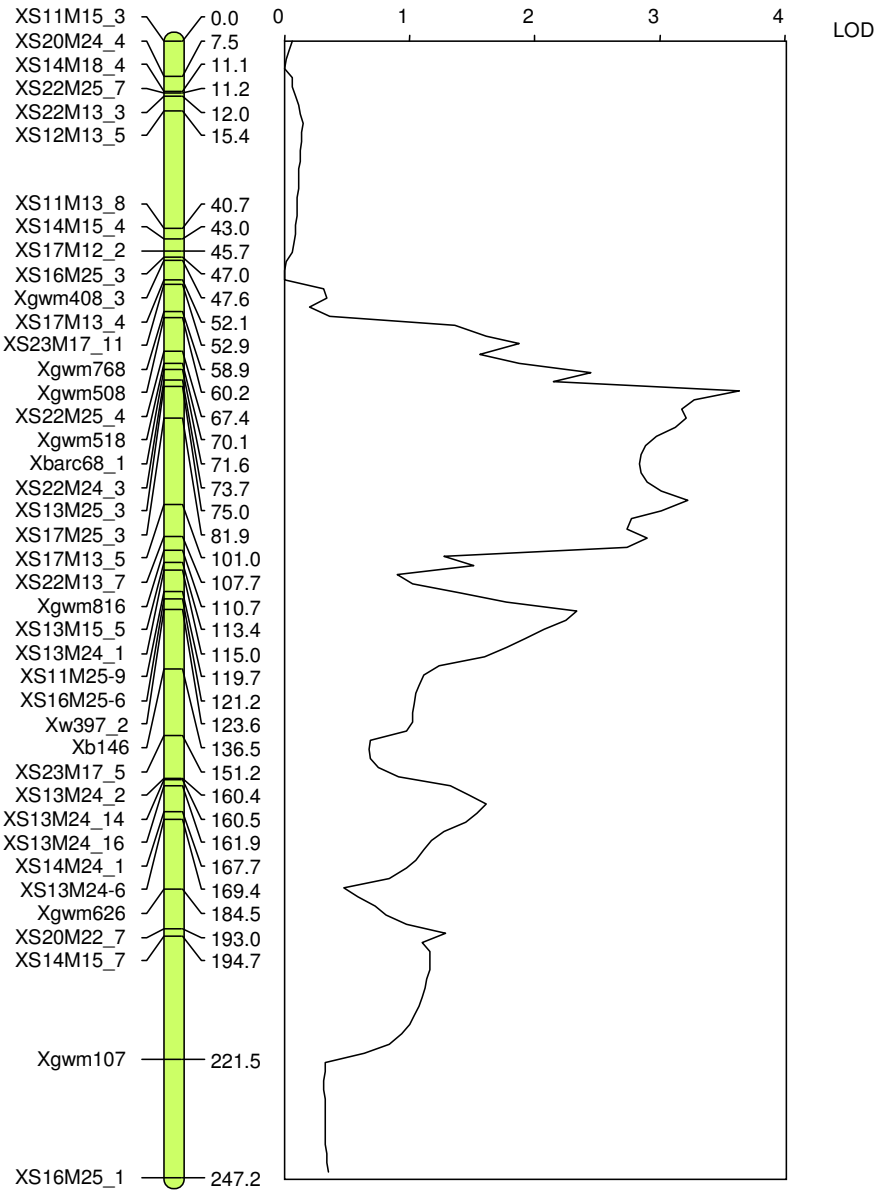


Figure 31: Interval analysis of a QTL for the mean FHB incidence over all experiments by simple interval mapping (SIM). The QTL is on a linkage group corresponding to a part of chromosome 6B.

3.10.5. Wilting

A QTL for the percent of wilted spikes (21 dai) was detected on chromosomes 3A (Table 33). This QTL (Figure 32) explained 14 % of the phenotypic variance. QTL analysis for the percent of wilted spikes was based on mean values over all experiments. The most-likely position for this QTL on chromosome 3A is between the flanking markers *Xgwm2 - Xgwm779*. This was significant at LOD = 3.3 (α <0.05).

Table 33: Flanking markers linked to the QTL estimated the percent of wilted spikes 21 dai. Chromosomal location, logarithm of odds (LOD), the percentage of explained phenotypic variance (R^2) and experiment means in field, GH1, GH2, GH3 and GH4 are shown here. QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM).

		field	GH1	GH2	GH3	GH4	mean	mean
Chromosome LOD	**Flanking markers**	**SIM**	**SIM**	**SIM**	**SIM**	**SIM**	**SIM**	**CIM**
3A - 1	*Xgwm2 - Xgwm779*	0.387	2.614	2.312	2.7	0.5	3.4*	3.5
R^2								
3A - 1	*Xgwm2 - Xgwm779*	0.017	0.11	0.096	0.113	0.023	0.14	0.15

** p < 0.01; * p < 0.05;

The phenotypic effect of the detected QTL associated with the percent of wilting spikes is illustrated in Table 34. Lines with the *T. diccocoides* allele of the QTL on chromosome 3A near *Xgwm2* showed on average 20% less wilted ears compared to lines with the Helidur allele.

Table 34: Numbers and mean values of the lines for alternative alleles of QTL for percent of wilting spikes 21 dai. (T. dic. *T. diccocoides*: N: Number of lines).

				T.dic		Helidur		Hetero.	
Traits	**chromosome**	**closest marker**	**Prob.**	**N**	**Mean**	**N**	**Mean**	**N**	**Mean**
Wilt	**3A -1**	*Xgwm2*	0.0011	16	40	69	60	11	50

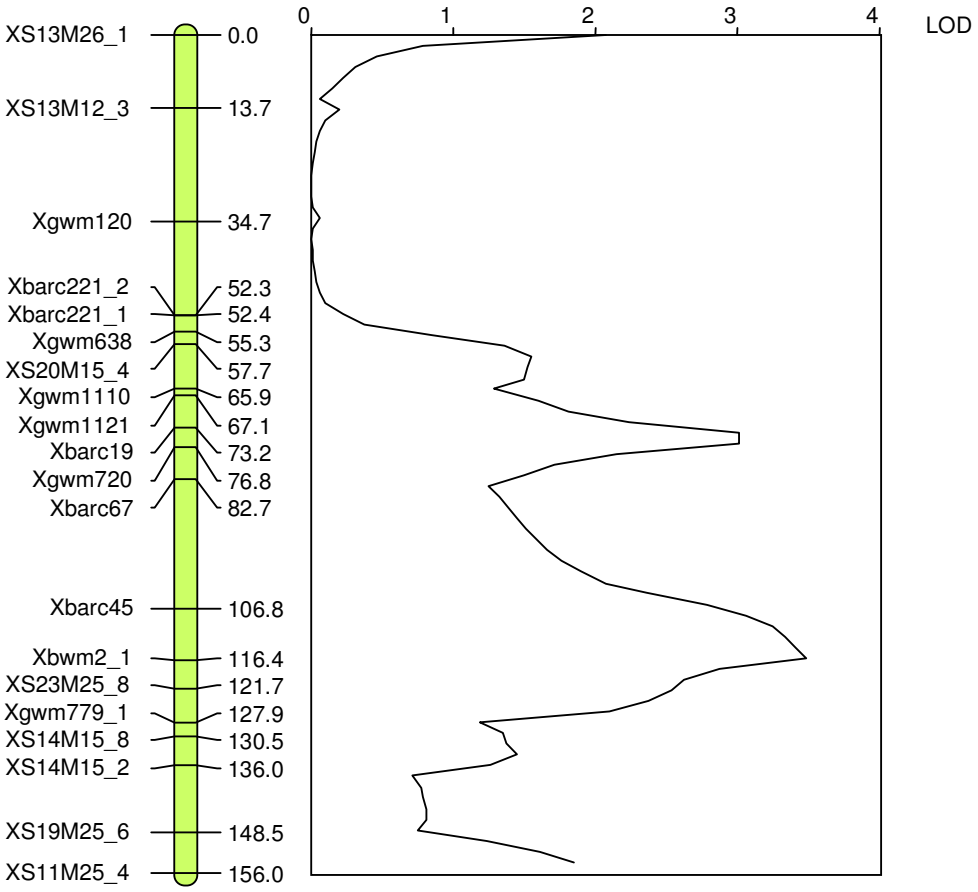


Figure 32: Interval analysis of a QTL of the mean for spikes wilt over all experiments by simple interval mapping (SIM). The QTL is on a linkage group corresponding to a part of chromosome 3A.

3.10.6. QTL analysis for other traits and powdery mildew

The results of the QTL analysis for plant height, spike length, ear type, waxiness, number of spikelets per spike, date of anthesis, brittle rachis, powdery mildew and colour of the spike for means are summarized in Table 35. The phenotypic effect of the detected QTL associated with developmental and morphological traits is illustrated in Table 36. Figure 33 shows total QTL for developmental and morphological traits.

Table 35: QTL for developmental and morphological traits as well as powdery mildew. Chromosomal location, logarithm of odds (LOD), percentage of explained phenotypic variance (R^2) and experiments mean. QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM).

Trait	Chrom.	marker	LOD SIM	R^2 SIM	LOD CIM	R^2 CIM
Plant height (4 experiments)	4B	*XS14M26_6*	12.0**	0.41	12.04	0.41
Date of anthesis (5 experiments)	X3	*XS20M24_3*	9.9**	0.35	9.9	0.35
Waxiness (3 experiments)	1B	*XS14M19_4*	8.4**	0.31	8.4	0.31
	2B	*Xgwm614*	9.0**	0.32	8.9	0.32
Spike length (3 experiments)	X6 or 2B	*XS20M17_3*	3.9*	0.16	3.9	0.16
Brittle rachis (3 experiments)	5A	*Xgwm179*	6.26**	0.24	6.259	0.24
Spikelets number (5 experiments)	6A	*XS22M15_5*	3.8*	0.15	3.79	0.15
	X3	*XS20M24_3*	5.1**	0.20	5.11	0.20
Spike colour (3 experiments)	5A	*XS13M18_2*	4.2**	0.19	4.23	0.19
Ear type (3 experiments)	-	-	-	-	-	-
Powdery mildew (PM) (2 experiments)	6B	*XS17M13_5*	3.6*	0.15	3.6	0.15

** $p < 0.01$; * $p < 0.05$;

Table 36: Numbers and mean values of the lines for alternative alleles of developmental and morphological traits as well as powdery mildew. (*T. dic. *T. diccocoides*: N: Number of lines).

			*T.dic		Helidur		Hetero.	
Traits	**marker**	**prob.**	**N**	**Mean**	**N**	**Mean**	**N**	**Mean**
Height	*XS14M26_6*	<.0001	24	96.1	69	74.8		
Date of anthesis	*XS20M24_3*	.000	26	14.5	63	11,4		
	XS14M19_4	<.0001	31	1.9	60	3.3		
Waxiness	*Xgwm614*	<.0001	19	1.7	64	3.2	4	1.9
Spike length	*XS20M17_3*	0.0001	15	1.0	76	1.5		
Brittle rachis	*Xgwm179*	<.0001	21	1.7	64	2.3	1	1.3
	XS22M15_5	<.0001	22	13.7	73	14.8		
Spikelets number	*XS20M24_3*	<.0001	22	15.3	61	14.2		
PM	*XS17M13_5*	0.001	22	1.9	66	2.5		

For plant height a QTL located on chromosomes 4B was detected. The effects of this QTL explained 41% of the total phenotypic variance. For this locus, *T. diccocoides* alleles contributed to higher plant height. QTL detection for waxiness revealed effects on chromosomes 1B and 2B. This explained 30% and 32% of the total phenotypic variance for the QTL on 1B and 2B, respectively. For all loci, Helidur alleles contributed to waxiness. For date of anthesis, a QTL, located on unknown linkage group X3, was detected. The effect of this QTL explained 35% of the total phenotypic variance. For this locus, *T. diccocoides* alleles contributed to late anthesis. QTL detection for spike height revealed effects on unknown linkage group X6. The effect of this QTL explained 16% of the total phenotypic variance. For this locus, Helidur alleles contributed to bigger spikes. Two loci, which directly affected the number of spikelets, were identified by QTL detection on chromosome 6A and unknown linkage group X3. The effect of these explained 15% and 20% of the total phenotypic variance for the QTL on chromosome 6A and unknown linkage group X3, respectively. For all loci, Helidur alleles contributed to higher spikelets numbers. For Brittle rachis QTL was detected on chromosomes 5A. These effects explained 24% of the total phenotypic variance. No QTL was detected for ear type. A powdery mildew resistance locus was detected on chromosome 6B. The effects explained 15% of the phenotypic variance. For this locus, *T. diccocoides* alleles contributed to mildew resistance (Figure 34).

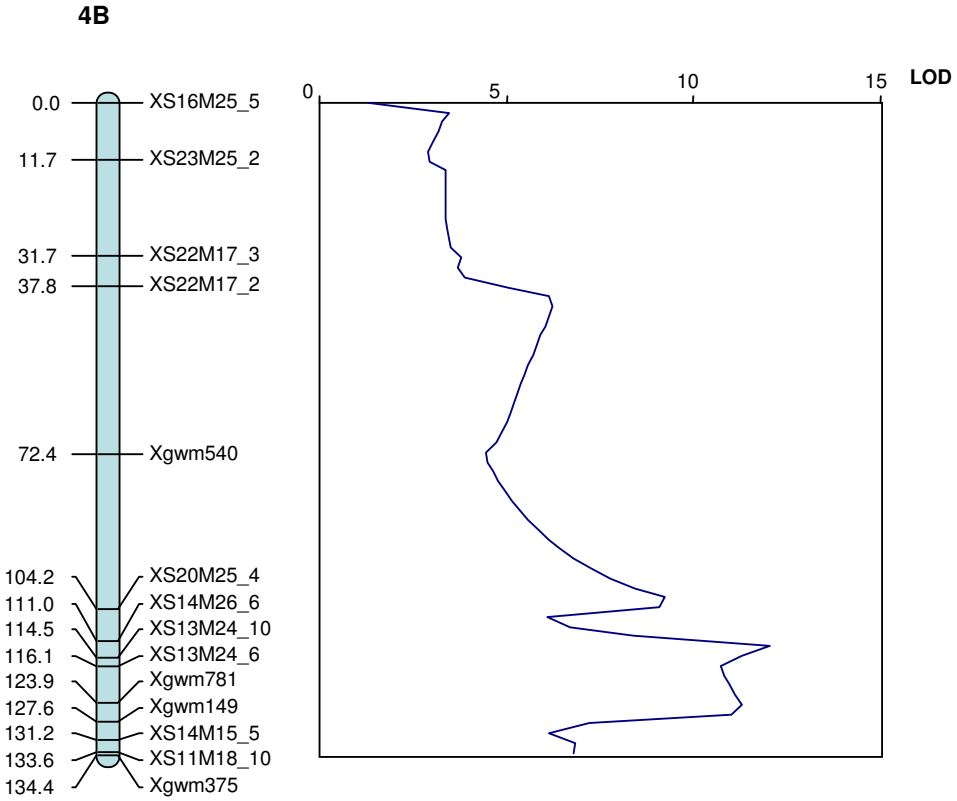


Figure 33-a: significant QTL for plant height on chromosome 4B.

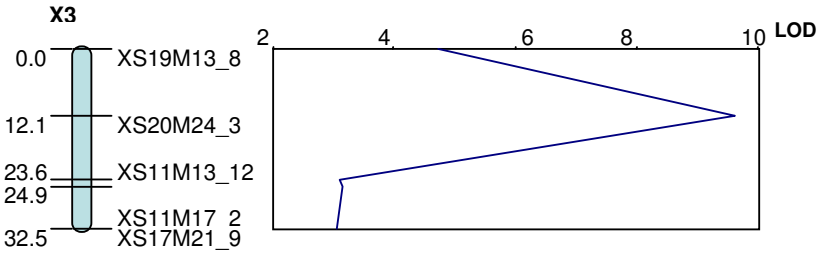
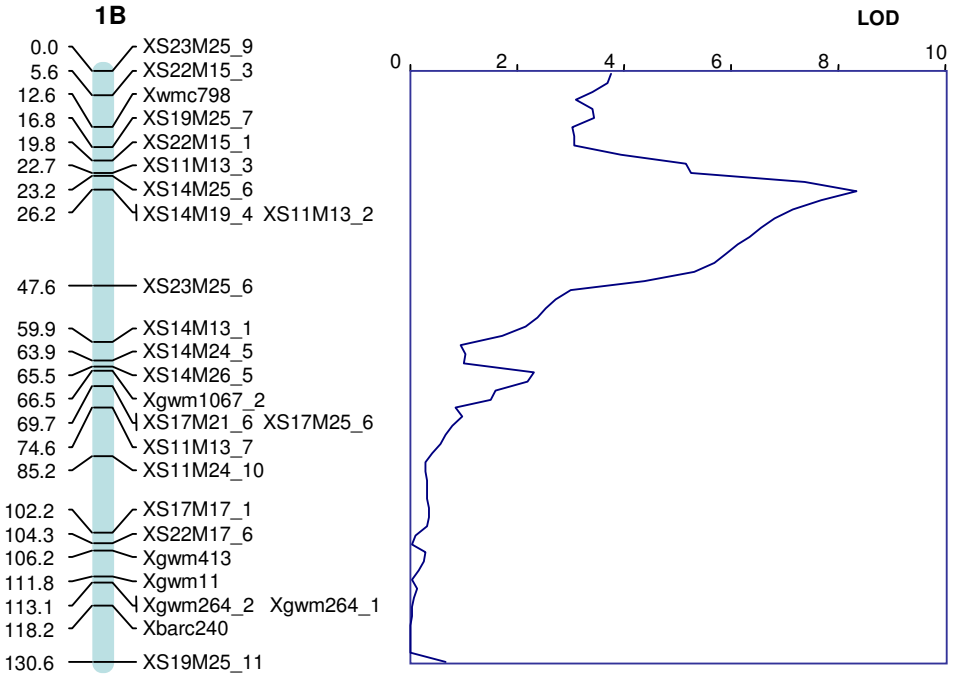


Figure 33-b: significant QTL for date of anthesis on unknown linkage group X3 .



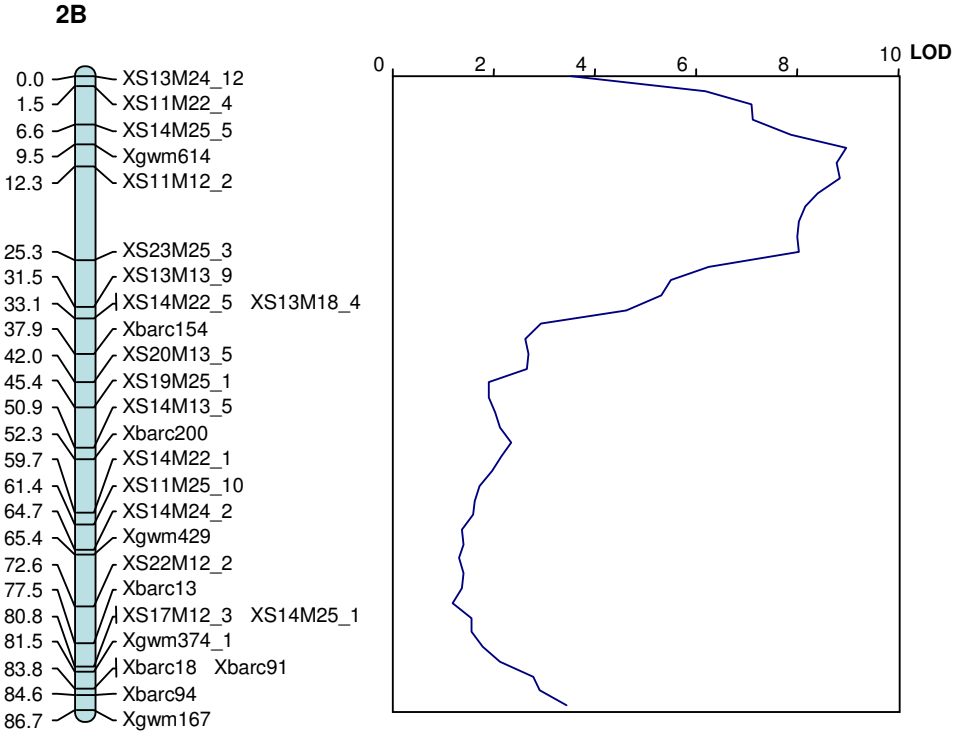


Figure 33-c: significant QTL for waxiness on chromosomes 1B and 2B.

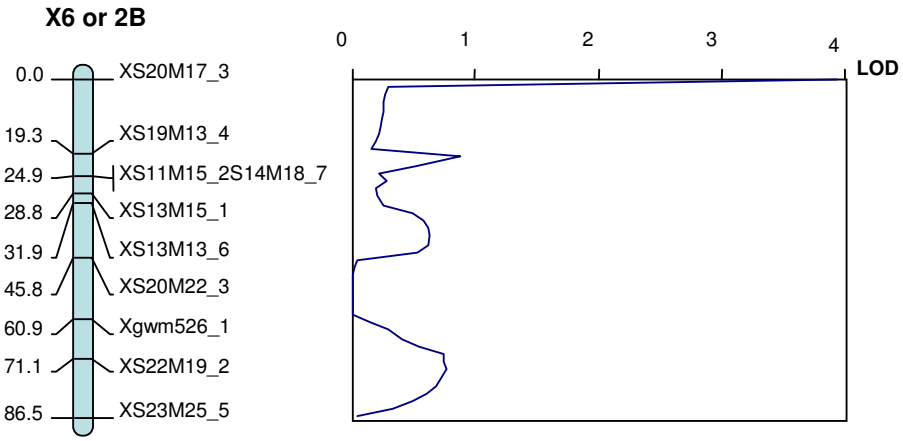


Figure 33-d: significant QTL for spike length on unknown linkage group X6.

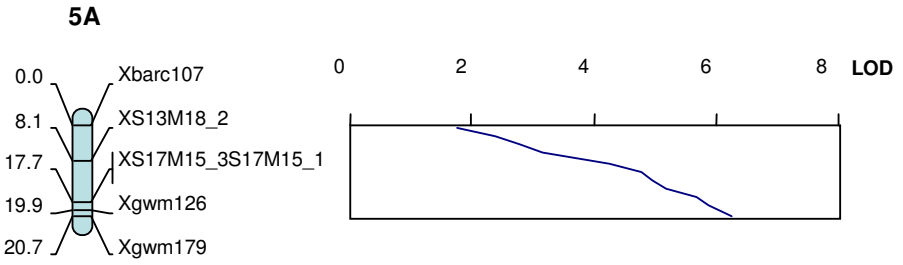
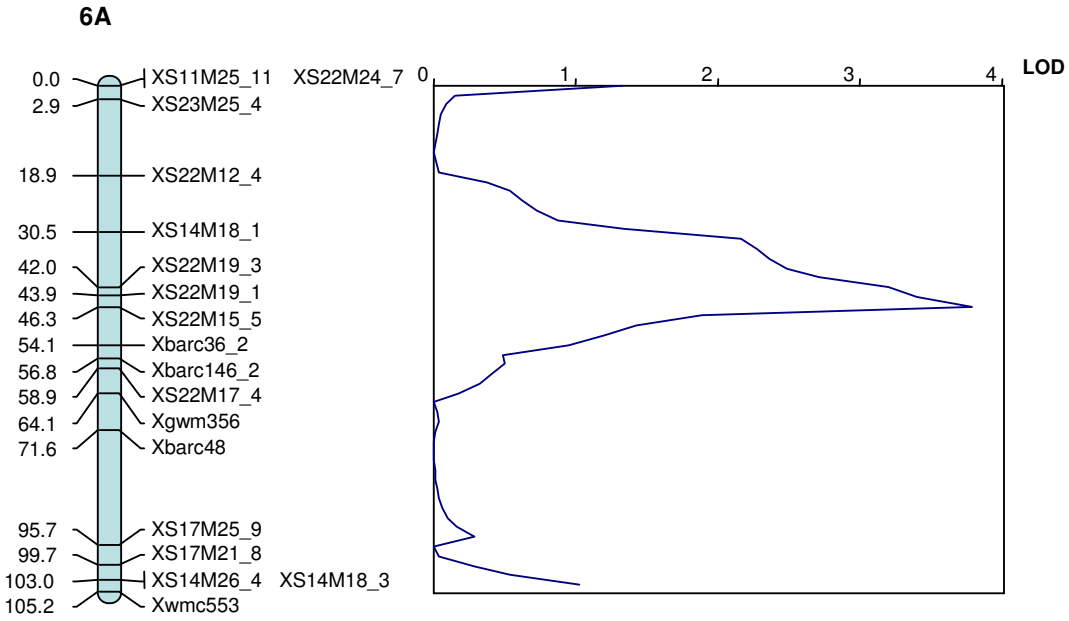


Figure 33-e: significant QTL for brittle rachis for on chromosome 5A.



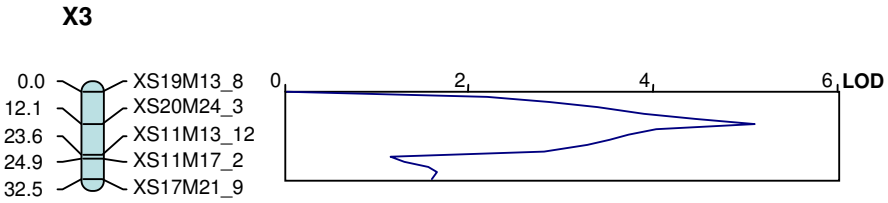


Figure 33-f: significant QTL for spikelets number on chromosome 6A and unknown linkage group X3.

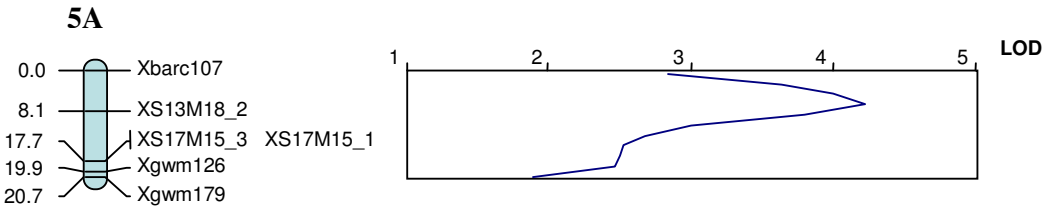


Figure 33-g: significant QTL for spike colour on chromosome 5A.

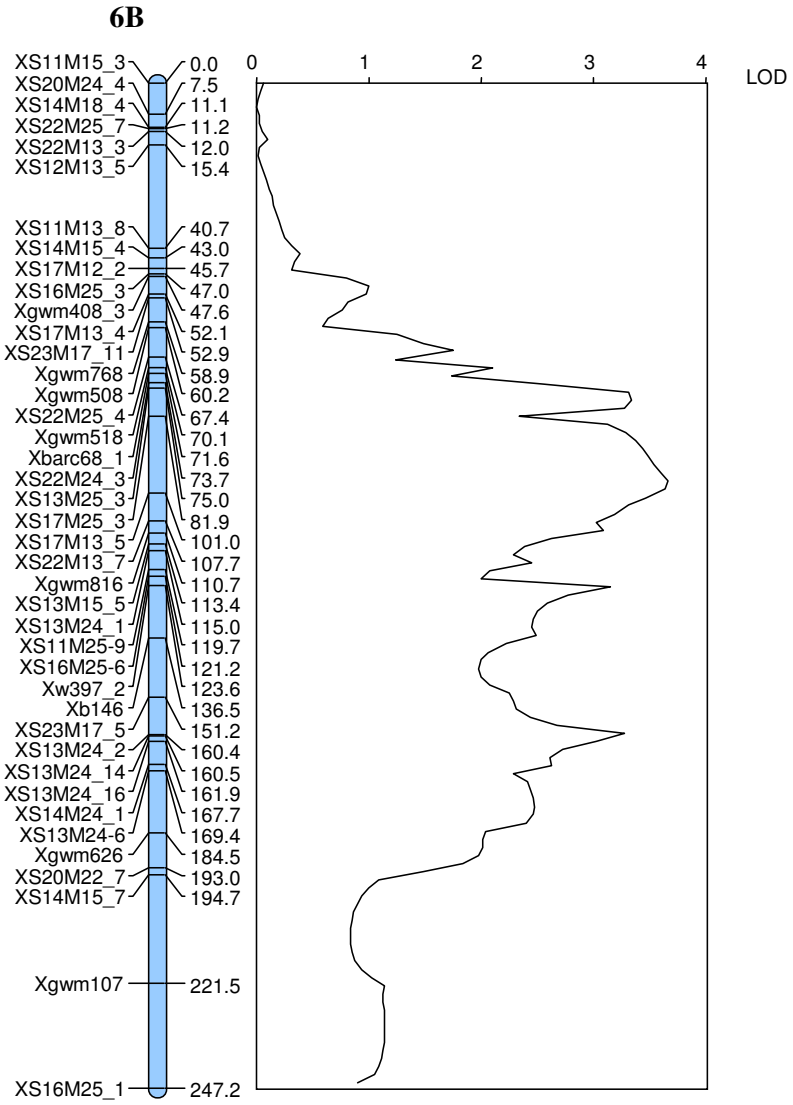


Figure 34: significant QTL for powdery mildew on chromosome 6B.

4. Discussion

4.1. Phenotypic analysis of the mapping population

4.1.1. Variation for FHB resistance

Wild emmer wheat (*T. dicoccoides*) that has the same genome (AABB) as durum wheat, represents an important gene pool for durum improvement (Buerstmayr et al. 2003, Oliver et al. 2007). Based on that, a population of 105 backcross lines (BC_1F_6) derived from the cross of the wild emmer with the *T. durum* cultivar Helidur was used for FHB resistance assessment. A continuous variation was observed among genotypes studied with respect to all FHB-related traits and the variation was significant ($\alpha < 0.05$) for all FHB-related traits. This result is in agreement with Stack et al. (2002) who tested a set of disomic lines derived from wild emmer (*T. dicoccoides*) for Type II resistance. Few lines showed resistance while several other lines showed a trend either for increased or reduced susceptibility to FHB. The result was also confirmed by the experiment of Chen et al. (2006). The lines derived from *T. dicoccoides* displayed a wide range of FHB response, from moderately resistant to very susceptible.

4.1.2. Assessment resistance for FHB-related traits (Type II)

The focus of this study was on resistance to fungal spread, also known as Type II Fusarium head blight resistance according to Schroeder and Christensen (1963). Different disease parameters were used to assess Type II resistance in the mapping population. These methods are: percent of infected spikelets (% DS), absolute number of infected spikelets (n.DS), speed of FHB spreading (SFS) and area under disease progress curve (AUDPC). Wilting and disease incidence are additional disease parameters. The correlation between them was significant ($\alpha < 0.05$) and high (r ranging from 0.73 to 0.96) and this means each of them could be used to assess Type II resistance. *T. diccocoides* showed lower FHB spread than Helidur at all evaluated time points after inoculation (14 dai, 21 dai and 28 dai). The mapping population is a result of a back cross of wild emmer with a modern cultivar. This wide cross lead to very large difference between the lines for many plant traits (for example spike length, number of spikelets and other traits). The assessment of FHB spread for the lines with large or small number of spikelets needs different methods of calculation. The % DS method enables us to assess the percent of infected spikelets compared to the total number of the spikelets on the same spike. The n.DS method calculates the mean of infected spikelets for all inoculated spikes of the same lines.

The spread of the FHB in the resistance lines with small number of spikelets will be clearly measured by n.DS, whereas spread of the FHB in the susceptible lines with large number of

spikelets appear more clearly with %DS. Broad-sense heritability was high (H^2 = 0.83 for % DS and 0.84 for n.DS), indicating a reproducible assessment of the FHB resistance level in this population. SFS and area under disease progress curve (AUDPC) are also additional methods to assess FHB severity. SFS calculates the average increase in numbers of infected spikelets per day during the period: 14 – 21 dai, 21 – 28 dai and 14 – 28 dai. The SFS reflects the plant's resistance for FHB. The highest correlation of %DS and n.DS with the speed of FHB spreading was during the period 14 -21 dai (r = 0 .83 and 0.82 respectively). In this period the increase in bleached spikelets is due to the infection of FHB more than other environment factors and this supported by the broad-sense heritability (H^2 = 0.77) during this period compared to the period 21 – 28 dai (H^2 = 0.30). The *T. diccocoides* had lower average speed of FHB spread than Helidur during the three mentioned periods. As an over all measure for FHB severity the area under disease progress carve (AUDPC) was used, and also for this trait the population displayed significant ($\alpha < 0.05$) and continuous variation. A low AUDPC score indicated the presence of resistance to FHB in the plant. The correlation of AUDPC with % DS, n.DS and SFS was high (r = 0.96, 0.95 and 0.73 respectively). Our heritability results are in the same range as reported by Otto et al. (2007): this group found a heritability of 66.9 for Type II FHB resistance for the Langdon-*T. dicoccoides* chromosome substitution lines based on the combined analysis of two seasons. Buerstmayr et al. (2002) reported a heritability of 66.0 for the assessments on 22 dai of visual FHB symptoms Type II FHB resistance.

A small proportion of inoculated heads remained without symptoms. This phenomenon was observed in all experiments. We considered it as a measure for disease incidence. The percentage of symptomatic heads among all inoculated heads was calculated as a measure for disease incidence. FHB incidence was high ranging from 73 to 100% infection. FHB incidence and FHB severity (as %DS) were correlated (r= 0.27). The correlation between FHB incidence and FHB severity indicates that low FHB incidence and FHB severity are under similar genetic control (Steiner et al. 2004).

Significant ($\alpha < 0.05$) transgressive segregation was found for the presence of wilted spikes within population. The correlation of the percent of wilted spikes with FHB related traits was high (for example the correlation between %DS and wilted spikes is 0.82).

In general transgressive segregates were observed in the mapping population indicating that parents, carry positive and negative alleles for FHB resistance and this in agreement with Buerstmayr et al. (2000).

4.1.3. Assessment of other traits and powdery mildew

Significant ($\alpha < 0.05$) variation was found among the BC_1F_6. lines for plant height, spike length, ear type, waxiness, number of spikelets, brittle riches, date of anthesis, colour of the spikes and powdery mildew severity. The phenotypic segregation of the previous traits was continuous instead of discrete, because several genes are involved in the expression of the trait and/or there are strong effects of the environment. Genetic studies of agronomic important traits in cereals have revealed that most of them are inherited quantitatively (Börner et al. 2002).

4.2. Genotyping analysis of the mapping population

4.2.1. Linkage map

In the present study, the total length of the molecular map for the mapping population is 2641cM which means an average distance between the markers of 5.1 cM and consists of 495 loci of SSR and AFLP markers on 36 linkage groups, resulting from 522 markers (495 linked and 27 unlinked markers). The markers order between our map and the consensus map (Somers et al. 2004) were in good agreement and with only few inversions of few markers on chromosome 1B, 2B and 5A. Our map covers more than 80% of the tetraploid wheat genome.

4.2.2. Markers and QTL analysis for FHB resistance

QTL analysis revealed associations of several genomic regions with FHB traits. QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM) based on permutation test for % DS and n.DS 21 dai, speed of FHB spreading (SFS) and area under disease progress curve (AUDPC) were detected on chromosomes 3A and 6B (Table 37).

Table 37: Summary of the QTL detected for 1) %DS (mean 5 experiments), 2) n.DS 21 dai.(means of five experiments), 3) AUDPC For the experiments GH1, GH2, GH3 and GH4. 4) SFS during the period 14-21 dai for experiments GH2, GH3 and GH4. 5) Incidence for mean values over all experiments. 6) Wilting for mean values over all experiments 21 dai. Chromosomal location, logarithm of odds (LOD), percentage of explained phenotypic variance (R^2) for over all mean. QTL analysis was carried out by simple interval mapping (SIM) and composite interval mapping (CIM).

parameter	Chromosome	Flanking markers	LOD		R^2	
			Mean			
			SIM	CIM	SIM	CIM
%DS dai	3A –1	*Xgwm1121 - Xgwm720*	4.8**	4.3	0.20	0.17
	3A –1	*Xgwm2 - Xgwm779*	4.5**	4.5	0.15	0.18
	6B	*XS23M17_5 - Xgwm626*	6.4**	6.4	0.26	0.24
n.DS 21 dai	3A –1	*Xgwm1121 - Xgwm720*	6.0**	5.5	0.23	0.21
	3A –1	*Xgwm2 - Xgwm779*	5.0**	4.8	0.18	0.19
	6B	*XS23M17_5 - Xgwm626*	5.5**	4.4	0.21	0.17
AUDPC	3A – 1	*Xgwm1121 - Xgwm720*	4.2**	4.2	0.17	0.17
	3A – 1	*Xgwm2 - Xgwm779*	3.7*	3.7	0.15	0.15
	6B	*XS23M17_5 - Xgwm626*	4.8**	4.8	0.19	0.19
SFS 14-21 dai	3A –1	*Xgwm1121 - Xgwm720*	4.5**	4.5	0.18	0.18
	3A –1	*Xgwm2 - Xgwm779*	4.5**	4.4	0.18	0.18
	6B	*XS13M25_3 - Xgwm626*	6.1**	6.1	0.23	0.23
Incidence	6B	*XS13M25_3 - XS17M25_3*	3.6*	3.5	0.15	0.15
Wilt 21 dai	3A – 1	*Xgwm2 - Xgwm779*	3.4*	3.5	0.14	0.15

** $p < 0.01$; * $p < 0.05$;

Two separate QTL were detected on chromosome 3A. The first QTL on 3A mapped to the flanking markers *Xgwm1121 - Xgwm720* and the second QTL on 3A mapped to the flanking markers *Xgwm2 - Xgwm779*. The most likely positions of these two QTL appeared at a distance of 39.6 cM. The effect of the first and second QTL was found significant ($\alpha < 0.05$) for the overall mean values and in different single experiments for FHB spread (%DS and n.DS), speed of FHB spreading during the period 14 -21 dai and AUDPC. One QTL was detected on chromosome 6B and the effect of the QTL was found significant ($\alpha < 0.05$) for the overall mean values and in different single experiments for FHB spread (%DS and n.DS), speed of FHB spreading (SFS) during the period 14 -21 dai and AUDPC. The position for 6B QTL is in the flanking markers *XS23M17_5 - Xgwm626*. The resistance was conferred by alleles of *T. diccocoides* in both chromosomes. The phenotypic variation on chromosome 3A explaining 15

to 21 % overall mean value for the two QTL and the phenotypic variation on chromosome 6B explaining 17 to 23 % overall mean value for the QTL. These results suggest that, the QTL for % DS and n.DS, SFS and AUDPC represent the same effect of the same loci with different methods of assessment.

Wheat chromosomes 3A and 6B have been reported to carry QTL for FHB resistance. QTL effects in similar regions of our map for 3A (tetraploid wheat) were reported by Otto et al. (2002). They used 'Langdon'-*T. dicoccoides* chromosome substitution lines to generate a linkage map of chromosome 3A. Analysis of 83 lines identified a single major quantitative trait locus, that explained 37% of the phenotypic variation for FHB resistance. The microsatellite locus, *Xgwm2*, was tightly linked to the highest point of the QTL peak. Gladysz et al. (2008) found the same *Xgwm2* marker on chromosome 3A, using 140 lines generated by back cross of durum wheat (Helidur) with *T. dicoccoides* (line Mt. Hermon#22). *Xgwm2* was also associated with the resistance in our QTL but with lower phenotypic variation for % DS, n.DS and AUDPC. For SFS of FHB symptoms marker *Xgwm2* was closely locus linked to the QTL peak. Chen et al. (2007) saturated the genomic region of 3A containing the QTL using EST, TRAP, STS and SSR markers. A QTL was positioned within flanking markers *Xfcp401* - *Xfcp397.2* including the *Xgwm2* marker. The second QTL on chromosome 3A was reported by Steiner et al. (2004). They used doubled-haploid lines from a cross of Frontana and Remus for evaluation of FHB resistance. The position of the QTL was flanked by the markers *Xgwm720–Xdupw227* and explained 16% of the phenotypic variation for Type I resistance (resistance to initial infection). Spray inoculations were used while our QTL was detected in the same position although in our case the plants were evaluated for Type II resistance using the single spikelet inoculation technique. A QTL effect in a similar region to our map for chromosome 6B was reported by Somers et al. (2006). They used a tetraploid cross of durum wheat (Strongfield x *T. carthlicum* cv. Blackbird) to generate a doubled haploid population. They identified two main QTL for FHB resistance on chromosomes 6B and 2B, respectively. The QTL in the Strongfield x Blackbird population on 6B is in the same region as the FHB resistance QTL called *Fhb2* derived from the hexaploid wheat cultivar Sumai-3 (Cuthbert et al. 2007). Based on common markers between the results of Somers et al. (2006) and our results (*Xwmc397)* it appears likely that the 6B QTL from *T. dicoccoides* is the same QTL as the 6B QTL reported by Somers et al. (2006) and Cuthbert et al (2007). Further QTL on chromosome 6B were also described by Yang et al. (2005); Draeger et al. (2007) Semagn et al. (2007).

A QTL for FHB incidence was detected on chromosomes 6B explaining 15 % of the phenotypic variation. The resistance for FHB incidence was conferred by alleles of *T. diccocoides* in

flanking markers *XS13M25_3 - XS17M25_3*. This locus is responsible for the reduction of FHB establishment. Most detected QTL for FHB incidence were detected using spray inoculation. They were located on different chromosomes, for example 2B and 7B (Gilsinger et al. 2005) and 5A, 4B and 2D (Lin et al. 2005). Few were found on chromosome 6B, for example Steiner et al. (2004) found marker *Xs23m14.4* and Yang et al. (2005) found marker *Xwmc397* which is close to our QTL for FHB incidence (but not in the peak of the our QTL). According to our results, a QTL for percentage of wilted spikes was detected on chromosomes 3A explaining 15 % of the phenotypic variation. The resistance for percentage of wilted spikes was conferred by alleles of *T. diccocoides* in the flanking markers *Xgwm2 - Xgwm779* in the same region as the QTL on 3A for FHB spread.

4.2.3. Markers and QTL analysis for other traits and powdery mildew

Information on genetic diversity of the developmental and morphological traits is of paramount important for breeders (Efremova et al. 1998). For this reason traits as plant height, spike length, waxiness, number of spikelets, brittle rachis, ear type, date of anthesis, colour of the spikes and powdery mildew severity have been studied. Plant traits could work as pre-formed defences of the plant (as cell walls and antimicrobial chemicals) or infection-induced responses (as antimicrobial enzymes and hypersensitive response) which prevent or reduce the growth of the pathogen. Some of these traits are associated with FHB resistance. In this study wheat traits were investigated to assess there relation with FHB. QTL were detected for waxiness, spike length, plant height, number of spikelets, brittle riches, date of anthesis, colour of the spikes and powdery mildew severity for the mean values over many experiments (see Table 35).

4.2.3.1. Waxiness

Leaf and spike waxiness are thought of as a heat avoidance mechanism in wheat. Higher epicuticular wax deposition in leaf increases reflectance and may help to reduce leaf temperatures, stomatal conductance, and improve water use efficiency. Highly significant ($\alpha < 0.05$) differences were found in the amount of epicuticular waxiness which associated with drought tolerance among the wheat cultivars (Nizam and Marshall 1987). Within the BC_1F_6 population evaluated here, high variation for waxiness was found. 2 QTL were detected on chromosome 2B and 1B explaining 32 and 30 % of the phenotypic variation respectively. Waxiness was conferred by alleles of Helidur. Mondal et al. (2009) identified 2 QTL associated with leaf waxiness on chromosome 2B and 6D. No correlation was found between waxiness and FHB related traits, suggesting that waxiness has no effect on FHB spread and severity (Type II resistance).

4.2.3.2. Plant height

Researchers found a relation between wheat height and disease resistance. Several studies reported negative correlation of wheat height with FHB incidence and severity. The shorter wheat genotypes tended to be more diseased than taller genotypes (Mesterhazy 1995; Steiner et al. 2004; Somers et al. 2003). Gervais et al. (2003) identified co-localisation between QTL for FHB resistance and plant height on chromosome 5A. Butler et al. (2005) found major genes, controlling reduced plant height and positioned on chromosomes 4B and 4D. Alleles located on 4B and 4D are known as Rht- genes. In the present study significant ($\alpha < 0.05$) variation for plant height were found among the BC_1F_6 population. QTL was detected on chromosome 4B explaining 41 % of the phenotypic variation and conferred by alleles of Helidur. Positive correlation was found between plant height and FHB severity. This result is in contrast with previous studies and a possible reason for this is that all of these studies measure the relation between plant height and FHB severity Type I and our study measures Type II resistance.

4.2.3.3. Date of anthesis

Significant ($\alpha < 0.05$) variation for date of anthesis was found in our mapping population. A QTL was detected on unknown linkage group X3 explaining 35 % of the phenotypic variation. *T. diccocoides* alleles contributed to late anthesis. No significant ($\alpha < 0.05$) correlation with FHB related traits was found which is in the contrast with Buerstmayr et al. (2000); Gervais et al. (2003); Steiner et al. (2004) who found correlation between FHB related traits and date of anthesis.

4.2.3.4. Spike length

Significant ($\alpha < 0.05$) variation for spike length was found. A QTL was detected on an unknown linkage group X6 explaining 16 % of the phenotypic variation and conferred by alleles of Helidur, which increases spike length. Few work was done regarding to the spike length. Gardner et al. (1985) found a relationship between leaf number and stage of spike development. Sharma et al. (2003) confirmed the major role of non-additive gene effects to control the inheritance of spike length in durum wheat. Few QTL were reported for spike length. Börner et al. (2002) found QTL on 1B, 4A and 5A, Mohammadi et al. (2005) reported a major QTL for spike length on chromosome 2D.

4.2.3.5. Number of spikelets

Significant ($\alpha < 0.05$) variation for number of spikelets per spike was detected in the mapping population. A QTL was detected on unknown linkage group X3 and chromosome 6A explaining 20 and 15 % of the phenotypic variation, respectively. Unknown linkage group X3 conferred alleles of Helidur while chromosome 6A conferred alleles of *T. diccocoides*. This means both parents carry positive and negative alleles for the number of spikelets per spike. The previous study of Börner et al. (2002) describes QTL on chromosome arms 2DS and 4AL. Dashti et al. (2007) found QTL for number of grains per ear on chromosomes 4A, 1B, 5B and 7A.

4.2.3.6. Colour of the spikes

Colour of the spikes is important taxonomic discriminators in wheat and is commonly used for the determination of homogeneity within or distinctness between wheat varieties (Khlestkina et al. 2006). In this study two loci were detected for colour of the spikes on chromosome 1B and 5A. This result fit partially with result of Efremova et al. (1998) who found loci for colour of the spikes on chromosome 1B and 1A. And Khlestkina et al. (2006) found genes representing a set of homoeoloci, designated as Rg-1A, Rg-1B and Rg-1D. Major genes for colour of the spikes were also found on chromosome arm 1DS and chromosome arm 2DS (Börner et al. 2002). Genetic analysis showed that the derived forms had inherited the Gli-D1 allele of the synthetic, which was found to be tightly linked to a gene for colour of the spikes (Pshenichnikova and Maystrenko 2009).

4.2.3.7. Brittle rachis

Brittle rachis is a domestication related trait of wild wheat including *T. dicoccoides*. One locus was detected for brittle rachis on chromosome 5A, explaining 24 % of the phenotypic variation. The Q locus on chromosome 5A is believed to be responsible for brittle rachis in bread wheat, *T. aestivum* (Chen et al. 1998). Nobuyoshi (2005) found that the gene for brittle rachis of Italian and Tunisian durum wheat from different areas was allelic to the brittle allele of the *Br-B1* locus on chromosome 3B. No correlation of brittle rachis with FHB severity was found in this study.

4.2.3.8. Powdery mildew

Powdery mildew (PM) is one of the most common diseases of wheat. The infection of the plant with PM has an effect on FHB sensitivity as described by Mesterhazy and Rowaished (1977). Plants with a high infection level of PM were more susceptible for FHB severity. This phenomenon is most probably due to reaction of the plants to another disease leading to

weakening of the plant defences against FHB. Also in our experiments in which a heavy infection with PM was observed (Field and GH2), the population mean for FHB severity was significantly higher as compared to the population mean in the other three experiments where a neglectable level of PM occurred. We think however, that the results on the presence and location of the QTL for FHB resistance presented in this work are not influenced by PM for the following reasons:

1) Independent QTL analyses of the individual experiments resulted in the identification of the same QTL for FHB resistance irrespective of the level of PM present.

2) In our experiments no correlation was found between PM mean values of the individual lines in the experiments Field and GH2 (heavy infection with PM) and FHB severity for the mean values over all experiments for the individual lines.

3) The QTL analysis detected one locus for powdery mildew on chromosome 6B, explaining 15 % of the phenotypic variation. But this QTL is not located in the same region as the QTL for FHB resistance detected on this chromosome.

Many genes for resistance to powdery mildew at several loci and different chromosomes have been identified in the previous studies. PmTm4 gene mapped on chromosome 7BL (Zhang et al. 2008) as example for mildew resistance and also QTL located on chromosomes 5D, 4A and 6A (Chantret et al. 2001). So far, no powdery mildew resistance genes or QTL have been reported on chromosome 6B. Therefore the powdery mildew marker *XS17M13_5* probably maps to a novel resistance QTL for powdery mildew.

4.3. Conclusion

The use of the wild emmer (*T. dicoccoides*) for durum wheat improvement is considered a very promising strategy by the wheat breeders because of the adaptive complexes of emmer to abiotic and biotic stresses. The resistance to spread of FHB symptoms (Type II resistance) is an important trait of *T. dicoccoides.* The inoculation of wheat with Fusarium spores using single floret injury is a good method to assess Type II resistance . The methods for assessments of Type II as %DS, n.DS, speed of FHB spreading and AUDPC gave similar results. The QTL analysis for FHB resistance Type II of a population constructed from the cross of *T. dicoccoides* with *T. durum* revealed two loci for FHB resistance on chromosomes 3A and one on 6B. The QTL on 3A reduced percent of wilted spikes and the QTL on 6B reduced the percent of infected spikes, which is considered as indicator of type one resistance. The QTL on 6B also reduced powdery mildew severity. Many other loci were found for morphological and developments

traits. Positive correlation was found for FHB resistance with plant height and number of spikelets per spike.

Finally the SSR and AFLP markers around the 3A and 6B QTL were identified and after validation could be used in marker assisted selection for FHB resistance and durum wheat improvement.

5. References

Ahmad J (2004) Improvements in wheat x maize crossing system of doubled haploid production in bread wheat. Dissertation, University of Agreculture, Faisalabd Pakistan.

Ambroz KH, Webb AC, Matthews RA, Li W, Gill SB, Fellers PJ (2006) Expression analysis and physical mapping of a cDNA library of Fusarium head blight infected wheat spikes. Crop Science 46:15-26.

Amersham Biosciences (2004) Handbook, Fluorescence Imaging, Principles and methods.

Austria statistics (2005/2006) Agriculture, Supply balance sheet for cereals. www.statistik.at. Accessed 10 August 2009.

Bai G and Shaner G, 1994: Scab of wheat: Prospects for control. Plant Disease 78:760-766.

Bernacchi D, Beck-Bunn T, Emmatty D, Eshed Y, Inai S, Lopez J, Petiard V, Sayama H, Uhlig J, Zamir D, Tanksley S (1998) Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from Lycopersicon hirsutum and L pimpinellifolium. Theoretical and Applied Genetics 97:170-180.

Bernardo A, Bai G, Guo P, Xiao K, Guenzi A, Ayoubi P (2007) *F. graminearum*-induced changes in gene expression between Fusarium head blight-resistant and susceptible wheat cultivars. Funct. Integr. Genomics 7:69-77.

Bonjean A, Angus W (2001) The world wheat book. Origin of cultivated wheat. Paris, p 5.

Booth C (1971) The Genus Fusarium. CMI Kew, Surrey, p 19-31.

Bordes J, Charmet G, Dumas R, Lapierre A, Pollacsek M, Beckert M, Gallais A (2007) Doubled-haploid versus single-seed descent and S1-family variation for testcross performance in a maize population. Euphytica 154:41-51.

Börner A, Schumann E, Furste A (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (Triticum aestivum L,) Theoretical and Applied Genetics 105:921-936.

Bottalico A, Perrone G (2002) Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. European Journal of Plant Pathology 108: 611-624.

Broman (2005)The Genomes of recombinant inbred lines. Genetics Society of America 169: 1133-1146.

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breeding 128:1- 26.

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P, (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theoretical and Applied Genetics 104: 84–91.

Buerstmayr H, Lemmens M, Patschka G, Grausgruber H, Ruckenbauer P (1996) Head blight (Fusarium spp.) resistance of wheat cultivars registered in Austria Die Bodenkultur.47 (3).

Buerstmayr H, Steiner B, Lemmens M, Ruckenbauer P (2000) Resistance to Fusarium head blight in two winter wheat crosses: heritability and trait associations. Crop Science 40: 1012-1018.

Buerstmayr H, Stierschneider M, Steiner B, Lemmens M, Griesser M, Nevo E, and Fahima T (2003) Variation for resistance to head blight caused by Fusarium graminearum in wild emmer (Triticum dicoccoides) originating from Israel. Euphytica 130:17-23.

Butler JD, Byrne PF, Mohammadi V, Chapman PL, Haley SD (2005) Agronomic performance of Rht alleles in a spring wheat population across a range of moisture levels. Crop Science 45:939-947.

Chantret N , Mingeot D, Sourdille P, Bernard M, Jacquemin J, Doussinault G (2001) A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat Theoretical and Applied Genetics 103:962-971.

Chen X, Faris J, Hu J, Stack R, Adhikari T, Elias E, Kianian S, Cai X (2006) Saturation and comparative mapping of a major Fusarium head blight resistance QTL in tetraploid wheat. Molecular Breeding 19:113-124.

Chen, Yen C, Yang J (1998) Chromosome location of the gene for brittle rachis in the Tibetan weedrace of common wheat. Genetic Resources and Crop Evolution 45:407-410.

Cuthbert P, Somers D, Brulé-Babel A (2007) Mapping of Fhb2 on chromosome 6BS: a gene controlling Fusarium head blight Weld resistance in bread wheat (Triticum aestivum L.) .Theoretical and Applied Genetics 114:429-437.

D'Mello F, Plcinta M, Mcdonald M (1999) Fusarium mycotoxins: a review of global implications for animal health, welfare and productivity. Animal Feed Science and Technology 80:183-205.

Dashti H, Yazdi B, Ghannadha M, NaghaviA MR, Quarri S (2007) QTL Analysis for drought resistance in wheat using doubled haploid lines. International journal of agriculture and Biology 1560-8530.

De Givry S, Bouchez M, Chabrier P, Milan D, Schiex T (2005) Multipopulation integrated genetic and radiated hybrid mapping. Bioinformatics 21:1703-1704.

Desjardins AE, Hohn TM, McCormick SP (1993) Trichothecene biosynthesis in Fusarium species: Chemistry, genetics, and significance. Microbiological Reviews 57:595–604.

DeWolf ED, Madden LV, Lipps PE (2003) Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology 93:428–435.

Doerge R (2001) Mapping and analysis of quantitative trait loci in expremental populaions .Department of Statistics, and Department of Agronomy, and Computational Genomics, Purdue University, West Lafayette, Indiana 47907-1399.

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285-294.

Dorofeev V, Filatenko A, Migushova E, Udaczin R, Jakubziner M (1979) Wheat. In: Dorofeev VF, Korovina ON (eds) Flora of Cultivated Plants, vol. 1., p. 346 (in Russian).

Draeger R, Gosman A, Steed, Chandler E, Thomsett M, Srinivasachary, Schondelmaier J, Buerstmayr H, Lemmens M, Schmolke M, Mesterhazy A, Nicholson P (2007) Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theoretical and Applied Genetics 115:617-625.

Dubcovsky J (2004) Marker-assisted selection in public breeding programs: The wheat experience. Crop Science 44:1895-1898.

Durner J, Shah J, Klessig D (1997) Salicylic acid and disease resistance in plants. Elsevier Science 2:266-274.

Efremova T, Maystrenko O, Arbuzova VS, Laikova LI (1998) Genetic analysis of glume colour in common wheat cultivars from the former USSR. Euphytica 102:211-218.

FAO statistics (2007) Production and yield of cereals and wheat worldwide and Austria. Source: www.faostat.fao.org. Accessed 10 June 2009.

Fertile_Crescent (2008) World Atlas, MiddleEast. http://en.wikipedia.org/wiki/Fertile_Crescent.

FOA (2008) Food and Agriculture in National and International Settings. http://www.fao.org/newsroom/en/news/2008/1000866/index.html. Accessed 10 June 2009.

Gallo G, Bianco M, Bognannia R, Saimbene G (2008) Mycotoxins in durum wheat grain: hygienic-health quality of sicilian production. Journal of food Science 73:42- 47.

Gardner J, Hess W, Trione EJ (1985) Development of the young wheat spike: A sem study of Chinese spring wheat . American Journal of Botany 72:548-559.

Geddes J, Eudes F, Laroche A, Selinger B (2008) Differential expression of proteins in response to the interaction between the pathogen *F. graminearum* and its host, Hordeum vulgare. Proteomics 8:545-554.

Gervais L, Dedryver F, Morlais JY, Bodusseau V, Negre S, Bilous M, Groos C, Trottet M (2003) Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. Theoretical and Applied Genetics 106:961-970.

Gilsinger, JL, Kong X, Shen, Ohm H (2005) DNA markers associated with low Fusarium head blight incidence and narrow flower opening in wheat. Theoretical and Applied Genetics 110:1218-1225.

Gladysz C, Lemmens M, Steiner B, Buerstmayer H (2008) Evaluation and genatic mapping of resistance to Fusarium head blight in *T. dicoccoides*. Israel Journal of plant sciences 55: 263-266.

Gold J, Harder D, Smith F, Aung T, Procunier J (1999) Development of a molecular marker for rust resistance genes SR39 and LR35 in wheat breeding lines. Biotechnology. http://ejb.ucv.cl/content/vol2/issue1/full/1/1.pdf. Accessed 10 August 2009.

Guenther, JC, Trail F (2005) The development and differentiation of Gibberella zeae (anamorph: Fusarium graminearum) during colonization of wheat. Mycologia 97: 229-237.

Guo PG, Bai GH, Shaner GE (2003) AFLP and STS tagging of a major QTL for Fusarium head blight resistance in wheat. Theoretical and Applied Genetics 106:1011-1017.

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breeding 118:369-390.

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002) Characterisation of polymorphic microsatellite markers from Aegilops tauschii and transferability to the D-genome of bread wheat. Theoretical and Applied Genetics 104:1164-1172.

Haldane J (1919) The combination of linkage values, and the calculation of distances between loci of linked factors. Genetics 8:299-309.

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315-324.

He P, Li JZ, Zheng XW, Shen LS, Lu CF, Chen Y, Zhu LH (2001) Comparison of molecular linkage maps and agronomic trait loci between DH and RIL populations derived from the same rice cross. Crop Science 41:1240-1246.

Holsinger K, (1998) Mapping quantitative trait loci. Sunderland, MA. http://darwin.eeb.uconn.edu/eeb348/lecture-notes/qtl-intro.pdf. Accessed 20 June 2009.

IFAD (2001) IFAD strategy for rural poverty reduction.http://www.ifad.org/operations/regional/2002/pi/pi.htm. Accessed 10 September 2009.

Joehanes R, Nelson J (2008) QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788-2789.

Johnson R (1992) past, present and future opportunities in breeding for disease resistance, with examples from wheat. Euphytica 63:1-2.

Jones N, Ougham H, Thomas H (1997) A Markers and mapping: we are all geneticists now. New Phytologist 137:165-177.

Josephides CM (2000) Breeding durum wheat for grain yield and quality. Agricultural Research Institute, Nicosia, Cyprus CIHEAM - Options Mediterraneennes. http://ressources.ciheam.org/om/pdf/a40/00600079.pdf. Accessed 25 September 2009.

Kang Z, Buchenauer H (1999) Immunocytochemical localization of Fusarium toxins in infected wheat spikes by Fusarium culmorum. Physiological and Molecular Plant Pathology 55: 275-288.

Kassa J, Kunesova G (2006) Comparison of the neuroprotective effects of the newly developed oximes (K027, K048) with trimedoxime in tabun-poisoned rats. Journal of Applied Biomidicine 4:123-134.

Kelly DJ, Miklas NP (1998) The role of RAPD markers in breeding for disease resistance in common bean. Molecular Breeding 4:1-11.

Khlestkina EK , Pshenichnikova TA , Röder MS , Salina EA , Arbuzova VS, Börner A (2006) Comparative mapping of genes for glume colouration and pubescence in hexaploid wheat (Triticum aestivum L.). Theoretical and Applied Genetics 113:801-807.

Kolb FL, Bai GH., Muehlbauer GJ, Anderson JA, Smith KP, Fedak G (2001) Host Plant Resistance Genes for Fusarium Head Blight: Mapping and Manipulation with Molecular Markers. Crop Science 41:611-619.

Kosack K, Jones J (1996) Resistance Gene-Dependent Plant Defense Responses. American Society of Plant Physiologists 8:1773-1791.

Kosambi DD (1944) The estimation of map distances from recombination values. Annual Eugen 12:172-175.

Kruger WM, Pritch C, Staggs R, Muehlbauer GJ (2002) Functional and comparative bioinformatic analysis of expressed genes from wheat spikes infected with Fusarium graminearum. Molecular Plant-Microb Interact 15:445- 455.

Kumar S, Stack RW, Friesen TL, Faris JD (2007) Identification of a Novel Fusarium Head Blight Resistance Quantitative Trait Locus on Chromosome 7A in Tetraploid. Wheat Phytopathology 97:592-597.

Lafferty J (2008) Durum breeder. Saatzucht Donau. Probstdorf, Austria. http://www.saatzucht-donau.at.

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) Mapmarker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Langridge P, Lagudah ES, Holton TA, Appels R, Sharp PJ, Chalmers KJ (2001) Trends in genetic and genome analyses in wheat: a review. Australian Journal l52:1043-1077.

Lemmens M, Scholz U, Berthiller F, Dall'Asta Ch, Koutnik A, Schuhmacher R, Adam G, Buerstmayr H, Mesterházy Á, Krska R, Ruckenbauer P (2005) The Ability to Detoxify the Mycotoxin Deoxynivalenol Colocalizes With a Major Quantitative Trait Locus for Fusarium Head Blight Resistance in Wheat. The American Phytopathological Society 18: 1318-1324.

Leonard KJ, Bushnell WR (2003) Fusarium head blight of wheat and barley. APS Press, St. Paul, Minnesota, p. 1-512.

Leslie JF, Summerell BA (2006) The Fusarium Laboratory manual. (Blackwell Publishing: Iowa, USA). http://www.infibeam.com/Books/info/John-F-Leslie/The-Fusarium-Laboratory-Manual/0813819199.html. Accessed 15 September 2009.

Li L, Xiang J, Liu X, Zhang Y, Dong B, Zhang X (2005) Construction of AFLP-based genetic linkage map for Zhikong scallop, Chlamys farreri Jones et Preston and mapping of sex-linked markers. Aquaculture 245:63-73.

Li L, Faris JD, Muthukrishnan S, Liu DJ, Chen PD, Gill BS (2001) Isolation and characterization of novel cDNA clones of acidic chitinases and β-1,3-glunanases from wheat spikes infected by Fusarium graminearum. Theoretical and Applied Genetics 102:353-362.

Lin F , Kong ZX , Zhu HL , Xue SL , Wu JZ , Tian DG , Wei JB , Zhang CQ, Ma ZQ (2005) Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419 × Wangshuibai population. I. Type II resistance. Theoretical and Applied Genetics 109:1504-1511.

Lotti C, Salve S, Pasqualoe A, Tuberosa R, Blanco A (2000) Integration of AFLP markers into an RFLP-based map of durum wheat. Plant Breeding 119:393-401.

Lvaro F, Isidro J, Villegas D, Garcı L, Royo C (2008) Old modern durum wheat varieties from Italy and Spain differ in main spike components. Field Crops Research 106:86-93.

Mauricio R (2001) Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. Nature Genetics 2:370-381.

McMullen PM, Stack RW (1999) Fusarium Head Blight (Scab) of Small Grains. County Commissions, North Dakota State University and U.S. Department of Agriculture cooperating 701:231-7881.

Mesterhazy A (1995) Types and components of resistance to Fusarium head blight of wheat. Plant Breeding 114:377-386.

Mesterhazy A (2002) Role of deoxynivalenol in aggressiveness of *F. graminearum* and *F. culmorum* and in resistance to Fusarium head blight. European Journal of Plant Pathology 108:675- 684.

Mesterhazy A, Rowaished AK (1977) Analysis of symptoms caused by Fusarium graminearum Schwabe and its relation to powdery mildew infection in wheat. Acta Phytopath. Academic Science Hungary 12:287-299.

Mohammadi V, Ghanadha MR, Zali AA, Yazdi-Samadi B, Byrne P (2005) Mapping QTLs for morphological traits in wheat. Iranian journal of agricultural sciences 36:145-157.

Mondal S, Mason R, Beecher F, Hays D (2009) Preliminary QTL analysis identified 2 QTL associated with leaf wax on chromosome 2B and 6D. Texas A&M Univ., College Station, TX. http://a-c-s.confex.com/crops/2009am/webprogram/Paper55531.html. Accessed 20 June 2009.

Mueller U, Wolfenbarger L (1999) AFLP genotyping and fingerprinting. Elsevier Science TREE 14.

Munns R, Hare RA, James RA, Rebetzke GJ (2000) Genetic variation for improving the salt tolerance of durum wheat. Australian Journal of Agricultural Research 51:69-74.

Nelson J (1997) Qgene: software for marker-based genomic analysis and breeding. Molecular Breeding 3:239-245.

Nelson P, Dignani C, Anaissie E (1994) Taxonomy, Biology, and Clinical Aspects of Fusarium Speciest. American Society for Microbiology 7:479-504.

Nizam M, Marshall DR (1987) Variation in epicuticular wax content in wheat. Biomedical and Life Sciences. http://www.springerlink.com/content/pg325180360191h3.

Nobuyoshi W (2005) The occurrence and inheritance of a brittle rachis phenotype in Italian durum wheat cultivars. Euphytica 142:247-251.

Nyquist WE (1991) Estimation of heritability and prediction of selection response in plant populations. Critical Reviews in Plant Science 10:235-322.

Oliver RE, Cai X, Friesen TL, Halley S, Stack RW, Xu SS (2008) Evaluation of Fusarium Head Blight Resistance in Tetraploid Wheat (Triticum turgidum L.). Crop Science 48: 213-222.

Oliver RE, Stack RW, Miller JD, Cai X (2007) Reaction of Wild Emmer Wheat Accessions to Fusarium Head Blight. Crop science 47:893-899.

Otto CD, Kianian1 SF, Elias1 E M, Stack RW, Joppa LR (2002) Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat Plant. Molecular Biology 48:625-632.

Ovevesna J, Polakova K, Lesova L (2002) DNA Analyses and their Applications in Plant Breeding Czech J. Genet. Plant Breeding 38:29- 40.

Pakendorf K, SGI A, Stellenbosch (2008) Plant breeding as a career. http://www.sapba.co.za/downloads/Pteeltkl.doc. Accessed 10 June 2009.

Pandeya R (2005) Winter Wheat Fusarium Research in Canada - Progress and Up-date. 4th Canadian Workshop on Fusarium Head Blight.

Parry DW, Jenkinson P, Mcleod L (1993) Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathology 44:207-238.

Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small grain cereals – a review. Plant Pathology 44:207-238.

Patil R, Oak M, Tamhankar S, Sourdille P, Rao V (2008) Mapping and validation of a major QTL for yellow pigmentcontent on 7AL in durum wheat (Triticum turgidum L. ssp. durum). Molecular Breeding 21:485-496.

Payne R, Murray D, Harding S, Baird D, Soutar D (2007) GenStat for Windows 10th edn. Hemel Hempstead, UK: VSN International.

Poehlman J (1990) Breeding Field Crops 724-pg hardcover published in 1987 Third Edition. New york.

Prang A, Modrow H, J, Kra Mer J, Kohler P (2005) Influence of Mycotoxin Producing Fungi, Fusarium, Aspergillus,Penicillium on Gluten Proteins during Suboptimal Storage of Wheat after Harvest and Competitive Interactions between Field and Storage Fungi. Agricultural and Food Chemistry 53:6930- 6938.

Pritsch C, Vance PC, Bushnell WR, Somers DA, Hohn TM, Muehlbauer GJ (2001) Systemic expression of defence response genes in wheat spikes as a response to *Fusarium graminearum* infection. Physiological and Molecular Plant Pathology 58: 1-12.

Pshenichnikova TA, Maystrenko OI (2009) Inheritance of genes coding for gliadin proteins and glume colour introgressed into Triticum aestivum from synthetic wheat. Plant Breeding 114:501-504.

Pshenichnikova TA, Maystrenko OI (1995) Inheritance of genes coding for gliadin proteins and glume colour introgressed into Triticum aestivum from a synthetic wheat. Plant Breeding 114:501-504.

Qu B, Li H, Zhang J, Huang T, Carter J, Liao Y, Nicholson P (2008) Comparison of genetic diversity and pathogenicity of fusarium head blight pathogens from China and Europe by SSCP and seedling assays on wheat. Plant Pathology 57:642-651.

Rajaram S (2005) Role of conventional plant breeding and biotechnology in future wheat production. Turk Agriculture 29:105-111.

Röder M , Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal M (1998) A microsatellite map of wheat .Genetics Society of America 149:2007-2023.

Royo C, A´ lvaro F, Martos V, Ramdani A, Isidro J, Villegas D, Moral L (2007) Genetic changes in durum wheat yield components and associated traits in Italian and Spanish varieties during the 20th century. Euphytica 155:259-270.

Rudd JC, Horsley RD, McKendry AL, Elias EM (2002) Host plant resistance genes for Fusarium head blight: sources, mechanisms, and utility in conventional breeding systems. Crop Science 41:620-627.

Saghai-Maroof MA, Soliman K, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location , and population dynamics. PNAS 81:8014-8018.

Saranga Y, Peleg Z, Fahima T (2008) Physiological-genetic dissection of drought resistance in wild emmer wheat Haifa 31905, Israel. http://ses.library.usyd.edu.au/bitstream/2123/3336/1/O35.pdf. Accessed 15 June 2009.

Schippers C, Gieffers W, Schäfer-Pregl R (1994) Quantitative resistance to Phytophthora infestans in potato: a case study for QTL mapping in an allogamous plant species. Genetics 137:67-77.

Schlatter J (2004) Toxicity data relevant for hazard characterization. ELSEVIER Toxicology Letters 153:83-89.

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by Gibberella zeae. Phytopathology 53:831-838.

Seeling K, Boguhn J, Strobel E, Dänicke S, Valenta H, Ueberschär KH, Rodehutscord M (2006) On the effects of Fusarium toxin contaminated wheat and wheat chaff on nutrient utilisation and turnover of deoxynivalenol and zearalenone in vitro (Rusitec). Toxicol in Vitro.20:703-11.

Semagn K, Bjørnstad Å, Ndjiondjop MN (2006) Principles, requirements and prospects of genetic mapping in plants. African Journal of Biotechnology 5:2569-2587.

Semagn, K, Skinnes H, Bjornstad A, Maroy AG, Tarkegne Y (2007) Quantitative trait loci controlling Fusarium head blight resistance and low deoxynivalenol content in hexaploid wheat population from _Arina_ and NK93604. Crop Science 47:294-303.

Sharma SN, Sain RS, Sharma RK (2003) Genetics of spike length in durum wheat. Euphytica130:155-161.

Singh RP, Rajaram S (2008) Breeding for disease resistance in wheat. FAO. http://www.fao.org/docrep/006/y4011e/y4011e0b.htm. Accessed 10 August 2009.

Šlikova S, GregovÁ E, Bartos P, Kraic J (2003) Marker-Assisted Selection for Leaf Rust Resistance in Wheat by Transfer of Gene Lr19 1Plant. Plant Protect. Sci. 39:13-17.

Snijders CH, Eeuwijk A, van FA (1990) Genotype by strain interactions for resistance to Fusarium head blight caused by Fusarium culmorum in winter wheat. Theoretical and Applied Genetics 81: 239-244.

Soliman MH, Rubiales' D, Cabrera' A (2001) A fertile amphiploid between durum wheat (Triticum turgidurn) and the x Agroticum amphiploid (Agropyron cristatum x T. tauschii) .Hereditas 135:183-186.

Somers D, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). Theoretical and Applied Genetics 109:1105-1114.

Somers DJ, Fedak G, Savard M (2003) Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46: 555-564.

Somers J, George F, John C, Wenguang C (2006) Mapping of FHB resistance QTLs in tetraploid wheat. Genome 49:1586-1593.

Sourdille P, Tavaud M, Charmet G, Bernard M (2001) Transferability of wheat microsatellite to diploid Triticeae species carrying the A, B and D genomes. Theoretical and Applied Genetics 103:346-352.

Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES (2005) Powdery mildew resistance and Lr34 / Yr18genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theoretical and Applied Genetics 111:731-735.

Šrobárová1 A, Šliková S, Šudyova V (2008) Diversity of the Fusarium species associated with head and seedling blight on wheat in Slovakia. Biologia 63: 332-337.

Stack W (2008) Return of an Old Problem: Fusarium Head Blight of Small Grains. Plant Pathology Dept. North Dakota State Univ. http://www.apsnet.org/education/feature/FHB/. Accessed 5 June 2009.

Stack W, Elias EM, Mitchell FJ, Miller JD, Joppa LR (2002) Fusarium head blight reaction of Langdon durum-Triticum dicoccoides chromosome substitution lines. Crop Science 42: 637- 642.

Stam P (1993) Construction of integrated genetic linkage maps by means of a computer package: Joinmap. Plant J. 5:739-744.

Steiner B, Lemmens M, Griesser M, Scholz U, Schondelmaier J, Buerstmayr H (2004) Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theoretical and Applied Genetics 109:215-224.

Suslow T, Thomas B, Bradford K (2002) Biotechnology provides new tools for plant breeding .Agricultural Biotechnology in aliforina Series Publication 8043.http://www.plantsciences.ucdavis.edu/bradford/8043.pdf. Accessed 25 June 2009.

Tadesse W, Schmolke M, Hsam SK, Mohler V, Wenzel G, Zelle FJ (2007) Molecular mapping of resistance genes to tan spot [Pyrenophora tritici-repentis race 1] in synthetic wheat lines. Theoretical and Applied Genetics 114:855-862.

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method of simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theoretical and Applied Genetics 92:191-203.

Tomás A, Solís I (2000) Effects of powdery mildew (Blumeria graminis) severity on durum wheat cultivars. CIHEAM – Options Mediterraneennes. http://ressources.ciheam.org/om/pdf/a40/00600069.pdf. Accessed 20 September 2009.

Topala A, Aydınb C, Akgu¨na N, Babaoglu M (2004) Diallel cross analysis in durum wheat (Triticum durum Desf.): identification of best parents for some kernel physical features .Field Crops Research 87:1-12.

Tyrka M (2002) A simplified AFLP method for fingerprinting of common wheat (Triticum aestivum L.) cultivars. Theoretical and Applied Genetics 43:131-143.

Utz HF, Melchinger AE (1996) PLABQTL: A program for composite interval mapping of QTL. J. Quant. Trait Loci 2:1. http://www.ncgr.org/jag/papers96/paper196/utz.html. Accessed 10 June 2009.

Vaughan T, Russal LC, Hill A, Frew TJ, Gilpin BJ (1997) DNA markers for disease resistance rreeding in peas (PISUM SATIVUM L.) Proc. 50th N.Z. Plant Protection Conf. 314-315.

Voorrips RE (2001) MapChart Version 2.0: Windows software for the graphical presentation of linkage maps and QTLs. Plant Research International, Wageningen, The Netherlands.

Vos P, Hogers R, Bleeker M, Reijans M, Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting .Nucleic Acids Research 21:4407- 4414.

Wang YZ, Miller JD (1987) Screening techniques and sources of resistance to Fusarium head blight. In: Wheat production constaints in tropical environments. A proceeding of the international conference, Jan. 19-23, Chiang Mai, Thailand, p. 239-250.

Welsh TS (2005) The literature of telemedicine: A bibliometric study. Science and Technology Libraries 25:21-34.

Windels CE (2000) Economic and social impacts of Fusarium Head Blight: Changing farms and rural communities in the Northern Great Plains. Phytopathology 90:17-21.

Xie W, Nevo E (2008) Wild emmer: genetic resources, gene mapping and potential for wheat improvement. Euphytica 164:603-614.

Yang, Z, Gilbert, Fedak G, Somers DJ (2005) A Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. Genome 48:187-196.

Zeng Z (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Genetics 90:10972-10976.

Zeng, ZB, Kao CH, Basten CJ (1999) Estimating the genetic architecture of quantitative traits. Genet Res 74:279-289.

Zhang, Zhao L, Hai Y, Chen G, Tian J (2008) QTL Mapping for Adult-Plant Resistance to Powdery Mildew, Lodging Resistance, and Internode Length Below Spike in Wheat. Acta Agronomica Sinica 34:1350-1357.

6. Appendix

6.1. Appendix 1

Large scale Genomic DNA Isolation

(Based on method of Saghai-Maroof et al., 1984*)

1. Weight 300-400 mg of ground, lyophilized tissue, into a 15 ml polypropylene centrifuge tube. DNA yields range from 50 to more than 100 µg DNA/ 100 mg dry tissue. If higher amounts are needed, start with 1 g lyophilized tissue into a 50 ml polypropylene centrifuge tube, and triple all the amounts given below.

2. Add 9.0 ml of warm (65°C) CTAB extraction buffer (Table 38**)** to the 300-400 mg ground, lyophilized tissue. It is best to distribute tissue along the sides of the tube before adding buffer, to avoid clumping of dry tissue in the bottom. Mix several times by gentle inversion.

3. Incubate for 60-90 min, with continuous gentle rocking in a 65°C oven or water bath.

4. Remove tubes from oven, wait 4-5 min for tubes to cool down, and then add 4.5 ml chloroform/isoamylalcohol (IAA) (24:1). Rock gently to mix for 5 -10 min.

5. Spin in a table-top centrifuge for 10 min at > 1300-1500 x g at RT.

NOTE: below 15°C the CTAB/nucleic acid complex may precipitate; this could ruin the preparation and cause damage to the centrifuge.

6. Pour off top aqueous layer into new 15 ml tubes. Add 4.5 ml chloroform/IAA and rock gently for 5-10 min.

7. Spin in a table-top centrifuge for 10 min at >1300-1500 x g1 at RT.

[8. Optional: Pipette off top aqueous layer into new 15 ml tubes containing 25-50 µl of 10 mg/ml RNase A (pre-boiled). Mix by gentle inversion and incubate for 30 min at RT.]

9. Add 6.0 ml of isopropanol (2-propanol). Mix by gentle inversion.

10. Remove precipitated DNA with glass hook.

11. Place hook with DNA in 5 ml plastic tube containing 3-4 ml of Wash1 (Table 39). Leave DNA on hook in tube for about 20 min.

12. Rinse DNA on hook briefly in 1-2 ml of Wash2 (Table 39) and transfer DNA to 5 ml plastic tube containing 0.5-1.0 ml TE; gently twirl hook until DNA slides off the hook. Cap tube and rock gently overnight at room temperature to dissolve DNA. Store samples at 4°C. For longer term storage store at -20°C.

---

* Saghai-Maroof, M.A., K. Soliman, R.A. Jorgensen and R.W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location , and population dynamics. *PNAS* **81**:8014-8018.

Table 38: CTAB Extraction Buffer.

STOCK	[FINAL]	1 RXN 10 ml	5 RXN 50 ml	10 RXN 100 ml	20 RXN 200 ml	50 RXN 500 ml	56 RXN 600 ml
dH_2O		6.5 ml	32.5 ml	65.0 ml	130.0 ml	325.0 ml	390.0 ml
1 M Tris-7.5	100 mM	1.0 ml	5.0 ml	10.0 ml	20.0 ml	50.0 ml	60.0 ml
5 M NaCl	700 mM	1.4 ml	7.0 ml	14.0 ml	28.0 ml	70.0 ml	84.0 ml
0.5 M EDTA-8.0	50 mM	1.0 ml	5.0 ml	10.0 ml	20.0 ml	50.0 ml	60.0 ml
CTAB[2]	1 %	0.1 g	0.5 g	1.0 g	2.0 g	5.0 g	6.0 g
14 M BME [3]	140 mM	0.1 ml	0.5 ml	1.0 ml	2.0 ml	5.0 ml	6.0 ml

[1] Use freshly made; warm buffer to 60-65°C before adding the CTAB and BME.
[2] CTAB = Mixed alkyltrimethyl-ammonium bromide (Sigma M-7635)
[3] Add BME (ß-mercaptoethanol) just prior to use, under a fume hood.

Table 39: Wash 1 (76% EtOH, 0.2 M NaOAc).

STOCK	100 ml	200 ml	300 ml	400 ml	500 ml
Absolute EtOH	76 ml	152 ml	228 ml	304 ml	380 ml
2.5 M NaOAc	8 ml	16 ml	24 ml	32 ml	40 ml
dH_2O	16 ml	32 ml	48 ml	64 ml	80 ml

Table 40: Wash 2 (76% EtOH, 10 mM NH4OAc).

STOCK	100 ml	200 ml	300 ml	400 ml	500 ml
Absolute EtOH	76 ml	152 ml	228 ml	304 ml	380 ml
1 M NH_4OAc	1 ml	2 ml	3 ml	4 ml	5 ml
dH_2O	23 ml	46 ml	69 ml	92 ml	115 ml

6.2. Appendix 2

PCR Amplification of Wheat Microsatellites[1]

(for separation on LI-COR 4200 machine)

1. Prepare a Bulk Mix (Table 41) containing all components except template DNA and primers.

Table 41: Bulk Mix for microsatellites.

	[stock]	[final]	for one 15 µl RXN	Bulk-mix for RXN
PCR buffer ($MgCl_2$-free)*	10 X	1 X	1.5 µl	µl
$MgCl_2$	50 mM	1.5 mM	0.45 µl	µl
dNTP Mix	2 mM (each)	0.2 mM each	1.5 µl	µl
F-Primer	10 µM	0.12 µM	0.18 µl	µl
R-Primer	10 µM	0.2 µM	0.3 µl	µl
Taq-Enzym	5 U/µl	0.04 U/µl	0.12 µl	µl
Template DNA	10 ng/µl	2 ng/µl	3 µl	µl
ddH2O		-	7.95 µl	µl
Total			15 µl	µl

*One may also use a PCR buffer which contains 15 (20 or 25) mM MgCl2 directly in the buffer.

2. Aliquot bulk mix into each labeled tube.

3. Add (primer and) template DNA sample to each tube. Mix briefly (optional: centrifuge).

4. Place in PCR machine.

5. Amplify using the following program:

1 Cycle	**35 Cycles**	**1 Cycle**
3 Min 94 °C	1 Min 94 °C	10 Min 72 °C
	1 Min 50 / 55 / 60 °C *	
	2 Min 72 °C	then store at 10 °C

** Depending on the microsatellite to amplify the optimal annealing temperature may be between 50°C and 60°C*

1) Roeder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. *Genetics* 149: 2007-2023

PCR Amplification of *M13-tailed* Microsatellites
(for separation on LI-COR 4200 machine)

In this case one microsatellite primer is extended by a M13 sequence at the 5' end. This serves a template for the fluorochrome labeled M13 primer. The final PCR product will thus be labeled with the fluorescent dye.

1. Prepare a Bulk Mix (Table 42) containing all components except template DNA and primers.

Table 42: Bulk Mix for microsatellites.

	[stock]		[final]		for one 10 µl RXN		Bulk-mix for RXN
PCR buffer 15 mM incl 15 mM MgCl2[a]	10	X	1	X	1	µl	µl
dNTP Mix (10X)	2	mM (each)	0.2	mM (each)	1	µl	µl
R-Primer (10µM)	10	µM	0.2	µM	0.2	µl	µl
F-Primer (10µM) [b]	10	µM	0.03	µM	0.03	µl	µl
M13-30 Primer (10µM)[c]	10	µM	0.18	µM	0.18	µl	µl
Taq-Enzym (5U/µl)	5	U/µl	0.05	U/µl	0.1	µl	µl
ddH2O			-		4.49	µl	µl
Template DNA (10ng/µl)	20-50	ng/µl	40-150	ng	3	µl	
				Total	10	µl	µl

a) One may also use a PCR buffer which contains 15 (20 or 25) mM MgCl2 directly in the buffer, b) The F-Primer has a M13-30 sequence at the 5'end, c) The M13-30 sequence we use is: 5' CCCAGTCACGACGTTG 3'. It is labeled with a fluorescent dye at the 5'end (in our case IRD-700 or IRD-800), for fragment detection on a LI-COR analyzer.

2. Aliquot bulk mix into each labeled tube.
3. Add (primer and) template DNA sample to each tube. Mix briefly (optional: centrifuge).
4. Place in PCR machine.
5. Amplify using the following program:

94°C for 2 min
95°C for 1 min
ramp 0.5°C/sec to 61°C or 51°C
61°C for 30 sec (or 51°C for 30 sec or other temperature, we also tried 57°C)

ramp 0.5°C/sec to 73°C
73°C for 1 min
repeat 30 times
73°C for 5 min
store at 8-10°C

6. Dilute amplicons (1:5 or 1:10) with ddH2O, add 6 µl formamide loading buffer (see AFLP protocol).
7. Denature 5 min at 95°C chill on ice and load on gel (see AFLP protocol for details of gel electrophoresis on the LI-COR DNA analyzer)

6.3. Appendix 3

AFLP on wheat*

* Protocol adapted based on a protocol from Dr. Lorenz HARTL, LBP-Freising, Germany; Important: the AFLP method is patent protected by the company *Keygene* (NL): Zabeau, M. 1992. Selective restriction fragment amplification: A general method for DNA fingerprinting. European patent 92402629.7. ; First publication describing the method : Vos, P., et al. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*. 23:4407-4414.

1. Preparation of Adapters

First one purchases single stranded oligos. From these the adapters can be made. The Mse-oligos are adjusted to a concentration of 500 µM and the Sse-oligos are adjusted to 50µM.

Mix for *Mse*-adapter

ADAMse1 (500µM) 2 µl

ADAMse2 (500µM) 2 µl

H2O 16 µl

total 20 µl ADAMse 50 µM

Mix for *Sse*-adapter

ADASse2 (50µM) 2 µl

ADASse1 (50µM) 2 µl

H2O 16 µl

total 20 µl ADASse 5 µM

These two mixtures can either: be incubated at room temperature for 30 minutes, and mixed gently several times or: the mix is heated in the cycler to 95°C and then cooled to room temperature within 10 minutes. Both procedures should lead to the double stranded adapters for AFLP.

2. Restriction and Ligation

For this reaction (Table 43) ATP (purchased from Amersham-Pharmacia) is dissolved in water to a final concentration of 10mM (aliquots can be stored frozen and used when needed, ATP should not be thawed and frozen several times).

Table 43: Restriction / Ligation.

	[Stock]		[Final]		per one	12.5	µl RXN	x		RXN
ATP	100	mM	1	mM		0.125	µl		0	µl
NEB2 buffer	10	X	1	X		1.25	µl		0	µl
BSA (10X Takara)	0.1	%	0.01	%		1.25	µl		0	µl
T4 Ligase	5	U/µl	1	Unit		0.2	µl		0	µl
***Sse*8387I** *	10	U/µl	2.5	Units		0.25	µl		0	µl
***Mse*I**	10	U/µl	**2**	Units		0.2	µl		0	µl
Sse-Adapter	5	µM	0.2	µM		0.5	µl		0	µl
Mse-Adapter	50	µM	2	µM		0.5	µl		0	µl
ddH2O						3.225	µl		0	µl
Genomic DNA	50	ng/µl	250	ng		5	µl		----	µl
			Checksumme			12.5			0	µl

* for genomic AFLP we mostly use Sse8387I, one may also use PstI or EcoRI, however one has to use the appropriate adapters then.

- Incubate for 2-3h at 37°C and then over night at room temperature

- Dilute with ddH2O four fold (add 37.5 µl H2O to 12.5 µl RL mix)

- For control, 5ml of the digestion can be loaded on a 2% agarose gel. A smear should appear at the range between 100 to 800 bp.

3. Pre-selective PCR Amplification: described in Table 44.

Table 44: Pre-selective PCR Amplification.

	[Stock]		[Final]		per one	12	µl RXN	x		RXN
PCR buffer incl 15 mM MgCl2	10	X	1	X		1.2	µl			µl
MgCl2	50	mM	**1.5**	mM		---	µl		---	µl
dNTP-Mix (each)	2	mM (each)	0.2	mM		1.2	µl			µl
Pre_Sse primer	10	µM	0.3	µM		0.36	µl			µl
Pre_Mse primer	10	µM	0.3	µM		0.36	µl			µl
Taq polymerase	5	U/µl	0.05	Units/µl		0.12	µl			µl
ddH2O						4.26	µl			µl
R/L DNA template			4.5	µl		**4.5**	**µl**		---	µl
			Checksumme			12	µl			µl

Amplify using the following program

2 min 720C

20 cycles:

30 sec 940C

60 sec 600C

2 min 720C

hold at 4°C

NOTE: All Temperature ramps must be 10C per second.

- For checking 5µl of the PCR product can be loaded on a 2% agarose gel. A smear should appear 100-800 bp range. Otherwise aliquot in 2 times 5µl and add 195 µl ddH2O to each sample.
- Label carefully and store at -20°C. Use this dilution as template for selective PCR

4. Selective PCR Amplification: described in Table 45.

Table 45: Selective PCR amplification

	[Stock]		[Final]		per one	10	µl RXN	x	RXN
PCR buffer incl 15 mM MgCl2	10	X	1	X		1	µl		µl
MgCl2	50	mM	**1.5**	mM		---	µl	---	µl
dNTP-Mix	2	mM (each)	0.2	mM		1	µl		µl
Sse-NN primer *	10	µM	0.15	µM		0.15	µl		µl
Mse-NN primer	10	µM	0.3	µM		0.3	µl		µl
Taq polymerase	5	U/µl	0.05	Units/µl		0.1	µl		µl
ddH2O						4.45	µl		µl
Preampl. DNA template			3	µl		**3**	**µl**	----	µl
			Checksumme			10	µl	0	µl

* We may use more Sse primer (e.g. 0.2 µM), depending on signal intensity
The Sse NN primer is labeled with a fluorescent dye on the 5' end.
NN stands for 2 ‘selective’ nucleotides

Amplify using the following program:

3 min 940C
10 cycles
    30 sec 940C
    30 sec 630C (touchdown 1°C per cycle to 540C)
    2min bei 720C
23 cycles
    30sec 940C
    30sec 540C
    2 min 720C
hold at 40C

NOTE: all ramps at 1°C per second

6.4. Appendix 4

Separation of DNA-Fragments using the CBS -System and Typhoon scanner

Operating Instruction and Gimmicks by (Matiasch L., Stift G., Alimari A.I. and Herzog K.)– IFA – TULLN 2009.

Gel preparation

- Cleaning and assembling of the glass plates.
- We use mostly 33 x 42 cm glass plates, one plate is 3 mm thick, spacer 0.4 mm.
- Carefully clean the glass plates. To get rid of dried gel-remainders, wash the plates with hot water. If they are rather clean, it is sufficient to spray the inner sides of the plates that will be in direct contact with the gel with aqua dest. and wipe off the water with "Roth" tissue. To improve later the filling with the liquid gel, clean the inner sides with some drops of ethanol abs. Use a plastic pasteur pipette and wipe off with the tissue.
- Clean spacer with aqua dest. and put them on the very left and right sides of the glass plate with the cavity for the sharktooth comb (as this is the broader of the two plates).
- Put the other glass plate on top and fix the two plates with 5 black clamps on each side. Leave a space for a sixth clamp on the down end of the plates. It is best to fix the clamps on the very outer part of the spacers to prevent the liquid gel from spilling.
- Pouring the gel
- Move to the fume hood for pouring the gel.
- Put the assembled glass plates in an angle of about 15° on a styrofoam plate.

In general we use a 7% acrylamide gel. For big gels (e.g. for AFLPs) you have to mix:

Urea+TBE 60 ml

Acrylamide 13 ml

APS 400 µl

TEMED 64 µl

Mix Urea+TBE and acrylamide in first thoroughly with a magnetic stirrer. Add APS and TEMED and be ready for pouring the gel. It polymerizes soon.

Pour the gel beginning at one side of the cavity. Whilst pouring, gently knock on the glass plates to prevent development of bubbles.

- Insert a red spacer at the cavity to get a straight edge, where later the sharktooth comb for loading the samples will be inserted. Fix the red spacer with two more black clamps and also add a sixth clamp on the left and right side of the very down part of the gel.
- Let the gel polymerize for at least half an hour.

- Remove gel particles on the outer parts by washing the glass plates. Dry the plates carefully.

Running-in the gel

- Insert the glass plates into the CBS unit. The plate with the cavity has to be at the side of the buffer reservoir.
- Fix it at both sides with two white spring clamps.
- Tighten the gasket of the upper reservoir.
- Fill both buffer reservoirs with running (1x TBE)-buffer up to the marks.
- Carefully clean the upper edge from all gel particles. These would later prevent loading of the samples. Washing with a 5ml-syringe with running buffer, or using the teeth of an old comb or an old wire for getting out bubbles may be helpful.
- Insert the sharktooth comb. If it efforts much strength to insert it, wait until the temperature of the gel has increased.
- Cover the buffer tanks and connect the other end of the cable to the power supply. Take care to use the correct adapters for the chosen power supply.
- Let the gel run for at least 40 minutes. The temperature of the gel should rise to about 45-50°C. It seems the longer you wait until loading the samples, the nicer the picture. But if a gel is running for a too long time, it will become damaged starting from the down side.

Settings:

2500 V (never go beyond this!)

150 mA (or maximum of the power supply)

100 W

Sample preparation

In general you get nice pictures when adding 5 µl loading buffer to the original PCR.

- Centrifuge mixture for some seconds at 3000 rpm.
- Denature at 95°C for 5 minutes.
- Immediately place on ice for fast cooling. After cooling down and centrifuge again.
- Loading the samples

It is possible to load up to 3 PCR labeled with different dyes.

Possible combinations:

Fluorescein and HEX or

Cy5, Fluorescein and TAMRA or

Cy5 and FAM or

Cy3 and FAM (But very intense FAM-band will be visible as very weak bands when scanning Cy3) or Cy5, Cy3 and FAM (But only if you want to use your data for mapping! When scanning Cy5, intense Cy3 bands will be visible too.)

If you are loading more than one PCR on the same gel, always take care, that the same sample will be at the same position

Depending on the used loading-comb you can load the following amounts:

126-comb: 1.4 – 1.5 µl

94-comb: 2.0 – 2.5 µl

62-comb: 2.0 – 3.0 µl

- Even despite loading from behind, you can always check, whether the needles are at the right places.
- Always clean the gel-loading syringe ("Hamilton") carefully by pipetting running buffer several times.
- After loading the first PCR, run the gel for several minutes until the samples are in the gel. Then no dye of the loading buffer should be seen above the gel.

Before loading the second PCR, wash the above edge of the gel again with running buffer.

Running the gel

Settings:

2500 V (never go beyond this!)

150 mA (or maximum of the power supply)

60 W (when running 1 gel)

80 W (when running 2 gels) Temperature should remain at about 45-50°C. If it is becoming too hot, you have to reduce to 65 W or less to prevent the gel from a smile or even more damage and the glass from breaking.

In general an AFLP-gel is scanned after 2 and 4 hours. After 2 hours, small fragments can be scored. After 4 hours, also the big fragments are well separated, but small fragments have already reached the end of the gel and escaped. Depending on the pattern of the special case, other times may be better.

Scanning

- Switch on computer and Typhoon scanner. It takes some time until the scanner is ready.
- Take out some buffer of the upper reservoir with a big syringe or a 50ml-tube until the level is deep enough not to float out when taking off the gasket and the glass plates.
- Carefully clean the glass plates with aqua dest. Before scanning, the gel should cool down for 10 minutes or you should accelerate cooling by washing with cold water first.

- Control the glass plate of the scanner. If necessary, clean with aqua dest. You may use alcohol too, but never use “70% Ethanol vergällt” (denatured ethanol).
- The thinner glass plate (the front one without the cavity) has to be the down one when scanning. Label your plates with a number and “left” or “right” that also on the scanned picture you can be sure that you did not change the gels or the side.
- Use KAPTON TM tape on the bottom side of the glass plate. This allows a tiny distance between the Scanner and the glass plate and avoids strange ring-patterns in the scan (see Typhoon instruction book for details how to apply these!).

To start the program

- Select the icon “Typhoon Scanner Control v 5.0” on the computer's desktop. With “Template” → “Load” you can select the suitable template (Table 46).
- If you want to change scanning intensity, choose “Setup” and “PMT”.
- Start with “Scan”.
- Settings:

Table 46: Typhoon scanner control.

Fluorochrome	Emission filter	PMT	Laser	Sensitivity	Beam Splitters
Fluorescein	526 SP	1000	Green (532)	Normal	
HEX				Normal	
Cy5	670 BP 30	800	Red (633)	Normal	580
Fluorescein	526 SP	1000	Green (532)	Normal	
TAMRA	580 BP 30	800	Green (532)	Normal	
Cy5	670 BP 30	800	Red (633)	Normal	630
Cy3	580 BP 30	800	Green (532)	Normal	
FAM	520 BP 40	700	Blue (488)	Normal	

The intensity of the bands is not the same for all possible AFLP-primer combinations. As a complete scan for 3 dyes takes about 20 minutes, it can save time, if you check the settings on a small part (about 4 rows) of the plate before scanning the entire one.

If your bands are too weak or too dark, adjust scanning intensity by choosing “Setup” and “PMT”.

7. Tables

Table **Page**

8. Figures

Figaure **Page**