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Microsatellite and AFLP mapping of a *T. diccocum* x *T. durum* population and QTL mapping of Fusarium resistance

Diploma Thesis

Written by

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Abstract

Fusarium head blight (FHB, scab) caused by fungi of the genus *Fusarium*. leads to tremendous losses in yield and quality and the contamination of the grains with mycotoxins. Especially the tetraploid durum wheat (*T. durum*) is highly susceptible to Fusarium head blight. Until now attempts to transfer resistance from hexaploid bread wheat into tetraploid durum wheat were met with limited success only. Close relatives of durum wheat, like wild emmer (*T. dicoccoides*) and cultivated emmer (*T. dicoccum*), appear as promising genetic resources for resistance breeding.

By back-crossing a resistant cultivated emmer line (*T. dicoccum* line 161.1) with an Austrian durum wheat line (DS-131621) a population of 118 BC_1F_4 lines was established. This population was evaluated for FHB resistance in replicated field experiments. The same population was genotyped with molecular markers: 69 SSR (simple sequence repeat, microsatellite) and 386 AFLP (amplified fragment length polymorphism) markers. The marker data were used for the calculation of a dense linkage map. The combined analysis of the marker data and the field data allowed the detection of QTL (quantitative trait loci) for FHB resistance and further plant traits.

The QTL analysis revealed five different QTL for FHB resistance mapping to chromosomes 1A, 3B, 6B, and two loci on 4B. Also QTL for different morphological traits like plant height (4B), date of anthesis (7B, 5A,6B,4A,3B), waxiness (1A, 1B), ear length (4B, 5A, 7A), ear compactness (5A), awn length (4A, 3B, 7A, 5A) and leaf chlorosis (5A,5B) were found.

Zusammenfassung

Ährenfusariose (Fusarium head blight, FHB) ist eine Weizenkrankheit hervorgerufen durch Pilze der Gattung *Fusarium*. Die Krankheit führt zu Verlusten bei Ertrag und Qualität und Verunreinigungen des Erntegutes mit Mykotoxinen. Speziell der tetraploide Durumweizen (*Triticum durum*) ist höchst anfällig für Ährenfusariosebefall. Bemühungen Resistenzgene aus dem hexaploiden Brotweizen in den tetraploiden Durumweizen zu übertragen zeigten bisher nur moderate Erfolge. Eine mögliche Resistenzquelle für Durumweizen stellen dessen verwandte Formen dar, wie zum Beispiel. der wilde Emmerweizen (*T. dicoccoides*) und der kultivierte Emmerweizen (*T. dicoccum*).

Durch Rückreuzung einer resistenten Linie der Art *T. dicoccum* (Linie 161.1) mit einer österreichischen Zuchtlinie der Art *T. durum* (Linie DS-131621) wurde eine Population von 118 BC_1F_4 Linien hergestellt. Diese Linien wurden in wiederholten Feldexperimenten auf

Fusariumresistenz überprüft. Dieselbe Population wurde mit molekularen Markern charakterisiert, und zwar mit 69 SSR (Mikrosatelliten) und 386 AFLP (amplifizierte Fragment-Längen-Polymorphismen) Markern. Mit den Markerdaten konnte eine ausreichend genaue Kopplungskarte berechnet werden. Die gemeinsame Analyse der Markerdaten mit den Resistenzdaten erlaubte die Kartierung von QTL (quantitative trait loci) für die Eigenschaft Ährenfusarioseresistenz sowie weiterer in der Population erhobener Pflanzenmerkmale.
Die QTL Analyse für Ährenfusarioseresistenz detektierte fünf QTL, und zwar auf den Chromsomen 1A, 3B, 6B, und zwei QTLs auf Chromosom 4B. Zudem wurden weitere QTL für morphologischen Eigenschaften wie Pflanzenhöhe (4B), Blühzeitpunkt (7B, 5A, 6B, 4A, 3B), Wachseinlagerung (1A, 1B), Ährenlänge (4B, 5A, 7A), Ährenkompaktheit (5A), Grannenlänge (4A, 3B, 7A, 5A) und Gelbblättrigkeit (5A, 5B) gefunden.

List of Abbreviations

PCR	polymerase chain reaction
DNA	desoxyribonucleicacid
DNase	desoxyribonuclease
dNTPs	deoxynucleoside 5'-triphosphates
RNA	ribonucleic acid
RNase	ribonuclease
fig.	Figure
AS	amino acid
bp	base pair
kb	kilobases
°C	degree Celsius
h	hour
min	minute
rpm	round per minute
ng	nanogram
mg	milligram
µg	microgram
Ta	annealing temperature
Tm	melting temperature
ca.	circa
ml	milliliter
RT	room temperture
SSR	simple sequence repeats
AFLP	amplified fragment length polymorphism
ph	ph - Value
mA	milli Amperes
ml	milliliter
µl	microliter
OD	optical density
ZON	Zearalenone
DON	deoxynivalenol
NIV	nivalenole
LOD	logarithm of odds

MAS	marker assisted selection
QTL	quantitative trait locus
SIM	simple interval mapping
CIM	Composite interval mapping

1. Introduction

Fusarium head blight (FHB) or scab is an infection of the wheat florets caused by the fungus *Fusarium sp*. This infection is one of the most destructive diseases in wheat (*Triticum sp*.) and leads to a significant loss of yield and quality (Qu et al. 2008). The yield and the quality losses are caused by mycotoxins and a shriveling of the seeds which generates a low thousand kernel weight.

By producing mycotoxins like DON and ZON infected seeds got a higher contamination and therefore they got a higher loss of quality which leads to a restriction of the processing of the seeds to products like food and fodder. Mycotoxins in higher concentration lead to vomiting and feed refusal in animals (DON) (Hollinger and Ekperigin, 1999) and to damages in fertility of animals (ZON) (D'Mello et al. 1999; Peraica et al. 1999).

F. graminearum, *F. avenaceum* and *F. culmorum* are the most devastating pathogens in cereals and infest mainly wheat, durum, oats, rye, barley, corn and other grass species. Dicots such as beans, potatoes etc., can also serve as a secondary host. The disease is prevalent all over the world. Although the disease is known since decades it still causes eminent damages.

Especially small farmers around the world are struggling to manage Fusarium head blight.

The most promising strategy to overcome the disease is to produce resistant cultivars against FHB.

For the tetraploid durum wheat (*Triticum durum* ; AABB), resistance breeding is quite difficult because of the lack of sufficient resistances sources in tetraploid wheat and until now, one hasn´t been successful to transfer resistance sources from hexaploid wheat (*T. aestivum* L. ;AABBDD) to tetraploid wheat. Although there hasn´t been found an effective resistance to FHB in durum wheat yet, tetraploid relatives (*T. dicoccoides*) describe an important gene pool for breeding and a potential source of FHB resistance (Miller et al. 1998; Bürstmayr et al. 2003; Kumar et al. 2007).

The resistance against FHB is a complex trait which is affected by several genes with additive effects (Kolb et al. 2001) also there are heavy environmental genotype interactions which make the breeding for this trait more difficult (Schmolke et al. 2005).

For FHB it is known that mostly a few major genes and minor genes are controlling this trait (Liu et al. 2005) and it is known that it is a quantitative and polygenic trait.

For this the genetic mapping for quantitative trait loci (QTL) became the method of choice to distinguish the localization of the QTL on the chromosomes.

By back crossing a resistant cultivated emmer (*T. dicoccoum*) *T. dicoccum 161* and a susceptible Austrian durum line *DS-131621* a new resistance against FHB could be inserted.

Analyzing the progenies in the BC_1F_4 generation by molecular markers could help to find resistance against FHB in new cultivars.

1.1 Wheat

Based on the FAO database (http://faostat.fao.org) wheat (*Triticum aestivum*, 2x = 6n = 42; AABBDD) is the most important cultivated plant worldwide (Feldman et al. 1995) for food, followed by maize and rice.

The earliest proof of *Triticum dicoccoides* (wild emmer; 2x = 4n = 28; AABB) and *Triticum monococcum* (einkorn ; 2x=4n=14; AA) wheat comes 19,000 years ago from Ohalo II a region near the Sea of Galilee in Israel, and is assumed to be the origin of wheat. At this time it is also known that the first settlements were established and the domestication of wheat started in this time (Kislev et al. 1992).

By continuous selection and breeding of this two wheat species, *Triticum monococcum* and *Triticum dicoccoides*, the today's known wheat (*Triticum aestivum)* derived.

At the same time another wheat species *Triticum durum* (2n = 4x = 28; AABB) derived independently from wild emmer (*T. dicoccoides*) and *T. urartu* (Bonjean and Angus 2001). Those two wheats *Triticum aestivum* and *Triticum durum* are now the most important cereals in the world.

Table 1: Acreage and production of wheat (*Triticum aestivum*) worldwide and in Austria (Source: http://faostat.fao.org)

Crop	Country	Area harvested (ha)	Yield (kg ha^{-1})	Production (t)
Cereals	Austria	845 036	6812,3	5 756 643
	World	713 443 557	3539,3	2 525 106 874
Wheat	Austria	296 775	5693,4	1 689 688
	World	223 564 097	3086,1	689 945 712

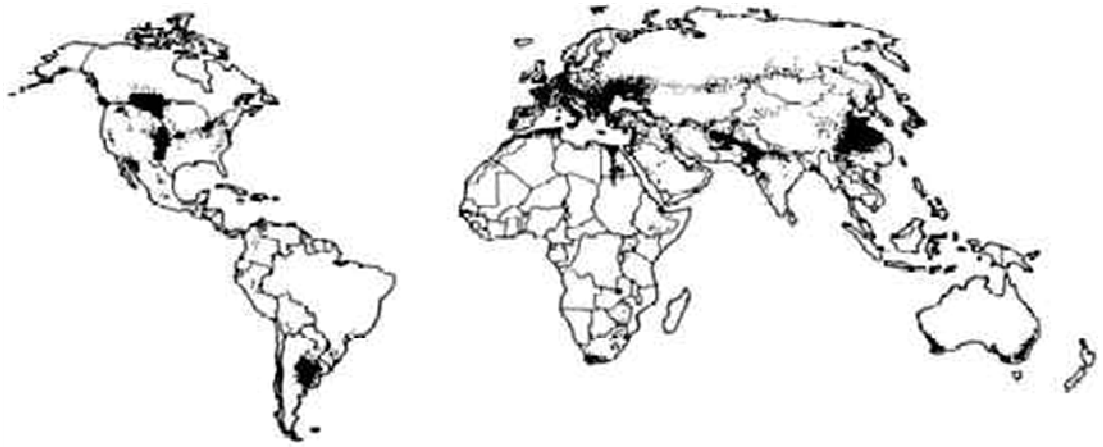


Figure 1. Major areas of wheat production around the world (Source: Goode´s World Atlas, 1975; updated by Cimmyt, 1984)

1.2 Durum wheat

As described above durum wheat (*T.durum*) is a plant belonging to the *Gramineae* family and is a result of crosses of wild emmer (*T. dicoccoides*) and *T. urartu* (Bonjean and Angus 2001). Durum wheat is mainly grown in regions with dry climate, with hot days and cool nights during the growing season, typical for Mediterranean and temperate climates.

The germination of the seed occurs as low as 2°C, but the optimal temperature is around 15°C (Bozzini et al. 1988).

The main cultivation areas are North Africa, Mediterranean Europe, the North American Great Plains and the Middle East (Cantrell et al. 1987) (Table 3 and 4).

The highest durum wheat production in the world is obtained in Canada, followed by Italy, Turkey and Syria (Table 4).

Physiologically durum wheat exists mainly as spring durum, which is sowed between January and April, but also it is partly cultivated as winter durum which is sowed in autumn mainly in mid-October (Kübler 1994).

Triticum durum is frost sensitive, thermophilic and most of them are drought resistant. Especially in dry years winter durum has a higher yield than summer durum (around 20%), because it can use the humidity of the soil more efficiently than summer durum (Figure 2).

Table 2: Most common spring and winter durum cultivars in Austria (Source: Österreichische Beschreibende Sortenliste; http://www.ages.at/ages/landwirtschaftliche-sachgebiete/sorte/bsl/getreide/durumweizen-hartweizen/).

Growth type	Cultivar name	Registration date	Breeding company
Spring	Ambrodur	20.12.2000	Probstdorf Saatzucht
	Helidur	16.12.1993	Probstdorf Saatzucht
	Floradur	18.12.2003	Probstdorf Saatzucht
	Calladur	21.12.2006	Probstdorf Saatzucht
	Rosadur	22.12.2004	Probstdorf Saatzucht
	Duramar	20.12.2000	Südwestdeutsche Saatzucht
	Durobonus	22.12.2004	Saatbau Linz
	Duroflavus	27.12.2007	Saatbau Linz
	Duroprimus	18.12.2003	Saatbau Linz
Winter	Auradur	22.12.2004	Probstdorf Saatzucht
	Coradur	21.12.2006	Probstdorf Saatzucht
	Inverdur	20.12.2002	Probstdorf Saatzucht
	Logidur	19.12.2008	Probstdorf Saatzucht
	Lunadur	21.12.2006	Saatbau Linz
	Prowidur	20.12.2000	Probstdorf Saatzucht
	Superdur	20.12.2000	Probstdorf Saatzucht
	Windur	22.12.2004	Saatbau Linz

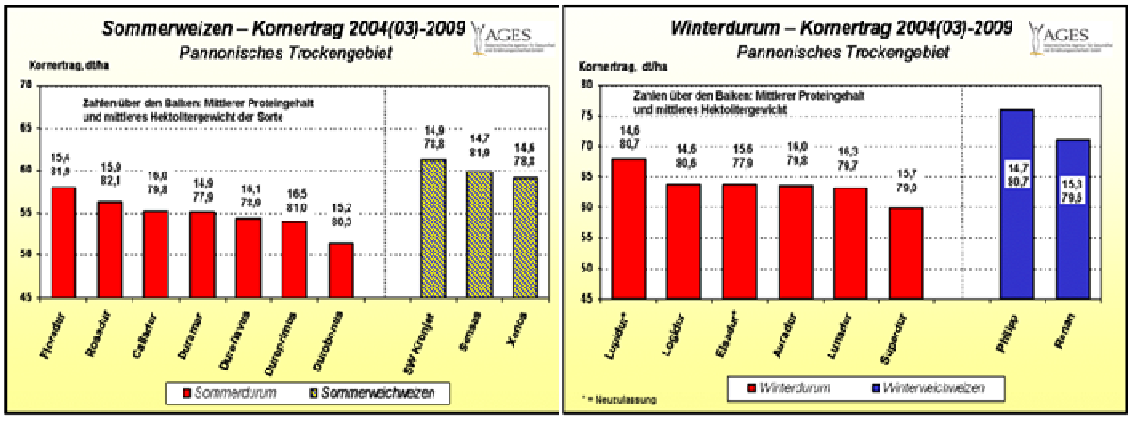


Figure 2: Comparison of the yields of spring and winter durum in Austria

Source: Bundesamt für Ernährungssicherheit (http://www.baes.gv.at/pflanzensorten/oesterreichische-beschreibende-sortenliste/getreide/durumweizen-hartweizen/)

Table 3: Durum wheat Area worldwide in thousand hectares (http://www.fas.usda.gov/)

Durum Wheat Area
(Thousand hectares)

	1992/93	1993/94	1994/95	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03	2003/04
Total	14975	13902	14582	14716	16112	14599	16091	13204	13774	13480	14847	14452
United States	991	850	1099	1358	1439	1286	1509	1444	1446	1129	1094	1161
Foreign	13984	13052	13483	13358	14673	13313	14582	11760	12328	12351	13753	13291
Algeria	1200	1000	683	1175	1585	590	1600	889	544	1112	880	1400
Argentina	41	34	42	55	83	81	73	70	68	48	45	46
Canada	1459	1441	2266	2125	2064	2212	2914	1769	2614	2036	2246	2430
France	426	222	235	229	270	264	296	327	338	306	335	350
Germany	16	10	11	8	8	7	7	12	9	5	5	4
Greece	674	450	480	450	450	470	550	425	450	450	670	400
Italy	1531	1410	1527	1623	1628	1665	1607	1691	1663	1664	1733	1680
Portugal	26	17	21	26	26	27	27	73	139	134	185	150
Spain	630	623	610	450	648	644	625	500	868	883	925	900
United Kingdom	2	2	2	2	1	1	1	1	1	1	1	1
European Union	3305	2734	2886	2788	3042	3090	3130	3049	3484	3455	3867	3500
Morocco	1088	1134	1336	800	1250	972	1127	1078	1079	977	882	1100
Syria	750	1000	1100	1100	1200	1300	1300	800	900	1300	1100	1200
Tunisia	835	780	400	560	900	600	600	670	600	630	600	700
Turkey	810	720	750	800	900	1100	1100	1100	1100	900	1100	1100
Russia	2000	2000	2000	2000	1800	1600	1400	1200	1000	1000	2100	1000
Kazakstan	1500	1200	1000	900	800	700	500	300	100	50	100	50
Australia	36	49	40	55	80	100	175	175	175	175	116	200
India	730	730	750	770	750	750	450	450	450	450	500	350
Mexico	230	230	230	230	230	230	230	230	230	230	230	230

37895 Production Estimates and Crop Assessment Division, FAS, USDA

Table 4: Global durum wheat production in 1,000 metric tons (http://www.fas.usda.gov/)

GLOBAL DURUM WHEAT PRODUCTION (1,000 Metric Tons)

	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Change in Production MMT	Change in Production Percent
Algeria	560	1190	2035	460	1500	900	486	1222	951	1809	1816	1000	**-816**	**-44,93**
Argentina	100	97	193	287	158	176	187	135	98	147	180	160	**-20**	**-11,11**
Australia	55	200	260	280	400	400	400	400	100	450	400	400	**0**	**0,00**
Austria	38	38	42	50	66	98	43	46	49	60	60	60	**0**	**0,00**
Canada	4635	4648	4627	4352	6042	4341	5709	2987	3877	4280	4962	4750	**-212**	**-4,27**
France	1044	1037	1255	851	1545	1541	1676	1338	1614	1428	2050	1650	**-400**	**-19,51**
Germany	58	37	47	34	60	65	43	24	26	20	50	46	**-4**	**-8,00**
Greece	1100	920	650	1000	1230	850	900	810	1005	700	1000	1000	**0**	**0,00**
India	1700	1900	1800	1800	1000	1000	1000	1200	1400	800	1200	1200	**0**	**0,00**
Italy	3900	3800	4000	3600	4500	4100	4310	3500	4400	3730	5700	3500	**-2200**	**-38,60**
Kazakhstan	700	500	500	500	300	400	100	50	100	100	100	100	**0**	**0,00**
Mexico	1100	1100	1100	1100	1100	1100	1100	1100	1100	1200	1200	1200	**0**	**0,00**
Morocco	2342	500	2270	882	1544	800	427	1039	1032	1766	2025	750	**-1275**	**-62,96**
Portugal	43	26	41	44	28	90	173	103	348	155	165	70	**-95**	**-57,58**
Russia	1800	1000	1300	2000	500	1000	1000	1300	1500	1200	1000	1200	**200**	**20,00**
Spain	963	300	1550	1114	1290	450	1917	1756	2073	2249	2825	1000	**-1825**	**-64,60**
Syria	1950	2350	2450	1900	2600	1000	1100	2400	2300	2300	2100	2100	**0**	**0,00**
Tunisia	440	470	1620	800	1100	1140	1100	935	370	1300	1400	1150	**-250**	**-17,86**
Turkey	1075	1300	1500	2200	2400	1600	2000	1600	2300	2300	2400	2300	**-100**	**-4,17**
UK	10	10	5	5	5	5	5	6	6	5	6	6	**0**	**0,00**
USA	2630	2780	3160	2390	3760	2700	2990	2270	2180	2630	2450	2560	**110**	**4,49**
EU	7156	6168	7590	6698	8724	7199	9067	7583	9521	8347	11856	7332	**-4524**	**-38,16**
World	26243	24203	30405	25649	31128	23756	26666	24221	26829	28629	33089	26202	**-6887**	**-20,81**

1.3 Products and quality of durum wheat

Durum wheat is mainly used for high-quality spaghetti and other pasta products but also for a special kind of durum bread.
In North African countries, such as Egypt, Libya, Tunisia, Algeria and Morocco couscous , bulgur , frekeh (Williams et al. 1985; Williams and El-Haramein, 1985) are the preferred products made of durum. These products are obtained when the hard, glassy kernels are grinded to flour and mixed with water.
In Italy the home country of pasta products the products are categorized in four main categories: (Dick and Matsuo, 1988)

- long goods which are products like spaghetti, vermicelli and linguine
- short goods like elbow macaroni, rigatoni and ziti
- egg noodles which is pasta made with eggs
- special items like lasagna, manicotti, jumbo shells and stuffed pasta

The unique characteristics like the glassy hard golden kernels, the protein content and gluten strength turns it into an economically important crop and those characteristics affecting directly the processing and culinary properties of pasta and the crumb and keeping the properties of bread (Liu et al. 1996; Marchylo et al. 1998).
For the quality of good food products made with durum wheat several quality traits play an important role

- hectolitre weight (defined as the density for 100 l of wheat in kg)
- protein content
- gluten strength
- falling number
- vitreousness (important fort the amount of semolina)
- yellow endosperm (indicator for the amount of carotenoids which gives the dough a special yellow color)

1.4 Fusarium head blight

One of the most concerning problems in wheat production is the infestation with Fusarium head blight (FHB) a fungal disease which causes important ear diseases in many wheat crops worldwide.

Especially durum wheat is heavily affected by this fungus. But also other wheat species like bread wheat, barley, oats and other plant species are host plants and are affected by this fungus.

The most important pathogens of head blight in wheat are *Fusarium graminearum, Fusarium culmorum*, and *Fusarium avenaceum.* In warmer climate regions predominantly *Fusarium graminearum*, in colder regions *Fusarium culmorum* is the most important pathogen (Parry et al.1995; Mc Mullen et al. 1997; Šrobárová1 et al. 2008). Worldwide FHB is considered to be the most important wheat disease at all.

The fungus is a natural soil fungus and is beneath other microorganisms responsible for the degradation of plant residues. The fungus can survive on dead as also on living material.

The fungus overwinters on plant residues in the soil, chaff or infected grain but can also outlast on non-host plants in a saprophytic way (*Fusarium culmorum*) (Nelson et al. 1994).

There are two ways how the inoculums can take place.

In the asexual (vegetative) way *Fusarium* produces conidia, chlamydospores or hyphal fragments by cell division as inoculums, or in the sexual (generative) way where *Fusarium graminearum* (*Gibberella zeae*) produces ascospores (Guenther et al. 2005). Those are mainly produced on the stem or on plant debris on the field. The infection can also take place at the root system or at the nodalities.

By humid weather the spores are spread by rain droplets or wind and access in this way onto the plants where they start the infection. Rainfall during the flowering time increases the amount of infection rapidly and also in flowering time plants are more susceptible to infections than in more earlier or later time points.

When *Fusarium* reaches the ears of the wheat and infects the flowers it causes Fusarium head blight (Parry et al. 1995) (Figure 3).

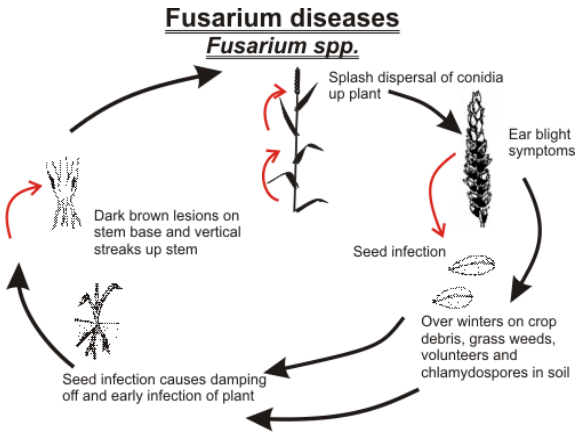


Figure 3: Life cycle of *Fusarium* sp.
Source: http://www.hgca.com/hgca/wde/diseases/Foliarfus/fusLifeCyc.html

Especially under humid and hot conditions the fungus of *Fusarium* has a large distribution. From the bottom it reaches fast the spikelet and infects the whole ear. There it causes white, green and pink heads which is a sign for FHB. When infecting the stem, the stem tissue appears in a brown and purple way (McMullen and Stack, 1999).The infection with FHB can lead to an immense loss of yield sometimes up to 80% (Bottalico et al. 2002) and this causes also a loss of money for farmers and means that million hectares are infected with the *Fusarium* fungus because it can remain a long time in the soil. But another serious problem besides the loss of yield and grain quality is that the fungus produces during the infection mycotoxins which are very dangerous for human and animal health.
Two of the most significant mycotoxins which are produced by this *Fusarium* are deoxynivalenol (DON) and zearalenone (ZON). DON, also known as vomitoxin, belongs to the trichothecenes which causes a weakening of the immune system in humans and animals when they are feed with contaminated grain. This can happen even with mycotoxins in low concentration. Animals will get more susceptible to any kind of diseases and will lose a lot of their power. This results in a skin irritation, denial of food, vomiting and in serious health problems sometimes till death (Hollinger and Ekperigin, 1999).
ZON is an estrogenic metabolite and thereby has a great impact on fertileness on animals. For female animals it can lead to infertility, for male animals it can lead to a less quality of sperms and in high concentration to malformed testicles (D'Mello et al. 1999; Peraica et al. 1999).

And because the appearance of the mycotoxins are widely spread the Scientific Committee on Food of the European Union decreed to establish a maximum value for mycotoxins in cereals, raw cereals, products made of cereals and also for cereal based food for babies and infants (Gallo et al. 2008) (Table 5).

Table 5: Maximum levels (µg/kg) for deoxynivalenol (DON) and zearalenone (ZON) in foodstuff (Source: COMMISSION REGULATION (EC) No1881/2006 of 19 December 2006; http://eur-ex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20090701:EN:PDF)

Deoxynivalenol (17)	
Unprocessed cereals (18) (19) other than durum wheat, oats and maize	1 250
Unprocessed durum wheat and oats (18) (19)	1 750
Unprocessed maize (18), with the exception of unprocessed maize intended to be processed by wet milling (37)	1 750 (20)
Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption, with the exception of foodstuffs listed in 2.4.7, 2.4.8 and 2.4.9	750
Pasta (dry) (22)	750
Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	500
Processed cereal-based foods and baby foods for infants and young children (3) (7)	200
Milling fractions of maize with particle size > 500 micron falling within CN code 1103 13 or 1103 20 40 and other maize milling products with particle size > 500 micron not used for direct human consumption falling within CN code 1904 10 10	750 (20)
Milling fractions of maize with particle size ≤ 500 micron falling within CN code 1102 20 and other maize milling products with particle size ≤ 500 micron not used for direct human consumption falling within CN code 1904 10 10	1 250 (20)

Zearalenone [17]	
Unprocessed cereals [18] [19] other than maize	100
Unprocessed maize [18] with the exception of unprocessed maize intended to be processed by wet milling [37]	350 [20]
Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption, with the exception of foodstuffs listed in 2.5.6, 2.5.7, 2.5.8, 2.5.9 and 2.5.10	75
Refined maize oil	400 [20]
Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize-snacks and maize-based breakfast cereals	50
Maize intended for direct human consumption, maize-based snacks and maize-based breakfast cereals	100 [20]
Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children [3] [7]	20
Processed maize-based foods for infants and young children [3] [7]	20 [20]
Milling fractions of maize with particle size > 500 micron falling within CN code 1103 13 or 1103 20 40 and other maize milling products with particle size > 500 micron not used for direct human consumption falling within CN code 1904 10 10	200 [20]

To avoid (high) infection with FHB there are several ways to act (Parry et al. 1995):

- use of fungicides, mainly triazoles, at the flowering time
- choice of a good preceding crop
    - the amount of DON can be reduced by choosing a good preceding crop
    - Maize, rice, soya, wheat are bad preceding crop because *Fusarium* can survive on the debris and can infect the plant in the following growing period
- soil cultivation
    - till plant debris into the soil by plowing to reduce the chance of infections in the next growing period
- choice of geographic location
    - hot and dry climates
- choice of tolerant/resistant plant material

1.5. Genetic resources and components of resistances

Genetic resistances in durum wheat are the most desirable breeding goals against Fusarium head blight (Miedaner et al. 1997; Mesterhazy et al. 1997). Especially because durum wheat is heavily affected by *Fusarium*. But the breeding for resistance is difficult because genetic resistances in tetraploid wheat are hard to find and sources of resistances from hexaploid wheat haven't been transferred successfully (Stack et al. 2002). Wild emmer (*T. dicoccoides*), cultivated emmer (*T. dicoccum*), einkorn (*T. monococcum*) and *T. turanicum* are the most promising tetraploid candidates for resistance breeding. Although a lot of research was done on crossing wild emmer and durum plants which increase the level of FHB resistance (Miller et al. 1998; Buerstmayr et al. 2003; Oliver et al. 2005, 2007, 2008)

The resistance against *Fusarium* is a non- race specific resistance and was described as a horizontal resistance (Snijders and Van Eeuwijk, 1991) and has great diversity within a population.

In any case the resistances of wheat against *Fusarium* are classified into five different types. The type I resistance described the resistance to initial infection, type II prevents the spread of the infection, type III prevents kernel infection, type IV tolerance during the infection, that means yield maintains the same during infection and type V is the capability of the plant to degrade DON (Lemmens et al. 2005, Mesterhazy et al. 2002, Mesterhazy et al. 1995)

1.6 Quantitative trait loci (QTL)

Most of the resistance successes in genetics are based on the detection of so called "major-genes". Major genes are genes which play an important role in one specific trait. If this major gene mutates the effect can directly be seen in the phenotype.

In case of resistances it means that e.g. a pathogen can harm the plant only if this major gene is absent or when the pathogen develops a strategy, mainly a mutation, to overcome the barrier of the major gene. The trait is also called qualitative trait and the distribution here is discontinuous. The inheritance of the major gene is called monogenic.

In the case of yield potential, quality, ear length and other quantitative traits and disease resistance not only one gene is involved but multiple genes.

Those genes are acting additive on a trait e.g. a resistance and therefore make this resistance more powerful but also more difficult to understand and to explore (Falconer et al. 1989).

For FHB it is known that mostly a few major genes and minor genes are controlling this traits (Liu et al. 2005).
QTL is the abbreviation of "quantitative trait loci" and this QTL-analysis is the method of choice to detect the genes which characterize together one specific trait.
QTL are special regions on a chromosome which are directly linked to a phenotypic trait. Quantitative means in this way that the trait has a continuous variation in a population which leads to a Gaussian distribution over the population. The inheritance of those quantitative traits is called polygenic (Tanksley et al. 1993).
Also the influence of the environment has a more or less influence on the continuous variation and makes the locating of the QTL more difficult.
Those quantitative trait loci are linked on one chromosome. That means that during meiosis chromosomes were rearranged by exchanging genetic material between the chromosomes. Although on both strands, breakings are generated which are connected again via crossing over (chiasmata) and thereby recombine.
By this crossing over genes which are close to each other on the chromosome do not assort independently and they are called to be linked. And so they will be inherited together from the parent to the progeny more frequently than genes which are not close together on one chromosome (Semagn et al. 2006). If in one case two genes were separated one time in one hundred meiosis (pl.), they have a distance of 1centiMorgan (cm).By the use of molecular markers QTL can be mapped into groups.
The bigger one population of analyzed plants are and the more molecular markers are used, the better and more detailed is the QTL map you are receiving (Vales et al. 2005; Beavis et al. 1998).

1.7 Molecular markers and marker assisted selection (MAS)

For hundreds of year's practical plant breeding by selection led to a high improvement of species. But breeding for quantitative traits is still difficult because they are affected by multiple genes and also by the environment.
Nowadays there is a tool to overcome these problems in the form of DNA markers.
DNA markers are used in plant breeding to fasten the process of identifying desired traits and thereby fasten the way of selection (MAS) (Ribaut et al. 1997; Van Berloo et al. 1998)

With molecular markers it is possible to follow genes in the breeding process although the sequences of the genes are not known. The only condition is that one knows which marker is linked to which trait.
This can be achieved by doing researches in breeding and the analysis of the progeny.
If plants with a certain trait always have the same allele of a certain marker, the gene will be close to the region of the marker.
So the marker is used as an orientation to localize certain traits in the genome, because the marker is arranged always on the same place in the genome.
This data of so called linkage groups are used for gene mapping. Especially in resistance breeding the use of molecular markers are wide spread.
The advantages of these DNA markers are that they are independent towards the environment and towards the phenotype. When DNA markers are available in high density it is possible to show most of the genome and so the estimation of the distance between different genotypes is more detailed and more accurate.
In this case the calculation of the genetic distance is shown in the binary code with a 1 for present and a 0 for absent.
Beneath the localization of monogenic traits DNA markers are also used to identify quantitative trait loci (QTL).
The most common used markers nowadays are RFLP (restriction fragment length polymorphism), PCR markers like SSR (Simple Sequence Repeats), RAPD (Random Amplified Polymorphic DNA), and AFLP (Amplified Fragment Length Polymorphism), SNP (single-nucleotide polymorphism) and DART (Diversity Arrays Technology) (Kassa et al. 2006).

For the appliance of molecular markers a few points have to be considered (Joshi et al. 1999).

- Highly polymorphic nature
- Codominant inheritance (determination of homozygous and heterozygous states of diploid organisms)
- Frequent occurrence in genome
- Neutral behaviour (should not be influenced by environmental conditions)
- Fast and simple appliance
- High reproducibility
- Low costs

In the case of *Fusarium* it is known from genetic studies that 2 up to 5 major QTL and an undefined number of modifying genes are involved in the resistance.
To investigate which genes are involved and where on the chromosome they are localized we analyzed during this diploma thesis 118 plants with 62 SSR markers and 386 AFLP markers which are described below (1.7.1; 1.7.2).

1.7.1 Simple sequence repeats markers (SSR) or microsatellites

Microsatellites (Tautz and Renz. 1984; Tautz et al. 1989) also called simple sequence repeat (SSR) are at this time the most frequently used markers of choice in plant genetics.
Simple sequence repeats indicates sequence repeats of 2-6 base pairs (bp) which was discovered by Condit and Hubbel in 1991. Most of the sequence repeats are AC and GA but also AAG, AAT, AATT, and AAAT (Gupta und Varshney, 2000). In wheat base pair combinations of AT and AG are most common (Ma et al. 1996).
SSR markers are visualized by PCR (*polymerase chain reaction*). By using known flanks as reverse and forward primers they amplify the repeating sequence between the primers.
By gel electrophoresis the different lengths of the amplified fragment can be detected by laser detectors. Most of the flanking regions are conserved and so those markers can be used for different species (Matsuoka et al. 2002).
The advantages of microsatellites are that they have a high level of polymorphism and so contain a lot of information, they are co-dominant, have a high reproducibility (Powell et al. 1996).
Because the number of the repeating bp is different between individuals those markers can be used to differ between individuals in a population or to distinguish the origin of an individual (Plaschke et al. 1995).

1.7.2 Amplified fragment length polymorphism markers (AFLP)

The AFLP (Amplified Fragment Length Polymorphism) (Vos et al. 1995) technique is a method, where you can generate specific fingerprints without knowing the sequence. This can be obtained with only little amount of DNA and with a high reproducibility.
Nowadays it is one of the most wide spread marker techniques in plant genetics.
AFLP is performed in a multilevel procedure. First the genomic DNA is digested with two appropriate restriction enzymes. One of them is a frequent cutter and the other is a rare cutter. By that you obtain fragments with two sticky ends.
Afterwards so called adapters are ligated at the end of the fragments (Vos et al.1995). This is made by adapter specific primers, which elongate the 3' end with selective nucleotides by PCR. The primer sequence is hereby obtained by the sequence of the restriction sites and the adapters.
You can vary the length of the selective nucleotides normally up to 2-4 bases.
During the PCR only a subset of all fragments are amplified, those which also contain the selective nucleotides (Figure 4). The amount of selective nucleotides increases the selectivity.
It was shown that the AFLP marker is one of the most efficient markers (Powell et al. 1996) and it showed that 8 times more polymorphisms were detected than compared with the RFLP method (Mackill et al. 1996).
The advantages of the AFLP method is to receive a lot of potential polymorphic fragments per PCR reaction. By choosing different bases for the selective PCR you got a lot of different primers and you don't need any knowledge about the sequence.
The only negative aspect is that the evaluation of the gel electrophoresis is much more complicated.

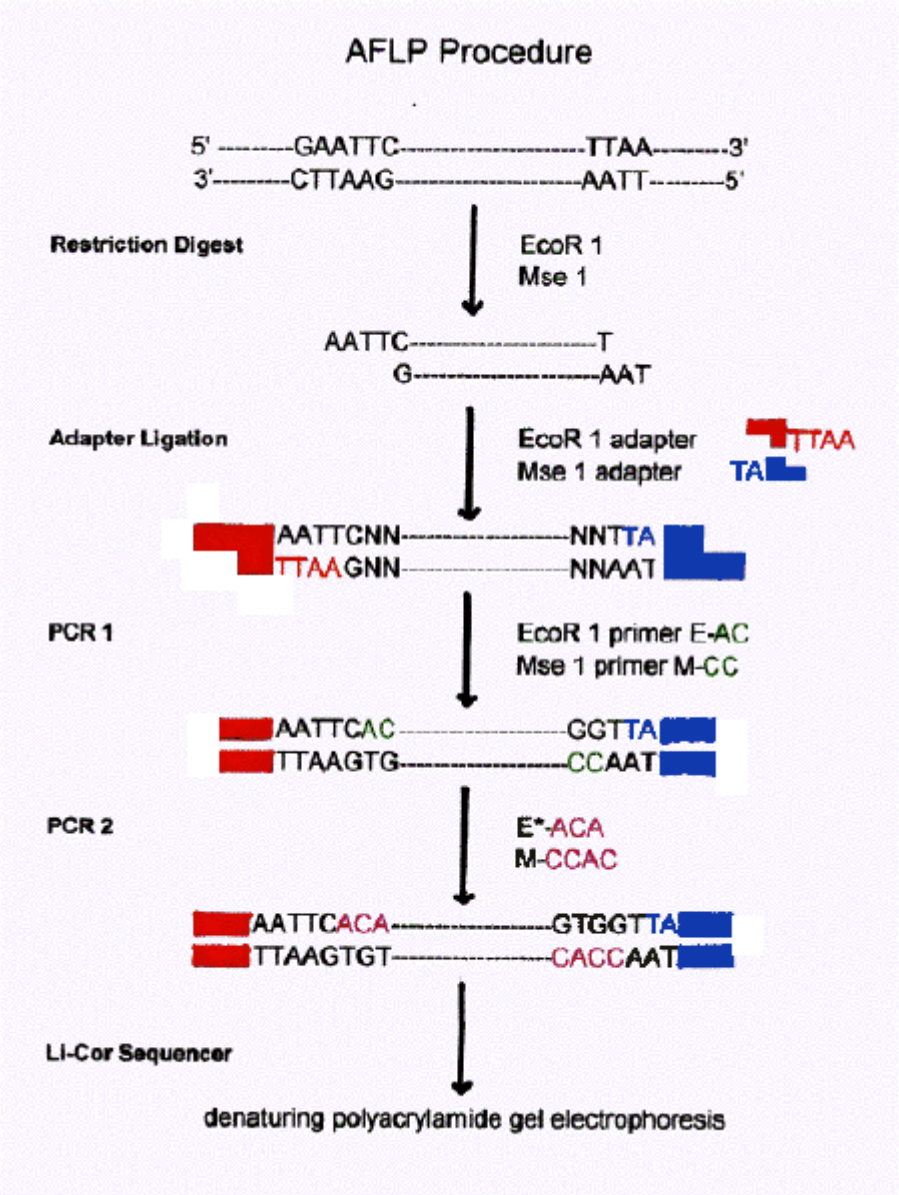


Figure 4: schematic AFLP procedure

Source: http://sorrel.humboldt.edu/~jlg21/AFLP/AFLP.GIF

2. Material and methods

2.1 List of chemicals

Chemical Product	Manufacturer
Sse8387I	Amersham-Pharmacia, Braunschweig (Germany)
ATP (adenosintriphosphate)	Amersham-Pharmacia, Braunschweig (Germany)
BSA (bovine serum albumin)	Amersham-Pharmacia, Braunschweig (Germany)
Long Ranger 50% Gel Solution	Cambrex Bio Science Rockland (NJ, USA)
Urea	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe (Germany)
Tris	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe (Germany)
Boric Acid	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe (Germany)
Ethylenediaminetetraacetic acid (EDTA)	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe (Germany)
Ammoniumperoxodisulphate (APS)	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe (Germany)
Ethanol 70 %	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe (Germany)
Sodium Acetate (NaOAc)	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe (Germany)
Ammonium Acetate (NH4OAc)	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe (Germany)
dNTP(deoxynucleoside 5'-triphosphates)	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe (Germany)
Ethidiumbromide	Carl-Roth GmbH+Co.Kg 76185 Karlsruhe

	(Germany)
Taq-polymerase	Eurogentec S.A.,Seraing (Belgium)
10x PCR buffer	Eurogentec S.A.,Seraing (Belgium)
$MgCl_2$ (magnesiumchlorid)	Eurogentec S.A.,Seraing (Belgium)
Ethanol	Merck KGaA 64293 Darmstadt (Germany)
Fuchsin	Merck KGaA 64293 Darmstadt (Germany)
NaCl (Sodium chloride)	Merck KGaA 64293 Darmstadt (Germany)
IAA (chloroform/isoamylalcohol)	Merck KGaA 64293 Darmstadt (Germany)
isopropanol (2-propanol)	Merck KGaA 64293 Darmstadt (Germany)
IAA (chloroform/isoamylalcohol (24:1))	Merck KGaA 64293 Darmstadt (Germany)
Agarose	Merck KGaA 64293 Darmstadt (Germany)
MseI	New England Biolabs, Ipswich,(England)
T4-DNA Ligase	New England Biolabs, Ipswich,(England)
Formamide	Sigma-Aldrich Handels Gmbh (St. Louis,USA)
dimethyl sulphoxide (DMSO)	Sigma-Aldrich Handels Gmbh (St. Louis,USA)
N,N,N',N'-Tetramethylethylenediamine (TEMED)	Sigma-Aldrich Handels Gmbh (St. Louis,USA)
NaOH	Sigma-Aldrich Handels Gmbh (St. Louis,USA)

2.2. Buffers and solutions

Urea 8M (Typhoon)

240,42g	Urea
59ml	10x TBE
add 500ml	dest. H20

Urea for LI-COR 7%

210g	Urea
50ml	10x TBE
add 420ml	dest. H20

10x TBE

162g	Tris
27,5g	boric acid
9,3g	EDTA
add 1l	dest. H20

Loading buffer

950µl	formamide
50µl	EDTA
3-5µl	fuchsin

10% APS

1 g Ammoniumpersulfat in 10 ml dH2O

agarose gel 2%

2g	agarose
100ml	1x TBE
10µl	ethidium bromide

WASH 1: 76% EtOH, 0.2 M NaOAc

100 ml	STOCK
76 ml	Absolute EtOH
8 ml	2.5 M NaOAc
16 ml	dH2O

WASH 2: 76% EtOH, 10 mM NH4OAc

100 ml	STOCK
76 ml	Absolute EtOH
1 ml	1 M NH4OAc
23 ml	dH2O

LI-COR Gel 7% acrylamide		Typhoon Gel 7% acrylamide	
3,5ml	50% acrylamide stock	12,4ml	50% acrylamide stock
10,5 g	Urea	60ml	Urea
2,5ml	10 X TBE	64µl	TEMED
250µl	DMSO	400µl	APS 10%
25µl	TEMED		
175µl	APS 10%		

2.3. Oligonucleotids

Oligonucleotids were ordered by MWG Biotech (85560 Ebersberg, Germany), Eurogenetec S.A. (4102 Seraing, Belgium) and VBC Genomics (1220 Wien, Austria) .
Most of the primers consisted of primers of the BARC (Song et al.2005), GDM (Pestsove et al. 2000) and GWM (Röder et al. 1995, 1998) database.
Primer sequences are available from the graingenes database (http://wheat.pw.usda.gov/GG2/index.shtml).

2.4. Plant material

During this project 118 back-cross lines were analyzed, deriving from crossing *T. dicoccum 161* and *DS-131621*.
The parent line *DS-131621*, which is a well-adapted Austrian durum line was given to the Department for Agrobiotechnology from Saatzucht-Donau. This line is highly susceptible to FHB.
T. dicoccum-161 was provided by Jeannie Gilbert (Agriculture and Agri-Food Canada, Winnipeg) and is resistant to FHB.

The F1- Generation of the crossing between *T. dicoccum 161* and *DS-131621* was back crossed with the *DS-131621* line to obtain BC_1F_1 seeds.
By doing a single seed descent, one seed per plant each generation, you obtained the BC_1F_4 generation.
Seeds from the BC_1F_4 generation were planted out to obtain plant material for the DNA isolation and 134 plants were developed. 118 out of 134 plants were analyzed by molecular markers to obtain a QTL map.

The following chapters about the field experiments, the inoculum production, the inoculation technique and disease evaluation and other traits were done Karin Huber. For more information please read the PHD thesis of Huber (2010).

2.5 Field experiments

The description of the field experiments was taken from the PHD thesis of Huber (2010). The lines of the mapping population and the parental lines were tested during 4 seasons at the experimental station of IFA-Tulln, 30 km west of Vienna, 180m above sea level. Soil type is meadow-czernosem. The average annual precipitation in this region is 620 mm and the average annual temperature is 9.2°C.

To control seed-borne diseases the seed was treated with 'Rovral-TS' (Rhone-Poulenc, Lyon France) seed dressing at a rate of 1g kg-1 of seed.

The experiments were arranged in a randomized complete block design with 1 (2005) to 4 (2006 - 2008) replications.

The replications were sown at two different dates, to account for variation in flowering time between the lines. In the year 2008, 2 replications where planted in fall to assure vernalization. Plots consisted of double rows with 17cm row spacing and 1m length. Sowing density was 5g of seed per plot.

Table 6 : Description of agronomic measures of field experiments for the years 2005-2008

		Experiment						
Trait	Scale	Fg05	Fg06	Fc06	Fg07	Fc07	Fg08	Fc08
Date of anthesis		x	x	x	x	x	x	x
Plant Height	cm	x	x	x	x	x	x	x
Leaf chlorosis	1-9		x	x	x	x		
Ear Compactness	1-9						x	x
Awn lenght	1-9						x	x
Waxiness	1-9						x	x
Ear length	1-9						x	x

[1] 1 low infected leaf area - 9 highly infected leaf area

[2] 1 loose ear, T. dicoccum - 5 compact ear - 9 very compact ear, Durum

[3] 1 short awns, T. dicoccum - 5 medium long awns - 9 long awns, Durum

[4] 1 green color of the glumes, T. dicoccum - 9 gray color of the glumes, Durum

[5] 1 *t. dicoccum* (long) -9 *t.durum* (short)

2.5.1 Inoculum production

For inoculation two single-spore isolates were applied: 1) *Fusarium graminearum* and 2) *F. culmorum*. Macroconidia of the *F. culmorum* isolate 'IPO 39-01' were prepared as described by Snijders and Van Eeuwijk (1991). A mixture of wheat and oat kernels (3:1) was soaked overnight in water and then autoclaved and inoculated. The mixture was incubated for 2 weeks at 25°C followed by 3 weeks at 5°C in the dark, leading to production of macroconidia. Macroconidia were washed off the colonized grain with deionised water. Macroconidia of *F. graminearum* isolate 'IFA 65' were produced in a liquid mungbean medium as described by Buerstmayr et al. (2002). Dry mungbean (*Vigna radiata L.*) seeds (20g l^{-1}) were boiled min distilled water for 20 min. The liquid phase was transferred to glass bottles and autoclaved. Following inoculation continuous aeration with sterile air at room temperature caused macroconidia development within one week. Conidia concentrations were determined using a Bürker-Türk counting chamber and adjusted to the desired concentration with deionized water. The final spore concentration used for inoculations was 2.5 x 10^4 spores ml^{-1} in all cases except for *F. graminearum* in 2008 where it was 5 x 10^4 spores ml^{-1}. The inoculum for both isolates *F. culmorum* and *F. graminearum* was stored at -18°C until use. The aggressiveness of the inoculum was monitored with a petri-dish infection test (Lemmens et al.1993) before and after the inoculation period.

2.5.2 Inoculation technique and disease evaluation

Spray inoculations were performed individually on each plot when 50% of the plants reach anthesis and repeated 2 days later. Using a motor driven back-pack sprayer, 50ml inoculum were sprayed on the heads. Inoculations were carried out in the evenings on alternate days. An automated mist-irrigation system switched by leaf wetness measurement, maintained humidity and kept the plants wet for 20h after inoculation.
In each plot the percentage of visually infected spikelets was scored according to a linear 0 to 100% scale on a whole plot basis as described in Figure 11. Percent FHB severity was recorded on days 10, 14, 18, 22, and 26 after inoculation. As an integrated measurement for FHB severity the area under the disease progress curve (AUDPC) was calculated.

Table 7: Linear scale for scoring visually infected spikelets

% infected spikelets per plot	
0	No visible infection
5	1 spikelet per head infected
10	2 spikelets per head infected
20	4 spikelets per head infected
40	8 spikelets per head infected
60	12 spikelets per head infected
80	16 spikelets per head infected
100	All spikelets per head infected

2.5.3 Other traits

Date of anthesis was recorded for each plot and used to calculate the number of days from 1 May to anthesis as a measure of earliness. Plant height was measured in cm from the soil surface to the top of the heads, excluding awns.
In each experiment one replication was scored to obtain the means for the phenotypic traits date of anthesis, plant height, and leaf chlorosis.
According to a linear 1 to 9 scale on a whole plot basis the morphological traits ear compactness (1 loose, *T. dicoccum* - 5 compact - 9 very compact, Durum), awn length (1 short - 5 medium - 9 long), spelt type (1 *T. dicoccum* - 9 Durum), and waxiness (1 green - 9 gray) were recorded in 2008.

2.6. DNA extraction, quantification and quality control

The genomic DNA from the analyzed plants was obtained from young leaves of the plants. The leaves were ground to powder with a ball mill and then extract with the method of Saghai-Maroof et al. (1984).
For this method 300-400mg of plant material were ground and transferred to a 15ml polypropylene centrifuge tube.
By adding 9ml of CTAB buffer to the plant powder and incubate it for 60-90 min by continuous inverting at a 65°C in a water bath, the high salt CTAB buffer separates DNA from unwanted substances like proteins.

Afterwards the tubes were cooled down and 4.5ml of chloroform/isoamylalcohol (IAA) (24:1) was added. The tubes were gently mixed for 10min and then centrifuged for 10min at a rotation of 1300-1500 x g at RT.
The liquid supernatant was transferred into new 15ml tubes, 4.5ml of chloroform/isoamylalcohol (IAA) (24:1) was added and gently inverted for 10min, followed by a centrifugation step for 10min at 1300-1500 x g at room temperature.
Again the liquid supernatant was transferred in a new 15ml tube and 25-50µg of 10mg/ml RNase A was added and well mixed during incubation for 30min at RT.
6 ml of isopropanol (2-propanol) was added by gentle inversion. By adding isopropanol the DNA will precipitate.
With a glass hook the precipitated DNA was removed and transferred to a 5ml plastic tube containing 1 ml of TE.
Overnight the tubes were incubated at RT by gentle mixing.
By adding 50µl of 5M NaCl and then 2.5 ml absolute EtOH the DNA was again precipitated and again transferred by a glass hook into a new tube containing 3-4 ml of WASH 1solution for about 20 min.
DNA was washed with 1-2ml of WASH 2 solution and transferred into new 5ml tubes containing 0.5 ml TE buffer.
The DNA was dissolved by gentle shaking overnight at RT.
The amount of DNA was quantified on a UV photometer and all the samples were diluted for further analyses to a concentration of 50ng/µl. Storage took place at -20°C.
The determination of the DNA concentration was done by 260nm and 280nm against a previous done nullification.
Nucleic acids absorb at 260nm, aromatic amino acids of proteins at 280nm wavelength.
The measurement was done in a 96well plate. The quotient of both measurements (260nm:280nm) was due to the purity of the nucleic acids.
Mainly the purity is influenced by proteins.

2.6.1 Polymerase Chain Reaction (PCR)

PCR is an enzyme regulated method for the amplification and detection of certain DNA fragments which are defined by specific oligonucleotides so called primers.
The sequences of the primers match complementary with certain DNA section on the desired DNA fragment one wants to amplify.
The primers bind to the complementary DNA and start the amplification.
The PCR is structured in several cycles, the denaturation, annealing of the primers and the elongation.
By heating the DNA up to 94°C the hydrogen bonds were divided and two single strands are generated.
Now the sample is heated up to the specific annealing temperature of the primers (forward and reverse) so they can attach to the complementary sequence.
The annealing temperature of the primers is dependent on the length and the sequence of the primer and can be calculated by different formulas.

69,3°C +(0,41 x (Guanin/Cytosin)%)-650/Amount of bases of the primer =T_m

$T_a = T_m - 5°C$
T_m: melting temperature
T_a: annealing temperature

The next step is to heat the whole sample up to 72°C the temperature where the *Taq*-polymerase can bind to the free hydroxyl groups of the oligonucleotides and start to synthesize a complementary strand by using the dNTP´s.
By repeating the steps it is possible to amplify the desired fragment within 30 cycles 10^6 times more.
The *Taq*-polymerase amplifies in 1min around 1.000bp.

Table 8: Standard PCR-approach and general PCR approach

20-50ng DNA
1 µl 10 x PCR-Puffer including 15Mm $MgCl_2$
1 µl dNTP (25 mM)
0,2 µl forward primer (10 pmol/µl)
0,2 µl reverse primer (10 pmol/µl)
0,1 µl *Taq* polymerase (5U/µl)
x µl H_2O
10 µl final volume

Initial denaturation	94°C	3 min	
Denaturation	94°C	30sec	
Annealing	T_a°C	45sec	29x
Elongation	72°C	synthesizes 1000bp in 1min	
	72°C	5min	
	4°C	endless	

T_a = annealing temperature of the forward and reverse Primer

2.6.2 SSR

At the IFA Tulln, 120 plants were tested with 69 microsatellites primers in 384-well plates („Primus 96-well Thermocycler“) for polymorphism.
Primers were chosen from former experiences, publications and databases and consisted of BARC (Song et al. 2005), GDM (Pestsove et al. 2000) and GWM (Röder et al. 1995, 1998).
For detection of the amplified fragments most of the GWM and BARC primers are directly labeled with a fluorochrome (IDR700 or IDR800), for primers with no labeling, special M13 primers were added which are also labeled with one of the fluorochromes.
For the samples which are amplified with the M13 primers a special PCR program must be used (Table 9).

The PCR product were diluted with a formamide buffer denatured for 5min at 95°C and then loaded on a 7 % polyacrylamide gel which was analyzed by a LI-COR 4200 DNA (MWG-Biotech dNA sequencer long reader 4200) dual-dye detector. Adjustments for the electrophoresis were set at a constant current of 65 W and 48°C until the patterns occur
In this method 64 samples could be tested at the same time. A digital image is captured on a computer.
The different patterns of the SSR markers were further analyzed manually with a standard image program.

Table 9: Standard PCR Amplification of *M13-tailed* microsatellites and schematic PCR process for *M13-tailed* microsatellites

	[stock]		one reaction	
PCR buffer 15 mM incl 15 mM MgCl2	10	X	1	µl
dNTP Mix (10X)	2	mM (each)	1	µl
R-Primer (10µM)	10	µM	0.2	µl
F-Primer (10µM)	10	µM	0.03	µl
M13-30 Primer (10µM)	10	µM	0.18	µl
Taq-Enzym (5U/µl)	5	U/µl	0.1	µl
ddH2O			4,49	µl
Template DNA (10ng/µl)	20-50	ng/µl	3	µl
Total			10	µl

Initial denaturation	94°C	3 min	
Denaturation	94°C	1min	← 35x
Annealing	T_a°C	1min	
Elongation	72°C	2min	
	72°C	10min	
	10°C	endless	

2.6.3 AFLP

To analyze the 118 durum plants, including the parents, the AFLP technique was used (Voss et al.1995) and carried out as described by Hartl et al. 1999. In this project 21 AFLP primer combinations were used which led to 386 AFLP polymorphic markers.
In the first step the genomic DNA is digested by two restriction enzymes, a frequent cutter *MseI* (5´-GACG-3‘) and a rare cutter *Sse8387I* (5’-CCTGCAGG-3’).

Restriction mix	
Genomic DNA	0.25 µg
Sse8387I	2.5 Units
MseI	2.5 Units
BSA (*optional*)	0.01 %
10X Restriction-buffer for Sse8387I	2µl (1X)
fill with H2O up to 20 µl	

The sample was incubated by 37°C for 90 minutes.
At the same time the purchased single stranded adapters are prepared by adjusting them to a concentration of 50µM for the *MseI* and 5µM for the *Sse8387I* adapter.

AFLP adapter sequences from 5’ to 3’:

Adapter*Mse*I-1: GACGATGAGTCCTGAG
Adapter*Mse*I-2: TACTCAGGACTCAT
Adapter*Sse8387*I-1:CTCGTAGACTGCGTACATGCA
Adapter*Sse8387*I-2: TGTACGCAGTCTAC
Preselective primers: Pre*Sse8387*I: GTAGACTGCGTACATGCAG
Pre*Mse*I: GATGAGTCCTGAGTAA
Selective primers: *Sse8387*I : **Cy3, Cy5, FAM** - GACTGCGTACATGCAG-**NN**
*Mse*I: GATGAGTCCTGAGTAA-**NN**

Mix for Mse-adapter

ADAMse1 (500µM)	6 µl
ADAMse2 (500µM)	6 µl
H2O	48 µl
total 60 µl ADAMse 50 µM	

Mix for Sse-adapter

ADASse2 (50µM)	6 µl
ADASse1 (50µM)	6 µl
H2O	48 µl
total 60 µl ADASse 5 µM	

Both were incubated for 30 minutes at RT by gently mixing them, which leads to double stranded adapters.
The next step is the ligation of the adapters to the sticky ends of the digested DNA.
For this a ligation mix is made and 5µl of the ligation mix is given to each restriction mix and incubated for 3 hours at 37°C.

Ligation mix

ADASse (5µM)	0.5 µl
ADAMse (50µM)	0.5 µl
ATP (10mM)	0.5 µl
10X Restriction-buffer for Sse8387I	0.5 µl
T4 Ligase (Biolabs 1U/µl)	1.0 µl
H2O	2.0 µl
total	**5.0 µl**

As a control 5ml of the final mix is loaded on a 2% agarose gel. If there is a smear between 100 and 800bp the reaction worked.
To the remaining sample 60µl of dest. H20 is added, mixed well and used as a template for the next step the pre-selective amplification.

In this step the first amplification of the fragments occurs. The sequence of the primers in this method is assembled by the complementary sequence of the adapters and the restriction enzymes (cutting sequence).

Pre-amplifikation mix

Pre*Sse*-Primer (10µM)	0.6 µl [no selektive Nucleotide]
Pre*Ms*e-Primer (10µM)	0.6 µl [no selektive Nucleotide]
dNTP(Pharmacia 2mM)	2.0 µl
PCR Puffer (10X)	2.0 µl [incl. MgCl2, final conc. 1.5mM]
Taq-Polymerase (5U/µl)	0.1 µl
ligated DNA	5.0 µl
H2O	9.7 µl
total	**20.0 µl**

Denaturation	94°C	30sec
Annealing	60°C	1min (ramp down 1°C per cycle)
Elongation	72°C	2min
	4°C	endless

20x

After the amplification the 5µl of the PCR product is loaded on a 2% Agarosegel as a control. A smear should appear 100-800 bp range. The remaining 15µl are diluted with 285µl H2O. These 300µl is the template for the next step the selective PCR amplification.

During this step primers are used which are elongated with two additional bases at the 3´- end. This reduces the amount of fragments extremely and the possibilities of primer combination increases. For a better detection the *Sse8387*I primer is labelled with a special dye (Cye3, Cy5, FAM…) at the 5´end. The different combinations are shown in the Table number 10.

Selective amplifikation mix

Sse-Primer (10µM)	0.15 µl [2 selective nucleotides, **5‘ Cy3 (or Cy5, FAM labeled)**
Mse-Primer (10µM)	0.3 µl [2 selective nucleotides]
dNTP (2mM)	1.0 µl
PCR Puffer (10X)	1.0 µl [incl. MgCl2, 1.5mM final]
Taq (5U/µl)	0.08 µl
Preamplified DNA	2.0 µl
H2O	5.47 µl
= total	10.0 µl

Initial denaturation	94°C	2 min	
Denaturation	94°C	30sec	← 10x
Annealing	63°C	30sec (ramp down 1°C per cycle to 54^0C)	
Elongation	72°C	2min	
Denaturation	94°C	30sec	← 23x
Annealing	54°C	30sec	
Elongation	72°C	2min	
	4°C	endless	

The PCR product were diluted with a formamide buffer denatured for 5min at 95°C and then loaded on a 7 % polyacrylamide gel which was analyzed by a Typhoon (GE Healthcare " Typhoon Trio Variable Mode Imager") detector.

Adjustments for the electrophoresis were set at a constant current of 65 W and 48°C until the patterns occur.

The different patterns of the AFLP markers were further analyzed manually using standard image processing software.

By analyzing the SSR and the AFLP pattern in the same way, it is possible to link the SSR marker, which are located on a specific position on the chromosome, with the unknown AFLP marker and group them. Experiences with this combination method were already obtained by former QTL mapping (Buerstmayr. et al 2002, 2003).

Table 10: 21 AFLP primer combinations with different selective bases and the according number of polymorphic bands

Sse8387I	**MseI**	**number of polymorphic bands**
S11 (AA)	M13 (AG)	22
	M14 (AT)	25
	M15 (CA)	18
	M17 (CG)	24
	M26 (TT)	20
S13 (AG)	M14 (AT)	14
S18 (CT)	M12 (AC)	10
	M14 (AT)	16
S20 (GC)	M14 (AT)	13
	M15 (CA)	16
S23 (TA)	M12 (AC)	18
	M13 (AG)	18
	M14 (AT)	34
	M17 (CG)	15
	M26 (TT)	29
S24 (TC)	M12 (AC)	15
	M14 (AT)	16
	M15 (CA)	13
	M26 (TT)	19
S25 (TG)	M12 (AC)	15
	M14 (AT)	16
sum	21 primer combinations →	386 polymorphic AFLP markers

2.7. Data Analyses

2.7.1. Linkage map construction

Linkage map construction was done with Carthagene (De Givry et al. 2005).
Carthagene is a computer program which allows you to build genetic maps of a single population or different populations by calculating the data for the maximum likelihood by using special ordering algorithms.
For this the data obtained and evaluated from the SSR markers and AFLP markers were prepared for the Carthagene program in .txt or .cvs file.
After uploading the file to the program you can use special commands which give you a lot of possibilities to handle your data.
The commands which were used for this evaluation will now be presented here.

The first thing to do is to group the SSR and AFLP markers into linkage groups. For this the command **group .3 3** is used which specifies a distance and LOD treshold.
In our case we choose a LOD threshold of 3.0 and a distance of 30cM.
Now you will obtain the linkage groups in our case we got 52 linkage groups.
To get a closer look at the single linkage groups we used the **groupget No.** command with the number of the interested linkage group and you will get a list of the markers belonging to this group. With the command **mrkselset [groupget No.]** you can select one of the interesting groups.
The next step is to build a map of the specific group we selected. With the command **sem** you assess the quality of the default order specified in the mrkselset command. Now you obtain a list with the markers ordered by a multipoint maximum likelihood.
To build more detailed maps we use the commands **nicemapd** and **nicemapl** which uses a 2-point LOD and 2-point distances as guide. More complex maps are obtained with the commands **mfmapd** and **mfmapl**.
With a more effective heuristic procedure you can now build another map which includes markers by choosing always the best loglikelihood and the best insertion point by using the command **build**.

To improve the map we use a verification algorithm, which flips the markers inside a window in Carthagene. For this you have to type in 3 parameters:

- The size of the flipping window
- A printing threshold on the difference of loglikelihood with the best map
- And a command which repeats the process if a better map is found

We used normally a window with 4-5 markers, all maps whose logkielihood is better or equal 1.0 LOD unit of the loglikelihood of the best map will be printed and it will be repeated if a better map is found. The whole command for this settings is **flips 4 1 1**.

To print the best map of all available maps you type in **bestprintd**.

The last step is to show all markers which have compatibles genotypes and to identify them with the command **mrkdouble**. This will show you the possible duplicated markers.

2.7.2. QTL mapping

The QTL analyses were carried out with the qgene program version-4.2.3 which was first described by Nelson JC (1997) (www.qgene.org)

SIM (single interval-mapping) (Haley and Knott, 1992), CIM (composite interval mapping) (Zeng et al. 1994), additive effects, coefficient of determination (R^2) and LOD (logarithm of odds) were regarded and the traits were calculated.

When a QTL got a LOD score above 2.5 it was appointed to be significant.

Also the overall mean overall years and the single means from every single year were taken into consideration for the calculation and a chromosome map was constructed.

2.7.3. Visualization of maps

The Visualization of maps was done with Mapchart 2.1 (Voorrips et al. 2002). Mapchart is a software for graphical presentation of linkage maps and QTL.

With this program it is possible to create diagrams of linkage maps and QTL. For the construction of these charts the program needs the chromosome information, the name of the markers and the positions of the markers on the chromosome in *cM* in one text file.

Out of this information it creates a vertical bar representing the linkage group or chromosome with the information of the position in *cM* on the left side and the associated marker information on the right side.

3. Results

3.1. Molecular genetic map

The backcross durum population of *T. dicoccum 161* and *DS-131621* showed after the evaluation QTL which might play an important role in the resistance against Fusarium head blight (FHB).
Also some phenotypic traits like date of anthesis, leaf chlorosis, ear compactness, ear length, waxiness, awn length and plant height were considered and evaluated.
The results from the field data Huber (2010) are not shown here, but the QTL analysis itself is shown in the following chapter.
For these results 118 lines of the population were evaluated, and a genetic linkage map was generated using 455 PCR markers, consisting of 69 SSR markers and 386 AFLP markers.

3.1.1. SSR

The parental lines and the progenies are analysed with 69 SSR markers which have a known position on the chromosome.
In the Figure 5 below three patterns of three different SSR markers are shown revealing the polymorphism between the two parents *T. dicoccum 161* and *DS- 131621*.

3.1.2. AFLP

Altogether 21 AFLP primer combinations are used which generated 386 polymorphic fragments. In the Figure 6 below as an example for one AFLP primer combination a part of the AFLP gel for the combination XS11M14 is shown.
The AFLP markers itself are in an unknown region on the chromosome but can be linked to the known SSR markers by comparing the polymorphism.

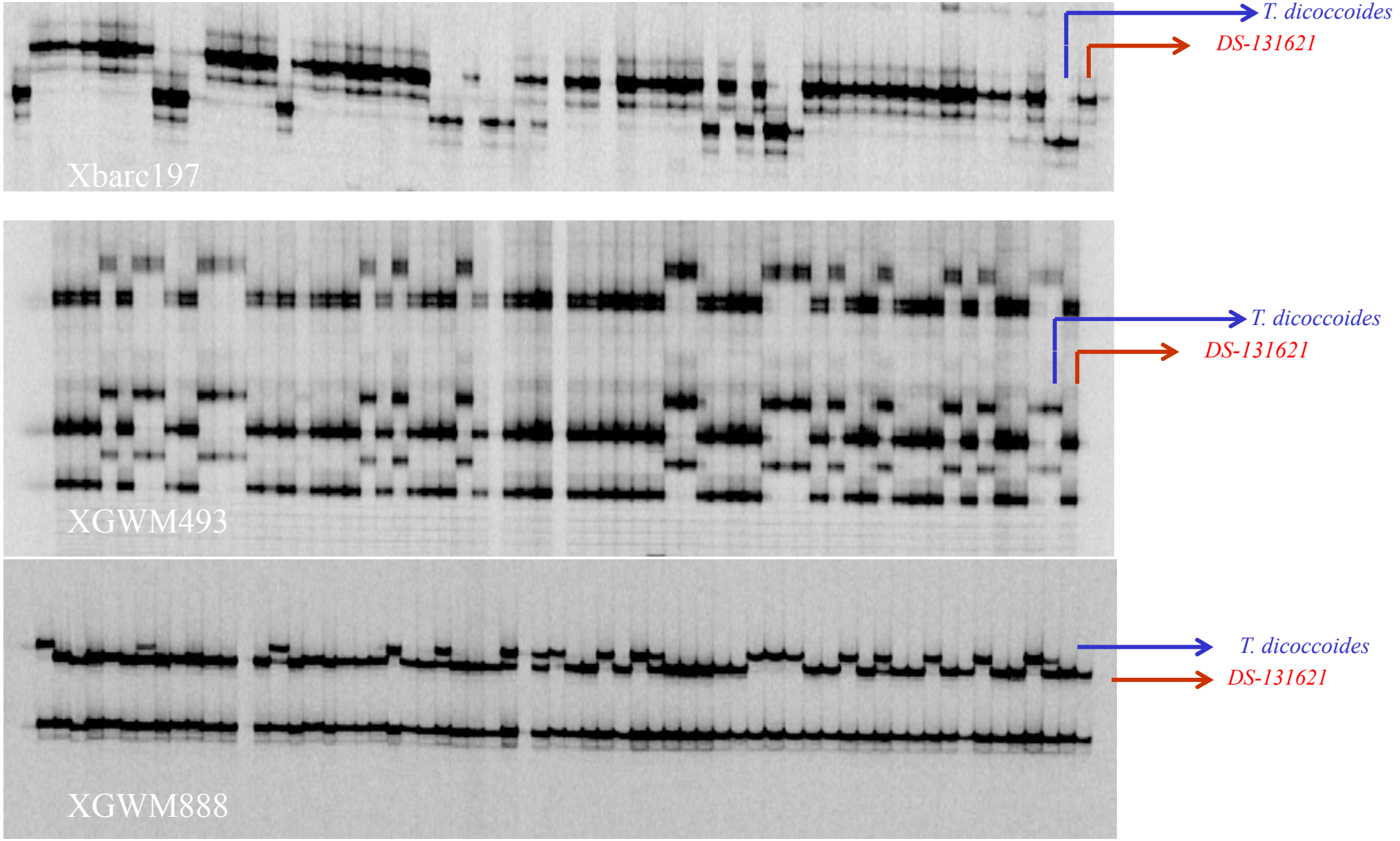


Figure 5: Three different SSR patterns from different SSR markers (Xbarc197; XGWM493; XGWM888)

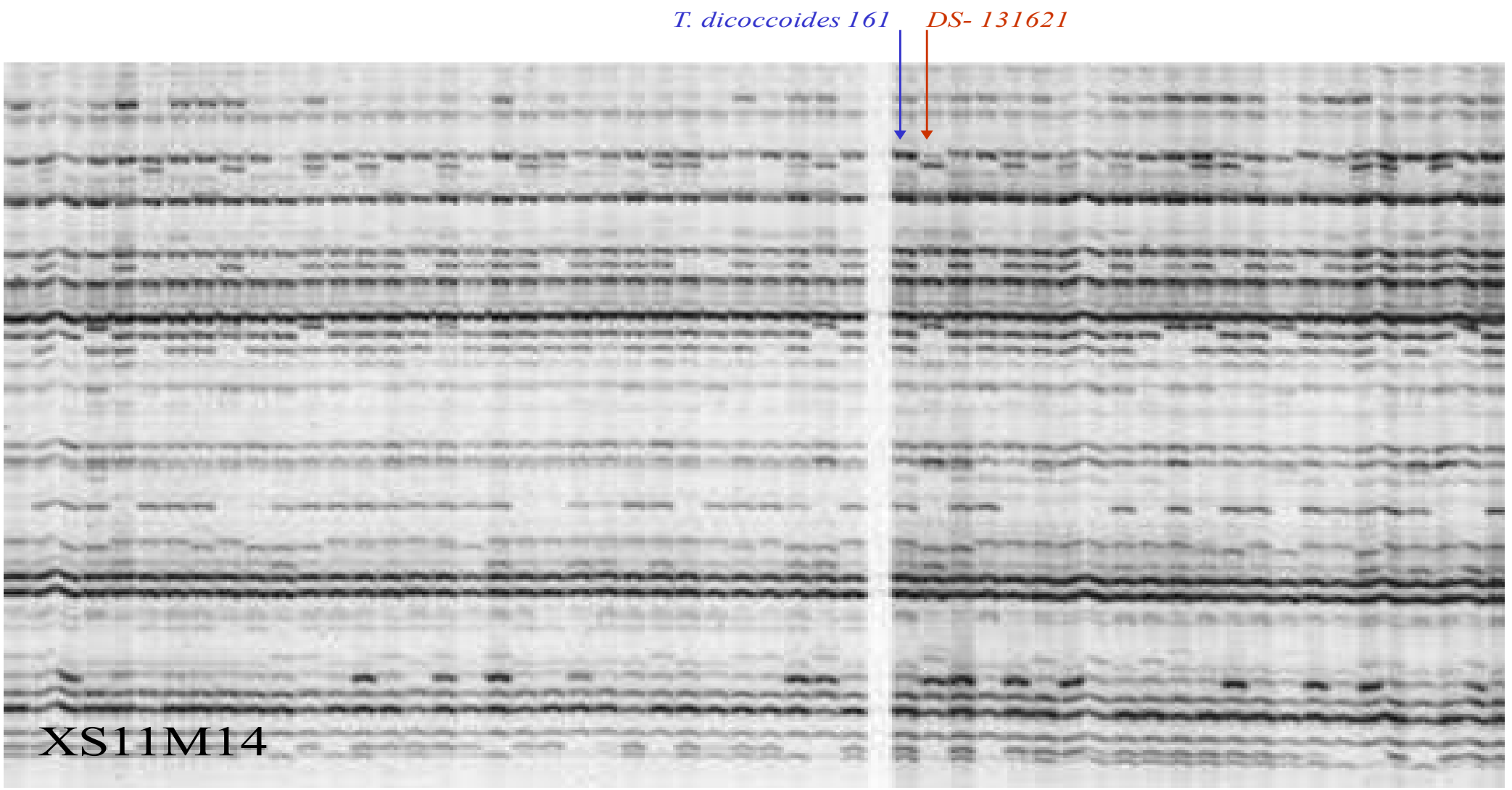
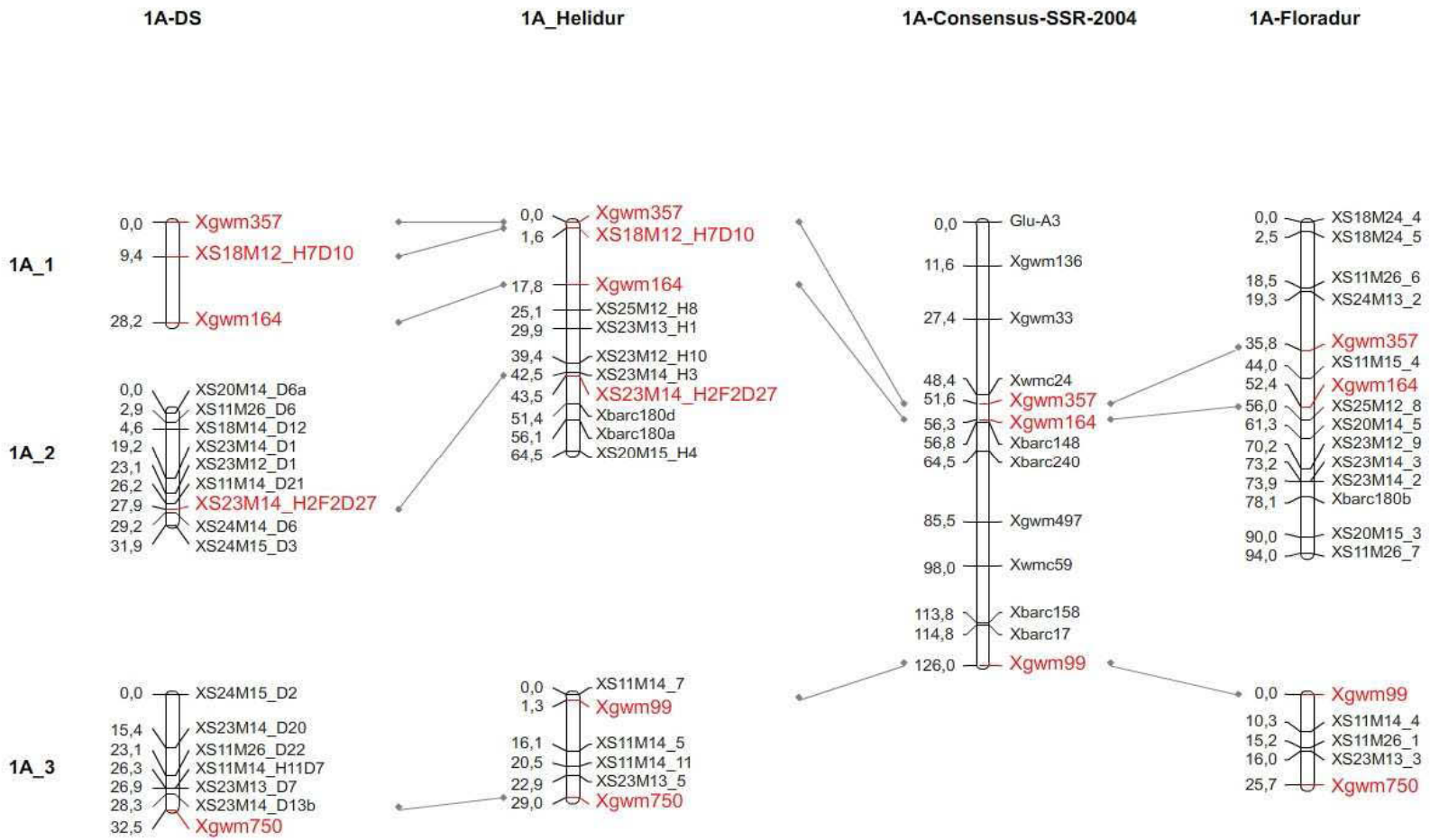


Figure 6: AFLP pattern of marker XS11M14 with a lot of polymorphisms

3.2. Construction of a molecular genetic map

The graphical illustration of the linkage groups with the loci are made for the *T. dicoccum 161* x *DS-131621* population and are compared to two former crosses (*T. dicoccum 161* x Helidur and *T. dicoccum 161* x Floradur) which were made at the IFA Tulln by Huber (2010). The similarity of this crosses are that one parent (*T. dicoccum 161*) is always the same.
From the 455 markers (69 SSR markers, 386 AFLP markers) 52 linkage groups could be mapped and 34 linkage groups could be assigned to the wheat consensus map from 2004 (Somers et al. 2004).
18 groups couldn't be determined to a chromosome and remained unassigned.
Apart from the 6A chromosome all durum wheat chromosomes are linked to a group.
The A chromosome is presented with 19 linkage groups from 168 markers, the B chromosome is presented with 15 linkage groups from 177 markers.
12 linkage groups couldn't be associated to a specific wheat chromosome and are shown as unassigned linkage groups, 6 out of them were single markers and are not shown in the Figure.
Figure 7 (next page) shows the complete linkage map with all linkage groups of the three populations (*T. dicoccum 161* x *DS-131621* is compared to two former crosses (*T. dicoccum 161* x Helidur and *T. dicoccum 161* x Floradur) and the unassigned groups.
All the genetic maps are compared to the wheat consensus map from Somers et al. (2004).
The molecular markers are shown on the right side, the cM on the left side.
Homolog AFLP and SSR markers are coloured in red and are connected between the linkage groups with a gray line. The cross *T. dicoccum 161* x *DS-131621* is marked as DS and is shown on the left side.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1A_Helidur
1A-Consensus-SSR-2004
1A-Floradur
1A_1
1A_2
1A_3
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9,4 XS18M12_H7D10
28,2 Xgwm164
0,0 XS20M14_D6a
2,9 XS11M26_D6
4,6 XS18M14_D12
19,2 XS23M14_D1
23,1 XS23M12_D1
26,2 XS11M14_D21
27,9 XS23M14_H2F2D27
29,2 XS24M14_D6
31,9 XS24M15_D3
0,0 XS24M15_D2
15,4 XS23M14_D20
23,1 XS11M26_D22
26,3 XS11M14_H11D7
26,9 XS23M13_D7
28,3 XS23M14_D13b
32,5 Xgwm750
0,0 Xgwm357
1,6 XS18M12_H7D10
17,8 Xgwm164
25,1 XS25M12_H8
29,9 XS23M13_H1
39,4 XS23M12_H10
42,5 XS23M14_H3
43,5 XS23M14_H2F2D27
51,4 Xbarc180d
56,1 Xbarc180a
64,5 XS20M15_H4
0,0 XS11M14_7
1,3 Xgwm99
16,1 XS11M14_5
20,5 XS11M14_11
22,9 XS23M13_5
29,0 Xgwm750
0,0 Glu-A3
11,6 Xgwm136
27,4 Xgwm33
48,4 Xwmc24
51,6 Xgwm357
56,3 Xgwm164
56,8 Xbarc148
64,5 Xbarc240
85,5 Xgwm497
98,0 Xwmc59
113,8 Xbarc158
114,8 Xbarc17
126,0 Xgwm99
0,0 XS18M24_4
2,5 XS18M24_5
18,5 XS11M26_6
19,3 XS24M13_2
35,8 Xgwm357
44,0 XS11M15_4
52,4 Xgwm164
56,0 XS25M12_8
61,3 XS20M14_5
70,2 XS23M12_9
73,2 XS23M14_3
73,9 XS23M14_2
78,1 Xbarc180b
90,0 XS20M15_3
94,0 XS11M26_7
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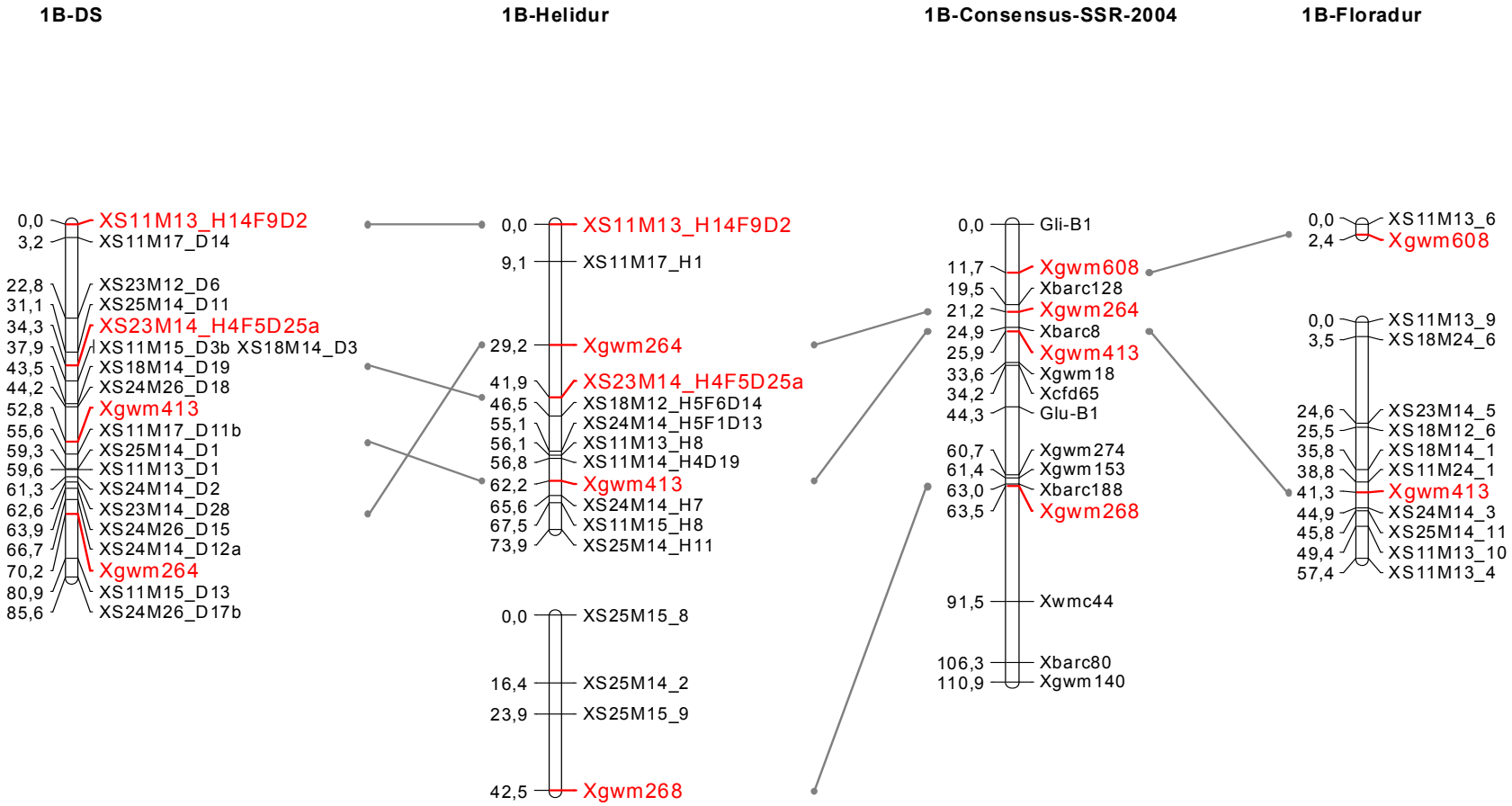
15,2 XS11M26_1
16,0 XS23M13_3
25,7 Xgwm750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1B-Helidur
1B-Consensus-SSR-2004
1B-Floradur
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22,8 XS23M12_D6
31,1 XS25M14_D11
34,3 XS23M14_H4F5D25a
37,9 XS11M15_D3b XS18M14_D3
43,5 XS18M14_D19
44,2 XS24M26_D18
52,8 Xgwm413
55,6 XS11M17_D11b
59,3 XS25M14_D1
59,6 XS11M13_D1
61,3 XS24M14_D2
62,6 XS23M14_D28
63,9 XS24M26_D15
66,7 XS24M14_D12a
70,2 Xgwm264
80,9 XS11M15_D13
85,6 XS24M26_D17b
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9,1 XS11M17_H1
29,2 Xgwm264
41,9 XS23M14_H4F5D25a
46,5 XS18M12_H5F6D14
55,1 XS24M14_H5F1D13
56,1 XS11M13_H8
56,8 XS11M14_H4D19
62,2 Xgwm413
65,6 XS24M14_H7
67,5 XS11M15_H8
73,9 XS25M14_H11
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16,4 XS25M14_2
23,9 XS25M15_9
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0,0 Gli-B1
11,7 Xgwm608
19,5 Xbarc128
21,2 Xgwm264
24,9 Xbarc8
25,9 Xgwm413
33,6 Xgwm18
34,2 Xcfd65
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61,4 Xgwm153
63,0 Xbarc188
63,5 Xgwm268
91,5 Xwmc44
106,3 Xbarc80
110,9 Xgwm140
0,0 XS11M13_6
2,4 Xgwm608
0,0 XS11M13_9
3,5 XS18M24_6
24,6 XS23M14_5
25,5 XS18M12_6
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38,8 XS11M24_1
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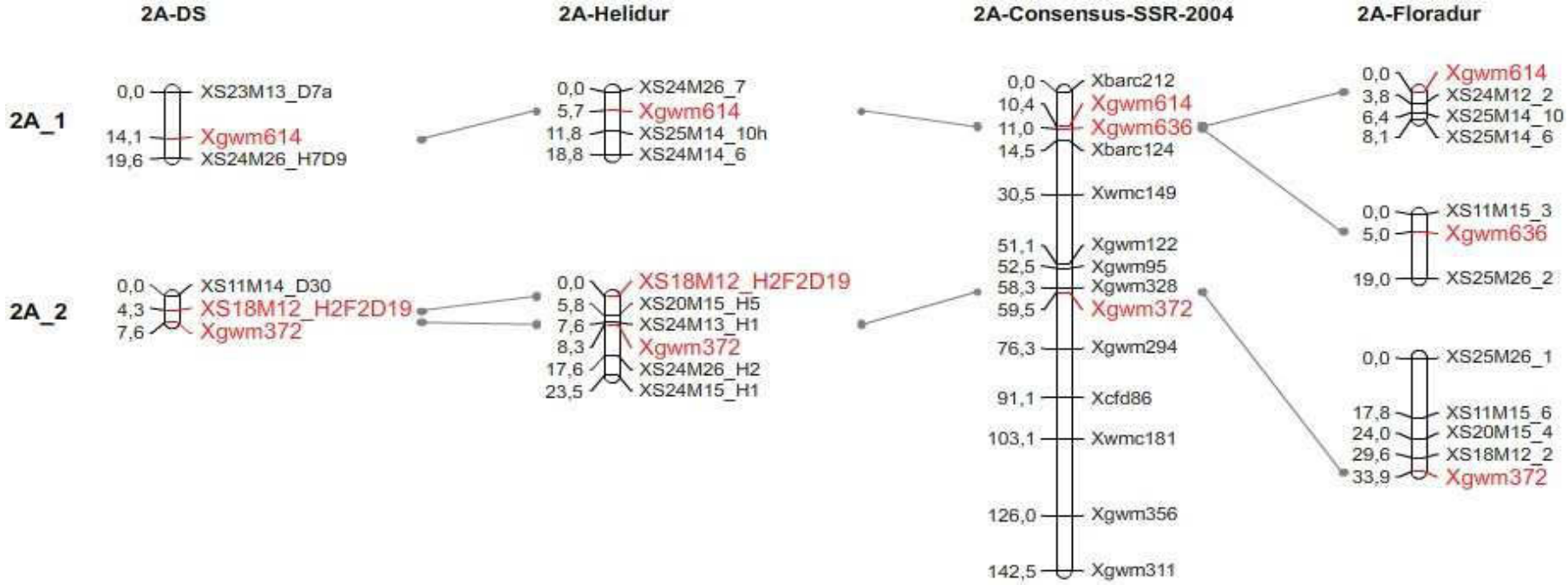
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19,6 XS24M26_H7D9
0,0 XS24M26_7
5,7 Xgwm614
11,8 XS25M14_10h
18,8 XS24M14_6
0,0 XS11M14_D30
4,3 XS18M12_H2F2D19
7,6 Xgwm372
0,0 XS18M12_H2F2D19
5,8 XS20M15_H5
7,6 XS24M13_H1
8,3 Xgwm372
17,6 XS24M26_H2
23,5 XS24M15_H1
0,0 Xbarc212
10,4 Xgwm614
11,0 Xgwm636
14,5 Xbarc124
30,5 Xwmc149
51,1 Xgwm122
52,5 Xgwm95
58,3 Xgwm328
59,5 Xgwm372
76,3 Xgwm294
91,1 Xcfd86
103,1 Xwmc181
126,0 Xgwm356
142,5 Xgwm311
0,0 Xgwm614
3,8 XS24M12_2
6,4 XS25M14_10
8,1 XS25M14_6
0,0 XS11M15_3
5,0 Xgwm636
19,0 XS25M26_2
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24,0 XS20M15_4
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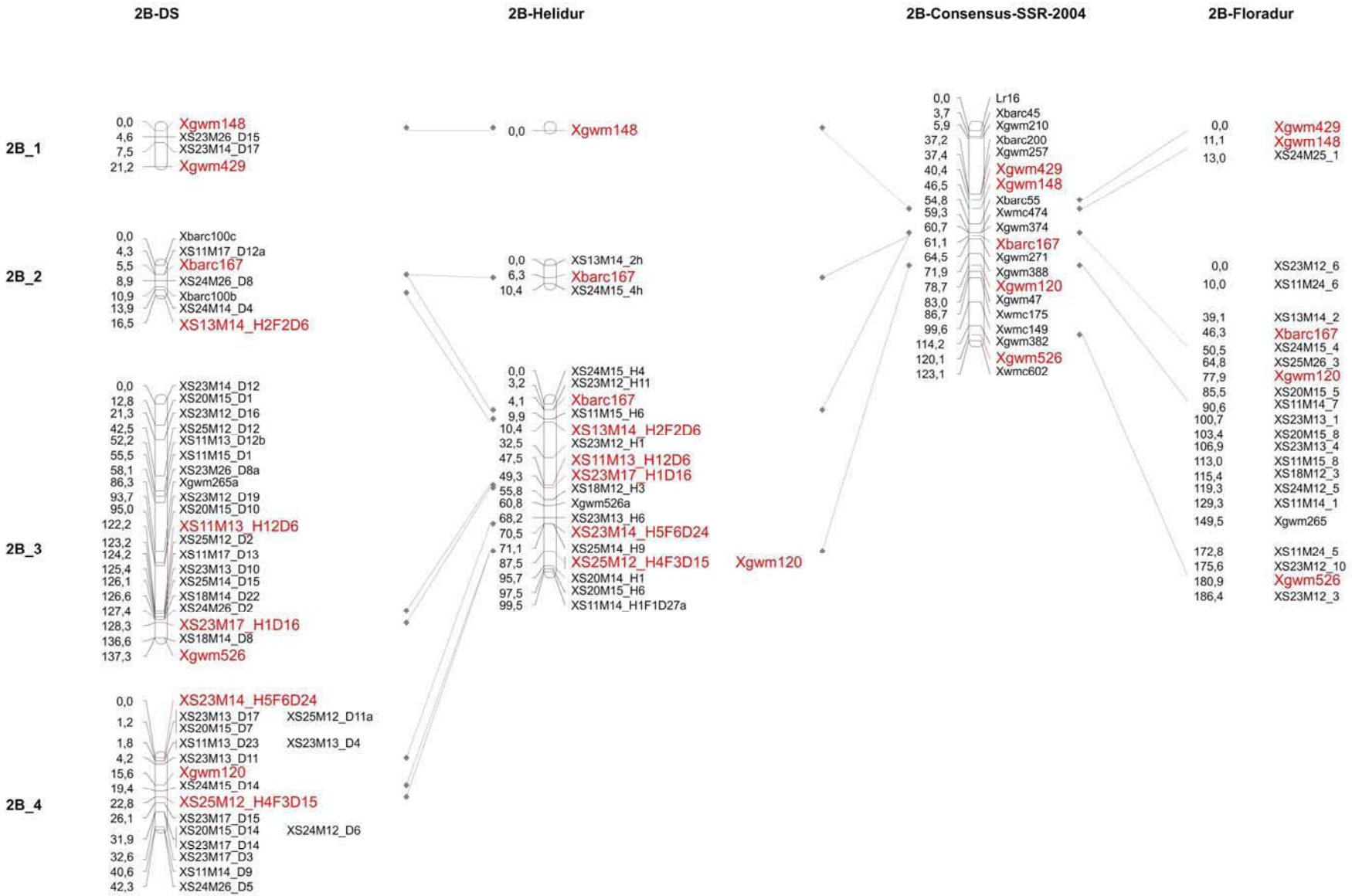
33,9 Xgwm372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2B-Helidur
2B-Consensus-SSR-2004
2B-Floradur
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2B_2
2B_3
2B_4
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7,5 XS23M14_D17
21,2 Xgwm429
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4,3 XS11M17_D12a
5,5 Xbarc167
8,9 XS24M26_D8
10,9 Xbarc100b
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21,3 XS23M12_D16
42,5 XS25M12_D12
52,2 XS11M13_D12b
55,5 XS11M15_D1
58,1 XS23M26_D8a
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95,0 XS20M15_D10
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124,2 XS11M17_D13
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127,4 XS24M26_D2
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XS25M12_D11a
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XS23M13_D11
XS23M13_D4
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XS24M12_D6
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Xgwm120
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40,4 Xgwm429
46,5 Xgwm148
54,8 Xbarc55
59,3 Xwmc474
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71,9 Xgwm388
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83,0 Xgwm47
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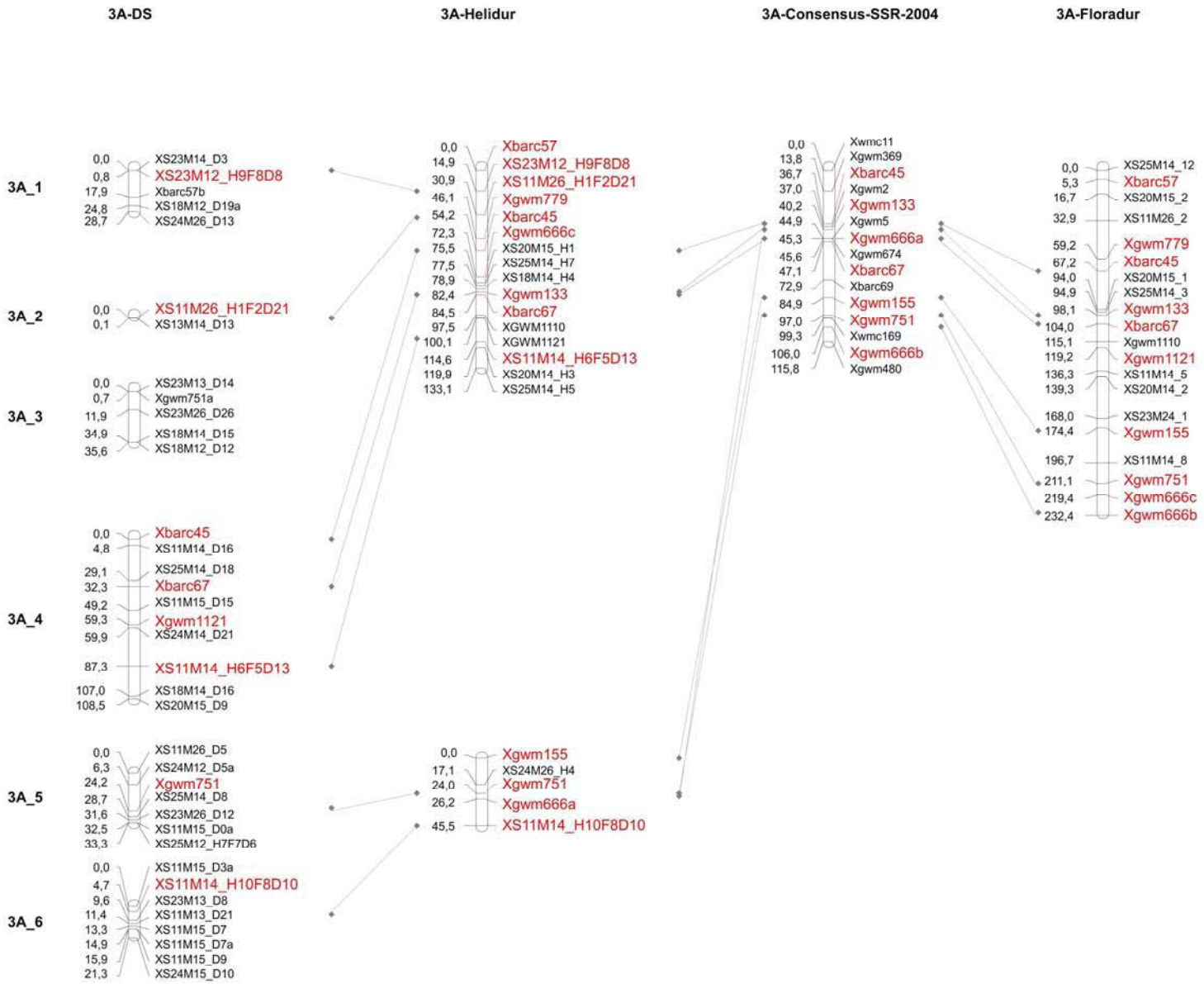
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3A-Helidur
3A-Consensus-SSR-2004
3A-Floradur
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3A_2
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0,1 XS13M14_D13
3A_3
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0,7 Xgwm751a
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34,9 XS18M14_D15
35,6 XS18M12_D12
3A_4
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29,1 XS25M14_D18
32,3 Xbarc67
49,2 XS11M15_D15
59,3 Xgwm1121
59,9 XS24M14_D21
87,3 XS11M14_H6F5D13
107,0 XS18M14_D16
108,5 XS20M15_D9
3A_5
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6,3 XS24M12_D5a
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3A_6
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11,4 XS11M13_D21
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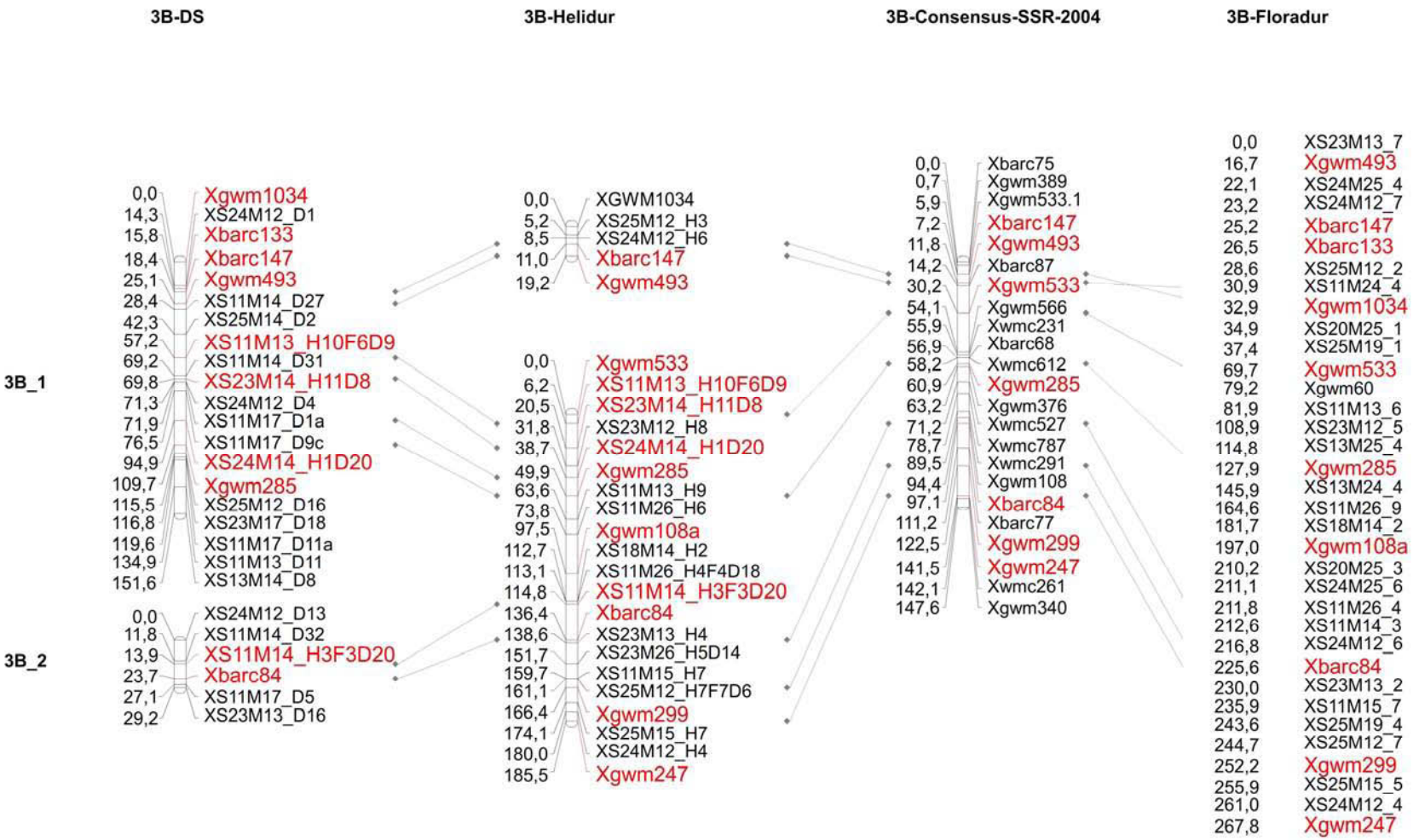
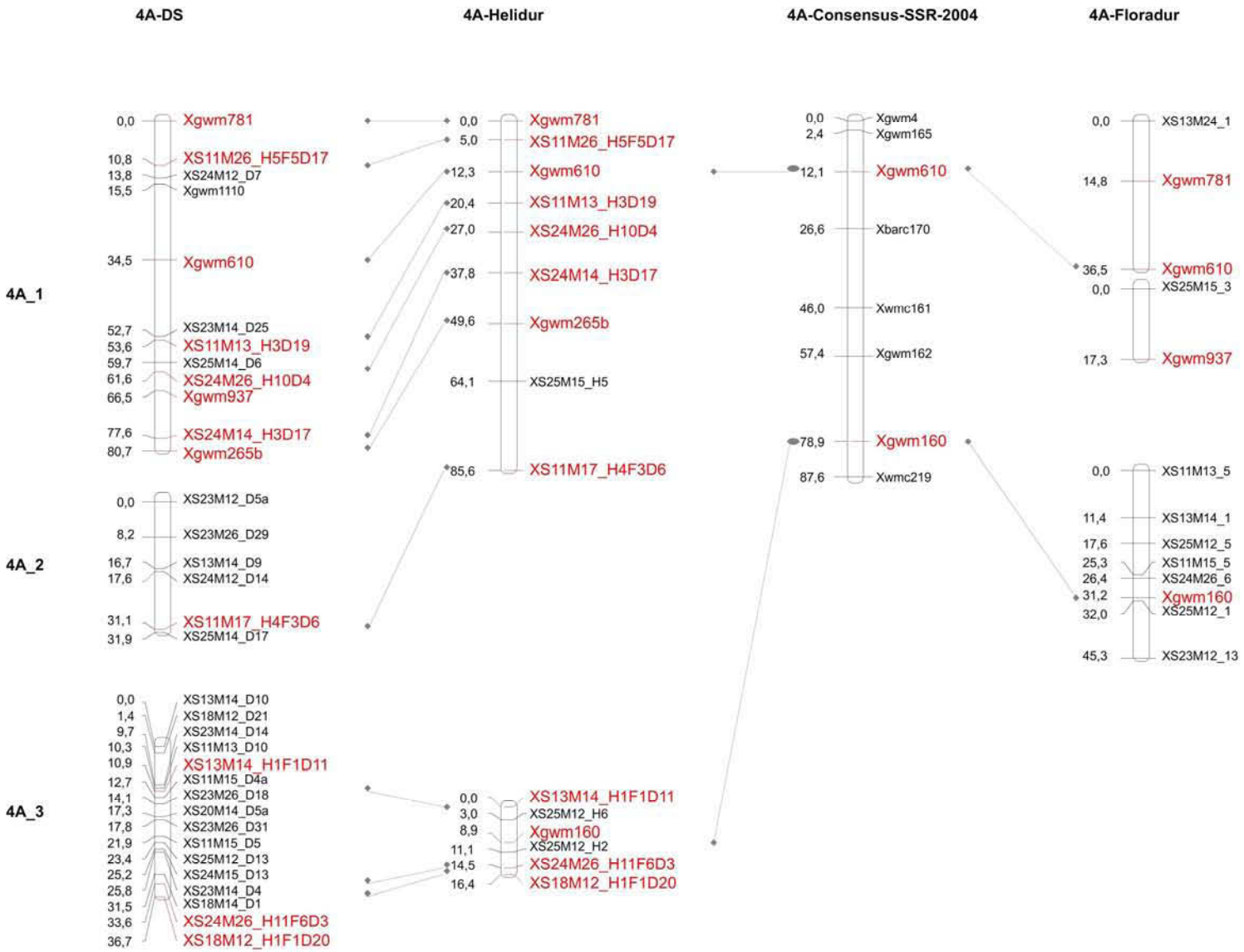
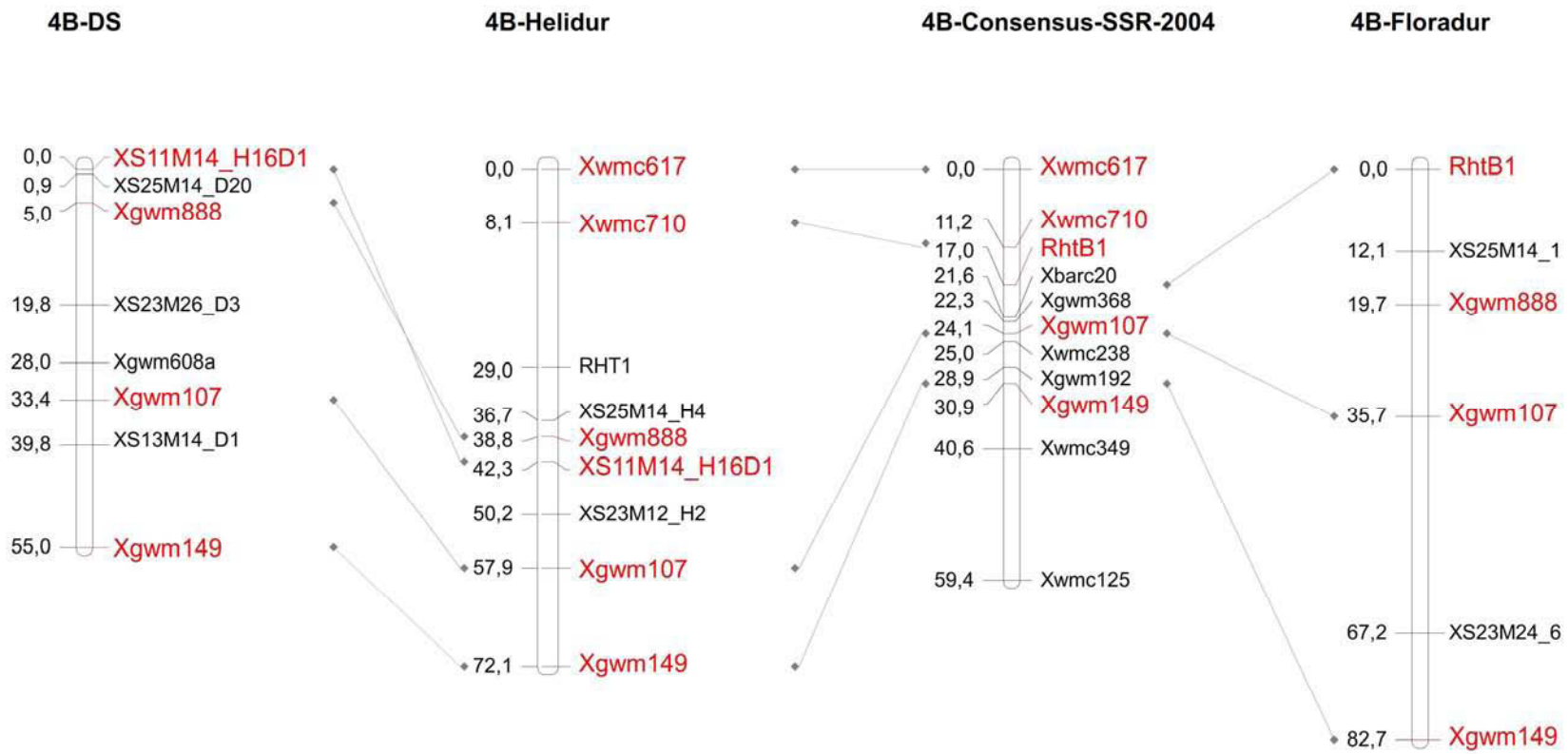
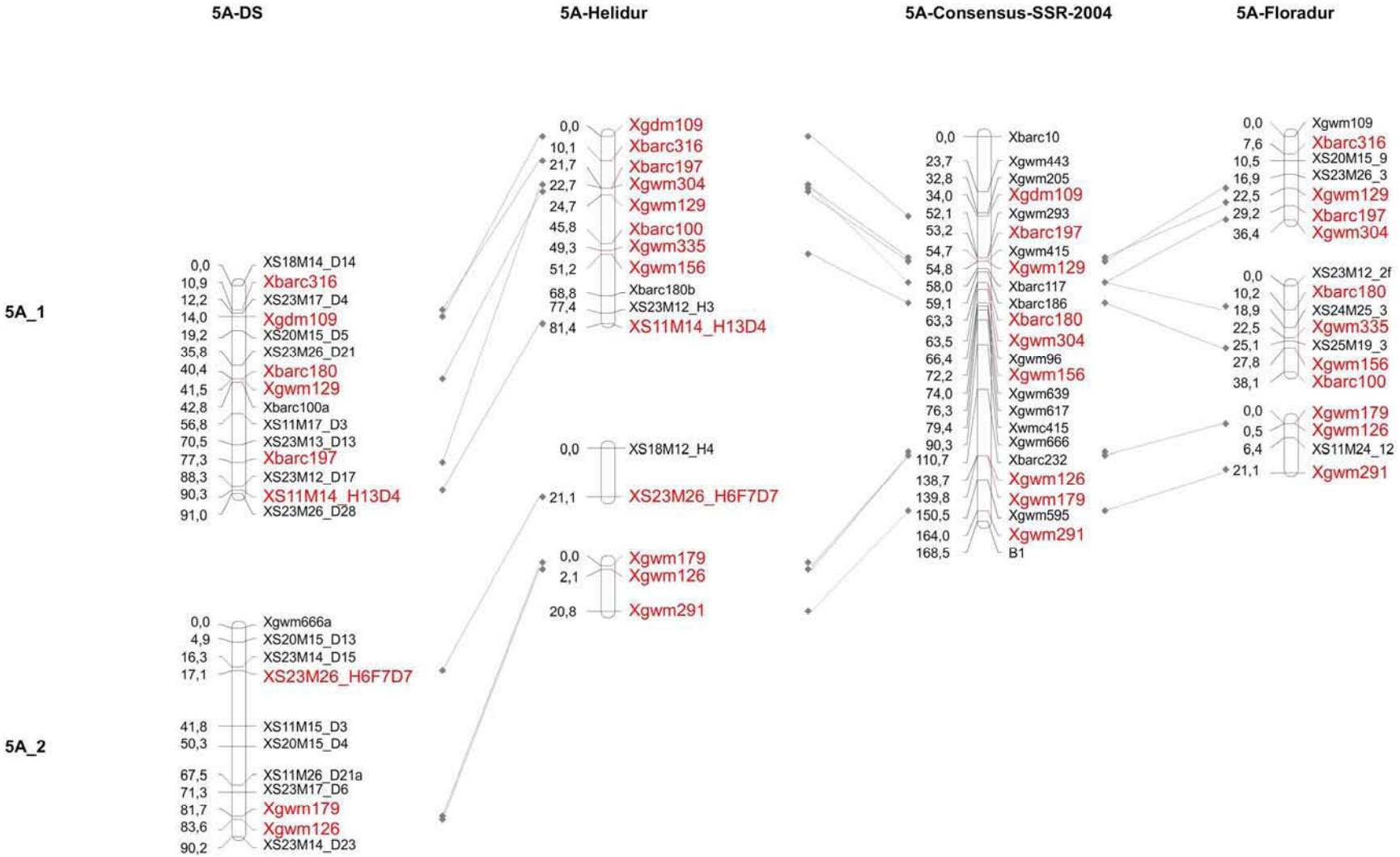
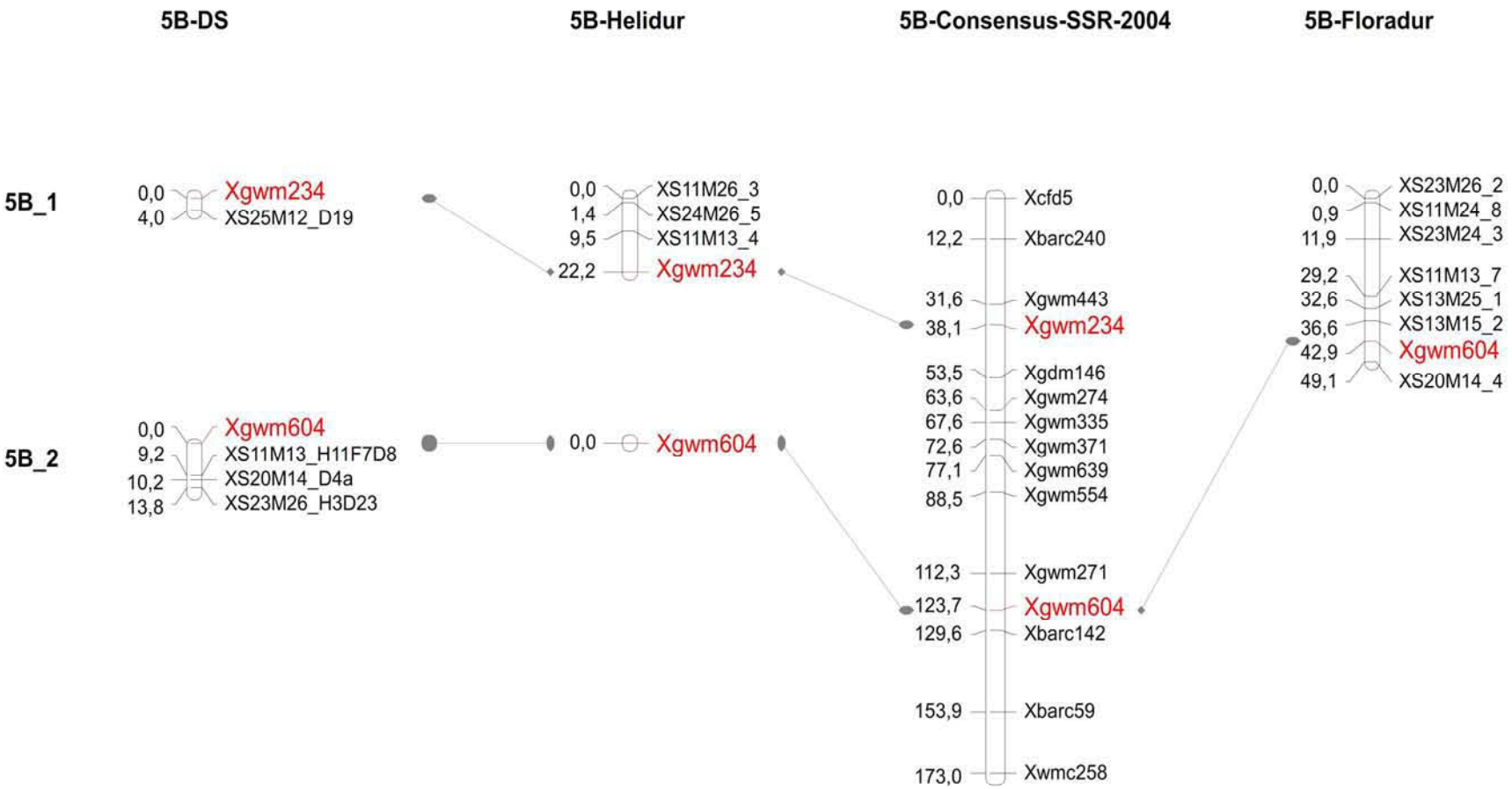
15,9 XS11M15_D9
21,3 XS24M15_D10
0,0 Xbarc57
14,9 XS23M12_H9F8D8
30,9 XS11M26_H1F2D21
46,1 Xgwm779
54,2 Xbarc45
72,3 Xgwm666c
75,5 XS20M15_H1
77,5 XS25M14_H7
78,9 XS18M14_H4
82,4 Xgwm133
84,5 Xbarc67
97,5 XGWM1110
100,1 XGWM1121
114,6 XS11M14_H6F5D13
119,9 XS20M14_H3
133,1 XS25M14_H5
0,0 Xgwm155
17,1 XS24M26_H4
24,0 Xgwm751
26,2 Xgwm666a
45,5 XS11M14_H10F8D10
0,0 Xwmc11
13,8 Xgwm369
36,7 Xbarc45
37,0 Xgwm2
40,2 Xgwm133
44,9 Xgwm5
45,3 Xgwm666a
45,6 Xgwm674
47,1 Xbarc67
72,9 Xbarc69
84,9 Xgwm155
97,0 Xgwm751
99,3 Xwmc169
106,0 Xgwm666b
115,8 Xgwm480
0,0 XS25M14_12
5,3 Xbarc57
16,7 XS20M15_2
32,9 XS11M26_2
59,2 Xgwm779
67,2 Xbarc45
94,0 XS20M15_1
94,9 XS25M14_3
98,1 Xgwm133
104,0 Xbarc67
115,1 Xgwm1110
119,2 Xgwm1121
136,3 XS11M14_5
139,3 XS20M14_2
168,0 XS23M24_1
174,4 Xgwm155
196,7 XS11M14_8
211,1 Xgwm751
219,4 Xgwm666c
232,4 Xgwm666b

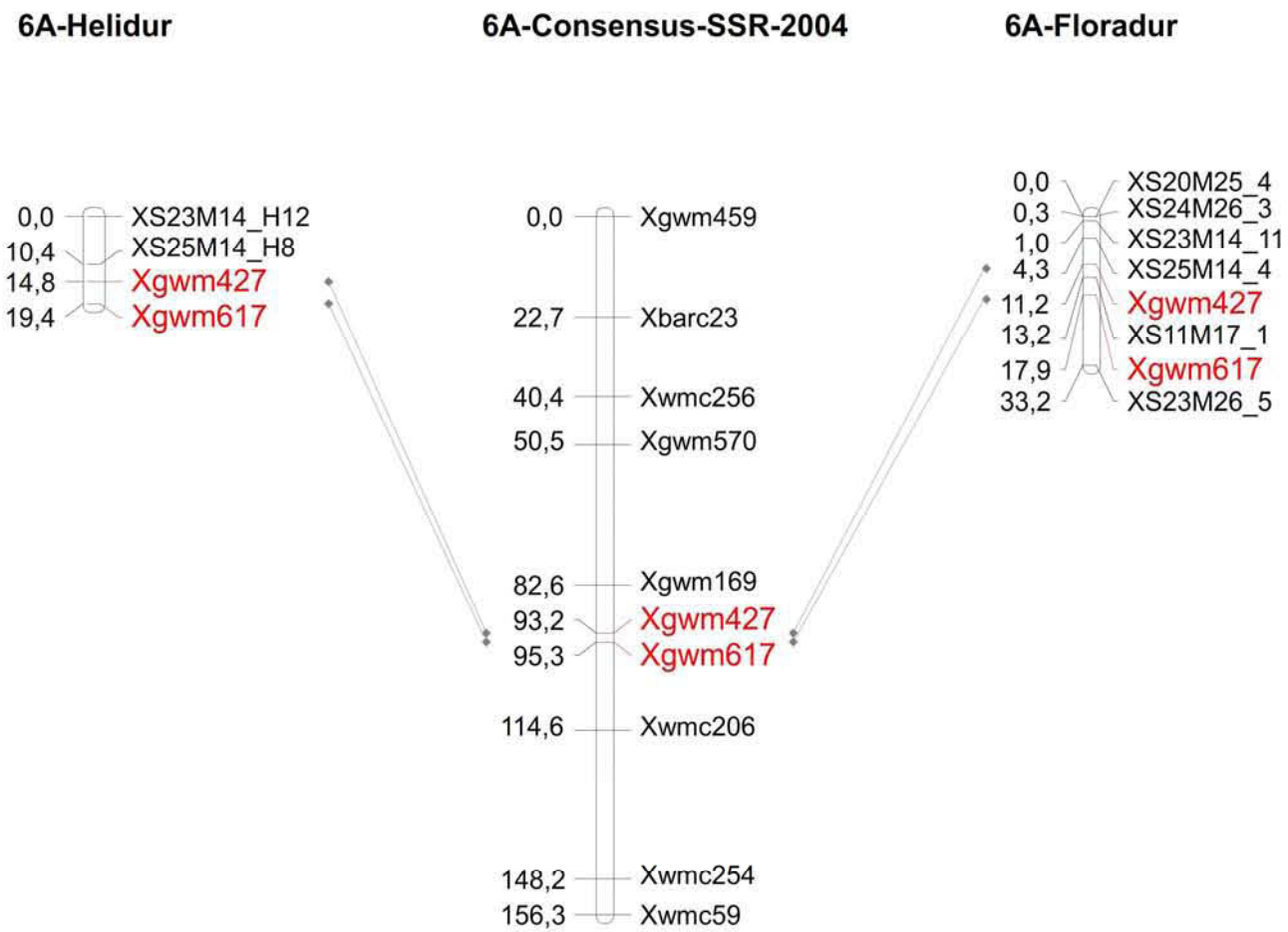

3B-DS
3B-Helidur
3B-Consensus-SSR-2004
3B-Floradur
3B_1
3B_2
0,0 Xgwm1034
14,3 XS24M12_D1
15,8 Xbarc133
18,4 Xbarc147
25,1 Xgwm493
28,4 XS11M14_D27
42,3 XS25M14_D2
57,2 XS11M13_H10F6D9
69,2 XS11M14_D31
69,8 XS23M14_H11D8
71,3 XS24M12_D4
71,9 XS11M17_D1a
76,5 XS11M17_D9c
94,9 XS24M14_H1D20
109,7 Xgwm285
115,5 XS25M12_D16
116,8 XS23M17_D18
119,6 XS11M17_D11a
134,9 XS11M13_D11
151,6 XS13M14_D8
0,0 XS24M12_D13
11,8 XS11M14_D32
13,9 XS11M14_H3F3D20
23,7 Xbarc84
27,1 XS11M17_D5
29,2 XS23M13_D16
0,0 XGWM1034
5,2 XS25M12_H3
8,5 XS24M12_H6
11,0 Xbarc147
19,2 Xgwm493
0,0 Xgwm533
6,2 XS11M13_H10F6D9
20,5 XS23M14_H11D8
31,8 XS23M12_H8
38,7 XS24M14_H1D20
49,9 Xgwm285
63,6 XS11M13_H9
73,8 XS11M26_H6
97,5 Xgwm108a
112,7 XS18M14_H2
113,1 XS11M26_H4F4D18
114,8 XS11M14_H3F3D20
136,4 Xbarc84
138,6 XS23M13_H4
151,7 XS23M26_H5D14
159,7 XS11M15_H7
161,1 XS25M12_H7F7D6
166,4 Xgwm299
174,1 XS25M15_H7
180,0 XS24M12_H4
185,5 Xgwm247
0,0 Xbarc75
0,7 Xgwm389
5,9 Xgwm533.1
7,2 Xbarc147
11,8 Xgwm493
14,2 Xbarc87
30,2 Xgwm533
54,1 Xgwm566
55,9 Xwmc231
56,9 Xbarc68
58,2 Xwmc612
60,9 Xgwm285
63,2 Xgwm376
71,2 Xwmc527
78,7 Xwmc787
89,5 Xwmc291
94,4 Xgwm108
97,1 Xbarc84
111,2 Xbarc77
122,5 Xgwm299
141,5 Xgwm247
142,1 Xwmc261
147,6 Xgwm340
0,0 XS23M13_7
16,7 Xgwm493
22,1 XS24M25_4
23,2 XS24M12_7
25,2 Xbarc147
26,5 Xbarc133
28,6 XS25M12_2
30,9 XS11M24_4
32,9 Xgwm1034
34,9 XS20M25_1
37,4 XS25M19_1
69,7 Xgwm533
79,2 Xgwm60
81,9 XS11M13_6
108,9 XS23M12_5
114,8 XS13M25_4
127,9 Xgwm285
145,9 XS13M24_4
164,6 XS11M26_9
181,7 XS18M14_2
197,0 Xgwm108a
210,2 XS20M25_3
211,1 XS24M25_6
211,8 XS11M26_4
212,6 XS11M14_3
216,8 XS24M12_6
225,6 Xbarc84
230,0 XS23M13_2
235,9 XS11M15_7
243,6 XS25M19_4
244,7 XS25M12_7
252,2 Xgwm299
255,9 XS25M15_5
261,0 XS24M12_4
267,8 Xgwm247

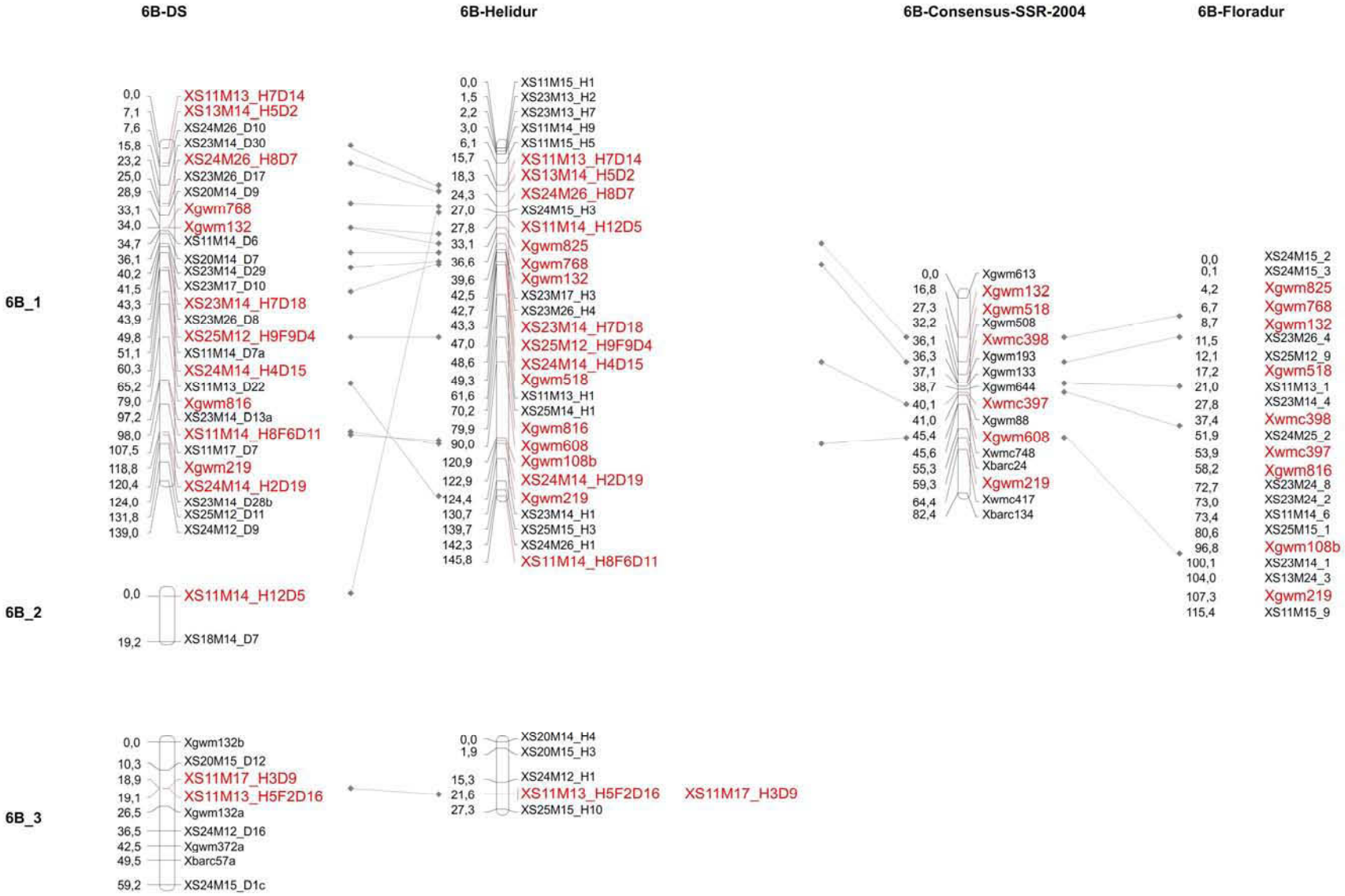

4A-DS
4A-Helidur
4A-Consensus-SSR-2004
4A-Floradur
4A_1
4A_2
4A_3
0,0 Xgwm781
10,8 XS11M26_H5F5D17
13,8 XS24M12_D7
15,5 Xgwm1110
34,5 Xgwm610
52,7 XS23M14_D25
53,6 XS11M13_H3D19
59,7 XS25M14_D6
61,6 XS24M26_H10D4
66,5 Xgwm937
77,6 XS24M14_H3D17
80,7 Xgwm265b
0,0 XS23M12_D5a
8,2 XS23M26_D29
16,7 XS13M14_D9
17,6 XS24M12_D14
31,1 XS11M17_H4F3D6
31,9 XS25M14_D17
0,0 XS13M14_D10
1,4 XS18M12_D21
9,7 XS23M14_D14
10,3 XS11M13_D10
10,9 XS13M14_H1F1D11
12,7 XS11M15_D4a
14,1 XS23M26_D18
17,3 XS20M14_D5a
17,8 XS23M26_D31
21,9 XS11M15_D5
23,4 XS25M12_D13
25,2 XS24M15_D13
25,8 XS23M14_D4
31,5 XS18M14_D1
33,6 XS24M26_H11F6D3
36,7 XS18M12_H1F1D20
0,0 Xgwm781
5,0 XS11M26_H5F5D17
12,3 Xgwm610
20,4 XS11M13_H3D19
27,0 XS24M26_H10D4
37,8 XS24M14_H3D17
49,6 Xgwm265b
64,1 XS25M15_H5
85,6 XS11M17_H4F3D6
0,0 XS13M14_H1F1D11
3,0 XS25M12_H6
8,9 Xgwm160
11,1 XS25M12_H2
14,5 XS24M26_H11F6D3
16,4 XS18M12_H1F1D20
0,0 Xgwm4
2,4 Xgwm165
12,1 Xgwm610
26,6 Xbarc170
46,0 Xwmc161
57,4 Xgwm162
78,9 Xgwm160
87,6 Xwmc219
0,0 XS13M24_1
14,8 Xgwm781
36,5 Xgwm610
0,0 XS25M15_3
17,3 Xgwm937
0,0 XS11M13_5
11,4 XS13M14_1
17,6 XS25M12_5
25,3 XS11M15_5
26,4 XS24M26_6
31,2 Xgwm160
32,0 XS25M12_1
45,3 XS23M12_13

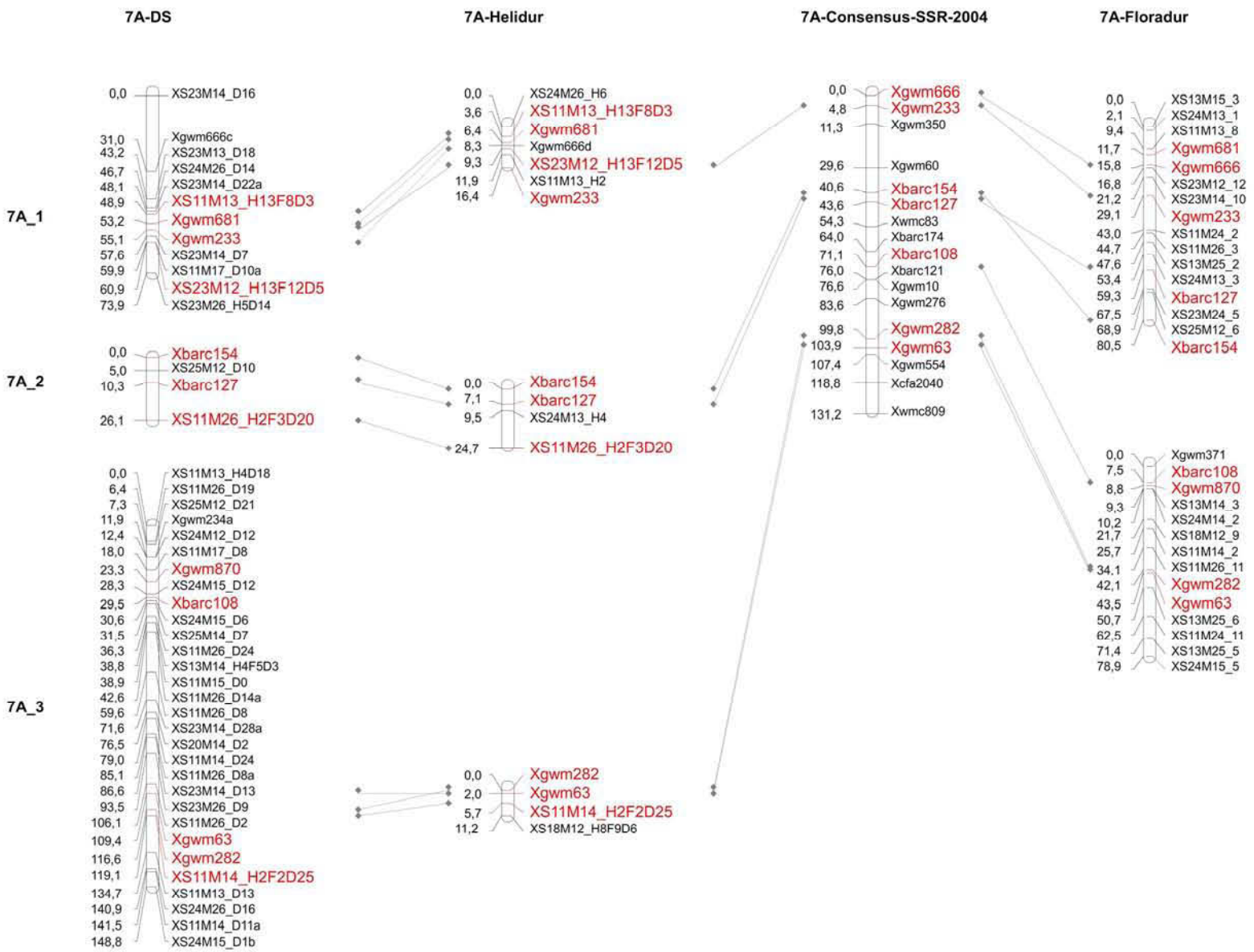

4B-DS
4B-Helidur
4B-Consensus-SSR-2004
4B-Floradur
0,0 XS11M14_H16D1
0,9 XS25M14_D20
5,0 Xgwm888
19,8 XS23M26_D3
28,0 Xgwm608a
33,4 Xgwm107
39,8 XS13M14_D1
55,0 Xgwm149
0,0 Xwmc617
8,1 Xwmc710
29,0 RHT1
36,7 XS25M14_H4
38,8 Xgwm888
42,3 XS11M14_H16D1
50,2 XS23M12_H2
57,9 Xgwm107
72,1 Xgwm149
0,0 Xwmc617
11,2 Xwmc710
17,0 RhtB1
21,6 Xbarc20
22,3 Xgwm368
24,1 Xgwm107
25,0 Xwmc238
28,9 Xgwm192
30,9 Xgwm149
40,6 Xwmc349
59,4 Xwmc125
0,0 RhtB1
12,1 XS25M14_1
19,7 Xgwm888
35,7 Xgwm107
67,2 XS23M24_6
82,7 Xgwm149


5A-DS
5A-Helidur
5A-Consensus-SSR-2004
5A-Floradur
5A_1
5A_2
0,0 XS18M14_D14
10,9 Xbarc316
12,2 XS23M17_D4
14,0 Xgdm109
19,2 XS20M15_D5
35,8 XS23M26_D21
40,4 Xbarc180
41,5 Xgwm129
42,8 Xbarc100a
56,8 XS11M17_D3
70,5 XS23M13_D13
77,3 Xbarc197
88,3 XS23M12_D17
90,3 XS11M14_H13D4
91,0 XS23M26_D28
0,0 Xgwm666a
4,9 XS20M15_D13
16,3 XS23M14_D15
17,1 XS23M26_H6F7D7
41,8 XS11M15_D3
50,3 XS20M15_D4
67,5 XS11M26_D21a
71,3 XS23M17_D6
81,7 Xgwm179
83,6 Xgwm126
90,2 XS23M14_D23
0,0 Xgdm109
10,1 Xbarc316
21,7 Xbarc197
22,7 Xgwm304
24,7 Xgwm129
45,8 Xbarc100
49,3 Xgwm335
51,2 Xgwm156
68,8 Xbarc180b
77,4 XS23M12_H3
81,4 XS11M14_H13D4
0,0 XS18M12_H4
21,1 XS23M26_H6F7D7
0,0 Xgwm179
2,1 Xgwm126
20,8 Xgwm291
0,0 Xbarc10
23,7 Xgwm443
32,8 Xgwm205
34,0 Xgdm109
52,1 Xgwm293
53,2 Xbarc197
54,7 Xgwm415
54,8 Xgwm129
58,0 Xbarc117
59,1 Xbarc186
63,3 Xbarc180
63,5 Xgwm304
66,4 Xgwm96
72,2 Xgwm156
74,0 Xgwm639
76,3 Xgwm617
79,4 Xwmc415
90,3 Xgwm666
110,7 Xbarc232
138,7 Xgwm126
139,8 Xgwm179
150,5 Xgwm595
164,0 Xgwm291
168,5 B1
0,0 Xgwm109
7,6 Xbarc316
10,5 XS20M15_9
16,9 XS23M26_3
22,5 Xgwm129
29,2 Xbarc197
36,4 Xgwm304
0,0 XS23M12_2f
10,2 Xbarc180
18,9 XS24M25_3
22,5 Xgwm335
25,1 XS25M19_3
27,8 Xgwm156
38,1 Xbarc100
0,0 Xgwm179
0,5 Xgwm126
6,4 XS11M24_12
21,1 Xgwm291


5B-DS
5B-Helidur
5B-Consensus-SSR-2004
5B-Floradur
5B_1
5B_2
0,0
4,0
Xgwm234
XS25M12_D19
0,0
9,2
10,2
13,8
Xgwm604
XS11M13_H11F7D8
XS20M14_D4a
XS23M26_H3D23
0,0
1,4
9,5
22,2
XS11M26_3
XS24M26_5
XS11M13_4
Xgwm234
0,0
Xgwm604
0,0
12,2
31,6
38,1
53,5
63,6
67,6
72,6
77,1
88,5
112,3
123,7
129,6
153,9
173,0
Xcfd5
Xbarc240
Xgwm443
Xgwm234
Xgdm146
Xgwm274
Xgwm335
Xgwm371
Xgwm639
Xgwm554
Xgwm271
Xgwm604
Xbarc142
Xbarc59
Xwmc258
0,0
0,9
11,9
29,2
32,6
36,6
42,9
49,1
XS23M26_2
XS11M24_8
XS23M24_3
XS11M13_7
XS13M25_1
XS13M15_2
Xgwm604
XS20M14_4


6A-Helidur
6A-Consensus-SSR-2004
6A-Floradur
0,0
10,4
14,8
19,4
XS23M14_H12
XS25M14_H8
Xgwm427
Xgwm617
0,0
22,7
40,4
50,5
82,6
93,2
95,3
114,6
148,2
156,3
Xgwm459
Xbarc23
Xwmc256
Xgwm570
Xgwm169
Xgwm427
Xgwm617
Xwmc206
Xwmc254
Xwmc59
0,0
0,3
1,0
4,3
11,2
13,2
17,9
33,2
XS20M25_4
XS24M26_3
XS23M14_11
XS25M14_4
Xgwm427
XS11M17_1
Xgwm617
XS23M26_5


6B-DS
6B-Helidur
6B-Consensus-SSR-2004
6B-Floradur
6B_1
6B_2
6B_3
0,0 XS11M13_H7D14
7,1 XS13M14_H5D2
7,6 XS24M26_D10
15,8 XS23M14_D30
23,2 XS24M26_H8D7
25,0 XS23M26_D17
28,9 XS20M14_D9
33,1 Xgwm768
34,0 Xgwm132
34,7 XS11M14_D6
36,1 XS20M14_D7
40,2 XS23M14_D29
41,5 XS23M17_D10
43,3 XS23M14_H7D18
43,9 XS23M26_D8
49,8 XS25M12_H9F9D4
51,1 XS11M14_D7a
60,3 XS24M14_H4D15
65,2 XS11M13_D22
79,0 Xgwm816
97,2 XS23M14_D13a
98,0 XS11M14_H8F6D11
107,5 XS11M17_D7
118,8 Xgwm219
120,4 XS24M14_H2D19
124,0 XS23M14_D28b
131,8 XS25M12_D11
139,0 XS24M12_D9
0,0 XS11M14_H12D5
19,2 XS18M14_D7
0,0 Xgwm132b
10,3 XS20M15_D12
18,9 XS11M17_H3D9
19,1 XS11M13_H5F2D16
26,5 Xgwm132a
36,5 XS24M12_D16
42,5 Xgwm372a
49,5 Xbarc57a
59,2 XS24M15_D1c
0,0 XS11M15_H1
1,5 XS23M13_H2
2,2 XS23M13_H7
3,0 XS11M14_H9
6,1 XS11M15_H5
15,7 XS11M13_H7D14
18,3 XS13M14_H5D2
24,3 XS24M26_H8D7
27,0 XS24M15_H3
27,8 XS11M14_H12D5
33,1 Xgwm825
36,6 Xgwm768
39,6 Xgwm132
42,5 XS23M17_H3
42,7 XS23M26_H4
43,3 XS23M14_H7D18
47,0 XS25M12_H9F9D4
48,6 XS24M14_H4D15
49,3 Xgwm518
61,6 XS11M13_H1
70,2 XS25M14_H1
79,9 Xgwm816
90,0 Xgwm608
120,9 Xgwm108b
122,9 XS24M14_H2D19
124,4 Xgwm219
130,7 XS23M14_H1
139,7 XS25M15_H3
142,3 XS24M26_H1
145,8 XS11M14_H8F6D11
0,0 XS20M14_H4
1,9 XS20M15_H3
15,3 XS24M12_H1
21,6 XS11M13_H5F2D16
27,3 XS25M15_H10
XS11M17_H3D9
0,0 Xgwm613
16,8 Xgwm132
27,3 Xgwm518
32,2 Xgwm508
36,1 Xwmc398
36,3 Xgwm193
37,1 Xgwm133
38,7 Xgwm644
40,1 Xwmc397
41,0 Xgwm88
45,4 Xgwm608
45,6 Xwmc748
55,3 Xbarc24
59,3 Xgwm219
64,4 Xwmc417
82,4 Xbarc134
0,0 XS24M15_2
0,1 XS24M15_3
4,2 Xgwm825
6,7 Xgwm768
8,7 Xgwm132
11,5 XS23M26_4
12,1 XS25M12_9
17,2 Xgwm518
21,0 XS11M13_1
27,8 XS23M14_4
37,4 Xwmc398
51,9 XS24M25_2
53,9 Xwmc397
58,2 Xgwm816
72,7 XS23M24_8
73,0 XS23M24_2
73,4 XS11M14_6
80,6 XS25M15_1
96,8 Xgwm108b
100,1 XS23M14_1
104,0 XS13M24_3
107,3 Xgwm219
115,4 XS11M15_9


7A-DS
7A-Helidur
7A-Consensus-SSR-2004
7A-Floradur
7A_1
7A_2
7A_3
0,0 XS23M14_D16
31,0 Xgwm666c
43,2 XS23M13_D18
46,7 XS24M26_D14
48,1 XS23M14_D22a
48,9 XS11M13_H13F8D3
53,2 Xgwm681
55,1 Xgwm233
57,6 XS23M14_D7
59,9 XS11M17_D10a
60,9 XS23M12_H13F12D5
73,9 XS23M26_H5D14
0,0 Xbarc154
5,0 XS25M12_D10
10,3 Xbarc127
26,1 XS11M26_H2F3D20
0,0 XS11M13_H4D18
6,4 XS11M26_D19
7,3 XS25M12_D21
11,9 Xgwm234a
12,4 XS24M12_D12
18,0 XS11M17_D8
23,3 Xgwm870
28,3 XS24M15_D12
29,5 Xbarc108
30,6 XS24M15_D6
31,5 XS25M14_D7
36,3 XS11M26_D24
38,8 XS13M14_H4F5D3
38,9 XS11M15_D0
42,6 XS11M26_D14a
59,6 XS11M26_D8
71,6 XS23M14_D28a
76,5 XS20M14_D2
79,0 XS11M14_D24
85,1 XS11M26_D8a
86,6 XS23M14_D13
93,5 XS23M26_D9
106,1 XS11M26_D2
109,4 Xgwm63
116,6 Xgwm282
119,1 XS11M14_H2F2D25
134,7 XS11M13_D13
140,9 XS24M26_D16
141,5 XS11M14_D11a
148,8 XS24M15_D1b
0,0 XS24M26_H6
3,6 XS11M13_H13F8D3
6,4 Xgwm681
8,3 Xgwm666d
9,3 XS23M12_H13F12D5
11,9 XS11M13_H2
16,4 Xgwm233
0,0 Xbarc154
7,1 Xbarc127
9,5 XS24M13_H4
24,7 XS11M26_H2F3D20
0,0 Xgwm282
2,0 Xgwm63
5,7 XS11M14_H2F2D25
11,2 XS18M12_H8F9D6
0,0 Xgwm666
4,8 Xgwm233
11,3 Xgwm350
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40,6 Xbarc154
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15,8 Xgwm666
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21,7 XS18M12_9
25,7 XS11M14_2
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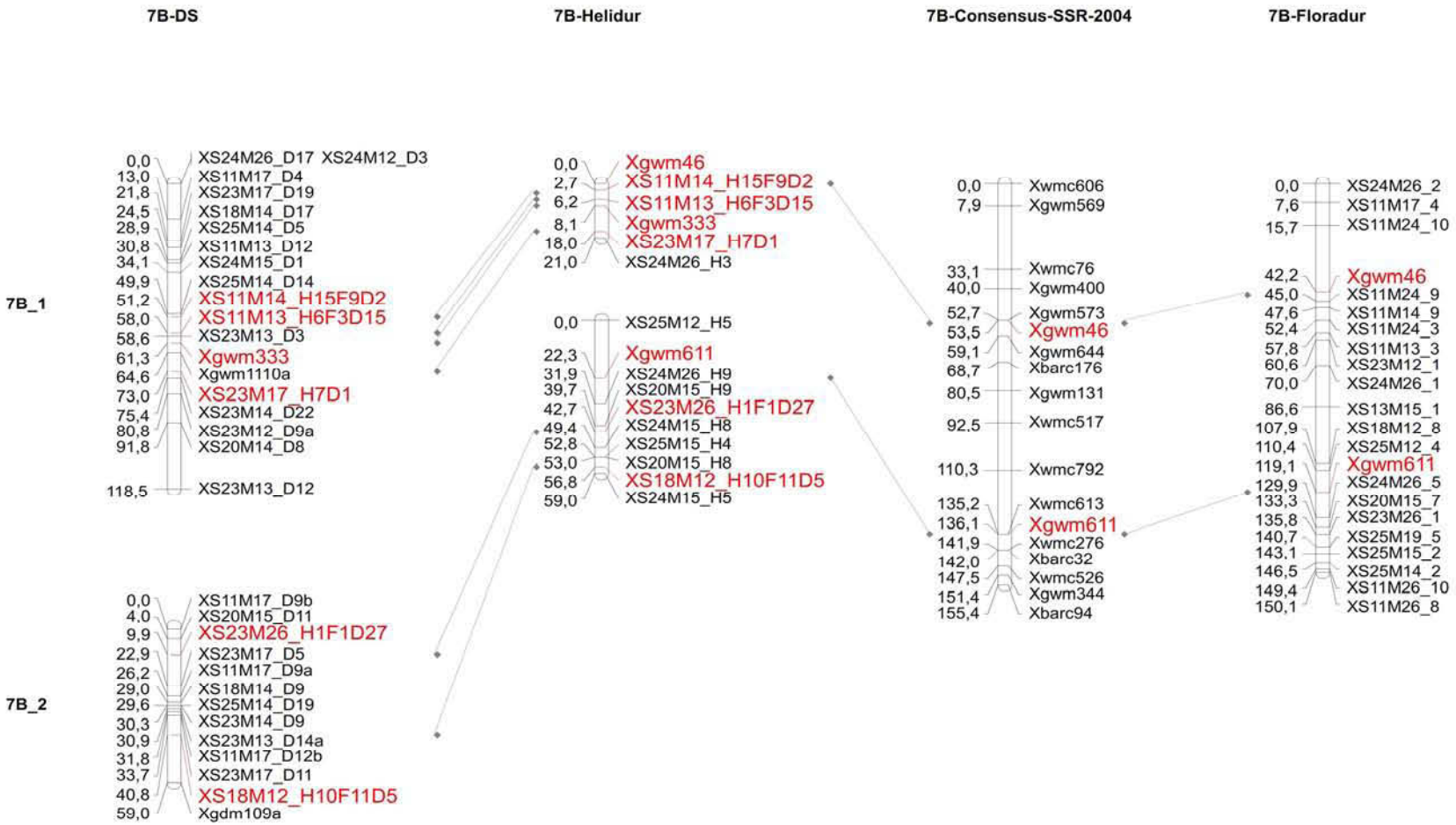
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7B-Consensus-SSR-2004
7B-Floradur
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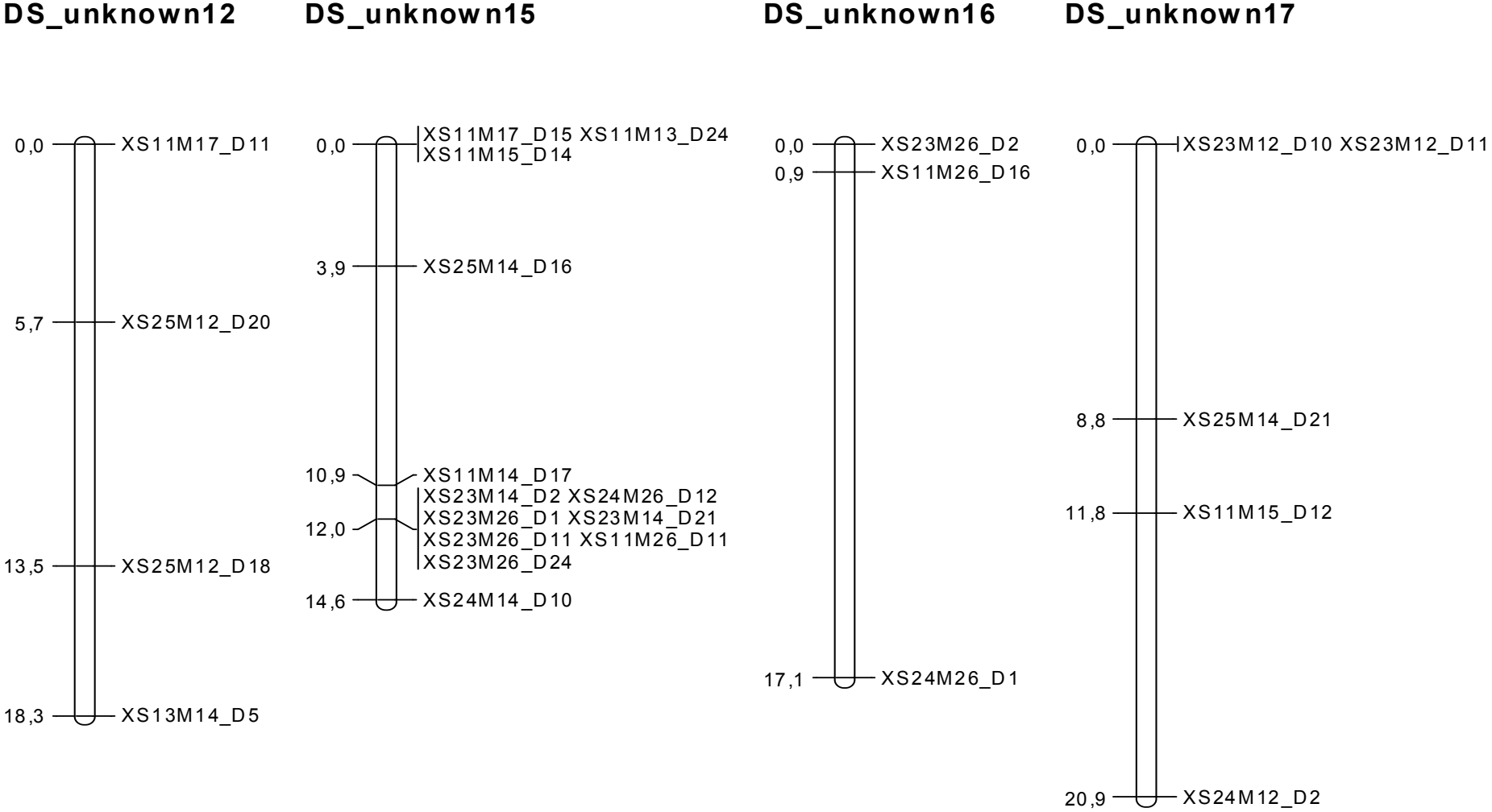
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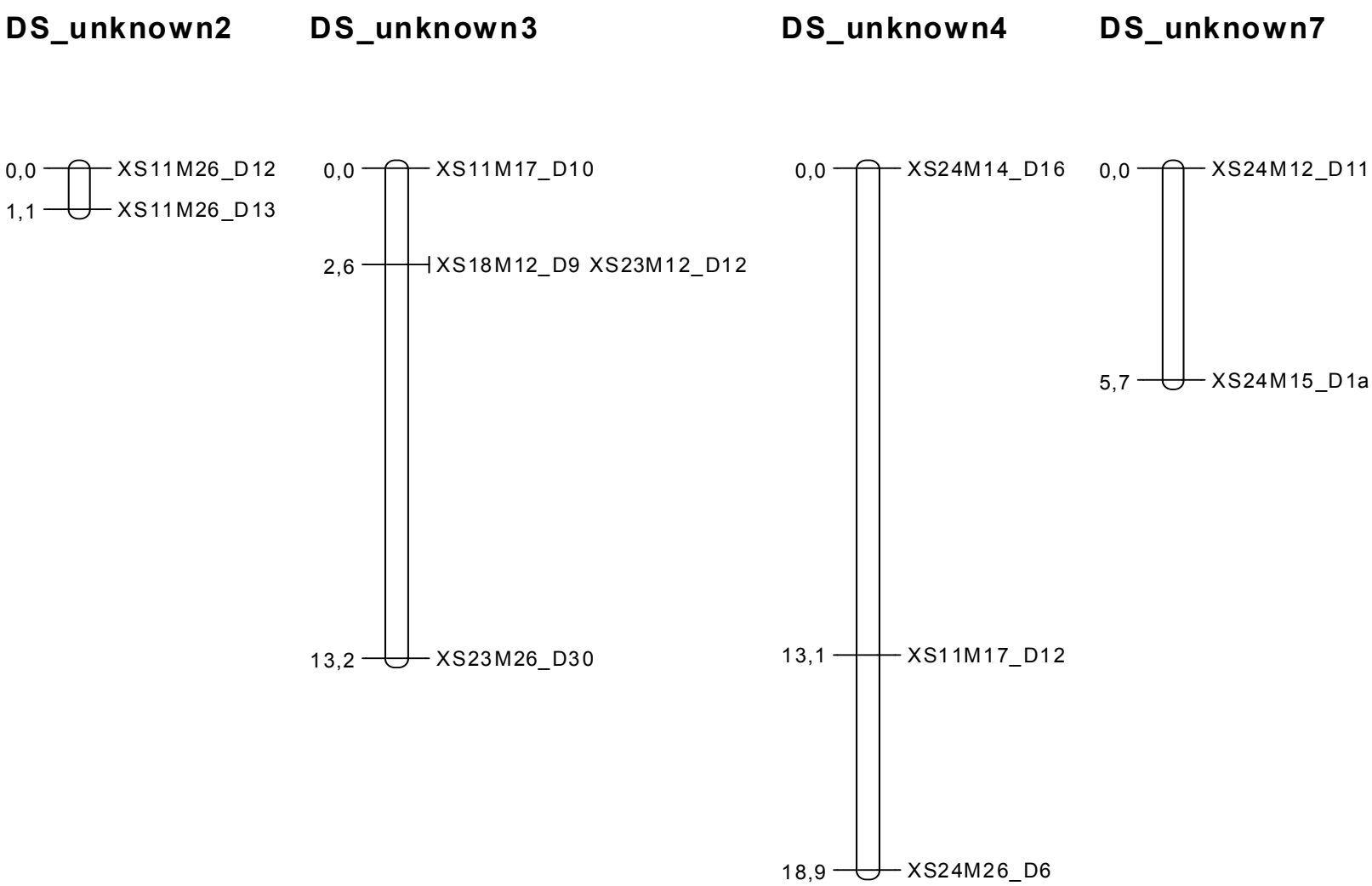
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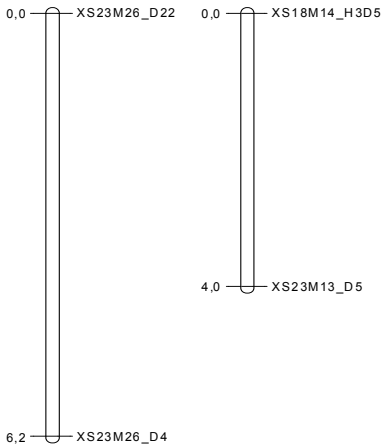

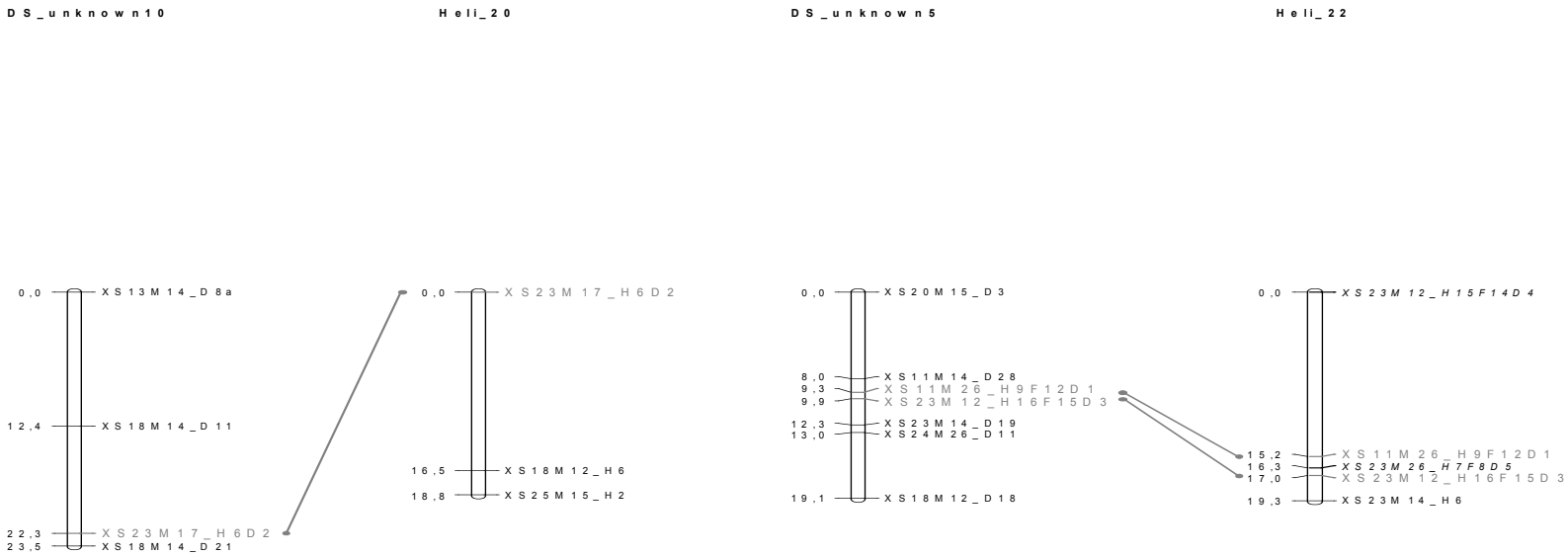

Figure 7: Genetic linkage map of the populations *DS-131621* x *T. dicoccum*, *T. dicoccum 161* x Helidur and *T. dicoccum 161* x Floradur compared to the reference map (wheat consensus map, Somers et al. 2004). Marker loci are listed to the right and centi Morgan (cM) distances are shown to the left.

3.3. FHB QTL analysis

The QTL analysis for FHB resistance is carried out by SIM (Simple Interval Mapping) and using qgene.
Unknown AFLP markers (18.38 polymorphic bands per primer in average) are assigned to known SSR. All in all 52 linkage groups with 386 AFLP markers and 69 SSR markers are constructed. 6 AFLP markers could not be linked to any group and remained unassigned.

QTL analysis by SIM detected 8 loci for Fusarium head blight resistance, 4 loci for *Fusarium culmorum* resistance and 4 loci for *Fusarium graminearum* resistance (Table 11).

Two loci for *Fusarium culmorum* resistance are on chromosome 4B; one is on linkage group 1A_1 and a QTL on linkage group 6B_3.
The QTL on 4B is only well expressed in the year 2008 (LOD= 2,9) and in the mean over the years 2005-2008 (LOD= 1,2 and accordingly 1,8). In the years 2006 and 2007 no significant QTL could be detected (Figure 10).
The QTL which might be on linkage group 6B_3 is well detected over all the years (2006: LOD=1,4 ; 2008: LOD=2,5) except in the year 2007. In average the QTL has a LOD score of 2,4 over the years 2005-2008 (Figure 9).The fourth QTL for *Fusarium culmorum* resistance could be detected on linkage group 1A_1 but only in the year 2007 (LOD=3,7). In the other years no significant QTL could be detected (Figure 8).
There are also four loci for *Fusarium graminearum* resistance two on chromosome 4B, one on 6B and one on the linkage group 3B_1.
The QTL on 4B is well expressed in the years 2005 (LOD=4,7 and 4,5), 2006 (LOD=1,8) and 2008 (LOD= 1,7 and 2) and in the mean over the years 2005-2008 (LOD= 4,4 and accordingly 2,4) (Figure 10).
In the years 2004 and 2007 no significant QTL could be detected.
The QTL on linkage group 6B_3, was well detected in the years 2006 (LOD=3) and 2008 (LOD=1,3). In average the QTL has a LOD score of 1,5 over the years 2004-2008 (Figure 9).
The fourth QTL for *Fusarium graminearum* could be detected on linkage group 3B_1 but only in the year 2005 (LOD=3,2). In the other years no QTL could be detected (Figure 11).
The additive effect, the LOD score and the coefficients of determination of each QTL are presented in the Tables below (Table 11).

Table 11: QTL identified with simple interval mapping (SIM) for FHB resistance measured by area under progress curve (AUDPC). For each QTL the closest markers, the chromosome, the additive effect, the logarithm of odds (LOD) and the percent of phenotypic variance (R2) are given. A positive additive effect indicates that T. dicoccoides contributed the resistant allele.

		F. culmorum														
		average mean			mean			2006			2007			2008		
Marker	**Chr**	**add**	**LOD**	**R²**	**add**	**LOD**	**R²**	**add**	**LOD**	**R²**	**add**	**LOD**	**R²**	**add**	**LOD**	**R²**
XS11M14_H16D11 – XS23M26_D3	4B	32,575	1,269	0,048	-11,846	0,079	0,003	-14,9	0,064	0,002	-27,745	0,404	0,017	16,7	0,105	0,004
Xgwm608a_ - Xgwm149	4B	35,383	1,813	0,068	27,084	0,473	0,018	-43,21	0,633	0,024	-36,79	0,905	0,038	86,26	**2,97**	0,109
XS20M15_D12 - Xgwm132a	6B_3	47,307	**2,423**	0,09	70,09	**2,707**	0,1	72,52	1,451	0,055	29,496	0,4	0,017	85,13	**2,53**	0,094
Xgwm357 - Xgwm164	1A_1										72,05	**3,718**	0,147			

		F.graminearum														
		mean			2004			2005			2006			2007			2008		
Marker	**Chr**	**add**	**LOD**	**R²**	**add**	**LOD**	**R²**	**add**	**LOD**	**R²**	**add**	**LOD**	**R²**	**add**	**LOD**	**R²**	**add**	**LOD**	**R²**
XS11M14_H16D11 – XS23M26_D3	4B	57.95	**4.42**	0.158	-7.07	0.14	0.02	130.01	**4.73**	0.17	51.43	1.86	0.07	4.14	0.18	0.01	27.56	1.74	0.07
Xgwm608a_ - Xgwm149	4B	40.58	**2.46**	0.091	42.63	0.24	0.03	120.55	**4.51**	0.16	37.97	1.09	0.04	-5.85	0.35	0.02	27.71	**2.08**	0.08
XS20M15_D12 - Xgwm132a	6B_3	41.69	1.51	0.057	89.11	0.75	0.08	68.07	0.84	0.03	72.74	**3.02**	0.11	1.90	0.28	0.01	29.54	1.38	0.05
XS11M17_D11a – XS13M14_D8	3B_1							131.75	**3.24**	0.12									

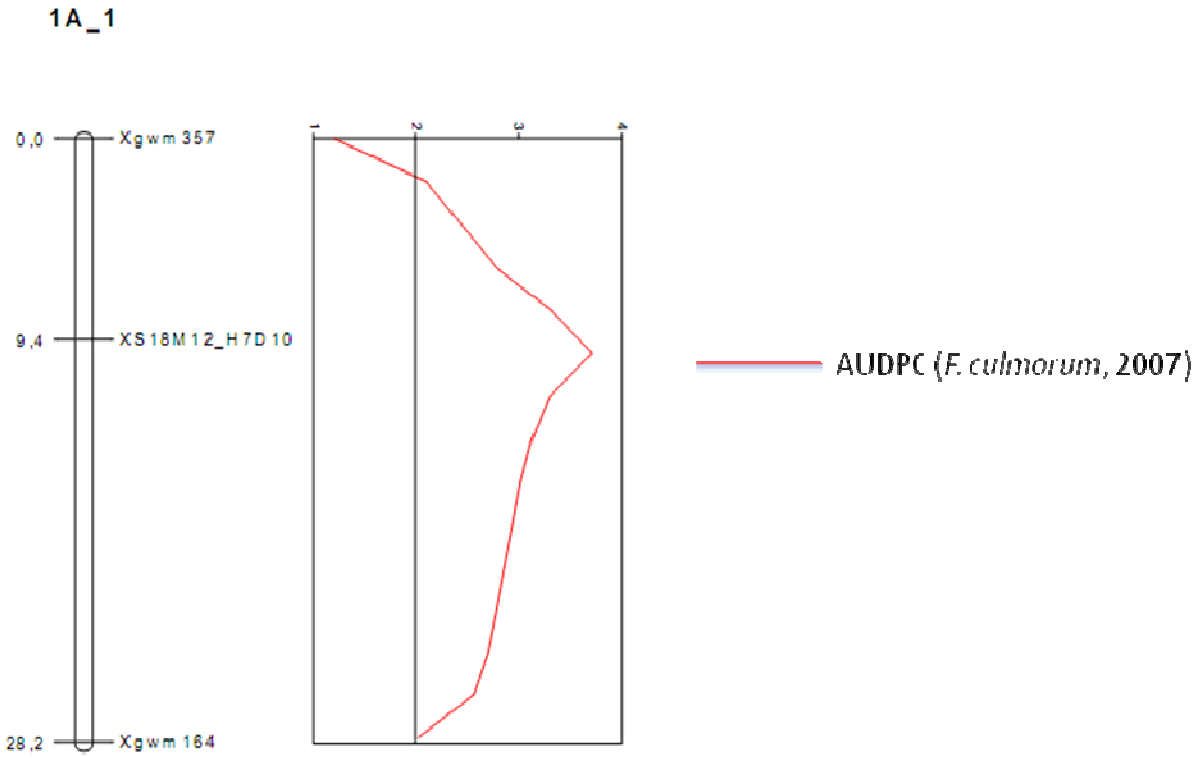

Figure 8: LOD curve for FHB resistance QTL measured by AUDPC in the 2007 experiment

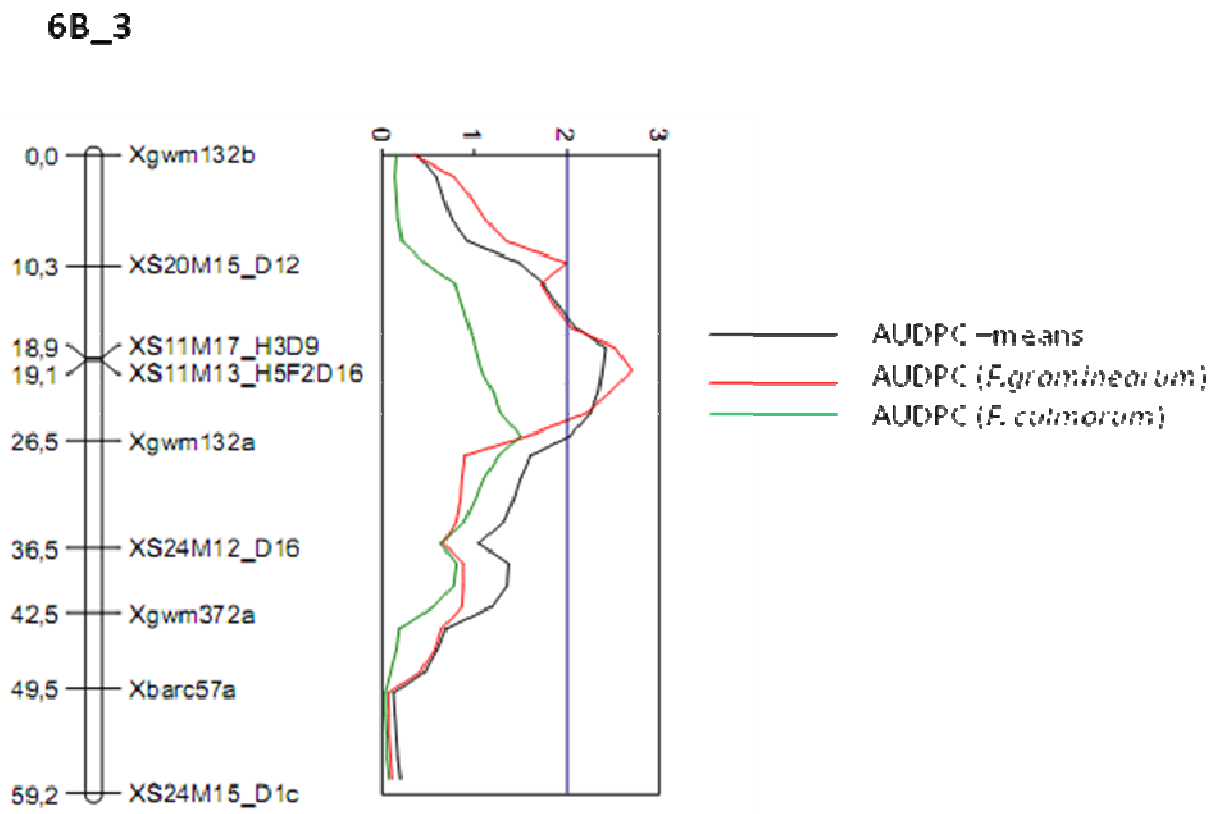

Figure 9: LOD curves for FHB resistance QTL on linkage group 6B_3 measured by AUDPC over all experiments, means from the *F.graminearum* inoculated experiments and the means from the *F. culmorum* inoculated experiments.

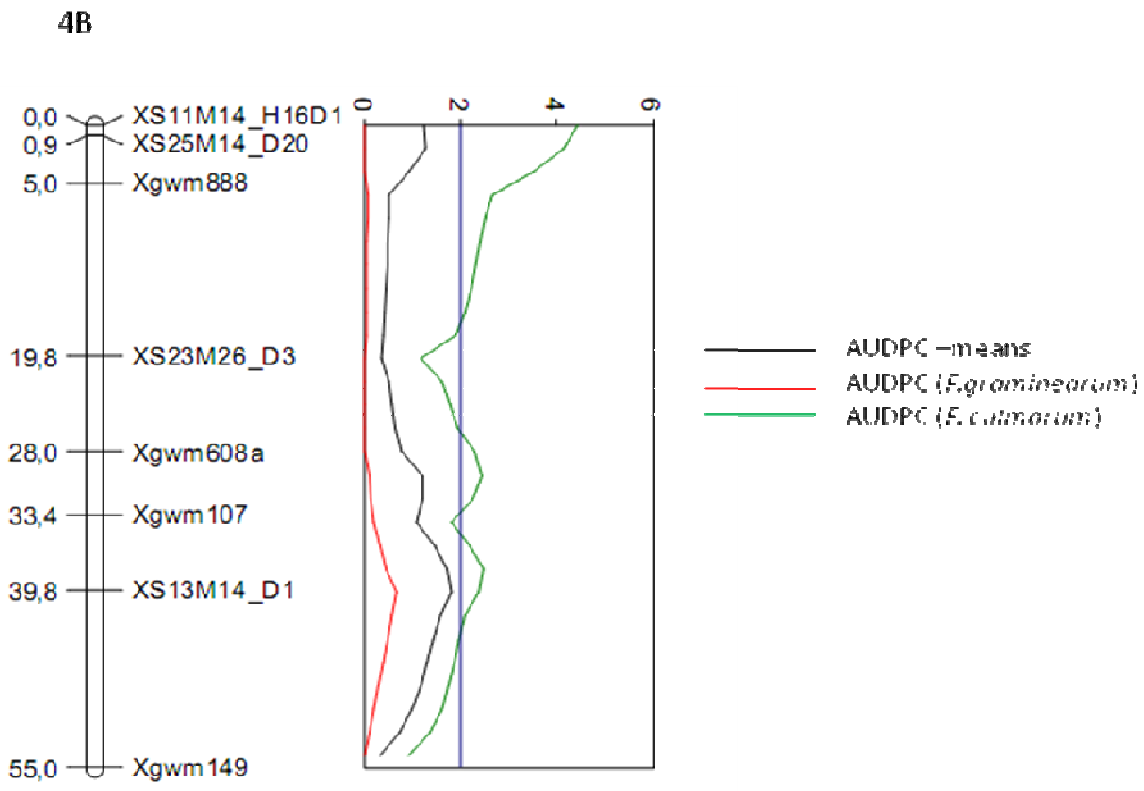

Figure 10: LOD curves for FHB resistance QTL on linkage group 4B measured by AUDPC over all experiments, means from the *F.graminearum* inoculated experiments and the means from the *F. culmorum* inoculated experiments.

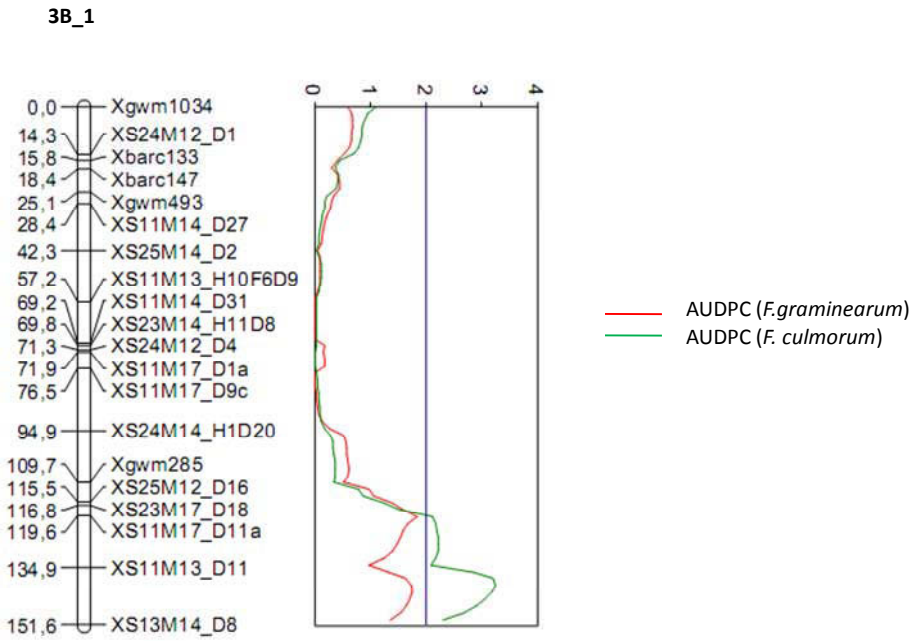

Figure 11: LOD curves for FHB resistance QTL on linkage group 3B_1 measured by AUDPC over all experiments, means from the *F.graminearum* inoculated experiments and the means from the *F. culmorum* inoculated experiments.

3.4 QTL analysis of the morphological traits

The QTL analysis for the morphological traits like ear compactness, ear length, waxiness, plant height, awn length, chlorosis and date of anthesis is also carried out with the same markers as described above.
For all morphological traits QTL are found. All QTL are identified with SIM and CIM between the years 2004 till 2008.

Ear compactness

One QTL is detected on the linkage group 5A_2 with the AFLP marker XS20M15_D4 and the SSR marker Xgwm179. With a LOD score of 4,7 this QTL explains 16,8% of the phenotypic variation (Figure 19).

Ear length

For the ear length four QTL are detected.
Two of the QTL are found on linkage group 4B, with a LOD score of 4,89 (XS11M14_H16D1- XS23M26_D3) and 4,88 (XS23M26_D3 - XS13M14_D1) (Figure 17) which explains 17,4% and 17,3% of the phenotypic variation.

The other two QTL are found on linkage group 5A_2 and 7A_1.
The QTL on linkage group 5A_2, localised by the markers XS20M15_D4 – Xgwm179 (Figure 19) explains a phenotypic variation of 12,4% with a LOD score of 3,4.
With a LOD score of 2,33 the QTL on the 7A_1 linkage group is detected by the Xgwm666c_1 – XS25M26_D14 marker (Figure 22).

Awn length

Five QTL associated with awn length are detected. The highest QTL is mapped on the linkage group 4A_1 with a LOD score of 5,3 which explains a phenotypic variation of 18,8%.
The SSR markers Xgwm1110 and Xgwm781 flanked this region (Figure 15).
On the same linkage group another QTL is detected by the markers Xgwm937 and XS23M14_D25 (Figure 15). With a LOD score of 2,3 it reaches a phenotypic variation of

8,7%.The QTL in the region between the markers XS18M12_H10F11D5 and Xgdm109a is detected on linkage group 7B_2 with a LOD score of 2,8 and a coefficient of determination of 10,5% (Figure 24).
The fourth QTL is found on linkage group 7A_1 with a LOD score of 5,29 which explains 18,7% of the phenotypic variation (Figure 22).
On linkage group 3B_1 another QTL is found with a LOD of 2,7 between the AFLP markers XS24M14_H1D20 and XS11M13_D11 (Figure 14).

Waxiness

Two QTL for the trait of waxiness are detected. One on linkage group 1A_2 with a LOD score of 2,6, flanked by the AFLP markers XS18M14_7 and XS23M14_26 (Figure 12). The other QTL is found on chromosome 1B with a LOD of 2,4 (Figure 13).

Chlorosis

Two QTL are found for chlorosis using the SIM and CIM method. The highest QTL on linkage group 5A_1 with a LOD score of 3,3 and a coefficient of determination of 12,4% is found with the barc markers Xbarc180 and Xbarc100a (Figure 18).
The second QTL with a LOD score of 2,6 is located on linkage group 5B_1 (Figure 20).

Date of anthesis

For this trait seven QTL are detected.
Three of them are located on linkage group 7B_1 and flanked by AFLP markers. The most significant QTL region with a LOD score of 4 and R^2 of 14,5% is followed by a QTL with a LOD of 3,8 and R^2 of 13,9%. The last one on linkage group 7B_1 is a QTL with a LOD of 2,4 (Figure 23).
On linkage group 5A_2 one QTL is detected with a LOD of 4,1 which explains a phenotypic variation of 14,8% (Figure 19).
The markers XS20M15_12 and Xgwm132a flanked a region on linkage group 6B_3 with a LOD of 2,5 (Figure 21).
Also one QTL on linkage group 4A_2 is detected with a LOD of 3,9 and a coefficient of determination of 14,2% (Figure 16).

The seventh QTL is located between the AFLP markers XS11M17_D9c and XS13M14_D8 with a LOD of 2,35 (Figure 14).

Plant height

For the trait plant height two QTL are found both on the linkage group 4B.
Both QTL are assigned with high LOD scores. One with a LOD of 17,1 which explains 48.8% of the phenotypic variation and the other with a LOD score of 6,5 and a R^2 of 22,7% (Figure 17).

Table 12: QTL of the morphological traits ear compactness, ear length, awn length, waxiness, chlorosis, day of anthesis and plant height located with SIM. Marker, chromosomal location, additive effects, LOD value and percent of the phenotypic variation are listed

			SIM		
Trait	**Marker**	**Chr**	**add**	**LOD**	**R^2**
Ear compactness (means)$_1$	XS20M15_D4 – Xgwm179	5A_1	0,74	4,7	0,16
Ear length (means)$_2$	XS11M14_H16D1- XS23M26_D3	4B	0,82	4,8	0,17
	XS23M26_D3 - XS13M14_D1	4B	0,73	4,8	0,17
	XS20M15_D4 – Xgwm179	5A_2	0,75	3,4	0,12
	Xgwm666c_1 – XS25M26_D14	7A_1	-0,57		0,08
Awn length (means)$_3$	Xgwm937 – XS23M14_D25	4A_1	0,39	2,3	0,08
	Xgwm1110 - Xgwm781	4A_1	0,59	5,3	0,18
	XS18M12_H10F11D5 -Xgdm109a	7B_2	-2,22	2,8	0,1
	Xgwm666c_1 – XS24M26_D14	7A_1	0,52	5,2	0,18
	XS24M14_H1D20 - XS11M13_D11	3B_1	0,45	2,7	0,1
Waxiness (means)$_4$	XS18M14_D12 – XS23M14_D26	1A_2	-0,29	2,6	0,09
	XS11M13_H14F9D2- XS23M12_D6	1B	0,3	2,3	0,08

Chlorosis (means)[5]	Xgwm234 – XS25M12_D19	5B_1	-0,53	2,6	0,09
	Xbarc180 - Xbarc100a	5A_1	-0,53	3,3	0,12
Day of anthesis (means)[6]	XS23M17_D6 – XS23M14_D23	5A_2	0,95	4,1	0,14
	XS20M15_D12 - Xgwm132a	6B_3	-0,81	2,5	0,09
	XS23M13_D12 – XS20M14_D8	7B_1	-0,67	2,4	0,09
	XS23M17_H7D1 – XS11M13_H6F3D15	7B_1	-0,89	3,8	0,13
	XS11M13_H6F3D15 – XS25M14_D14	7B_1	-0,9	4	0,14
	XS13M14_D10 – XS18M12_D21	4A_2	-0,89	3,9	0,14
	XS11M17_D9c - XS13M14_D8	3B_1	-0,69	2,3	0,08
Plant height (means)[7]	XS11M14_H16D1- XS23M26_D3	4B	-13,52	17,1	0,48
	XS23M26_D3 - XS13M14_D1	4B	-8,73	6,5	0,22

[1]Positive add values indicate that the T. dicoccum alleles induce less compact ears
[2]Positive add values indicate that the T. dicoccum alleles induce longer ears
[3]Positive add values indicate that the T. dicoccum alleles induce shorter awns
[4]Positive add values indicate that the T. dicoccum alleles induce more waxiness
[5]Positive add values indicate that the T. dicoccum alleles do not induce leaf chlorosis
[6]Positive add values indicate that the T. dicoccum alleles induce later flowering
[7]Positive add values indicate that the T. dicoccum alleles induce higher plants

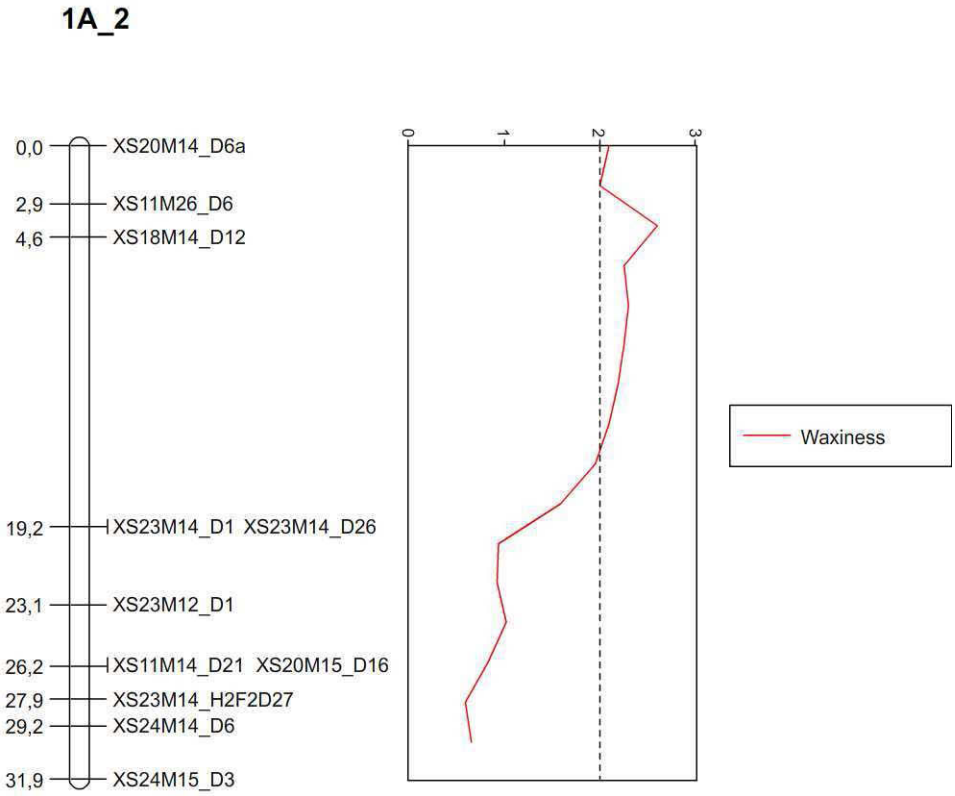

Figure 12: LOD curve for waxiness QTL on linkage group 1A_2.

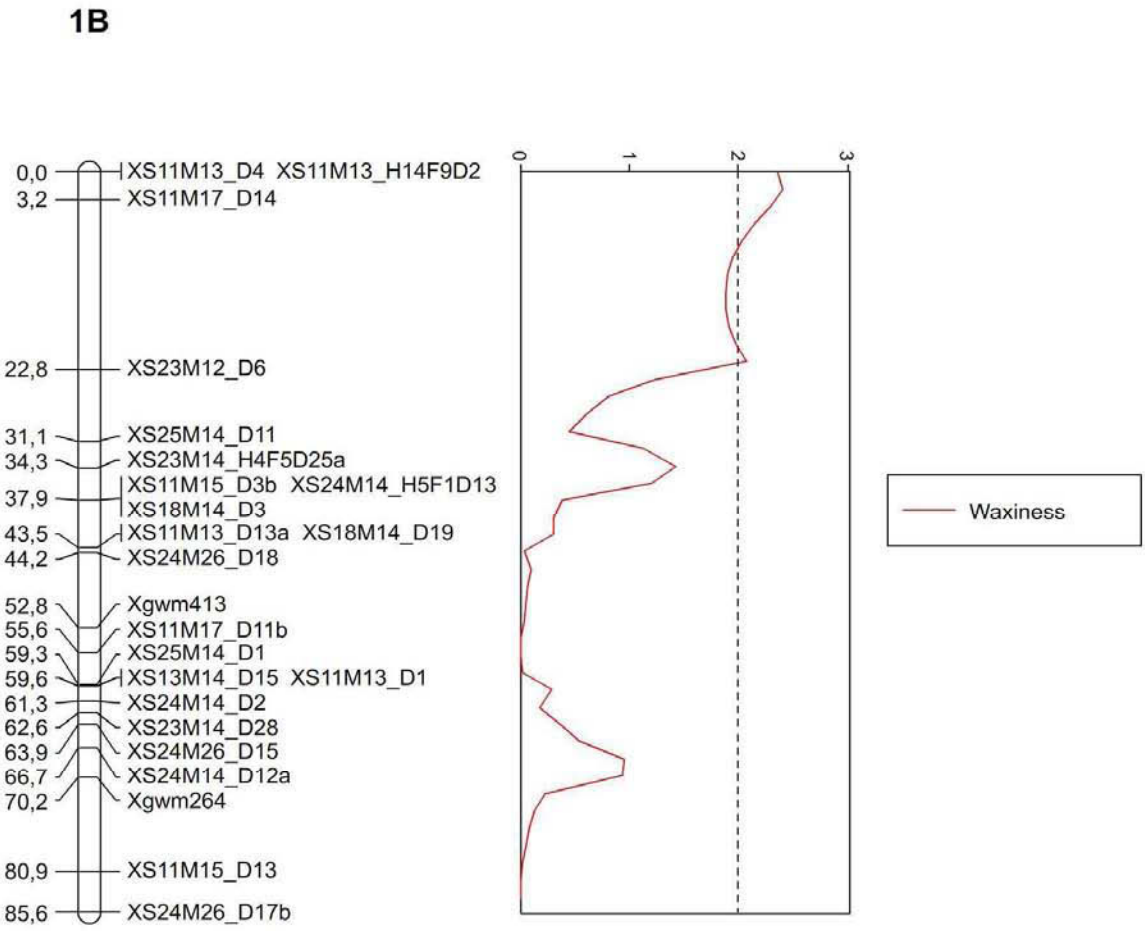

Figure 13: LOD curve for waxiness QTL on linkage group 1B.

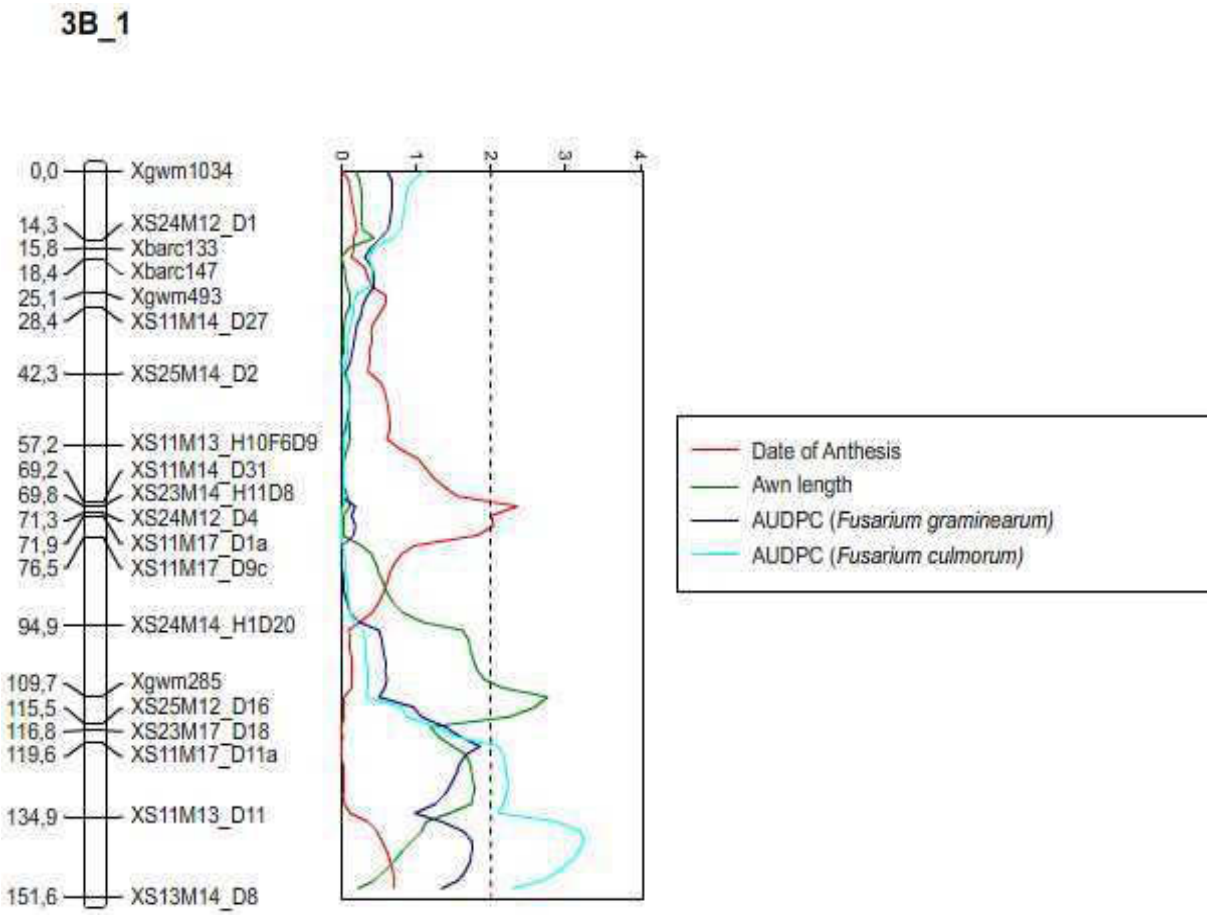

Figure 14 : LOD curves for of anthesis, awn length and FHB resistance QTL on linkage group 3B_1. FHB resistance QTL on linkage group 3B_1 measured by AUDPC from *F.culmorum* inoculated experiments and means from the *F.graminearum* inoculated experiments.

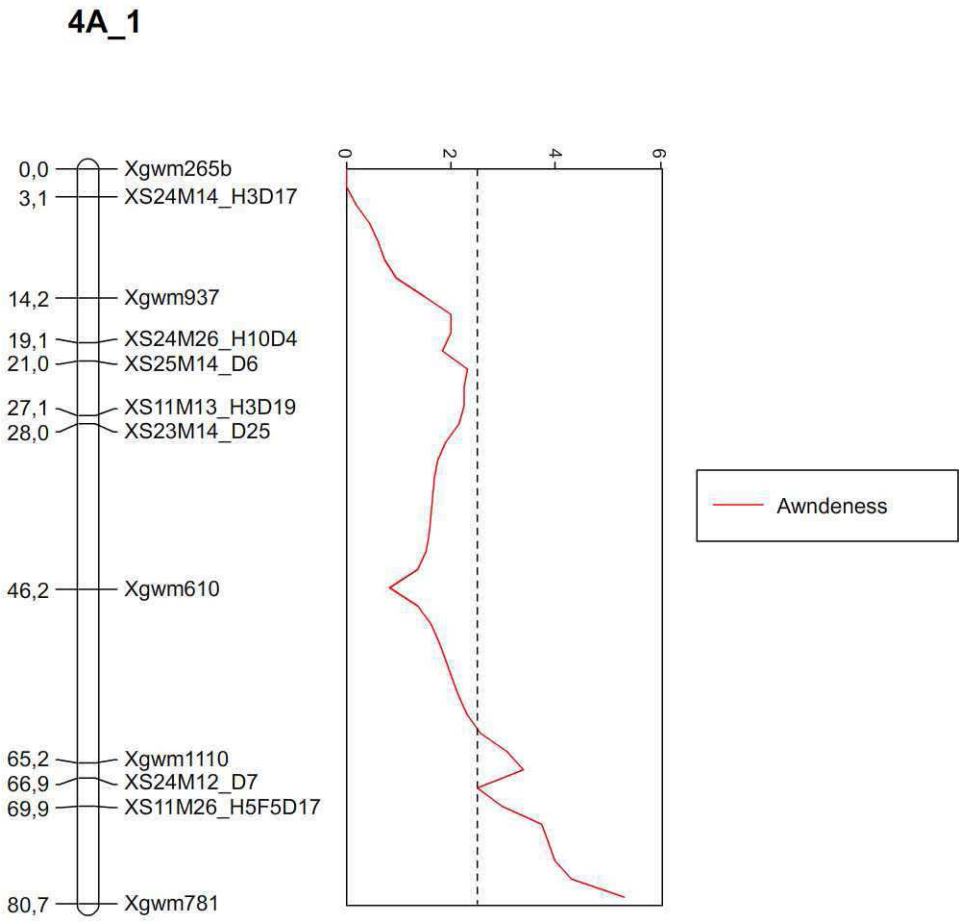

Figure 15: LOD curve for awn length QTL on linkage group 4A_1.

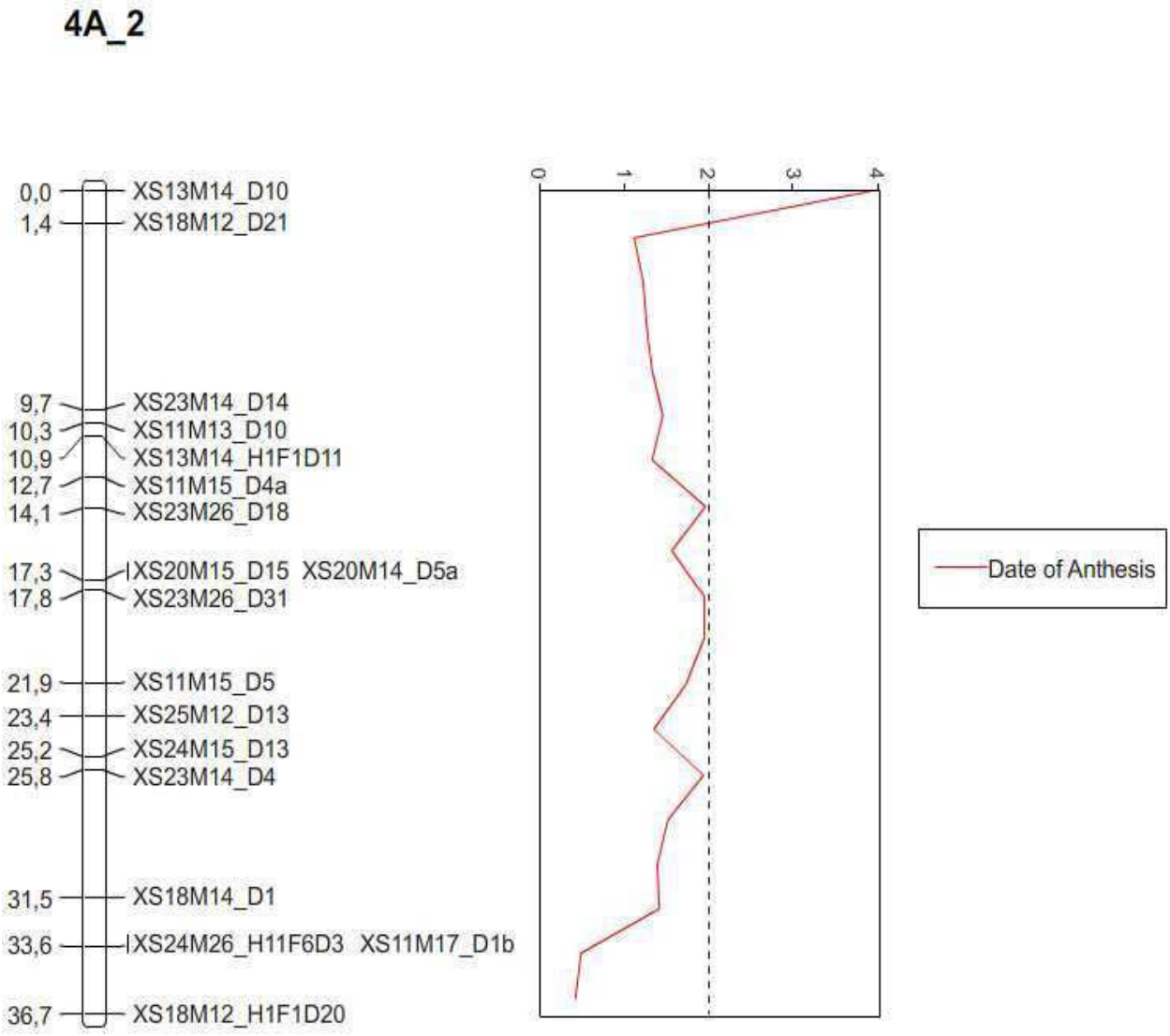

Figure 16: LOD curve for anthesis QTL on linkage group 4A_2.

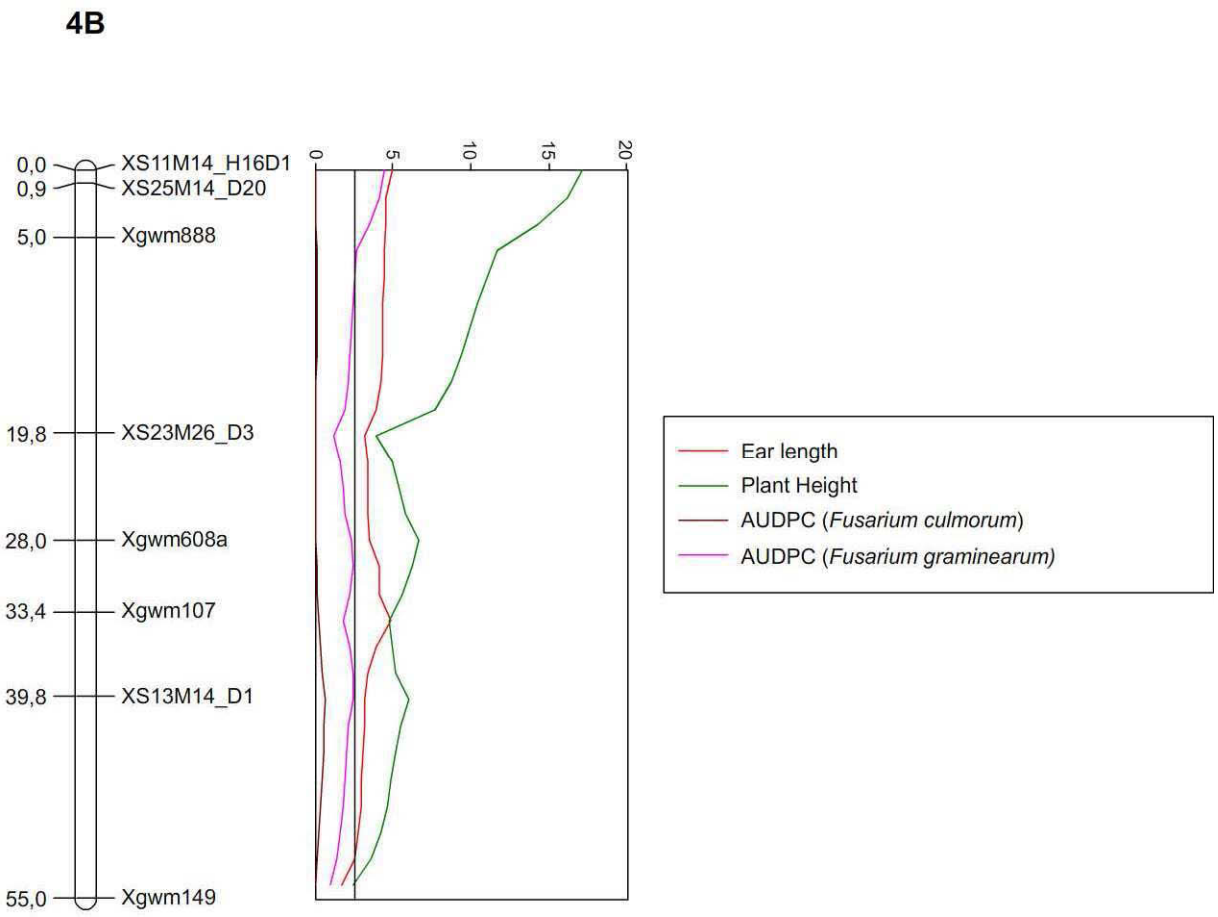

Figure 17: LOD curves for ear length, plant height and FHB resistance QTL on chromosome 4B. FHB resistance QTL on chromosome 4B measured by AUDPC from *F.culmorum* inoculated experiments and means from the *F.graminearum* inoculated experiments.

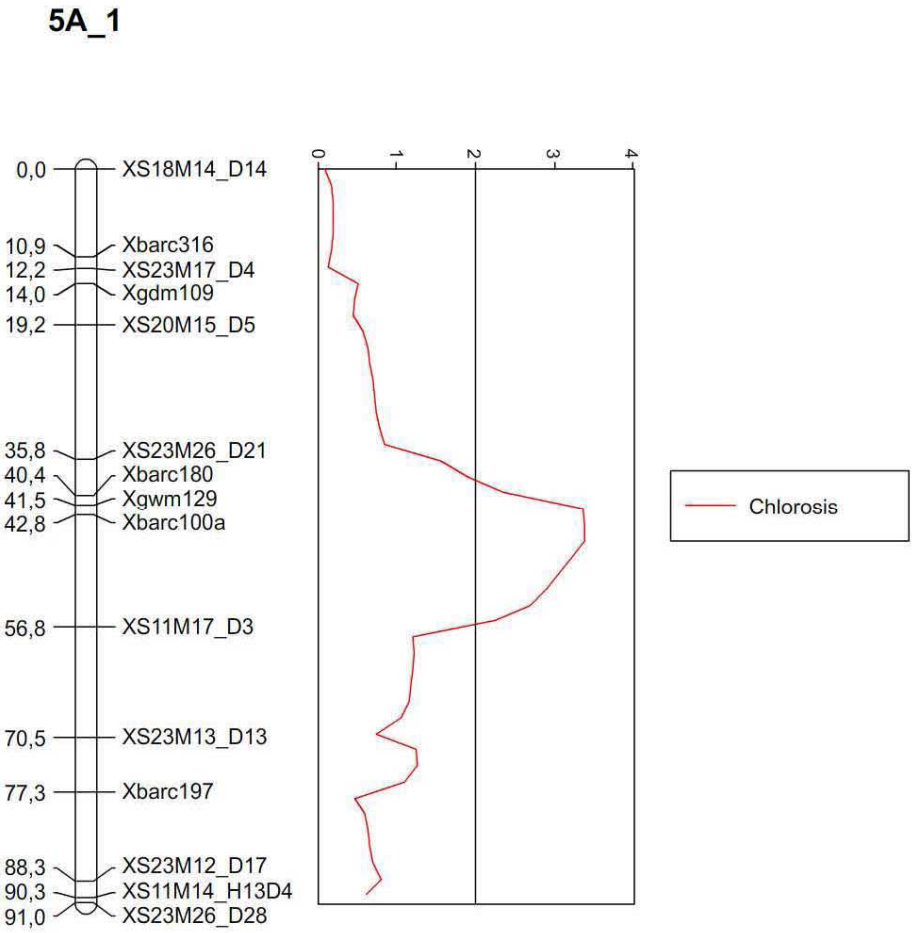

Figure 19: LOD curve for chlorosis QTL on linkage group 5A_1.

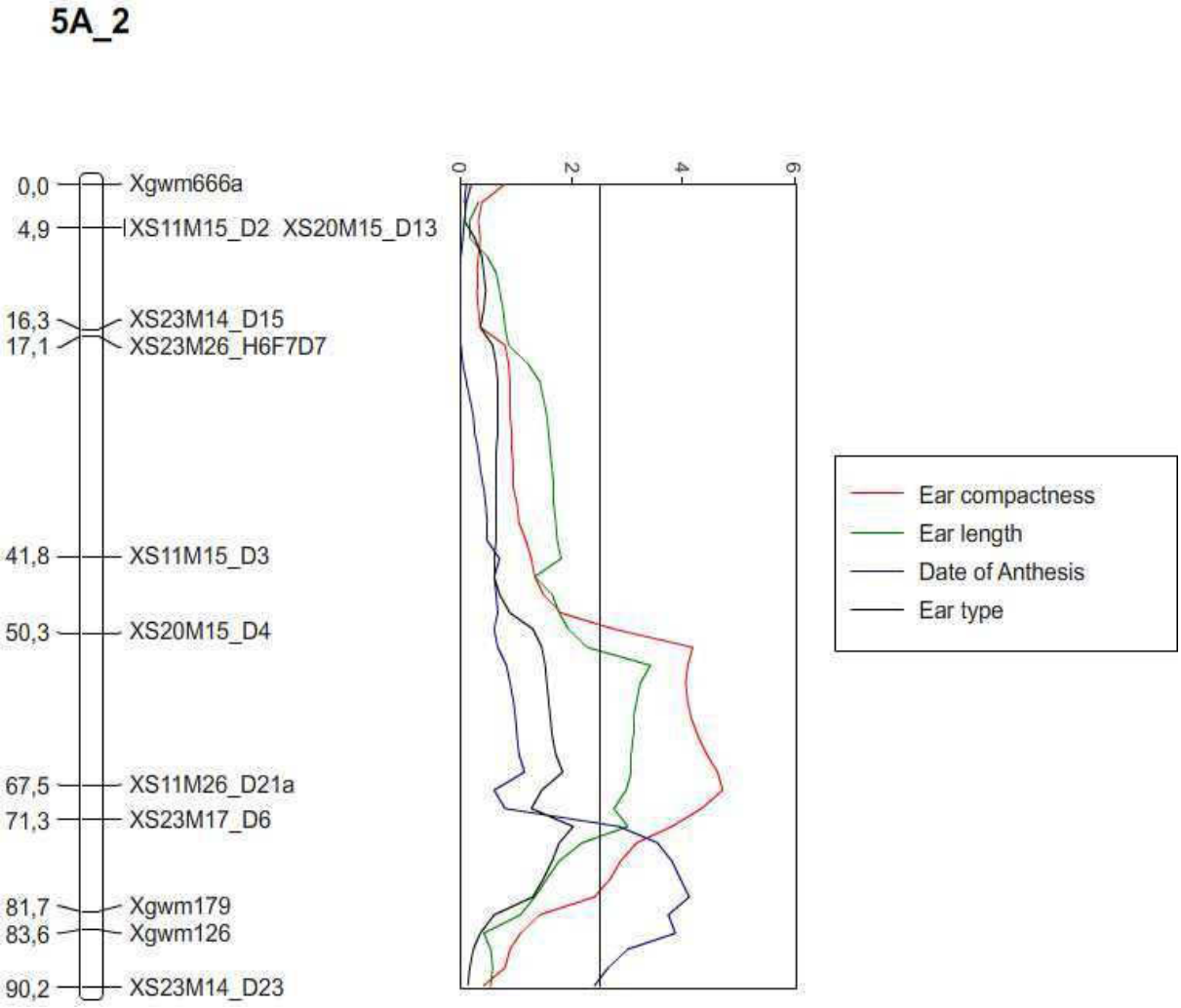

Figure 18: LOD curves for ear length, ear compactness and day of anthesis QTL on linkage group 5A_2.

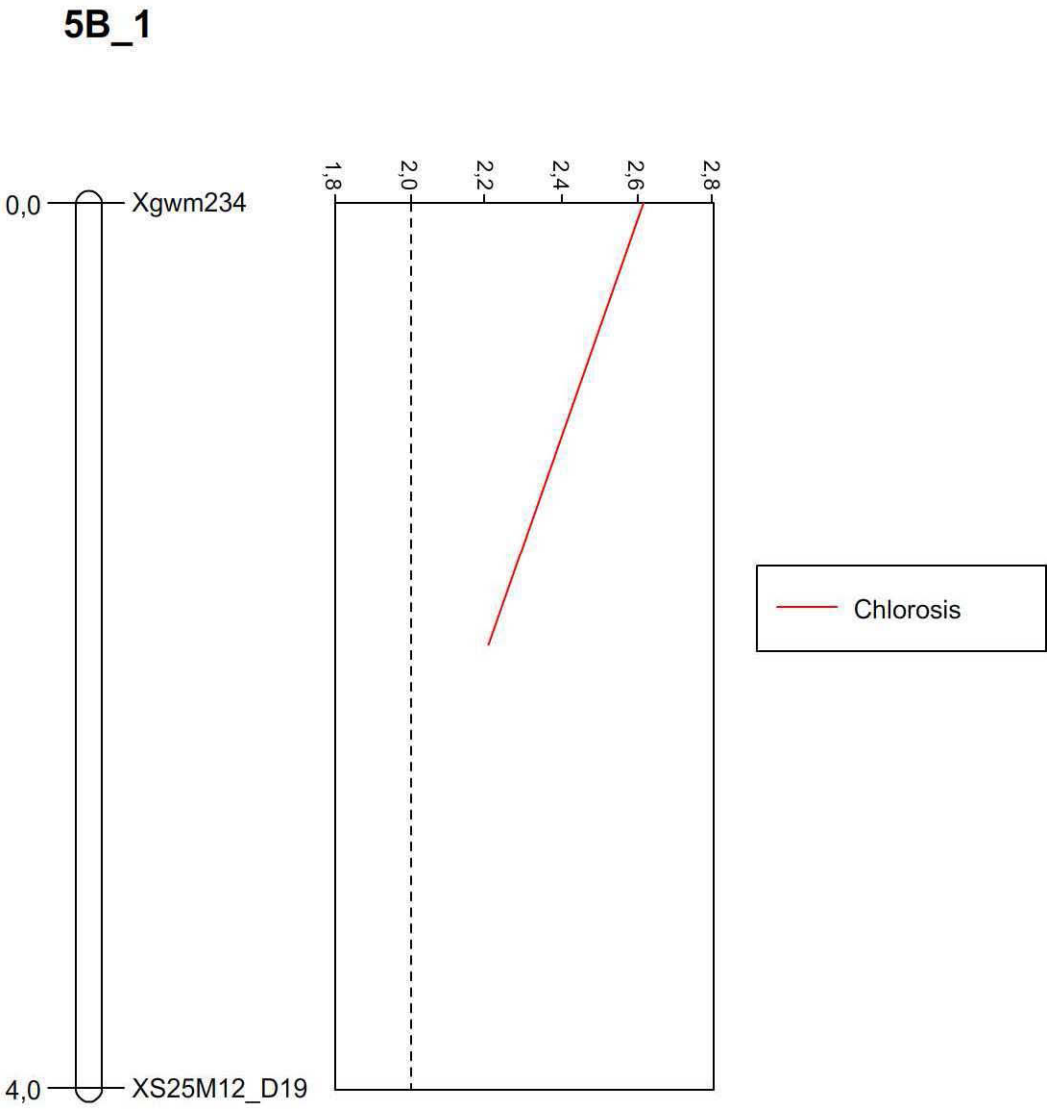

Figure 21: LOD curve for chlorosis QTL on linkage group 5B_1.

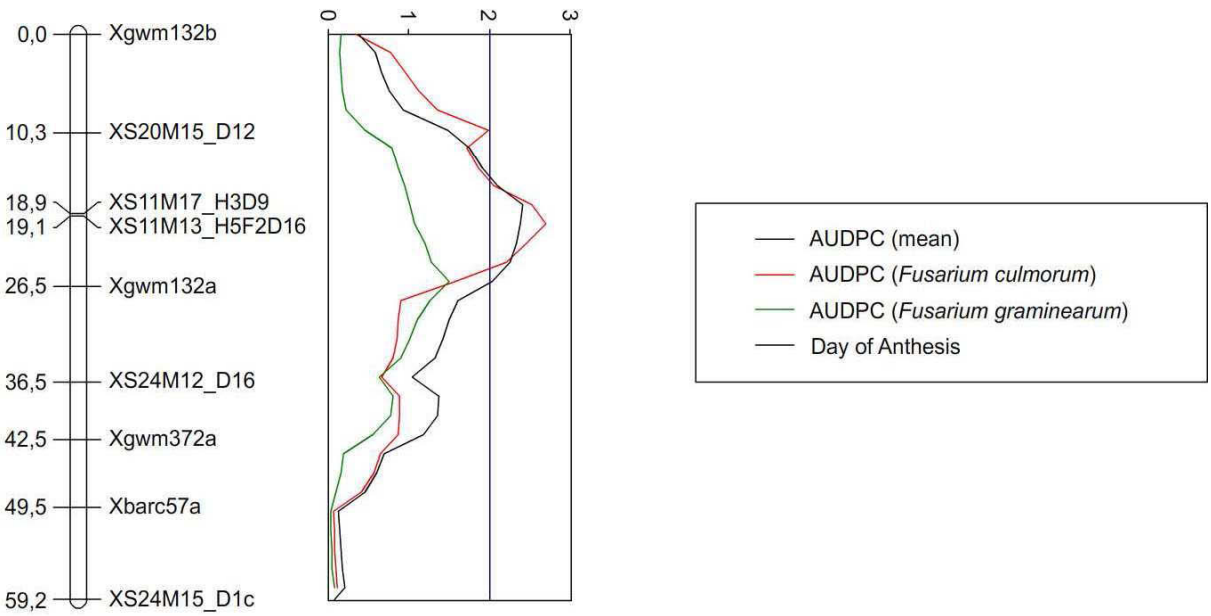

Figure 22: LOD curves for anthesis and FHB resistance QTL on linkage group 6B_3. FHB resistance QTL on linkage group 6B_3 measured by AUDPC means over all experiments, means from *F.culmorum* inoculated experiments and means from the *F.graminearum* inoculated experiments.

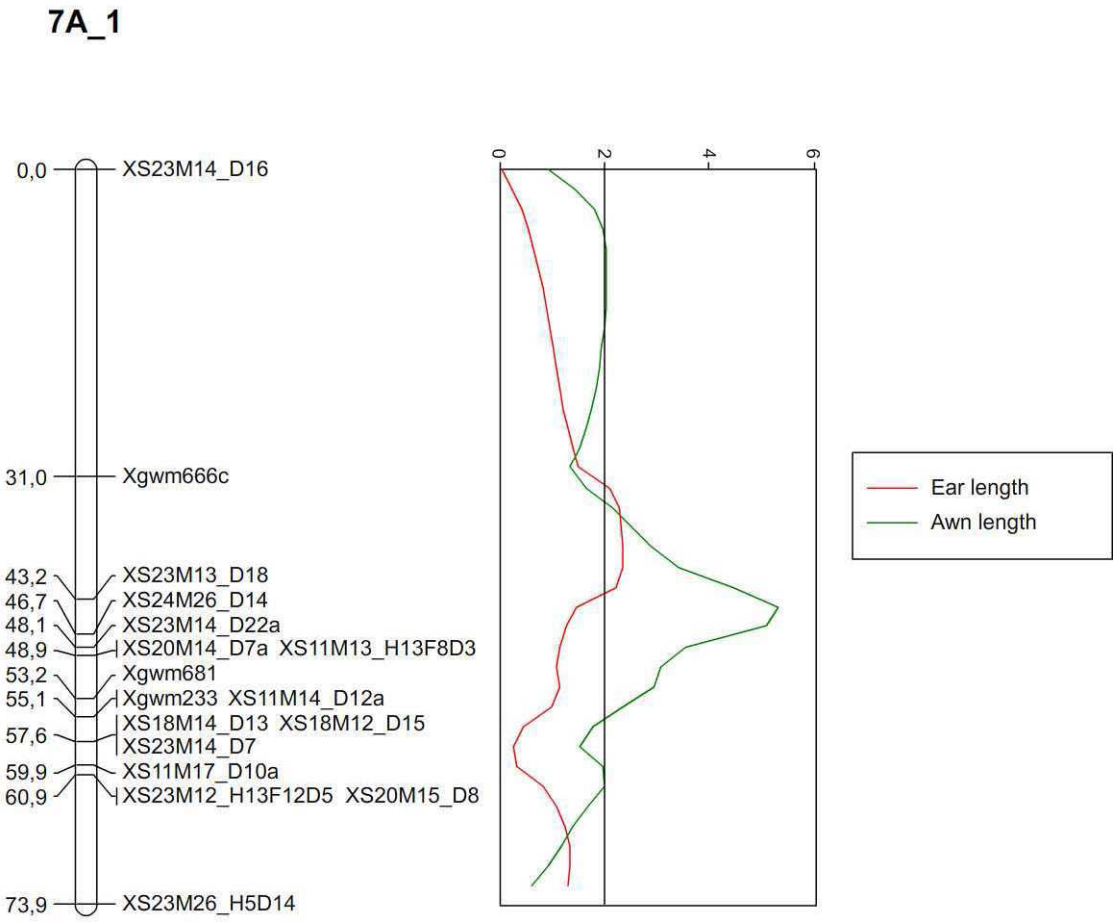

Figure 23: LOD curves for awn length and ear length QTL on linkage group 7A_1.

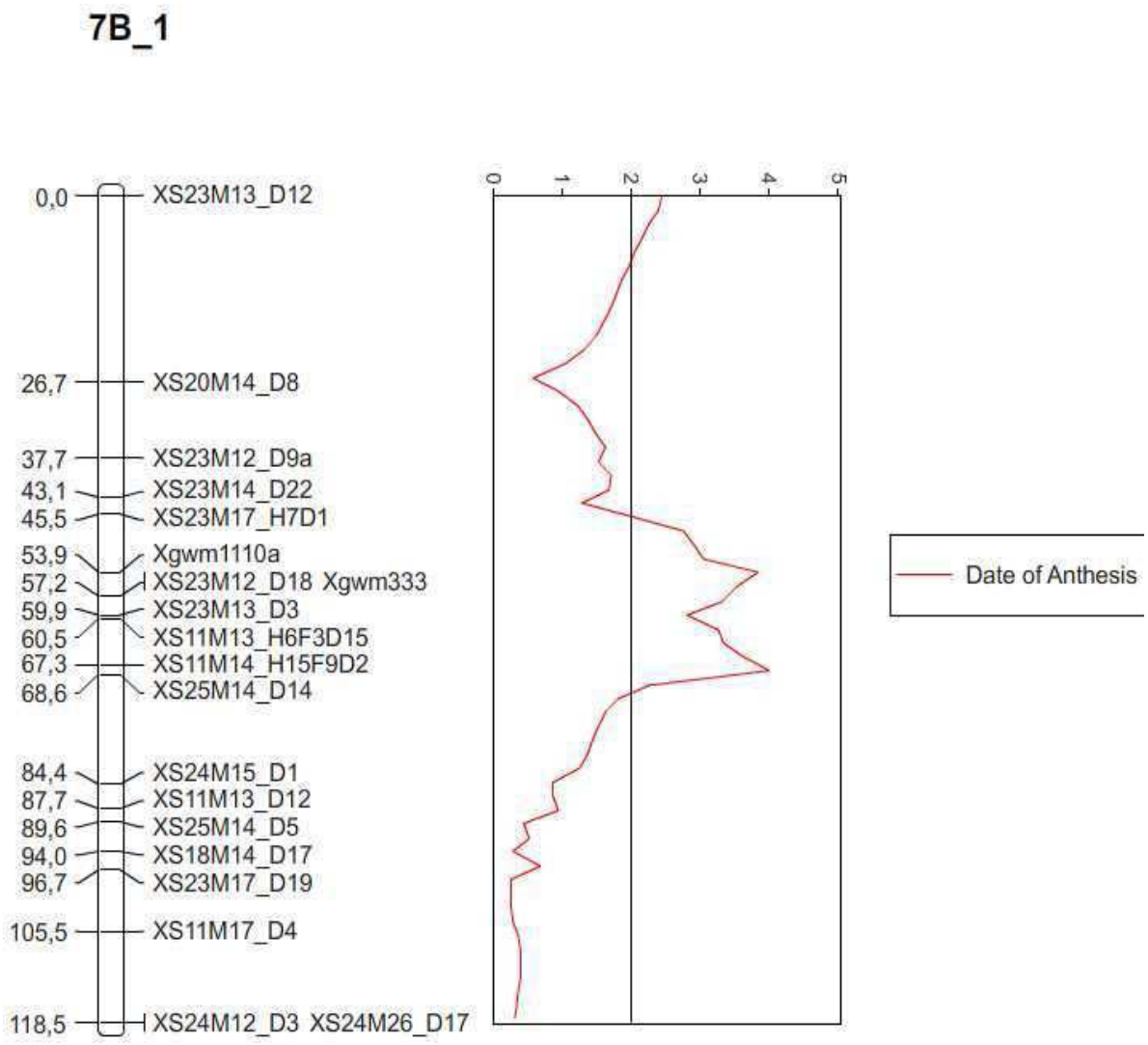

Figure 20: LOD curve for anthesis QTL on linkage group 7B_1.

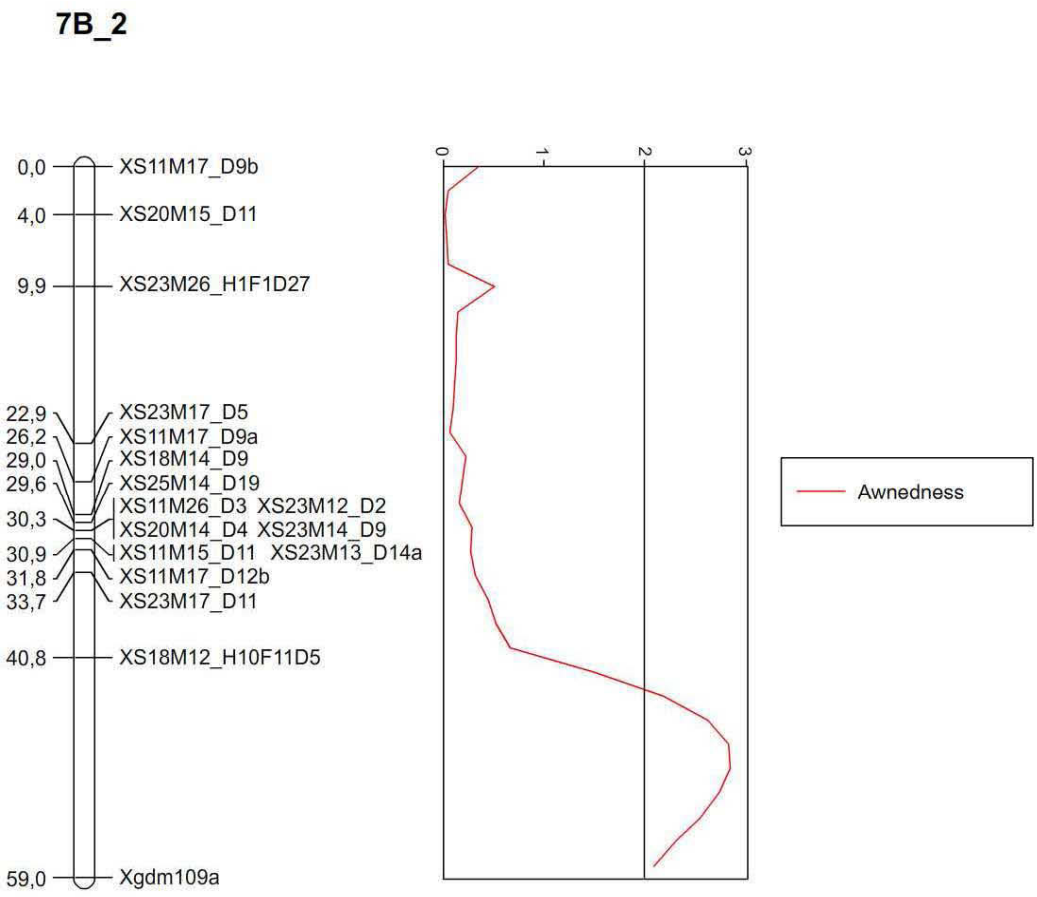

Figure 24: LOD curve for awn length QTL on linkage group 7B_2.

4. Discussion

The breeding for FHB resistance in durum wheat is an important and serious goal because *Fusarium sp*. causes tremendous losses in yield and quality. Until now one wasn´t successful to achieve resistance. But by looking for unknown resistance sources in other wheat species and inserting them into an established wheat cultivar seems to be the most promising and sustainable method.

The transference of FHB resistance from hexaploid wheat to tetraploid wheat didn´t expose to work properly (Stack et al. 2002).

So in the case of durum wheat the most promising way to achieve a resistance is to cross the susceptible durum wheat with emmer wheat (*T. dicoccocum*) which has the same genome (AABB) and gains access to a wide and mostly unknown gene pool (Buerstmayr et al. 2003, Oliver et al. 2007). But crosses with wild species, like the emmer wheat brings also problems with it because wild species often have a lack of distinctive morphological traits like plant height, stability and others.

By back crossing the resistant wild type lines with the ergonomically adapted but FHB susceptible durum cultivar the influence of this undesired traits can be reduced by every back cross by 50%.

The focus of this diploma thesis was to establish a genetic chromosomal map of a back cross population derived from a resistant emmer (*T. dicoccocum*) *T. dicoccum 161* and a susceptible Austrian durum line *DS-13162* to detect minor and major QTL against FHB.

The population, 118 BC_1F_4 lines, were analyzed with 69 SSR markers and 386 AFLP markers and a chromosomal linkage map was established.

The data from the field experiments were carried out by Huber (2010) and by that also QTL results concerning plant height, date of anthesis, waxiness, ear length, ear compactness, awn length and chlorosis could be obtained**.**

4.1 QTL analysis of FHB resistance

By simple interval mapping five different QTL were detected which are linked to FHB resistance.

For resistance to *Fusarium culmorum* four QTL was detected. One significant QTL on linkage group 6B_3 which was detected during all the years (2006-2008) with an average LOD of 2,42. On chromosome 4B for resistance to *Fusarium culmorum* two QTL were detected and one of them is also associated with the trait plant height. The last QTL which was detected for FHB resistance against *Fusarium culmorum* was located on the linkage group 1A_1 but only in the year 2007 with a LOD score of 3,7 which explained 14,7% of the phenotypic variation.

For the resistance against *Fusarium graminearum* also four QTL were detected over the years 2004-2008.

This time the QTL, which were described above for the chromosome 4B, are much more distinct with LOD scores of 4,42 which explained 15,8% of the phenotypic variation and a LOD score of 2,46 in average. For the QTL for resistance to *Fusarium graminearum* on linkage group 6B_3 which is flanked by the same primers like the QTL against resistance to *Fusarium culmorum* a LOD of 1.5 was calculated. On linkage group 3B_1 a QTL with a LOD of 3.24 was calculated which explained 12% of the phenotypic variation.

The QTL on chromsome 4B was also found by Huber (2010) but couldn't be confirmed by other publications but it has to be considered that only little research is done with *T. durum.* , and maybe the QTL on linkage group 4B are brought out to be an unknown QTL for FHB resistance. Huber (2010) crossed two Austrian durum wheat lines with a resistant *T. dicoccum* line and also found one QTL located on linkage group 3B likewise did Ban et al. (2001) who mapped the cross between *T.turgidum ssp. dicoccoides* and a substitution line of a durum wheat cultivar "Langdon". The cross also revealed a QTL on chromsome 3A, which was also found by other research groups (Joppa et al .1993, Otto et al. 2002, Gladysz et al .2007, Alimari et al. 2009).

By crossing a Brazilian wheat cultivar Frontana (resistant) and a German cultivar Remus Steiner et al. (2004) found two QTL on chromosomes 3A and 5A.

Singh et al. (2008) found QTL on chromosome 1A in a cross from Strongfield (*T. durum*) x Blackbird (*t. turgidum spp. carthlicum*) and QTL on linkage group 5A in a cross of two advanced Canadian durum breeding lines DT707 x DT696.

Kumar et al. (2007) found a QTL on chromosome 7A by crossing Langdon durum and a Langdon *T. turgidum spp*. dicoccoides PI478742.

Somers et al. (2006) and Alimari et al. (2009) found QTL on chromsome 6B. Somers performed a crossing of tetraploid cultivars Stronghold x Blackbird and detected QTL for FHB resistance on chromosomes group 2B and 6B. The same QTL was found in the hexaploid wheat cultivar Sumai-3 (Cuthbert et al. 2006). In the Swiss winter cultivar Arina the same QTL was found by Semagn et al. (2007) and Draeger et al. (2007).

4.2 QTL analysis of morphological traits

Beside the analysis of FHB resistance also morphological and developmental traits were analyzed because some qualitative morphological traits affect the expression of quantitative traits like the FHB resistance (Mesterhazy et al.1995).

4.2.1 Plant height

Type I resistance for FHB, resistance to initial penetration, is influenced by environmental conditions and plants with a small plant height are more often and more highly infected then higher plants. So the plant height and the FHB resistance are negatively correlated (Mesterhazy et al. 1995, Steiner et al. 2004). In this case plant height was measured in cm.
In our case two QTL for plant height were found on chromosome 4B. One with a LOD of 17,1 which explains 48.8% of the phenotypic variation and the other with a LOD score of 6,5 and a R^2 of 22,7% *(*Table 13*)*. The genes on chromosome 4B in durum wheat (*Triticum durum*) and on chromosome 4D in wheat (*Triticum aestivum L.*) are also called Rht genes and are semi-dwarfing genes which are responsible for the reduced height in wheat cultivars and it was shown that these genes have major effects. The origin of the Rht gene is a Japanese wheat cultivar Norin#10 (Börner et al. 1996).
These results agree with former publications of Cadalen et al. (1998), Blanco et al. (1982) and Somers et al. (2004).

4.2.2 Date of anthesis

The time from seed germination to the date of anthesis was measured in days. For this trait seven Loci were detected. Three of them were located on linkage group 7B_1 and flanked by AFLP markers. The most significant Locus region with a LOD score of 4 and R^2 of 14,5% is followed by a Locus with a LOD of 3,8 and R^2 of 13,9%. The last one on chromosome 7B_1 was a locus with a LOD of 2,4 (*Table 13*). On linkage group 5A_2 one Locus was detected with a LOD of 4,1 which explained a phenotypic variation of 14,8% (Figure 23).The markers XS20M15_12 and Xgwm132a flanked a region on linkage group 6B_3 with a LOD of 2,5 (Table 12). Also one Locus on chromosome 4A_2 was detected with a LOD of 3,9 and a coefficient of determination of 14,2% *(*Table 13*).*The seventh Locus is located between the AFLP markers XS11M17_D9c and XS13M14_D8 with a LOD of 2,35 (Table 13).
Results from Lin et al. (2008) described also one QTL for flowering time on chromosome 7B and one on chromosome 1B in wheat. T´oth et al. (2003) reported one QTL for flowering time on chromosome 5B.
The relationship between flowering time and the infestation with *Fusarium* was already shown in former publication (Buerstmayr et al. 2000; Gervais et al.2003; Steiner et al. 2004). Other studies in our department revealed QTL in similar populations. Huber (2010) found also one QTL on chromosome 7B and one QTL in an unassigned chromosome. Alimari et al. (2009) also found one QTL in an unassigned chromosome.

4.2.3 Chlorosis

Leaf chlorosis was scaled from 1 (low infected leaf area) till 9 (highly infected leaf area).
For chlorosis two QTL were found, one flanked by the marker Xgwm234 and the AFLP marker XS25M12_D19 on linkage group 5B_1 with a LOD score of 2,6 and one QTL found on linkage group 5A_1.
The same QTL for chlorosis on chromosome 5B with the same flanking markers was also found in former studies done by Huber (2010). Other cases of QTL for chlorosis in *T. durum* couldn´t be found.

4.2.4 Waxiness

The waxiness of a plant, especially the epicuticular wax, is known to play an important role for yield in wheat. Also in dry areas with a lack of water waxiness affects the water economy of the plant. (Johnson et al. 1983; Richards et al. 1984).
In this work waxiness was scaled from 1 (green color of the glumes, *T. dicoccum*) to 9 (gray color of the glumes, *T. durum*)
Two loci for the trait of waxiness were detected on linkage group 1A_2 with a LOD score of 2,6, flanked by the AFLP markers XS18M14_7 and XS23M14_26 (Table 12). The other locus was found on chromosome 1B with a LOD of 2,4 (Table 12).
Other studies revealed QTL on chromosomes 1B and 2B (Alimari et al. 2009), 5B and 7B (Huber (2010) in durum wheat , 2B and 6B (Mondal et al.2009) in wheat and on chromosomes 1A, 1D, 2B, 2D, 6A, 7A, 7D in bread wheat (Kulwal et al. 2003).

4.2.5 Awn length

Awn length was determined on the field and categorized in a scale from 1 (short awns) till 9 (long awns). *T.durum* is here the donor for long awns. Awns are not only responsible for a higher photosynthesis rate but also for the distribution of the seeds. Plants with longer awns are more infected by FHB in contrast to plants with no awns (Mesterhazy et al. 1995 and 1989), the reason for that is that water is kept longer and so the conditions for fungal spread increases rapidly.
Five QTL associated with awn length were detected during this work. The highest QTL (was mapped on linkage group 4A_1 with a LOD score of 5,3 which explains a phenotypic variation of 18,8%. The other QTL were found on linkage groups 4A_1, 7B_2, 7A_1 and 3B_1.
Huber at al. (2010) found out that only a moderate negative correlation between awn length and FHB severity could be detected and found one significant QTL also on chromosome 4A. Sourdille et al (2002) detected two QTL in bread wheat on chromosomes 4A and 6B.

4.2.6 Ear compactness

In general it is said that plants with compact ears a more susceptible to FHB than plants with loose ears, because they hold the humidity longer and develop a special micro-climatic atmosphere which is perfect for fungal spread (Mesterhazy 1995). The plants were scaled from 1 (loose, *T. dicoccum*) to 9 (compact/dense *T. durum*).
On linkage group 5A_2 one QTL with a LOD of 4.7 was found which explains a phenotypic variation of 16,8%. Jantasuriyarat et al (2003) found five QTL on chromosomes 1B, 2B, 4A, 5A, and 6A in wheat (*Triticum aestivum L.*) affecting ear compactness whereas the QTL on linkage group 5A explained 14% of the phenotypic variance.
On chromosome 5A one of the three minor genes (Q, C, S1) which are affecting the morphology of the ear Q is located (Sourdille et al 2000). It shortens the length of the rachis, increases the amount of spikes per ear and it affects if the rachis is freethreshing or not.

4.2.7 Ear length

Ear length was scaled from 1(*T. dicoccom*) to 9 (*T.durum*). QTL analysis revealed four QTL for this trait. Two QTL on chromosome 4B with a LOD score of 4,89 and 4,88. The other two QTL were found on linkage groups 5A_2 and 7A_1.
Alimari et al (2009) found in a back-cross population of *T. dicoccoides* x *T. durum* only one QTL in an unknown linkage group.

Similar results are only made in bread wheat *(T. aestivum L*). Börner et al (2002) mapped 114 recombinant inbred lines and described QTL on chromosome 1B, two QTL on 4A and one QTL on 5A.
Jantasuriyarat et al (2003) also analyzed RIL for the International Triticeae Mapping Initiative (ITMI) like Börner et al (2002) did and found four QTL on 1B, 4A, 4D, and 7A.
Sourdille et al. (2000) mapped a DH population of two wheat cultivars Courtot x Chinese Spring and detected on 1A, 2D, 4A, 2B and 5A.
As expected most of the studies revealed one QTL on chromosome 5A which is also the location of the major gene Q which is affecting the ear morphology. In bread wheat two more major genes are known S1 gene on 3D and the C gene linkage group 2D.

4.3 Conclusions

The continuous increase of the fungal spread of *Fusarium* worldwide and for it the increase of yield losses and the contamination with mycotoxins like DON and ZON in all wheat species is not to be underestimated.

The resistance breeding for bread wheat (*T. aestivum*) is since years a principal matter for researchers but the durum wheat which plays an important role in the livelihood for nutrition especially in countries around the Mediterranean Sea and in big parts of the Middle East but nowadays also in Europe and in North America pushes along in the focus of breeders worldwide.

The tetraploid wheat *T. durum* where until now no durable resistance against FHB could be inserted displays a challenge which only can be overcome by resistance breeding with closely related species, which describe a mostly unknown source of resistances, like the wild emmer (*T. dicoccoides*) and the cultivated emmer (*T. dicoccum*).

In this study the back-crossing of a resistant cultivated emmer *(T. dicoccoides*) *T. dicoccums 161* and an Austrian susceptible *T. durum DS-131621* and the analysis of QTL by SSR and AFLP markers was successfully performed.

The analysis revealed five different QTL against FHB on linkage groups 1A_1, 3B_1, 6B_3 and two QTL on chromosome 4B. In addition QTL for different morphological traits like plant height, date of anthesis, waxiness, ear length, ear compactness, awn length and chlorosis were also revealed.

The proceeding breeding for resistance against FHB by crossing wild, cultivated or unknown landraces with cultivated already used wheat species plays an important role and can only be promoted by research and the collecting as much data as possible worldwide to obtain a durable and reliable resistance.

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