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Genetic linkage map construction and identification of Quantitative Trait Loci (QTLs) determining post-anthesis drought tolerance and other agronomic traits in bread wheat

Dissertation

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M.Sc. in Plant Breeding Khalil Zaynali Nezhad

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Gutachter: 1. Prof. Dr. W. Eberhard Weber

2. Priv. Doz. Dr. Andreas Börner

3. Prof. Dr. Jens Leon

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This thesis is dedicated with deep gratitude to the memory of
my late mother, my father, my family, and my wife
for their unconditional love, endless support and encouragement.

It is also dedicated to all those who taught me and directed
me towards my goals.

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List of Abbreviations:

h^2	Heritability
R^2	the explained phenotypic variance based on partial correlation of coefficient
%	percent
*	significant at 5% level of probability
**	significant at 1% level of probability
AFLP	amplified fragment length polymorphism
ALF	automated laser fluorescence express DNA sequencer
ANOVA	analysis of variance
BARC	acronym of the USDA-ARS Beltsville Agricultural Research Center
bp	base pair
CIM	composite interval mapping
cm	centimeter
cM	centiMorgans
CV	coefficient of variation
DH	doubled haploid
DNA	deoxyribonucleic acid
dTTP	deoxythymidinetriphosphate
EDTA	ethylenediaminetetraacetic acid
EtBr	ethidium bromide
EtOH	ethanol
F1	first filial generation, produced by crossing two parental lines
F2	second filial generation, produced by selfing the F1 plant
F2:3	third filial generation, produced by selfing each single F2 plant
FMC	field mean data under control
FMS	field mean data under stress
GDM	Gatersleben D-genome Microsatellite
GMC	greenhouse mean data under control
GMS	greenhouse mean data under stress
GWM	Gatersleben Wheat Microsatellite
IPK	Leibniz Institute of Plant Genetics and Crop Plant Research
ITMI	International Triticeae Mapping Initiative
KI	potassium iodide
L	long arm of chromosome
LOD	logarithm of odds
MC	mean data under control
ml	milliliter
mm	millimeters
mM	millimolar
MS	mean data under stress
ng	nanogram
NIL	Near Isogenic Lines

List of Abbreviations

ns	not significant
NT	nulli-tetrasomic
PCR	polymerase chain reaction
QAwn	QTL for awn
QDtf	QTL for Days to flowering
QNfs	QTL for No. fertile spikes per plant
QNsp	QTL for No. seeds per spikes
QNut	QTL for No. unfertile tillers per plant
QPhe	QTL for Plant height
QSea	QTL for Seed area
QSel	QTL for Seed length
QSew	QTL for Seed width
QSpl	QTL for Spike length
QTgw	QTL for thousand-grain weight
QTL	quantitative trait loci
QWas	QTL for Weight of all spikes per plant
QWsp	QTL for Seed weight per spikes
QWts	QTL for Weight of three spikes per plant
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RIL	recombinant inbred line
Rnase	ribonuclease
rpm	rounds per minute
S	short arm of chromosome
SDS	sodium dodecyl sulfate
SIM	simple interval mapping
SNP	single nucleotide polymorphism
SSR	simple sequence repeats or microsatellite
TBE	Tris-Borate-EDTA buffer
TE	Tris-EDTA (TE) buffer
Tris	tris(hydroxymethyl) aminomethane
V	volt
v/v	volume/volume
W	Watt
W/V	weight/volume
X	locus for marker
µg	microgram
µl	microliter

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1 Introduction

1.1 Econ omic importance of wheat

Wheat is the most widely grown and consumed food crop world-wide with total harvested area of 223,564,097 hectares and production of 689,945,712 tones in 2008 (FAO, 2009). It is a staple food for nearly 35 percent of population in the world and requirement for wheat will grow faster than for any other major crop (Rajaram and Ginkel, 2000). Demand for wheat has been projected to increase by 1.3 and 1.8 percent per year on world-wide and developing countries, respectively for the period up to 2018 (Reynolds et al., 1999). Much of this requirement for wheat will have to be met by increases in yield, since there will be little extension in the total wheat grown area (Hall, 2001).

Wheat importance derives from the properties of wheat gluten proteins, a cohesive network of tough endosperm proteins that stretch with the expansion of fermenting dough, yet coagulate and hold together when heated to produce a risen loaf of bread. Only wheat, and to a lesser extent rye and triticale, have these properties. Wheat is utilized for making bread, confectionery products, semolina, breakfast cereals, etc. Its diversity of use, nutritive content, and storage qualities has made it a staple food for more than one third of the world's population (Poehlman and Sleper, 1995; Zohary and Hopf, 2000). Bread is the preferred staple food of traditional farming communities throughout the Old World from the Atlantic coast of Europe to the northern parts of the Indian subcontinent including Iran, and from Scandinavia and Russia to Egypt. Thus, it is not surprising that in numerous cultures food has been equated with bread (Zohary and Hopf, 2000).

1.2 Origin and genome composition of wheat

Wheat has been cultivated in southwestern Asia, its geographic center of origin, for more than 10,000 years. The genus of *Triticum* is divided into three ploidy groups: diploids ($2n = 2x = 14$), tetraploids ($2n = 4x = 28$), and hexaploids ($2n = 6x = 42$). Currently, 11 diploid, 11(or 12) tetraploid, and six hexaploid species of *Triticum* are recognized (Poehlman and Sleper, 1995). Only two species are commercially important: the hexaploid species, *T. aestivum*, the bread wheat, and the tetraploid species, *T. turgidum*, the durum wheat (Poehlman and Sleper, 1995; Zohary and Hopf, 2000).

In wheat, evidence for homoeology between the three genomes had been provided by cytogeneticists, particularly by Ernie Sears (Sears, 1954) who developed the first set of aneuploid genetic stocks in wheat. Hexaploid bread wheat (*Triticum aestivum*, 2n = 6x = 42) originated approximately 10,000 years ago after the domestication of tetraploid wheat. It is an allopolyploid with genome constitution AABBDD, formed through hybridization of *T. urartu* (AA) with a B genome diploid of unknown origin but related to *Aegilops speltoides*, and subsequent hybridization, only about 8,000 years ago, with a D genome diploid, *Ae. tauschii*. The development of aneuploid stocks in wheat led to the discovery that an extra dose of a particular chromosome could compensate for the absence of another. This compensating ability of chromosomes of different ancestral origin defined their relationship, and resulted in the classification of the 21 wheat chromosomes into seven homoeologous groups each group having three partially homologous chromosomes, one from each of the A, B, and D genomes. Therefore, in wheat, chromosomes are identified by the homoeologous group number (number 1 to 7), and the genome (A, B, or D) from which the chromosome originated (Poehlman and Sleper, 1995)

1.3 Drought stress effect on wheat production in world-wide and in Iran

The distribution of wheat growing area in the world is broad and contains a range of temperature and radiation regimes in irrigated and non-irrigated as well as rainfed environments including the Mediterranean region where the annual precipitation varies between 200 to 900 mm. Therefore, in the regions with low precipitation, plants are exposed to periods of water deficit that have a negative impact on growth and yield (Merah et al., 2001). Drought is generally accepted to be the most widespread abiotic stress experienced by crop plants (Figure 1.1). It is a serious problem in many parts of the world where wheat, barley, and other cereals form the staple food (Quarrie et al., 1999). Furthermore, changing precipitation patterns with global warming are predicted to reduce rainfall in many regions, increase rainfall variability, and reduce total water available for dryland cropping (Rebetzke et al., 2009). Drought affects an estimated of 65 million hectares of wheat grown area world-wide and in these water-limited environments, wheat yields are commonly reduced to 50% or less of the irrigated yield potential (Byerlee and Morris, 1993).

In Iran, wheat is grown in different ecological areas with range of elevation from -25 m below to 2,800 m above sea level. Based on the amount and variation of precipitation, minimum temperature and elevation above sea level, these regions could be categorized into several different climatic zones (Saidi et al., 2000). Iran with wheat production of about 15 million tones per year is among the 15 largest wheat producers in the world (FAO, 2009). Total wheat production in the last five years averaged 10-12 million tones. Wheat is sown on 7 million hectares, of which 2.5 million hectares are irrigated (Figure 1.2). Although irrigated wheat occupies only one third of the area under wheat production, two thirds of the total wheat production comes from irrigated areas (Figure 1.2). The average grain yield from irrigated and rainfed areas is 3.1 and 0.9 tones per hectares, respectively. Ninety percent of wheat production is used for bread making which plays a major role in supplying the daily calories of Iranian people. On average, Iran imports about three to five million tones of its wheat consumption. Dry conditions and irregular rainfall result in higher levels of wheat import (Saidi et al., 2000).

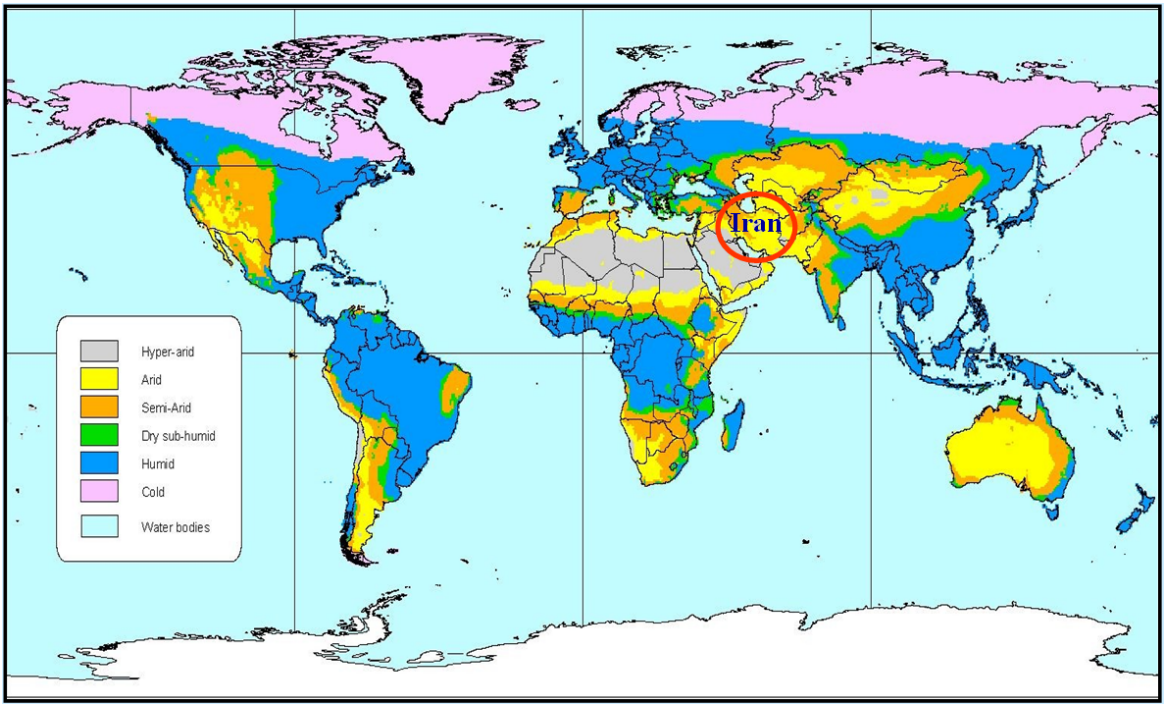


Figure 1.1 Global extent of dry land (WTP, 2007a)

Wheat is the major cereal grown in dry regions of Iran. Severe drought stress, especially terminal drought stress, because of low and irregular rainfall, which is the characteristic of the

Mediterranean environment (Figure 1.2), is one of the major abiotic factors reducing wheat yield in Iran. In many rainfed wheat growing area the amount of precipitation during grain filling period in May and June is reduced which causes post anthesis drought stress (Table 1, Figure 1, and Figure 2 in Appendix 1). Even in irrigated areas wheat production is not stable because sometimes farmers prefer to use the available source of water to irrigate vegetables which gives more income instead of cereals like wheat. The last few years have coincided with dry climatic patterns reducing total wheat yield significantly. Special national projects have been started to develop bread wheat cultivars for dry areas, particularly those genotypes that require less irrigation and bearing tolerance against terminal drought but still produce a relatively high yield (Saidi et al., 2000).

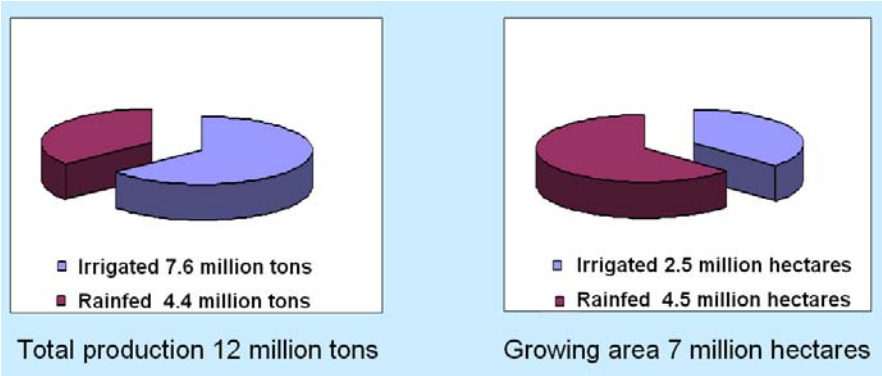


Figure 1.2 Wheat production and growing area in Iran

1.4 Drought tolerance as a wheat breeding objective

Drought is defined as where a dry soil (due to lack of rain or delayed irrigation) causes a substantial reduction in crop performance in terms of either plant survival or economic yield or crop quality (Hall, 2001). Drought tolerance is defined as the ability of a cultivar to produce a greater economic yield than another cultivar when they are subjected to drought (Quarrie et al., 1999; Hall, 2001).

Drought tolerance is one of the most ambiguous concepts in the literature and its definition strongly depends on the phenotyping methods used (Collins et al., 2008). Blum (2006) believed that the definitions of drought resistance are linked to the drought stress presented by the targeted environment. Therefore, a specific description of a field stress can be found for

each given crop and region, for example, seedling related stress, vegetative stage stress, pre-flowering stress, flowering stress, post-flowering stress, terminal stress, and so forth.

It is important to distinguish between what is meant by drought resistance and by drought associated traits. The only criterion to be considered in terms of defining drought resistance should be economic yield (e.g. grain yield in wheat). Thus, a drought resistant variety is one, which gives a significantly higher yield than average under specified conditions where yield is limited by water availability. Traits associated with drought resistance, such as early leaf rolling, does not necessarily lead to a more drought resistant plant. Such a plant may be excellent at surviving a drought but agriculturally useless if; as a result, it yields very poorly (Quarrie et al., 1999).

The goal of the wheat breeder is to create new genotypes improved in characters that contribute to greater yield potential, increased yield stability, and improved product quality. Yield potential affects the amount of the product harvested. Yield stability is important to obtain a uniform high yield over a wide range of environments and to assure a broad adaptation of the cultivar. Yield stability is increased with optimum maturity and resistance to lodging, drought, disease pathogens, and insect pest (Poehlman and Sleper, 1995).

Wheat plant mechanisms that contribute to drought resistance are early maturity to ripen the crop ahead of periods of drought stress, vigorous and deep root systems to utilize available soil moisture efficiently, ability to close stomata during periods of drought stress to decrease water loss, and a wax bloom on the leaf surface to reduce transpiration loss. Drought resistance is a complex quantitative character and is not subjected to measurement by a single laboratory procedure (Poehlman and Sleper, 1995).

Of all the abiotic stresses that limit crop productivity, drought is the most devastating one and the most difficult to breeders' efforts. In the past, breeding efforts to improve drought tolerance have been hindered by its quantitative genetic basis and the poor understanding of the physiological basis of yield under water-limited conditions (Tuberosa and Salvi, 2006). It seems clear that current breeding programs continue to make progress through commonly used breeding approaches. However, marker assisted selection could greatly assist plant breeders in reaching this goal although, to date, the impact on variety development has been minimal (Collard and Mackill, 2008). Therefore, genomics-assisted improvement of abiotic stress tolerance of crops will increasingly rely on the QTL approach. The systematic

dissection of QTLs governing the naturally occurring variation relevant for crop yield and its sustainability will facilitate a more targeted and effective tailoring of cultivars with an improved performance under abiotic constraints (Collins et al., 2008).

1.5 The effect of post anthesis drought stress on wheat yield components

Figure 1.3 shows a schematic diagram of wheat growth and developmental stages. As indicated, there are three major growth stages (GS1, GS2 and GS3). GS1: the vegetative phase, when the leaves are initiated. GS2: the reproductive phase, when floret development occurs until the number of fertile florets, virtually the number of grains, is determined. GS3: the grain filling phase, when the grain first develops the endosperm cells and then grows to determine the final grain weight (Miralles and Slafer, 1999).

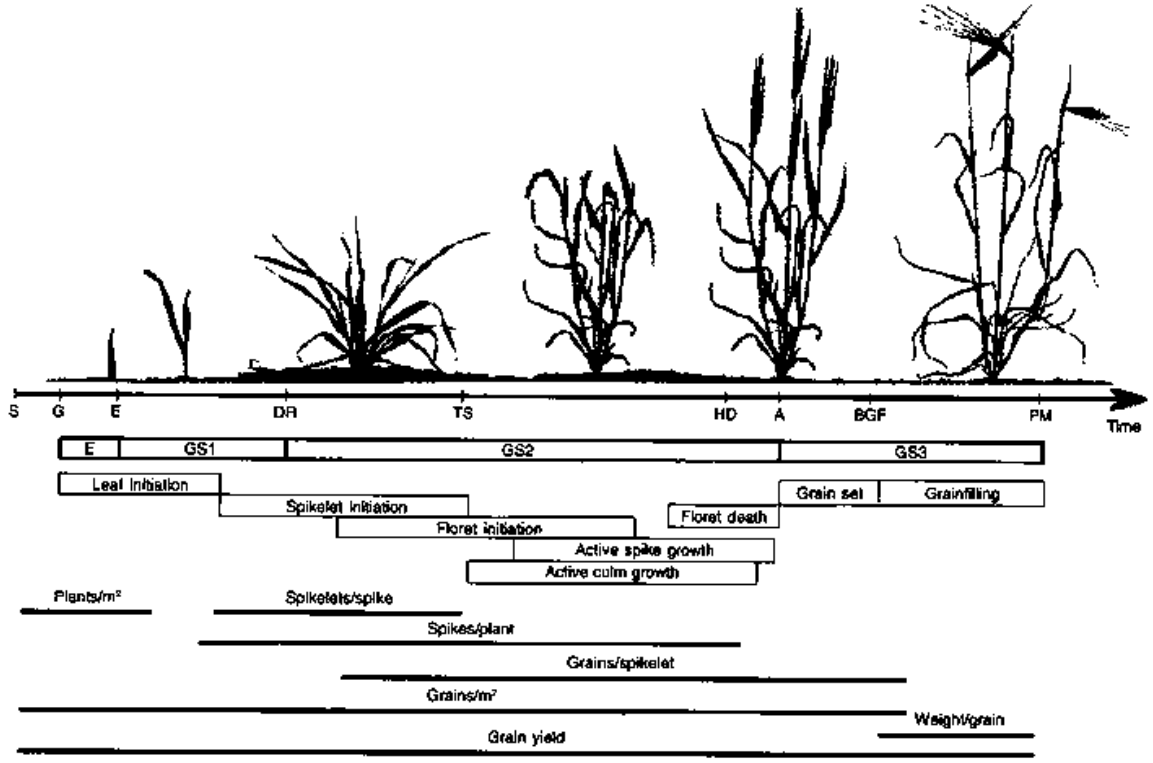


Figure 1.3 Wheat plant developmental stages S=sowing; G=germination; E=emergence; DR=double ridge appearance; TS=terminal spikelet initiation; HD=heading; A=anthesis; BGF=beginning of grain filling period; PM=physiological maturity; GS=growth stage (Reynolds, 2002)

The number of kernels per square meter is determined by the number of kernel bearing tillers per square meter and the number of kernels per spike. Many factors affect tiller initiation and

survival, such as genotype, growth habit of wheat, cultural practices, and growing conditions. However, number of spikes per unit of land area and number of seeds per spike as two wheat yield components are determined in the vegetative and reproductive phase and the third component, weight of seed, in the grain filling phase. In other words, before anthesis mainly the number of grains is determined while after anthesis the grains are actually filled and the individual grain weight is established (Frederick and Bauer, 1999; Miralles and Slafer, 1999). Reductions in leaf photosynthesis and leaf area index due to drought stress occurring prior to anthesis are correlated with reductions in the number of kernels per spike (Frederick and Bauer, 1999). The influence of post-anthesis water stress on grain yield reflects direct and indirect changes on kernel size. Reduction in kernel size contribute to an increase in the proportion of shriveled kernels, lower harvest index and grain yield, and decreased grain quality (Ruuska et al., 2006).

1.6 Improving stem reserve utilization for grain filling as a breeding strategy

In order to increase the application of the genetic mechanisms for traits offering drought resistance in wheat, reported in the literature, Reynolds et al. (2005) suggested a conceptual model Figure 1.4 which is a systematic trait orientation into the four groups in a way that the physiological effects among groups are likely being relatively independent. The following groups of candidate traits are being considered for drought adaptation in wheat namely traits relating to: 1- pre-anthesis growth 2- water extraction 3- water use efficiency 4- photo-protection. Therefore, it will be possible to bring together the adaptive traits from different sources via selection in a segregating population derived from contrasting parental lines.

Early vigor and stem carbohydrates reserves are among the traits related to early growth, while the former helps to shade the soil and suppress weeds that compete for water, the later can be helpful by re-mobilization during grain-filling to supplement assimilates generated in the drier post-anthesis period (Reynolds et al., 2005; Reynolds and Tuberosa, 2008).

Plant carbohydrates take many forms, ranging from simple soluble sugars (e.g., sucrose, glucose, and fructose) to the polysaccharides that are used to build the cell wall (e.g. cellulose), to storage reserves (e.g., starch and fructans). Together they can account for up to 90 percent of the total dry mass of plants. The major carbohydrates can be reversibly converted from one to another by a metabolic pathway involving several enzymes. In plants

only the synthesis of cellulose by cellulose synthase seems to be a one-way route (Tiessen et al., 2006).

The production of fruits and seeds generally requires a large supply of carbohydrate that cannot always be met by current photosynthesis. To overcome this problem many plants, including cereals, accumulate temporary stores of carbohydrate in their stems during their vegetative growth phase, and then re-mobilize them when needed for synthesis of long-term storage reserves. Maize and rice store starch, while wheat and barley store fructans. Fructans are soluble high molecular-weight polymers of fructose units derived from sucrose. They are synthesized and accumulated in the vacuoles of many plants, including wheat and barley (Tiessen et al., 2006).

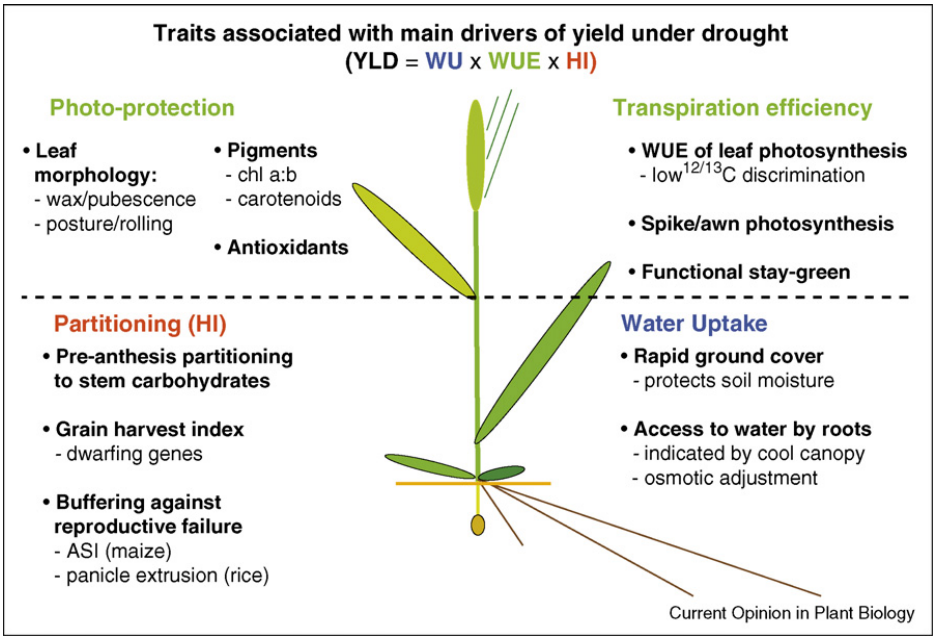


Figure 1.4 Conceptual model for traits in wheat and other cereals associated with adaptation to drought-prone environments (Reynolds and Tuberosa, 2008).

Photosynthesis is inhibited under water stress because of the closure of stomata to prevent excessive transpiration and desiccation of the cell. The increased stomatal resistance not only reduces the escape of water vapor but also blocks the entry of CO_2 that can be used by the Rubisco enzyme. Thus, plants face a dilemma in their efforts to save water, because if they close the stomata too tightly, then photosynthesis will be inhibited (Tiessen et al., 2006). However, experiments with sorghum (Sung and Krieg, 1979) have shown that translocation is

unaffected until late in the water stress period, when other processes, such as photosynthesis, have already been strongly inhibited Figure 1.5. This relative insensitivity of translocation to stress allows plants to mobilize and use reserves where they are needed e.g. in seed growth, even when stress is extremely severe. Therefore, if photosynthesis is inhibited during grain filling period under any type of stress, which inhibits current assimilation, stem reserves offer a powerful resource for grain filling. The ability to continue translocating assimilates is a key factor in almost all aspects of plant resistance to drought (Taiz and Zeiger, 2002).

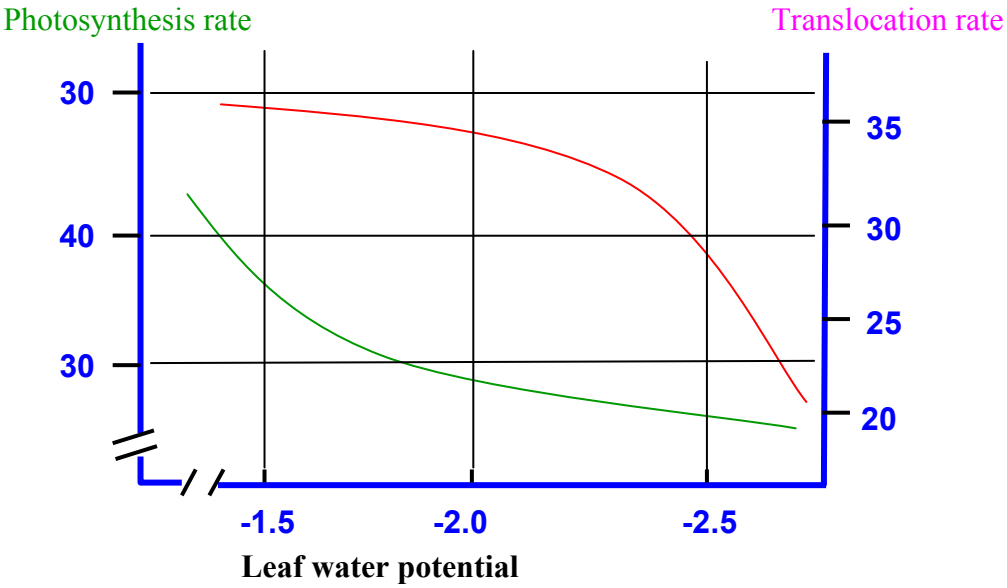


Figure 1.5 Relative effects of water stress on photosynthesis and translocation in sorghum. Photosynthesis was affected by mild stress, whereas, translocation was unaffected until stress was severe. After Sung and Krieg (1979).

Furthermore, drought stress during grain filling period may induce reserve mobilization. In spring wheat post anthesis drought stress increases the proportion of the grain weight originating from stem reserves, with values ranging from nearly 10 percent under normal condition to greater than 40 percent under drought condition (Davidson and Chevalier, 1992). Ehdaie (Ehdaie and Waines, 1996) reported these values range from nearly 30 percent under normal condition to 46.6 percent for drought stress.

However, it is recognized that reserve mobilization depends on species and genotype and genotypic variation exists in wheat accessions for the proportion of re-mobilized stem reserves in final grain weight (Blum, 1998; Ehdaie et al., 2006). Therefore, improving grain filling capacity by stem reserves is an important breeding target in cereals subjected to

terminal environmental or biotic stresses (Blum, 1998). Australian crop physiologists were the first to call attention for the potential of stem reserves as a yield supporting mechanism in small grains subjected to late season drought stress (Blum, 2006).

1.7 QTL mapping as a genomics approach for drought tolerance improvement

Little is known or understood of the genetic basis of wheat performance under drought (Rebetzke et al., 2009). Improving the tolerance of crops against drought, compared with other abiotic stresses, requires a broader interdisciplinary approach, involving an understanding of the factors (e.g. availability of water during the crop cycle) determining yield in a particular target population of environments (Collins et al., 2008). Drought stress is as complicated and difficult to plant biology as cancer is to mammalian biology (Pennisi, 2008). Therefore, wheat genomics project has the overall goal to transfer new developments in genomics to wheat improvement by combining the expertise of genomics researchers, wheat breeders, and end-users. However, one constant that will never change is that breeding progress depends on accurate selection of rare genotypes that posses new or improved attributes due to superior combinations of alleles at multiple loci, in the context of a target set of environments (Sorrells, 2007).

Given that the morpho-physiological traits that affect the tolerance of crops to drought are quantitatively inherited, the discovery of Quantitative Trait Loci (QTLs) plays a central role in crop improvement through MAS (Figure 1.6). The increasing number of studies reporting QTLs for drought-related traits and yield in drought-stressed crops indicates a growing interest in this approach (Tuberosa and Salvi, 2006). Perhaps the greatest opportunity for use of linked markers, resulted from QTL mapping, in breeding for performance under drought stress will be their application for selection of difficult, low heritable and expensive-to-measure traits (Tuberosa et al., 2002). A range of different types of molecular markers provides greater opportunity for trait dissection into component QTLs, and insight into the underlying genetic basis for variation in the trait (Rebetzke et al., 2009).

While plant breeding relies heavily on the science of genetics, the primary goal of a plant breeder is fundamentally different from the primary goal of a plant geneticist. Regarding the QTL mapping in plant there are two general goals. The first one, which is related to plant breeding, is to identify markers that can be used to select for a complex trait. The second aim,

which focuses on pure genetics, is increasing biological knowledge of the inheritance and genetic architecture of quantitative inherited traits. If the genes underlying the QTL have been cloned, then transgenic approaches can also be used to directly introduce beneficial alleles across wide species. While these two goals are not mutually exclusive, they require different levels of stringency for declaring the presence of a QTL and different levels of resolution for detecting QTL (Borevitz and Chory, 2004; Bernardo and Bohn, 2007; Bernardo, 2008).

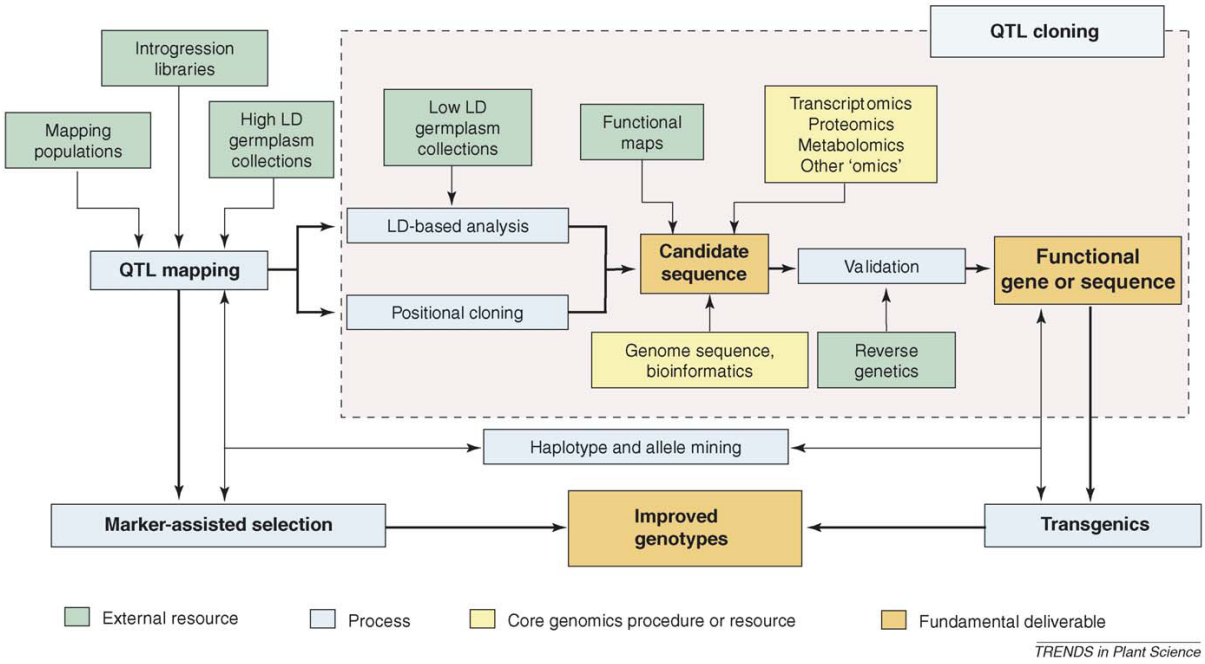


Figure 1.6 QTL mapping position in the genomics (Tuberosa and Salvi, 2006)

1.8 QTL analysis and its requirements

The goal of QTL mapping is to determine the loci that are responsible for variation in a quantitative trait. This approach dissects the variation of a quantitative trait into its component QTLs. Later on, each of the mapped QTL can be evaluated separately for more details. The loci controlling quantitative traits have commonly been referred to as Quantitative Trait Loci (QTLs). The procedures for finding and locating the QTLs are called QTL analysis, which involves systematic search for linkage disequilibrium between traits and polymorphic marker loci and a significant association may be evidence of a QTL near the markers (Kearsey and Pooni, 1996; Liu, 1998; de Vienne, 2003; Collard et al., 2005). QTLs can be assayed indirectly by using linked marker loci. This indirect approach has long been recognized, but

until recently, it has been regarded as of minor importance because of the lack of sufficient genetic markers (Lynch and Walsh, 1998).

The idea behind using marker information to map and characterize QTLs is quite simple and based on crossing two inbred lines in order to create linkage disequilibrium between loci that differ between the lines, and this in turn creates associations between marker loci and linked segregating QTLs. For this, we need to create a segregating population to determine the genotype of individuals with a series of marker loci, to measure the value of the quantitative trait on each individual of mapping population and finally to use biometric methods to find the marker whose genotype is correlated with the traits. A large number of experimental designs and statistical methodologies have been developed to exploit this information. These designs can be categorized by the type of mapping population used for generating disequilibrium and the unit of marker analysis used (e.g., single marker, interval mapping) (Kearsey and Pooni, 1996; Lynch and Walsh, 1998; Doerge, 2002; de Vienne and Causse, 2003; Collard et al., 2005). Because of the broadness of this area, only an introduction to the concepts and techniques is provided here.

1.8.1 Mapping populations and their development

In plant, the conditions are generally favorable for the construction of genetic maps because controlled crosses can be done, homozygous parents are often available or can be developed, and large populations can be used. The most commonly used mapping populations in plants generally originate from a cross between two homozygous inbred parental lines (Figure 1.7) to produce F_1 individuals, identical and heterozygous for all loci that have been fixed different alleles in the parents. From the F_1 plant, different population such as backcross population, F_2 intercross, Double Haploid (DH), and Recombinant Inbred Lines (RIL) can be generated (Doerge, 2002; de Vienne, 2003; Collard et al., 2005).

A backcross population results from crossing of an F_1 with one of its parents called recurrent parent. If recurrent parent is genetically fixed, its meiosis does not lead to segregation, and its gametes are genetically identical and the F_1 will be the only one responsible for the segregation observed in the population. An F_2 population results from the self-pollination of individuals and since the male and female gametes are subject to recombination, two effective meioses cause the segregation. RILs are obtained by successive self pollination from F_2

individuals. At each generation, a single individual is chosen which will be the parent of the following generation. However, several generations of selfing are required to generate a set of RILs, so this process can be quite time consuming. Despite the succession of generations, the genome is not broken into numerous fragments, because at each meiosis the number of crossover is limited (de Vienne, 2003; Collard et al., 2005). Moreover, some regions of genome tend to stay heterozygous longer then expected from the theory (Burr and Burr, 1991).

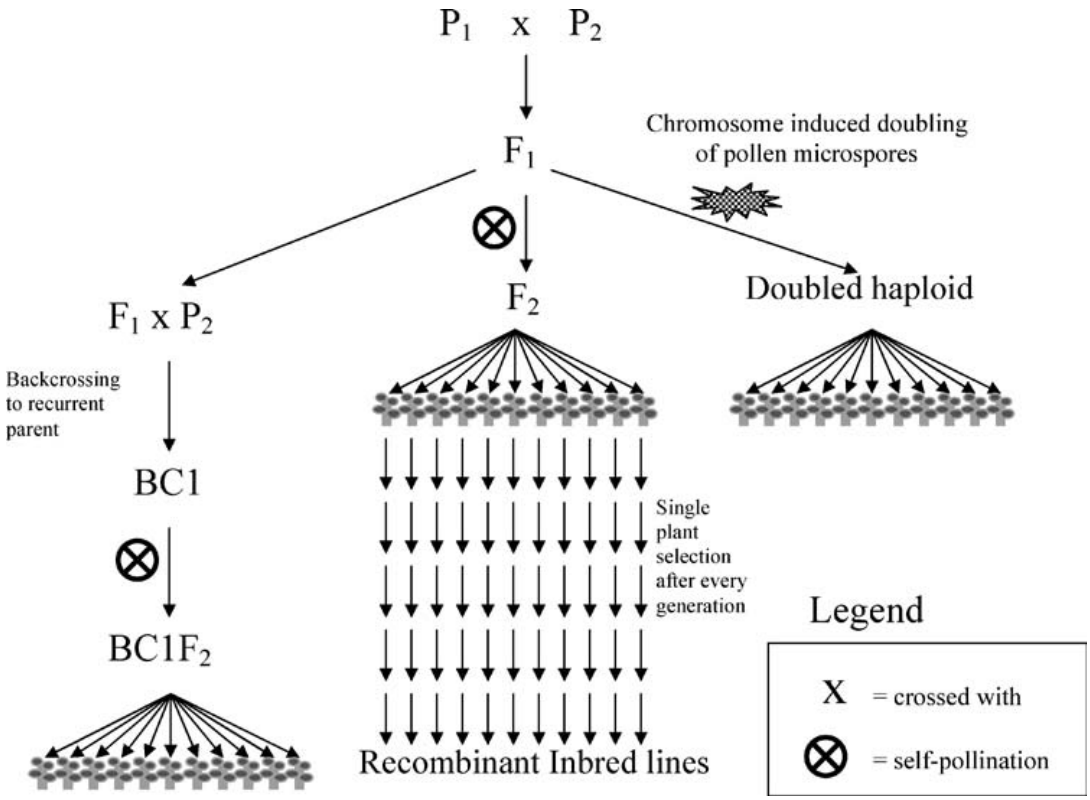


Figure 1.7 Diagram of main types of mapping populations in plants (Collard et al., 2005)

The mapping populations can be classified into two categories, temporary populations and permanent or immortalized populations. In a permanent population such as Recombinant Inbred Lines (RILs) and Doubled Haploid (DH), each individual in the population is genetically homozygous at all loci, (for RIL some heterozygosity may be retained). Therefore, the genetic composition will not change during self-pollination and they constitute a reference genetic material on which as many molecular marker can be accumulated as desired (de Vienne, 2003; Bernardo, 2008). The permanent populations also allow precisely determining the phenotype of complex quantitative traits through replicated experiments, and the same

genotype can be repeatedly tested under different environments for more accurate measurement of the quantitative trait (Tanksley, 1993).

Once an appropriate mapping population has been chosen, the appropriate population size must be determined. Since the resolution of a map and the ability to determine marker order is largely dependent on population size, this is a critical decision. Whenever possible, the larger the mapping population the better the map resolution (Young, 2001).

Only the F_2 populations which have the heterozygous genotype together with the two homozygous ones allow estimating the degree of dominance for loci linked to markers. The backcross population only with reciprocal crosses allows this estimating, since in this case the three genotypes represented can also be used to determine the degree of dominance. For both cases co-dominant markers must be used. For a given size of the progeny, the accuracy of estimation of *r* (recombination fraction) differs according to the type of progeny used. The F_2 with co-dominant markers provide the most accurate estimations of *r* and to obtain a given precision, twice as many individual are necessary in backcross or double haploids as for F_2 at least when the map is quite dense (de Vienne, 2003).

The main advantages of backcross population and F_2 intercross are that they are easy to generate and require only a short time to produce. The length of time needed for producing RILs is the major disadvantage, because usually six to eight generations are required. Doubled haploid (DH) populations may be produced by regenerating plants by the induction of chromosome doubling from pollen grains, however, the production of DH populations is only possible in species that are amenable to tissue culture (Collard et al., 2005).

1.8.2 Population genotyping and estimation of recombination rates

The second step in the construction of a linkage map is to identify DNA markers that reveal differences between parents. Several reviews have described molecular marker methods required to identify polymorphic markers and population genotyping (de Vienne, et al., 2003; Weising et al., 2005; Doveri et al., 2008; Maccaferri et al., 2009). It is critical that sufficient polymorphism exists between parents in order to construct a linkage map. Once polymorphic markers have been identified, they must be screened across the entire mapping population, including the parents and F_1 hybrid, if possible. This is known as population genotyping.

In a segregating population, there is a mixture of parental and recombinant genotypes. The frequency of recombinant genotypes can be used to calculate recombination fractions, which may be used to infer the genetic distance between markers. Even though the recombination rate *r* is defined as the ratio of the number of recombinant gametes to the total number of gametes (Kearsey and Pooni, 1996), and though we do not have access to the genotype of the gametes, in some certain population like backcross and double haploid *r* can be estimated directly from the population genotyping data. Because in these certain population the segregation occurs only by one meiosis so it is possible to reach to the haploid phase exactly. In other cases, especially in F_2 populations, it is necessary to use the maximum likelihood method to estimate the most probable value of *r* for a given pair of loci because two effective meioses cause the segregation. In RILs for a given pair of loci, the proportion *R* of lines that have recombined can be calculated but this is not an estimate of *r*. With successive generation, there are several chances for recombination in a given pair of loci. Therefore, *R* (The proportion of lines that have recombined in a given pair of loci with successive generation) can be higher than *r*. Therefore, a formula must be used to deduce *r* from the number of lines that have recombined. A statistical test is needed before declaring linkage between two loci. In practice the χ^2 test is sometimes used but most often the LOD score, derived from Maximum likelihood method is applied (Liu, 1998; de Vienne, 2003; Collard et al., 2005).

1.8.3 Genetic linkage map analysis

Genetic linkage maps are the fundamental tool to identify features of phenotypes that are linked to specific genetic loci (Lehmensiek et al., 2009). The tendency for genes or markers to be inherited in groups is known as genetic linkage, and the collection of genes or markers within a single chromosome is called a genetic linkage group (Poehlman and Sleper, 1995). By analyzing the segregation of markers, the relative order and distances between markers can be determined, the lower the frequency of recombination between two markers, the closer they are situated on a chromosome (conversely, the higher the frequency of recombination between two markers, the further away they are situated on a chromosome). Markers that have a recombination frequency of 50% are described as ‘unlinked’ and assumed to be located far apart on the same chromosome or on different chromosomes.

For a data set composed of a small number of genetic marker, a genetic map can be constructed using classical linkage analysis, for example, by estimation of pair wise recombination fraction among three loci from genotypic data of these loci in a doubled-haploid population. However, for a large data set with a large number of genetic markers, the markers are first grouped into different linkage groups. The number of linkage group should be close to the haploid number of chromosomes for the organism. In the next step which is called "three-locus ordering" markers in the same linkage group are ordered and their relative position on the map is determined. "Double crossover approach", "Two-locus recombination fraction approach", and "log likelihood approach" are some of the commonly used methods to order markers in each group. Linkage between markers is usually calculated using odds ratios (i.e. the ratio of linkage versus no linkage). This ratio is more conveniently expressed as the logarithm of the ratio, and is called a logarithm of odds (LOD) value or LOD score. LOD values greater than 3 are typically used to construct linkage maps. A LOD value of 3 between two markers indicates that linkage is 1000 times more likely (i.e. 1000:1) than no linkage (null hypothesis). LOD values may be lowered in order to detect a greater level of linkage or to place additional markers within maps constructed at higher LOD values. The unit for expressing the genetic distance between markers on a chromosome is the Morgan (or, more usually, the centiMorgan, cM) and is defined as the distance along which one recombinational event is expected to occur per gamete per generation (Liu, 1998; de Vienne, 2003; Collard et al., 2005).

In order to relate DNA markers to specific chromosome, aneuploid and substitution lines are applied. Both analysis have similar concept which are based on testing marker of interest on each chromosome of a complete set of aneuploid lines (such as nullisomics) or a substitution line and lack of the band on a line without specific chromosome identifies the location of the marker loci of interest (Young, 2001).

All linkage maps are unique and are the product of a mapping population derived from two specific parents and the types of markers used. However, previous linkage maps may provide an indication of which markers are polymorphic, as well as provide an indication of linkage groups and the order of markers within linkage groups. In case of access to previous published maps, they can be used as reference map, there is no need to work with aneuploid and substitution lines, and pervious information can be applied to develop a new map. In this

case markers can be added to a new map with an optimum number, either by focusing on markers evenly distributed on the genome, or by targeting specific regions of interest (Young, 2001; Collard et al., 2005).

1.8.4 Population phenotyping

Given plentiful markers and high-throughput genotyping technologies, QTL studies are limited by reliable phenotypic measures and multiple observations. The three main factors affecting QTL analysis include size of the population, density of markers on the genetic map, and quality of the phenotypic data. The size of the population determines the distribution of crossover points in the genetic map and thus directly affects the resolution of the QTL analysis. Likewise, the marker density will directly affect the resolution of the QTL. Both of them can be controlled easily. Hence, the success of QTL analysis is most affected by the quality of the phenotypic data. Poor data can lead to detection of spurious QTLs or improper estimation of the effect of a real QTL. It follows that complex traits such as yield require a high degree of replication and careful measurement of trait within each replication (Somers and Humphreys, 2009).

Experimental design is of great importance for appropriate phenotyping. With unlimited resources, replication of the experiment over time and space decreases all sources of variation. However, an appropriate design that minimizes the sources of variation has most impact on the experimental goals. If one would like to conclude that QTLs are repeatable across several independent experiments (perhaps across seasons or locations), then several studies must be performed (Borevitz and Chory, 2004; Somers and Humphreys, 2009).

1.8.5 Methods to detect QTL

There are several statistical procedures to determine association of markers to a quantitative trait. Three widely-used methods for detecting QTLs are single-marker analysis, simple interval mapping (SIM), made popular by Lander and Botstein (1989) and composite interval mapping (CIM) introduced by Zeng (Zeng, 1993, 1994). These biometric techniques can be classified into two categories, those that are based on marker-by-marker analysis, which is called “single marker analysis”, and those that simultaneously take into account two or more markers, which is called “interval mapping”.

All of the biometric techniques share the same basic principle: to partition the population into different genotypic classes based on genotypes at the marker locus and then to use correlative statistics to determine whether the individuals of one genotype differ significantly compared with individuals of other genotypes with respect to the trait being measured. If the phenotypes differ significantly, it is interpreted that a gene(s) affecting the trait is linked to the marker locus used to subdivide the population (Figure 1.8). The procedure is then repeated for additional marker loci throughout the genome to detect as many QTL as possible (Tanksley, 1993; Liu, 1998).

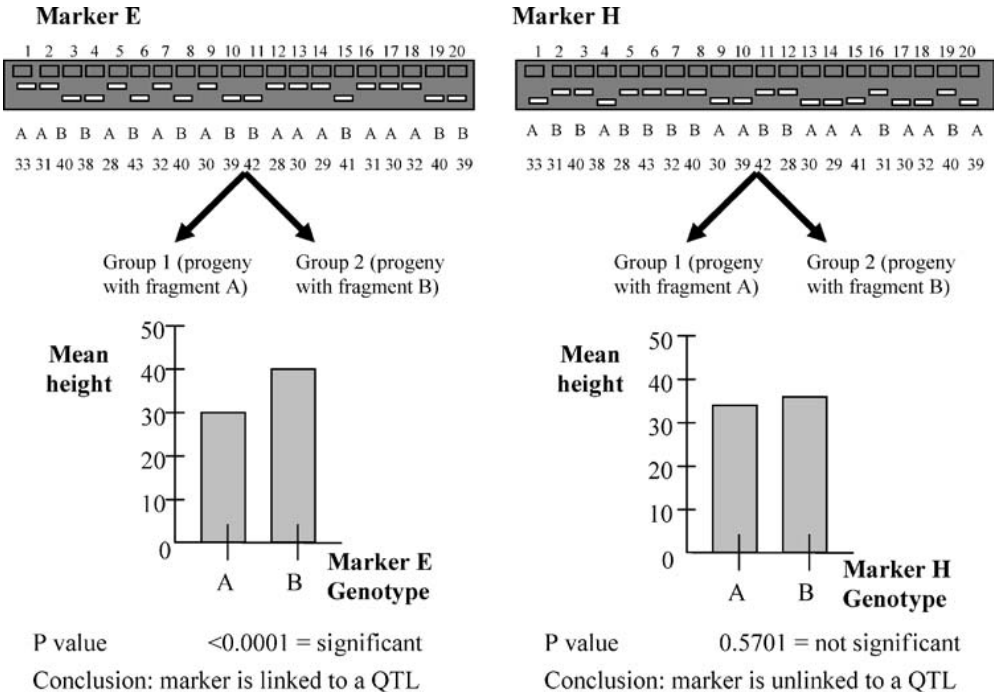


Figure 1.8 Principle of QTL analysis. Markers that are linked to a gene or QTL controlling a particular trait will indicate significant differences when the mapping population is partitioned according to the genotype of the marker. Based on the results in this diagram, Marker E is linked to a QTL because there is a significant difference between means. Marker H is unlinked to a QTL because there is no significant difference between means (Collard et al., 2005).

Single marker analysis can be implemented as a t-test, an analysis of variance, and a linear regression and it is simple in terms of data analysis and implementation. In single marker analysis gene order and a complete linkage map are not required. However, linkage map will help in interpretation and presentation of results. The disadvantage of this method is that it is ineffective in estimating the actual position and effects of QTL.

Simple interval mapping (SIM), using the likelihood approach or the regression approach, is the most commonly used methodology in QTL analysis. However, when there are several QTL in a large genome segment, the precise location of QTL cannot be determined. It is limited by both the model that defines it as a single QTL method, and by the one-dimensional search that does not allow interactions between multiple QTL to be considered. To resolve the problem, "Composite interval mapping" (CIM) (Zeng, 1993) and "Multiple QTL Mapping" (Jansen, 1993) were introduced in the same year. Both methods extend the ideas of interval mapping to include additional markers as cofactors outside a defined window of analysis. Compared to simple interval mapping, composite interval mapping approach increases the resolution of QTL location by controlling residual noise in the model using markers other than the ones immediately flank the segment (Jansen, 1993; Kearsey and Pooni, 1996; Liu, 1998; Lynch and Walsh, 1998; de Vienne and Causse, 2003).

Interval mapping methods produce a profile of the likely sites for a QTL between adjacent linked markers. The results of the test statistic for SIM and CIM are typically presented using a logarithmic of odds (LOD). The LOD profile is used to identify the most likely position for a QTL in relation to the linkage map, which is the position where the highest LOD value is obtained. A typical output from interval mapping is a graph (Figure 1.9) with markers comprising linkage groups on the X-axis and the test statistic on the Y-axis.

The peak or maximum must also exceed a specified significance level in order for the QTL to be declared as statistically significant. The determination of significant thresholds is most commonly performed using permutation tests. Briefly, the phenotypic values of the population are shuffled while the marker genotypic values are held constant that means all marker-trait associations are broken and QTL analysis is performed to assess the level of false positive marker-trait associations and the maximum value for the test statistic are recorded. Finally, these maximum values are sorted and a threshold declared as the percentile above which only a chosen proportion of scores fall: say the 95th, for a 0.05 significance threshold. This critical value is valid simultaneously for all analysis points in the linkage map. Many factors can vary from experiment to experiment and have influence on the critical value. These include, but are not limited to, the sample size, the genome size of the organism under study, the genetic map density, segregation ratio distortions, the proportion and pattern of missing data, and the number and magnitude of segregating QTL. Therefore, the critical value

is limited specifically to the data set on which permutation is performed (Churchill and Doerge, 1994; Hackett, 2002; Collard et al., 2005).

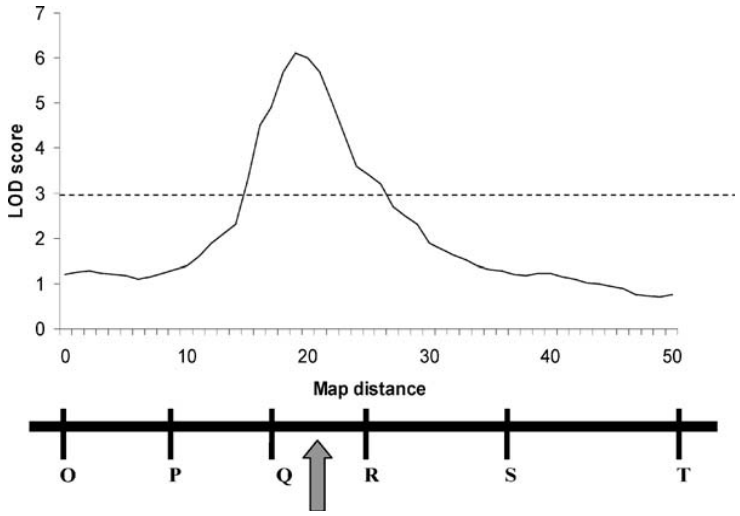


Figure 1.9 Hypothetical output showing a LOD profile for a chromosome. The dotted line represents the significance threshold determined by permutation tests. The output indicates that the best flanking markers for this QTL would be Q and R (Collard et al., 2005).

The accuracy of locating QTL is limited by the information, in particular the number of recombinants that is gained from observing the genotypic status of the markers. These observed recombinants can be limited by both small sample size and missing genotypic data (Doerge, 2002).

Previously, it was assumed that most markers associated with QTLs from preliminary mapping studies were directly useful in MAS. However, it has become widely accepted that QTL confirmation, QTL validation may be required (Langridge et al., 2001). Such confirmation studies may involve independent populations constructed from the same parental genotypes or closely related genotypes used in the primary QTL mapping study and sometimes, larger population sizes may be used (Melchinger et al., 1998; Collard et al., 2005). However, knowledge of the approximate locations of QTL has been used as a starting point for fine mapping or for studying candidate genes that are close to the identified QTL and that may be the actual genes that affect the quantitative trait (Bernardo, 2008).

Finally, QTL analysis itself is largely a statistical exercise combining genotype and phenotype data. However, it will play an important role in future genome analysis, since whole-genome sequencing for gene identification is still difficult especially for crop like wheat bearing large

genome size, the trend will continue for using QTLs as a staring point to high resolution mapping and then leading to gene cloning (Somers and Humphreys, 2009).

There are approaches like high resolution mapping, map based cloning, association mapping, comparative mapping which take data and material resulted from QTL analysis and continues the way from phenotype to reach more closely to the targeted gene.

1.9 Genetic linkage map construction in wheat

The first molecular marker genetic map in plants was made in tomato using RFLP markers and consisted of 57 loci (Bernatzky and Tanksley, 1986). Since then maps have been constructed for nearly all crop plants. The first wheat RFLPs genetic maps was created by Chao et al. (1989) for the chromosomes of the homoeologous group 7 and they demonstrated an approach how to map the wheat genome. Due to the low level of RFLP polymorphism in bread wheat (Chao et al., 1989) and in order to increase the level of polymorphism between parents of mapping populations, genome mapping using populations derived from wide crosses, was proposed as strategy. A standard hexaploid variety such 'Opata' was crossed with a synthetic hexaploid wheat, a chromosome-doubled hybrid of the wide cross tetraploid *T. turgidum* × diploid *Aegilops tauschii,* and resulted in a RILs mapping population known as ITMI (International Triticeace Mapping Initiative) consists of 114 lines derived by single-seed descent (Van Deynze et al., 1995).

With introducing microsatellite or Simple Sequence Repeat (SSR) which detect a much higher level of variability compared with RFLPs, Röder et al.(1998) using 70 lines of the ITMI population developed the first SSR-based genetic map in wheat. After that, attempts have been made world-wide to develop and map additional microsatellite markers on wheat. In common wheat (*Triticum aestivum* L.) SSR markers have been developed and mapped on the ITMI population (Röder et al., 1998b; Pestsova et al., 2000; Gupta et al., 2002; Somers et al., 2004; Song et al., 2005). Alternatively, the constituent genomes have been analyzed at the diploid level and for the D genome the isolation and development of microsatellite markers specifically derived from *Aegilops tauschii* significantly improved the coverage of the existing D-genome wheat microsatellite map (Boyko et al., 1999; Pestsova et al., 2000; Boyko et al., 2002). On the other hand, significant efforts have recently been placed on generating substantial EST-SSRs for plant species including wheat (Sourdille et al., 2004).

Ganal and Röder (2007) improved in collaboration between IPK-Gatersleben and TraitGenetics the ITMI map with 1169 SSR loci which currently bears the largest number of wheat SSR markers.

In order to accelerate map construction and also to fill the region of map bearing gap or showing low density of markers, AFLP marker which reveal polymorphism simultaneously at higher number of loci, compared with SSR, have been used. They were incorporated in many genetic maps in accompany with RFLP or SSR markers (Nachit et al., 2001; Sourdille et al., 2003; Verma et al., 2004).

Diversity arrays technology (DArT) (Jaccoud et al., 2001) which is a hybridization based marker technology and is capable to genotype simultaneously several thousand loci in a single assay were tested successfully by Akbari et al. (2006) in hexaploid wheat for genetic map construction. After that, several genetic maps of wheat published which used SSR markers in combination with DArT markers (Semagn et al., 2006; Peleg et al., 2008). Varshney et al. (2006) and Lenhmensiek et al. (2009) provided a summary of some maps, which were developed on wheat. The details of these genetic maps and the updated version of them are available at GrainGenes (http://wheat.pq.usda.gov.GG2/maps.shtml).

Although many mapping populations and their genetic maps including bread wheat are available, the wheat breeders and geneticists are primarily interested in their own mapping population. Therefore, the number of mapping populations is increasing. However, from a plant breeding point of view, an important pitfall of most mapping populations and corresponding QTL studies is that the parental lines have mainly been selected based on differences in target traits rather than on their overall agronomic value, which is often poor. Although this approach maximizes the possibility of identifying QTLs for the traits of interest, it does not guarantee any real progress in terms of field performance when the identified QTL allele is introgressed in the elite cultivars (Cadalen et al., 1998; Tuberosa and Salvi, 2006). Because of this, the intervarietal cross between cultivated bread wheat is of interest that means continuing genetic linkage map construction. The production of genetic maps of wheat is moving towards higher resolution mapping of specific regions associated with important agronomic or quality traits. Coincident with the need for higher resolution is a trend toward analyzing large F_2 populations because of high cost of producing DH lines (Lehmensiek et al., 2009).

1.10 QTL analysis of agronomic traits and drought tolerance in wheat

Varshney et al. (2006) summarized achievements of QTL studies in identification of QTLs for agronomic traits and resistance to biotic stress in wheat. Maccaferri et al. (2009) compiled the results of QTL studies against abiotic stress in Triticeae including wheat. These summaries together with other recent mapping works show that a variety of traits including resistance against biotic and abiotic stress, grain quality, morphologic and agronomic traits have been evaluated using numerous QTL analysis in wheat.

Some of the primary QTL studies took forward into next step in order to dissect the mapped QTLs. Quarrie et al. (2006) quantified a yield QTL using a set of near-isogenec lines. Lu et al. (2006) developed a SSR marker for a gene conferring sensitivity to the host-selective toxin produced by the tan spot fungus using EST-based genetic map and establishing co-linearity with rice. Röder et al. (2008) fine mapped a QTL for grain weight. Cuthbert et al. (2006) fine mapped a QTL for a fusarium head blight disease resistance. Ling et al. (2003) physically mapped a QTL for a leaf rust disease resistance, and Distelfeld et al. (2006) physically mapped a QTL for grain protein content. In these studies, wheat scientists were able to develop successfully closer flanking markers to the genes of interest and provided tools for selection in wheat breeding.

This considerable amount of QTL studies, first, shows the growing interest of wheat breeders and geneticists to the QTL mapping approach as powerful tool in the genomics era (Tuberosa and Salvi, 2006). Secondly, the researcher tested some traits more than other traits. Flowering time, grain protein content, grain weight, seed size, pre-harvest sprouting tolerance, and plant height were among the more investigated agronomic traits. There were intensive works on biotic and abiotic stresses, too. Thirdly, traits that were evaluated by several independent experiments revealed more chromosomal regions bearing the responsible QTLs. This is because of the fact that any QTL mapping can reveal only some of the QTL that were polymorphic among parental lines and therefore just a small fraction from QTL pool in the species is usually detected in each study. This is especially true for wheat with large genome size and higher number of linkage groups compared to other cereals that increases the probability for a QTL to be undetected because of low marker coverage in the genetic map. Even with the same mapping population, because of genotype by environment interaction, a QTL may be present only in some environments. Therefore, in order to identify all loci

responsible for the trait of interest and to find their increasing alleles, there should be more mapping populations from different parental lines and being tested on different environments. It means that increase in QTL studies needed to be continued and more traits should be evaluated.

In wheat, regarding drought stress, QTL mapping have been applied at several plant developmental stages including germination stage, seedling stage, vegetative stage, and grain filling stage. On the other side, in order to measure the effects of drought stress at each developmental stage several morpho-physiological traits in different mapping populations were recorded such as growth of roots, coleoptiles and shoots (Mohammadi et al., 2006; Landjeva et al., 2008), leaf senescence (Verma et al., 2004; Snape et al., 2007), abscisic acid (Quarrie et al., 1994), water-soluble carbohydrates (Snape et al., 2007; Yang et al., 2007; Rebetzke et al., 2008b), osmotic adjustment (Morgan and Tan, 1996), carbon isotope discrimination (Rebetzke et al., 2008a), and grain yield (Kirigwi et al., 2007; Salem et al., 2007; Kordenaeej et al., 2008; Maccaferri et al., 2008; Mathews et al., 2008). It should be mentioned that, although there are large number of QTL analyses against drought stress at whole plant or cell level, however, only few QTL studies have been conducted for grain yield under field trials.

As mentioned above several traits were considered in order to identify QTL with potential for selection against drought stress. They show the complexity of drought because it seems there is not a single, magical drought tolerance trait (Reynolds et al., 2005; Pennisi, 2008) and also no clear consensus exists on key traits conferring wheat productivity in drought stress condition. In contrary, and being optimistic, this is because of the effort and contribution of researchers from different field of science including pure science where research predominantly have been made on the traits associated with survival under extreme stress than those related with agronomic productivity (Reynolds et al., 2005).

In wheat most QTL studies has not been extended beyond their QTLs detection for a given trait under drought therefore the work still is on primary level and no QTL for drought tolerance has been fine mapped or cloned (Cattivelli et al., 2008). The limited success in improving drought resistance is primarily due to the difficulty in identifying and accurately measuring the key physiological determinants of yield under drought condition in the field (Maccaferri et al., 2009) and also because of mapping populations which have been

developed especially for drought adaptive traits and not yield performance under drought stress (Tuberosa and Salvi, 2006). Moreover, in most of the QTL mapping experiments that have been conducted especially in the beginning, researchers used the available mapping population and did not develop populations suitable for drought stress.

Above all, QTL mapping only shows the differences of the alleles in the parental lines, and do not show which loci and genes are the most important for the breeding of the trait of interest e.g. water stress improvement. Therefore, because of this theoretical limitation of QTL analysis, the application of the identified marker for improving traits in other crosses, like high yielding cultivars, is limited (Tiessen et al., 2006).

Finally, the road ahead of wheat breeders to improve wheat lines against drought stress through QTL study which is, actually, exploitation of natural variation of drought adaptive traits (Collins et al., 2008) may be long, but should be approached with optimism. The improvement is slow, however it is clear (Collins et al., 2008) and the resulting knowledge will be a piece of a drought puzzle. Therefore finding QTLs should be continued which increase yield performance and adaptation of wheat plant under drought stress with appropriate mapping populations accompanied by accurate phenotyping.

1.11 The objectives of this study

Yield under stress condition is one of the most complex traits that are inherited quantitatively. Considering the QTL analysis as a powerful method for dealing with this kind of complex characters, the present study entitled “Linkage map construction and identification of Quantitative Trait Loci (QTLs) determining post-anthesis drought tolerance and other agronomic traits in bread wheat” was conducted.

Therefore, and with consideration of the value of developing own mapping population, the main objectives of this study are as following:

1- Establish a molecular genetic linkage map for bread wheat using an intraspecific cross between two bread wheat accessions HTRI 11712 and HTRI 105.

2- Perform a QTL analysis of agronomic traits under control and post-anthesis drought stress conditions especially for trait like thousand-grain weight as one of the wheat yield components affected by stress during grain filling period.

2 Material and Methods

2.1 Plant material - Parents and mapping population development

Screening for parental lines was carried out based upon a primary investigation of 100 bread wheat accessions, collected from different parts of the world and representing enough diversity, selected from wheat collection of IPK-Gatersleben genebank. It was performed through field experiment at IPK-Gatersleben (elevation 112 m above sea level) via chemical desiccation (see below) in 2000 (unpublished data). Finally, two accessions were chosen according to their behavior against post-anthesis drought stress. A tolerant accession HTRI 11712 (*T. aestivum* L.) originated from Pakistan and a sensitive HTRI 105 (*T. aestivum* L.) from Sweden. Table 2.1 and Figure 2.1 show that these two accessions were similar regarding growth habit, spike density, and plant height. They were different for awnedness and glume hairiness but the main reason for their selection was difference to response against post-anthesis drought stress.

Table 2.1 Characters of parental lines

Parents	Variety	Origin	Growth habit	Spike density	Awnedness	Glume hairiness	Plant height	Thousand -grain weight under (Control-Stress)
A = HTRI 11712	heraticum (Kob.) Mansf.	Pakistan	winter	loose	short awns	high	140 cm	27.7 - 23.8
B = HTRI 105	lutescens (Alef.) Mansf.	Sweden	winter	loose	awnless	absent	150 cm	31.2 – 14.0

The two parental lines, HTRI 11712 as male parent and HTRI 105 as female parent, were crossed to develop F_1 plant in 2001. In the two successive years 2002 and 2003 the mapping population was developed. By selfing of only one F_1 plant 143 F_2 plants were generated and then all seeds from each F_2 plants were harvested and considered as $F_{2:3}$ family. Ten of the F_2 plants did not produce enough seed to be used as $F_{2:3}$ families, therefore they were removed from population phenotyping. However, they were applied for population genotyping and genetic linkage map construction. Finally, population genotyping and phenotyping were conducted on 143 F_2 plants and 133 $F_{2:3}$ families, respectively.

Figure 2.1 Spikes, seeds, and plants of HTRI 105 and HTRI 11712 (left and right, respectively)

2.2 Methods

2.2.1 Genotyping and genetic linkage map construction

2.2.1.1 Enzymes, buffers and solutions

Enzymes, buffers and solutions as well as DNA isolation buffers are given in Appendix 2.

2.2.1.2 Genomic DNA extraction

Total genomic DNA was isolated according to the protocol described previously by Anderson et al. (1993). Briefly, 3-5 g of leaf tissue per sample (each sample was collected from each single eight-weeks-old F_2 plant, 143 plants in total) were ground in liquid nitrogen and its powder was added to 50 ml polypropylene tubes. Extraction buffer, (100 mM Tris-HCl, 500 M NaCl, 50 mM EDTA, 1.25 % SDS) was added to the 50 ml tubes. Just before use 3.8 g/l Nabisulfite was added to readjust pH. Then they were incubated at 60°C for 45 minutes in a water bath. After cell disruption and incubation with hot isolation buffer, proteins were removed by chloroform : iso-amyl alcohol (24:1, *v:v*). Samples were incubated for 30 minutes by shaking and then centrifuged at 3,000 rpm for 30 minutes. The aqueous layer was transferred to a new tube and 20 µl RNase A (10 mg/ml) was added. Samples were incubated for 30 minutes at room temperature. One volume of cold ethanol was added to precipitate DNA. After 30 minutes incubation at 4°C, precipitated DNA was hooked out and placed in a 2 ml reaction tube containing 1 ml of 75% ethanol. After washing twice with 75% ethanol, the washing solution was removed and the DNA pellet was dried thoroughly and dissolved in TE

buffer. The DNA samples were diluted and stored at -20 °C. The DNA was diluted to a concentration of 50 ng/µl for SSR experiment.

Genomic DNA was run on 1% agarose gel at 100v for 90 minutes in 1x TAE buffer. A molecular ladder (1Kb) was run as standard for comparison. After run, the gel was stained with ethidium bromide (a florescence dye which intercalates between the base pairs of DNA) and visualized under UV light in a transilluminator. The quality and quantity of DNA were measured by comparison of band-intensity on stained agarose gels with a DNA molecular weight standard. According to the band width, DNA was diluted for further steps of PCR experiment.

2.2.1.3 Simple sequence repeats (SSRs) primer pairs

Primer pairs from three different sources were applied such as 'GWM' stands for 'Gatersleben Wheat Microsatellite' (Röder et al.1995; Röder et al.1998b), 'GDM' stands for 'Gatersleben D-genome Microsatellite'(Pestsova et al., 2000), and 'BARC' stands for the acronym of the USDA-ARS Beltsville Agricultural Research Center (Song et al., 2005) which were developed for the US Wheat and Barley Scab Initiative to map and characterize genes for fusarium resistance.

Nearly all primer pairs from GWM, ranging the name from GWM0002 to GWM1303 including 612 primer pairs, were investigated in order to identify the polymorphic ones, mainly to develop the map and later on 10 primer pairs from GDM and 40 from BARC were used to fill the gaps. The information for the primer pairs which were applied in the present study are in Appendix 3.

2.2.1.4 Reference genetic maps

The ITMI (International Triticeae Mapping Initiative) map (Röder et al., 1998b; Ganal and Röder, 2007) was mainly used to find the position and the order of SSR loci for the GWM and GDM primer pairs. In order to find some proper BARC loci to fill the gaps, the microsatellite consensus map (Somers et al., 2004) was used. The ITMI map was developed on the ITMI population including 68 recombinant inbred lines which was derived from a cross between 'W-7984', a Synthetic amphi-hexaploid wheat and the Mexican wheat variety 'Opata 85' from CIMMYT (Centro Internacional de Me joramiento de Maizy Trigo). The

ITMI population was developed for the purpose of having a shared mapping population among wheat scientists. The microsatellite consensus map (Somers et al., 2004) was constructed by joining four independent genetic maps of bread wheat bearing microsatellite markers from different research groups including the Wheat Microsatellite Consortium, GWM, GDM, and BARC.

2.2.1.5 Polymerase chain reaction (PCR)

SSR procedures were as described by Röder et al. (1998). Each Polymerase Chain Reaction (PCR) was performed in a volume of 25 µl in Perkin-Elmer (Norwalk, Ct.) thermo cycles. Table 2.2 shows the reaction mixture that was used in this experiment. Amplification for all microsatellites carried out according to the Table 2.3. After the final extension (polymerization) step, the samples were transferred to 4 °C or to -20 °C if they were not going to be used immediately.

Table 2.2 The reaction mixture for one, 50, and 100 samples

Stock	PCR reaction in 25 µl	1X	50X	100X
10X PCR buffer	1 M Tris-HCl 1 M KCl 1.5 mM MgCl2	2.5 µl	125 µl	250 µl
Left primer	250 nM	0.65 µl	32.5 µl	65 µl
Right primer	250 nM	0.65 µl	32.5 µl	65 µl
***Taq* DNA polymerase**	1 U	0.1 µl	5 µl	10 µl
dNTPs	0.2 nM	50 µl	100 µl	200 µl
Genomic DNA	50-100 ng			
dd H20	Variable	14.1 µl	705 µl	1410 µl

Table 2.3 The program for thermocycler which was applied for PCR amplification

Steps	Temperature	Time	No. of cycles
Initial denaturing	94 °C	3 min	1 (First cycle)
Denaturing	94 °C	1 min	45 Cycles
Annealing	50, 55 or 60 °C	1 min	
Elongation	72 °C	2 min	
Final elongation	72 °C	8 min	1 (Last cycle)

2.2.1.6 Electrophoresis and fragment analysis

The PCR products were separated with the help of the Automated Laser Fluorescence (ALF) express DNA sequencer machine (Amersham Biosciences, Freiburg, Germany) (Biosciences,

2004b, a) using short gel cassettes Figure 2.2. The technique involves using of labeled 5` primer with a fluorescent tag, Cy5, for the PCR reaction. A sample of about 1.5 µl of PCR product were mixed with 2µl internal size standard, denatured at 96°C for 2 min and chilled on ice. Denaturing 6% polyacrylamide gels, 0.35 mm thick, were prepared using ReproGel™ .The gels were run about 60 to 90 minutes depending on the expected fragment size, in 1 X Tris-borate-EDTA (TBE) buffer (pH 8.0) with 800 V, 50 mA and 50 W with 2 mW laser power and a sampling interval of 1.00 sec. The gels were re-used four to five times depending on the quality of the gel that was determined by a parameter called Laser value indication on the machine. During electrophoresis, the fluorescently labeled fragments in each lane migrate downwards through the gel. The fixed laser beam passes through the glass spacer located between the glass plate and the thermoplate of the gel cassette. The beam is directed into the gel perpendicular to the direction of band migration. The laser beam excites the fluorescently labeled PCR product and the light emitted is detected by photodetectors located behind the gel.

Figure 2.2 Automated Laser Fluorescence (ALF) express DNA sequencer

Since, there were 40 photodetectors, one for each lane; for each run forty samples were loaded including one well for external standard. On the first well external standard is loaded which contained a mixture of four DNA fragment like 73, 122, 196, and 231 bp. For rest of the 39 wells, depending on the expected size of PCR product, two fragments with known size like 73, 122, 196, 231, and 304 bp were included as internal standards. Microsatellite fragment sizes were calculated using the computer program Fragment Analyser Version 1.02 ‘Amersham Bioscience, Freiburg, Germany’ (Biosciences, 2004b, a). Firstly all peaks

including external standard, internal standard and expected SSR fragments were marked and all the rest unspecified peaks were deleted from the output of ALFexpress. Secondly, three or four peaks as external standard, which was loaded on the well number one and were detected on first line of output were defined as external size standard and then the internal standard peaks on the rest 39 wells were adjusted to external size standards. Then the fragment sizes of PCR products were calculated by comparison them with the internal standard peaks (Röder et al., 1998).

2.2.1.7 Genotypic data scoring

Co-dominant marker data was scored as A, B and H to mark F_2 genotypes like parent HTRI 11712, parent HTRI 105, and heterozygote, respectively. An allele was considered as null if it showed amplification in Chinese Spring wheat cultivar but no amplification in parental lines even after several enquiries with fresh material. In F_2 population since at locus bearing null allele, the heterozygous individuals can not be detected from one of the homozygous parent, dominant scoring was applied. The dominant scoring was also used for co-dominant loci when because of unclear peak there was some difficulty to distinguish between the heterozygous and homozygous individuals.

Dominant markers were scored as following: A and C scoring when parent HTRI 11712 had null allele. Therefore, individuals showing null allele are scored as A. C means the individuals correspond to the parent HTRI 105 or they are heterozygous. B and D scoring when parent HTRI 105 had null allele. Therefore, individuals showing null allele are scored as B. D means the individuals correspond to the parent HTRI 11712 or they are heterozygous. Finally '-' was used for missing data. Whenever there were several peaks from a primer pair, each peak was scored separately as dominant marker and after constructing the linkage groups, alleles which co-segregate were considered as alleles of the same loci and then their separate dominant scoring were converted to the co-dominant score (Collard et al., 2005). More explanation with graph in Appendix 4.

2.2.1.8 SSR loci nomenclature

Detected loci were marked with an '*X*' as the basic symbol for molecular marker loci of unknown function in wheat followed by SSR primer pair name, GWM, GDM, and BARC.

The loci that were mapped for first time in the present study were marked with red color, extra loci were marked by blue color. Extra of the first time mapped loci were marked by a bold red exclamatory mark (**!**). Markers with more than one locus (Multilocus) were marked by a blue capital m (M). Loci with null allele were marked by "0". Loci with distorted segregation were marked by an asterisk (*) in parenthesis.

First time mapped locus was defined as the locus corresponding to the primer pair which was mapped for the first time in the present study. Extra locus was defined as the locus corresponding to the primer pair which has already been mapped in other studies but showed a extra locus on the same linkage group or another linkage group in the present study. Multiple loci or multilocus was defined as the loci corresponding to a primer pair which has several fragments and each could be separately mapped by dominant scoring.

2.2.1.9 Segregation distortion evaluation

Chi-square (χ^2) test was performed to check the segregation pattern of alleles of each locus against the expected Mendelian ratios like 1:2:1 for co-dominant and 3:1 or 1:3 for dominant loci. Distorted loci were again checked precisely to correct any kind of possible mistake in the scoring. The distorted loci were marked by an asterisk (*) in parenthesis.

2.2.1.10 Genetic linkage map construction

Genetic linkage groups construction analysis was performed using the computer program MAPMAKER (Lander et al., 1987) through evaluating the genotypic data generated from the 143 F_2 plants of mapping population. Data file was arranged according to the position and order of the loci displayed on the published genetic linkage map (Röder et al., 1998) of the ITMI population. Markers were grouped by applying two-point analyses with 'Grouping' command with minimum LOD = 3.0 and recombination ratio maximum $\theta = 0.40$. The linkage group and position of the loci which were first time mapped and also BARC loci were added to the map by 'Grouping' command and followed by 'Try' command. The obtained order of markers was then analyzed further using a three-point linkage analysis 'Ripple' command. Chromosome assignment were determined by comparing the map to the previously published wheat maps, especially to the wheat SSR map from Röder et al. (1998). Marker loci and linkage group that were more than 50 cM apart were considered to be not significantly linked.

Kosambi function (Kosambi, 1944) was used to convert the recombination frequency to genetic distances in centimorgans (cM). The approximate position of centromere on the linkage maps were estimated according to the published physical map by Sourdille et al. (2004).

2.2.2 Experiments for the phenotypic traits evaluation

The phenotypic evaluation of HTRI 11712 × HTRI 105 mapping population consisting of 133 $F_{2:3}$ families was performed in four different environments at IPK-Gatersleben including field experiments in 2004 and 2005 and greenhouse experiments in 2004 and 2007. The soil type at the experimental site was silty loam and a common cultivation was practiced.

2.2.2.1 Field experiment in 2004

$F_{2:3}$ families and the parents were grown on the experimental field of IPK-Gatersleben during the wheat growing season 2003/2004 with one replication in which each plot contains four rows with 100 cm length and 20 cm width between the rows. Two rows were considered as control and the two others as treatment. Characters were measured from the control rows and all spikes from control rows in each plot were harvested as bulk when the plants reached maturity. For the two treatment rows, drought stress was conducted via chemical desiccation and seed harvested only from labeled spikes (see 2.2.3.1).

2.2.2.2 Greenhouse experiment in 2004

$F_{2:3}$ families and the parents after treatment for eight weeks at 4˚C in growth chamber, exposing vernalization, were transferred to greenhouse at IPK-Gatersleben in which there were six pots including three controls and three treatments, each containing one plant, per each family. On the three treatment pots, drought stress was conducted via chemical desiccation and seed harvested only from labeled spikes (see 2.2.3.1).

2.2.2.3 Field experiment in 2005

$F_{2:3}$ families and the parents were evaluated based on an experimental design called *Augmented* Randomized Complete Block Design (RCBD) (Federer, 1961) with 10 blocks and 18 plots per block. Each line was present only once in the experiment and a set of three

German wheat cultivars as standard cultivars was repeated on each block. So there were 15 $F_{2:3}$ families plus 3 standard cultivars in each block. [Originally, in this experiment 143 $F_{2:3}$ families plus parents were tested therefore ten blocks were considered. However, ten $F_{2:3}$ families were removed from further data analysis]. Each plot contains four rows with 100 cm length and 20 cm width between the rows. Two rows were considered as control and the two others as treatment. Characters were measured from the control rows and all spikes from control rows in each plot were harvested as bulk when the plants reached maturity. For the two treatment rows, drought stress was conducted via chemical desiccation and seed harvested only from labeled spikes (see 2.2.3.1).

2.2.2.4 Greenhouse experiment in 2007

$F_{2:3}$ families and the parents after exposing vernalization period by spending eight weeks at a temperature of 4 ˚C in growth chamber were grown in greenhouse at IPK-Gatersleben in which there were 12 pots, each containing one plant, per each $F_{2:3}$ family. Six pots were treated as control and the other six pots as treatment. Drought stress was applied by stopping irrigation on treatment pots two weeks after flowering.

2.2.3 Phenotyping methods

2.2.3.1 Chemical desiccation as a simulator for post-anthesis drought stress

In order to simulated post-anthesis drought stress Blum et al. (1983) proposed chemical desiccation of whole wheat plants which can be used for revealing genetic variation among wheat accessions. In this simulation, spraying the wheat plants with Magnesium chlorate 14 days after anthesis would rapidly kill the main photosynthetic tissues and then subsequent grain growth would be entirely supported by the remobilization of vegetative reserves. After that, Nicolas and Turner (1993) confirmed the utility of chemical desiccation as a post anthesis simulator and also they compared Magnesium chlorate with some other desiccants and sensing agents on wheat plant and found Potassium iodide (KI) as a desiccant having the least toxic effect on grain growth. This method has been confirmed by several independent studies which show its usefulness and efficiency (Regan et al., 1993; Blum, 1998; Royo and Blanco, 1998) and was, therefore, used in the present study. Anthesis was recorded when about 50% of the plants showed spikes with exerted anthers in the central third of the spikes

as shown in Figure 2.3. Spikes with 50% of anthesis were labeled and fourteen days after anthesis, chemical desiccation was applied to the plants on the two treatment rows of each plot, while the other two rows were kept untreated (without desiccation) by using a plastic cover Figure 2.4, Figure 2.5, and 2.6 compare control and stressed wheat plants (via chemical desiccation) one week after sparing and on the time of harvesting, respectively.

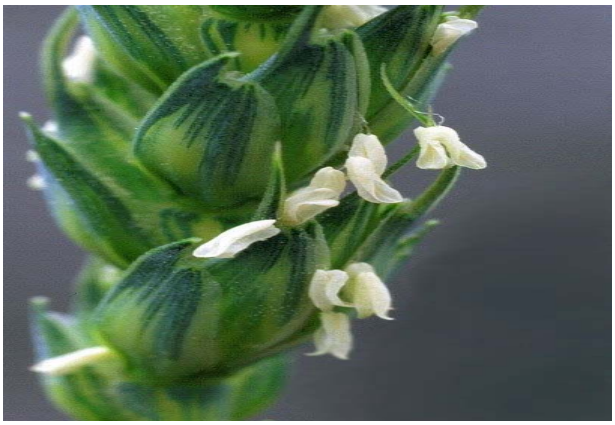


Figure 2.3 Wheat spike with exerted anthers

Figure 2.4 Treatment with KI in field experiment

Figure 2.5 Plants one week after KI treatment

Figure 2.6 Plant at the time of harvesting

The desiccation treatment was applied by spraying the whole plant canopy to full wetting with an aqueous solution of potassium iodide (KI, 0.5 % *w/v*). The desiccant was applied using a hand-held boom sprayer allowing spray penetration to the whole plant canopy. However, the desiccation treatment in greenhouse experiment 2005 was applied by spraying the whole plant on the three pots, which were considered as treatment.

2.2.3.2 Targeted traits

In total 14 characters were scored in different experiments. Not all characters were recorded in each experiment (Table 2.4).

Table 2.4 Trait recorded in each experiment

Traits	Experiments			
	Field 2004	Greenhouse 2004	Field 2005	Greenhouse 2007
Thousand-grain weight(C)	X	X	X	X
Thousand-grain weight(S)	X	X	X	X
Seed area(C)	X	X	X	X
Seed area(S)	X	X	X	X
Seed width(C)	X	X	X	X
Seed width(S)	X	X	X	X
Seed length(C)	X	X	X	X
Seed length(S)	X	X	X	X
Days to flowering	X	X	X	X
No. seeds per spike(C)		X	X	X
No. seeds per spike(S)				X
Seed weight per spike (C)		X	X	X
Seed weight per spike(S)				X
Spike length(C)			X	X
Spike length(S)				X
Plant height(C)			X	X
Plant height(S)				X
Weight of 3 spikes per plant(C)			X	X
Weight of 3 spikes per plant(S)				X
No. of fertile spikes per plant(C)				X
No. of fertile spikes per plant(S)				X
Weight of all spikes per plant(C)				X
Weight of all spikes per plant(S)				X
No. of unfertile tillers per plant(C)				X
No. of unfertile tillers per plant(S)				X
Awnedness			X	X

C = Control, S = Stress, X = Trait was recorded

Targeted traits:

Thousand-grain weight and grain characters: Thousand-grain weight, Seed area, seed width, and seed length were recorded using a grain analyzer called Marvin equipment (GTA Sensorik GmbH, Neubrandenburg, Germany) which is a kind of scanning machine. These characters were measured from a random sample of about 250 to 300 seeds from each $F_{2:3}$ family.

Days to flowering: It was determined as the number of days from date of sowing to the date of the first anthers exertion of 50% of the ears.

Spike length: Length of 10 spikes per each plot and three spikes per each plant were recorded in field and greenhouse experiments, respectively.

Plant height: Length of the main culm (in centimeters) was measured from the soil surface to the tip of the main ear at maturity, excluding awns.

Number of fertile spikes per plant: Number of spikes bearing seeds per each plant was counted

Number of unfertile tillers per plant: Number of tillers with unfertile spike per each plant was counted.

Weight of three spikes per plant: Weight of three spikes per each plant was recorded.

Weight of all spikes per plant: Weight of all spikes per each plant was measured

Number of seeds per spike: Number of seed from 10 spikes per each plot and three spikes per each plant was recorded in field and greenhouse experiments, respectively.

Seed weight per spike: Weight of seed from 10 spikes per each plot and three spikes per each plant was recorded in field and greenhouse experiments, respectively.

Awnedness: Awnedness was considered as qualitative trait and recorded as scale of which 0 for awnless, 2 for short awns and 4 for long awned under field experiment 2005 and greenhouse experiment in 2007. Moreover, awn length was also recorded in centimeters on each single plant at greenhouse experiment in 2007.

2.2.4 Descriptive statistics and analysis of variance for the experiments

The normality of data was checked graphically by histogram of the observed frequency distribution. The existence of extreme single variates, or outliers due to errors of recording was tested graphically and for each suspected outlier, the raw data were checked. Errors were eliminated, but otherwise the data were not eliminated. Descriptive statistics like minimum, maximum, range, mean, variance, and coefficient of variation (CV) were calculated for each trait. Pearson's correlation coefficient was calculated to investigate relationships between the quantitative traits observed in each environment separately and for the mean of all experiments (Sokal and Rohlf, 2001). Moreover, correlation between each pair of the environments regarding the same trait was calculated and served as rough estimate of

heritability in these experiments (Falconer, 1952; Lynch and Walsh, 1998; Börner et al., 2002). These parts of data analysis were performed using SPSS version 9.0 (SPSS-Inc, 1999). Analysis of variance was carried out for the experiments. In addition, an *Augmented* Randomized Complete Block Design (RCBD) (Federer, 1961) was conducted in 2005 at IPK-Gatersleben experimental field station in order to check the null hypothesis regarding the homogeneity of field condition (Steel et al., 1997; Sokal and Rohlf, 2001).

A linear mixed model analysis of variance without replication (Hicks and Turner, 1999) was conducted for the data from quantitative traits which were collected on the control and stress condition, each one separately and for the combined experiments including both control and drought stress. For each of the separate analysis for the control and stress conditions, a nested three-factor factorial analysis of variance without replication was conducted. A two-tailed test was made (Table 2.5) in which genotypes (133 $F_{2:3}$ families) and locations (field and greenhouse) were considered as two fixed factors crossed with each others and years as random factor nested within locations.

Table 2.5 Source of variation (SOV), Degree of freedom (df), Expected mean square (EMS), and *F* test for the linear mixed model of the nested three-factor factorial analysis of variance without replication

	SOV	df	EMS	*F* test
1	G_i	(G-1)=132	$\sigma^2_{GY} + 4 Ø_G$	MS1/MS5
2	L_j	(L-1)=1	$\sigma^2_{GY} + 133 \sigma^2_Y + 266 Ø_L$	MS2/MS4
3	$G*L_{ij}$	(G-1)(L-1)=132	$\sigma^2_{GY} + 2 Ø_{LG}$	MS3/MS5
4	$Y(L)_{k(j)}$	L(Y-1)=2	$\sigma^2_{GY} + 133 \sigma^2_Y$	MS4/MS5
5	$G*Y(L)_{ik(j)}$	(G-1)L(Y-1)=264-X	σ^2_{GY}	

G=genotype, L=location, Y=year, X=number of missing data (0 for control and 1 for stress)

This analysis was applied under control condition for the following traits: thousand grain weight, seed area, seed width, seed length, days to flowering, number of seeds per spike, weight of seeds per spike, spike length, and plant height. The last five traits were not measured in all four experiments, so that for some sources of variation the number of degree of freedom is reduced. Under stress condition the analysis of variance was applied for the four traits thousand-grain weight, seed area, seed width, and seed length because the other traits were not recorded or were recorded only at one experiment.

In the combined analysis of variance of the seed related traits, a nested four-factor factorial analysis of variance without replication was conducted (Table 2.6) in which genotypes (133 $F_{2:3}$ families), locations (field and greenhouse), and treatments (control and stress) were considered as three fixed factors crossed with each others and years as random factor nested within location.

Table 2.6 Source of variation (SOV), Degree of freedom (df), Expected mean square (EMS), and *F* test for the linear mixed model of the nested four-factor factorial analysis of variance without replication

	SOV	df	EMS	*F* test
1	G_i	(G-1)=132	$\sigma^2_{GTY} + 2\,\sigma^2_{GY} + 8\,Ø_G$	MS1 / MS9
2	L_j	(L-1)=1	$\sigma^2_{GTY} + 266\,\sigma^2_{Y} + 532\,Ø_L$	MS2 / MS8
3	T_k	(T-1)=1	$\sigma^2_{GTY} + 133\,\sigma^2_{TY} + 532\,Ø_T$	MS3 / MS10
4	$G*L_{ij}$	(G-1)(L-1)=132	$\sigma^2_{GTY} + 2\,\sigma^2_{GY} + 4\,Ø_{GL}$	MS4 / MS9
5	$G*T_{ik}$	(G-1)(T-1)=132	$\sigma^2_{GTY} + 4\,Ø_{GT}$	MS5 / MS11
6	$L*T_{jk}$	(L-1)(T-1)=1	$\sigma^2_{GTY} + 133\,\sigma^2_{TY} + 266\,Ø_{TL}$	MS6 / MS10
7	$G*L*T_{ijk}$	(G-1)(L-1)(T-1)=132	$\sigma^2_{GTY} + 2\,Ø_{GLT}$	MS7 / MS11
8	$Y(L)_{m(j)}$	L(Y-1)=2	$\sigma^2_{GTY} + 266\,\sigma^2_{Y}$	-
9	$G*Y(L)_{im(j)}$	(G-1)L(Y-1)=264	$\sigma^2_{GTY} + 2\,\sigma^2_{GY}$	-
10	$T*Y(L)_{km(j)}$	(T-1)L(Y-1)=2	$\sigma^2_{GTY} + 133\,\sigma^2_{TY}$	-
11	$G*T*Y(L)_{ikm(j)}$	(G-1)(T-1)L(Y-1) =264-X	σ^2_{GTY}	-

G=genotype, L=location, T=treatment, Y=year, X=number of missing data (0 for control and 1 for stress)

In order to verify the effect of drought stress on the measured traits a one-tailed test was applied for stress treatment factor, since it could be expected the superiority of the traits under control condition over stress condition. This analysis was applied for thousand grain weight, seed area, seed width, and seed length.

There were 2 years 2004 and 2005 for field experiments, and 2 years 2004 and 2007 for greenhouse experiments. Since the result of specific year was not interested, therefore year was treated as nested within locations as a random factor. There was no replication for each

combination of factors. SAS software (SAS-Institute, 2000) was applied to carry out the analysis of variance. The SAS procedures applied for the two above mentioned analysis and the test for *F*-values are given in Appendix 7.

Heritability (h^2) of the traits per each control and stress condition was calculated based on the components of the expected mean square from the corresponding linear mixed model analysis of variance (Table 2.5). Heritabilities were calculated as $h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_e)$ where σ^2_g is the genetic variance among $F_{2:3}$ families and σ^2_e is the error variance.

2.2.5 Quantitative Trait Loci (QTLs) analysis

PLABQTL program, version 1.2 (Utz and Melchinger, 2007), was applied to localize and characterize QTLs. The program employs the interval mapping approach (Lander and Botstein, 1989) based on multiple regression analysis with flanking markers according to the procedure described by Haley and Knott (1992). Marker loci with a distance smaller than 1.1 cM were excluded from the QTL analysis to prevent ill-conditioned equation systems and the generation of "synthetic" new markers by the PLABQTL program (Utz and Melchinger, 2007). Furthermore, marker loci with high segregation distortion were also removed.

Bonferroni chi-square approximation (Zeng, 1994) was applied to the overall exploratory QTL experiments and a genome wide type-I error rate of 0.25 (Beavis, 1998) with 2 degrees of freedom because of additive effects fitted for QTL in the model. In order to check the repetition of the detected QTL, the mapping analysis was conducted with a lower LOD and only the QTL which were detected at the same position from the other measurements in the present study were considered as repetition of the QTL and no other new QTL were included in the result.

The whole-genome scan with CIM (Composite Interval Mapping) was conducted using the automatic covariate selection statement ('cov select'). The LOD curves were checked visually as recommended in the user's manual, because in very seldom cases peaks or QTL is not detected by the program and is missed in the LIST OF DETECTED QTL. In all CIM runs, only additive genetic effects were considered in the model. The minimum distance between two putative QTLs to be listed as separate QTL was 10 cM.

Since the CIM method was applied for QTL mapping, the coefficient of determination or the percentage of the phenotypic variation (R^2) explained by a single QTL was based on partial coefficient of correlation of the QTL with the phenotypic observed variable (Utz and

Melchinger, 2007). Permutation test (Churchill and Doerge, 1994) was applied to determine the appropriate threshold value for declaring significant QTL effects. A 1000 permutations were carried out to estimate critical thresholds at different level of α including α = 0.50, α = 0.30, α = 0.25, α = 0.05, and α = 0.01. The locations of individual QTLs were drawn on genetic maps using MapChart 2.1 (Voorrips, 2002).

The symbolization of the QTLs follows the rules of McIntosh et al.(2008). However, in the present study in order to distinguish QTLs from different experiments or different stress conditions, two letters and one number were included to the name of each QTL pear each experiment as following. F and G as abbreviation for the two locations field and greenhouse, C and S as abbreviation for control and stress condition, and finally 4, 5, and 7 indicating the year when the experiment was conducted. For example, FC4 in QTgw.ipk-4B-FC4 indicates a QTL that was identified at field experiment (F) under control condition (C) in 2004 (4). The QTL analysis also was applied on the mean of all experiments, the mean of field, and the mean of greenhouse experiments for each of the control and stress conditions. Therefore the corresponding detected QTLs were named by MC, MS for the overall mean under control and stress condition, respectively and MGC and MGS for mean of field under control and stress condition, respectively, and finally MFC and MFS for the mean of field under control and stress condition, respectively. QTgw.ipk-7A-FMS, for example, shows a QTL that was detected based on the mean of field data under stress condition.

QTLs of a trait from different experiments that their peaks placed on regions of a chromosome with less than 20 cM distance and showing increasing allele from the same parent was considered as the same QTL that mapped at different experiments. The efficiency of alleles in a given identified QTL to discriminate $F_{2:3}$ families for the trait of interest were verified by Boxplots using left or right markers of the identified QTL (Sokal and Rohlf, 2001).

3 Results

3.1 Genetic linkage map construction

3.1.1 Microsatellite loci assessment in parental lines

3.1.1.1 Degree of polymorphism

From the total of 666 SSR primer pairs including 612 GWM, 10 GDM, and 44 BARC, which were applied to survey polymorphism between the parental lines, 398 and 243 revealed to be polymorphic and monomorphic, respectively and 25 were without any amplification. On average about 60% of tested primer pairs revealed polymorphism. The 394 polymorphic primer pairs contained 372 GWM, 5 GDM, and 21 BARC markers whereas the 243 monomorphic primer pairs compiled 215 GWM, 5 GDM, and 23 BARC markers.

Although the majority of the primer pairs showed amplification at least on one of the two parents, 25 (3.8%) (Table 1 in Appendix 3) did not show any amplification even after several PCR amplification attempts with fresh material. Since these primer pairs were already mapped in the ITMI population, the lack of amplification in the present study could be due to the presence of null alleles in both parental lines. Furthermore, there were about 76 null allele loci detected within the 398 polymorphic primer pairs (19%), in which parent A and parent B were 35 and 39 times responsible, respectively.

Since the majority of primer pairs tested in the present study, were from GWM (587 out of 612, with ignoring the 25 primer pairs without amplification), the screening information of these markers is given in Table 3.1. Regarding the 372 polymorphic GWM primer pairs that correspond to 383 loci, based on the reference map (Röder et al., 1998b; Ganal and Röder, 2007), 140 were located on genome A, 163 on genome B, and 80 on genome D (Table 3.1). For the 215 monomorphic GWM primer pairs (Table 2 in Appendix 3) the 235 corresponding loci were located on genomes A (69), B (89), and D (77).

While on average about 62 percent of tested GWM primer pairs revealed polymorphism, these ratios were 67, 65, and 51 percent for genomes A, B, and D, respectively. Among the seven homoeologous groups, a maximum ratio of polymorphism (0.69) exhibited by the three chromosomes of homoeologous groups 5 and a minimum of this ratio (0.57) belonged to the chromosomes of homoeologous groups 1 and 2. However, the polymorphism ratio per individual chromosomes revealed that chromosome 6B with the ratio of 0.81 and

chromosome 6D with the ratio of 0.29 had the highest and lowest polymorphisms, respectively.

Table 3.1 Polymorphism of the loci corresponding to 587 GWM primer pairs

	Polymorphic loci	Monomorphic loci	Total loci	Polymorphism ratio
1A	11	12	23	**0.48**
2A	35	14	49	**0.71**
3A	16	7	23	**0.70**
4A	17	13	30	**0.57**
5A	16	7	23	**0.70**
6A	16	6	22	**0.73**
7A	29	10	39	**0.74**
Genome A	**140**	**69**	**209**	**0.67**
1B	24	11	35	**0.69**
2B	15	19	34	**0.44**
3B	26	11	37	**0.70**
4B	20	9	29	**0.69**
5B	27	11	38	**0.71**
6B	29	7	36	**0.81**
7B	22	21	43	**0.51**
Genome B	**163**	**89**	**252**	**0.65**
1D	10	11	21	**0.48**
2D	16	17	33	**0.48**
3D	8	11	19	**0.42**
4D	6	7	13	**0.46**
5D	15	8	23	**0.65**
6D	4	10	14	**0.29**
7D	21	13	34	**0.62**
Genome D	**80**	**77**	**157**	**0.51**
Total	**383**	**235**	**618**	**0.62**

Regarding the number of polymorphic primer pairs detected per chromosome, on genome A chromosome 2A with 35 loci, on genome B chromosome 6B with 29 loci, and on genome D chromosome 7D with 21 loci showed the highest number of polymorphic loci, while chromosome 1A with 11 loci, 2B with 15 loci, and 6D with 4 loci showed the lowest. Therefore, overall 2A had the highest number of polymorphic loci while 6D showed the

lowest. Interestingly, chromosome 2A, with 35 polymorphic loci out of 49 tested ones showed about 71% of polymorphism and was not the most polymorphic chromosome

This result showed that the number of polymorphic microsatellite primer pairs and also the ratio of polymorphism were not uniformly distributed among 21 chromosomes, three genomes, and seven homoeologous groups. It means that there was no equal chance to find the same number of polymorphic loci among the 21 wheat chromosomes.

3.1.1.2 Allelic variation and allelic difference in parental lines

Figures 3.1 a and b show variation of microsatellite allele sizes on each of the parental lines ignoring null alleles. Two hundred and ninety two microsatellite alleles from parent A had a range of 215 bases with a minimum of 78 at *Xgwm0003-3D* and maximum of 293 at *Xgwm0140-1B*. The mean, mode, median, and standard deviation were 163.7, 119, 154 and 44.66 bases, respectively. Two hundred and ninety one microsatellite alleles from parent B showed a range of 248 bases with the minimum of 72 at *Xgwm1070-2B* and maximum of 320 at *Xgwm0372-2A*. Mean, mode, median, and standard deviation were 164.3, 108, 154 and 44.79, respectively. Although the range of allele size in parent B was larger than in parent A both had nearly the same means, medians and variances. It can be seen from Figure 3.1 a and b that the distributions of microsatellite allele sizes on both parents showed a shift towards the left of the curves.

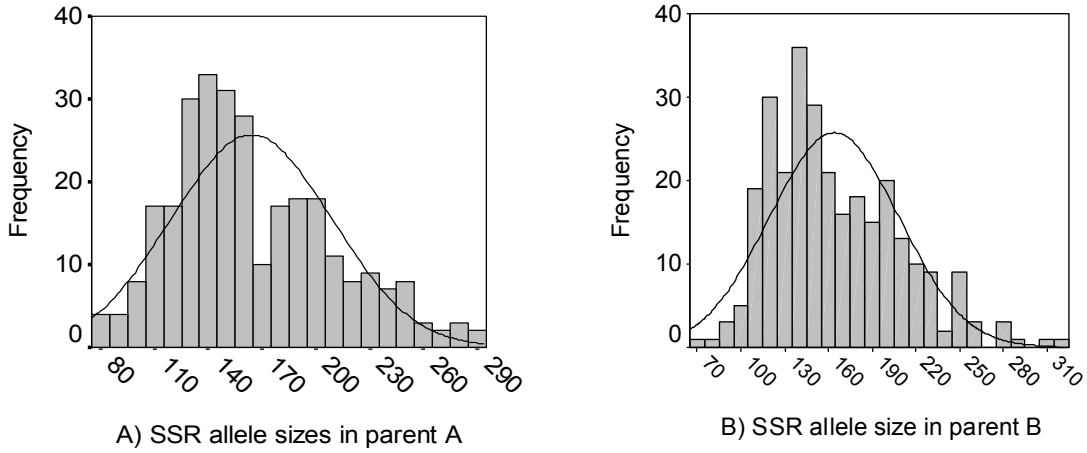


Figure 3.1 The distribution of allele sizes in parent A and parent B

Regard to SSR repeat motifs on the applied polymorphic primer pairs, ignoring 43 dominant loci and considering only the 270 co-dominant loci, it mainly (255 out of 270) consisted of dinucleotide repeat (NN) and there were nine primer pairs with trinucleotide (NNN) repeat motifs and finally seven primer pairs with tetranucleotide (NNNN) repeat motifs or more. Observed polymorphism between alleles from the parental lines (Figure 3.2) ranged from two to 80 bases and mainly with even number of differences.

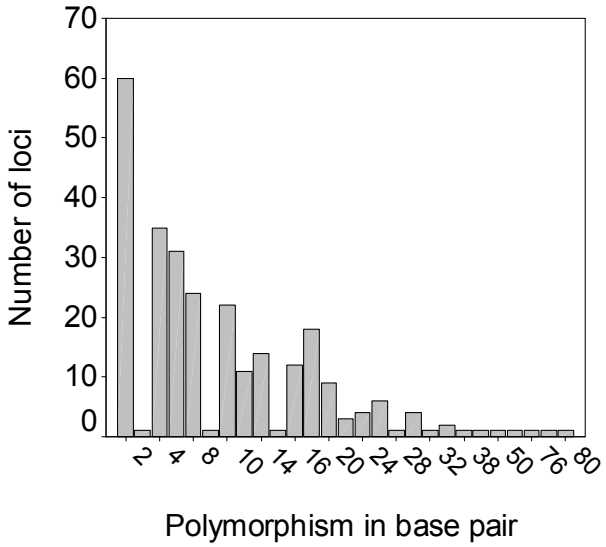


Figure 3.2 Bar chart of polymorphisms between parental lines

The bar chart with L-shape shows, the amount of loci decreasing rapidly with increasing the amount of base pair polymorphism in the parental lines. As it was expected, most of the polymorphisms were 2 bases or a multiple of 2 bases and few of them were 3, 9, and 15 bases. Importantly, around 31 percent of loci showed polymorphism of 4 or less than 4 bases. Since in the population genotyping, whenever was possible primer pairs with higher number of base pairs polymorphism were applied.

3.1.2 Population genotyping

According to the positions and genetic distances of polymorphic loci displayed on the published ITMI map (Röder et al., 1998b) and consensus map (Somers et al., 2004) a total of 273 microsatellite primers pairs (Table 3 in Appendix 3) including 247 GWM, 21 BARC, and 5 GDM were selected to genotype all of the 143 F_2 plants. Moreover, there are 111 polymorphic primer pairs (Table 4 in Appendix 3) still remaining, which were distributed

among different chromosome regions. Due to their very close loci on the ITMI map, the close loci in the present study were expected. Therefore, they could not be so informative in this study and thus they were not considered for population genotyping. However, for more simplicity and accuracy always the loci with sharper and clear peak and with bigger polymorphism were applied. Loci with null allele were ignored as much as possible except for the loci that were going to be mapped for the first time in this study. Only 43 null allele loci were applied for population genotyping so the data set contained 43 dominant loci out of 313 loci (14%), with nearly equal number of null alleles for parents A (21) and B (22). From the remaining 111 polymorphic primer pairs, the ones that are located in the region of a putative QTL can be used later for fine mapping studies with large number of individuals in order to find a closer interval for the given QTL.

By application of these 273 polymorphic primer pairs, about 417 loci were amplified but 313 loci out of them showed polymorphism. Table 5 in Appendix 3 shows information regarding these loci including monomorphic and polymorphic fragment size in parental lines and Chinese Spring, mode of inheritance, expected loci in the ITMI map, extra loci, first time mapped loci and segregation distortion for the loci. Two hundred and ninety three (293) loci out of these polymorphic loci could be mapped but the rest of 20 remained unmapped (Table 6 in Appendix 3). [Since there were no proper genetic maps for 4D and 6D, it can be possible that some of the unmapped loci belong to 4D and 6D chromosomes]. Finally, 14 primer pairs (Table 7 in Appendix 3) showed polymorphism on parental lines, but were not easy to score or did not behave as expected. Therefore, they were ignored for further analysis.

3.1.3 Genetic linkage map

This chapter gives general information about the new constructed genetic linkage map. Detailed information of the map considering the homoeologous groups and chromosomes are given in the Appendix 5.

The genetic map of 21 linkage groups associated with 19 chromosomes, consisting of 293 loci is shown in Figure 3.3. The approximate position of centromere on the genetic maps were estimated according to the published physical map by Sourdille et al. (Sourdille et al., 2004). The 293 loci were mapped on 21 linkage groups in which 110 loci on genome A, 121 on genome B, and 62 on genome D. Table 3.2 shows map data with respect to genomes and

chromosomes. The highest number of mapped loci belonged to homoeologous group 2 with 60 loci followed by homoeologous group 7 with 54 loci while the lowest number belonged to homoeologous group 4 with 29 loci by considering the lack of map for chromosome 4D.

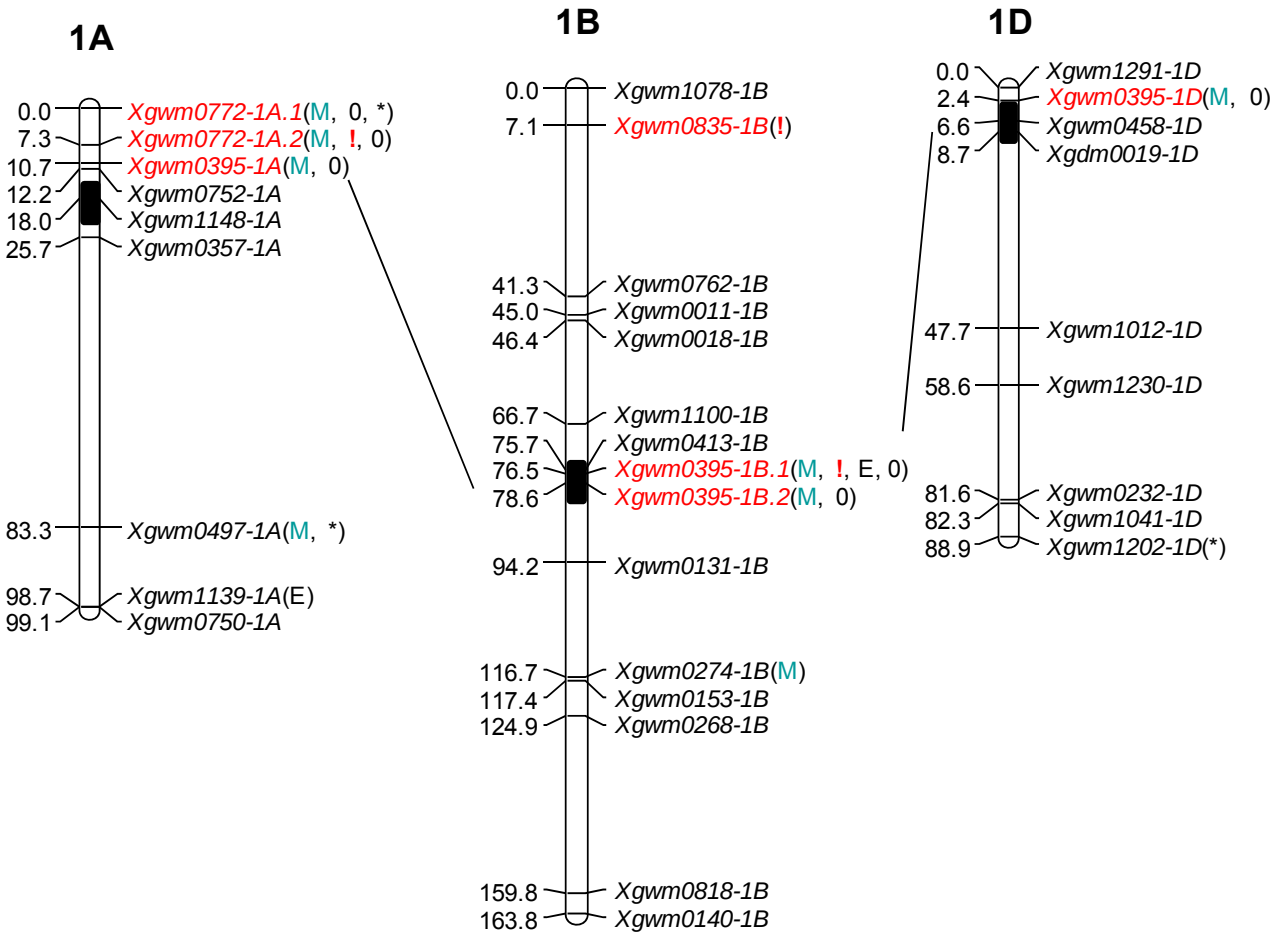


Figure 3.3 The genetic linkage map of hexaploid wheat HTRI 11712 × HTRI105. Map distance were shown in centiMorgans (cM) using the Kosambi mapping function (Kosambi, 1944). The short arms of chromosomes are at the top. The approximate centromere locations are indicated in black color. The loci which were mapped for first time are marked in red color, markers with more than one locus (Multilocus) were marked by M, loci with null allele by 0, distorted locus by an asterisk (*). Extra loci were indicated by blue color and in case it was also an extra locus of a first time mapped locus by red color and a bold red color exclamatory mark (**!**), and loci which were excluded from QTL analysis (loci with a distance smaller than 1.1 cM, see 2.2.5) by E. Lines connected the homoeologous loci.

Figure 3.3 Continued

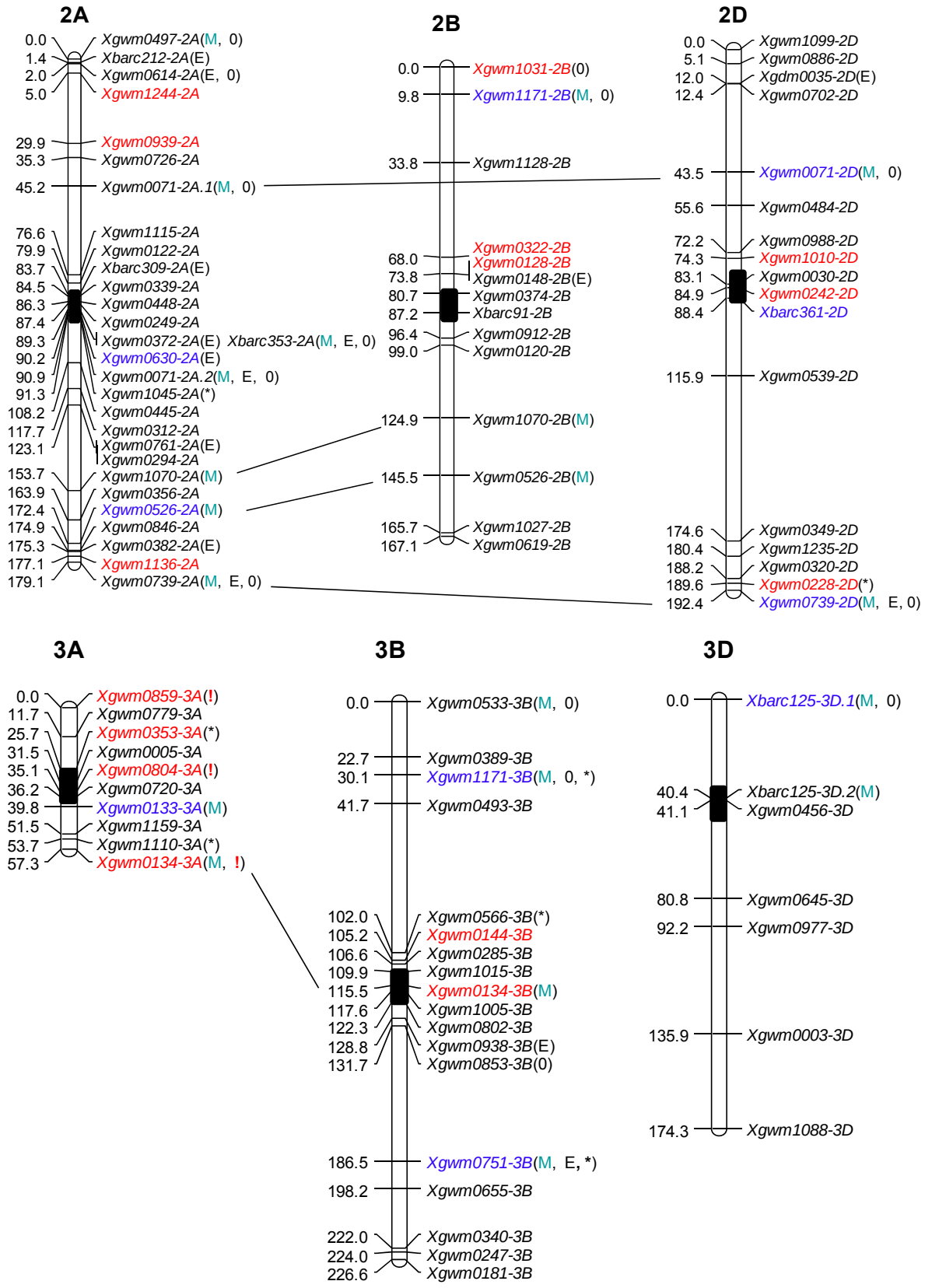


Figure 3.3 Continued

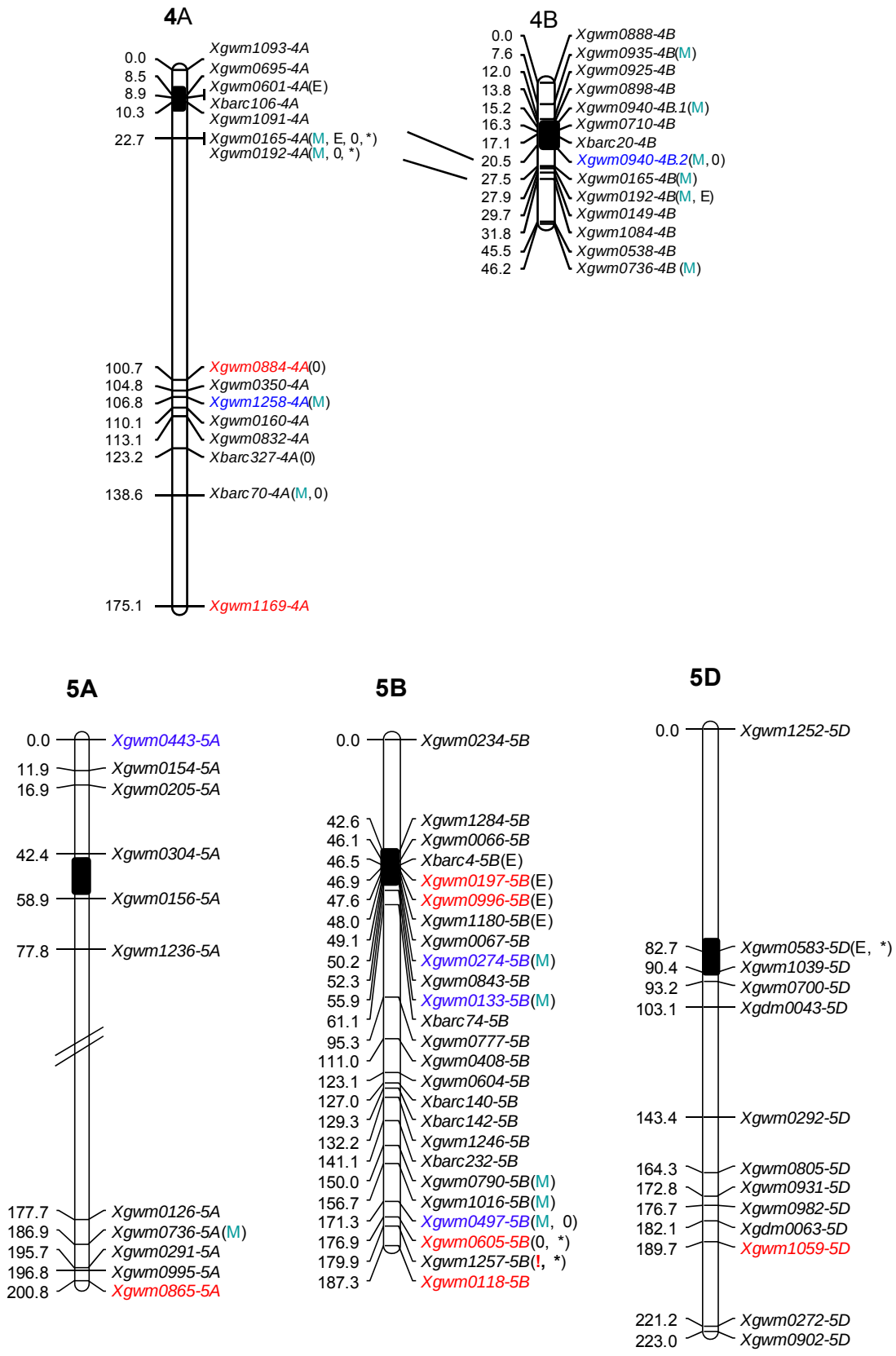


Figure 3.3 Continued

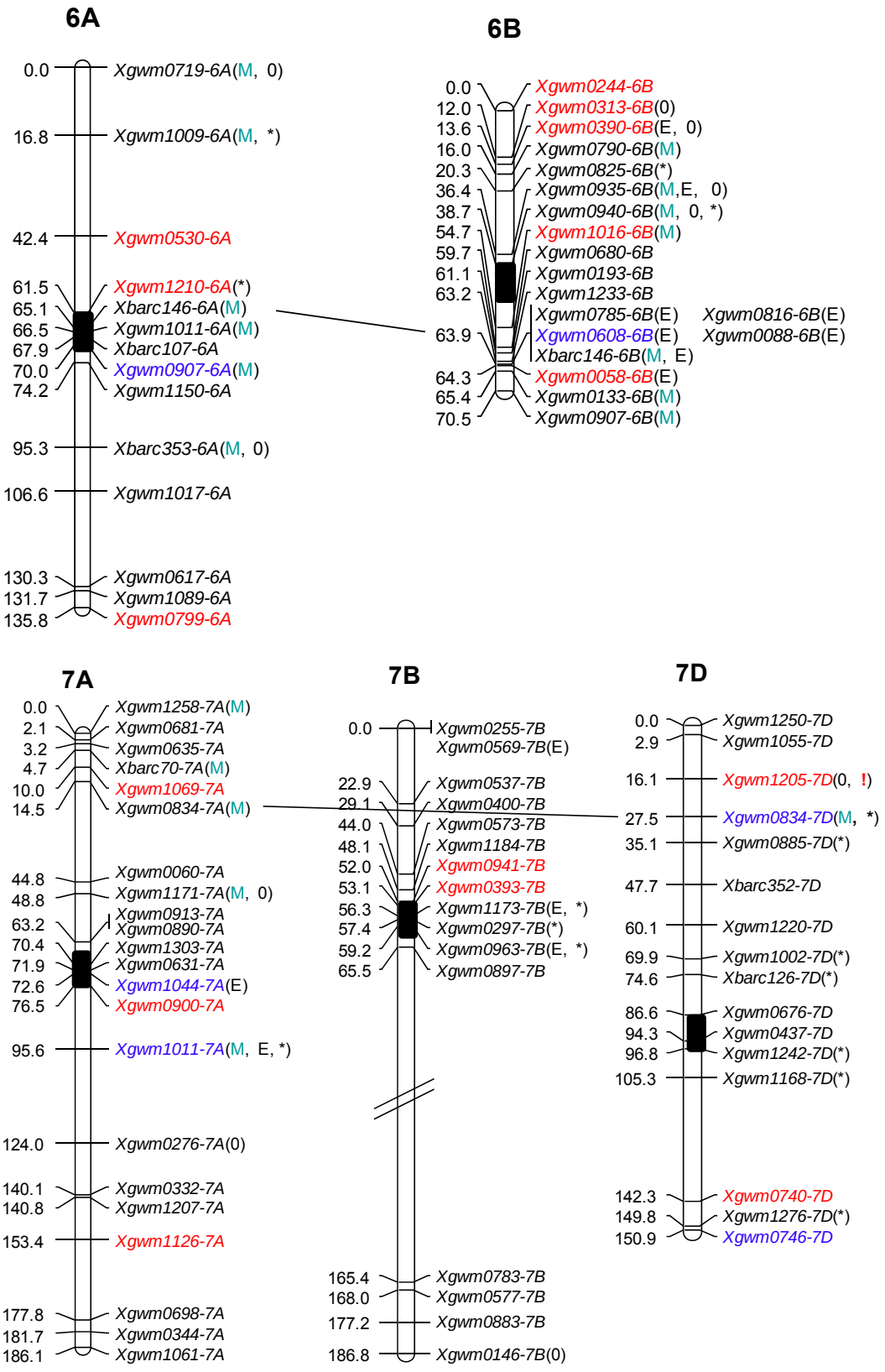


Table 3.2 Mapping data, considering genomes and chromosomes

Linkage group	Total mapped Loci	No. co-dominant loci	No. dominant loci	Map Length (cM)	Marker density (per cM)	No. first time mapped	Percent mapped loci	No. extra loci	No. missing data	No. multiple loci
1A	9	6	3	99.1	11.1	3	3.1	1	18	4
2A	29	23	6	179.1	6.2	3	10.1	2	40	7
3A	10	10	0	57.3	5.7	4	3.5	4	17	2
4A	15	10	5	175.1	17.7	2	5.1	1	25	4
5A	11	11	0	77.8 + 23.3	9.2	1	3.8	1	34	1
6A	14	12	2	135.8	9.7	3	4.9	1	23	6
7A	22	20	2	186.3	8.5	3	7.5	3	34	5
Total genome A	**110**	**92**	**18**	**933.8**	**8.5**	**19**	**37.5**	**13**	**191**	**29**
1B	15	13	2	163.8	10.9	3	5.2	2	29	3
2B	14	12	2	167.1	11.9	3	4.9	1	14	3
3B	18	16	2	226.6	12.6	2	6.3	2	23	4
4B	14	13	1	46.2	3.3	0	4.8	1	17	6
5B	25	23	2	187.3	7.5	4	8.7	4	46	5
6B	19	15	4	70.5	3.7	5	6.6	1	17	7
7B	16	15	1	65.4 + 21.2	5.4	2	5.6	0	23	0
Total genome B	**121**	**107**	**14**	**948.1**	**7.8**	**19**	**41.3**	**11**	**169**	**28**
1D	9	8	1	88.9	9.9	1	3.1	0	8	1
2D	17	15	2	192.4	11.3	3	5.9	3	29	2
3D	7	6	1	174.3	24.9	0	2.4	1	10	2
5D	13	13	0	223.0	17.1	1	4.5	0	14	0
7D	16	16	0	150.9	9.4	2	5.6	3	22	1
Total genome D	**62**	**58**	**4**	**829.5**	**13.4**	**7**	**21.2**	**7**	**83**	**6**
Total genome A, B, and D	**293**	**257**	**36**	**2711.4**	**9.2**	**45**	**100**	**31**	**443**	**63**

In genome A with seven linkage groups, chromosome 2A with 29 mapped loci (Figure3.3) had the highest number, while chromosome 1A with nine mapped loci showed the lowest. In genome B also seven linkage groups were constructed and the highest and lowest number of mapped loci belonged to chromosomes 5B and 4B with 25 and 14 loci, respectively. In D genome five linkage groups were constructed and linkage groups for chromosome 4D and 6D are missing since there were not so much segregating loci for them. Chromosome 2D with 17 loci had the highest and chromosome 3D with seven loci showed the lowest number of mapped loci on genome D. Overall, chromosome 2A showed the highest number of mapped loci while chromosome 3D had the lowest.

This genetic map with 293 mapped loci, including 257 co-dominant and 36 dominant loci, had a total length of 2711 cM (Table 3.2). There were two gaps on chromosomes 7B and 5A (Figure 3.3). The chromosome length ranged from 46 cM for chromosome 4B to 226 cM for chromosome 3B. The number of loci per chromosome ranged from seven to 29 for chromosome 3D and 2A, respectively. The average of chromosome length and number of loci per chromosome was 141.14 cM and 15.15, respectively. Therefore, there was on average one locus per each 9.2 cM. From 293 mapped loci there were 45 and 31 loci as first time mapped and extra loci, respectively.

[First time mapped locus: The locus corresponding to the primer pair that was mapped for the first time in the present study].

[Extra locus: The locus corresponding to the primer pair that already was mapped in other study but showed a new locus in the present study.]

The linear order of marker loci for each chromosome was verified from the ITMI reference map (Röder et al., 1998b; Ganal and Röder, 2007). Only few discrepancies were observed between the genetic maps obtained in the present study and the ITMI map. Most of the discrepancies were observed close to the centromeres where it is difficult to order the loci accurately (Sourdille et al., 2004)

Genome A with 110 mapped loci (Table 3.2) bearing 36.8 percent of all mapped loci including 92 co-dominant and 18 dominant loci, had a total length of 933.2 cM with 8 linkage groups associated with all the seven chromosomes of this genome. Twenty nine out of 110 mapped loci (26.4 %) in genome A were multiple loci and the rest were specific loci. The average of chromosome length and number of loci per chromosome was 130.22 cM and

15.14, respectively. So there was on average one locus per each 8.6 cM. From 106 mapped loci there were 19 loci and 13 loci as first time and extra loci, respectively

Genome B with 121 mapped loci (Table 3.2) bearing 41.7 percent of all mapped loci including 107 co-dominant and 14 dominant ones, had a total length of 947.8 cM with eight linkage groups associated with all the seven chromosomes of this genome. Twenty eight out of 121 mapped loci (23.3%) in genome B were multiple loci and the rest were specific loci. The average of chromosome length and number of loci per chromosome was 134.39 cM and 17.14, respectively. Therefore, there was on average one locus per each 7.8 cM. From the 121 mapped loci there were 19 and 11 loci as first time mapped and extra ones, respectively.

Genome D with 62 mapped loci (Table 3.2) bearing 21.5 percent of all mapped loci, including 58 co-dominant and four dominant loci, had a total length of 829.3 cM with five linkage groups corresponding to the five chromosomes of this genome except 4D and 6D. Six out of 62 mapped loci (9.7%) in genome D were multiple loci. The average of chromosome length and number of loci per chromosome was 130.22 cM and 17.14, respectively. So there was on average one locus per each 13.37 cM. From 62 mapped loci there were seven and eight loci as first time mapped and extra loci, respectively

3.1.4 Features of the genetic map

3.1.4.1 The accuracy of the genetic map regard to missing data

The genotypic data set contained 44,759 data points (143 × 313) which were compiled from genotyping of 143 individuals with 313 loci. Four hundred and sixty seven (467) of these data, equal to one percent, were recorded as missing data including either no amplification or any suspicious peak. Therefore, about 99 percent of this data was recorded without any discrepancy, showing the high level of the accuracy of this data set.

To the 19 constructed linkage groups 41,899 (143 × 293) genotypic data were applied, which were compiled from genotyping of 143 individuals with 293 loci and from this data set 443 data, about one percent was as missing data.

Regarding the loci, 122 loci out of 313 loci did not have any missing data and there were 191 loci bearing 467 missing data, which ranged from a minimum of one on 75 loci to maximum of 10 on only three loci. Figure 3.4 shows the distribution of number of missing data on loci, most of loci had less than four missing data and in rare case the number of missing data per

each locus increased to 10. Regarding the number of missing data per individual (Figure 3.5), 14 individuals did not have any missing data and 129 individuals had missing data ranging from a minimum of one at 22 individuals to 12 from only one individual.

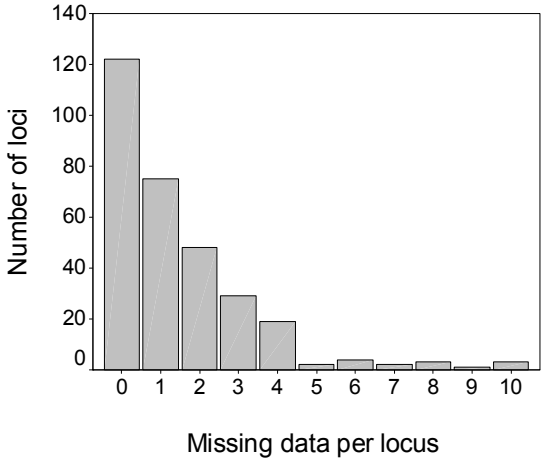


Figure 3.4 Bar chart for missing data per locus

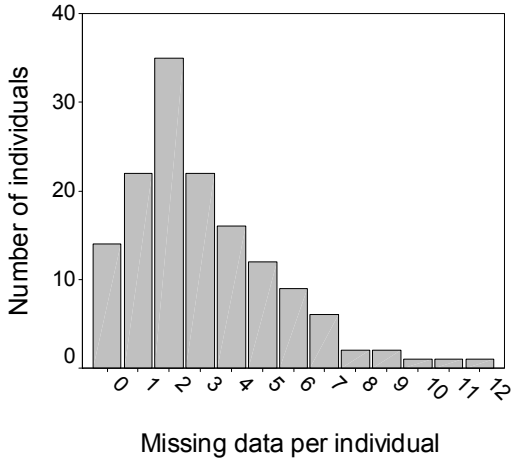


Figure 3.5 Bar chart for missing data per individual

As can be concluded from Figure 3.4 and Figure 3.5 the one percent of missing data were shared both among many individuals and also over many loci. It is therefore clear that the missing data did not accumulate on some specific individuals or few loci to reduce the quality of data or accuracy of the constructed map.

3.1.4.2 Segregation distortion

In order to check the segregation pattern of alleles in each locus, Chi square (χ^2) test was applied and 32 segregated distorted loci, about 10 percent of the loci in this study, were detected. Table 3.3 shows the list of distorted loci, their chromosomes, segregation pattern and also their Chi-square values (χ^2). Among the 32 distorted loci, 14, 15, and 3 loci showed distortion at $P = 0.05$, 0.01 and 0.001, respectively.

Dominant loci showed higher proportion of distortion (17%; seven out of 43 loci) than co-dominant loci (9.3%; 25 out of 270 loci). Interestingly, all the dominant distorted loci except *Xgwm0605-5B* were multiple loci. The rate of distorted loci among primer pairs with multiple loci was about 16% (11 out of 70 loci) compared to 9% (21 out of 243 loci) for primer pairs with specific loci which indicate double amount of distortion in primer pairs with multiple loci.

Table 3.3 List of segregation distorted loci

No.	Mapped loci	Expected Chromosome	Other mapped loci	SSR fragments on ChS (HTRI 11712 – HTRI 105)	(A:H:B:C:D:-) χ^2
1	*Xgwm1011-7A*	6A,2A	6A	122(119-125)	(10:100:32:0:0:1) 30.51*****
2	*Xgwm1173-7B*	7B		250(245-249)	(49:58:36:0:0:0) 7.50**
3	*Xgwm0297-7B*	7B		150(155-149)	(49:58:36:0:0:0) 7.50**
4	*Xgwm0963-7B*	7B		254(252-254)	(50:57:36:0:0:0) 8.60**
5	*Xgwm0834-7D*	7A	7A	225(229-219)	(25:87:30:0:0:1) 7.56**
6	*Xgwm0885-7D*	7D		173(162-180)	(37:73:21:10:0:2) 5.63*
7	*Xgwm1002-7D*	7D		182(200-166)	(43:55:43:0:0:2) 6.68*
8	*Xbarc126-7D*	7D,7B		118(118-124)	(40:56:47:0:0:0) 7.41**
9	*Xgwm1242-7D*	7D		143(147-153)	(32:64:47:0:0:0) 4.72*
10	*Xgwm1168-7D*	7D		125(126-124)	(32:60:49:0:0:2) 7.23**
11	*Xgwm1276-7D*	7D		204(184-198)	(23:83:37:0:0:0) 6.44**
12	*Xgwm1009-6A*	6A,6D	unlink	97,130(119-127)	(24:85:34:0:0:0) 6.50**
13	*Xgwm1210-6A*	6A		99(101-99)	(24:76:43:0:0:0) 5.62*
14	*Xgwm0825-6B*	6B		113,130(114-130)	(24:83:36:0:0:0) 5.71*
15	*Xgwm0940-6B*	6B,4B,2B	4B,4B	138,161,178(null-150)	(25:0:0:118:0:0) 4.31**
16	*Xgwm0605-5B*	5B		138(136-null)	(0:0:48:0:94:1) 5.87**
17	*Xgwm1257-5B*	6B		252(251-247)	(28:65:46:0:0:4) 5.24*
18	*Xgwm0583-5D*	5D		164(162-166)	(29:58:56:0:0:0) 15.29 ******
19	*Xgwm0165-4A*	4A,4A,4D	4B ,4D	186,200,249(192,null)	(0:0:46:0:97:0) 3.92**
20	*Xgwm0192-4A*	4A,4B,4D	4B ,4D	130,140,189(132,null)	(0:0:46:0:97:0) 3.92**
21	*Xgwm0353-3A*	3A(3D)		189(187-161)	(40:57:44:0:0:2) 5.40*
22	*Xgwm1110-3A*	3A		196(196-200)	(40:54:46:2:0:1) 7.83**
23	*Xgwm1171-3B*	7A,5A	7A, 2B!	146,152,158(null-147)	(27:0:0:115:0:1) 2.71*
24	*Xgwm0566-3B*	3B		131(125-123)	(46:57:37:0:0:3) 5.99*
25	*Xgwm0751-3B*	3A	unlink	124,141(136-138)	(35:55:52:0:0:1) 11.30***
26	*Xgwm1045-2A*	2A		194(191-185)	(44:69:26:3:0:1) 4.67*
27	*Xgwm0228-2D*	2D		210(204-214)	(32:84:35:0:0:2) 5.87*
28	*Xgwm0772-1A.1*	1A	1A!	195(241-null)	(0:0:43:0:94:6) 2.98*
29	*Xgwm0497-1A*	1A,2A,3D	2A, 5B!	84,91,98,153,170(154-130)	(36:83:24:0:0:0) 5.71*
30	*Xgwm1202-1D*	1D		?(274-262)	(38:57:46:0:0:2) 6.08**
31	*Xgwm0161-3D*	3D		154(152-154)	(47:54:38:4:0:0) 8.08**
32	*Xgwm0533-3B*	3B,	3B	?(null,120)	(45:0:0:98:0:0) 3.9*

Red color show the unlinked distorted loci, highlighted are the most distorted loci, and exclamatory mark (!) shows the extra loci.

Three of these most distorted loci, *Xgwm1011-7A*, *Xgwm0583-5D* and *Xgwm0751-3B* showed calculated Chi-square values about 30.5, 15.2, and 11.3, respectively. Since these three loci were too distorted and their integration into the map led to strong artefactual increases of genetic distances, they were removed from further QTL analysis. The rest of 29 distorted loci were distributed over 12 chromosomes, namely 7B, 7D, 6A, 6B, 5B, 4A, 3A, 3B, 2A, 2D, 1A, and 1D, and finally, there were two distorted alleles that stayed unlinked. The Chi-square

values for these 29 distorted loci ranged from 2.71 for *Xgwm1171-3B* to 8.60 for *Xgwm0963-7B*.

Seven loci namely *Xgwm1173-7B, Xgwm0297-7B, Xgwm0963-7B, Xgwm0566-3B, Xgwm1045-2A, Xgwm0161-3D,* and *Xgwm0533-3B* showed distortion in favor of the parent A allele whereas 12 loci including *Xgwm1242-7D, Xgwm1168-7D, Xgwm1210-6A, Xgwm0605-5B, Xgwm1257-5B, Xgwm0583-5D, Xgwm0165-4A, Xgwm0192-4A, Xgwm0772-1A.1, Xgwm1171-3B, Xgwm0751-3B,* and *Xgwm1202-1D* showed distortion in favor of allele from parent B. The four loci *Xgwm1002-7D, Xbarc126-7D, Xgwm0353-3A,* and *Xgwm1110-3A* showed distortion in favor of both parents where number of heterozygous individuals were reduced in favor of homozygous individuals. In contrast, eight loci including *Xgwm1011-7A, Xgwm0834-7D, Xgwm0885-7D, Xgwm1276-7D, Xgwm1009-6A, Xgwm0825-6B, Xgwm0940-6B, Xgwm0497-1A* illustrated an increased in heterozygotes and a decrease in both homozygotes. Finally, it should be mentioned that the two distorted loci *Xgwm0533-3B* and *Xgwm0161-3D* were in favor of parent A but stayed unlinked.

The two chromosomes 7B (3 loci: *Xgwm1173-7B, Xgwm0297-7B,* and *Xgwm0963-7B*), and 2A (one locus: *Xgwm1045-2A*) were identified bearing distorted loci only in favor of alleles from parent A. The three distorted loci on chromosome 7B were placed close to each other in centromere region (Figure 3.3). They showed an interesting pattern: the number of alleles from parent B (HTRI 105) was as expected whereas the number of individuals bearing alleles from parent A (HTRI 11712) was increased on expense heterozygous individuals.

Four chromosomes, 5B (two loci: *Xgwm0605-5B, Xgwm1257-5B*), 5D (one locus *Xgwm0583-5D),* 4A (two loci: *Xgwm0165-4A, Xgwm0192-4A* and 1D (one locus: *Xgwm1202-1D*) were detected as bearing distorted loci only in favor of alleles from parent B. Finally, out of the 19 chromosomes of genome A, B, and D of wheat, five chromosomes (5A, 4B, 3D, 2B, and 1B) showed no segregation distortion.

3.1.4.3 Primer pairs with multiple loci

The 273 polymorphic SSR primer pairs revealed 313 loci representing a mean of 1.14 polymorphic loci per primer pair (Details in Table 5 Appendix 3). Based on data from the reference map (Röder et al., 1998b; Ganal and Röder, 2007; Somers et al., 2004), 216 out of the 273 polymorphic primer pairs were locus specific. However, in the present study, the

single locus specificity was confirmed only for 163 out of these 216 polymorphic primer pairs, where 154 out of these 163 single locus primer pairs were mapped as expected from the reference maps and nine remained unlinked. For the 53 remaining loci supposed to be locus specific based on data from the reference map (Röder et al., 1998b; Ganal and Röder, 2007), an additional fragment being monomorphic or polymorphic or a extra locus was detected in the present study.

Two hundred and forty (243) out of the 273 polymorphic primer pairs, about 89 percent, amplified only one polymorphic locus. The 30 remaining ones (11%) were multilocus primer pairs. From the 30 multilocus primer pairs, 22 primer pairs amplified two polymorphic loci, six primer pairs amplified three polymorphic loci, and two primer pairs amplified four polymorphic loci. Therefore, all together 70 loci were considered as multiple polymorphic loci. Sixty three of these multiple loci were mapped and seven stayed unlinked. The mapped multiple loci were not equally distributed among the three genome in which genomes A and genome B with 29 and 28 loci, respectively bear nearly equal number of them while genome D contained only 6 multiple loci. The ratio of the mapped multiple loci to the total mapped loci in genome A, B, and D were 27.3, 23.3, and 9.7, respectively which showed the differences among the genomes for having multiple loci. The multilocus was indicated in the Figure 3.3 by a blue M in parenthesis.

3.1.4.4 Co-dominant and dominant loci

Two hundred and seventy loci out of 313 (86%) behaved as co-dominant loci and only 43 loci were dominant (For details see Table 5 Appendix 3). Dominant loci were indicated in the Figure 3.3 by null sign (0) in parenthesis. For 21 of the dominant loci, (*Xgwm0276-7A, Xgwm0935-6B*, *Xgwm0940-6B*, *Xgwm0884-4A*, *Xbarc70-4A, Xgwm1171-3B*, *Xgwm0533-3B, Xbarc125-3D.1*, *Xgwm0497-2A*, *Xgwm0614-2A, Xgwm0739-2A*, *Xbarc353-2A, Xgwm1031-2B, Xgwm0071-2D*, *Xgwm0739-2D*, *Xgwm0772-1A.2*, *Xgwm0395-1B.1*, *Xgwm0395-1D, Xgdm0098-6D, Xgwm0165-4D,* and *Xgwm0192-4D*) the null allele came from parent A and for the 22 others (*Xgwm1171-7A*, *Xgwm0146-7B, Xgwm1205-7D, Xgwm0719-6A*, *Xbarc353-6A*, *Xgwm0313-6B, Xgwm0390-6B, Xgwm0605-5B, Xgwm0497-5B*, *Xgwm0165-4A, Xgwm0192-4A, Xbarc327-4A, Xgwm0940-4B.2*, *Xgwm0853-3B*, *Xgwm0533-3B*, *Xgwm0071-*

3B, *Xgwm0071-2A.1*, *Xgwm0071-2A.2*, *Xgwm1171-2B*, *Xgwm0772-1A.1*, *Xgwm0395-1A*, *Xgwm0395-1B.2*) parent B was responsible for null allele.

Both parents showed almost equal incidence of null alleles and these dominant loci were distributed over 16 linkage groups and not accumulated on few specific ones. Therefore, the majority of genotypic data (about 86%) was generated from co-dominant loci, which were suitable to be used for the F_2 type of mapping population.

Eleven of these dominant loci namely *Xgwm0276-7A, Xgwm0146-7B, Xgwm0313-6B, Xgwm0390-6B, Xgwm0605-5B, Xgwm0884-4A, Xbarc327-4A, Xgwm0853-3B, Xgwm0614-2A, Xgwm1031-2B*, and *Xgdm0098-6D* were locus specific and these loci were located as expected from the ITMI reference map. The remaining 32 dominant loci were multiple loci. Six of these dominant loci, *Xgwm1205-7D, Xgwm0497-5B, Xgwm1171-3B, Xgwm1171-2B, Xgwm0071-2D,* and *Xgwm0739-2D,* were detected as extra loci. Primer pairs GWM0739 amplified two dominant loci on chromosomes 2A and 2D (*Xgwm0739-2A* and *Xgwm0739-2D*) and interestingly on both loci parent A had null alleles. Primer pair GWM0395 also showed an interesting behavior by amplifying four loci *Xgwm0395-1D, Xgwm0395-1B.1*, *Xgwm0395-1B.2,* and *Xgwm0395-1A* that all were as dominant loci.

3.1.4.5 First time mapped loci

Among the applied 273 polymorphic primer pairs 45 from GWM were not mapped in the ITMI map, because they did not reveal polymorphism on parental lines of that population (Table 3.4) (For more details Table 5 in Appendix 3). However, they were assigned to specific wheat chromosomes, utilizing nulli-tetrasomic lines of Chinese Spring (Dr. M. Röder, personal communication). As these primer pairs distinguished between the two parents utilized in this study, it become possible to locate their loci on the present map. These primer pairs amplified 50 loci and 46 of them (92%), were mapped on the same chromosome as detected by nulli-tetrasomic analysis by Röder et al. (Personal communication). The first time mapped loci were indicated in the Figure 3.3 by a red color.

The only four discrepancies between genetic and nulli-tetrasomic mapping occurred for the following loci *Xgwm0859* (mapped on 3A instead of 2D), *Xgwm0835* (mapped on 1B instead 1A), *Xgwm0804* (mapped on 3A instead of 7D), and *Xgwm1205* (mapped on *7D* instead of 6B, 5A, and 4B). Since there was also monomorphic locus on each of the last three above

Table 3.4 Details of SSR loci that were first time mapped

No.	Mapped loci in the present study	Expected on chromosome[1]	Other mapped loci in the present study	Monomorphic peak	ChS (HTRI 11712 - HTRI 105)	Motif
1	*Xgwm1069-7A*	7A			138(129-138)	GT
2	*Xgwm0900-7A*	7A			250(244-246)	GTimp
3	*Xgwm1126-7A*	7A			110(102-110)	CT, CA
4	*Xgwm0941-7B*	7B		90	120(117-121)	CA
5	*Xgwm0393-7B*	7B, 7B			114(113-117)	CA
6	*Xgwm1205-7D!*	6B, 5A, 4B		90, 112, 122, 124, 148	114, 124, 126, 139, 142(137-null)	GA
7	*Xgwm0740-7D*	7D			129(127-97)	GT
8	*Xgwm0530-6A*	6A, 6B, 6D		136,148	188(204-198)	CT
9	*Xgwm1210-6A*	6A			99(101-99)	CT
10	*Xgwm0799-6A*	1B, 6A			188(183-196)	GT
11	*Xgwm0244-6B*	6B			226(230-200)	CAimp
12	*Xgwm0313-6B*	6B			160(160-null)	CT, GT
13	*Xgwm0390-6B*	6B			147(149-null)	CT, GT
14	*Xgwm1016-6B*	6B, 5B	5B	114	128, 147(128-135)	GA
15	*Xgwm0058-6B*	6B			114(125-112)	CA
16	*Xgwm0865-5A*	5A			170(156-144)	GA
17	*Xgwm0197-5B*	5B		112	125(119-125)	CT
18	*Xgwm0996-5B*	5B			192(200-196)	GA
19	*Xgwm0605-5B*	5B			138(136-null)	GA
20	*Xgwm0118-5B*	5B, 5D, 4A		80	107(107-118)	CA
21	*Xgwm1059-5D*	5D			205(214-206)	GT
22	*Xgwm0884-4A*	4A			147, 161(null-163)	GAimp
23	*Xgwm1169-4A*	4A			227(214-223)	AT, GT
24	*Xgwm0859-3A !*	2D			175(175-173)	GA

Exclamatory mark (***!***) shows extra loci,

1= based on the analysis of the nulli-tetrasomic lines of Chinese Spring conducted by Dr. M. Röder (Personal communication).

Table 3.4 (Continued)

No.	Mapped loci in the present study	Expected chromosome	Other mapped loci in the present study	Monomorphic peak	ChS (TRI 11712 - TRI 105)	Motif
25	*Xgwm0353-3A*	3A, 3D			189(187-161)	GCGT, GT
26	*Xgwm0804-3A!*	7D		90	144(148-144)	GT
27	*Xgwm0134-3A!*	3B	3B		109(98-108)	CA
28	*Xgwm0144-3B*	3B			202(240-234)	GT
29	*Xgwm0134-3B*	3B	3A!		109(113-122)	CA
30	*Xgwm1244-2A*	2A			139(145-134,142)	GA
31	*Xgwm0939-2A*	2A, 2D			231(234-231)	CA
32	*Xgwm1136-2A*	2A, 2B			112(112-110)	GT
33	*Xgwm1031-2B*	2B			161(null-161)	GT
34	*Xgwm0322-2B*	2B		96	87, 119(87-131)	GA
35	*Xgwm0128-2B*	2B			175(187-189)	CA
36	*Xgwm1010-2D*	2D			203(203-193)	GT
37	*Xgwm0242-2D*	2D, 2A, 2B		116	140(144-152)	GA
38	*Xgwm0228-2D*	2D			210(204-214)	CT, CA
39	*Xgwm0772-1A.1*	1A	1A!	202	195(241-null)	AT
40	*Xgwm0772-1A.2!*	1A	1A	202	195(null-216)	AT
41	*Xgwm0395-1A*	1A, 1B, 1D	1B, 1B!, 1D		137, 144, 148(144-null)	CA
42	*Xgwm0835-1B!*	1A		204	196(196-194)	CT
43	*Xgwm0395-1B.1!*	1A, 1D	1A, 1B, 1D		137, 144, 148(null-150)	CA
44	*Xgwm0395-1B.2*	1B, 1A, 1D	1A, 1B!, 1D		137, 144, 148(148-null)	CA
45	*Xgwm0395-1D*	1D, 1B, 1A	1A, 1B, 1B!		137, 44, 148(null-137)	CA

mentioned PCR products, the polymorphic ones could be considered as extra loci and the monomorphic ones are probably the loci previously mapped by nulli-tetrasomics. Therefore, there was only one locus (*Xgwm0859-3A*), which was located on linkage group 3A and not on 2D as expected. In summary, 45 GWM primer pairs, which were not located to the ITMI map were mapped in the present study.

3.1.4.6 Extra loci

Thirty one out of the 293 mapped loci were considered as extra loci (Table 3.5 and more details Table 5 in Appendix 3) because they were mapped on a different linkage group or as extra loci on the same linkage group compared to the ITMI map (Röder et al., 1998) or consensus map (Somers et al., 2004). Extra locus was indicated by blue color in the Figure 3.3 and in case it was also an extra locus of a first time mapped locus by red color and red bold exclamatory mark (**!**) in parenthesis. The following 18 loci, *Xgwm1011-7A*, *Xgwm0834-7D*, *Xgwm0907-6A*, *Xgwm0274-5B*, *Xgwm0133-5B*, *Xgwm0497-5B*, *Xgwm1258-4A*, *Xgwm0940-4B.2*, *Xgwm0133-3A*, *Xgwm0134-3A*, *Xgwm1171-3B*, *Xbarc125-3D.1*, *Xgwm0526-2A*, *Xgwm1171-2B*, *Xgwm0071-2D*, *Xgwm0739-2D*, *Xgwm0772-1A.2*, and *Xgwm0395-1B.1* were considered as extra loci because their corresponding primer pairs amplified several loci including some of the previous reported ones.

There were nine loci (*Xgwm1044-7A*, *Xgwm1205-7D*, *Xgwm0746-7D*, *Xgwm0608-6B*, *Xgwm1257-5B*, *Xgwm0804-3A*, *Xgwm0630-2A*, *Xbarc361-2D*, and *Xgwm0835-1B*) where corresponding primer pairs amplified mono as well as polymorphic loci and probably the previously mapped loci were monomorphic on the parental lines used in the present study. Primer pair GWM0751 amplified two loci, one as extra locus *Xgwm0751-3B* and the other remained unlinked in the current map. However, it did not amplified any zoomorphic locus. Finally, regarding the remaining three extra loci (*Xgwm0344-7A*, *Xgwm0443-5A*, *Xgwm0859-3A*) only one locus per primer pair was amplified and mapped and there was no monomorphic locus. Since these three loci were located on another linkage group, they were considered as extra loci. Among the 31 extra loci, 19 came from multiple loci and 12 were locus specific.

The following 16 extra loci : *Xgwm1044-7A, Xgwm0344-7A, Xgwm834-7D, Xgwm0746-7D, Xgwm907-6A, Xgwm0443-5A, Xgwm134-3A, Xgwm751-3B, Xgwm0630-2A, Xgwm526-2A,*

Xgwm71-2D, *Xgwm739-2D*, *Xbarc361-2D*, *Xgwm0772-1A.2*, *Xgwm0835-1B*, and *Xgwm0395-1B.1* were homoeoloci of loci on the ITMI map (Röder et al., 1998), as were detected on the homoeologous chromosomal regions.

Table 3.5 List of extra SSR loci, which were found in the present study

No.	Mapped loci in present study	Expected based on ITMI map	Other mapped loci	Monomorphic Peak bp	Fragments on ChS (HTRI 11712 - HTRI 105)
1	*Xgwm1044-7A*	7D		140, 278	141(128-139)
2	*Xgwm1011-7A*	6A, 2A	6A	100, 117	121(107-105)&(136-null)
3	*Xgwm0344-7A*	7B			131(121- null)
4	*Xgwm1205-7D*	6B, 5A, 4B		90, 112, 122, 124, 148	114, 124, 126, 139, 142(137-null)
5	*Xgwm0834-7D*	7A	7A		225(229-219)
6	*Xgwm0746-7D*	7A		112	104, 143(142-138)
7	*Xgwm0907-6A*	6B	6B		154(154-158)
8	*Xgwm0608-6B*	4D, 2D		112	126(122-126)
9	*Xgwm0443-5A*	5B			135(127-121)
10	*Xgwm0274-5B*	1B, 7B	1B	140	167, 186(162-166)
11	*Xgwm0133-5B*	6B	6B, 3A!		111, 117, 134(138-187)
12	*Xgwm0497-5B*	1A, 2A, 3D	1A, 2A	85, 92	84, 91, 98, 153, 170(169-null)
13	*Xgwm1257-5B*	6B		202	252(251-247)
14	*Xgwm1258-4A*	7A, 7D	7A	195	197(157-171)
15	*Xgwm0940-4B.2*	6B, 2B	4B, 6B		138, 161, 178(138-null)
16	*Xgwm0859-3A*	2D			175(175-173)
17	*Xgwm0804-3A*	7D		90	144(148-144)
18	*Xgwm0133-3A*	6B	5B!, 6B		89, 111, 117, 134(111-117)
19	*Xgwm0134-3A*	3B	3B		109(98-108)
20	*Xgwm1171-3B*	7A, 5A	7A, 2B!	145	146, 152, 158(null-147)
21	*Xgwm0751-3B*	3A	unlink		124, 141(136-138)
22	*Xbarc125-3D.1*	2B, 3D, 4B, 5A, 7D	3D	132	130, 134, 146, 150(null,144)
23	*Xgwm0630-2A*	2B		125	106(107-109)
24	*Xgwm0526-2A*	2B	2B	141	141, 151, 155(137-131)
25	*Xgwm1171-2B*	7A, 5A	7A, 3B!	145	146, 152, 158(156-null)
26	*Xgwm0071-2D*	2A, 2A, 3D	2A, 2A, unlink	96, 128	102, 111, 131(null,116)
27	*Xbarc361-2D*	2B, 5D, 6B		195	195, 227, 268, 237(228-226)
28	*Xgwm0739-2D*	2A, 2B	2A		154(null-154)
29	*Xgwm0772-1A.2*	1A	1A	202	195(null-216)
30	*Xgwm0835-1B*	1A		204	196(196-194)
31	*Xgwm0395-1B.1*	1A, 1D	1A, 1B, 1D		137, 144, 148(null-150)

Exclamatory mark shows extra loci, Red color show the first time mapped loci

In contrary 13 extra loci *Xgwm1011-7A*, *Xgwm1205-7D*, *Xgwm0608-6B*, *Xgwm0274-5B*, *Xgwm0133-5B*, *Xgwm0497-5B*, *Xgwm1257-5B*, *Xgwm1258-4A*, *Xgwm0859-3A*, *Xgwm0804-*

3A, *Xgwm0133-3A*, *Xgwm1171-3B*, and *Xgwm1171-2B* were found to be non-homoeologous with the loci on the ITMI map as were detected on the non-homoeologous chromosomal regions.

The remaining two extra loci *Xbarc125-3D.1* and *Xgwm0772-1A.2* were identified in the present study as extra loci on the same reported chromosome of reference maps. Ten of the extra loci were supposed to be locus specific based on the ITMI map (Röder et al., 1998a; Röder et al., 1998b). However, in the present study their primer pairs amplified fragments from different loci in which six times the amplifications were from homoeologous chromosomes (*Xgwm1044-7A, Xgwm0344-7A, Xgwm746-7D, Xgwm0443-5A, Xgwm0630-2A, and Xgwm0835-1B)* and four times from non-homoeologous chromosomes (*Xgwm1257-5B, Xgwm1163-6D, Xgwm0859-3A, and Xgwm0804-3A*). Three of the extra loci (*Xgwm0940-4B.2, Xgwm0772-1A.2, Xgwm0395-1B.1*) had a locus on the same linkage group. Ten of the extra loci (*Xgwm1011-7A, Xgwm1205-7D, Xgwm0497-5B, Xgwm0940-4B.2, Xgwm1171-3B, Xgwm1171-2B, Xgwm0071-2D, Xgwm0739-2D, Xgwm1205-7D, and Xgwm0395-1B.1*) behaved as dominant and the 21 as co-dominant loci.

3.2 Phenotypic evaluations

The mapping population, including 133 $F_{2:3}$ families, was evaluated under four experiments as explained in material and methods (section 2.2.2). Frequency distributions of the traits from the four experiments recorded under control and drought stress conditions showed continuous and approximately normal distribution with transgressive segregation, indicating polygenic inheritance with partial gene association (Figures 11 to 18 in Appendix 7).

In the following, the descriptive statistics and Pearson correlation coefficients between pairs of traits for each experiment are given. Then correlation coefficients of single traits between pairs of experiments, analysis variance and heritability are presented.

3.2.1 Field experiment in 2004

Table 3.6 shows descriptive statistics including range, minimum, maximum, mean, standard deviation and coefficient of variation (CV) for the nine measured traits on the mapping population and the parental lines. As the table shows, parental lines were different for the measured traits except for seed width for both control and stress conditions. Comparing the

minimum and maximum values of the traits of the mapping population to parental lines the existence of individuals with lower and higher value compared to parental lines appeared.

Regarding the four seed related traits, their minimum, maximum, and mean showed lower value under stress condition than those under control condition that showed clearly the effect of stress treatment. Thousand-grain weight had the highest CV under both control (6.33%) and stress conditions (13.39%) while days to flowering had the lowest (0.89%). Interestingly, the seed related traits had higher CVs under stress compared to the control, especially for thousand-grain weight, which increased about two times. However, by looking to the table it can be found that the main reason for these results was reduction in the mean of these traits under stress condition and not because of the increase in variation per se.

Table 3.7 shows the Pearson correlation coefficient between pairs of traits. All correlation coefficient except those with days to flowering were positive. Both under control and stress conditions, high correlation existed between the seed related traits, except between seed length and seed width. Thousand-grain weight showed high correlation with seed area and seed width under both conditions. While seed related traits had high correlation with each other under the same condition, they showed lower correlation between conditions. The correlations of the same traits between control and stress conditions ranged from 0.30 in thousand-grain weight to 0.59 for seed length. Interestingly, thousand-grain weight under stress condition showed higher correlation with the thousand-grain weight (0.30), seed area (0.28), and seed width (0.31) compared to seed length (0.16) under control condition.

3.2.2 Greenhouse experiment in 2004

Table 3.8 shows descriptive statistics for the ten measured traits on segregating population and parental lines. As the table shows, parental lines were different for most of the measured traits under both control and stress conditions. Comparing the minimum and maximum values of the traits of mapping population to parental lines the existence of individuals with lower and higher values compared to the parental lines appeared for most of the traits.

Number of seeds per spike and seed weight per spike had larger CVs than seed related traits. Regarding the four seed related traits, their minimum, maximum, and mean showed lower values under stress condition compared to the control, which showed obviously the effect of stress treatment. Considering the seed related traits, thousand-grain weight showed the

highest amount of CVs under both control (13.78) and stress conditions (21.37). Finally, the seed related traits had higher CVs under stress condition compared to control condition, especially for thousand-grain weight because of reduction on the means of traits under stress condition.

Table 3.9 shows the Pearson correlation coefficient of the traits. Under each of both control and stress conditions, there were high correlations between the seed related traits except between seed length and seed width and also seed length and thousand-grain weight. Thousand-grain weight showed high correlations with seed area and seed width under both conditions. Seed related traits had higher correlations with each other under the same condition than those between conditions. The correlations of the same traits between control and stress conditions ranged from 0.29 for thousand-grain weight to 0.57 for seed length and were more or less higher than correlation between different traits. Number of seeds per spike and weight of seed per spike showed a high correlation of 0.89 under control condition.

3.2.3 Field experiment in 2005

The analysis of variance on data from three standard cultivars (Appendix 7) showed that there was no significant difference between blocks for thousand-grain weight, no. of seeds per spike, spike length, seed area, seed width, and seed length. Therefore, it can be deduced that the $F_{2:3}$ lines were grown under homogenous field condition.

Table 3.10 shows descriptive statistics for the measured traits on segregating population and parental lines. As the table shows, parental lines were different for most of the measured traits except for seed width under both control and stress conditions. Comparing the minimum and maximum values of the traits in mapping population to parental lines the existence of individuals with lower and higher value than those in parental lines appeared for all traits.

Traits number of seeds per spike and seed weight per spike that were recorded only under control condition had larger CVs than those from seed related traits on the same condition. Regarding the four seed related traits, their minimum, maximum, and mean showed lower value under stress condition than those under control, which confirmed the effect of drought stress. The seed related traits had higher CVs under stress condition compared to control

Table 3.6 Descriptive Statistics for the traits from the field experiment in 2004

Traits	Parents		Mapping population					
	P. A	P. B	Range	Minimum	Maximum	Mean	Std. Deviation	CV%
(1)Days to flowering	256	261	12	252	264	258.02	2.30	0.89
(2)Thousand-grain weight(C)	54.22	48.96	16.84	43.43	60.27	52.84	3.34	6.33
(3)Seed area(C)	20.4	19.0	5.4	17.0	22.4	19.60	0.99	5.05
(4)Seed width(C)	3.6	3.6	0.6	3.3	3.9	3.57	0.11	3.17
(5)Seed length(C)	6.4	6.0	1.1	5.7	6.8	6.18	0.2212	3.58
(6)Thousand-grain weight(S)	21.49	16.26	14.52	12.26	26.78	19.50	2.61	13.39
(7)Seed area(S)	15.1	13.4	5.0	12.3	17.3	14.89	0.93	6.23
(8)Seed width(S)	2.7	2.7	0.9	2.4	3.3	2.79	0.14	5.07
(9)Seed length(S)	5.9	5.2	1.1	5.0	6.1	5.60	0.23	4.05

C = control, S = stress, P. A = HTRI 11712, P. B = HTRI 105

Table 3.7 Pearson correlation between traits from the field experiment in 2004

Traits	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1)Days to flowering	1.00	-0.27	-0.17	-0.16	-0.09	-0.50	-0.26	-0.24	-0.25
(2)Thousand-grain weight(C)	-0.27	1.00	0.80	0.70	0.65	0.30	0.31	0.17	0.36
(3)Seed area(C)	-0.17	0.80	1.00	0.74	0.88	0.28	0.47	0.28	0.51
(4)Seed width(C)	-0.16	0.70	0.74	1.00	0.39	0.31	0.40	0.46	0.23
(5)Seed length(C)	-0.09	0.65	0.88	0.39	1.00	0.16	0.40	0.09	0.59
(6)Thousand-grain weight(S)	-0.50	0.30	0.28	0.31	0.16	1.00	0.77	0.73	0.64
(7)Seed area(S)	-0.26	0.31	0.47	0.40	0.40	0.77	1.00	0.83	0.87
(8)Seed width(S)	-0.24	0.17	0.28	0.46	0.09	0.73	0.83	1.00	0.49
(9)Seed length(S)	-0.25	0.36	0.51	0.23	0.59	0.64	0.87	0.49	1.00

C = control, S = stress, red color shows correlation between seed related traits

Table 3.8 Descriptive Statistics for the traits from the greenhouse experiment in 2004

Traits	Parents			Mapping population				
	P. A	P. B	Range	Minimum	Maximum	Mean	Std. Deviation	CV%
(1)No. seeds per spike(C)	16.0	28.5	69.0	11.0	80.0	43.84	12.82	29.23
(2)Seed weight per spike(C)	0.96	1.05	2.64	0.53	3.17	1.98	0.58	29.25
(3)Thousand-grain weight(C)	60.31	47.72	37.68	27.42	65.10	47.17	6.50	13.78
(4)Seed area(C)	22.6	17.1	6.7	15.6	22.3	18.23	1.24	6.81
(5)Seed width(C)	3.7	3.3	1.1	2.9	4.0	3.46	0.19	5.58
(6)Seed length(C)	6.8	5.8	1.3	5.3	6.6	5.92	0.27	4.59
(7)Thousand-grain weight(S)	16.56	18.13	21.39	11.23	32.62	18.29	3.91	21.37
(8)Seed area(S)	14.8	13.8	5.8	11.1	16.9	14.12	1.11	7.87
(9)Seed width(S)	2.6	2.7	0.9	2.3	3.2	2.67	0.19	7.18
(10)Seed length(S)	6.0	5.3	1.4	4.7	6.1	5.50	0.26	4.77

C = control, S = stress, P. A = HTRI 11712, P. B = HTRI 105

Table 3.9 Pearson correlation between traits from greenhouse experiment in 2004

Traits	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1)No. seeds per spike(C)	1.00	0.89	-0.31	-0.22	-0.41	0.10	-0.05	-0.02	-0.12	0.10
(2)Seed weight per spike(C)	0.89	1.00	0.14	0.15	-0.03	0.28	0.11	0.14	0.02	0.24
(3)Thousand-grain weight(C)	-0.31	0.14	1.00	0.82	0.84	0.41	0.29	0.34	0.28	0.30
(4)Seed area(C)	-0.22	0.15	0.82	1.00	0.72	0.77	0.21	0.42	0.22	0.48
(5)Seed width(C)	-0.41	-0.03	0.84	0.72	1.00	0.13	0.31	0.35	0.42	0.15
(6)Seed length(C)	0.10	0.28	0.41	0.77	0.13	1.00	0.03	0.29	-0.08	0.57
(7)Thousand-grain weight(S)	-0.05	0.11	0.29	0.21	0.31	0.03	1.00	0.83	0.84	0.53
(8)Seed area(S)	-0.02	0.14	0.34	0.42	0.35	0.29	0.83	1.00	0.84	0.82
(9)Seed width(S)	-0.12	0.02	0.28	0.22	0.42	-0.08	0.84	0.84	1.00	0.40
(10)Seed length(S)	0.10	0.24	0.30	0.48	0.15	0.57	0.53	0.82	0.40	1.00

C = control, S = stress, red color shows correlation between seed related traits

condition and especially thousand-grain weight which showed the highest amount of CV under control (7.34) and stress conditions (15.40). Table 3.11 shows the Pearson correlation coefficient of the traits. Number of seeds per spike and seed weight per spike, which were recorded only under control condition showed high correlation (0.77). Seed related traits did not show high correlations with other measured traits under both control and stress conditions. However, within each condition, there were high correlations between the seed related traits except between seed length and seed width and seed length and thousand-grain weight. Thousand-grain weight showed high correlation with seed area and seed width under both conditions. Seed related traits had higher correlations with each other within condition than between conditions.

The correlations of the same traits between conditions ranged from 0.40 for thousand-grain weight to 0.70 for seed length and were higher than correlation between different traits.

3.2.4 Greenhouse experiment in 2007

Table 3.12 shows descriptive statistics for the greenhouse experiment in 2007. Parental lines were different for most of the measured traits except for seed width under both control and stress conditions. Comparing the minimum and maximum values of the traits in mapping population to parents individuals with lower and higher values than those in parental lines were identified for all traits except number of unfertile tiller per plant under stress condition which showed no transgressive segregation in the direction of the higher parent.

Plant height and spike length showed nearly the same amount of mean and CV under both control and stress conditions indicating no effect of the imposed stress on these two characters. However, all the 10 remaining traits had decreasing mean and increasing CVs under stress compared to the control condition, showing the consequence of imposed stress on these traits. Considering the seed related traits, thousand-grain weight showed the highest amount of variation under both control (6.81) and stress conditions (18.32). Regarding the number of fertile spikes per plant and number of unfertile tillers per plant, as Table 3.12 shows drought stress reduced the mean for both of them.

Table 3.13 shows the Pearson correlation coefficients of traits (the whole dada as Table 1 in Appendix 7). In each of control and stress conditions, there were high correlations between the seed related traits except between seed length and seed width under control condition.

Table 3.10 Descriptive Statistics for the traits from the field experiment in 2005

Traits	Parents		Mapping population					
	P. A	P. B	Range	Minimum	Maximum	Mean	Std. Deviation	CV
(1)Days to flowering	253	259	12	248	260	254.10	2.88	1.13
(2)Plant height(C)	138.3	152.6	50.4	116.7	167.1	146.14	10.40	7.11
(3)Spike length(C)	12.7	14.0	7.0	11.3	18.3	14.56	1.20	8.23
(4)No. seeds per spike(C)	76.2	66.9	46.1	61.5	107.6	79.41	8.97	11.29
(5)Seed weight per spike(C)	4.11	3.80	3.10	3.00	6.10	4.15	0.46	11.03
(6)Thousand-grain weight(C)	54.04	54.29	18	44.1	62.1	52.47	3.85	7.34
(7)Seed area(C)	21.0	20.2	6.0	18.3	24.3	21.23	1.11	5.23
(8)Seed width(C)	3.5	3.7	0.6	3.4	4.0	3.64	0.13	3.58
(9)Seed length(C)	6.7	6.2	1.5	5.8	7.3	6.56	0.24	3.72
(10)Thousand-grain weight(S)	24.8	23.8	21.6	13.4	35.0	23.82	3.67	15.40
(11)Seed area(S)	17.2	16.6	7.8	12.5	20.3	16.91	1.36	8.04
(12)Seed width(S)	2.8	2.9	0.8	2.5	3.3	2.88	0.18	6.10
(13)Seed length(S)	6.4	6.0	1.9	5.1	7.0	6.18	0.29	4.75

C = control, S = stress, P. A = HTRI 11712, P. B = HTRI 105

Table 3.11 Pearson correlation between traits from the field experiment in 2005

Traits	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
(1)Days to flowering	1.00	0.22	0.14	0.05	0.01	-0.06	-0.05	0.05	-0.13	0.37	0.25	0.32	0.13
(2)Plant height(C)	0.22	1.00	0.42	0.01	0.08	0.10	0.09	-0.08	0.14	-0.03	0.04	-0.10	0.12
(3)Spike length(C)	0.14	0.42	1.00	0.32	0.43	0.16	0.39	0.04	0.48	0.05	0.28	0.09	0.37
(4)No. seeds per spike(C)	0.05	0.01	0.32	1.00	0.77	-0.37	-0.10	-0.26	0.09	-0.27	-0.15	-0.19	-0.08
(5)Seed weight per spike(C)	0.01	0.08	0.43	0.77	1.00	0.31	0.45	0.24	0.45	0.00	0.12	0.04	0.17
(6)Thousand-grain weight(C)	-0.06	0.10	0.16	-0.37	0.31	1.00	0.80	0.75	0.52	0.40	0.39	0.34	0.35
(7)Seed area(C)	-0.05	0.09	0.39	-0.10	0.45	0.80	1.00	0.67	0.85	0.43	0.57	0.39	0.61
(8)Seed width(C)	0.05	-0.08	0.04	-0.26	0.24	0.75	0.67	1.00	0.24	0.41	0.35	0.46	0.19
(9)Seed length(C)	-0.13	0.14	0.48	0.09	0.45	0.52	0.85	0.24	1.00	0.25	0.52	0.20	0.70
(10)Thousand-grain weight(S)	0.37	-0.03	0.05	-0.27	0.00	0.40	0.43	0.41	0.25	1.00	0.81	0.86	0.64
(11)Seed area(S)	0.25	0.04	0.28	-0.15	0.12	0.39	0.57	0.35	0.52	0.81	1.00	0.86	0.90
(12)Seed width(S)	0.32	-0.10	0.09	-0.19	0.04	0.34	0.39	0.46	0.20	0.86	0.86	1.00	0.58
(13)Seed length(S)	0.13	0.12	0.37	-0.08	0.17	0.35	0.61	0.19	0.70	0.64	0.90	0.58	1.00

C = control, S = stress, red color shows correlation between seed related traits

Table 3.12 Descriptive Statistics for traits from greenhouse experiment in 2007

Traits	Parents		Mapping population					
	P. A	P. B	Range	Minimum	Maximum	Mean	Std. Deviation	CV
(1)Plant height(C)	87.0	95..8	48.6	85.2	133.8	110.02	9.75	8.87
(2)No. fertile spikes per plant(C)	8.8	7.8	5.3	6.5	11.8	8.48	1.10	12.97
(3)No. unfertile tillers per plant(C)	2.5	6.5	6.7	2.0	8.7	4.16	1.18	28.32
(4)Spike length(C)	10.5	11.4	4.6	8.1	12.7	10.32	0.81	7.89
5)Weight of all spikes per plant(C)	18.0	16.6	16.1	13.8	29.9	18.88	2.82	14.96
(6)Weight of three spikes per plant(C)	8.1	7.9	4.6	6.6	11.2	8.95	0.98	10.96
(7)No. of seeds per spikes (C)	51.9	50.7	30.7	38.8	69.5	52.60	6.83	12.98
(8)Seed weight per spikes(C)	2.3	2.2	1.5	1.6	3.1	2.40	0.32	13.11
(9)Thousand-grain weight(C)	46.74	43.76	14.30	40.20	54.50	45.77	3.12	6.81
(10)Seed area(C)	19.0	17.4	4.9	16.4	21.3	18.56	0.94	5.05
(11)Seed width(C)	3.3	3.4	0.6	3.1	3.7	3.37	0.12	3.59
(12)Seed length(C)	6.3	5.7	1.3	5.6	6.9	6.21	0.25	4.02
(13)Plant height(S)	83.7	87.0	52.8	83.5	136.3	102.01	8.40	8.23
(14)No. fertile spikes per plant(S)	4.8	4.3	4.8	3.7	8.5	6.08	0.96	15.76
(15)No. unfertile tillers per plant(S)	2.7	6.7	4.5	1.5	6.0	3.29	0.99	30.12
(16)Spike length(S)	9.6	10.2	4.5	7.8	12.3	10.48	0.75	7.16
(17)Weight of all spikes per plant(S)	5.7	5.2	5.1	3.0	8.1	5.37	1.13	20.96
(18)Weight of three spikes per plant(S)	3.7	3.5	1.9	2.3	4.2	3.33	0.45	13.47
(19)No. seeds per spikes(S)	45.3	41.6	28.8	23.1	51.9	38.71	6.14	15.87
(20)Seed weight per spikes(S)	0.7	0.8	1.0	0.3	1.3	0.68	0.19	28.28
(21)Thousand-grain weight(S)	15.86	18.98	11.80	7.30	19.10	11.56	2.12	18.32
(22)Seed area(S)	15.3	14.2	5.0	9.1	14.1	11.67	1.01	8.70
(23)Seed width(S)	2.7	2.8	0.7	2.0	2.7	2.29	0.15	6.56
(24)Seed length(S)	6.0	5.4	1.6	4.2	5.8	5.08	0.30	5.89

C = control, S = stress, P. A = HTRI 11712, P. B = HTRI 105

Table 3.13 Pearson correlation between traits from the greenhouse experiment in 2007

Traits	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1)Plant height	**0.72**	0.14	0.17	0.26	0.11	0.05	-0.02	0.05	0.13	0.14	0.09	0.07
(2)No. fertile spikes per plant	-0.31	**-0.07**	0.05	0.01	0.61	-0.04	-0.13	-0.09	0.07	-0.01	0.06	-0.08
(3)No. unfertile tillers per plant	0.14	-0.46	**0.38**	0.19	0.01	-0.10	-0.01	-0.02	0.01	-0.01	0.00	-0.03
(4)Spike length	0.33	-0.12	0.24	**0.76**	0.41	0.48	0.25	0.37	0.24	0.23	0.08	0.23
(5)Weight of all spikes per plant	-0.39	0.83	-0.56	-0.10	**-0.01**	0.70	0.43	0.54	0.25	0.15	0.01	0.15
(6)Weight of three spikes per plant	-0.33	0.52	-0.50	-0.03	0.87	**0.33**	0.70	0.83	0.28	0.22	-0.04	0.30
(7)No. seeds per spikes	-0.18	0.38	-0.44	0.02	0.65	0.78	**0.38**	0.86	-0.21	-0.21	-0.43	0.03
(8)Seed weight per spikes	-0.21	0.49	-0.48	-0.19	0.77	0.83	0.77	**0.06**	0.31	0.23	-0.04	0.33
(9)Thousand-grain weight	-0.18	0.47	-0.35	-0.24	0.67	0.64	0.38	0.85	**-0.01**	0.85	0.73	0.57
(10)Seed area	-0.12	0.43	-0.33	-0.18	0.62	0.58	0.34	0.77	0.92	**0.14**	0.64	0.83
(11)Seed width	-0.09	0.33	-0.32	-0.24	0.49	0.43	0.21	0.62	0.81	0.88	**0.23**	0.15
(12)Seed length	-0.09	0.44	-0.32	-0.10	0.62	0.60	0.42	0.76	0.85	0.93	0.67	**0.30**

Values on the diagonal (bold) are correlation of the same trait between control and stress conditions. Values above and below the diagonal are correlation of traits under each control and stress conditions, respectively. red color shows correlation between seed related traits

Thousand-grain weight showed high correlation with seed area and seed width under control conditions. However, under stress condition thousand-grain weight showed high correlations with seed area, seed width, as well as seed length. Seed related traits had higher correlations with each others within condition than between conditions. The correlations of seed related traits between control and stress conditions ranged from -0.01 for thousand-grain weight to 0.30 for seed length. However, there was high correlation for spike length (0.76) and plant height (0.72) between the two conditions. While seed related traits showed low correlation with other traits under control condition, under the stress condition these traits had high correlation especially with some traits like weight of all spikes per plant, weight of three spikes per plant, and seed weight per spike per.

3.2.5 Correlation of traits based on the mean of four experiments

Table 3.14 shows the Pearson correlation coefficients of the seed related traits based on the mean of the four experiments within each control and stress condition and also between the two conditions. Under each of the control and stress conditions, there were high correlations between the seed related traits except between seed length and seed width and also seed length and thousand-grain weight. Seed related traits had higher correlation with each other within condition than between conditions. The correlations of the same traits between two control and stress conditions ranged from 0.53 for thousand-grain weight to 0.78 for seed

length. Regarding the correlation of thousand-grain weight under stress condition and the traits at control condition, the highest correlation was identified with thousand-grain weight 0.53 which was nearly the same as in each single experiment. The pattern of correlation within and between conditions was comparable to each of the separate experiment.

Table 3.14 Correlation coefficient between mean of seed related traits from four experiments

Traits	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1)Thousand-grain weight(C)	1	0.83	0.72	0.54	0.53	0.61	0.44	0.54
2)Seed area(C)	0.83	1	0.61	0.84	0.44	0.70	0.36	0.71
3)Seed width(C)	0.72	0.61	1	0.13	0.52	0.51	0.66	0.19
4)Seed length(C)	0.54	0.84	0.13	1	0.20	0.55	0.02	0.78
5)Thousand-grain weight(S)	0.53	0.44	0.52	0.20	1	0.81	0.80	0.54
6)Seed area(S)	0.61	0.70	0.51	0.55	0.81	1	0.75	0.83
7)Seed width(S)	0.43	0.35	0.66	0.02	0.80	0.75	1	0.32
8)Seed length(S)	0.54	0.71	0.19	0.78	0.54	0.83	0.32	1

C = control, S = stress

3.2.6 Traits correlation between pairs of the experiments

Pearson correlation coefficients between seed related traits in pairs of experiments under both control and stress conditions was calculated (Details in Table 2 in Appendix 7) as rough estimates of heritability in these experiments. These coefficients were not equal under the control and stress conditions and they were usually higher under control compared to the stress condition. Table 3.15 (was extracted from Table 2 in Appendix 7) shows these correlation coefficients and also range, minimum, mean, and maximum of them. No negative correlations were observed for these traits. Among these traits, thousand-grain weight and seed length, respectively showed the lowest and the highest amount of correlation under both control and stress conditions. Under control condition the range of correlation coefficients for thousand-grain weight ranged from 0.13 to 0.41 and for seed length from 0.62 to 0.74.

The correlation coefficient of seed related traits under control condition and thousand-grain weight under stress condition were checked in order to find a trait under control condition as a suitable trait for indirect selection of thousand-grain weight under stress condition. Table 3.16 which was extracted from Table 2 Appendix 7 shows the minimum, maximum, and mean of the correlation coefficients between thousand-grain weight, seed area, seed width, and seed length under control condition and thousand-grain weight under stress condition.

Table 3.15 Correlation coefficients for seed related traits between pairs of experiments

Traits	Correlation between pair of experiments						Range	Minimum.	Mean	Maximum
	F4-G4	F4-F5	F4-G7	G4-F5	G4-G7	F5-G7				
Thousand-grain weight(C)	0.34	0.39	0.41	0.29	0.13	0.28	0.28	0.13	0.31	0.41
Thousand-grain weight(S)	0.04	0.21	0.42	0.19	0.10	0.07	0.38	0.04	0.17	0.42
Seed area(C)	0.52	0.60	0.53	0.46	0.40	0.59	0.20	0.40	0.52	0.60
Seed area(S)	0.15	0.40	0.38	0.35	0.21	0.20	0.25	0.15	0.28	0.40
Seed width(C)	0.52	0.42	0.42	0.41	0.35	0.42	0.17	0.35	0.42	0.52
Seed width(S)	0.24	0.44	0.37	0.33	0.27	0.29	0.20	0.24	0.32	0.44
Seed length(C)	0.62	0.73	0.68	0.63	0.64	0.74	0.12	0.62	0.67	0.74
Seed length(S)	0.26	0.47	0.46	0.48	0.32	0.27	0.22	0.26	0.38	0.48

C = control, S = stress, F4 = Field 2004, G4 = Greenhouse 2004, F5 = Field 2005, G7= Greenhouse 2007

Table 3.16 Correlation coefficients between the seed related traits under control condition and thousand-grain weight under stress condition

Traits	Range	Minimum	Mean	Maximum
Thousand-grain weight	0.49	-0.09	0.21	0.40
Seed area	0.47	-0.04	0.20	0.43
Seed width	0.44	0.00	0.21	0.44
Seed length	0.28	-0.02	0.10	0.26

As the Table 3.16 shows, there were low correlations between seed related traits under control condition and thousand-grain weight under stress. Therefore, based on this result, seed related traits under control condition were no good estimators for thousand-grain weight under stress.

3.3 Analysis of variance and heritability

The linear mixed model factorial analysis of variance was conducted for the three sets of data including control condition, stress condition, and finally the combination of both conditions. In the following, the main points of each analysis are given.

Control condition

The details of these three analysis of variances are given in Table 3, Table 4, and Table 5 in Appendix 7. However, in the following parts the main points of the results are presented.

Under control condition (Table 3.17) genotypes showed highly significant differences for all nine traits considered. The effect of location, interaction between genotype and location, and year (location) were assayed for the following six traits: thousand-grain weight, seed area, seed width, seed length, number of seeds per spike, and seed weight spike. The interaction between genotype and location for none of them was significant (Table 3.17). For trait thousand-grain weight location and year (location) showed significant difference. However, for the other traits while location did not show any significant difference, years (location) had highly significant effect (Table 3 in Appendix 7) which shows the importance of year in the evaluation of this population compared to the applied locations in the present study.

Table 3.17 Summary of the analysis of variance for genotype and genotype × location for the traits tested under control condition

Traits	**Error** [1]		**Genotype**			**Genotype × Location**		
	df	m.s.	df	m.s.	*F*-value	df	m.s.	*F*-value
Thousand-grain weight	264	15.451	132	35.092	2.85 **	132	12.286	0.80 ns
Seed area	264	0.593	132	2.962	5.64 **	132	0.525	0.89 ns
Seed width	264	0.013	132	0.044	4.19 **	132	0.011	0.79 ns
Seed length	264	0.020	132	0.185	8.81 **	132	0.021	1.09 ns
Days to flowering	132	2.663	132	10.856	4.07 **	-	-	-
No. seeds per spike	132	85.831	132	136.585	1.98 **	132	68.860	0.8 ns
Seed weight per spike	132	0.175	132	0.288	1.58 **	132	0.182	1.04 ns
Spike length	132	0.456	132	1.643	3.60 **	-	-	-
Plant height	132	58.580	132	144.670	2.47 **	-	-	-

1 = Genotype and genotype × location were tested against the same source of error.
** = Significant at the $\alpha = 0.01$ level (2-tailed).
ns = not significant

Stress condition

Under stress condition genotypes showed significant differences for thousand-grain weight whereas there were highly significant differences among the genotypes for traits like seed area, seed width, seed length. The interaction between genotype and location for these traits were not significant (Table 3.18). For the same traits, while location was not significant, year (location) showed highly significant difference (Table 4 in Appendix 7) which shows the importance of year for the evaluation of this population compared to the applied locations.

Table 3.18 Summary of the analysis of variance for genotype and genotype × location for the traits tested under stress condition

Traits	Error[1]		Genotype			Genotype × location		
	df	m.s.	df	m.s.	*F*-value	df	m.s.	*F*-value
Thousand-grain weight	263	8.697	132	14.558	1.71 *	132	8.472	0.97 ns
Seed area	263	0.874	132	2.321	2.34 **	132	0.989	1.13 ns
Seed width	263	0.018	132	0.054	2.7 **	132	0.020	1.12 ns
Seed length	263	0.046	132	0.159	3.24 **	132	0.049	1.08 ns

1 = Genotype and genotype × location were tested against the same source of error.
* and ** = Significant at the α = 0.05 and α = 0.01 level (2-tailed), respectively. ns = not significant

Combined experiment

In order to verify the effect of drought stress on the seed related traits, combined analysis of variance was conducted. Since it could be expected the superiority of the traits under control condition over the stress condition, a one-tailed *F*-test was applied for stress treatment. Post-anthesis drought stress showed significant difference on seed area and highly significant difference on thousand-grain weight and seed width. However, it was no significant on seed length (Table 19). Result from the combined analysis of variance showed the stability of seed length against post-anthesis drought stress compared to other seed related traits.

Table 3.19 Combined analysis of variance for drought treatment on seed related traits

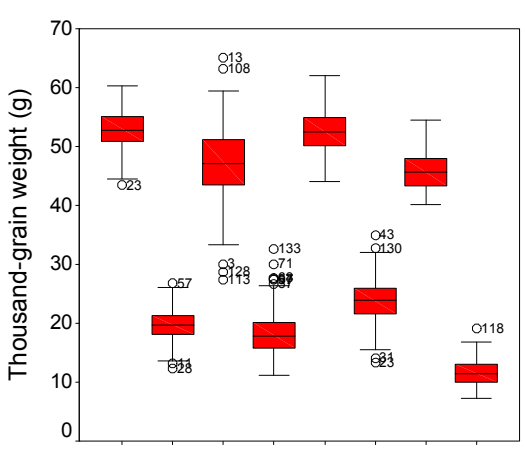
Traits	Error		Stress treatment		
	df	m.s	df	m.s	*F*-value
Thousand-grain weight	2	814.210	1	259444.810	318.64 **
Seed area	2	130.880	1	6651.174	50.80 *
Seed width	2	1.375	1	193.083	140.42 **
Seed length	2	9.075	1	103.909	11.45 ns

* and ** = Significant at the α = 0.05 and α = 0.01 level (1-tailed), respectively.
ns = not significant

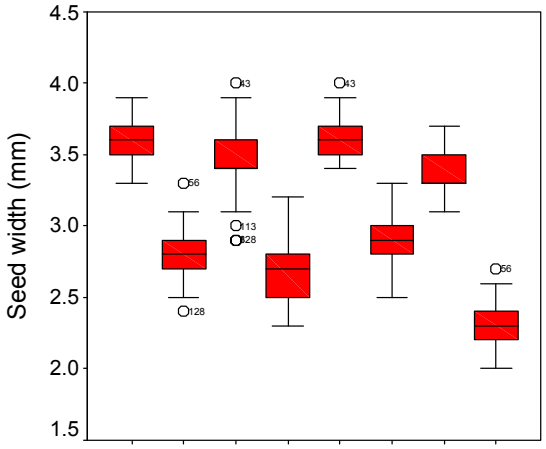
Genotype was significant for all the seed related traits. However, location, interaction between genotype and location, and interaction between genotype and treatment was not significant for the all traits (Table 5 in Appendix 7).

Figure 3.6 shows graphically the effect of the applied stress on the four seed related traits. Larger difference between means of $F_{2:3}$ families under control and stress conditions can be seen for thousand-grain weight followed by seed width, and seed area compared to seed

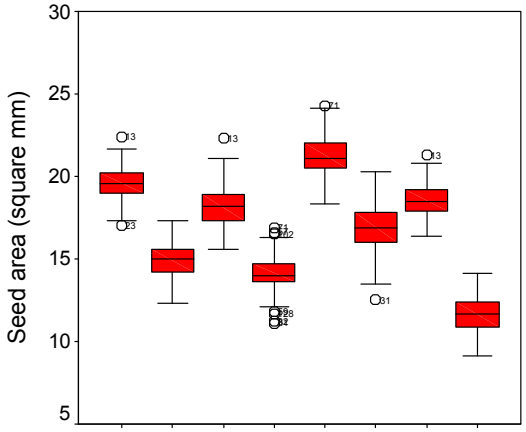
length. Regarding only means of $F_{2:3}$ families for seed length, the largest difference appeared at green house experiment 2007. It could be due to sever drought stress by water stress compared to chemical desiccation.



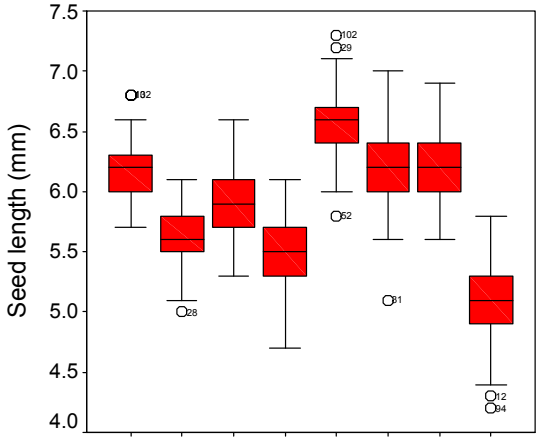
a) FC4, FS4, GC4, GS4, FC5, FS5, GC7, GS7



b) FC4, FS4, GC4, GS4, FC5, FS5, GC7, GS7



c) FC4, FS4, GC4, GS4, FC5, FS5, GC7, GS7



d) FC4, FS4, GC4, GS4, FC5, FS5, GC7, GS7

Figure 3.6 a, b, c, and d Boxplot for thousand-grain weight, seed area, seed width, and seed length, respectively, under control and stress conditions for each of the four experiments

Heritability

The heritability (h^2) of the traits under each control and stress condition was calculated based on the data that resulted from the analysis of variance at each condition. As Table 3.20, shows the values of heritability for the traits under control condition were higher than the values for the same traits under the stress condition. Interestingly, for both conditions thousand-grain weight and seed length had the lowest and the highest values, respectively. The experiments under control conditions had lower CVs compared to the experiments

under stress conditions. The experiments for thousand-grain weight showed the highest CVs under both control and stress conditions.

Table 3.20 Coefficient of variation (CV) for each experiments and heritability (h^2) of the traits under each control and stress condition

Traits	Control		Stress	
	C.V%	h^2 %	C.V%	h^2 %
Thousand-grain weight	7.93	24.11	16.11	14.42
Seed area	3.96	49.96	6.49	29.27
Seed width	3.29	36.59	5.07	32.97
Seed length	2.25	67.84	3.84	38.05
Days to flowering	0.63	43.47	-	-
Spike length	5.43	39.44	-	-
Plant height	5.97	26.86	-	-

3.4 QTL analysis

Forty three marker loci with a distance smaller than 1.1 cM and three segregated distorted loci with chi-square (χ^2) higher than ten were excluded from the linkage map for the QTL analysis. Therefore, a map consisting of the 248 remaining informative marker loci was recalculated and was applied in QTL analysis. The analysis was performed for each experiment independently, and also for three averages of data including average over all data of field and greenhouse experiments, average of data over the field experiments, and average of data over the greenhouse experiments. In the following, general overview of QTL analysis and also QTL analysis in trait point of view are given.

Bonferroni chi-square approximation (Zeng, 1994) was applied to the overall exploratory QTL experiments with 229 marker intervals (Beavis, 1998) with 2 degrees of freedom because of additive effects fitted for QTL in the model. It showed a LOD = 2.96 as equal to a genome wide type-I error rate of 0.25. Therefore, QTLs with LOD score of three and more were considered. However, to check the repeatability of identified QTLs, LOD score was reduced to 2.5. In the following, L refers to the long arm, S to the short arm of chromosomes, and C refers approximately to the centromere region.

3.4.1 General overview

Awnedness as the only morphologic trait with two records at field experiment in 2005 and greenhouse experiment in 2007 showed one QTL on chromosome arm 5AL in 2005 and two QTLs on chromosome arms 4AS and 5AL in 2007. The increasing allele for the QTL on chromosome arm 5AL originated, not surprisingly, from parent A (HTRI 11712) which bears awns in medium size. However, the increasing allele for the QTL on chromosome 4AS originated from parent B (HTRI 105), which does not bears awns. The LOD score and explained phenotypic variance (R^2%) value for the QTL on chromosome arm 5AL were 57.93 and 86.26 in 2005 and 45.15 and 77.97 in 2007, respectively. These values for QTL on chromosome arm 4AS were 8.14 and 18.68, respectively. As table 3.21 shows both type of data scoring (M = metric and O = ordinal) in greenhouse experiment in 2007 revealed peaks and interval for the awn loci.

Table 3.21 QTL analysis for awns

Awnedness	Left / Right interval markers	1-LOD interval	QTL peak	LOD score	Partial R^2	Additive effect
QAwn.ipk-5A-GC7(M)	Xgwm0995-5A / Xgwm0865-5A	194-198	196	45.15	77.97	-0.746
QAwn.ipk-4A-GC7(M)	Xgwm1093-4A / Xgwm0695-4A	0-12	6	8.14	18.69	0.195
QAwn.ipk-5A-GC7(O)	Xgwm0995-5A / Xgwm0865-5A	194-198	196	32.28	65.82	-2.602
QAwn.ipk-4A-GC7(O)	Xgwm1093-4A / Xgwm0695-4A	0-12	8	4.10	12.75	0.707
QAwn.ipk-5A-FC5(O)	Xgwm0995-5A / Xgwm865-5A	194- 198	196	57.93	86.26	-1.873

M = Metric, O = Ordinal scoring of awns.

From the 13 other quantitative characters 54 records were analyzed of which 31 and 24 were measured under control and stress conditions, respectively. Composite Interval Mapping (CIM) analysis by PLABQTL (Utz and Melchinger, 2007) revealed 88 and 60 QTLs under control and stress conditions, respectively, having a LOD score higher than 3. Therefore, 148 QTLs were identified from 54 records of the traits with an average of 2.7 QTLs per each trait per experiment. There was at least one QTL for each trait per measurement except for thousand-grain weight under stress at greenhouse experiment in 2004. Because of the identification of the same QTLs in different experiments, the 88 and 60 mapped QTLs under control and stress condition represented 64 and 53 unique QTLs, respectively, and in total 117. There were 23 QTLs which appeared under both control and stress conditions for the same trait.

As Figure 3.7 shows the number of QTLs in most of the trait measurements per experiment, ranged from one to four. However, in the seven following trait measurements number of QTLs exceed four and present higher numbers: thousand-grain weight under stress condition with five QTLs at field experiment in 2004, seed length with seven and five QTLs under control and stress conditions, respectively in field experiment 2005, followed by days to flowering with six QTLs in field experiment 2004, seed weight per spike with seven QTLs under stress condition in 2007, number of seeds per spike with five QTLs under control condition in 2005, and plant height with five QTLs under control condition in 2005.

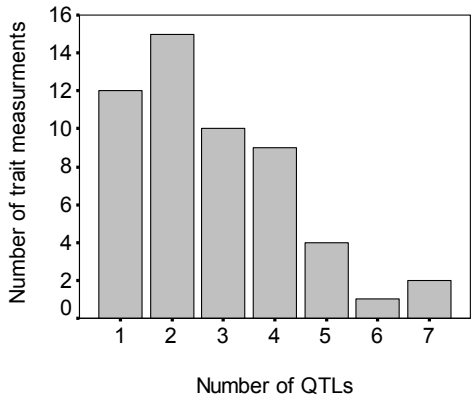


Figure 3.7 Number of QTLs per each trait measurement

The detailed information for all identified QTLs based on each separate experiment and also by three kind of averages of data including QTL name, flanking markers for the QTL, LOD score, the explained phenotypic variance (R^2%) based on partial correlation of coefficient, one LOD support intervals, and additive effect are presented in Table 2 in Appendix 8. In the above mentioned tables, the same QTLs for each trait were marked with similar color to easy of recognition. The entire identified QTLs were presented graphically in Figure 3.8 that show one LOD support interval of the QTLs. In these graphical views information such as location, condition, year of the experiment in which the QTLs were detected, the LOD score, and the origin of increasing allele (A: positive allele from HTRI 1171, B: from HTRI 105) are given behind each.

Table 3.22 shows the QTLs for investigated traits detected on each single chromosome. QTLs were identified on most of the constructed linkage groups except those representing chromosomes 6A, 6B, 3B, and 3D. However, the numbers of QTLs on chromosomes were not equal and ranged from one QTL at chromosomes 1D to 16 QTLs on chromosome 7D.

Table 3.22 QTLs for the traits under study detected on single chromosomes

Traits	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	5A	5B	5D	6A	6B	7A	7B	7D	No. QTLs per trait	P. A	P. B
Thousand-grain weight(C)		**A***									**B/*B****						B			**4**	1	3
Thousand-grain weight(S)		A								B*/A	*B**						**B**		**A**	**6**	3	3
Seed area(C)		A/***A***		A						B	*B*/**B***		**A***							**7**	4	3
Seed area(S)		*A**/A/A				B				B*	*B**		B						A	**8**	4	4
Seed width(C)		A									***B*/B***		B/B							**5**	1	4
Seed width(S)				A		B				B/A	*B**								A	**6**	3	3
Seed length(C)	*A**	A/A*	B	***A**/A/A*						B	***B****	*A*	***A****	A				A		**13**	10	3
Seed length(S)	*A*	***A***/A		*A*		B					***B***	*A*	***A****							**8**	6	2
Days to flowering		B*				B	A*					B/B*		B				B	**B***	**8**	1	7
No. seeds per spike(C)				***A****							**A***		*A**				**A***		**B***	**5**	4	1
No. seeds per spike(S)				*A*								A	*A*							**3**	3	0
Seed weight per spike (C)				***A****									*A*						B	**3**	2	1
Seed weight per spike(S)				*A*	A	A						A	*A*				B		A	**7**	6	1
Spike length(C)						B	*A**			B			**A***						***B****	**5**	2	3
Spike length(S)							*A*												*B*	**2**	1	1
Plant height(C)					*B*					***A****	**B***		*A**					B	***B****	**6**	2	4
Plant height(S)					*B*					*A*			*A*						*B*	**4**	2	2
Weight of three spikes per plant(C)				*A*						B										**2**	1	1
Weight of three spikes per plant(S)				*A*		A						A							A	**4**	4	0
Weight of all spikes per plant(C)																	A			**1**	1	0
Weight of all spikes per plant(S)												A							A	**2**	2	0
No. of fertile spikes per plant(C)																	A			**1**	1	0
No. of fertile spikes per plant(S)																			A	**1**	1	0
No. of unfertile tillers per plant(C)		B			B													B	*B*	**4**	0	4
No. of unfertile tillers per plant(S)												B							*B*	**2**	0	2
No. QTLs per chromosome	**2**	**14**	**1**	**12**	**4**	**7**	**3**	**0**	**0**	**11**	**13**	**9**	**13**	**2**	**0**	**0**	**6**	**4**	**16**	**117**		
P. A	2	12	0	12	1	2	3	0	0	4	1	6	10	1	0	0	3	1	7		**65**	
P. B	0	2	1	0	3	5	0	0	0	7	12	3	3	1	0	0	3	3	9			**52**

P. A = Parent A (HTRI 11712) and P. B = Parent B (HTRI 105) show the origin of increasing alleles, Bold shows QTL mapped at least two times. * = Identified also by over all mean, Italic= Identified under both control and stress conditions, / = Different QTLs on chromosome

Interestingly, the increasing alleles for most of the QTLs that were mapped on chromosomes 1B, 2A, and 5B originated from parent A whereas parent B was responsible for most of the increasing alleles for the QTLs on chromosomes 4A and 4B. However, on chromosome 7D with 16 QTLs, showing the highest number of QTLs, parent A and parent B were sources of increasing alleles for 7 and 9 QTLs, respectively.

Both parents were important for most of the measured agronomic traits and harbor alleles which increase the characters in which 65 and 52 out of 117 mapped QTL, the increasing alleles originated from parent A and parent B, respectively. However, in some traits, parents did not share equally the increasing alleles, for example, parent A contributed most of the increasing alleles for seed length and number of seeds per spike, under both control and stress conditions whereas parent B showed more responsibility for the traits like days to flowering and number of unfertile tillers per plant.

As Figure 3.8 shows QTLs from different traits co-localized and formed clusters. Clusters including QTLs for at least four traits were found on chromosomes 1B, 2A, 4B, 4AL, 5BC, 5A, 7DS, and 7AS. Chromosome 1B showed three clusters on the short arm, centromere region as well as on the long arm. The two former regions included QTLs for thousand-grain weight, seed area, seed width, and seed length, while the later contained QTLs for thousand-grain weight, seed area, seed length, days to flowering, and number of unfertile spikes per plant. QTLs for the traits like seed area, seed length, number of seeds per spike, weight of seed per spike, and weight of three spikes per plant formed a cluster on chromosome 2AC.

On chromosome 4B QTLs of traits like thousand-grain weight, seed area, seed weight, seed length, number of seeds per spike, and plant height coincided. On chromosome 4AL, QTLs of five traits co-located: thousand-grain weight, seed area, seed width, seed length, and plant height. The QTLs on chromosome 5B formed two clusters, one on centromere region including QTLs for seed area, seed width, seed length, spike length, and plant height. The other QTL cluster on chromosome 5B located on the long arm and contained QTLs for seed area, seed width, number of seeds per spike, and weight of seeds per spike. On the long arm of chromosome 5A near to centromere region, QTLs of seed length, days to flowering, number of seeds per spike, weight of seeds per spike, and number of unfertile spikes per plant were co-located.

QTL cluster on chromosome 7DS included QTLs for the 12 following traits : thousand-grain weight, seed area, seed width, days to flowering, number of seeds per spike, weight of seeds per spike, spike length, weight of three spikes per plant, weight of all spikes per plant, plant height, number of fertile spikes per plant, and number of unfertile tiller per plant. The cluster on chromosome 7AS contained thousand-grain weight, number of seeds per spike, weight of seeds per spike, and weight of all spikes per plant.

As Table 3.22 shows analysis revealed different numbers of QTLs per trait. Number of QTLs per trait ranged from one QTL for weight of all spikes per plant under control condition and number of fertile spikes per plant under both control and stress conditions to 13 QTLs for seed length under control condition. Comparing only thousands-grain weight and seed size related traits, in order to make an appropriate comparison among traits with the same number of trials, seed length showed the highest number of QTLs followed by seed area, seed width, and finally thousand-grain weight under both control and stress conditions.

Mapping of the 13 traits recorded under control condition showed all together 64 QTLs. Since four of these traits were recorded only at one experiment (under greenhouse experiment in 2007), therefore the remaining nine traits were taken into account to find the repeatability of the identified QTLs at different experiments. QTL analysis of these nine traits under control condition showed all together 56 QTLs [of which 20 were mapped repeatedly in different experiments of the present study, too]. Under stress condition, there were four traits which records at more than one experiment and showed all together 28 QTLs of which five were identified repeatedly in different experiments of the present study. Therefore from the 84 identified QTLs in the repeated experiments, 25 QTLs were detected under the same either control or stress condition in at least two experiments.

From the 12 traits which were measured under both control and stress conditions, 56 and 52 QTLs were identified, respectively of which 23 were detected under both conditions. Eleven out of these 23 common QTLs were also mapped repeatedly under at least one of the two stress or control conditions.

Finally, 37 QTLs (including 20 QTLs repeated under control, 5 QTLs repeated under stress, and 12 QTLs common under control and stress conditions and not repeated on the same condition) out of 117 identified QTL were classified as repeated QTLs in the current study.

Figure 3.8 Graphical view of the all detected QTLs including one LOD support interval, the experiment location, condition, and year in which the QTLs were detected plus the LOD score and origin of the increasing allele behind each QTL. QTgw = thousand-grain weight, QSea = seed area, QSew = seed width, QSel = seed length, QDtf = days to flowering, QAwn = awn, QNsp = No. of seeds per spike, QWsp = weight of seeds per spike, QSpl = spike length, QWts = weight of three spikes per plant, QPhe = plant height, QNfs = no. of fertile spikes per plant, QWas = weight of all spikes per plant, QNus = no. of unfertile tillers per plant. There are two kinds of treatments: stress (S) and control (C) and two locations: field (F) and greenhouse (G) and three years: 2004 (4), 2005 (5), and 2007 (7). QTL also mapped for mean of field (FM) and mean of greenhouse (GM) and mean of both location (M). The first time mapped loci were marked in red color and extra loci were indicated by blue color. Color was applied just to separate QTL of different group of traits. Filled bars show QTLs from separate experiment whereas not filled bars with pattern inside represent QTL based on one of three set of means of data.

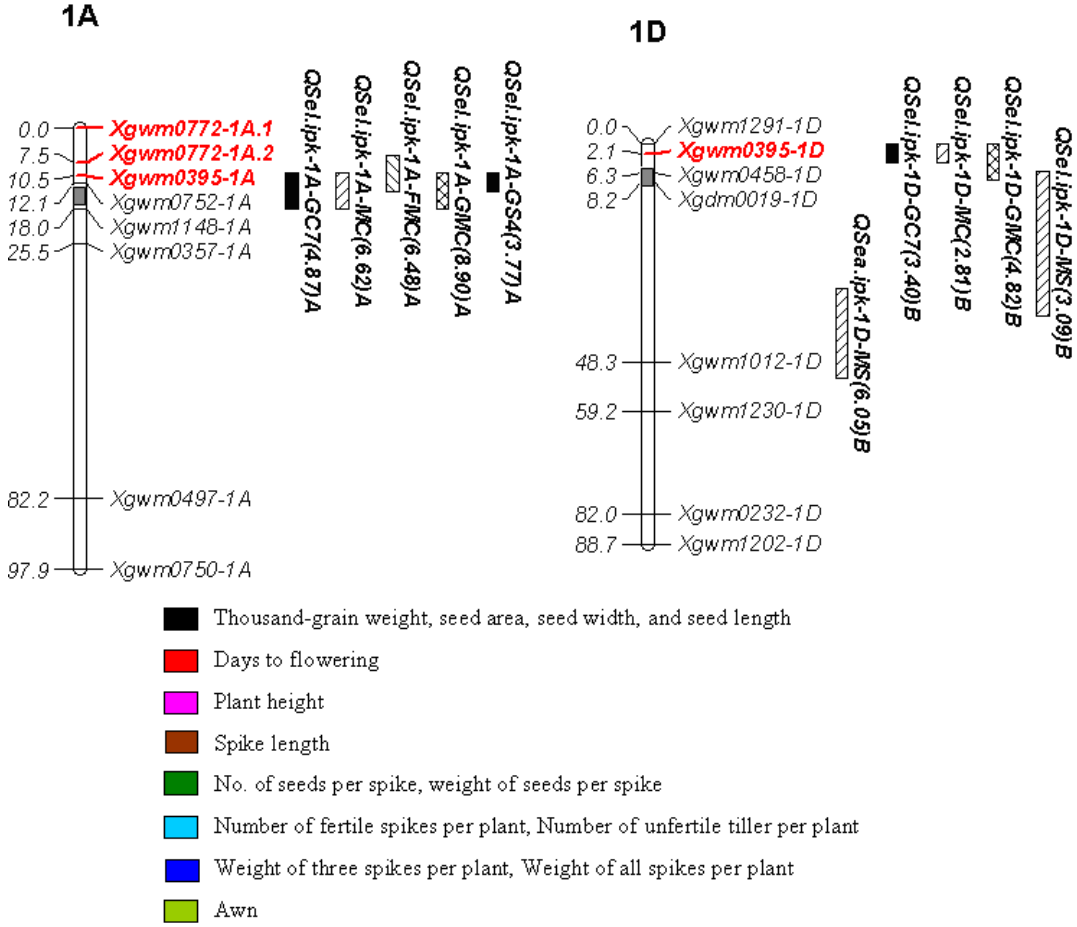
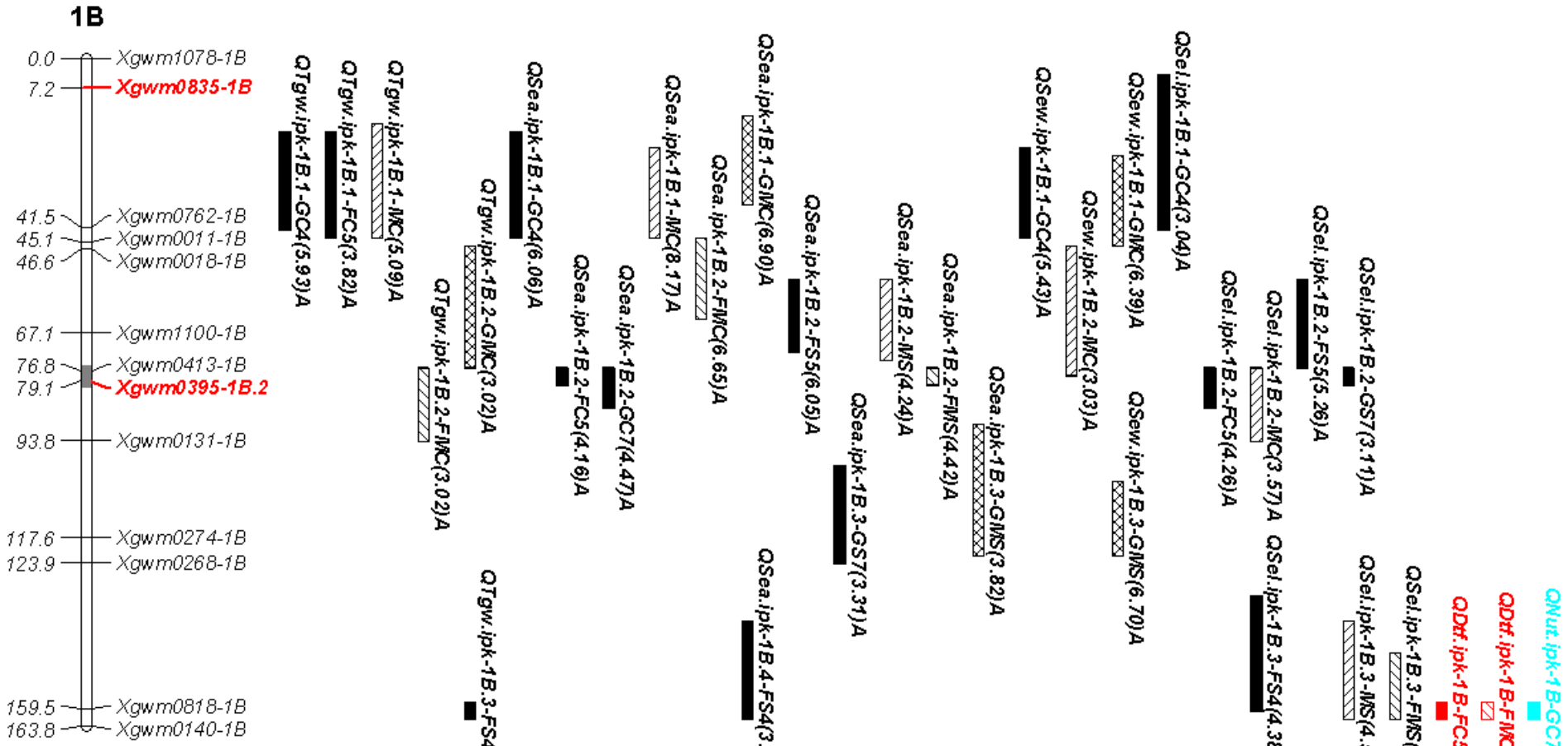


Figure 3.8 continued



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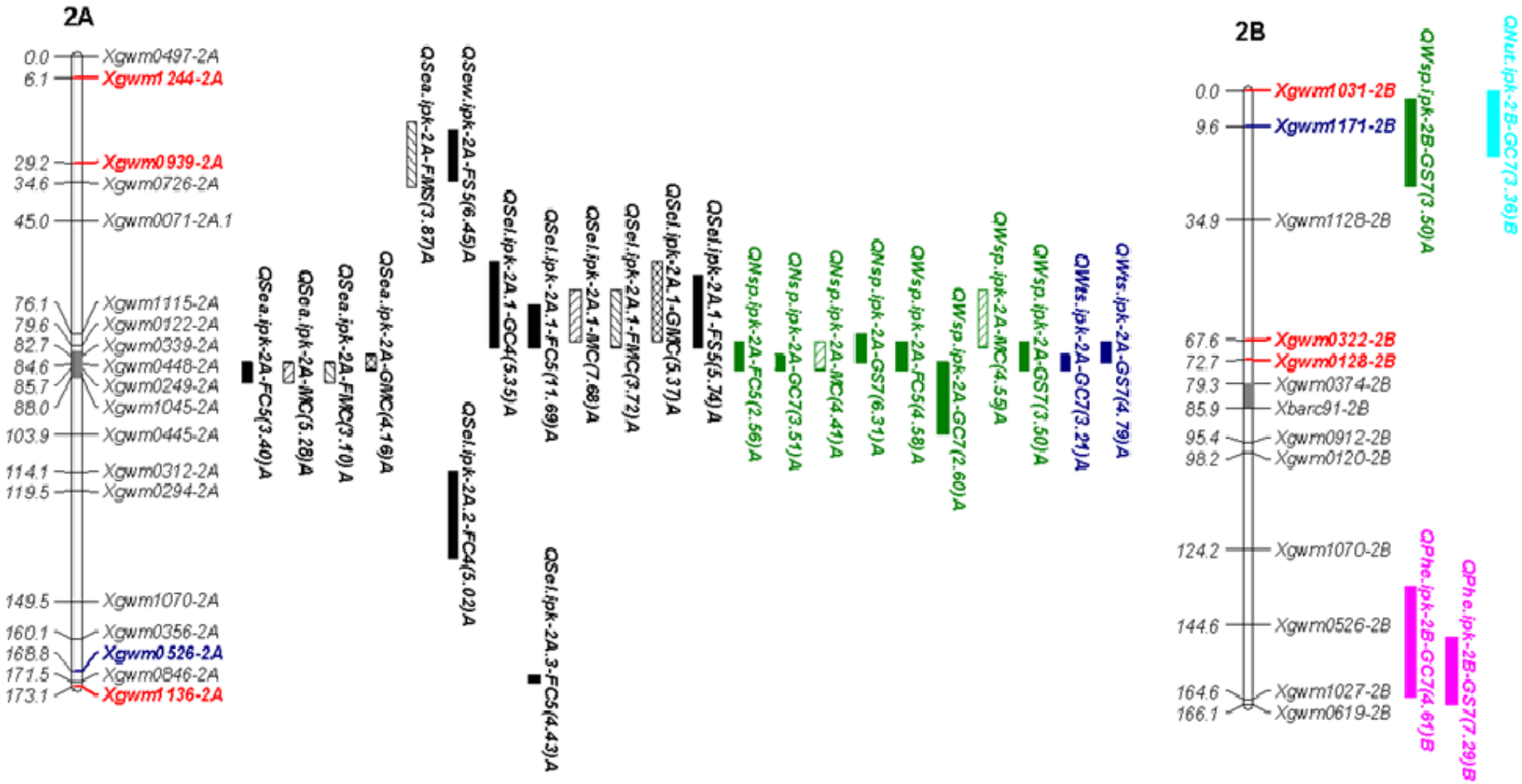

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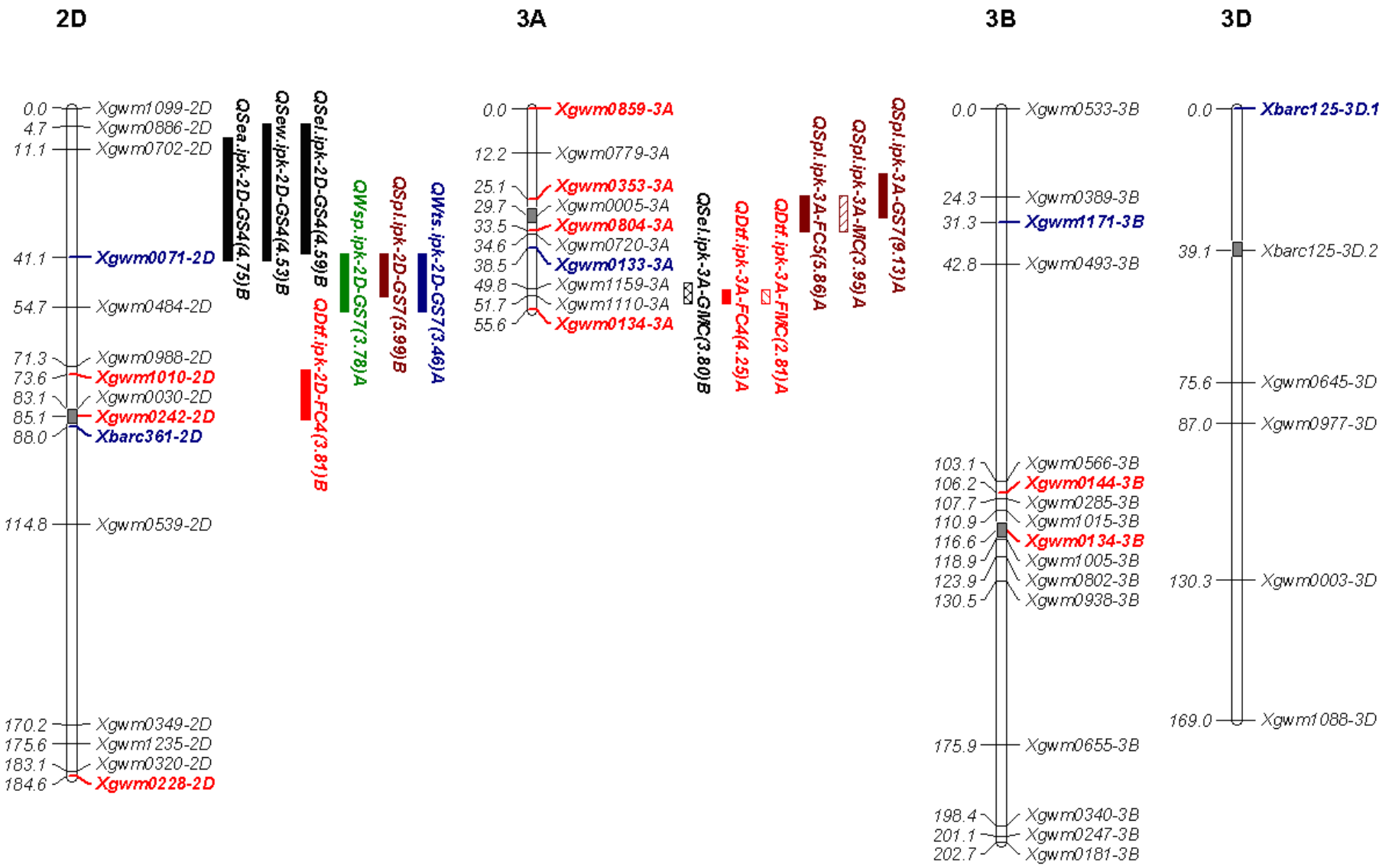

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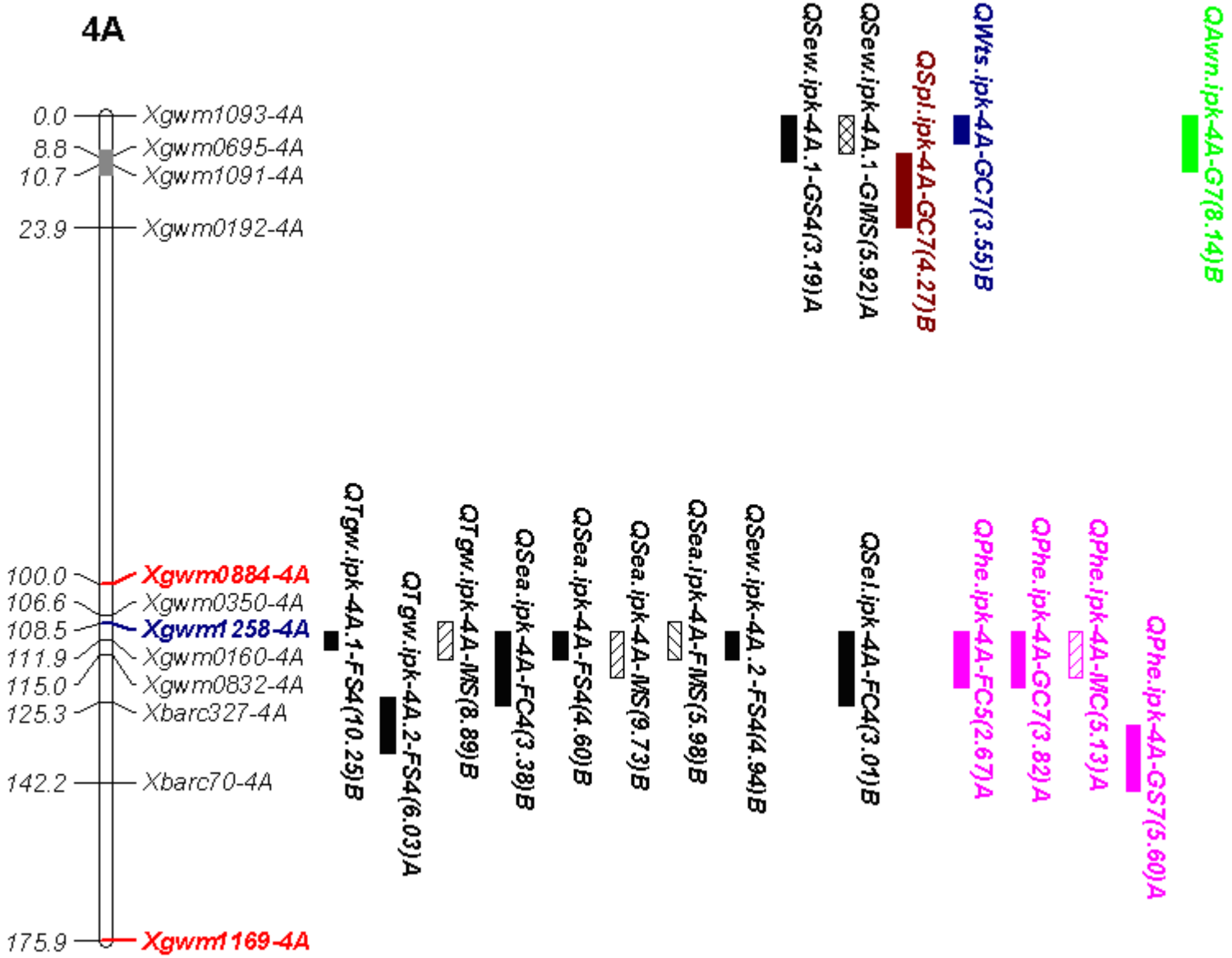

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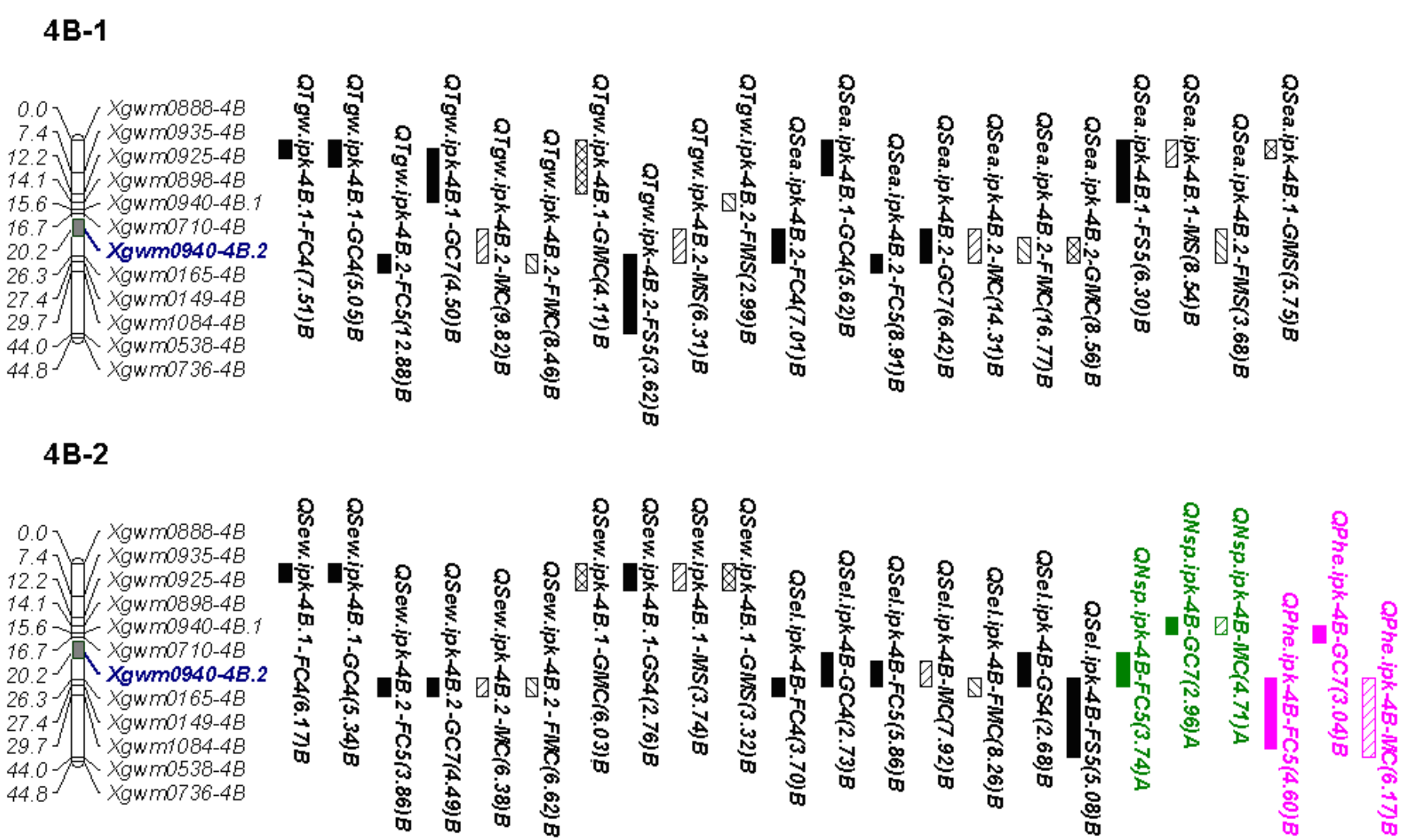

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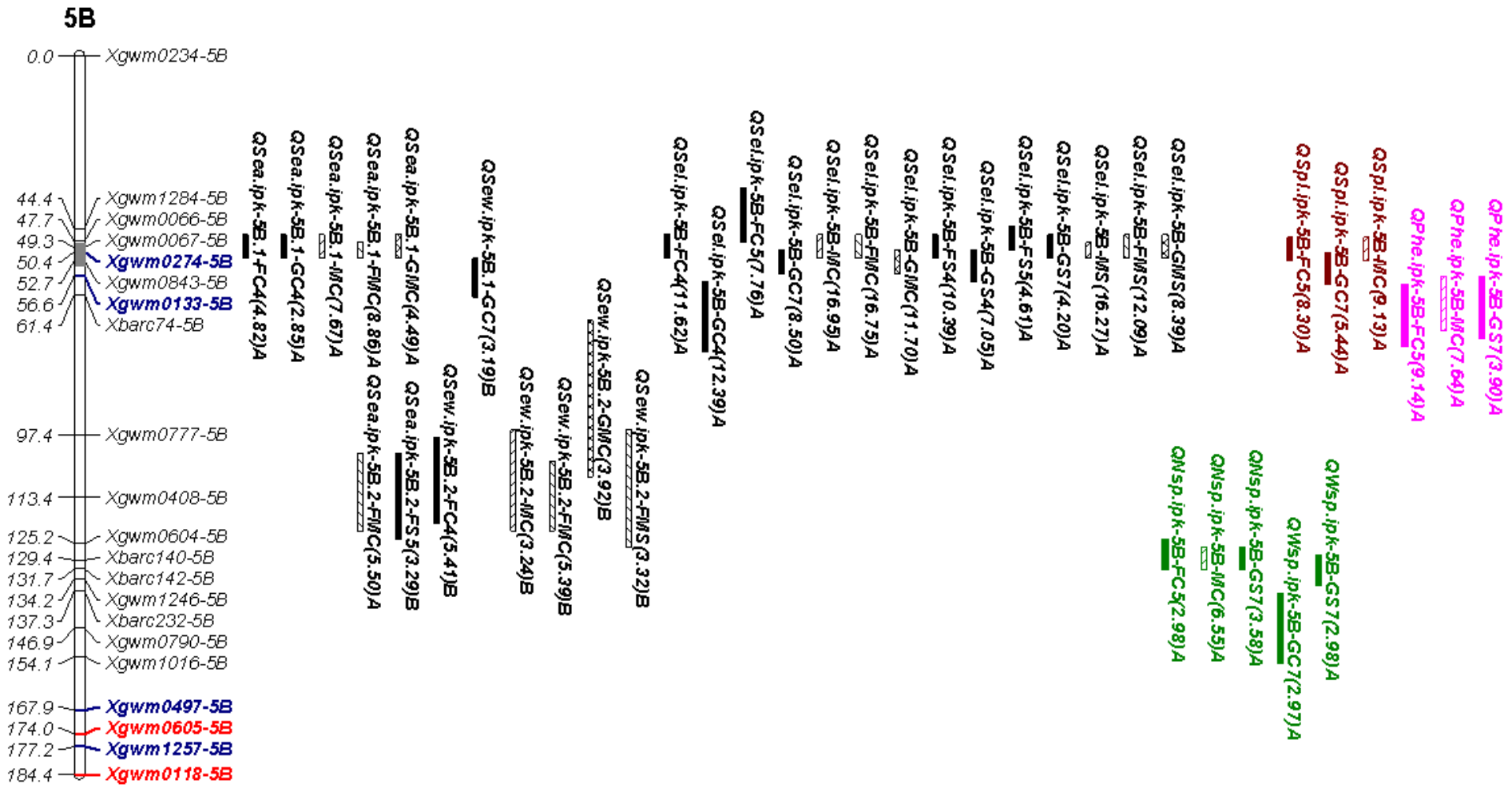

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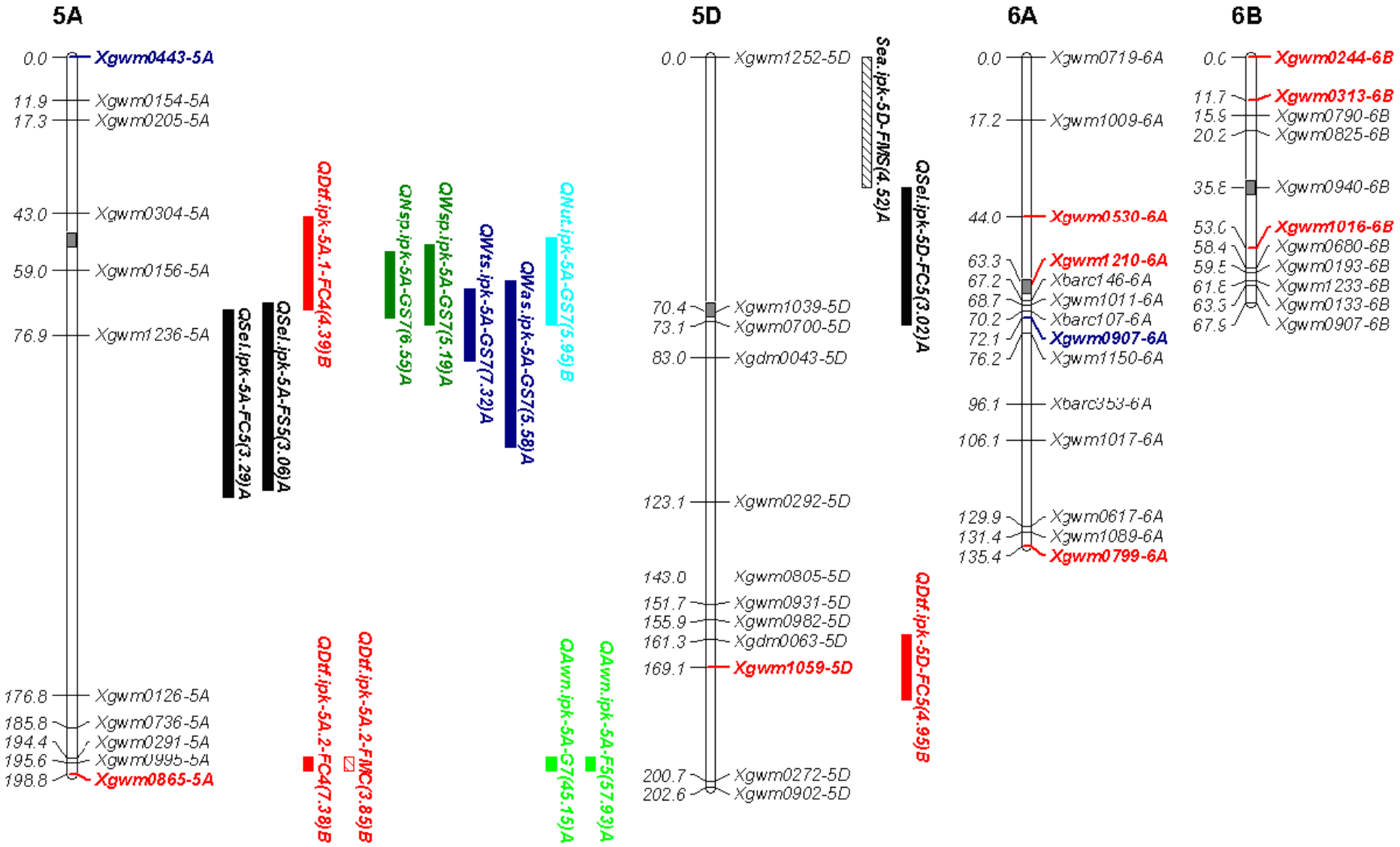

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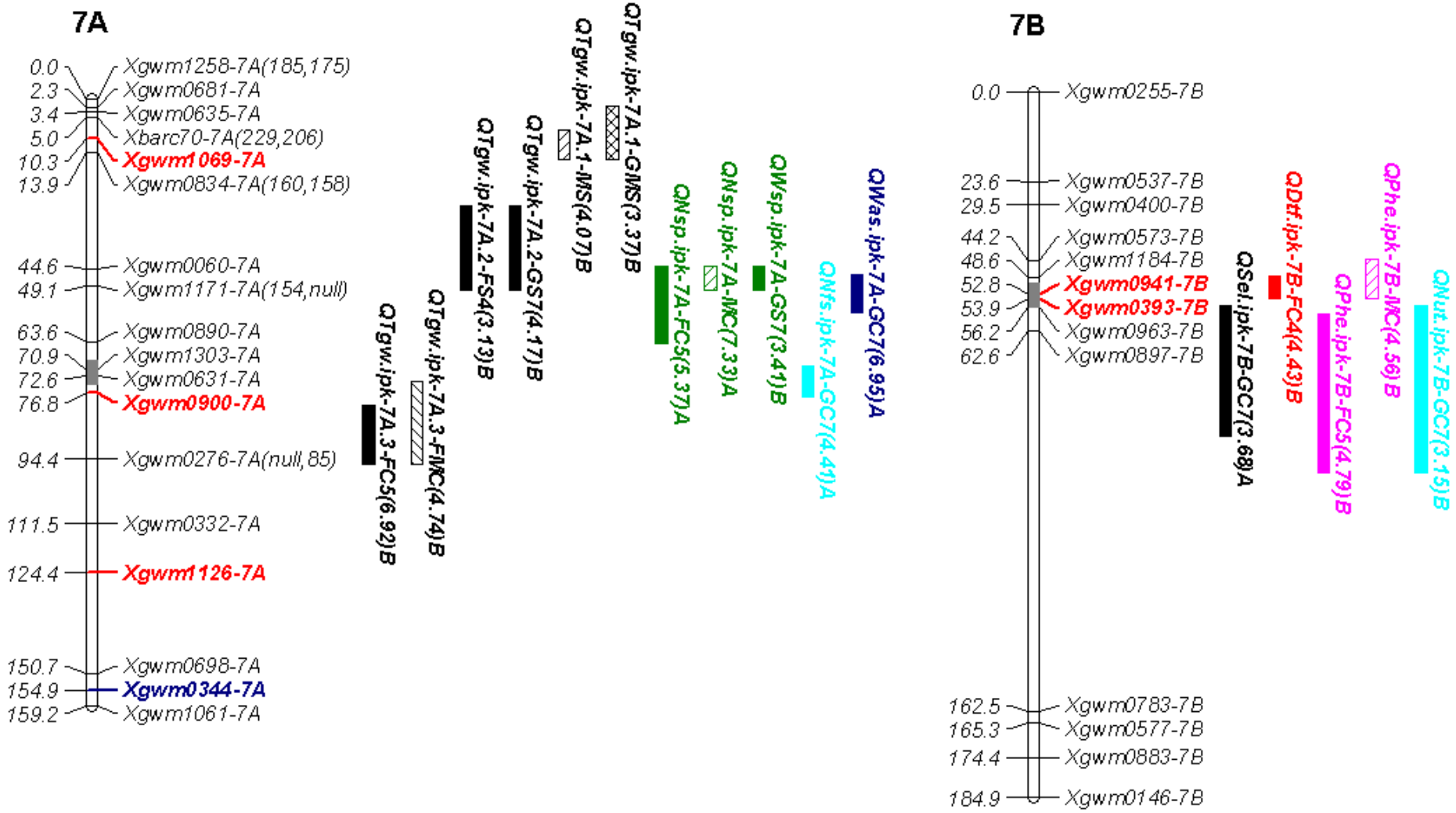

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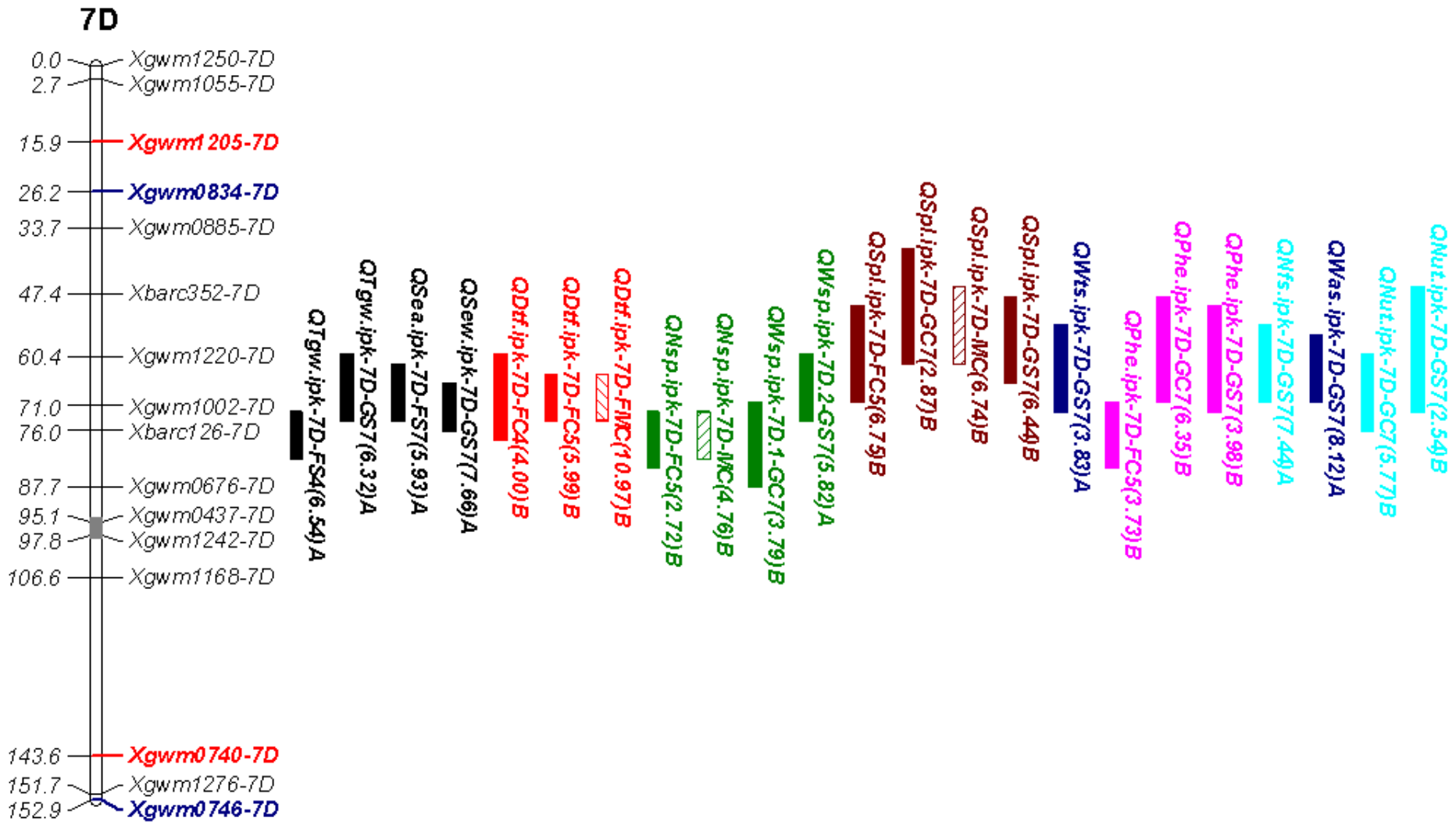

12 QTLs were identified under both control and stress condition as well as were mapped repeatedly at least under one of the two conditions. The repeated QTLs had consistency in the direction of additive effects between experiments, although the LOD and R^2 were varied. Figure 1 to 14 in Appendix 8 show Boxplots using left or right interval markers (the closest linked one) for some of the identified QTLs (Table 2 in Appendix 8) and verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait under control and stress condition. As can be seen in the graphs, the group bearing increasing alleles always had higher average value for the trait.

3.4.2 QTL analysis considering the traits under study

Thousand-grain weight

Mapping analysis under control conditions revealed four QTLs on chromosomes 7A, 4B (two QTLs), and 1B of which three were detected repeatedly in two experiments (Figure 3.8). The explained phenotypic variation (R^2%) by a single QTL ranged from 8.7 to 26.5% (Table 2 in Appendix 8) and both parents contributed the increasing alleles. Two of the repeated QTLs were detected on the short and long arms of chromosome 4B (increasing allele from parent B) whereas the third QTL was mapped on chromosome 1B (increasing allele from parent A). Two of the repeated QTLs were found by mean of over all data, too. The QTL on chromosome 7A which was not repeated in any other experiment, showed an R^2 of 14.7% and LOD value of 6.92 which is higher than the critical LOD threshold (6.62) of α =1 % based on permutation test (Table 1 in Appendix 8). The *QTgw.ipk-4B-FC5* with increasing allele from parent B showed the highest value for both the LOD score (12.8) and R^2 (26.5%).

Under stress conditions six QTLs were detected as responsible for thousand-grain weight of which two were detected repeatedly in two experiments (Table 2 in Appendix 8). The R^2 for each single QTL ranged from 8.93 to 21.04%. The repeated QTLs were mapped, interestingly, in homoeologous positions of group seven on chromosome 7AS (increasing allele from parent B) and 7DS (increasing allele from parent A). Interestingly, parent B, the drought sensitive parent, also contributed alleles for drought tolerance. At field experiment in 2004, two QTLs in repulsion were identified on chromosome 4AL with 16 cM distance in their peaks. The *QTgw.ipk-4A-FS4* (increasing allele from patent B) showed the highest values for LOD (10.25) and R^2 (21.04%). There was only one QTL on chromosome arm 4BL

which was mapped under both control and stress conditions and it was one of the three QTLs which was identified repeatedly under control conditions.

Seed area

Mapping analysis under control conditions showed seven QTLs of which three were mapped repeatedly in at least two experiments. The R^2 for a single QTL ranged from 8.2 to 23.4% (Table 2 in Appendix 8) and the increasing alleles originated from both parents. One of the repeated QTLs was detected on chromosome 5B (increasing allele from parent A), the other QTL was mapped on chromosome 4B (increasing allele from parent B) and the third QTL located on chromosome 1B (increasing allele from parent A). The repeated QTLs on chromosomes 5B and 4B were found by mean of over all data, too. The *QSea.ipk-4B-FC5* (increasing allele from parent B) showed highest values for LOD (8.91) and R^2 (23.4 %).

Under stress conditions eight QTLs were detected for seed area but none of them repeated in other experiments (Table 2 in Appendix 8). The R^2 for each single QTL ranged from 7.7 to 14.4 %. Both parents shared increasing alleles. Regard to the repeatability, three of the eight QTLs were identified by mean of over all experiments under stress condition and also were detected under control conditions. These repeated QTLs were mapped on chromosome 4A (increasing allele from parent B), chromosome 4B (increasing allele from parent B), and chromosome 1B (increasing allele from parent A). The *QSea.ipk-4B-FS5* (increasing allele from parent B) showed the highest value for LOD score (6.30) and *QSea.ipk-2D-GS4* (increasing allele from parent B) had the highest value of R^2 (14.4%).

Seed width

Seed width under control conditions showed five QTLs of which two were identified repeatedly in at least two experiments. The R^2 of a single QTL ranged from 7.9 to 15.7% (Table 2 in Appendix 8). Parent A contributed the increasing allele only at one QTL on chromosome 1B. Both of the repeated QTLs were mapped on the short and long arms of chromosome 4B (increasing allele from parent B). The repeated QTL on chromosome 4BL was identified by mean of over all data, too. The *QSew.ipk-4B-FC4* showed highest value for LOD score (6.17) whereas *QSew.ipk-4B-FC5* had the highest value of R^2 (15.7%).

Under stress conditions six QTLs were detected for seed width and none of them repeated in other experiments (Table 2 in Appendix 8). However, one QTL was identified by over all mean of data on chromosome 4BS (increasing allele from parent B) which was common

under control and stress conditions and notably it was one of the two repeated QTLs for seed width under control conditions. The R^2 for each single QTL ranged from 6.7 to 12.9%. The *QSew.ipk-7D-GS7* (increasing allele from parent A) showed highest values for LOD score (7.66) and R^2 (12.9%).

Seed length

Mapping analysis under control conditions showed 13 QTLs of which three were identified repeatedly in at least two experiments. The R^2 for a single QTL ranged from 8.3 to 26.9% (Table 2 in Appendix 8) and the increasing alleles originated from both parents. One of the repeated QTLs was mapped on chromosome 5B (increasing allele from parent A), the second QTL was mapped on chromosome 4B (increasing allele from parent B) whereas the third one was found on chromosome 2A (increasing allele from parent A). The repeated QTLs were found by mean of over all data, too. Interestingly, on chromosome 2A, two QTLs (*QSel.ipk-2A.1-FC5* and *QSel.ipk-2A.3-FC5*) (increasing allele from parent A) simultaneously were identified on field experiment in 2005. However, only one of them (*QSel.ipk-2A.1-FC5*) repeated. The *QSel.ipk-5B-GC4* (increasing allele from parent A) showed the highest value for LOD (12.39) whereas *QSel.ipk-5B-FC4* (increasing allele from parent A) had the highest value of R^2 (26.9%).

Under stress conditions eight QTLs were detected for seed length of which three were identified repeatedly in other experiments (Table 2 in Appendix 8). The R^2 for each single QTL ranged from 8.6 to 22.4% and the increasing alleles originated form both parents. The repeated QTLs were mapped on chromosome 5B (increasing allele from parent A), on chromosome 4B (increasing allele from parent B), and on chromosome 1B (increasing allele from parent A). Only the repeated QTL on chromosome 5B was found by mean of over all data, too. There were six QTLs on chromosomes 5AL, 5BC, 4BL, 2AS, 1AC, and 1BC common under control and stress conditions and in each condition two of them were mapped repeatedly. The *QSel.ipk-5B-FS4* (increasing allele from parent A) showed the highest values for LOD (10.39) and R^2 (22.4 %). Interestingly, this QTL was the only one that repeated in all the experiments under both control and stress condition, means appeared at all the eight measurements of the conducted experiments.

Days to flowering

Days to flowering were analyzed under field experiments in the two years and showed eight QTLs. However, only one QTL located on chromosome 7DS was mapped repeatedly and four of them including the repeated QTL were detected by mean of the two years data, too. Interestingly, under field experiment in 2004 two QTLs (*QDtf.ipk-5A.1-FC4* and *QDtf.ipk-5A.2-FC4*) were identified on chromosome 5A (increasing allele both from parent B). Parent A contributed increasing allele only for one QTL which mapped on chromosome 3A. The R^2 for a single QTL ranged from 7.7 to 14.7% (Table 2 in Appendix 8). The *QDtf.ipk-5A.2-FC4* (increasing allele from parent B) showed the highest value for LOD score (7.38) whereas *QDtf.ipk-7D-FC4* showed the highest amount for R^2 (24.7%).

Number of seeds per spike

Under control conditions showed five QTLs of which two were found repeatedly in two experiments. All of the mapped QTLs were found by mean of over all data, too. The R^2 with a single QTL ranged from 7.96 to 16.3% (Table 2 in Appendix 8). The repeated QTLs located on chromosome 5B and 2A (both with increasing allele from parent A). The *QNsp.ipk-7A-FC5* (increasing allele from parent A) showed the highest values for LOD score (5.37) and R^2 (16.3%). Under stress condition three QTLs were detected. The R^2 for each single QTL ranged from 12.1 to 14.1%. There were two QTLs common between the control and stress conditions and one of them was one of the two repeated QTLs in control conditions (Table 2 in Appendix 8).

Seed weight per spike

Under control conditions showed three QTLs on chromosomes 7D, 5B, and 2A. The R^2 of a single QTL ranged from 7.61 to 10.66% (Table 2 in Appendix 8). The repeated QTL located on chromosome 2A (increasing allele from parent A) was mapped by mean of data, too. This QTL showed the highest LOD score (4.58) at field experiment in 2005 and the highest value of R^2 (10.66%) at greenhouse experiment in 2007. Under stress condition seven QTLs were detected. The R^2 for each single QTL ranged from 7.9 to 21.7% (Table 2 in Appendix 8). There were two QTLs on chromosomes 5BL and 2AC, which were common between the control and stress conditions and interestingly the QTLs on chromosome 2AC was the only QTL which repeated at the two experiments under control conditions. Interestingly, chromosome 7D showed two QTLs one per each condition and with a genetic distance of

about 18 cM between their peaks. However, while under control condition the increasing allele originated from parent A, under the stress condition it came from parent B. This showed the existence of different genes for this trait in nearby regions on chromosome 7D and confirmed the value of both parents for this trait but each one under specific condition.

Spike length

Under control conditions showed five QTLs of which two were repeated in two experiments. Three of the mapped QTLs were found by mean of over all data, too. The R^2 of a single QTL ranged from 8.3 to 15.4% (Table 2 in Appendix 8). Both repeated QTLs located on chromosomes 7D (increasing allele from parent B) and 5B (increasing allele from parent A) were mapped by mean of data, too. The *QSpl.ipk-5B-FC5* showed the highest LOD value (8.30) and *QSpl.ipk-2D-GC7* had the highest value of R^2 (15.4 %). Under stress condition two QTLs were detected on chromosomes 7DS and 3AC with R^2 of 12.7 and 13.5%, respectively (Table 2 in Appendix 8). The *QSpl.ipk-3A-GS7* with increasing allele from patent A showed the highest value for LOD (9.13) and R^2 (13.5 %). The identified QTLs under stress condition were detected also under control conditions and QTL on chromosome 7DS was the one that repeated at each of the two experiments under control.

Plant height

Analysis showed six QTLs of which three were repeated in the two experiments. The R^2 for a single QTL ranged from 8.7 to 20.9% (Table 2 in Appendix 8). The repeated QTLs were mapped on chromosomes 7D (increasing allele from parent B), 4A (increasing allele from parent A), and 4B (increasing allele from parent B). The *QPhe.ipk-5B-FC5* (increasing allele from parent A) showed the highest value for LOD (9.14) and *QPhe.ipk-7D-FC5* (increasing allele from parent B) had the highest value of R^2 (20.9%). Under stress condition, four QTLs were detected and all of them were common with control condition. Two of them on chromosome 7D and 4A were the ones which mapped repeatedly at each of the two experiments under control conditions (Table 2 in Appendix 8). The R^2 for each single QTL under stress condition ranged from 9.75 to 17.55%. The *QPhe.ipk-2B-GS7* (increasing allele from parent A) showed the highest value for LOD (5.75) and R^2 (16.52%).

Weight of three spikes per plant

Mapping of the trait revealed two QTLs, one on chromosome 4A (increasing allele from parent B) with R^2 of 8 % and the other on chromosome 2A (increasing allele from parent A)

with R^2 of 9.8% (Table 2 in Appendix 8). The *QWts.ipk-4A-GC7* showed the highest value for LOD (3.55) and *QWts.ipk-2A-GC7* had the highest value of R^2 (9.8%). Under stress condition four QTLs were mapped with R^2 of 12.1 to 21.6% for single QTL and notably, parent A was the source of alleles for all them. The *QWts.ipk-5A-GS7* showed the highest value for LOD score (7.32) and R^2 (21.6%). The QTL on chromosome 2A was common between the control and stress conditions.

Weight of all spikes per plant

Weight of all spikes per plant was recoded at greenhouse experiment in 2007 under both control and stress conditions. Mapping analysis revealed one QTL on chromosome 7A under control condition with LOD score of 6.95 and R^2 of 8.7% (Table 2 in Appendix 8). Two QTLs were identified under stress conditions on chromosomes 7D and 5A. Interestingly, parent A was the origin of all the increasing under both conditions. The QTL on chromosome 7D had the highest value of both LOD score (8.12) and R^2(19.4%).

Number of fertile spikes per plant

QTL analysis under control condition revealed a QTL on chromosome 7A with R^2 of 9.7% while under stress condition a QTL with R^2 16.3% on chromosomes 7D was detected. For both QTLs increasing alleles originated from parent A (Table 2 in Appendix 8).

Weight of all spikes per plant

Mapping analysis revealed one QTL under control condition on chromosome 7A with R^2 of 8.7%. Two QTLs were found under stress condition on chromosome 7D and 5A with R^2 19.4 and 16.3%, respectively. Under both control and stress conditions the increasing allele originated from parent B (Table 2 in Appendix 8).

Number of unfertile tillers per plant

Under control condition QTL analysis revealed four QTLs on chromosomes 7B, 7D, 2B, and 1B with R^2 from 9.5 to 20% and for all loci increasing alleles came from parent B. The *QNus.ipk-7D-GC7* showed the highest values for both LOD (5.77) and R^2 (20.0%) (Table 2 in Appendix 8).Under stress condition two QTLs (both increasing allele from parent B) were identified. One QTL with R^2 of 10.7% was mapped on chromosome 5A whereas another QTL with R^2 of 8.75% was found on chromosome 7D. The later QTL was detected also under control condition and it showed the highest values for LOD and R^2 under both control and stress condition (Table 2 in Appendix 8).

4 Discussion

4.1 Genetic linkage map construction

A new SSR-based genetic linkage map of bread wheat was constructed using based on 143 F_2 individuals derived from an intraspecific cross between HTRI 11712 × HTRI 105, two winter wheat accessions from Pakistan and Sweden, respectively. The parental lines were analyzed with 666 simple sequence repeat (SSR) primer pairs, including 612 GWM, 44 BARC, and 10 GDM primer pairs. Out of 666 tested SSRs, 25 (3.8%) primer pairs did not show amplification in both parents. From the remaining 641 primer pairs with amplification, 398 SSR primer pairs, on average about 62 percent, revealed polymorphism. Overall and with considering only GWM, because of its majority in the present study, chromosome 2A had the highest number of polymorphic loci while 6D and 4D showed the lowest. However, chromosome 2A, with 35 loci out of 49 tested ones had the ratio 0.71 but was not the most polymorphic chromosome. This result showed that the number of polymorphic microsatellite primer pairs and the ratio of polymorphism were not uniformly distributed among three genomes and seven homoeologous groups. Therefore, the extent of polymorphism exhibited was different across the genome.

The 398 polymorphic primer pairs were divided into three parts. First part contained 273 ones, which were applied for population genotyping. Second part including 14 ones that showed some difficulties in data scoring and were removed form data set. Finally the third part had 111 one which were not applied because of being so close to other loci with no recombination and hence could not be informative but they can be useful later for fine mapping studies with large number of individuals in order to find a closer interval for a given QTL. The 273 polymorphic primer pairs that were applied for population genotyping yielded amplification in 417 loci including 313 polymorphic and 104 monomorphic ones.

Two hundred and ninety three (293) of the polymorphic loci were mapped on 21 linkage groups associated with 19 wheat chromosomes, with a total map length of 2,711 cM, which corresponded to approximately 67% of genome coverage based on the estimation by Sourdille et al., (2003) who suggested that the entire map length of common wheat was about 4,000 cM in the case of an intraspecific population. The average of chromosome length and number of loci per chromosome was 141 cM and 15 cM, respectively. Therefore, there was on average one locus per each 9.2 cM on this map. Chromosomes 6D and 4D failed to have proper maps because of low amount of detected polymorphism. Less loci were mapped on the D genome

(21.2%) compared to those on A (37.5%) or B (41.2%) genomes as is observed in other genetic mapping studies.

Most of the markers maintained their position and order along the linkage group as presented in the reference linkage map (Röder et al., 1998). There were some discrepancies in loci order and mostly observed close to the centromere regions or the regions bearing many loci where it was difficult to order the loci accurately. The order of loci within linkage groups is of great importance for robust QTL detection. However, there is no perfect algorithm or analysis method for ordering loci on linkage maps. Different methods may provide different orders, and most methods may provide multiple alternative orders in a repeated analysis. Therefore, there is always some level of uncertainty associated with any marker order, especially when markers occur in clusters, i.e. large number of markers located within 10 cM distance or less (Sourdille et al., 2004; Lehmensiek et al., 2009). The consistency of marker order and distances with previously published maps is also good evidence for the validity of the population.

Furthermore, 31 loci were map at different positions compared to the ITMI linkage map (Röder et al., 1998). Therefore, they were recorded as extra loci. This could be due to amplification of SSR at multiple loci. Zhang et al. (2008) reported 20 SSR loci, which their chromosomal locations differed from the ones in previously published maps. In the current study, among these 31 extra loci, 19 came from multiple loci but 12 were locus specific. Sixteen extra loci were homoeoloci with the loci on the ITMI map, as were detected on the homoeologous chromosomes and 13 extra loci were detected as non-homoeologous. The remaining two extra loci *Xbarc125-3D.1* and *Xgwm0772-1A.2* were identified in present study as extra loci on the same reported chromosomes on reference maps (Röder et al., 1998b; Ganal and Röder, 2007).

The present study confirmed the single locus specificity for 163 out of the 216 polymorphic primer pairs which were reported as single specific primer pairs for the reference maps. For 53 out of 216, about 25 percent, amplification of more then one locus including mono or polymorphic or from one single locus but an extra one were identified. While in genetic map construction primer pairs with multiple loci by accelerating genotyping task are useful and valuable, there are attractions for single locus primer pairs when researchers are planning to study diversity among wheat accessions and especially performing association mapping for studies because of accurate genotypic data scoring. In bread wheat, Gupta et al. (2002)

classified molecular markers that have been used for mapping into three groups: (1) those having triplicate homoeoloci, one locus each on three chromosomes of a homoeologous group, (2) those having multiple loci, but not on homoeologous chromosomes, and (3) those which are chromosome specific, each with a single locus. If it is assumed that multiple loci in bread wheat should be either homoeoloci or duplicated loci, then the loci for the same microsatellite on non-homoeologous chromosomes may be either due to translocations or duplications between non-homoeologous chromosomes (Gupta et al., 2002).

There were 45 GWM primer pairs which were not located on the ITMI map but were mapped on the present study (Table 3.4). The genetic mapping of the majority of these loci (92%) was in agreement with those of chromosome assignment done by Röder et al. (1998a) using Chinese Spring nulli-tetrasomic lines. Three of them (*Xgwm0058-6B*, *Xgwm0118-5B*, and *Xgwm0144-3B*) were mapped in wheat by Korzun et al. (1997) and their locations confirm the result of the present map, too. Therefore, 42 loci were mapped for first time. There were 31 extra loci in the present map compared to the ITMI (Table 3.5). Eleven of these extra found loci were detected on the same chromosome arm as reported by other genetic mapping studies like *Xgwm0630-2A* (Quarrie et al., 2005), *Xgwm0344-7A* (Singh et al., 2007), *Xgwm0526-2A,* and *Xgwm0071-2D* (Paillard et al., 2003) *Xgwm0608-6B*, *Xgwm0443-5A*, *Xgwm0497-5B*, *Xgwm0133-5B,* and *Xgwm0133-3A* (Somers et al., 2004), *Xgwm0274-5B,* and *Xgwm0443-5A* (Paillard et al., 2003) and (Somers et al., 2004). The detection and mapping of these common loci can be a sign of accuracy of co-linearity of this genetic linkage map to other already published ones. Finally, there were no reports about the other 20 extra loci and these are reported for the first time in the present study. Importantly, in total 76 loci (including 31 extra loci and 45 first time mapped loci) out of 293 mapped loci were considered as new reported loci compared to the ITMI map. Since 14 of them were already reported by others in different maps, it can be said that this map contain 62 loci (including 20 extra loci and 42 first time mapped loci) which were mapped and reported for the first time. However, bearing all 76 new reported loci together in one map is of the advantage of present map compared to others. The newly reported loci increase the coverage and density of existing wheat microsatellite genetic maps.

The described linkage map could be useful to enrich the bread wheat genetic map by specially constructing a consensus map and incorporating the new 62 reported loci on it. This is especially important when different genetic maps are fused to derive a consensus map which

is a significant improvement over single-population genetic maps and provides a new tool for wheat breeding and genomics research. Such a map was developed in wheat by joining four independent genetic maps of bread wheat (Somers et al., 2004) and in barley by joining six independent genetic maps (Varshney et al., 2007). Ideally, all markers should be mapped in the same mapping population. However, the limited polymorphism in a single mapping population has not allowed all possible SSR markers to be mapped onto a single genetic map. An alternative way to prepare a dense SSR genetic map is to combine different and available genetic maps by exploiting common bridging markers and constructing a consensus map that can include various types of molecular markers (Varshney et al., 2007).

Considering the whole set of primer pairs tested with clear amplification (666-25=641) and resulting of about 62% polymorphism (398 out of 641) in parents showed that HTRI 11712 and HTRI 105 were good candidates for the construction of an intraspecific mapping population coming from Sweden and Pakistan, respectively.

Although microsatellites are supposed to be locus specific, several primer pairs amplified more than one fragment and interestingly there were informative primer pairs which showed polymorphism for two genomes and in several cases even for three genomes. Cadalen et al. (1997) reported RFLP polymorphism from an intervarietal cross in bread wheat only from one genome or, more rarely, for two genomes out of three and polymorphic probe/enzyme combination for all three genomes was never detected. Paillard et al. (2003) found a higher level of polymorphism on SSR markers compared to RFLP probes in an intervarietal genetic linkage map of 250 RILs resulting from a cross between two Swiss winter wheat varieties. However, he found nearly the same level of polymorphism (61%) on SSR primer pairs compared to the present study. Twenty nine (16%) of the 179 microsatellites primer pairs in his study revealed two or three polymorphic loci. The maximum number of polymorphic loci revealed by one marker was six for RFLP probes and three for SSR markers which confirm the locus specificity of SSRs compared to RFLP markers. Gao et al. (2004) in developing a consensus map in bread wheat by using of EST-SSR markers, reported less polymorphic loci in an interspecific cross (the ITMI mapping population) compared to the loci in an intraspecific cross. He also reported that 10 out of 88 primer pairs amplified more than one locus, and the highest number of loci was four, which were mapped to the non-homoeologous groups.

Regarding to the occurrence of null alleles simultaneously on both parents, which was found 3.8 percent in this study, Paillard et al. (2003) reported a similar situation in an intervarietal cross in bread wheat demonstrating 7 percent (24 out of the 329) of their tested SSR primer pairs yielding no amplification. Singh et al. (2007) reported the existence of higher amount of null alleles for SSR primer pairs in a diploid A genome species of wheat. They found that out of 306 microsatellite primer pairs tested for polymorphism, 98 (32.0%) were null alleles in *T. monococcum,* 105 (34.3%) in *T. boeoticum,* and 74 (24.2%) did not show any amplification (null) in both parents. This difference can be due to the fact that the microsatellite primer pairs on A genome diploid wheat species, did not have the chance to amplify loci from other B and D genome as in bread wheat. Therefore, the rate of null alleles was higher than in hexaploid wheat like in the present study.

The primer pairs screening showed the existence of 76 loci having null allele out of 398 polymorphic ones. Since part of the loci with null allele were applied for population genotyping, therefore, the genetic data set contained only 43 loci (13.3%) having null allele out of 313 loci and 36 of them were mapped. In an intervarietal bread wheat cross with RFLP markers Cadalen et al. (1997) found higher degree (24%, 72 out of 293) of dominant loci compared to the present study. Paillard et al. (2003) reported that among 396 loci, 105 loci showed dominant inheritance (26.5%) including 54 RFLP loci (28.7%) and 51 SSR loci (24.5%).

The map length spanned 2,711 cM, which corresponded to approximately 67 percent genome coverage based on an estimation (Sourdille et al., 2003) that the entire map length of common hexaploid wheat was about 4,000 cM in case of an intraspecific population. The linkage map generated in the present study has two gaps in linkage groups 7B and 5A. Cadalen et al. (1997) reported a map with 266 RFLP markers that covered 1,772 cM of bread wheat genome and contained gaps on seven linkage groups notably in 3A, 4A, 4B, 5A, 6A, 6D, and 7D. Similar cases were reported in several mapping studies with hexaploid wheat. Gaps on chromosomes 5A, 5B, and 7B were found for the linkage maps of a DH population derived from a cross between two elite common wheat varieties widely grown in China (Zhang et al., 2008). Quarrie et al. (2005) developed a genetic map comprising of 567 markers including SSR, RFLP, and AFLP assigned to 21 linkage groups, giving a total map length of 3,521.7 cM, with an average chromosome length of 168 cM. The map length was divided approximately equally amongst the three genomes: 1,148.0 cM, 1,204.8 cM and 1,168.9 cM

for the A, B and D genomes, respectively. However, the distribution of markers across the genome was not uniform, and there were gaps of more than 40 cM on chromosome 1A, 2D, 3B, 3D, 4A, 5D, and 5B. Paillard et al. (2003) reported six gaps on chromosomes 2D, 3A, 3B, 3D, 5B, and 5D and no or partial coverage of some chromosome arms like 1AL, 4AS, 4BS, 4DL, 4DS, 6BL, and 6DL. Gaps have been reported in intraspecific (Torada et al., 2006) or interspecific (Messmer et al., 1999) crosses used for generating linkage maps in wheat species. Röder et al. (1998b) and Gupta et al. (2002) reported gaps by mapping GWM, WMC primer pairs, respectively, on the ITMI map. The region of gaps can be similar or dissimilar in comparison of different maps. Dissimilar region may occur, because of differences on homozygous regions between pair of parents used as parental lines. However, similar region, because of the lack of SSR marker sources on specific parts of the genome.

Two regions on chromosomes 7BL and 5AL were found to be genetically independent in the current map suggesting the occurrence of a recombination hot spot in the cross between parental lines used in the present study. However, comparing the genetic and physical maps, higher resolution mapping or eventually partial sequencing of these regions will provide definite answers for this suggestion (Sourdille et al., 2004).

Less loci due to the low level of polymorphism were mapped on the D genome (21.5%) compared to those on the A (36.9%) or B (41.6%) genomes. Chao et al. (1989) reported differences in the three chromosomes of homoeologous group 7 of wheat regarding RFLP loci, with the 7B chromosome loci being approximately three times as variable as their homoeoloci on 7A and 7D. Zhang et al. (2008) reported nearly the same situation in a doubled haploid population derived from an intraspecific cross between two elite common wheat varieties widely grown in China. Torada et al. (2006) found less mapped loci also on the D genome from a doubled haploid population derived from an intraspecific cross between ‘Kitamoe’, a Japanese winter wheat cultivar, and ‘Münstertaler’, a line from Switzerland. Paillard et al. (2003) found low level of polymorphism on the D genome compared to A and B in an intervarietal cross. Song et al. (2005), Röder et al. (1998), and Gupta et al. (2002) reported mapping of less BARC, GWM, and WMC primer pairs, respectively, on the D genome of the ITMI population. The low level of polymorphism in the D genome compared to A and B is well known and is in agreement with the hypothesis of a more recent, monophyletic introduction of the D genome into bread wheat (Lagudah et al., 1991). The reasons for the higher level of polymorphism in genome B are yet unclear. However, there are

two non-exclusive possibilities (Chao et al., 1989). One supposes that the B genome is more mutable, the other is that the B genome progenitor species was more variable a priori and that this variability has been maintained through the allopolyploidization events, which have led to the evolution of hexaploid wheat. However, whatever might be the reason for the difference in the levels of polymorphisms, it indicates that it will be easier to construct detailed genetic maps for the wheat B genome chromosomes.

A similar lack of coverage for chromosome 4D as detected here was as observed in some of the populations derived from other intervarietal crosses. Torada et al. (2006) found the minimum number of mapped loci (11) on chromosome 4D. In the maps published by Paillard et al. (2003), Gao et al. (2004), Cadalen et al. (1997), Sourdille et al. (2003) and Groos et al. (2003) chromosome 4D is either missing or only partially covered. Hai et al., (2008) reported a map of doubled haploid lines of bread wheat derived from a cross between two Chinese winter wheat cultivars**,** representing 19 chromosomes of hexaploid wheat, except 4D and 6D due to a lack of polymorphic SSRs.

The number of loci did not distribute uniformly among the A, B, and D genomes and on the seven homoeologous groups. Such predominance of mapped microsatellites on the B and A genomes were also observed in the ITMI map (Röder et al., 1998b; Gupta et al., 2002). Quarrie et al. (Quarrie et al., 2005) reported that the distribution of markers amongst the genomes was not uniform, with twice as many polymorphic markers mapping to the A (224), and B (228) genomes as to the D (115) genome. Paillard et al. (2003) found less mapped loci on the D genome (26%) compared to the A (36%) or B (38%) genomes.

Therefore, attempts have been made by wheat breeders and geneticists to utilize wild germplasem via developing synthetic wheat using different sources for D genome in order to broaden the diversity of D genome in wheat. Huang et al. (2003) reported the highest polymorphism of SSR markers in D genome compared to A and B in a cross between the synthetic wheat W-7984 which developed in CIMMYT and a German winter wheat cultivar. Chu et al. (2008) in a cross between a synthetic wheat and an elite hard red spring wheat found almost the same number of SSR markers in B and D genome which were higher compared to the A genome.

In the present study, 32 out of 313 loci (about 10%) showed segregation distortion. There were three distorted loci close to each other on centromere region in the chromosome 7B (Figure 3.3) which was also reported by Khlestkina et al. (2009) in the same region of a

genetic map constructed from a set of 46 winter wheat single chromosome recombinant lines. Segregation distortion seems a common phenomenon in many populations types as F_2, DH or RILs, with RILs having highest probability of distortions due to continued selfing for 5 to 6 generations (Singh et al., 2007). Xu et al. (1997) compared segregation distortion in 56 populations of different species showing that recombinant inbred populations had significant higher frequencies of distorted markers than those of DH and backcross populations, whereas F_2 populations tended to have lower frequencies of distorted markers and higher variability between individual crosses. Sayed et al. (2002) compared segregation distortion ratios in a DH population and an F_2 population of the same cross between two-row barley varieties. They found considerable differences in the two populations in which segregation distortion in the DH population was with 44.2% of the loci, much higher than in the F_2 population (16.3%). Yu et al (2004) identified about 13 percent of segregation distortion in EST-derived SSR loci on the ITMI population.

Segregation distortions have been reported in all the interspecific and intraspecific crosses used for generating linkage maps in wheat species. From an intraspecific cross in *Ae. tauschii,* the D-genome progenitor of bread wheat, Boyko et al. (1999) reported 24% of AFLP loci (132 out of 546 loci) showing significant deviation ($P < 0.05$) from the expected segregation ratios. On the same segregating population but using 194 co-dominant RFLP markers, Faris et al. (1998) reported 57 loci with segregation distortion phenomenon. Segregation distortion was reported by Cadalen et al. (1997) in 27 percent (81 out of 293 loci) of the RFLP loci in a DH population resulting from anther culture in an intervarietal cross of hexaploid bread wheat. Quarrie et al. (2005) reported several regions of the genetic map of DH population developed from the maize pollination method in an intervarietal cross of hexaploid bread wheat showing significant segregation distortion, these were restricted to only 17 of 567 markers (3.0%) and were distributed among eight regions of the genome. The authors compared their result with Cadalen et al. (1997) and concluded that since both DH populations were generated using chromosome doubling techniques to produce doubled haploids, the much smaller proportion of distorted segregations in his population implies that the maize pollination method has advantages over anther culture. Singh (2007) reported segregation distortion for 58 of 188 loci from 93 RILs developed from a cross of diploid A genome species *T. boeoticum* × *T. monococcum*. Chu et al. (2008) reported that in a doubled haploid population derived from the synthetic hexaploid wheat line 'TA4152-60' and the

North Dakota hard red spring wheat line 'ND495' of 632 SSR and TRAP markers, 94 (14.9%) had segregation distortion and clusters of markers with skewed segregation ratios were observed on several chromosomes.

In the present study, dominant loci showed higher proportion of distortion than co-dominant loci and interestingly, all the dominant distorted loci except *Xgwm0605-5B* were multiple loci. The rate of distorted loci among multiple loci was about 16% compared to 8% in specific loci which indicate double amount of distortion in multiple loci. Messmer et al. (1999) on mapping 176 RFLP and 9 SSR markers in an interspecific cross between a Swiss winter wheat variety (*Triticum aestivum* L.) and the Swiss winter spelt variety (*Triticum spelta* L.) found 84 loci with distorted segregation. They found that these loci were not randomly distributed and clustered on certain chromosome regions, i.e. some chromosome segments of 3A, 5B, and 7A showed an excess of alleles from one parent, while alleles from the other parent were more frequent on chromosomes 1BS, 3B, 3DS, 4A, 4B, 7A, and 7B. However, there was no correlation between the segregation distortion and the mode of inheritance (co-dominant vs dominant). Other studies also reported segregation distortion in intraspecific crosses. Zhang et al. (2008) found segregation distortion at 77 loci (24.4%) from the 315 loci analyzed using 168 DH lines. Paillard et al. (2003) reported 67 (17%) loci with distorted segregation out of the 396 markers including RFLP and SSR markers sharing more or less equals proportion of distortion (16% and 18%, respectively). They found chromosome 2B as the most affected by segregation distortion while in the present study there was no distorted locus on this chromosome. One reason for segregation distortion can be linkage between the loci and sterility genes, due to gametophytic selection or due to physiological and environmental effects. Preferential transmission of one of the alleles can have both positive and negative impacts on interspecific gene transfers (Singh et al., 2007).

In the present study, 20 unlinked loci appeared**.** Song et al. (2005) found that 12 loci out of the 255 polymorphic markers could not be unambiguously positioned on a chromosome. Their assumption was that the corresponding primer pairs amplified homoeologous sites with identical or very similar PCR fragment sizes or amplified cytoplasmic organelle DNA. Paillard et al. (2003) reported 14 unlinked loci in an intervarietal cross of hexaploid bread wheat. Boyko et al. (1999) in an intraspecific cross in *Ae. tauschii,* the D-genome progenitor of bread wheat, reported 12 unlinked loci out of 546 AFLP loci.

In this study around 31 percent of the mapped loci showed 2 or 4 bp polymorphism. These were out of the used primer pairs and if the whole polymorphic primer pairs were considered, this portion would be increased. Somers et al. (2004) in construction of a microsatellite consensus map by joining four independent genetic maps of bread wheat reported allele pairs differing by 4 bp or less between mapping parents average at 38% of the mapped loci, with a tendency of genetically narrow crosses to have a larger fraction of parent allele pairs at 4 bp or less. Their results showed a substantial proportion of the microsatellites (38%) having parent allele pair differences of four bp or less. Thus high resolution polyacrylamide or capillary electrophoresis is essential to make use of the SSR markers in map construction.

Regarding locus specificity of primer pairs, the result from current study is comparable to those reported from the ITMI mapping population (Röder et al., 1998b; Varshney et al., 2000a; Song et al., 2005), and the map developed by Gupta et al. (2002) with more than eighty percent of primer pairs reported as locus specific. High locus specificity could be of value when chromosome specificity is desired like for genetic diversity or association mapping studies. However, some microsatellite markers amplified two or three polymorphic products and, consequently, were mapped to more than one position in the genome. In genetic linkage mapping studies, the amplification of several loci with one primer pair even can be of advantage because it accelerates the genotyping work. Song et al. (2005) found 347 SSR marker loci from 315 BARC microsatellite primer pairs.

Dataset of SSR markers produced here included all monomorphic and polymorphic loci in the Chinese Spring (CS) wheat, the parental lines, and ‘Synthetic’/‘Opata’ the parents of the ITMI mapping populations. This information allowed us to explain the possible reason for some discrepancy about the positions of some loci between the present and the ITMI map and why they were considered as extra loci. The present study provides insight into the variation in allele sizes between mapping parents, which may be extended to other parents and breeding populations. The allele database directs the user towards the expected PCR fragment size to be detected by electrophoresis. Many of the microsatellite primer pairs amplified complex, multilocus profiles, and the allele size become important information in order to know which fragment maps to which locus (Somers et al., 2004).

The availability of plentiful polymorphisms is a benefit for fine-mapping because marker discovery is often rate-limiting at this stage (Borevitz and Chory, 2004; Collard et al., 2005; Lehmensiek et al., 2009). There were bout 100 polymorphic primer pairs which were not used

for linkage map construction and also 45 redundant loci which were not used for mapping analysis. However, they can be useful for further work like fine-mapping.

Nonhomoeologous translocations of 4A/5A, and 4A/7B are well known in wheat. Liu et al. (1992) using previous reports of two reciprocal translocations (Naranjo et al., 1987) via constructing genetic linkage maps, showed evidence for the presence of reciprocal interchromosomal translocations of 4AL/5AL and 4AL/7BS. Devos et al. (1995) explained the structural evolution of the chromosomes in these translocations (Figure 4.1).

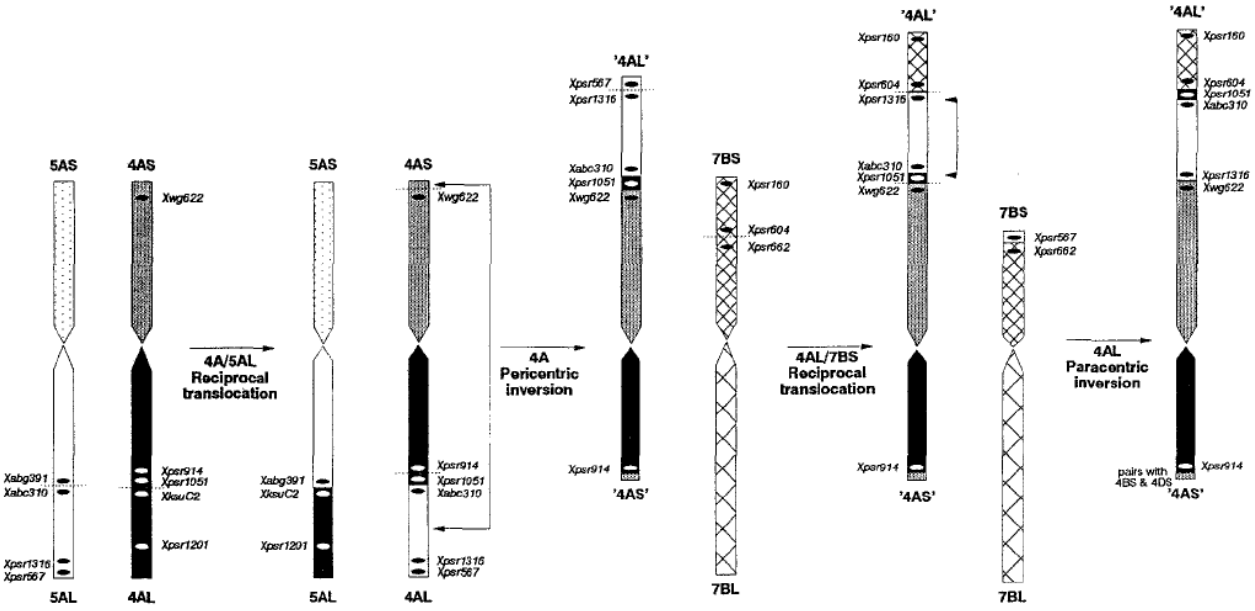

Figure 4.1 The evolution of modern-day chromosome 4A of wheat (Devos et al., 1995)

In the present study SSR markers showed these translocations, too (Figure 3.3). Firstly, markers *Xgwm0834-7A* and *Xgwm0834-7D* were located on homoeologous chromosomes 7AS and 7DS and lacking of locus on chromosome 7BS, at least showed that this region probably belonged to the reciprocal translocation segment. Secondly, the existence of common markers (*Xgwm1258-7A*, *Xgwm1258-4A* and *Xbarc70-7A*, *Xbarc70-4A*) between chromosomes 7AS and 4AL, suggested that the loci on chromosome 4AL were located originally on chromosome 7BS as a homoeologous loci to those on chromosome 7AS and were moved into chromosome 4AL by reciprocal translocation. Thirdly, presence of common markers between chromosome 4BL (*Xgwm0736-4B*) and 5AL (*Xgwm0736-5A*), showed that the locus on 5AL originally was on 4AL as a homoeologous to the locus on chromosome 4BL

and by reciprocal translocation moved into 5AL. Finally, the above mentioned markers all together verified the two already known translocations in the current genetic map.

4.2 Phenotypic evaluations

The parents (parent A = HTRI 11712, parent B = HTRI 105) of the mapping population were originally chosen because of differences in the post-anthesis drought stress tolerance. However, the progeny of the cross were diverse for many other morphological traits (spike color, awn length, and glume hairiness) and agronomic traits (thousand-grain weight, seed size, flowering date, plant height, spike length, number of spikes per plant, and weight and number of seeds per spike). Therefore, the possibility was used to record and analyze some of the above mentioned traits.

All the traits showed approximately normal distribution (Figures 11 to 18 in Appendix 7). It means the distribution of the traits met the assumption analysis of variance and QTL analysis in the present study. Frequency distribution of the traits except number of unfertile tillers per plant under stress condition showed transgressive segregation in both directions. The existence of individuals with higher and lower values compared to the parents, indicated polygenic inheritance with partial gene association (Kearsey and Pooni, 1996). Continuous variation and transgressive segregation are the two obvious characters of multiple genes inheritance (Poehlman and Sleper, 1995). However, the continuous distribution of a quantitative trait does not exclude the possibility that only one gene is involved in any particular instance but simply imply that, if it is so controlled, then the phenotypic differences among the genotypes at that locus are small relative to variation caused by non-genetical, or environmental influences (Kearsey and Pooni, 1996).

In general, the range of variation was higher under the stressed compared to the control condition. Descriptive statistics of the traits that were recorded under both control and stress conditions clearly showed the effect of drought stress by reducing the means that resulted in increasing the CVs. In other words, more fluctuations or higher variation of traits were found under stress conditions. Higher variation of drought related traits such as Relative Water Content and leaf osmotic potential were reported by Teulat et al. (2001) in RILs population of barley grown in a growth-chamber under two water regimes.

Regarding correlation between traits within experiments, seed related traits showed higher correlations to each other compared to other traits whenever they were measured. Considering

only the seed related traits under both control and stress conditions, there were high correlations between the traits except between seed length and seed width. However, seed length and seed width showed higher correlation under stress conditions. In all the four experiments seed length showed the lowest correlation to thousand-grain weight under both control and stress condition. However, the correlation was higher under stress condition.

Breseghello and Sorrels (2007) in a study of kernel morphology in two hexaploid wheat mapping populations found low correlation between kernel length and kernel width in both populations (r = 0.30 and 0.22), giving an indication that these traits are controlled independently.

Generally, thousand-grain weight showed higher correlation with seed area and seed width compared to seed length under both control and stress conditions except under stress condition at greenhouse experiment in 2007, where there was also high correlation between thousand-grain weight and seed length. The exception may be because of different methods which were used to impose the post-anthesis drought stress. Sun et al. (2009) found higher correlation between thousand-grain weight and seed width compared to seed length in the analysis of grain shape and weight in common winter wheat using a set of 131 recombinant inbred lines.

Weight of seeds per spike and number of seeds per spike which were recorded at three experiments showed high correlations with each other under both control and stress conditions. Weight of seeds per spike and number of seeds per spike showed low positive and negative correlations, respectively, with thousand-grain weight under control condition at three experiments where they were recorded. Hai et al. (2008) found the same relationship among these traits in a winter wheat mapping population consisting of 113 DH lines based on means of two years field trails.

QTLs can be detected only if the parents carry different alleles. Therefore, the first stage in QTL analysis is to determine if there are indeed significant differences between the progeny lines. This is done by carrying out an analysis of variance (Brown and Caligari, 2008). In the present study, analysis of variance showed significant differences between $F_{2:3}$ families under both control and stress conditions which justified the following QTL analysis.

The interaction between genotype and location were not significant for the assayed traits. However, year (location) showed highly significant differences for all the traits under both conditions. Under both control and stress conditions, location was significant only for

thousand grain weight. This shows the importance of years compared to the location. Therefore, further evaluation of this mapping population should be planed on different years instead of different locations.

The Pearson coefficient of correlation for the same trait between pairs of experiments (Table 3.15) considering the mean values) which were calculated as a rough estimates of the heritability showed that the correlations were not equal under control and stress conditions and they were usually higher under control conditions. Under both control and stress conditions, seed length had the highest correlation whereas thousand-grain weight showed the lowest. However, while seed area had higher correlation than seed width under control condition, the situation was reverse under stress condition and seed width showed more correlation compared to seed area.

Heritability based on analysis of variance for all the seed related traits were higher under control compared to the stress condition. Lower heritabilities of traits under stressed condition was reported by Quarrie et al. (2005). Under both control and stress conditions, seed length had the highest heritability and thousand-grain weight showed the lowest. Similarly Sun et al. (2009) found higher heritability of seed length compared to seed width and thousand-grain weight in the analysis of grain shape and weight in common winter wheat using a set of 131 recombinant inbred lines. Interestingly, under both control and stress conditions there were high comparabilities between the heritabilities that were calculated based on correlation of the same trait between pairs of experiments and the heritabilities based on analysis of variance.

Quantitatively inherited characters differ in heritability. A character such as yield that is greatly influenced by the environment has a low heritability. Characters not greatly influenced by environment usually have high heritability. This may influence the choice of selection procedure used by plant breeders (Poehlman and Sleper, 1995).

The comparison of the coefficient of variation of the experiment under control with stress condition shows that the analysis of variance for all the seed related traits were conducted more precisely under control condition than those under stress condition. Furthermore, the experiment for seed length had the highest accuracy followed by seed width, seed area, and thousand-grain weight under both control and stress conditions. These accuracies were comparable with the amount of the observed heritability of the traits of which with increasing the heritability of trait the coefficient of variation of the experiments reduced. Appropriate

experimental design should be applied for traits with low heritability in order to reduce error variance and to increase the accuracy of the experiment for the given traits.

Regarding the correlation of the same traits between stress and control condition, and considering only seed related traits, thousand-grain weight showed the lowest and seed length the highest correlations, respectively. This is in agreement with higher heritability of seed length and shows the stability of seed length compared to other traits between conditions.

Since the performance of a trait in two environments is regarded as two different characters which are genetically correlated (Falconer, 1952) the measurements of the traits with significant effect from drought, under control and stress condition were considered as records of correlated traits and not considered as records from the same traits. Although the heritabilities of seed related traits were higher under control condition compared to stress condition, they were not great enough to justify favoring selection under control condition instead of selection under stress condition. Therefore, any improvement of these traits should be conducted in primary environment, stress condition, and the breeding via correlated response could not be useful.

4.3 QTL analysis

Under both control and stress conditions QTL analysis showed significant results for all traits in the present study. Therefore, the results demonstrated the utility of our maps for identifying QTLs associated with agronomic traits. QTL analysis also revealed QTL per trait per each measurement under both control and stress conditions except for the measurement of thousand-grain weight under stress condition at greenhouse experiment in 2004. QTLs may not be detected in all experiments. The main reasons are the interaction between genotypes and environments, and the experimental error (Börner et al., 2002). The existence of QTLs for all measured traits in the present study, confirmed the value of parents, and the resulted segregating population.

QTL analysis revealed significant results on 15 out of 19 constructed linkage groups and, however, the analysis failed to find significant regions on linkage groups representing chromosomes 3B, 3D, 6A, and 6B. In a QTL analysis of six yield related traits Hai et al. (2008) analyzed 19 linkage groups of a winter wheat mapping population (lack of chromosomes 4D and 6D) and reported 30 putative QTLs that distributed over all chromosomes except 3A, 3B, 5A, and 6B. Li et al. (2007) in a whole genome QTL analysis

using recombinant inbred lines derived from a cross between Chinese winter wheat varieties, mapped 46 QTLs for tiller and spike characters on 12 chromosomes, lacking of significant results for chromosomes 1B, 2B, 3A, 3D, 4D, 5A, 5B, 6D, and 7B. Sourdille et al. (2003) on a whole genome QTL analysis for several agronomic traits mapped 46 QTLs on all chromosomes except on chromosomes 1D, 3A, and 3D.

QTL analysis revealed that the parents contributed nearly equally the increasing alleles for the most measured traits. Therefore, from this population it will be possible to find theoretically individuals bearing all the increasing alleles, and individuals with all the decreasing alleles for the studied traits. This constitution of alleles at their loci results in contrasting lines that is important for further genetics or QTL analysis. These types of contrasting individuals can be used as parents of a new mapping population in order to study the given traits. This type of individuals will also be very important in wheat improvement if they have other favorable traits and allow selecting individuals bearing favorable alleles for the traits of interest.

QTL analysis of nine traits that were recorded at more than one experiment under control conditions showed all together 58 QTLs of which 20 were mapped repeatedly in the present study. Regard to the four seed related traits under control conditions, 29 QTLs were identified in which 11 of them were detected repeatedly in two or more experiments. Eight of these repeated QTLs (*QTgw.ipk-1B.1, QTgw.ipk-4B.1, QTgw.ipk-4B.2, QSea.ipk-5B, QSea.ipk-1B.2, QSew.ipk-4B.1, QSea.ipk-4B.2,* and *QSel.ipk-2A.1*) were identified in two environments, two (*QSea.ipk-4B.2*, *QSel.ipk-4B*) in three environments and one *(QSel.ipk-5B*) in all four environments. Under stress condition, the four seed related traits were recorded in more than one experiment and showed all together 28 QTLs of which five were identified repeatedly in other experiments of the present study. These five QTLs belonged to thousand-grain weight (*QTgw.ipk-7A* and *QTgw.ipk-7D*) and seed length (*QSel.ipk-5B, QSel.ipk-5B*.2, and *QSea.ipk-4B*). Sun et al. (Sun et al., 2009) in a QTL analysis of grain shape and weight in common wheat in four different environments, identified 20 QTLs of which six were detected in two, one in three, and one in all four environments. Hai et al. (2008) reported the consistency of 46 QTLs mapped in four experiments of which 18 were detected in two or three, but none in all the four environments.

Agronomic traits are among the most important however, least understood traits of wheat. Understanding the genetic control of these traits is crucial for the sustained improvement of wheat (McCartney et al., 2005).

Inheritance of awnedness in wheat has been well studied since the beginning of the twentieth century. As well known in barley, awns may play a major role in the elaboration of the yield in wheat, especially under terminal drought condition (Sourdille et al., 2002). The genetic control of this trait was generally found to be simple, and three dominant inhibitor genes *Hd* (Hooded), *B1,* and *B2* (tipped 1 and 2) are involved in the differences between awned and awnless cultivars. The *Hd* is located on the short arm of chromosome 4A; *B1* and *B2* on the long arm of chromosomes 5A and 6B, respectively (Sears, 1954). Awnedness with two records at field experiment in 2005 and greenhouse experiment in 2007 showed one QTL on chromosome 5AL in 2005 and two QTLs on chromosomes 4AS and 5AL in 2007. Interestingly, both parents contributed the increasing alleles for this trait, however, QTL on chromosome 5AL showed much stronger additive effect, explained phenotypic variance (R^2) value, and LOD score compared to the QTL on chromosome 4AS. One explanation for failure of detection of QTL on chromosome 4AS in 2005 could be due to the way of data scoring for this trait in $F_{2:3}$ families in which while 12 single plants were recorded in 2007, the over all view of plants in each plot was recorded at field experiment in 2005. Araki et al. (1999) mapped the *Hd* locus on chromosome 4AS in a genetic analysis of chromosome 4A using 98 single-chromosome recombinant substitution lines. Sourdille et al. (2002) mapped the *Hd* locus on chromosome 4AS and *B2* locus on chromosome 6BL using a doubled haploid line population derived from the cross between the cultivars 'Courtot' (awned) and Chinese Spring (awnless).

The two detected QTLs on chromosomes 4AS and 5AL correspond to the *Hd* locus and *B1* locus. Lack of QTL on chromosome 6B suggests that both parents have the same allelic constitution at *B2* locus. So the two QTLs can explain the observation of transgressive segregation in the $F_{2:3}$ families in the direction of parent HTRI 11712 which bears awns in medium size while there were some individuals bearing longer awns compared to parent HTRI 11712 (Figure 1 in Appendix 6).

Cereal plants were among the first to be used for the study of the physiology and genetics of **flowering time**. Understanding of the genetics of the flowering process in cereals is important because of the effects of flowering in adaptation and optimizing the yield. A flowering time inappropriate for the local environment may subject a crop at critical growth stages to the influences of extreme weather conditions such as frost, drought or heat stress, and significantly reducing the yield potential (Law and Worland, 1997; Kuchel et al., 2006).

Three genetic systems control flowering time in wheat namely vernalization sensitivity, photoperiod sensitivity, and developmental rate or earliness *per se*. The first two systems response differentially to different lengths of cold treatment and photoperiod, respectively, while the third affect life cycle timing independent of environmental signals (Snape et al., 2001).

A summary of previous studies (Law and Worland, 1997; Snape et al., 2001) particularly in the hexaploid bread wheat, which derived from chromosome assays and the analysis of aneuploids and substitution lines, has shown that long arms of chromosomes homoeologous group 5 contain three major genes controlling vernalization sensitivity (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*) whereas short arms of chromosomes homoeologous group 2 contain major genes that control photoperiod sensitivity (*Ppd-A1*, *Ppd-B1* and *Ppd-D1*) and loci of earliness *per se* are generally identified as QTLs rather than as major genes and they would be expected to be present on a number of wheat chromosomes.

QTL analysis for flowering time or heading date in wheat were reported in several populations (Araki et al., 1999; Shah et al., 1999; Sourdille et al., 2000b; Börner et al., 2002; Li et al., 2002; Campbell et al., 2003; Gervais et al., 2003; Hanocq et al., 2004; Paillard et al., 2004; McCartney et al., 2005; Xu et al., 2005; Kuchel et al., 2006; Narasimhamoorthy et al., 2006; Chu et al., 2008; Lin et al., 2008; Pankova et al., 2008; Griffiths et al., 2009) and QTLs were identified on all of the wheat chromosomes, except chromosome 6B.

In the present study, days to flowering were analyzed under two field experiments in 2004 and 2005. On both years parent B flowered about five days later than parent A. However, their progenies showed a range of 12 days in both years. The trait was normally distributed in both years suggesting that they were under polygenic control and could be considered as quantitative trait. The presence of numerous transgressive segregants indicated that alleles shortening and lengthening the flowering time are dispersed between the parents.

QTL analysis revealed eight QTLs on chromosomes 1B, 2D, 3A, 5A (two QTLs on 5AL), 5D, 7D, and 7B and all of them, except QTLs on chromosome 5AL (*QDtf.ipk-5A.2*) and 7DS (*QDtf.ipk-7D*) were reported earlier. As expected, the allele from parent B, later in flowering, had a major effect on days to flowering over the two years field experiments.

The previous studies which reported QTLs similar to the six mapped QTLs in the current work are as following: QTLs on long arm of chromosome 1B (*QDtf.ipk-1B*) (Lin et al., 2008)(Griffiths et al., 2009), on short arm of chromosome 2D (*QDtf.ipk-2D*) (Sourdille et al.,

2000a; Börner et al., 2002; Li et al., 2002; Gervais et al., 2003; Huang et al., 2003; Hanocq et al., 2004; Xu et al., 2005; Narasimhamoorthy et al., 2006; Liu et al., 2007; Kordenaeej et al., 2008; Lin et al., 2008), on long arm of chromosome 3A (*QDtf.ipk-3A*) (Börner et al., 2002)(Griffiths et al., 2009), on long arm of chromosome 5D (*QDtf.ipk-5D*) (Börner et al., 2002), on long arm near to centromere region of chromosome 5A (*QDtf.ipk-5A.1*) (Kato et al., 1999; Huang et al., 2003; Hanocq et al., 2004; Kuchel et al., 2006; Chu et al., 2008), and short arm of chromosome 7B (*QDtf.ipk-7B*)(Huang et al., 2003; Kuchel et al., 2006; Lin et al., 2008; Khlestkina et al., 2009).

The mapped QTL on chromosome 2DS (*QDtf.ipk-2D*) probably reflect photoperiod response and QTLs on chromosomes 5DL (*QDtf.ipk-5D*) and 5AL (*QDtf.ipk-5A.1*) are corresponding to vernalization response (Snape et al., 2001). No QTL was detected close to the photoperiod sensitivity genes *Ppd-A1*, and *Ppd-B1* on chromosomes 2A and 2B, respectively, and also for the vernalization sensitivity gene (*Vrn-B1*) on chromosome 5B, suggesting that the parents have the same alleles at these loci.

For the QTL on chromosome 7DS near to centromere region, however, Börner et al. (2002) with applying the ITMI mapping population and phenotyping for both ear emergence time and days to flowering, reported only a QTL for ear emergence time distally on chromosome 7DS and did not find any QTL for days to flowering on chromosome 7D. QTL on distal part of chromosome 7DS was also mapped in another spring wheat mapping population (McCartney et al., 2005). Therefore, the QTL on short arm of chromosome 7D (*QDtf.ipk.7D*) near to centromere region has not been detected and interestingly this was the only QTL for this trait which was consistent across the years (increasing allele from parent B) and explained on average 21% of the variability and with mean additive value of 1.35.

Lin et al. (2008) suggested the existence of two loci associated with heading date or flowering time on chromosome 7BS. Khlestkina et al. (2009) mapped both vernalization and photoperiod response genes on chromosomes 7BS of two different mapping populations sharing common markers (Figure 4.2).

They showed while photoperiodic response gene located near to the end of chromosome 7BS, the vernalization gene was located more close to centromere similar to the mapped QTL on homoeologous chromosome 7DS in the present study, the region far from 7BS/4AL translocation segment (Figure 4.1). Therefore, the QTL on 7DS (*QDtf.ipk-7D*) probably is for vernalization response. If it is so, then this is the first report of QTL for vernalization response

(*Vrn*) on chromosome 7DS. However, special experiment needed to uncover the type of response.

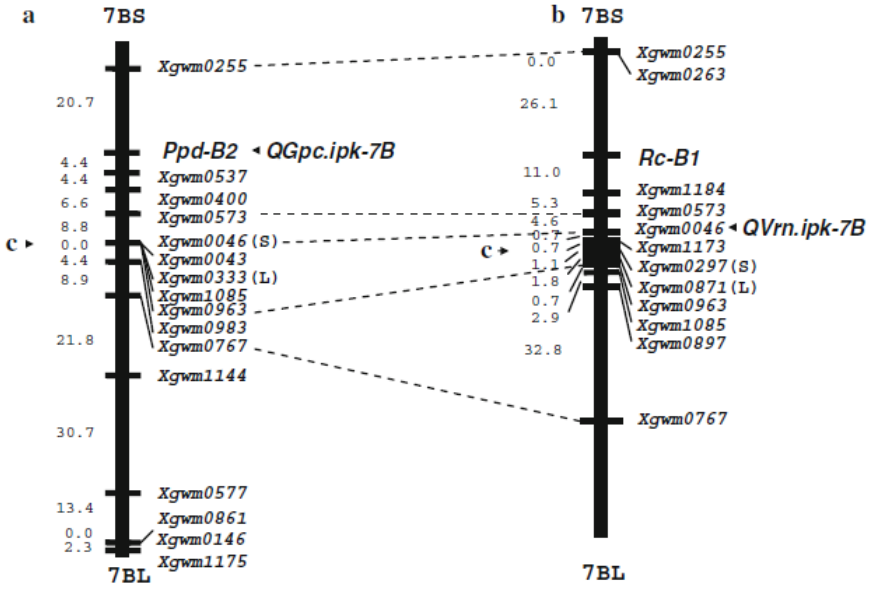

Figure 4.2 Genetic maps of chromosome 7B from two different population showing loci of vernalization and photoperiod response gene. After Khlestkina et al. (Khlestkina et al., 2009)

Two QTLs (*QDtf.ipk-5A.1* and *QDtf.ipk-5A.2*) in coupling were identified on chromosome 5AL from field experiment in 2004 (increasing allele for both from parent B). *QDtf.ipk-5A.2* was mapped by overall mean of data, too. It co-localized with QTL for awnedness on distal part of the long arm of chromosome 5A, on the *B1* locus. Kato et al. (1999) using recombinant substitution lines derived from a cross between Chinese Spring (Cappelle-Desprez 5A)[A substitution lines for chromosome 5A from winter wheat cultivar 'Cappelle-Desprez' into a 'Chinese Spring' background] and Chinese Spring (*T. spelta* 5A)[A substitution lines for chromosome 5A from a spring accession of *T. spelta* into a 'Chinese Spring' background] mapped two QTLs with large effects in coupling on 5AL associated with ear emergence time. They mapped one of them at the *vrn-A1* locus and the other near to *Q* locus (a major gene for spike morphology (Galiba et al., 1995)).

The QTL which were mapped distally on the long arm of chromosome 5A (*QDtf.ipk-5A.2*), in the same region as the *B1* locus has not been yet mapped. However, Snape et al.(2001) suggested the existence of loci for vernalization on the long arm of chromosomes 4B, 4D, and 5A (because of the 4AL/5AL translocation) due to the synteny with locus *Vrn-H2* on

chromosome 4H of barley and also the work of (Dubcovsky et al., 1998) who mapped two loci *Vrn-A*^{m}*1* and *Vrn-A*^{m}*2* on the proximal and distal regions of chromosome 5A^{m} of *T. monococcum*, respectively (Figure 4.3) suggesting the existence of polymorphisms for *Vrn-2* series loci in wheat, the orthologous of *Vrn-H2* from barley (Dubcovsky et al., 1998).

Because of the 4AL/5AL translocation, *Vrn-A1* and *Vrn-A2* are located on the same chromosome arm (Figure 4.3) but belong to different homoeologous groups. *Vrn-A1* is proximal to the 5A.L/4A.L translocation break point in the region that is homoeologous to group-5 chromosomes, whereas *Vrn-A2* is distal to this translocation break point in a region that is homoeologous to group-4 chromosomes in other wheat and Triticeae genomes (Dubcovsky et al., 1998).

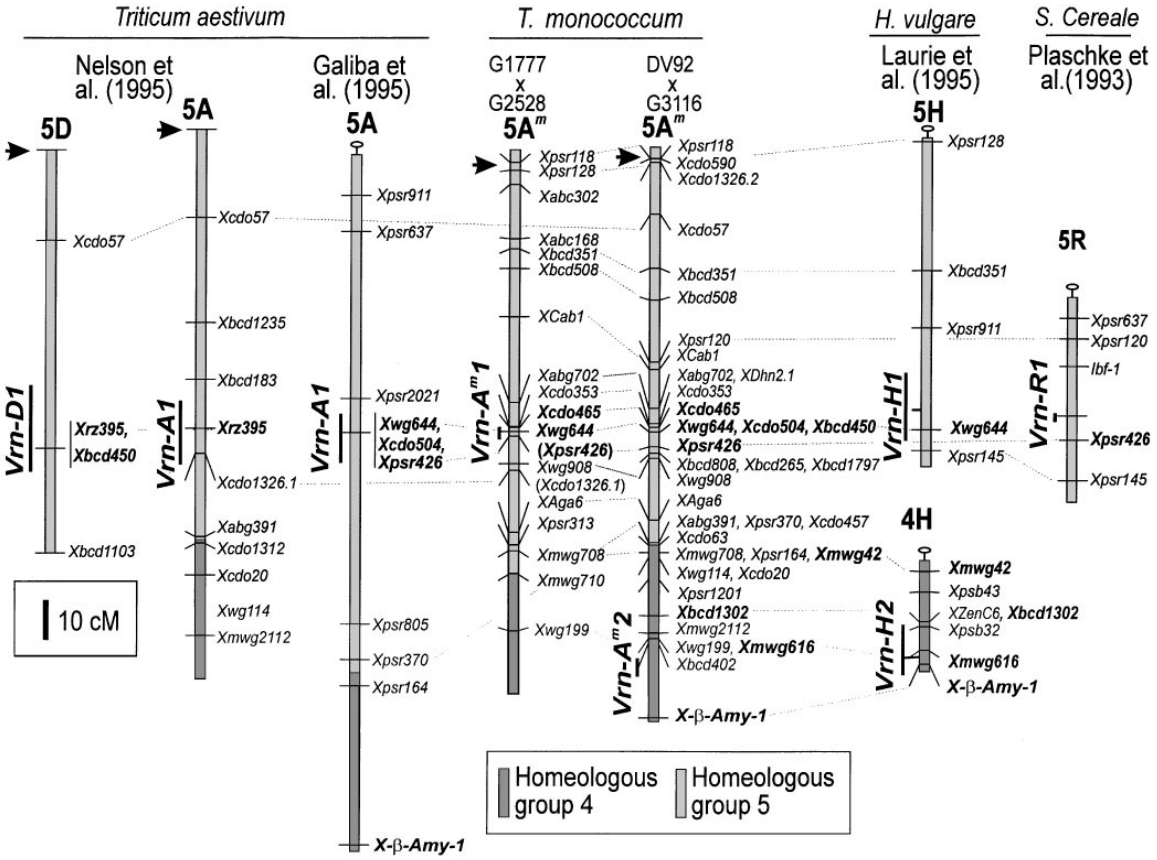

Figure 4.3 Comparative RFLP maps of *T. monococcum* chromosome arms 5AL and homoeologous chromosome regions in other Triticeae species (Dubcovsky et al., 1998).

Griffiths et al. (2009) conducted a meta-QTL analysis to identify genes controlling ear emergence time in four doubled haploid populations. They found 19 meta-QTLs including two on chromosome 5A (above part in Figure 4.4). Their map of chromosome 5A contains three SSR markers in common to the map in the present study: *Xgwm0156-5A* (near to

centromere), *Xgwm0126-5A* (the distal part of long arm), and *Xgwm0291-5A* (the most distal part of 5AL). These three common loci were connected between the two maps via lines Figure 4.4. As the map shows (Figure 4.4) they found meta-QTLs close to the two former SSR markers but not to the later one (*Xgwm0291-5A*).

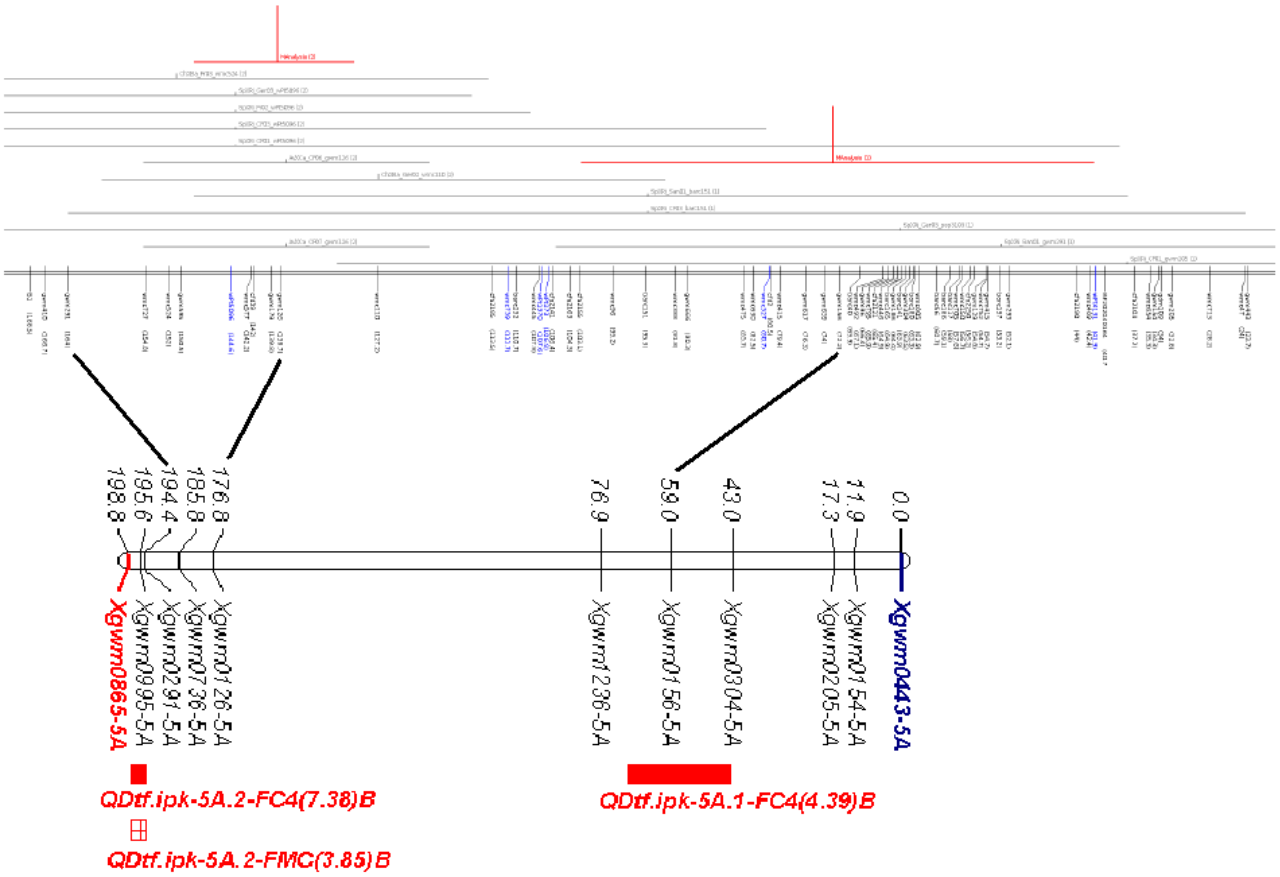

Figure 4.4 Chromosomal location of QTLs for flowering time in the present study (bottom) and two meta-QTLs for heading date (above) identified on chromosome 5A by Griffiths et al. (2009)

The QTL (*QDtf.ipk-5A.2*) in the present study mapped in a narrow interval of 4 cM between *Xgwm0995-5A* and *Xgwm8651-5A* with a peak at the most distal part of the chromosome. In the present study, however, no QTL was mapped on chromosomes 4B (4D was failed to have a linkage group) probably because of no polymorphism at this locus. On the long arm of chromosome 5A one QTL was mapped by (Chu et al., 2008) and two QTLs by Kato et al. (1999) but none of them were located on distal part of chromosome where the *B1* locus was mapped, too (Figure 3.8). Therefore, this locus (*QDtf.ipk-5A.2*) was mapped genetically for

the first time in the present study is most likely the one that was reported by Dubcovsky et al. (1998) but not mapped yet in bread wheat.

Kuchel et al. (2006) were the first to map a photoperiod-sensitive gene(s) homoeologous to *Ppd-H2* by QTL analysis on the long arm of chromosome 1A in wheat using a doubled haploid population. However, they did not find any evidence of further QTL associated with ear emergence time on chromosomes 1B and 1D in this population. Interestingly, one QTL in the present study was identified on chromosome 1BL which support the idea of existence of photoperiod-sensitive gene(s) on long arms of the homoeologous group1 chromosomes suggested by Kuchel et al. (2006).

Plant height seems to be affected by many genetic factors which regulate development, morphology and vigour. The dwarfing genes of wheat are classified according to their response to exogenously applied gibberellic acid into two groups, insensitive and sensitive (Gale et al., 1975). Plant breeders have paid considerable attention to plant height in order to achieve the best compromise between an adequate lodging resistance and acceptable yield levels. A reduction in plant height is usually obtained by introducing specific dwarfing genes into the genotype (Cadalen et al., 1998). The semi-dwarf genotypes became particularly important with the use of chemical fertilizers, since they are able to respond to fertilizer application without lodging (Worland et al., 1994). Furthermore, positive pleiotropic effects of gibberellic acid insensitive dwarfing genes were demonstrated on the increased number of grains per spike which result in higher yields (Börner et al., 1993). Therefore, it is important to evaluate new sources of dwarfism that may be of potential use to breeders (Worland et al., 1994). The *Rht-B1b* and *Rht-D1b* alleles, have been the most widely used as dwarfing genes in plant breeding schemes for the last 50 years (Cadalen et al., 1998). The two *Rht-B1* and *Rht-D1* loci were first mapped genetically in 1997 by Börner et al. (1997) using three F_2 mapping populations on short arms of chromosomes 4B and 4D, respectively. In addition to semi-dwarfing *Rht* genes, allelic variation at other loci associated with height reductions could have breeding potential for lodging resistance and yield increase (Kato et al., 1999).

QTLs for plant height were reported for all wheat chromosomes (Cadalen et al., 1998; Araki et al., 1999; Kato et al., 1999; Shah et al., 1999; Börner et al., 2002; Campbell et al., 2003; Gervais et al., 2003; Huang et al., 2003; McCartney et al., 2005; Spielmeyer et al., 2007; Chu et al., 2008; Hai et al., 2008; Kordenaeej et al., 2008; Röder et al., 2008).

In the present study, plant height of parents and $F_{2:3}$ families were recorded at field experiment in 2005 and greenhouse experiment in 2007. In both cases parent B was a little taller than parent A (Figure 5 in Appendix 6). The height of plants was higher under field conditions compared to greenhouse experiment with a mean value of 146.14 cm and 110.02 cm, respectively. However, they showed nearly the same amount of range and standard deviations (Table 3.10 and 3.12).

Plant height was also recorded under stress condition which showed the effect of post anthesis drought stress in reduction of plant height of about eight cm and also decreasing the amount of variation of the trait (Table 3.12). Although plant height was reduced due to the applied post-anthesis stress, this trait showed, however, the least amount of reduction compared to other measured traits.

From the five mapped QTLs under field experiment, three were also mapped in greenhouse. Four QTLs were identified under stress of which all were also mapped under control conditions as well. There was only one QTL that was mapped in greenhouse experiment under both conditions but not in field. The probable reason can be the way of data recording in greenhouse, (six single plants under each control and stress condition), compared to the field (random selection of ten plants per plot).

Seven QTLs for plant height were mapped in the present study and both parents contributed the increasing alleles. All the identified QTLs except QTL on chromosome 5B were reported earlier: QTL on 7B (Huang et al., 2003; McCartney et al., 2005), 7D (Röder et al., 2008), 4B (Huang et al., 2003; McCartney et al., 2005), 4A (Araki et al., 1999; Börner et al., 2002; Gervais et al., 2003), and 2B (Huang et al., 2003; Kordenaeej et al., 2008).

The QTL on chromosome 5B had the highest amount of LOD score (9.14) and additive effect (-5.576) with increasing allele from parent A. QTLs for plant height were reported on chromosomes 5B (Cadalen et al., 1998; McCartney et al., 2005) however they were mapped in different regions compared to the mapped QTL in present study. Therefore, the QTL (*QPhe.ipk-5B*) is reported for the first time here.

Spike is probably the part of the wheat plant which differ most in shape, length, width, and density among cultivars. Variation in spike morphology is one of the most widely used criterion for species determination and is extensively investigated. Spike morphology is known to be influenced by three major genes: *Q*, *C* and *S1*. These genes are located on the long arm of chromosomes 5A, chromosome 2D, and near to centromere of the short arm of

chromosome 3D, respectively (Sourdille et al., 2000a). There is no evidence that allelic variation at these three loci exists within *T. aestivum* (Sourdille et al., 2000b).

QTLs for spike length were reported on chromosomes 1AL, 2DS, 2BS, 4AS, and 5AL (Sourdille et al., 2000b), on chromosomes 1AL, 1BS, 4AL, 7BL, and 7AL (Li et al., 2002), on chromosomes 3DS, 4AL, and 5AL (Chu et al., 2008), on chromosomes 1B, 2D, 4A, 5A, 5D, 6A, 6B, 6D, and 7A (Börner et al., 2002), and on chromosome 7D (Röder et al., 2008).

Spike length of parent B was a little bit longer compared to parent A at all measurements of trait. While parents differ less than two cm in spike length, the $F_{2:3}$ families show a range of 7 and 4.5 cm at field and greenhouse experiments (Figure 2 in Appendix 6), respectively. Spike length at field experiment was longer compared to greenhouse with mean value of 14.56 and 10.32, respectively. The trait showed slightly more variation at field (standard deviation of 1.20) than greenhouse (standard deviation of 0.81) (Table 3.12). Not surprising, the post-anthesis drought stress did not show any effect on spike length. Five QTLs were mapped under control conditions on chromosomes 7D, 5B, 4A, 3A, and 2D and two of them were identified on chromosomes 7D and 3A under stress condition, too.

Three out of the five mapped QTLs in the present study were reported earlier in similar regions of chromosomes 2DS (Sourdille et al., 2000a; Sourdille et al., 2003), 4AS (Börner et al., 2002; Sourdille et al., 2003), and 7D (Röder et al., 2008). Therefore two QTLs for spike length were identified for the first time in the present study on chromosomes 5B (*QSpl.ipk-5B*) and 3A (*QSpl.ipk-3A*).

The QTL on chromosome 7D (increasing allele from parent B) was mapped at all the three trait measurements and also by mean of data from control conditions whereas QTL on chromosome 5B was identified only under control conditions. Interestingly, both QTLs were mapped on the same location as the QTLs for plant height and, more interestingly, with the same origin of increasing alleles. Based on phenotypic evaluation, there were positive coefficient of correlations between these two traits (0.42 at field and 0.26 at greenhouse experiment) (Table 3.11 and 3.13). Therefore, the two common QTLs can explain the positive correlations.

Number of seeds per spike is one of the wheat yield components and has high positive correlation with weight of seeds per spike as demonstrated in the present study both under control and stress conditions (Table 3.11 and 3.13). Parent A had ten more seeds per spike compared to parent B under field experiment in 2005 whereas in greenhouse experiment

parents had nearly the same number of seeds. The $F_{2:3}$ families showed higher range, mean and standard deviation in the field compared to greenhouse experiment (Table 3.10 and 3.12). QTLs for number of seeds per spike were reported on chromosomes 1A, 2B, 2D, 5D, 7A, and 7D (Hai et al., 2008), 1AS, 1BL, 2BS, 4AL, 4AS, 4BC, 4BL, 5BS/L, 5DS, 5DL, 5AL, 7BS, 7BL and 7AL (Quarrie et al., 2005), 1D, 2AC, 3B, 6B and 6A (Li et al., 2007), on chromosome 3A (Campbell et al., 2003), 1AC, 2DS, 3A, 4AL, 6DS, 7DL and 7AL (Börner et al., 2002), 3B, 4A, 5B and 7A (Kordenaeej et al., 2008), and 3DS (Narasimhamoorthy et al., 2006).

QTL for spikelet number per ear as a correlated trait with number of seeds per spike were mapped on chromosomes 5AS (Kato et al., 2000), 2A, 5D, 6B, and 7DL (Li et al., 2007), and 4DL (Chu et al., 2008). The previous work showed the presence of genes responsible for the traits number of seeds per spike and spikelet number per ear on most of wheat chromosomes except chromosomes 4D, 5A, and 6D which confirm the polygenic inheritance of the traits.

In the present study under control conditions five QTLs on chromosomes 7AS, 7DS, 5BL, 4BC, and 2AC were identified for number of seeds per spike and all were mapped by means of data, too. Parent B was the origin of increasing allele only for one QTL (*QNsp.ipk-7D*). Under stress condition QTLs on chromosomes 5B, and 2A plus a new QTL on chromosome 5AC were mapped. Two out of the six mapped QTLs were revealed by previous studies in similar regions of chromosomes 2A (Li et al., 2007), 4BC (Quarrie et al., 2005), and 7AS (Kordenaeej et al., 2008). A QTL on chromosome 5B near to centromere region was reported (Quarrie et al., 2005). However, the existence of a SSR marker (*Xbarc74-5B*) in both maps clearly showed the distance between the two QTLs and confirmed the QTL in the present study as an extra and new QTL on chromosome 5BL. Therefore, three QTLs on chromosomes 5B (*QNsp.ipk-5B*), 5A (*QNsp.ipk-5A*), and 7D (*QNsp.ipk-7D*) are reported for the first time in the present study. The result shows the importance of homoeologous chromosomes groups 5 and 7 on this trait.

Not surprisingly, **weight of seeds per spike**, the product of the two yield components, grain number per spike and thousand-grain weight, in the present study showed a pattern of variation similar to the number of seeds per spike. Three and seven QTLs were mapped under control and stress conditions, respectively. There were two QTLs on chromosomes 2A and 5B (both increasing alleles from parent A) which revealed for number and weight of seeds per spike under both control and stress conditions. The two common and consistence QTLs can

explain the high correlation between the two traits. However, each trait showed some independent QTLs not only on different chromosomes but also in similar regions such as short arm of chromosome 7A containing QTLs for number of seeds per spike (*QNsp.ipk-7A*) under control condition (increasing allele from parent A) and weight of seeds per spike (*QWsp.ipk-7A*) under stress condition (increasing allele from parent B). Mapping of these two QTLs with different sources of additive effect show the presence of two loci in a similar region of chromosome 7AS for the two correlated trait.

On chromosomes 7AS and 7DS, the QTLs for weight of seeds per spike under stress condition co-located with QTLs for thousand-grain weight under stress condition with the same origin for the increasing allele on each location. Therefore, it may be one QTL per each region and related to both traits.

Two QTLs (*QWsp.ipk-7D.1-GC7* and *QWsp.ipk-7D.2-GS7*) were mapped on chromosome 7DS for weight of seeds per spike, one for each control and stress condition, but with different origins for the increasing alleles. This shows the existence of two loci for these correlated traits, weight of seeds per spike under stress and control condition, on the short arm of chromosome 7D of which each one expressed under specific conditions. This can be validated by further analysis like fine mapping.

For **grain weight per spike** there are few QTL studies published. Hai et al. (2008) identified three QTLs on chromosomes 1A, 2B, and 2D. Kato et al. (2000) using single-chromosome recombinant lines mapped three QTLs on chromosome 5A (*QEgw.ocs-5A.1*, *QEgw.ocs-5A.2*, and *QEgw.ocs-5A.3*), and Börner et al. (2002) detected QTLs on chromosomes 1A, 1BL, 2AL, 2BS, 2DS, 2DL, 4AL, 5AL, 6BL, and 7AS.

Four of the mapped QTLs in the present study on chromosomes 2AL, 2BS, 2DS, and 7AS were reported earlier by Börner et al. (2002) and the QTL on chromosome 5AC was mapped by Kato et al. (2000). Therefore QTLs on chromosomes 5BL and 7DS are reported here for the first time. It is worth mentioning here that, all of the previous reports of QTL for this trait were from experiments conducted under non-stress conditions. Therefore, this is first report of QTLs for weight of seeds per spike under post-anthesis drought stress condition.

Araki et al. (1999) found QTL of the yield components, spikelet number per ear, and grain weight per ear, which were highly genetically correlated, to very similar positions to the QTL for yield on chromosome 4AS in their study. Börner et al. (2002) found co-localization of QTLs for spikelet number per ear, and grain weight per ear, on chromosomes 1AC, 2DS, and

4AL. Campbell et al. (2003) using a population of 3A recombinant inbred chromosome lines mapped QTLs for plant height, thousand-grain weight, spike number per square meter, and kernel number per spike. Four regions were revealed for the trait bearing QTL for one to all four traits. They found co-localization of QTLs on chromosome 3AS for yield, thousand-grain weight, and kernel per spike.

Regard to **number of fertile spikes per plant** and **number of unfertile tillers per plant**, QTL analysis showed interesting results about the sources of increasing alleles in which parents did not share the increasing alleles and always one parent was the source for each of the two traits. For number of fertile spikes per plant parent A was the origin of increasing alleles for the two QTLs which were mapped in similar regions on homoeologous chromosomes 7A and 7D under control and stress conditions, respectively. Parent A had one fertile spike more than parent B and one QTL for this trait per each condition was mapped. However $F_{2:3}$ families showed considerable amount of variation with a range of 6.7 and 4.8 under control and stress conditions, respectively (Table 3.12 and Figures 3 left one, in Appendix 6). The variation can be due to the existence of other undetected segregating QTLs in the population or because of large environmental effects on the trait.

There were more QTLs for number of unfertile tillers per plant compared to number of fertile spikes per plant. Parent B was the sources for all the five QTLs for the number of unfertile tillers per plant on chromosomes 1B, 2B, 5A, 7D, and 7A. It means that the identified QTLs for this trait were in association and since parent B has all the increasing alleles, there was no transgressive segregation in the direction of higher parent. However, the observed transgressive segregation can be because of the environmental effect or undetected segregating QTLs.

There are few studies about number of tillers or spikes per plant. Li et al. (2002) using the ITMI population detected QTLs for the **number of tillers per plant** on chromosomes 1DS, 2DS, and 6AS. Araki et al. (1999) found one QTL associated with tiller number per plant on chromosome 4AS. Quarrie et al. (2005) reported QTLs for number of spikes per plant on chromosomes 1AS, 1BL, 2BS, 3DL, 4BC, 4AL, 5DL, and 5AL. It seems no earlier report exists for the two mapped QTLs on chromosomes 7A (*QNfs.ipk-7A*) and 7D (*QNfs.ipk-7D*) in the present study and they were reported for the first time here. There is no QTL study for number of unfertile tillers per plant. Therefore, all of the identified QTLs in the current study

(*QNut.ipk-1B, QNut.ipk-2B, QNut.ipk-5A, QNut.ipk-7D,* and *QNut.ipk-7B*) remained as reported for the first time.

Spike number per square meter as one of the wheat yield component have also been studied through QTL analysis and revealed several regions as responsible loci for this trait. Huang et al. (2003) using advanced backcross QTL analysis mapped eight QTLs for spike number per square meter on chromosomes 1B, 2A, 2D, 3B, 4D, 5D, 6D, and 7AS. Li et al. (2007) using recombinant inbred lines mapped eight QTLs for spike number per square meter on chromosomes 1D, 1A, 2D, 2A, 3B, 4B, and 7DL. Shah et al. (1999) using a population of 3A recombinant inbred chromosome lines, revealed a QTL on the long arm of chromosome 3A which was tightly linked to QTL for plant height and kernel number per spike. Campbell et al. (2003) mapped QTLs for plant height, grain yield, thousand-grain weight, spike number per square meter, and kernel number per spike on chromosome 3A using a population of 98 chromosome 3A recombinant inbred lines.

QTL analysis for weight of three spikes per plant was not reported; however, since it was a high correlated trait with number of seeds per spike and weight of seeds per spike $r = 0.70$ and 0.83, respectively under control conditions, and $r = 0.78$ and 0.83, respectively, under stress conditions co-localization of their QTLs can be evaluated. On chromosome 2D, QTLs for weight of three spikes and weight of seeds per spike both under stress condition were found. On chromosomes 2A, 5A, and 7D QTLs for the three traits were mapped.

Weight of all spikes per plant and weight of three spikes per plant, both under stress conditions, showed two common QTLs on chromosomes 7D and 5A (both increasing alleles from parent A) which could explain the high correlation between these traits under stress (0.77) conditions. Under control conditions there was no common QTL between these traits, however, the correlation was even higher (0.86).

The end-use quality of wheat is greatly influenced by **seed size** and large seeds usually command consumer preference and thereby represent an important factor in controlling the economic value of wheat. Kernel shape and size are key components of kernel visual distinguishability which Canadian breeding lines must meet as requirements to be registered as cultivars in a particular marketing class (McCartney et al., 2005). From plant breeding perspective, grain size and shape are important for their relationship with yield potential and product quality (Breseghello and Sorrells, 2007). The development of improved seed size specific cultivars is thus an important breeding objective in wheat agriculture (Campbell et al.

1999). Grain size in wheat is a complex character and any information on its genetic control is useful for increasing breeding efficiency. However, few QTL mapping studies have been conducted for kernel shape (Sun et al., 2009).

A study using two mapping populations including the ITMI and 'AC Reed' × 'Grandin' (Breseghello and Sorrells, 2007) indicated that many regions across the wheat genome were related to kernel size and shape. Stronger signals were found on chromosomes 1B, 2D, and 5B, in the ITMI population, and on chromosomes 1A, 1D, 2B, 2D, 4B, 5B, and 6B, in 'AC Reed' × 'Grandin' population. The authors referred the differences in the two populations to the complex inheritance of kernel size and shape, the differences between mapping populations, and the error related to limited population size. Breseghello and Sorrels (Breseghello and Sorrells, 2007) in the ITMI population found QTLs for seed length on chromosomes 5B, and 5DL but QTL on chromosome 5B was the most significant one. In the same population, they found QTLs for seed width and seed area on chromosomes 1B and 2A, respectively. In 'AC Reed' × 'Grandin' population they found QTLs for seed length on chromosome 4B, and for seed area on chromosomes 1BL, 2BL, 2DS, and 7BC.

Campbell et al. (1999) using recombinant inbred lines mapped QTLs for kernel length on chromosomes 1B, 2A, 2B, 2D, 3B, and 7B, for kernel width on chromosomes 1A, 2A, 2B, 2DL, 3D, 5A, and 6B and kernel area on chromosomes 1A, 2A, 2B, 2D, 3B, 3D, 6B, and 7B. Several of the QTLs for kernel length and kernel width co-located with the QTLs for kernel area. Co-located QTLs for kernel area and width were found on chromosomes 1A, 2AS, 2B, and 3D but for kernel area and length on chromosomes 2B, 3B, and, 7BL. However, kernel length and kernel width did not show any co-located QTLs, indicating that these traits are independent. Lack of coincidence of QTLs for kernel width and kernel length were also reported by Sun et al. (2009) in a QTL analysis of grain shape and weight in common wheat using recombinant inbred lines in four different environments. They identified three QTLs for seed width on chromosomes 2A, 5D, and 6A while six QTLs for seed length on chromosomes 1A, 1B (*QKl.sdau-1B.1* and *QKl.sdau-1B.2*), 2B, 4A, and 4B.

Two of the seven revealed QTLs on chromosomes 1BL and 2A for seed area under control condition in the present study were reported earlier (Breseghello and Sorrells, 2007). There was no study of this trait under stress condition for comparison, however, one QTL from none stress condition in similar region of the chromosome 2DS was reported (Breseghello and Sorrells, 2007). From 13 QTLs for seed length the ones on chromosomes 1B and 2A were

reported earlier (Campbell et al., 1999). Two QTLs for seed width similar to the ones which were mapped in the present study on chromosomes 1B and 2A were reported by (Breseghello and Sorrells, 2007) and (Campbell et al., 1999), respectively.

Several regions on chromosome 1B responded to seed related traits in the current study. Similar case was reported by Sun et al. (2009) and QTLs in similar regions compared to present study were detected in which the QTLs identified by Sun et al (2009) *QKl.sdau-1B.1* and *QKl.sdau-1B.2* are similar to *QSel.ipk-1B.1* under control and *QSel.ipk-1B.3* under stress condition, respectively.

This study, combined with the results of (Breseghello and Sorrells, 2007) point out the loci on chromosomes of homoeologous groups 1, 2, 4, and 5. However, the QTL for seed length on chromosome 5B was mapped for all trait measurements under both conditions that showed its high consistency. Its R^2 ranged from 16.2 to 26.9% under control conditions and 11.3 to 22.4% under stress conditions. Analysis of overall mean of data for this trait showed even higher values of R^2 under control (29.6%) and stress (24.9%) conditions. This result showed the major effects of this QTL on seed length in the present study. However, the effect of this locus on other seed related traits like thousand-grain weight, seed area, and seed width was no significant. Interestingly, in the same region, QTLs for spike length and plant height were mapped repeatedly and with the same parent for the increasing alleles, the parent A which was even a little lower in plant height and spike length. Therefore, this is the first report of co-localization of QTLs for plant height, spike length, and seed length in a similar region of chromosome 5B. Röder et al. (2008) reported the same situation (co-localization of QTLs for plant height, spike length, and seed length) on chromosome 7DS in the genetic dissection of a QTL for thousand-grain weight (*QTgw.ipk-7D*).

In a study using the ITMI population (Breseghello and Sorrells, 2007) reported the most significant QTL for seed length on the linkage group 5B, near the locus *Xpsr574*. They also found no significant effect of this locus on kernel weight or volume that agrees totally with the result in the current study. In another study (Börner et al., 2002) plant height and spike length were evaluated in the ITMI population but no QTL was found for these traits on chromosome 5B. Result of the two above mentioned studies suggest the existence of a separate locus for seed length compared to the plant height, spike length.

From five QTLs for seed width and 13 QTLs for seed length, and considering only the repeated QTLs, only one QTL was detected on chromosome 4BL as common QTL between

these traits. The only one common QTL plus low correlations between seed length and seed width (Tables 3.7, 3.9, 3.11, 3.13, and 3.14), suggested that the loci controlling the two traits were independent. This agrees with a previous report indicating that the genetic control of kernel length and width was largely independent in which Sun et al. (2009) found no co-located QTL and also low correlation between these traits. Campbell et al. (1999) reported that kernel width and kernel length did not share any significant markers indicating that these two traits were probably under the control of different genes.

Regard to the co-localization of the QTLs for seeds related traits and considering only the repeated QTLs in the present study, there was only one QTL on chromosome 4BL common among all the traits. However, seed width showed another QTL on chromosomes 4BS in common with thousand-grain weight. This is in agreement with the coefficient of correlation observed in the present study between these traits in control conditions (Tables 3.7, 3.9, 3.11, 3.13, and 3.14) which show higher correlation of thousand-grain weight with seed width compared to seed length. Interestingly, there was only one QTL in common between seed area and thousand-grain weight. However, correlation between thousand-grain weight and seed area were similar to the correlation between thousand-grain and seed width which had two QTLs in common. This may be due to the fact that, the seed area was under the effect of both seed length and seed width characters. Therefore, like seed width, seed area showed high correlation with thousand-grain weight. This might suggest that thousand-grain weight is determined more by seed width rather than seed length. Therefore, in order to increase thousand-grain weight, seed width should be enhanced, in accordance with practical wheat breeding. This agrees with the previous report indicating genetic control of thousand-grain weight and grain width was largely dependent in which Sun et al. (2009) found three co-located QTL and also high correlation between these traits (Breseghello and Sorrells 2007). Campbell et al. (1999) found markers associated with thousand-grain weight on chromosomes 1A, 1B, 3B, 3DL, 6B, 7B, and 7AC. All markers also exhibited significant associations with either kernel length, width, or area. In other word, QTLs for seed width and seed length influenced seed area and thousand-grain weight, this was verified by the present study, too. However, QTLs for seed width and seed length did not influence each other. This was not confirmed in the present study.

Grain yield in cereals is generally controlled by a number of QTLs and is affected by environmental factors, making it difficult to manipulate and improve in breeding programs.

The genetic analysis of such complex traits becomes possible only by performing QTL analyses (Börner et al., 2002). Grain yield can be dissected into a number of component traits such as spike number per plant, ear grain weight, spikelet number per ear, and thousand-grain weight (Kato et al., 2000). Some of them, however, are less environmentally sensitive and have higher heritabilities than grain yield itself. Therefore, while looking for QTLs controlling grain yield, QTLs for yield components should also be determined to provide more useful information (Kato et al., 2000). Grain yield is a major target for wheat breeding programmes around the world. However, due to its complex nature, little is known regarding the genetic control of grain yield (Kuchel et al., 2007).

There are many QTL studies regarding **thousand-grain weight** under control condition in wheat. All wheat chromosomes were reported as harboring loci responsible for this trait (Campbell et al., 1999; Kato et al., 2000; Varshney et al., 2000b; Zanetti et al., 2001; Börner et al., 2002; Campbell et al., 2003; Groos et al., 2003; Huang et al., 2003; Gonzalez-Hernandez et al., 2004; McCartney et al., 2005; Quarrie et al., 2005; Kumar et al., 2006; Li et al., 2007; Hai et al., 2008; Kordenaeej et al., 2008; Sun et al., 2009).

Many agronomic traits were recorded and analyzed in the present study, however, the primary aim was to identify QTLs for the main trait, thousand-grain weight as the part of wheat yield components which is reduced under post-anthesis drought stress. Parents were selected based on the primary investigation and due to their difference at thousand-grain weight under post-anthesis drought stress, which was imposed by chemical desiccation. However, when they were tested at experiments for population phenotyping they did not behave the same as the primary investigation regarding tolerance against stress. It means there was no clear difference between them. This showed that parental lines selection for a quantitative trait is not an easy job because of large influence from environment.

The present study revealed four QTLs under control and six QTLs under stress conditions. Only one QTL on chromosome 4BL (*QTgw.ipk-4B.2*) was common between control and stress conditions and had the highest value of LOD score and R^2 under control condition. Therefore, there were three QTLs specific to control whereas five QTLs specific to stress condition. Parent A was the source of increasing alleles for one QTL (*QTgw.ipk-1B.1*) under control condition and three QTLs (*QTgw.ipk-7D*, *QTgw.ipk-4A.2*, and *QTgw.ipk-1B.2*) under stress condition. Indeed, QTL analysis confirmed transmission of alleles of both positive and negative genetic effect from each parent. At field experiment in 2004, two QTLs in repulsion

were identified on chromosome 4AL with 16 cM distance in their peaks. However, they did not identified on the other experiments of the present study. QTLs in repulsion in close distance can hide the effect of each other. Therefore, it is difficult to map this type of QTLs. This can be a possible reason that why these two QTLs were not mapped repeatedly.

Previous studies revealed QTLs in similar regions to ones that were identified under control condition on chromosomes 1BS (Zanetti et al., 2001), 4B (McCartney et al., 2005; Quarrie et al., 2005), and 7AL (Campbell et al., 1999; Groos et al., 2003). Therefore, there was no new QTL for this trait under control condition, which simultaneously confirmed the mapped QTLs in the present study.

There are few QTL studies for thousand-grain weight or grain yield under post-anthesis drought stress in wheat. The first report of the QTL analysis for grain yield under terminal drought stress belonged to Kirigwi et al., (2007) who used a mapping population including 127 recombinant inbred lines, with two years field evaluation. The authors mapped a QTL on chromosome 4A close to marker *Xwmc420*, which accounted for 20% of the observed phenotypic variation. Nearly the same time, Salem et al. (Salem et al., 2007) using the ITMI population and applying chemical desiccation treatment, as simulator of post-anthesis drought stress, mapped three QTLs on chromosomes 2DC, 5DC, and 7DL. Kordenaeej et al. (2008) mapped QTLs for thousand-grain weight on chromosomes 2BL, 3DC, 3BS, 3BL, 4DS, 4BL, 5DL, 5BL, 5AS, 5AL, and 7BL. They applied 118 recombinant inbred lines derived from a cross between a drought tolerant Iranian landrace 'Tabassi' and a non-drought tolerant European wheat variety, 'Taifun' and evaluated the lines under post-anthesis drought stress in Iran and but applying chemical desiccant, via spraying KI (potassium iodide) over the whole plants, in Hungary and Austria. McCartney et al. (2008) using a population of 249 recombinant inbred lines of durum wheat derived from a cross between cultivars 'Kofa' and 'Svevo' and evaluation under 16 environments characterized by a broad range of water availability, found two major QTL on chromosomes 2BL and 3BS.

Kordenaeej et al., (2008) reported a QTL for thousand-grain weight under post-anthesis drought stress on chromosome 4BL on similar region to the mapped QTL here. Regard to the five remaining QTLs, previous studies under non-stress conditions showed QTLs in similar region of chromosomes 4AL (McCartney et al., 2005; Quarrie et al., 2005), 7AS (Huang et al., 2003; Kumar et al., 2006) and 7DS (Börner et al., 2002; Huang et al., 2003). Since in the present study there was no QTL for thousand-grain weight in the same regions under control

condition, therefore, these QTLs were considered as specific to post-anthesis drought stress condition and differ from the ones which reported earlier but under non-stress condition.

Teulat et al. (1997) using a population of 187 recombinant inbred lines of barley (*Hordeum vulgare* L.) mapped a QTL for relative water content on chromosome 7H, however, they did not measure yield component traits. Morgan (1991) using substitution lines found chromosome 7A in response to the osmoregulation. Later on and based on the result from the substitution lines, Morgan and Tan (1996) developed an F_2 mapping population derived from a cross between the cultivar 'Songlen' (high response) and a breeding line (low response) and constructed RFLP linkage groups only for chromosomes 7A and 7D. They found a single locus influencing osmotic adjustment in wheat on short arm of chromosome 7A. Again, yield component traits were not measured. Literature reviews by Quarrie et al. (1999) and Cattivelli et al. (2002) also showed the importance of these regions, however, no QTL for any of the yield components was reported in Triticeae. Therefore, Quarrie et al. (1999) concluded "It remains to be seen whether this gene for osmotic adjustment is co-located with any QTL for yield under drought in wheat, barley or rice", the thing that happened in the present study. Rebetzke et al. (2008) using doubled haploid bread wheat population derived from a cross between 'CD87' and 'Katepwa' identified QTLs for water-soluble carbohydrates concentration on chromosomes 1BL and 7DS and water-soluble carbohydrates content on chromosome 1BL, on the same regions where QTLs were mapped in the presents study. However, further studies are necessary to reveal the function of the mapped QTLs and to show whether they are related to traits such as relative water content, osmotic adjustment, and water-soluble carbohydrates, which their QTLs reported on the regions of QTLs in the present study.

Two QTLs for thousand-grain weight under stress condition on homoeologous regions of chromosomes 7AS and 7DS were identified. However, no QTL was mapped on chromosome 7BS. Considering the QTLs for the same trait on chromosome 4AL and due to the known reciprocal translocation of 4AL/7BS in wheat (Figure 4.1) (Liu et al., 1992; Devos et al., 1995) QTL on chromosome 4AL can be related to the ones on chromosomes 7AS and 7DS. Therefore, the existence of homoeologous series genes in these regions in Triticeae can be suggested (Figure 4.5) and these QTLs may be donated from the original three diploid species involved in the evolution of bread wheat. Each of the QTL on chromosomes 7DS, 7AS, and 4AL, gives insight into the existence of QTL for the same trait on other homoeologous

chromosome. Moreover, mapping QTLs simultaneously on these homoeologous regions confirmed the identified QTLs on these regions, too.

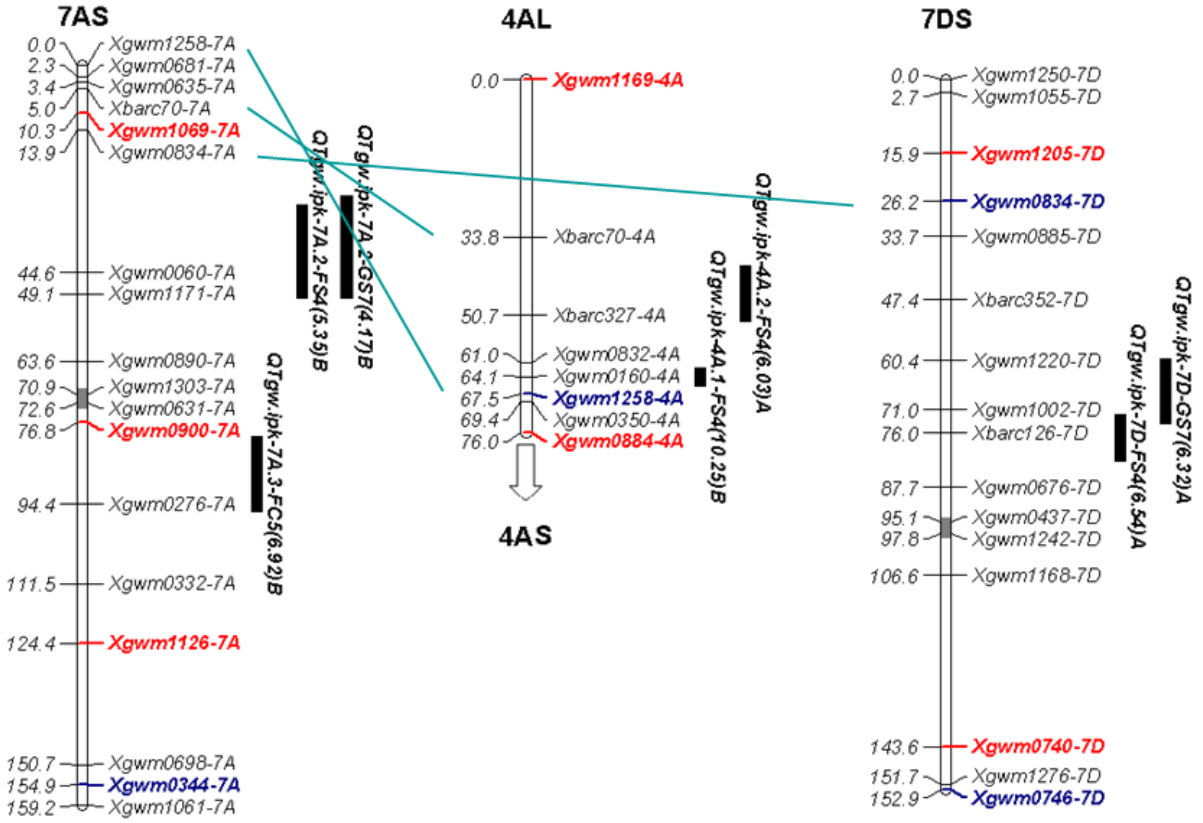

Figure 4.5 The proposed homoeologous relationships among QTLs for thousand-grain weight under stress condition on chromosomes 7AS, 4AL, and 7DS.

Khlestkina et al (2010) via performing partial gene cloning and comparative structural and mapping analysis among three *Ent*-kaurenoic acid oxidase (Kao) genes in bread wheat (*T. aestivum* L.) demonstrated that the Kao loci map to the distal ends of the chromosome arms 7AS, 4AL and 7DS represent a homoeoloci set. There were four SSR loci namely *Xgwm0832*, *Xgwm0160*, *Xgwm1258*, and *Xgwm0350* in the region corresponding to the reciprocal translocation of 7BS/4AL common between their map and the map in the present study. These four common SSR loci verified that the QTLs on chromosome 4AL in the present study belong to the translocation region. Howevere, more study is required to confirm the homoeoloci relationship among the QTLs for thousand-grain weight under stress condition on chromosomes 7AS, 4AL, and 7DS, which is proposed for the first time here.

Interestingly, there were co-location of QTLs for thousand-grain weight under stress condition with QTLs for adaptive traits such as plant height on chromosome 4AL, 4BL, and 7DS and days to flowering on chromosomes 1BL, and 7DS. These co-located QTLs increase the importance of these regions with regard to the post-anthesis drought stress. Future study via fine mapping will reveal the genetic control of these traits.

Röder et al. (2008) report genetic dissection of a QTL for thousand-grain weight (*QTgw.ipk-7D*) associated with SSR marker *Xgwm1002-7D*. Huang et al. (2004) originally detected this QTL in a BC_2F_3 advanced backcross population of the German winter wheat variety 'Prinz' and the synthetic wheat line 'W-7984'. Röder et al. (2008) dissected it into a single Mendelian gene via developing a defined introgression lines which showed a significant increase in grain weight of about 10% compared to 'Prinz' and the control group. The increase in grain weight was caused by a significant difference in grain length, whereas the grain width was not significantly different.

In the present study, the QTL for thousand-grain weight on chromosome 7DS had linkage with SSR marker *Xgwm1002-7D*, too. However, the QTL in the current study appeared only under the stress condition whereas, Huang et al. (2004) and Röder et al. (2008) mapped and fine mapped, respectively their QTL under control conditions. Moreover, Röder et al. (2008) reported that the increase in grain weight was connected with increase to seed length. This seems not to be the case for the mapped QTL in the present study because there were QTLs for seed area and seed width co-located with the given QTL but there was no QTL for seed length in this region under stress and control conditions.

It has been demonstrated that correlated traits or components of plant yield often have QTLs mapped at similar locations (Kato et al., 2000). Li et al. (2007) found agreement between the co-located QTLs with the results of simple correlation analysis and identified co-localization of QTLs conditioning days to anthesis and number of spikelets on chromosome 2DS. Huang et al. (2003) reported in the 2.9 cM interval between *Xgdm61* and *Xgdm129* on chromosome 4DS, four QTLs for plant height, thousand-grain weight, tiller number per square meter and yield. The QTL clusters for yield related traits were identified on chromosomes 2AL, 5DS, and 6AS (Sun et al., 2009), 2DS, 2BS, and 4AL (Börner et al., 2002), 1DS, 2AS, 6BL, and 7DL (Li et al., 2007), 4DS (McCartney et al., 2005). The QTL clusters also were found on distal parts of 7AL, and 7BL (Quarrie et al., 2005) and 2BS, 3AS, 5BL, 7AL, and 7DL (Groos et al., 2003).

As was indicated in the phenotypic evaluation of the measured traits (section 3.2) the traits were correlated. This resulted in the coincidence of QTLs for more than one trait at the same position and formed clusters of QTLs that may be due to closely linked loci or the pleiotropic effect. The large majority of yield QTLs were coincident with QTLs for one or more yield components. In the presents study, clusters of QTLs for at least four traits were found on eight chromosomes including 1B, 2A, 4B, 4AL, 5BC, 5A, 7DS, and 7AS. Three out of the eight QTL clusters in the present study were reported earlier on chromosomes 2A (Li et al., 2007), 4AL (Börner et al., 2002), and 7DS (Röder et al., 2008). Therefore, the present study distinguished five new QTL clusters on chromosomes 1B, 4B, 5B, 5A, and 7AS.

Cluster on chromosome 7DS had four QTLs in common with each of the cluster on chromosomes 7AS and 4AL. The existence of QTLs for common traits on the short arms of homoeologous group-7, (7AS and 7DS) could be due to homoeologous genes on two of the genomes and on 4AL because of the 7BS/4AL translocation. QTL cluster on chromosome 7DS with QTLs from 12 traits showed the highest number of QTL co-localization which make this chromosome arm of especial interest.

While on chromosome 4D of bread wheat QTLs for several traits like plant height, grain yield, thousand-grain weight, and time to maturity under control (McCartney et al., 2005) and post-anthesis stress drought stress (Kordenaeej et al., 2008) were identified, this chromosome failed to have a proper linkage group here. Considering many mapped QTLs on chromosome 4B in the present study, as homoeologous chromosome of 4D, constructing a linkage map for 4D will probably reveal more QTL for the measured traits in the present study.

Fine mapping of QTLs for thousand-grain weight under stress condition, plus the QTLs for adaptive traits, especially the co-localized ones, will generate markers more close to these genes. These markers can be applied for wheat breeding program against drought stress and will improve our knowledge about it genetics mechanism.

4.4 Outlook

Based on the results of the present study the following works should be continued:

1- Fine mapping of the detected QTLs for thousand-grain weight under stress condition and the QTLs for adaptive traits, especially the co-localized ones.

2- Validation of the new identified QTLs for days to flowering on chromosomes 7D and 5AL.

3- Validation of the homoeologous relationship among the three mapped QTLs for thousand-grain weight under stress condition on chromosomes 7DS, 7AS, and 4AL via applying more markers for these chromosomes.

4- Map saturation for the regions bearing gaps and especially for chromosomes 4D and 6D will be necessary to detect QTLs of these regions and chromosomes.

5- Conducting association mapping to the regions where QTLs were identified in order to reveal other alleles of the gene of interest.

5 Summary

Grain yield under post-anthesis drought stress is one of the most complex traits, which is inherited quantitatively. Considering Quantitative Trait Loci (QTLs) analysis as a powerful method for identifying genes determining this kind of complex characters, the present study was conducted in order to dissect the variation of the trait into its QTL components. Two cultivated bread wheat (*T. aestivum* L.) accessions were selected as parental lines; the drought tolerant accession HTRI 11712 originated from Pakistan and the sensitive one HTRI 105 from Sweden. Mapping population was developed from a single F_1 plant. Population genotyping was conducted on 143 F_2 plants. Phenotyping was carried out on 133 $F_{2:3}$ families through four experiments at field and greenhouse at IPK-Gatersleben. Several agronomic and adaptive traits were evaluated. However, thousand-grain weight was considered as the main trait because it is the only part of wheat yield components, which is reduced by post-anthesis drought stress. Not all traits were measured in all experiments. Chemical desiccation was applied in three experiments as simulator of post-anthesis drought stress whereas water stress was applied in one experiment.

Analysis of variance showed significant difference among the $F_{2:3}$ families for all the measured traits. The $F_{2:3}$ families showed transgressive segregation for most of the traits showing the existence of increasing alleles in both parents. Regarding correlation between traits within experiments, seed related traits showed higher correlations to each other compared to other traits whenever they were measured. Considering only the seed related traits under both control and stress conditions, there were high correlations between the traits except between seed length and seed width. However, seed length and seed width showed higher correlation under stress conditions. In all experiments seed length showed the lowest correlation to thousand-grain weight under both control and stress condition. However, the correlation was higher under stress condition.

A total of 666 SSR primer pairs, mainly from Gatersleben Wheat Microsatellite (GWM) collection were applied to survey polymorphism between the parental lines and out of them 398 (60%) revealed to be polymorphic. Mainly the ITMI map was used as a reference map to select well distribute SSR markers. Therefore, 273 of polymorphic SSR primer pairs were applied for population genotyping and 313 polymorphic loci were amplified. The molecular genetic linkage map was constructed including 293 loci associated to 19 wheat chromosomes.

Twenty loci stayed unlinked and chromosomes 4D and 6D failed to have proper linkage groups. There are 76 new compared to the ITMI map. Thirty two loci (about 10%) showed segregation distortion. Three of the most distorted loci were removed from QTL analysis because their integration into the map led to strong artefactual increase in genetic distances. Forty three loci also were removed because of close distance to other loci. Therefore, a map including 248 loci for 133 individuals were recalculated and applied for QTL analysis. Composite Interval Mapping revealed 88 and 60 QTLs under control and stress conditions, respectively, having a LOD score higher than three. Therefore, 148 QTLs were identified from the 54 records of the traits with an average of 2.7 and representing 117 unique QTLs. The number of QTLs in most of the trait measurements per experiment, ranged from one to four. However, in seven cases, numbers of QTLs exceed four and present higher numbers and reach seven. Both parents were important for most of the measured traits and shared nearly equal number of increasing alleles in which 65 and 52 out of 117 unique QTLs, the increasing alleles originated from parent A and parent B, respectively.

QTLs were identified on most of the constructed linkage groups except those representing chromosomes 6A, 6B, 3B, and 3D. However, the numbers of QTLs on chromosomes were not equal and ranged from one QTL at chromosomes 1D to 16 QTLs on chromosome 7D. QTLs from different traits co-localized and formed clusters. Clusters including QTLs for at least four traits were found on the eight following chromosomes: 1B, 2A, 4B, 4AL, 5BC, 5A, 7DS and 7AS. The analysis revealed eight QTLs for days to flowering on chromosomes 1B, 2D, 3A, 5A (two QTLs on 5AL), 5D, 7D, and 7B and all of them, except QTLs on chromosome 5AL (*QDtf.ipk-5A.2*) and 7DS (*QDtf.ipk-7D*) were reported earlier. Seven QTLs for plant height including a new one (*QPhe.ipk-5B*) were mapped.

Five QTLs for spike length were identified. Two of them on chromosomes 5B (*QSpl.ipk-5B*) and 3A (*QSpl.ipk-3A*) were found for the first time in the present study. Five QTLs for number of seeds per spike including three new ones on chromosomes 5B (*QNsp.ipk-5B*), 5A (*QNsp.ipk-5A*), and 7D (*QNsp.ipk-7D*) were identified. This study point out the loci on chromosomes of homoeologous groups 1, 2, 4, and 5 for seed related traits. The QTL for seed length on chromosome 5B was mapped for all trait measurements under both conditions that showed high stability of this QTL. However, the effect of this locus on other seed related traits like thousand-grain weight, seed area, and seed width was not significant. Interestingly,

in the same region, QTLs for spike length and plant height were mapped repeatedly, too, with the same parent for the increasing alleles. From the five QTLs for seed width and 13 QTLs for seed length, and considering only the repeated QTLs, only one QTL was detected on chromosome 4BL as common QTL between these two traits. The only one common QTL plus low correlations between seed length and seed width suggested that the loci controlling the two traits were probably independent.

The present study revealed four and six QTLs for thousand-grain weight under control and stress conditions, respectively. Only one QTL on chromosome 4BL (*QTgw.ipk-4B.2*) was common between conditions. Previous study revealed QTLs in similar regions to ones that were identified under control condition on chromosomes 1BS, 4B (two QTLs), and 7AL. Therefore, there was no new QTL for this trait under control condition, which simultaneously confirmed the mapped QTLs in the present study. Five QTLs for thousand-grain weight were found to be specific to stress condition on chromosomes 1B (*QTgw.ipk-1B.1*), 4AL (*QTgw.ipk-4A.1, QTgw.ipk-4A.2*), 7AS (*QTgw.ipk-7A.2*), and 7DS (*QTgw.ipk-7D*). On chromosomes 7AS and 7DS, QTLs for thousand-grain weight under stress condition co-located with the QTLs for weight of seeds per spike under stress condition, with the same origin for the increasing allele. It may be one QTL per each region and related to both traits. Identifying QTLs for thousand-grain weight under post-anthesis drought stress on chromosomes 7DS, 7AS, and 4AL and considering the known reciprocal translocation of 4AL/7BS in wheat, revealed the importance of the chromosomes from the homoeologous group 7 of Triticeae. Based on these results the homoeologous relationship among these QTLs is proposed which needs to be verified.

Finally, here we report a new intraspesific SSR-based genetic linkage map of bread wheat including 76 new loci, compared to the ITMI map that can be useful for map saturation in other studies. The new linkage map was applied successfully for QTLs analysis for agronomic traits and revealed QTLs under both control and stress conditions. Fine mapping of the QTLs for thousand-grain weight under stress condition, plus the QTLs for adaptive traits, especially the co-localized ones, will generate markers more close to these genes. These markers can be applied for breeding against drought stress and also will improve our knowledge about the genetic mechanism determining post-anthesis drought stress tolerance in wheat.

6 Zusamenfasssung

Kornertrag unter Trockestressbedingungen ist ein sehr komplexes Merkmal, welches quantitativ vererbt wird. Die vorliegende Studie nutzte eine ‘Quantitative Trait Locus’ (QTL) Analyse als wirksame Methode zur Identifizierung von Genen, die solche komplexe Merkmale steuern. Zwei Brotweizen (*T. aestivum* L.) Akzessionen dienten als Elternformen; die gegen Trockenheit tolerante Akzession HTRI 11712, stammend aus Pakistan und das sensitive Muster HTRI 105 aus Schweden. Ausgehend von einer einzelnen F_1-Pflanze wurde eine Kartierungspopulation entwickelt. Die Genotypisierung erfolgte an 143 F_2-Pflanzen. Phänotypisiert wurden 133 $F_{2:3}$-Familien im Freiland und im Gewächshaus des IPK Gatersleben. Agronomische und umweltabhängige Merkmale wurden erfasst, wobei nicht alle Merkmale in allen Experimenten bonitiert wurden. Schwerpunkt war das Tausendkorngewicht, die Ertragskomponente, welche durch Trockenstress nach der Blüte beeinflusst wird. Stress wurde in drei Experimenten mittels chemischer Desikkation simuliert während in einem Versuch die Wasserzufuhr reduziert wurde.

Die durchgeführte Varianzanalyse wies signifikante Unterschiede zwischen den $F_{2:3}$-Familien für alle gemessenen Merkmale auf. Für die meisten der Merkmale zeigte sich eine transgressive Aufspaltung mit positiven Allelen stammend von beiden Eltern. Die Korrelationsanalyse für die Merkmale innerhalb der Experimente zeigte immer höhere Werte zwischen Samen-Eigenschaften im Vergleich zu anderen erfassten Merkmalen. Die Korrelationen innerhalb der Samen-Merkmale waren sowohl unter Kontroll- als auch unter Stressbedingungen hoch, ausgenommen die Beziehung zwischen Samenlänge und Samenbreite. Die Korrelation zwischen der Länge der Samen und dem Tausendkorngewicht war in allen Experimenten am geringsten, unter Stress jedoch höher als unter Kontrollbedingungen.

Insgesamt wurden 666 SSR Primer-Paare, meist stammend aus der Gatersleben Wheat Microsatellite (GWM) Kollektion verwendet, um nach Polymorphismus zwischen den Eltern zu screenen. 398 (60%) der Primer-Paare waren polymorph. Die ITMI-Referenzkarte wurde verwendet, um gleichmäßig gut verteilte SSR Marker auszuwählen. Daraufhin wurden 273 polymorphe Marker verwendet, die insgesamt 313 Loci amplifizierten. Die Molekulare Kopplungskarte bestand schließlich aus 293 Loci die 19 Chromosomen zugeordnet werden konnten. Für zwanzig Loci konnte keine Kopplung gefunden werden. Für die Chromosomen

4D und 6D konnten keine ausreichend gute Kopplungsgruppen gefunden werden. Im Vergleich zur ITMI Karte wurden 76 neue Loci gefunden. 32 Loci (~ 10%) zeigten eine abweichende Aufspaltung. Drei der am meisten abweichenden Loci wurden nicht in der QTL-Analyse berücksichtigt, da sie zu einer ungerechtfertigten Veränderung der genetischen Abstände geführt hätten. Weitere 40 Loci wurden auf Grund sehr enger Kopplung ebenfalls verworfen. Die endgültige Karte basiert auf 248 Loci für 133 Individuen. Mittels Composite Interval Mapping wurden 88 beziehungsweise 58 QTLs mit einem LOD-Wert > 3 unter Kontroll- und Stressbedingungen gefunden. Die insgesamt 148 QTLs (117 Einzelloci) entstammen 54 Merkmalsbonituren was einem Durchschnitt von 2,7 entspricht. Die Anzahl der meisten QTLs pro Merkmal und Experiment lag zwischen eins und vier. In sechs Fällen war die Zahl jedoch höher und erreichte einen Maximalwert von sieben. Beide Eltern trugen zu nahezu gleichen Teilen an der Ausprägung der Merkmale bei. Positive Allele stammten für 63 und 52 der 115 QTLs vom Elter A beziehungsweise B.

QTLs befanden sich auf nahezu allen Kopplungsgruppen, ausgenommen die Chromosomen 6A, 6B, 3B, und 3D. Die Anzahl der QTLs pro Chromosom war unterschiedlich und schwankte zwischen 1 (Chromosom 1D) und 16 (Chromosom 7D). QTLs für verschiedene Merkmale befanden sich in gleichen Regionen und bildeten Cluster. Cluster mit QTLs für mindestens 4 Merkmale befanden sich auf den Chromosomen 1B, 2A, 4B, 4AL, 5BC, 5A, 7DS und 7AS.

Acht QTLs wurden für das Merkmal Blühzeitpunkt gefunden, die sich auf den Chromosomen 1B, 2D, 3A, 5A (zwei QTLs auf 5AL), 5D, 7D, and 7B befanden. Mit Ausnahme der QTLs auf den Chromosomen 5AL (*QDtf.ipk-5A.2*) und 7DS (*QDtf.ipk-7D*) wurden vergleichbare Loci bereits in früheren Studien beschrieben. Für das Merkmal Pflanzenlänge wurden sieben QTLs gefunden, davon erstmals beschrieben *QPhe.ipk-5B*.

Fünf Loci wurden für das Merkmal Ährenlänge detektiert, von denen zwei auf Chromosomen 5B (*QSpl.ipk-5B*) und 3A (*QSpl.ipk-3A*) neu sind. Ebenfalls fünf QTLs wurden für das Merkmal Kornzahl pro Ähre nachgewiesen, eingeschlossen drei erstmals beschrieben Loci auf den Chromosomen 5B (*QNsp.ipk-5B*), 5A (*QNsp.ipk-5A*) und 7D (*QNsp.ipk-7D*). QTLs für Samen-Merkmale befinden sich auf Chromosomen der homöologen Gruppen 1, 2, 4, and 5. Ein QTL für Samenlänge auf Chromosom 5B wurde für alle durchgeführten Messungen und unter Stress- und Kontrollbedingungen gefunden, was für eine sehr hohe Stabilität

spricht. Allerdings waren die Effekte dieser Region für andere Samen-Merkmale wie Samenbreite oder Tausendkorngewicht nicht signifikant. Interessanterweise befanden sich in dieser Region aber auch QTLs für Ährenlänge und Pflanzenhöhe, die wiederholt detektiert wurden und deren positiven Allele vom selben Elter stammen. Unter den insgesamt mehrfach aufgetretenen 5 QTLs für Samenbreite und 13 QTLs für Samenlänge fand sich nur ein gemeinsamer Lokus auf Chromosome 4BL. Das Auftreten nur eines gemeinsamen QTLs sowie die geringe Korrelation zwischen beiden Merkmalen lassen eine unabhängige genetische Kontrolle vermuten.

In der vorliegenden Studie wurden sechs QTLs unter Stress- und vier QTLs unter Kontrollbedingungen für das Merkmal Tausendkorngewicht aufgefunden, von denen einer auf Chromosom 4BL (*QTgw.ipk-4B.2*) gemeinsam auftrat. Vorangegangene Studien bestätigten die hier unter Kontrollbedingungen gefundenen QTLs in vergleichbaren Regionen auf den Chromosomen 1BS, 4B (zwei QTLs) und 7AL. Fünf Stress-spezifische QTLs für Tausendkorngewicht (*QTgw.ipk-1B.1*, *QTgw.ipk-4A.1, QTgw.ipk-4A.2, QTgw.ipk-7A.2,* und *QTgw.ipk-7D*) wurden auf den Chromosomen 1BL, 4AL 7AS, und 7DS gefunden. Die Loci auf den Chromosomen 7AS und 7DS befanden sich in der gleichen Region in der QTLs für das Merkmal Korngewicht pro Ähre unter Stressbedingungen gefunden wurden. Möglicherweise beeinflusst ein QTL beide Merkmale. Das Auffinden von QTLs für Tausenkorngewicht unter Stressbedingungen auf den Chromosomen 7DS, 7AS und 4AL unter Berücksichtigung der reziproken 4AL/7BS Translokation unterstreicht die Bedeutung der homöologen Gruppe 7 des hexaploiden Weizens für Trockenstress. Ob die aufgefundenen QTLs wirklich homöologe Loci darstellen, muss durch weitere Untersuchungen verifiziert werden.

Die hier beschriebene neue SSR-Marker basierte Kopplungskarte mit insgesamt 76 neuen Loci kann für künftige Kartierungen (Markerabsättigung) verwendet werden. Die Kopplungskarte wurde erfolgreich genutzt, um QTLs für agronomische Merkmale unter Kontroll- und Stressbedingungen zu identifizieren. Eine künftige Feinkartierung der aufgefundenen QTLs für Tausendkorngewicht unter Stressbedingungen wird zu eng gekoppelten Markern führen, die für die Züchtung trockentoleranter Sorten aber auch zur weiteren Aufklärung der genetischen Mechanismen der Trockenstresstoleranz nach der Blüte genutzt werden können.

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8 Supplementary material

Appendix 1

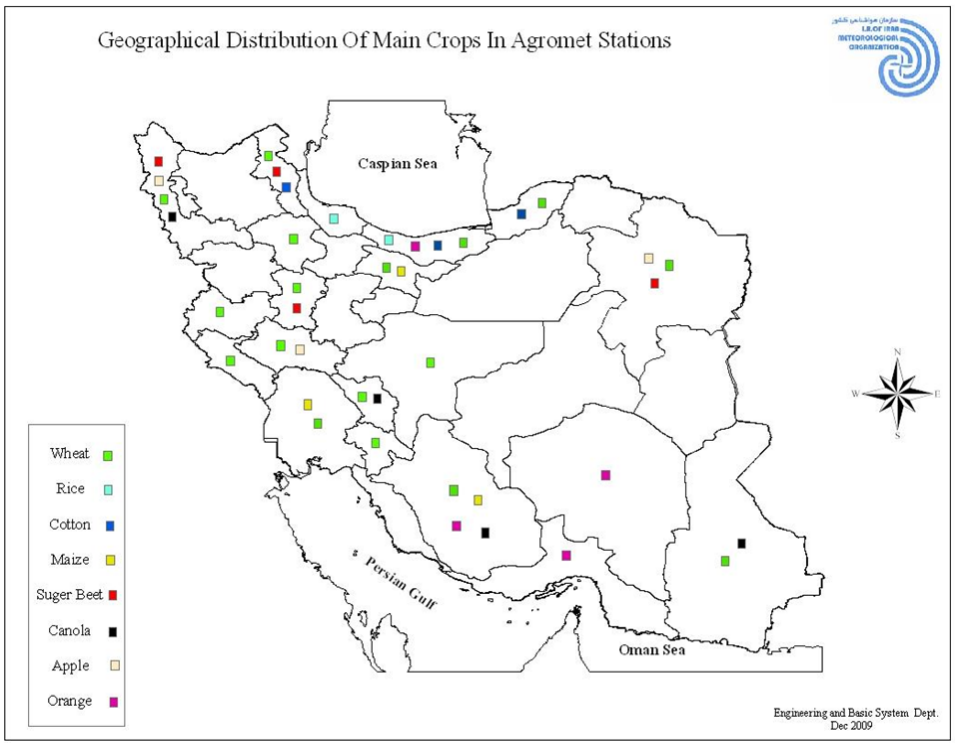
Table 1 Monthly precipitation (mm) from different wheat growing area in Iran (IRIMO, 2010).

	Mean	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEP.	OCT.	NOV.	DEC.	Annual	From
1	**BUSHEHR**	81.6	33.2	23.7	9.0	2.8	0.0	0.0	0.4	0.0	5.9	41.1	81.4	**279.1**	**1951**
2	**AHWAZ**	49.8	27.3	28.3	15.3	4.8	0.4	0.1	0.0	0.1	6.6	31.9	48.5	**213.4**	**1951**
3	**SHIRAZ**	90.2	52.6	56.2	26.7	7.0	0.2	1.2	0.7	0.0	3.9	28.9	78.4	**346.0**	**1951**
4	**ESFAHAN**	19.9	14.2	21.7	18.9	8.7	1.2	1.7	0.3	0.1	3.9	12.5	19.7	**122.8**	**1951**
5	**SEMNAN**	20.3	19.5	25.8	16.9	14.0	3.1	3.3	2.6	1.5	6.2	9.5	18.1	**140.8**	**1965**
6	**SHAHRE KORD**	60.8	47.9	60.3	37.5	14.1	0.9	1.9	0.4	0.0	6.9	32.2	58.6	**321.5**	**1955**
7	**YASOUJ**	178.3	155.9	165.8	65.0	14.7	0.6	1.2	1.8	0.4	11.1	65.0	205.1	**864.9**	**1987**
8	**TEHRAN**	34.6	32.2	40.8	30.7	15.4	3.0	2.3	1.8	1.1	10.9	26.0	34.0	**232.8**	**1951**
9	**ILAM**	116.0	90.1	122.6	62.9	17.5	0.2	0.5	0.1	0.2	23.2	84.1	98.6	**616.0**	**1986**
10	**HAMEDAN**	37.7	40.6	55.2	42.4	26.4	2.7	3.3	2.6	0.5	24.9	35.1	46.3	**317.7**	**1976**
11	**MASHHAD**	33.0	35.2	55.6	46.3	27.6	4.2	1.1	0.8	1.7	8.6	16.4	24.7	**255.2**	**1951**
12	**KHORRAMABAD**	80.1	74.4	87.0	71.7	30.0	0.9	0.3	0.3	0.8	18.6	60.5	84.4	**509.0**	**1951**
13	**KERMANSHAH**	61.7	57.2	83.9	64.7	30.2	1.1	0.6	0.2	1.8	24.0	56.2	63.5	**445.1**	**1951**
14	**ARAK**	52.7	44.0	58.6	54.6	30.8	2.3	1.0	1.7	0.5	16.3	33.9	45.3	**341.7**	**1955**
15	**GHAZVIN**	40.9	39.7	53.1	45.5	34.3	4.9	2.1	1.7	0.8	18.3	31.8	42.9	**316.0**	**1959**
16	**SANANDAJ**	65.1	60.4	79.9	72.4	37.7	2.1	1.0	0.5	0.8	24.6	58.2	55.7	**458.4**	**1959**
17	**ZANJAN**	30.3	28.4	48.1	56.5	42.3	11.0	5.6	3.7	4.3	21.7	32.9	28.3	**313.1**	**1955**
18	**TABRIZ**	22.3	24.2	40.6	52.7	42.6	16.9	5.8	3.2	7.6	21.9	27.9	23.2	**288.9**	**1951**
19	**GORGAN**	55.0	55.8	79.4	52.8	44.1	33.4	22.2	27.3	38.9	66.1	68.5	57.5	**601.0**	**1952**
20	**ARDEBIL**	24.7	21.8	37.4	38.3	45.1	19.4	6.7	5.4	9.9	33.0	37.1	25.1	**303.9**	**1976**
21	**OROOMIEH**	30.2	33.2	52.3	62.0	45.6	14.2	5.5	2.1	4.4	21.8	40.0	29.7	**341.0**	**1951**
22	**SARI**	59.4	50.6	76.8	54.3	56.2	40.8	18.8	39.3	71.2	86.8	147.1	87.9	**789.2**	**1999**

Appendix 1

Figure 1 Distribution of annual precipitation in Iran (WTP, 2007b).

Figure 2 Geographical Distribution of Main Crops in Iran (IRIMO, 2009)



Appendix 2

Enzymes, buffers and solutions

Enzymes

• RNase A (10 mg/ml)

100 mg of RNase was dissolved in a sterile solution of 10 mM Tris-HCL (pH 7.5) and 15 mM NaCl and boiled for 15 min in a water bath. After cooling RNase was tested and stored at -20 °C.

• *Taq*-DNA Polymerase

Buffers and solutions

• **0.5 M Ethylene diamine tetra acetate (EDTA, $Na_2EDTA.\ 2H_2O$) pH 8**

186.12 g Na2EDTA. 2H2O (MW = 372.2) was dissolved in 800 ml distilled water by stirring vigorously and pH was adjusted to 8.0 with NaOH (~20 g of NaOH pellets). Solution was sterilized by autoclaving.

(24:1, *v/v*) Chloroform: Iso-amyl alcohol ($CHCl_3$: IAA)

40 ml *iso*-amyl alcohol was added to 960 ml chloroform. Store the mixture at 4°C in dark glass bottles.

• **Ethidium bromide (Et Br, 10 mg/ml) Stock**

1g ethidium bromide was added to 100 ml of H_2O and stirred vigorously on a magnetic stirrer for several hours to ensure that the dye has dissolved. The container was wrapped in aluminium foil and stored at 4°C.

• **3 M Sodium acetate (NaOAc) pH 5.2**

408.1 g of sodium acetate was dissolved in 800 ml of distilled water; pH was adjusted to 5.2 with glacial acetic acid and sterilized by autoclaving.

• **5 M Sodium chloride (NaCl)**

292.2 g of NaCl (MW = 58.44) was dissolved in 800 ml of distilled water (dH_2O) and volume adjusted to 1000 ml and solution was sterilized by autoclaving.

• **(20%, *w/v*) Sodium dodecyl sulphate (SDS)**

20 g of lauryl dodecyl sulphate sodium salt (SDS) was dissolved in 100 ml of distilled

water by heating at 70 °C and pH was adjusted to 7.2 by adding a few drops of concentrated HCl.

• **1 M Tris-HCl pH 8**

121.1 g of Tris base was dissolved in 800 ml of distilled water and the pH to desired value (pH 8) was adjusted by adding concentrated HCl. The volume was made up to 1L and sterilized by autoclaving.

• **Tris-EDTA (TE) buffer**

(1,2114 g) of 1M Tris-HCl pH 8.0 and (0,37224 g) of 0.5M Na_2 EDTA pH 8.0 was added to 900 ml of distilled water (dH_2O). The volume was made up to 1L and sterilized by autoclaving.

• **(50 X) Tris-acetate-EDTA (TAE)**

242 g Tris, 57.1 ml glacial acetic acid and 37.2 g Na_2EDTA. 2H2O was added to 900 ml of distilled water (dH_2O), pH was adjusted to 8. The volume was made up to 1L.

1X TAE: 20ml 50X TAE/l H_2O

• **(10 X) Tris-Borate-EDTA (TBE) buffer**

(108.0 g) of 0.89M Tris, (55.0 g) of 0.89M boric acid and (8.3 g) of 20mM EDTA was added to 900 ml of distilled water (dH_2O). The volume was made up to 1L.

• **10 mM dNTPs**

Equal amounts of 10mM dATP, 10mM dCTP, 10mM dGTP and 10 mMdTTP was mixed. Store in 10 µl aliquots at -20 °C.

• **(10 X) PCR buffer**

1 ml of 1M Tris-HCl pH 8.0, 5 ml of 1M KCl, 150 µl of 1M MgCl2 and 10 mg of gelatin (Sigma G-2500) was added to 3.85 ml of distilled water (dH2O). The volume was made up to 10 ml and sterilized by autoclaving.

Solution for DNA isolation

Mix from 100 ml of 5M NaCl, 100 ml of 1M Tris-HCl pH 8.0, 100 ml of 0.5M EDTA pH 8.0 and 62.5 ml of 20% SDS was added to 500 ml of distilled water (dH2O). The volume was made up to 1L and sterilized by autoclaving.

Just before use 3.8 g/l Nabisulfite was added to readjust pH

• 75% EtOH Absolute EtOH was diluted to 75% with distilled water (dH2O).

Appendix 3

Table 1 Primer pairs without amplification in both parents (HTRI 11712 × HTRI 105)

	GWM No.	Tm	Length(bp)	Mapped	Chromosome
1	GWM0002	50 °C	132	ITMI	3D,3A
2	GWM0112	55 °C	101	ITMI	7B, 3B
3	GWM0121	50 °C	143	ITMI	7D
4	GWM0162	60 °C	208	ITMI	3A
5	GWM0180	50 °C	84	NT	1D?, 4B
6	GWM0224	50 °C	142	NT	2D?
7	GWM0231	60 °C	130	NT	1D
8	GWM0284	60 °C	123	ITMI	3B
9	GWM0346	55 °C	203	NT	3A?
10	GWM0358	55 °C	164	ITMI	5D
11	GWM0384	55 °C	204	NT	7A?
12	GWM0476	60 °C	>194	NT	2B or 6A
13	GWM0553	60 °C	121	NT	4D
14	GWM0570	60 °C	143	ITMI	6A
15	GWM0613	60 °C	130	ITMI	6B
16	GWM0760	50 °C	101	ITMI	6D
17	GWM0784	50 °C	199	ITMI	1B
18	GWM0789	60 °C	257	ITMI	1D
19	GWM0821	60 °C	155	NT	7B
20	GWM0911	55 °C	272	ITMI	1B, 5D
21	GWM0919	60 °C	241	NT	6B
22	GWM1000	50 °C	114	ITMI	7D/3D
23	GWM1142	55 °C	108	NT	3A?
24	GWM1186	50 °C	228	ITMI	2D
25	GWM1267	60 °C	146	ITMI	7B

Appendix 3

Table 2 List of monomorphic SSR primer pairs

	SSR primer pair	Tm	Length(bp) in parental lines	Length(bp) in ChS (bp)	Mapped	Chromosome
1	GWM0004	55 °C	237	294	ITMI	4A
2	GWM0006	55 °C	194	205	ITMI	4B
3	GWM0010	50 °C	136	166	ITMI	2A
4	GWM0016	50 °C	181	227	ITMI	7B, 5D, 2B
5	GWM0032	55 °C	171	176	ITMI	3A
6	GWM0033	60 °C	126	123	ITMI	1A, 1B, 1D
7	GWM0037	60 °C	180	193	ITMI	2D
8	GWM0043	60 °C	146	180	ITMI	7B
9	GWM0044	60 °C	192	182	ITMI	7D
10	GWM0047	60 °C	161	166	ITMI	2B, 2A, 2A
11	GWM0052	60 °C	156	150	ITMI	3D
12	GWM0055	60 °C	97	127	ITMI	2B, 6D, 2B
13	GWM0057	60 °C	200, 224	224	NT	4A,(1B, 6B)
14	GWM0063	60 °C	268	271	ITMI	7A
15	GWM0068	60 °C	125	182	ITMI	5B, 7B
16	GWM0072	55 °C	156	144	ITMI	3B
17	GWM0077	55 °C	135	153	ITMI	3B
18	GWM0082	60 °C	149	152	NT	6A
19	GWM0099	60 °C	108	119	ITMI	1A
20	GWM0106	60 °C	156	139	ITMI	1D
21	GWM0111	55 °C	196	205	ITMI	7D
22	GWM0113	60 °C	149	148	ITMI	4B
23	GWM0114	60 °C	202	206(177)	ITMI	3D, 3B
24	GWM0119	55 °C	186	181	NT	5B
25	GWM0130	60 °C	139	113	ITMI	7A
26	GWM0135	55 °C	138	143	ITMI	1A
27	GWM0140	55 °C	237	251(?)	ITMI	1B
28	GWM0157	60 °C	103	106	ITMI	2D
29	GWM0163	55 °C	128	127	NT	7D?
30	GWM0164	55 °C	117	120	ITMI	1A
31	GWM0174	55 °C	177	173	ITMI	5D
32	GWM0179	55 °C	187	181	ITMI	5A
33	GWM0182	60 °C	162	165	ITMI	5D
34	GWM0186	60 °C	121	140	ITMI	5A
35	GWM0189	55 °C	104	117	NT	2B
36	GWM0195	60 °C	106	108	NT	7B

ChS- cultivar Chinese Spring

Appendix 3

Table 2 (Continued) List of monomorphic SSR primer pairs

	SSR primer pair	Tm	Length(bp) in parental lines	Length(bp) in ChS (bp)	Mapped	Chromosome
37	GWM0198	60 °C	123	130	NT	4A
38	GWM0210	60 °C	191	192	ITMI	2B, 2D
39	GWM0212	60 °C	100	104	ITMI	5D
40	GWM0213	60 °C	172	184	ITMI	5B
41	GWM0218	60 °C	150	149	NT	3A
42	GWM0237	55 °C	110	137	NT	5A?
43	GWM0238	55 °C	210	204	NT	7B
44	GWM0241	55 °C	155	146	NT	5A?
45	GWM0251	55 °C	111	103	ITMI	4B
46	GWM0260	55 °C	166	157	ITMI	7A
47	GWM0261	55 °C	170	192	ITMI	2D
48	GWM0264	60 °C	212	219	ITMI	1B, 3B
49	GWM0265	55 °C	194	200	ITMI	2A
50	GWM0269	60 °C	107	>148	ITMI	5D
51	GWM0271	60 °C	200	162	ITMI	5D
52	GWM0273	55 °C	193	167	ITMI	1B
53	GWM0293	55 °C	195	201+10bp	ITMI	5A
54	GWM0295	60 °C	208	258	ITMI	7D
55	GWM0296	55 °C	140	149	ITMI	2D, 2A
56	GWM0299	55 °C	207	208	ITMI	3B
57	GWM0301	55 °C	206	204	ITMI	2D
58	GWM0311	60 °C	117	151	ITMI	2A, 2D
59	GWM0314	55 °C	124	170	ITMI	3D
60	GWM0316	55 °C	176	176	NT	2A(2B)
61	GWM0319	55 °C	196	200	ITMI	2B
62	GWM0328	55 °C	209	193	ITMI	2A
63	GWM0333	55 °C	148	150	ITMI	7B
64	GWM0335	55 °C	220	187 (225)	ITMI	5B
65	GWM0336	55 °C	107	108	NT	4D
66	GWM0337	55 °C	179	183	ITMI	1D
67	GWM0238	55 °C	204	211	NT	7B
68	GWM0342	55 °C	196	169	NT	7B/D (7A)
69	GWM0369	60 °C	190	188	ITMI	3A
70	GWM0371	60 °C	182	170	ITMI	5B
71	GWM0375	55 °C	164	156	NT	4B?
72	GWM0383	60 °C	192	195	ITMI	3D

Appendix 3

Table 2 (Continued) List of monomorphic SSR primer pairs

	SSR primer pair	Tm	Length(bp) in parental lines	Length(bp) in ChS (bp)	Mapped	Chromosome
73	GWM0388	60 °C	161	162	ITMI	2B
74	GWM0391	55 °C	78	150	ITMI	3A
75	GWM0397	55 °C	176	179	ITMI	4A
76	GWM0403	55 °C	111	133	ITMI	1B
77	GWM0410	55 °C	331	334	ITMI	5A, 2B
78	GWM0411	55 °C	147	148	NT	3D, (6D)
79	GWM0420	55 °C	210	223	NT	3B
80	GWM0428	60 °C	139	143	ITMI	7D
81	GWM0429	50 °C	209	221 (290)	ITMI	2B
82	GWM0455	55 °C	156	151	ITMI	2D
83	GWM0494	60 °C	200	198	ITMI	6A
84	GWM0498	55 °C	157	159	ITMI	1B
85	GWM0499	60 °C	128	145	ITMI	5B
86	GWM0501	60 °C	158	172	ITMI	2B
87	GWM0512	60 °C	182	185	ITMI	2A
88	GWM0515	60 °C	133	134	ITMI	2A, 2D
89	GWM0518	55 °C	166	166	ITMI	6B
90	GWM0547	60 °C	182	185	ITMI	3B
91	GWM0554	60 °C	134	160	ITMI	5B
92	GWM0564	60 °C	184	177	NT	2D
93	GWM0565	60 °C	138	142	ITMI	5D
94	GWM0609	50 °C	106	92	ITMI	4D
95	GWM0611	55 °C	189	168	ITMI	7B
96	GWM0626	50 °C	106	102?	ITMI	6B
97	GWM0633	60 °C	144	136	ITMI	1A
98	GWM0636	50 °C	103	110	ITMI	2A
99	GWM0637	60 °C	199	173	ITMI	4A
100	GWM0639	55 °C	133	134	ITMI	5D, 5A, 5B
101	GWM0642	60 °C	192	187	ITMI	1D
102	GWM0646	50 °C	166	165	NT	6A
103	GWM0656	55 °C	147	140	NT	1A
104	GWM0664	55 °C	152	150	ITMI	3D
105	GWM0674	60 °C	162	164	ITMI	3A
106	GWM0682	55 °C	124	124	ITMI	2B
107	GWM0691	60 °C	160	154	ITMI	1A
108	GWM0705	50 °C	94	97	ITMI	3B
109	GWM0707	60 °C	118	108	ITMI	3D

Appendix 3

Table 2 (Continued) List of monomorphic SSR primer pairs

	SSR primer pair	Tm	Length(bp) in parental lines	Length(bp) in ChS (bp)	Mapped	Chromosome
110	GWM0715	60 °C	144	147	ITMI	6D?
111	GWM0735	50 °C	129	136	ITMI	7A, 7D
112	GWM0744	60 °C	180, 196	194	NT	3B, 3D
113	GWM0748	60 °C	129	131	ITMI	7A
114	GWM0756	55 °C	120	121	NT	5B
115	GWM0757	60 °C	102	102	ITMI	3A
116	GWM0759	60 °C	113	122	ITMI	1B
117	GWM0778	60 °C	204	207	ITMI	1A
118	GWM0781	55 °C	133	104	ITMI	4A
119	GWM0787	60 °C	149	152	NT	7B
120	GWM0795	60 °C	177	175	ITMI	3D
121	GWM0801	55 °C	151	147	NT	4D
122	GWM0808	60 °C	112	108	ITMI	7B
123	GWM0819	50 °C	174	177	ITMI	4D
124	GWM0820	60 °C	146	142	ITMI	1D
125	GWM0823	60 °C	135	132	ITMI	2D
126	GWM0827	50 °C	83	102	NT	?
127	GWM0830	60 °C	127	130	ITMI	2A
128	GWM0831	50 °C	110	129	ITMI	5B
129	GWM0845	60 °C	196	196	ITMI	3B
130	GWM0848	55 °C	192	187	ITMI	1D
131	GWM0858	55 °C	108	177	ITMI	3D
132	GWM0861	60 °C	126	128	ITMI	7B, 7A
133	GWM0862	60 °C	172, 213	211	NT	6B,(6D)
134	GWM0871	60 °C	151	148	ITMI	7B
135	GWM0876	60 °C	104	108	NT	3D
136	GWM0877	55 °C	100	101	ITMI	2B, 2D
137	GWM0889	60 °C	142	142	ITMI	6B
138	GWM0891	55 °C	96	100	ITMI	4B
139	GWM0893	60 °C	137	139	NT	?
140	GWM0894	60 °C	121	125	ITMI	4A
141	GWM0903	60 °C	79	103	ITMI	1B, 1D
142	GWM0904	60 °C	155	159	ITMI	6D
143	GWM0910	55 °C	151	148	ITMI	4B
144	GWM0914	55 °C	182, 220, 224	231	NT	1A, 1B , 1D
145	GWM0928	55 °C	177	120	ITMI	7D

Appendix 3

Table 2 (Continued) List of monomorphic SSR primer pairs

	SSR primer pair	Tm	Length(bp) in parental lines	Length(bp) in ChS (bp)	Mapped	Chromosome
146	GWM0930	50°C	123	186	ITMI	4B
147	GWM0936	60°C	250	250	ITMI	7B
148	GWM0937	60 °C	159	162	ITMI	4A
149	GWM0945	50°C	171	173	NT	?
150	GWM0947	55°C	128	(138)243	ITMI	5B
151	GWM0951	60 °C	172	155	ITMI	7B
152	GWM0959	50 °C	218	215	ITMI	4A
153	GWM0960	55 °C	190	186	ITMI	5D
154	GWM0965	50 °C	192	50	NT	?
155	GWM0969	60 °C	175	173	NT	2D or 6A
156	GWM0973	55 °C	147	146	ITMI	3D
157	GWM0974	50°C	97	101	ITMI(NT)	7D(7A,7B)
158	GWM0976	60°C	247	244	ITMI	4D
159	GWM0983	55 °C	131	134	ITMI	7B
160	GWM0998	55°C	205	199	ITMI	4B
161	GWM1023	60°C	211	232	ITMI(NT)	?(5D)
162	GWM1037	55°C	207	140	ITMI	3B
163	GWM1043	60°C	139	146	ITMI	5B
164	GWM1048	60°C	130	143	NT	2A, 2B, 2D
165	GWM1049	55°C	209	206	ITMI	1D
166	GWM1050	60°C	218	222	ITMI	1B
167	GWM1052	50°C	254	253	ITMI	2A,7D
168	GWM1058	60°C	238	246	NT	7D?
169	GWM1065	60°C	118	119	ITMI	7A
170	GWM1067	55°C	178	179	ITMI	2B
171	GWM1077	60°C	135	133	ITMI, NT	4A?
172	GWM1076	55°C	133	131	ITMI	6B
173	GWM1081	60°C	140	137	ITMI	4A
174	GWM1085	50°C	123	128	ITMI	7B
175	GWM1096	60°C	216	214	NT	3A
176	GWM1097	60°C	167	~155	ITMI	1A
177	GWM1104	50°C	167	167	ITMI	1A
178	GWM1111	55°C	144	150	ITMI	1A
179	GWM1123	60°C	152	151	ITMI	7D
180	GWM1124	60°C	114	117	NT	2B, 2A
181	GWM1130	60°C	117	116	ITMI	1B

Appendix 3

Table 2 (Continued) List of monomorphic SSR primer pairs

	SSR primer pair	Tm	Length(bp) in parental lines	Length(bp) in ChS (bp)	Mapped	Chromosome
182	GWM1134	60°C	101	103	NT	7D,7A,7B
183	GWM1144	60°C	127	118	ITMI	7B
184	GWM1151	60°C	132	257	ITMI	2A
185	GWM1166	60°C	139	142	ITMI	6D
186	GWM1167	50°C	140	142	ITMI	4B, 6D
187	GWM1175	60°C	165	248	ITMI	7B
188	GWM1176	60°C	259	263	ITMI	2A
189	GWM1177	60°C	108	115	ITMI	2B
190	GWM1179	55°C	252	247	ITMI	4A
191	GWM1185	55°C	255	224	ITMI	6A
192	GWM1191	55°C	107	108	ITMI	5A
193	GWM1198	60°C	153	147	ITMI	2A
194	GWM1199	50°C	245	210	ITMI	6B
195	GWM1203	55°C	158	153	NT	2B
196	GWM1209	60°C	129	130	NT	1D
197	GWM1212	60°C	215	214	NT	5D
198	GWM1213	60°C	161	~167?	NT	2D or 5A
199	GWM1219	60°C	118	116	NT	7B
200	GWM1226	55°C	135, 149	145	NT	5D
201	GWM1234	60°C	124	123	NT	4D?4A
202	GWM1241	60°C	133	131	ITMI	6D
203	GWM1249	55°C	120	116	ITMI	2B
204	GWM1251	55°C	222	228	ITMI	4A
205	GWM1253	55°C	95	118	ITMI	5D
206	GWM1264	55°C	103	100	ITMI	2D
207	GWM1265	60°C	107	111	NT	?
208	GWM1266	60°C	177	157	ITMI	3B
209	GWM1268	55°C	147	145	ITMI	6D
210	GWM1271	60°C	127	125	NT	3D(3B)
211	GWM1273	50°C	112	109	ITMI	2B
212	GWM1274	50°C	160	168	ITMI	2D
213	GWM1286	55°C	116	116	NT	1D?, 3D
214	GWM1296	60°C	281	274	ITMI	6A
215	GWM1302	60°C	180	228	ITMI	4D

Appendix 3

Table 3 Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
1	GWM0003	55	84	CA	18	ITMI	3D
2	GWM0005	50	172	TC, T, GT	23, 4, 12	ITMI	3A
3	GWM0011	50	196	Tai, CA, TA	8, 19, 6	ITMI	1B
4	GWM0018	50	186	CA, TA	17, 4	ITMI	1B
5	GWM0030	60	206	AT, GT	19, 15	ITMI	2D, 3A
6	GWM0058	60	118	CA	17	NT	6B
7	GWM0060	60	211	CA	30	ITMI	7A
8	GWM0066	60	150	CA, TA	30, 21	ITMI	5B ,4B
9	GWM0067	60	85	CA	10	ITMI	5B
10	GWM0071	60	128	GT	20	ITMI	2A ,2A, 3D
11	GWM0088	60	121	GT	18	ITMI	6B
12	GWM0118	60	110	CA	14	NT	5B (5D, 4A)
13	GWM0120	55	139	CT, CA	11, 17	ITMI	2B
14	GWM0122	60	149	CT, CA	11, 31	ITMI	2A
15	GWM0126	60	196	CA	15	ITMI	5A
16	GWM0128	50	176	CA	20	NT	2B
17	GWM0131	60	131	CT	22	ITMI	1B, 3B
18	GWM0133	60	118	CT	36	ITMI	6B
19	GWM0134	60	111	CA	15	NT	3B?
20	GWM0140	55	251 (?)	CT	>42	ITMI	1B
21	GWM0144	50	200	GT	15	NT	3B
22	GWM0146	60	162	Gaimp	26	ITMI	7B
23	GWM0148	60	163	CA	22	ITMI	2B

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Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
24	GWM0149	55	161	GA	25	ITMI	4B
25	GWM0153	60	188	GA	19	ITMI	1B
26	GWM0154	55	102	GA	32	ITMI	5A
27	GWM0155	60	141	CT	19	ITMI	3A
28	GWM0156	60	277	GT	14	ITMI	5A
29	GWM0160	60	184	GA	21	ITMI	4A
30	GWM0161	60	154	CT	15	ITMI	3D
31	GWM0165	60	199	GA	20	ITMI	4A, 4D, 4B
32	GWM0181	50	135	GA	29	ITMI	3B
33	GWM0192	60	191	CT	44	ITMI	4A ,4B, 4D
34	GWM0193	60	171	CT, CA	22, 9	ITMI	6B
35	GWM0197	60	126	CT	17	NT	5B?
36	GWM0203	55	139	CA, GA	13, 21	NT	4D, (4A)
37	GWM0205	60	152	CT	21	ITMI	5A, 5D
38	GWM0228	60	210	CT, CA	17, 10	NT	2D
39	GWM0232	55	141	GA	19	ITMI	1D
40	GWM0234	55	241	CT, CA	16, 2	ITMI	5B
41	GWM0242	55	142	GA	19	NT	2D, (2A, 2B)
42	GWM0244	60	227	Caimp	45	NT	6B
43	GWM0247	60	158	GA	24	ITMI	3B
44	GWM0249	60	177	GAimp	11	ITMI	2D, 2A
45	GWM0255	55	148	GA	24	NT	7B, (7A)
46	GWM0268	55	241	GAimp	>44	ITMI	1B

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Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
47	GWM0272	55	140	CA	17	ITMI	5D
48	GWM0274	50	179	GT	27	ITMI	1B,7B
49	GWM0276	55	99	CT	24	ITMI	7A
50	GWM0285	60	243	GA	27	ITMI	3B
51	GWM0291	60	>158	CA	35	ITMI	5A
52	GWM0292	60	220	CT	38	ITMI	5D
53	GWM0294	55	100	GAimp	5, 15	ITMI	2A
54	GWM0297	55	150	GT, GA	12, 18	ITMI	7B
55	GWM0304	55	217	CT	22	ITMI	5A
56	GWM0312	60	235	GA	37	ITMI	2A
57	GWM0313	55	156	CT, GT	12, 28	NT	6B
58	GWM0320	55	>263	GT, GA	9, >15	ITMI	2D
59	GWM0322	55	119	GA	25	NT	2B
60	GWM0332	60	231	GA	36	ITMI	7A
61	GWM0339	50	159	CT	22	ITMI	2A
62	GWM0340	60	132	GA	26	ITMI	3B
63	GWM0344	55	131	GT	24	ITMI	7B
64	GWM0349	55	230	GA	34	ITMI	2D
65	GWM0350	55	146	GT	14	ITMI	7D, 7A
66	GWM0353	60	179	GCGT, GT	4, 14	NT	3A (3D)
67	GWM0356	55	224	GA	36	ITMI	2A
68	GWM0357	55	123	GA	18	ITMI	1A
69	GWM0372	60	>329	GA	> 51	ITMI	2A

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Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
70	GWM0374	60	213	GT	17	ITMI	2B
71	GWM0382	60	115	GA	26	ITMI	2D, 2A, 2B
72	GWM0389	60	130	CT, GT	14, 16	ITMI	3B
73	GWM0390	55	143	CT, GT	12, 28	NT	6B?
74	GWM0393	55	107	CA	25	NT	7B?
75	GWM0395	60	147	CA	13	NT	1B(1A, 1D)
76	GWM0400	60	139	CA	21	ITMI	7B
77	GWM0408	55	176	CAimp, TA	30, 9	ITMI	5B
78	GWM0413	60	94	GA	18	ITMI	1B
79	GWM0437	50	109	CT	24	ITMI	7D
80	GWM0443	55	134	CA, GA	20, 22	ITMI	5B
81	GWM0445	55	192	CT	19	ITMI	2A
82	GWM0448	60	231	GA	29	ITMI	2A
83	GWM0456	55	132	GA	21	ITMI	3D
84	GWM0458	60	113	CA	13	ITMI	1D
85	GWM0480	60	188	CT, CA	16, 13	ITMI	3A
86	GWM0484	55	145	CT	29	ITMI	2D
87	GWM0493	60	208	CAi	43	ITMI	3B
88	GWM0497	55	>106	GTimp	24	ITMI	1A, 2A, 3D
89	GWM0526	55	140	CT	16	ITMI	2B
90	GWM0530	55	186	CT	29	NT	6A (6B, 6D)
91	GWM0533	60	147	CT, CA	18, 20	ITMI	3B, 3B
92	GWM0537	60	209	CA, TA	18, 13	ITMI	7B

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Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
93	GWM0538	60	147	GTimp	16	ITMI	4B
94	GWM0539	60	147	GA	27	ITMI	2D
95	GWM0566	60	130	CA, TA	21, 8	ITMI	3B
96	GWM0569	47	134	GT	36	ITMI	7B
97	GWM0573	50	212	CA	30	ITMI	7B, 7A
98	GWM0577	55	133	CA, TA	14, 6	ITMI	7B
99	GWM0583	60	165	CA	27	ITMI	5D
100	GWM0601	60	148	CT	17	ITMI	4A
101	GWM0604	50	119	GA	29	ITMI	5B
102	GWM0605	55	140	GA	18	NT	5B
103	GWM0608	60	126	GA	16	ITMI	4D, 2D
104	GWM0614	60	152	Gai	23	ITMI	2A
105	GWM0617	60	131	GA	43	ITMI	6A, 5A
106	GWM0619	50	148	CT	19	ITMI	2B
107	GWM0630	60	120	GT	16	ITMI	2B
108	GWM0631	60	197	GT	23	ITMI	7A
109	GWM0635	60	107	CA, GA	10, 14	ITMI	7D, 7A
110	GWM0645	55	149	CTimp	23	ITMI	3D
111	GWM0655	60	177	CA	37	ITMI	3B
112	GWM0663	50	163	CT	39	ITMI	4A
113	GWM0676	50	119	GA	28	ITMI	7D
114	GWM0680	55	123	GT, Gaimp	8, 24	ITMI	6B
115	GWM0681	60	188	CT	16	ITMI	7A

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Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
116	GWM0695	60	258	GAimp	32	ITMI	4A
117	GWM0698	60	213	GA	44	ITMI	7A
118	GWM0700	60	143	CA	27	ITMI	5D
119	GWM0702	60	151	CA	23	ITMI	2D
120	GWM0710	50	143	GA	18	ITMI	4B
121	GWM0719	55	186	GA	28	ITMI	6A
122	GWM0720	60	162	GA	33	ITMI	3A
123	GWM0726	50	136	GT	35	ITMI	2A
124	GWM0736	60	187	TA, GA	5, 4	ITMI	4B, 5A
125	GWM0739	50	158	Caimp	> 49	ITMI	2B, 2A
126	GWM0740	50	131	GT	31, 5	NT	7D
127	GWM0746	55	143	CA, GA	13, 18	ITMI	7A
128	GWM0750	60	217	GA	21	ITMI	1A
129	GWM0751	50	126	CA, GA	13, 24	ITMI	3A
130	GWM0752	55	125	GT	26?	ITMI	1A
131	GWM0761	55	100	Ctimp	15+8	ITMI	2A
132	GWM0762	50	147	CA, TA	27, 14	ITMI	1B
133	GWM0772	50	184	AT	10	NT	1A
134	GWM0777	60	113	CAimp	24	ITMI	5B
135	GWM0779	60	215	CA, GAimp	31, 33	ITMI	3B, 3A
136	GWM0783	50	103	GA	21	ITMI	7B
137	GWM0785	60	134	GAimp	17	ITMI	6B
138	GWM0790	55	215	CT	53	ITMI	2D, 6B, 5B

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Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
139	GWM0799	50	181	GT	24	NT	1B, 6A?
140	GWM0802	60	132	CA	25	ITMI	3B
141	GWM0804	60	139	GT	25	NT	7D?
142	GWM0805	55	243	CA	27	ITMI	5D
143	GWM0816	60	194	GT	21	ITMI	6B
144	GWM0818	50	149	CA	16	ITMI	1B
145	GWM0825	55	110	GA	28	ITMI	6B
146	GWM0832	55	147	CTimp	38	ITMI	4A
147	GWM0834	55	225	CTimp, GT	42, 6	ITMI	7A
148	GWM0835	55	194	CT	19	NT	1A
149	GWM0843	55	107	5A	26	ITMI	5B
150	GWM0846	60	121	GA	30	ITMI	2A, 2B, 2D
151	GWM0853	60	112	GT	20	ITMI	3B
152	GWM0859	60	159	GA	> 14	NT	2D
153	GWM0865	55	171	GA	24	NT	5A
154	GWM0883	50	204	CT	-	ITMI	7B
155	GWM0884	60	150	GAimp	34	NT	4A
156	GWM0885	60	172	GA	25	ITMI	7D
157	GWM0886	60	125	Ctimp	31	ITMI	2D
158	GWM0888	60	197	GTimp	20	ITMI	4B
159	GWM0890	50	131	GT	39	ITMI	7A
160	GWM0897	50	148	CA, GA	23, 18	ITMI	7B
161	GWM0898	55	104	GAimp	16	ITMI	4B

Appendix 3

Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
162	GWM0900	60	243	Gtimp	25	NT	7A
163	GWM0902	55	148	CA	20	ITMI	5D
164	GWM0907	60	139	GT	13	ITMI	6B
165	GWM0912	55	179	GTimp	23	ITMI	2B, 2A
166	GWM0913	55	182	GT	41	ITMI	7A
167	GWM0925	55	186	GT	9	ITMI	4B
168	GWM0931	55	274	GAimp	42	ITMI	5D
169	GWM0935	60	143	GA	28	ITMI	6B, 4B, 2B
170	GWM0938	55	156	GA	29	ITMI	3B
171	GWM0939	50	236	CA	39	NT	2A (2D)
172	GWM0940	60	177	GAimp	22	ITMI	6B, 4B, 2B
173	GWM0941	60	124	CA	25	NT	7B
174	GWM0944	60	102	GT	20	NT	4D
175	GWM0961	60	180	CA	33	ITMI, NT	2D?
176	GWM0963	50	254	CA	20	ITMI	7B
177	GWM0977	55	108	CT	24	ITMI	3D
178	GWM0982	55	131	GT, GA	7, 19	ITMI	5D not 5A
179	GWM0988	50	183	CT, CTG	?	ITMI	2D
180	GWM0995	60	163	GT	42	ITMI	5A
181	GWM0996	60	192	GA	19	NT	5B
182	GWM0999	60	150	GA	17	NT	6D (6B)
183	GWM1002	60	176	CT	>60	ITMI	7D
184	GWM1005	60	152	CA	13	ITMI	3B

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Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
185	GWM1009	55	95	CT	18	ITMI	6A, 6D
186	GWM1010	60	170	GT	11	NT	2D ?
187	GWM1011	60	121	GA	20	ITMI	6A, 2A
188	GWM1012	60	113	GT	15	ITMI	1D
189	GWM1015-	50	149	GT	20	ITMI	3B
190	GWM1016	60	147	GA	18	ITMI, NT	5B (6B)
191	GWM1017	55	262	GT	26	ITMI	6A
192	GWM1027	60	135	CA	15	ITMI	2B
193	GWM1031	50	158	GT	15	NT	2B?
194	GWM1039	60	135	GA	25	ITMI	5D
195	GWM1041	60	243	GA	47	ITMI	1D
196	GWM1044	60	141	CA	13	ITMI	7D
197	GWM1045	55	189	GT, GC, GA	17, 3, 19	ITMI	2A
198	GWM1055	60	150	CT	27	ITMI	7D
199	GWM1059	60	202	GT	19	NT	5D
200	GWM1061	60	165	Gti	29	ITMI	7A
201	GWM1069	60	134	GT	15	NT	7A
202	GWM1070	60	120	CA	31	ITMI	2B, 2A
203	GWM1078	55	144	GT	20	ITMI	1B
204	GWM1084	60	179	CT	37	ITMI	4B
205	GWM1088	60	246	GT, GA	22+5+9, 16	ITMI	3D
206	GWM1089	60	150	CA	27	ITMI	6A
207	GWM1091	60	229	CT	30	ITMI	4A

Appendix 3

Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
208	GWM1093	60	115	CA, GA	17, 13	ITMI	4A
209	GWM1099	50	131	CT	-	ITMI	2D
210	GWM1100	50	227	CA (CGCA)	9(7)	ITMI	1B
211	GWM1110	55	194	TA,TG	6, 15	ITMI	3A
212	GWM1115	60	132	CT, GT	20, 15	ITMI	2A
213	GWM1126	55	122	CT, CA	12, 22	NT	7A?
214	GWM1128	55	156	CT	14	ITMI	2B
215	GWM1136	60	109	GT	11	NT	2A? / 2B?
216	GWM1139	60	235	CT, CA, TA	9, 12, 29	ITMI	1A, 1B
217	GWM1148	60	133	GA	31	ITMI	1A
218	GWM1150	60	174	GT, GA	12, >24	ITMI	6A
219	GWM1159	55	199	CA	15	ITMI	3A
220	GWM1163	60	138	CA	13	ITMI	4D
221	GWM1168	60	125	GT	12	ITMI	7D
222	GWM1169	50	220	AT, GT	16, 37	NT	4A
223	GWM1171	55	149	GT	13	ITMI	5A, 7A
224	GWM1173	55	249	GT, GA	12, 18	ITMI	7B
225	GWM1180	60	148	CA	20	ITMI	5B
226	GWM1184	55	142	CA, TA	17, 7	ITMI	7B
227	GWM1202	55	269	GT	48	ITMI	1D
228	GWM1205	60	139	GA	21	NT	6B (5A, 4B)
229	GWM1207	60	249	GA	42	ITMI	7A
230	GWM1210	55	100	CT	17	NT	6A

Appendix 3

Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
231	GWM1220	60	139	CT	25	ITMI	7D
232	GWM1229	55	143	GT	29	ITMI	3A
233	GWM1230	55	114	GT	13	ITMI	1D
234	GWM1233	60	142	CT	12	ITMI	6B
235	GWM1235	60	129	CA, CT	9, 2	ITMI	2D
236	GWM1236	60	147	GA	29	ITMI	5A
237	GWM1242	60	142	GA	22	ITMI	7D
238	GWM1244	60	140	GA	19	NT	2A
239	GWM1246	55	232	GA	40	ITMI	5B, 5D
240	GWM1250	50	156	GA	27	ITMI	7D
241	GWM1252	50	114	CT	24	ITMI	5D
242	GWM1257	60	244	GT	32	ITMI	6B
243	GWM1258	60	196	CCT, CAT	28	ITMI	7A, 7D
244	GWM1276	60	190	CT	?	ITMI	7D
245	GWM1284	60	150	GCACAC	6	ITMI	5B
246	GWM1291	50	187	GT	25	ITMI	1D
247	GWM1303	50	244	CA	15	ITMI	7A
248	GDM0019	55	199	(CA)24(TA)7	-	ITMI	1D, 2D
249	GDM0035	55	189	CA	26	ITMI	2D
250	GDM0043	55	142	GA	24	ITMI	3A, 5D
251	GDM0063	60	150	CT	20	ITMI	5D
252	GDM0098	60	146	GT	21	ITMI	6D
253	BARC4	52	158	TTA	15	ITMI	5B
254	BARC20	52	186	ATT	21	ITMI	7BL, 4AL

Appendix 3

Table 3 (Continued) Information of the assayed polymorphic SSR primer pairs

	SSR primer pair	TM	bp	Motif	Repeats	Map	Chromosome
255	BARC48	55	198	TATC	11	ITMI	1A, 4D, 6A, 6B
256	BARC70	55	193	(TATCTA)3(TCTA)7	-	ITMI	7AL, 4AL
257	BARC74	60	175	(GA)13(GATA)7(GA)9	-	ITMI	5B
258	BARC91	50	129	TAA	15+11	ITMI	2B, 4D
259	BARC106	50	142	ATT	21	ITMI	4AL
260	BARC107	50	224	ATT	16	ITMI	6A
261	BARC125	52	175	CT	29	ITMI	2B, 3D, 4B, 5A, 7D
262	BARC126	52	164	CTT	17	ITMI	7D, 7B
263	BARC140	52	133	CT	12	ITMI	5B, 2B, 5D
264	BARC142	52	243	CT	13	ITMI	5B, 2D, 5A, 6A
265	BARC146	52	130	CT	21	ITMI	6A, 6B, 6D
266	BARC212	52	190	CT	14	ITMI	2A
267	BARC232	65	189	CT	18	ITMI	5B, 5A, 5D
268	BARC273	52	225	ATT	13	ITMI	6D
269	BARC309	55	149	CT	11	ITMI	2A
270	BARC327	52	248	(TAGA)11(CCAT)4	-	ITMI	4AL
271	BARC352	60	248	CT	14	ITMI	7D
272	BARC353	55	226	CT	16	ITMI	6A, 2D
273	BARC361	60	261	(CT)10 CT(10)	-	ITMI	2B, 5D, 6B

Appendix 3

Table 4 List of the rest of polymorphic SSR primer pairs (not applied in population genotyping)

	SSR primer pair	Tm	Length(bp) ChS	Mapped	Chromosome	Length(bp) (HTRI 11712, HTRI 105)
1	GWM0046	60 °C	187	ITMI	7B	(173, 184)
2	GWM0070	60 °C	194	ITMI	6B	(190, 201)
3	GWM0095	60 °C	121	ITMI	2A	(106, 116)
4	GWM0102	60 °C	143	ITMI	2D	(145, null)
5	GWM0107	60 °C	195	ITMI	4B	(201, 217)
6	GWM0108	60 °C	132	ITMI	3B	(136, null)
7	GWM0129	55 °C	221	ITMI	5A, 2B	(null, 222)
8	GWM0132	60 °C	119	ITMI	6B	(119, 123)&(126, 132)
9	GWM0159	60 °C	192	ITMI	5B	(200, 198)
10	GWM0183	55 °C	158	ITMI	3D	(193, 195)
11	GWM0190	60 °C	>201	ITMI	5D	(205, 207)
12	GWM0191	60 °C	128	ITMI	5B, 2B, 6B	(124,128)&(144,133)
13	GWM0194	50 °C	131	ITMI	4D	(134, 132)
14	GWM0200	60 °C	250	NT	6B, (6A)	(235, null)
15	GWM0210	60 °C	192	ITMI	2B, 2D	(183, 191)
16	GWM0213	60 °C	184	ITMI	5B	(172, 160)
17	GWM0219	60 °C	181	ITMI	6B	(196, 188)
18	GWM0233	60 °C	261	ITMI	7A	(250, 256)
19	GWM0257	60 °C	192	ITMI	2B	(200, 198)
20	GWM0259	55 °C	105	ITMI	1B	(null, 109)
21	GWM0263	55 °C	134	NT	7B?	(132, 130)
22	GWM0264	60 °C	219	ITMI	1B, 3B	(212, null)
23	GWM0271	60 °C	162	ITMI	5D	(200, 202)
24	GWM0275	50 °C	107	ITMI	2A	(106, 114)&(119, null)

ChS- cultivar Chinese Spring

Appendix 3

Table 4 (Continued) List of the rest of polymorphic SSR primer pairs (not applied in population genotyping)

	SSR primer pair	Tm	Length(bp) ChS	Mapped	Chromosome	Length(bp) (HTRI 11712, HTRI 105)
25	GWM0282	55 °C	220	ITMI	7A	(201, 203)
26	GWM0302	60 °C	180 (340)	ITMI	7B	(null, 255)
27	GWM0325	55 °C	131	ITMI	6D	(140, 142)
28	GWM0330	55 °C	165	NT	5A	(168, 195)
29	GWM0341	55 °C	133 (150)	ITMI	3D	(143, 141)
30	GWM0359	55 °C	217	ITMI	2A	(213, 215)
31	GWM0368	60 °C	249	ITMI	4B	(247, null)
32	GWM0376	60 °C	147	ITMI	3B	(103,101)&(148, 144)
33	GWM0415	55 °C	131	ITMI	5A	(131, 132)
34	GWM0425	60 °C	>143	ITMI	2A	(145, 141)
35	GWM0427	50 °C	215	ITMI	6A	(196, 213)
36	GWM0459	55 °C	>138	ITMI	6A	(null, 133)
37	GWM0471	60 °C	149	ITMI	7A	(142, 126)
38	GWM0473	55 °C	220	ITMI	2A	(214, 212)
39	GWM0508	50 °C	165	ITMI	6B	(null, 133)
40	GWM0513	60 °C	144	ITMI	4B	(144, 146)
41	GWM0540	55 °C	129	ITMI	5B	(125, 115)
42	GWM0544	55 °C	167	ITMI	5B	(166, null)
43	GWM0550	55 °C	150	ITMI	1B	(150, 160)
44	GWM0558	55 °C	125	ITMI	2A	(119, 121)
45	GWM0582	50 °C	151	ITMI	1B	(143, 146)
46	GWM0588	60 °C	102	NT	7A	(87, 81)
47	GWM0595	60 °C	188	ITMI	5A	(145, null)
48	GWM0610	60 °C	168	ITMI	4A	(172, 170)

Appendix 3

Table 4 (Continued) List of the rest of polymorphic SSR primer pairs (not applied in population genotyping)

	SSR primer pair	Tm	Length(bp) ChS	Mapped	Chromosome	Length(bp) (HTRI 11712, HTRI 105)
49	GWM0624	50 °C	119	ITMI	4D	(136, 132)&(null, 138)
50	GWM0644	60 °C	166	ITMI	6B, 7B	(153, 155)&(182, 179)
51	GWM0659	60 °C	159	ITMI	1B	(null,167)
52	GWM0666	60 °C	107	ITMI	7A, 3A, 1A, 3A, 5A	(88, null)
53	GWM0731	55 °C	147	ITMI	4A	(82, 76)
54	GWM0732	60 °C	177	ITMI	6D	(146, 148)
55	GWM0742	55 °C	150	ITMI	4A	(133, null)
56	GWM0745	50 °C	147	ITMI	7B	(152, 154)&(null, 207)
57	GWM0771	50 °C	100	ITMI	6B	(null, 99)
58	GWM0774	50 °C	138	ITMI	6D	(102, 100)
59	GWM0780	50 °C	102	ITMI	7D	(114, 116)
60	GWM0786	60 °C	139	ITMI	6A	(117, null)
61	GWM0791	60 °C	177	ITMI	1A	(173, 167)
62	GWM0793	55 °C	140	ITMI	1D	(null,138)
63	GWM0810	60 °C	ca. 131	ITMI	5B	(161, 153)
64	GWM0814	60 °C	140	ITMI	6B	(148, 152)
65	GWM0855	50 °C	155	ITMI	4A	(176, null)
66	GWM0856	50 °C	112	ITMI	4B	(117, 119)
67	GWM0857	60 °C	186	ITMI	4B	(190, 188)
68	GWM0870	50 °C	122	ITMI	7A	(129, 133)
69	GWM0895	55 °C	150	ITMI	2A	(145, 147)
70	GWM0896	55 °C	156	ITMI	3B	(162, null)
71	GWM0903	60 °C	103	ITMI	1B, 1D	(100, 131)
72	GWM0905	55°C	250	ITMI	1A	(240, 227)&(272, 268)

Appendix 3

Table 4 (Continued) List of the rest of polymorphic SSR primer pairs (not applied in population genotyping)

	SSR primer pair	Tm	Length(bp) ChS	Mapped	Chromosome	Length(bp) (HTRI 11712, HTRI 105)
73	GWM0921	60°C	213	ITMI	6B	(145, null)&(217, null)
74	GWM0928	55 °C	120	ITMI	7D	(173, 177)
75	GWM0929	55 °C	138	ITMI	4A	(136, 134)
76	GWM0934	60 °C	119	ITMI	1B, 1D	(119, null)
77	GWM0946	60°C	-	ITMI	4B	(180, 184)&(222, 237)
78	GWM0957	55°C	156	ITMI	1D	(160, 162)
79	GWM0980	55 °C	148	ITMI	3B	(166, 172)
80	GWM0984	50 °C	228	ITMI	7B, 7A	(205, null)&(220, null)&(260, null)
81	GWM1004	60°C	150	NT	7D	(154, 152)
82	GWM1007	60°C	193	ITMI	7D	(194, 197)
83	GWM1014	55°C	202	ITMI	7D	(202, 200)
84	GWM1028	50°C	116	ITMI(NT)	1B(1D)	(null, 109)
85	GWM1029	60°C	217	ITMI	3B	(218, 220)
86	GWM1034	55°C	115	ITMI	3B	(129, null)&(135, 137)
87	GWM1038	55°C	230	ITMI	3A	(222, 211)
88	GWM1040	60°C	141	ITMI	6A	(145, 149)
89	GWM1042	50°C	99	ITMI	3A	(97, 95)
90	GWM1051	55°C	250	NT	6B	(261, 259)
91	GWM1053	60°C	136	ITMI	2A	(131, 133)
92	GWM1054	60°C	121	ITMI	5B	(138, 142)
93	GWM1057	60°C	247	ITMI	5A	(257, 255)
94	GWM1063	60°C	120	ITMI	3A	(121, 123)
95	GWM1066	60°C	139	ITMI	7A	(136, 138)
96	GWM1071	55°C	148	ITMI	3A	(150, 148)

Appendix 3

Table 4 (Continued) List of the rest of polymorphic SSR primer pairs (not applied in population genotyping)

	SSR primer pair	Tm	Length(bp) ChS	Mapped	Chromosome	Length(bp) (HTRI 11712, HTRI 105)
97	GWM1072	60°C	198	ITMI	5D,5B	(199, 201)
98	GWM1073	55°C	228	ITMI	1B	(1039, null)
99	GWM1083	50°C	108	ITMI	7A	(97, 95)
100	GWM1102	55°C	105(198)	ITMI	7D	(null, 151)
101	GWM1107	50°C	113	NT	?	(117, 119)
102	GWM1121	50°C	131	ITMI, NT	3A	(null, 108)&(121, 119)&(141, null)
103	GWM1122	60°C	113	ITMI	5D	(117, 119)
104	GWM1154	60°C	118	ITMI	7D	(129, 131)
105	GWM1200	50°C	219	ITMI	3D	(175, 177)&(null,226)
106	GWM1217	60°C	149	ITMI	3A	(147, 150)
107	GWM1240	55°C	225	NT	3B?	(null, 224)
108	GWM1255	50°C	250	ITMI	6B	(250, 264)
109	GWM1263	55°C	248	ITMI	2A	(null,254)
110	GWM1278	60°C	100	ITMI	4B	(null, 104)
111	GWM1293	60°C	113	ITMI	6A	(97, 95)

Appendix 3

Table 5 List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-synthetic)
1	1	*Xgwm1258-7A*	7A, 7D	4A!	195	197(185-175)	196(null-165)
2	2	*Xgwm0681-7A*	7A		-	185(181-199)	188(184-189)
3	3	*Xgwm0635-7A*	7A, 7D		-	100,109(93-111)	107(109-null)
4	4	*Xgwm1069-7A*	7A		-	138(129-138)	NT (not yet)
5	5	*Xbarc70-7A*	4AL	4A	183	183, 200, 204(229 - 206)	193
6	6	*Xgwm0834-7A*	7A	7D!	-	255(160-158)	225(156-154)
7	7	*Xgwm0060-7A*	7A		150	207(203-188)	211(190-224)
8	8	*Xgwm1171-7A*	7A, 5A	2B!, 3B!	145	146,152,158(154-null)	149(155-null)
9	9	*Xgwm0913-7A*	7A, 7D		-	193(175-171)	182(167,169-164,167)
10	10	*Xgwm0890-7A*	7A		-	135?(118-114)	131(113-111)
11	11	*Xgwm1303-7A*	7A		-	250(256-248)?	244(248-250)
12	12	*Xgwm0631-7A*	7A		-	203(194-192)	197(192-183)
13	13	*Xgwm1044-7A!*	7D		140, 278	252(252-173)	141(128-139)
14	14	*Xgwm0900-7A*	7A		-	250(244-246)	NT (not yet)
15	15	*Xgwm1011-7A!*	6A, 2A	6A	100, 117	122(119-125)	121(107-105)&(136-null)
16	16	*Xgwm0276-7A*	7A	Locus specific	-	97(null-85)	99(109-101)
17	17	*Xgwm0332-7A*	7A		194	196(197-199)	231(290-211)
18	18	*Xgwm1207-7A*	7A		-	247(231-208)	249(208-312)
19	19	*Xgwm1126-7A*	7A?		-	110(102-110)	NT (not yet)
20	20	*Xgwm0698-7A*	7A		-	214(160-213)	213(210-160)
21	21	*Xgwm0344-7A!*	7B		-	135(128-134)	131(121- null)
22	22	*Xgwm1061-7A*	7A		136	171(171-169)?	165(168-170)
23	1	*Xgwm0255-7B*	7B (7A)		120	149(141-143)	-
24	2	*Xgwm0569-7B*	7B		-	141(159-145)	134(130-126)
25	3	*Xgwm0537-7B*	7B		-	210(212-210)	209(207-203)
26	4	*Xgwm0400-7B*	7B		-	136(131-145)	139(143-150)
27	5	*Xgwm0573-7B*	7B, 7A		180	?(215-213)	212(210-212)
28	6	*Xgwm1184-7B*	7B		-	139(141-145)	142(146-140)

ChS = cultivar 'Chinese Spring', extra loci indicated by cell in yellow color, first time mapped loci by red color and extra loci of the first time mapped loci by red color in yellow cell

Loci in the table were ordered based on the constructed linkage map, from short arm of chromosome 7A to long arm of chromosome 1D

First column shows number of loci in the linkage map of the present study and in total 293 loci, second column shows number of loci per each chromosome. Different colors in second culumn separate different chromosomes.

Appendix 3

Table 5 (Continued) List of mapped SSR loci

		Loci	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Sentetic)
29	7	*Xgwm0941-7B*	7B		90	120(117-121)	NT (not yet)
30	8	*Xgwm0393-7B*	7B?		-	114(113-117)	NT (not yet)
31	9	*Xgwm1173-7B*	7B		210	250(245-249)	249(250-269)
32	10	*Xgwm0297-7B*	7B		-	150(155-149)	150(150-168)
33	11	*Xgwm0963-7B*	7B		230, 236	254(252-254)	254(null-247)
34	12	*Xgwm0897-7B*	7B		-	146(145-143)	148(141-123?)
35	13	*Xgwm0783-7B*	7B		-	104(134-108)	103(131,133-null)
36	14	*Xgwm0577-7B*	7B		-	131(136-162)	133(164-155)
37	15	*Xgwm0883-7B*	7B		196 and 198 co-segr.	198, 204(208-202)	204(224-197)
38	16	*Xgwm0146-7B*	7B	Locus specific	-	160(157-null)	162(174-null)
39	1	*Xgwm1250-7D*	7D		-	157(150-168)	156(147-117)
40	2	*Xgwm1055-7D*	7D		-	150(147-145)	150(147-null)
41	3	*Xgwm1205-7D!*	6B(5A, 4B)		90,112,122,124,148	114,124,126,139,142(137-null)	NT (not yet)
42	4	*Xgwm0834-7D!*	7A	7A	-	225(229-219)	225(156-154)
43	5	*Xgwm0885-7D*	7D		-	173(162-180)	172(181-null)
44	6	*XBARC352-7D*	7D		-	248(249-247)	248( )
45	7	*Xgwm1220-7D*	7D		-	138(149-143)	139(143-null)
46	8	*Xgwm1002-7D*	7D		184	182(200-166)	176(171-126)
47	9	*XBARC126-7D*	7D, 7B		-	118(118-124)	(122-116)
48	10	*Xgwm0676-7D*	7D		78, 88	140(146-152)	119(-)
49	11	*Xgwm0437-7D*	7D		-	106(90-108)	109(109-111)
50	12	*Xgwm1242-7D*	7D		98	143(147-153)	142(151-147)
51	13	*Xgwm1168-7D*	7D		-	125(126-124)	125(126-130)
52	14	*Xgwm0740-7D*	7D		-	129(127-97)	NT (not yet)
53	15	*Xgwm1276-7D*	7D		-	204(184-198)	190(199-185)
54	16	*Xgwm0746-7D!*	7A		112	104,143(142-138)	143(142-150)&(115-106)
55	1	*Xgwm0719-6A*	6A	unlink	-	153,164,189(153-null)	186(155-162)

Appendix 3

Table 5 (Continued) List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
56	2	*Xgwm1009-6A*	6A, 6D	unlink	-	97,130(119-127)	95(92-86)
57	3	*Xgwm0530-6A*	6A (6B, 6D)		136, 148	188(204-198)	NT (not yet)
58	4	*Xgwm1210-6A*	6A		-	99(101-99)	NT (not yet)
59	5	*Xbarc146-6A*	6A, 6B, 6D	6B	144	130,138,152(162-156)	(163-151)
60	6	*Xgwm1011-6A*	6A, 2A	7A!	100, 117	122(108-110)	121(107-105)
61	7	*Xbarc107-6A*	6A		-	187(187-184)	190
62	8	*Xgwm0907-6A!*	6B	6B	-	154(154-158)	139(136-134)
63	9	*Xgwm1150-6A*	6A		-	171(185-192)	174(173-199)
64	10	*Xbarc353-6A*	6A, 2D	2A	224	228(228-null)	226( )
65	11	*Xgwm1017-6A*	6A		-	272(279-283)	262(270-null)
66	12	*Xgwm0617-6A*	6A, 5A		-	125(117-137)	131(133-null)
67	13	*Xgwm1089-6A*	6A		-	148(148-164)	150(null-139)
68	14	*Xgwm0799-6A*	1B, 6A?		-	188(183-196)	NT (not yet)
69	1	*Xgwm0244-6B*	6B		-	226(230-200)	NT (not yet)
70	2	*Xgwm0313-6B*	6B	Locus specific	-	160(160-null)	NT (not yet)
71	3	*Xgwm0390-6B*	6B?	Locus specific	-	147(149-null)	NT (not yet)
72	4	*Xgwm0790-6B*	6B, 2D, 5B	5B	194	167,183,213(173,206)	215(162-165)
73	5	*Xgwm0825-6B*	6B	nice peak	-	113,130(114-130)	110(135-122)
74	7	*Xgwm0935-6B*	6B, 4B, 2B	4B	103	104,127,144(null-115)	143(143-null)
75	6	*Xgwm0940-6B*	6B, 4B, 2B	4B,4B	-	138,161,178(null-150)	177(178-null)
76	8	*Xgwm1016-6B*	6B, 5B	5B	114	128,147(128-135)	NT (not yet)
77	9	*Xgwm0680-6B*	6B		-	?(125-110)	123(123-133)
78	10	*Xgwm0193-6B*	6B		-	169(169-155)	171(171-182)
79	11	*Xgwm1233-6B*	6B		-	143(170-141)	142(140-150)
80	12	*Xgwm0785-6B*	6B		-	141(144-134)	134(134-138)
81	13	*Xgwm0816-6B*	6B		-	198(193-180)	194(180-190)
82	14	*Xgwm0608-6B!*	4D, 2D		112	126(122-126)	126(151-144)&(166-181)

Appendix 3

Table 5 (Continued) List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
83	15	*Xgwm0088-6B*	6B		-	125(138-123)	121(162-null)
84	16	*Xbarc146-6B*	6B, 6A, 6D	6A	144	130,138,152(134-128)	130
85	17	*Xgwm0058-6B*	6B		-	114(125-112)	NT
86	18	*Xgwm0133-6B*	6B	3A!, 5B!	-	89,111,117,134(129-142)	118(128-124)
87	19	*Xgwm0907-6B*	6B	6A!	-	136,154(137-135)	139(136-134)
88	1	*Xgwm0443-5A!*	5B		-	135(127-121)	134(209-null)
89	2	*Xgwm0154-5A*	5A		84	102(103-123)	102(102-120)
90	3	*Xgwm0205-5A*	5A, 5D		-	152(153-155)	152(158-152)
91	4	*Xgwm0304-5A*	5A		-	206,216(196-201)	217(202-208)
92	5	*Xgwm0156-5A*	5A		266	316(283-313)	277(300-279)
93	6	*Xgwm1236-5A*	5A		-	?(151-149)	147(127-122)
94	7	*Xgwm0126-5A*	5A		-	193(199-193)	196(196-null)
95	8	*Xgwm0736-5A*	5A, 4B	4B	-	143,174,184(143-176)	187
96	9	*Xgwm0291-5A*	5A		-	?(131-113)	>158(160-158)
97	10	*Xgwm0995-5A*	5A		-	163(131-112)	163(170-167)
98	11	*Xgwm0865-5A*	5A		-	170(156-144)	NT (not yet)
99	1	*Xgwm0234-5B*	5B		-	231(236-232)	241(250-229)
100	2	*Xgwm1284-5B*	5B		-	148(142-137)	150(142-148)
101	3	*Xgwm0066-5B*	5B, 4B		-	145(146-138)	150(158-137)
102	4	*Xbarc4-5B*	5B		-	161(178-187)	(175-190)
103	5	*Xgwm0197-5B*	5B?		112	125(119-125)	NT (not yet)
104	6	*Xgwm0996-5B*	5B		-	192(200-196)	NT (not yet)
105	7	*Xgwm1180-5B*	5B		-	145(147-139)	148(137-135)
106	8	*Xgwm0067-5B*	5B		-	84(84-92)	85(94-92)
107	9	*Xgwm0274-5B!*	1B, 7B	1B	140	167,186(162-166)	179(184-177)&(null-154)
108	10	*Xgwm0843-5B*	5B		80	107(97-103)	107
109	11	*Xgwm0133-5B!*	6B	6B, 3A!	-	111,117,134(138-187)	118(128-124)

Appendix 3

Table 5 (Continued) List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
110	12	*Xbarc74-5B*	5B		-	180(184-177)	-
111	13	*Xgwm0777-5B*	5B		-	110(114-110)	113(117-115)
112	14	*Xgwm0408-5B*	5B		-	175(177-179)	176(182-148)
113	15	*Xgwm0604-5B*	5B		-	117(127-123)	119(133-127)
114	16	*Xbarc140-5B*	5B, 2B, 5D		-	134(134-141)	(139-132)
115	17	*Xbarc142-5B*	5B, 2D, 5A, 6A		-	264(251-288)	(276-299)
116	18	*Xgwm1246-5B*	5B, 5D		134	234(221-236)	232(232-242)
117	19	*Xbarc232-5B*	5B, 5A, 5D		-	191(204-222)	135( )
118	20	*Xgwm0790-5B*	5B, 2D, 6B	6B	194	167,1183,205(228,153)	215(null-150)
119	21	*Xgwm1016-5B*	5B, 6B	6B	114	128,147(145-155)	147(144-158)
120	22	*Xgwm0497-5B!*	1A, 2A, 3D	1A, 2A	85, 92 and many co segr.	84,91,98,153,170(169-null)	>106(null-147)&(137-null)&(null-103)
121	23	*Xgwm0605-5B*	5B	Locus specific	-	138(136-null)	NT (not yet)
122	24	*Xgwm1257-5B!*	6B		202	252(251-247)	244(248-256)
123	25	*Xgwm0118-5B*	5B (5D, 4A)		80	107(107-118)	NT
124	1	*Xgwm1252-5D*	5D		-	114(111-107)	114(103-128)
125	2	*Xgwm0583-5D*	5D		-	164(162-166)	165(165-161)
126	3	*Xgwm1039-5D*	5D		-	136(128-122)	135(119-121)
127	4	*Xgwm0700-5D*	5D		-	140(138-142)	143(136-133)
128	5	*Xgdm0043-5D*	5D, 3A		-	146(136-146)	-
129	6	*Xgwm0292-5D*	5D		-	219(215-213)	220(214-188)
130	7	*Xgwm0805-5D*	5D		157	243(241-245)	243(242-240)
131	8	*Xgwm0931-5D*	5D		-	274(275-277)	274(274-271)
132	9	*Xgwm0982-5D*	5D		134	130(127-125)	131(131-null)
133	10	*Xgdm0063-5D*	5D		128	152(132-148)	-
134	11	*Xgwm1059-5D*	5D		-	205(214-206)	NT (not yet)
135	12	*Xgwm0272-5D*	5D		-	135(131-133)	140(138-140)

Appendix 3

Table 5 (Continued) List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
136	13	*Xgwm0902-5D*	5D		-	147(148-146)	148(146-132)
137	1	*Xgwm1093-4A*	4A		-	114(132-130)	115(141-175)
138	2	*Xgwm0695-4A*	4A		-	265(267-253)	258(252-248)
139	3	*Xgwm0601-4A*	4A		-	163(155-159)	148(152-142)
140	4	*Xbarc106-4A*	4AL			141(135 - 129)	142
141	5	*Xgwm1091-4A*	4A		140	227(213-199)	229(213-null)
142	6	*Xgwm0165-4A*	4A, 4B, 4D	4B, 4D	196 segr. But not clear	186,200,249(192,null)	199(257-261)
143	7	*Xgwm0192-4A*	4A, 4B, 4D	4B, 4D	134 segr. But not clear	130,140,189(132,null)	191(197-201)
144	8	*Xgwm0884-4A*	4A	Locus specific	-	147,161(null-163)	NT (not yet)
145	9	*Xgwm0350-4A*	4A, 7A, 7D		154	?(192-196)	146(215-209)&(178-null)
146	10	*Xgwm1258-4A!*	7A, 7D	7A	195	197(157-171)	196(null,165)&(203-187)
147	11	*Xgwm0160-4A*	4A		co-segr. Loci (181-186)	184(191-208)	184(184-196)
148	12	*Xgwm0832-4A*	4A		-	148(157-147)	147(162-null)
149	13	*Xbarc327-4A*	4AL			248(240 - null)	248
150	14	*Xbarc70-4A*	4AL	7A		183, 200, 204(null - 219)	193
151	15	*Xgwm1169-4A*	4A		-	227(214-223)	NT (not yet)
152	1	*Xgwm0888-4B*	4B		192, 222	183(184-182)	197(195-192)
153	2	*Xgwm0935-4B*	4B, 6B, 2B	6B	103	104,127,144(127-105)	143(103-null)
154	3	*Xgwm0925-4B*	4B		-	185(189-179)	186(183-191)
155	4	*Xgwm0898-4B*	4B		-	106(111-121)	104(107-105)
156	5	*Xgwm0940-4B.1*	4B, 6B, 2B	4B!, 6B	-	138,161,178(160-140)	177(165-153)
157	6	*Xgwm0710-4B*	4B		132, 134	142(139-141)	143(140-142)
158	7	*Xbarc20-4B*	186			189(192 - 196)	186
159	8	*Xgwm0940-4B.2!*	6B, 2B	4B, 6B	-	138,161,178(138-null)	177(165-153)
160	9	*Xgwm0165-4B*	4B, 4A, 4D	4A, 4D	196 segr. But not clear	186,200,249(255-261)	199(257-261)
161	10	*Xgwm0192-4B*	4B, 4A, 4D	4A, 4D	134 segr. But not clear	130,140,189(196-203)	191(197-201)
162	11	*Xgwm0149-4B*	4B		150	164(176-164)	161(161-152)
163	12	*Xgwm1084-4B*	4B		-	189(176-169,182)	179(164,177-144)
164	13	*Xgwm0538-4B*	4B		140 (152, 150 co-segr.)	150,180(165-156) ?	147(168-149)
165	14	*Xgwm0736-4B*	4B, 5A	5A	-	143,174,184(166-172)	187(170-165)
166	1	*Xgwm0859-3A!*	2D		-	175(175-173)	NT (not yet)

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Table 5 (Continued) List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
167	2	*Xgwm0779-3A*	3A, 3B		-	209(210-206)	215(201-209)
168	3	*Xgwm0353-3A*	3A (3D)		-	189(187-161)	NT (not yet)
169	4	*Xgwm0005-3A*	3A		-	180(190-178)	172(171-158)
170	5	*Xgwm0804-3A!*	7D?		90	144(148-144)	NT (not yet)
171	6	*Xgwm0720-3A*	3A		-	165(161-149)	162(146-130)
172	7	*Xgwm0133-3A!*	6B	5B!, 6B	-	89,111,117,134(111-117)	118(128-124)
173	8	*Xgwm1159-3A*	3A		-	196(200-212)	199(197-201)
174	9	*Xgwm1110-3A*	3A		152, 158	196(196-200)	194(201-196)
175	10	*Xgwm0134-3A!*	3B?	3B	-	109(98-108)	NT (not yet)
176	1	*Xgwm0533-3B*	3B, 3B	unlink	-	…(142-null)	226( )
177	2	*Xgwm0389-3B*	3B		-	132(135-115)	130(117-128)
178	3	*Xgwm1171-3B!*	7A, 5A	7A, 2B!	145	146,152,158(null-147)	149(155-null)&(146-144)
179	4	*Xgwm0493-3B*	3B		-	143(144-142)	208(179-171)
180	5	*Xgwm0566-3B*	3B		-	131(125-123)	130(131-122)
181	6	*Xgwm0144-3B*	3B		-	202(240-234)	NT
182	7	*Xgwm0285-3B*	3B		-	244(239-223)	243(222-227)
183	8	*Xgwm1015-3B*	3B		-	154(156-158)	149(141-150)
184	9	*Xgwm0134-3B*	3B?	3A!	-	109(113-122)	NT (not yet)
185	10	*Xgwm1005-3B*	3B		-	152(165-151)	152(168-null)
186	11	*Xgwm0802-3B*	3B		-	132(119-134)	132(138-145)
187	12	*Xgwm0938-3B*	3B		98, 102	154(162-181)	156(132-null)
188	13	*Xgwm0853-3B*	3B	Locus specific	134	121,134(119-null)	112(124-131)
189	14	*Xgwm0751-3B!*	3A	unlink	-	124,141(136-138)	126(129,195-142)
190	15	*Xgwm0655-3B*	3B		-	173(150-172)	177(172-201)
191	16	*Xgwm0340-3B*	3B		-	135(147-137)	132(159-null)
192	17	*Xgwm0247-3B*	3B		-	160(177-166)	158(187-198)
193	18	*Xgwm0181-3B*	3B		75, 99	128(144-135)	158(150-168)

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Table 5 (Continued) List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
194	2	*Xbarc125-3D.1!*	2B, 4B, 5A, 7D	3D	132	130,134,146,150(null,144)	175
195	3	*Xbarc125-3D.2*	3D, 2B, 4B, 5A, 7D	3D!	132	130,134,146,150(148,152)	(145-134)
196	1	*Xgwm0456-3D*	3D		108	132(132-134)	132(138-165)
197	4	*Xgwm0645-3D*	3D		-	149(154-175)	149(161-145)
198	5	*Xgwm0977-3D*	3D		-	108(106-104)	108(103-90)
199	6	*Xgwm0003-3D*	3D		-	80(78-80)	84(84-null)
200	7	*Xgwm1088-3D*	3D		-	253(253-255)	246(250-248)
201	1	*Xgwm0497-2A*	2A, 1A, 3D	1A, 5B!	85, 92 and many co-segr.	84,91,98,153,170(null-143)	>106(137-null)
202	2	*Xbarc212-2A*	2A		-	188(213-205)	190
203	3	*Xgwm0614-2A*	2A	Locus specific	153	?(null-127)	152(126-null)
204	4	*Xgwm1244-2A*	2A		-	139(145-134,142)	NT (not yet)
205	5	*Xgwm0939-2A*	2A (2D)		-	231(234-231)	NT (not yet)
206	6	*Xgwm0726-2A*	2A		-	140(122-130)	136(124/125-129)
207	7	*Xgwm0071-2A.1*	2A, 2A, 3D	2A, 2D!, unlink	96, 128	102,111,131(128-null)	128(126-124)
208	8	*Xgwm1115-2A*	2A		co-segr. Loci (136-122)	98,135(104-100)	132(129-127)
209	9	*Xgwm0122-2A*	2A		-	147(124-132)	149(147-131)
210	10	*Xbarc309-2A*	2A		-	146(146-148)	149
211	11	*Xgwm0339-2A*	2A		-	161(159 or 173-167)	159(162-166)
212	12	*Xgwm0448-2A*	2A		-	243(245-228)	231(203-243)
213	13	*Xgwm0249-2A*	2A, 2D		138	186(171-186)	177(177-180)
214	14	*Xgwm0372-2A*	2A		-	331(288-320)	>329(310-309)
215	15	*Xbarc353-2A*	2A, 2D	6A	224	228(null-234)	226( )
216	16	*Xgwm0630-2A!*	2B		125	106(107-109)	120(120-null)
217	17	*Xgwm0071-2A.2*	2A, 2A, 3D	2A, 2D!, unlink	96, 128	102,111,131(111-null)	128(120-118)
218	18	*Xgwm1045-2A*	2A		-	194(191-185)	189(185-183)

Appendix 3

Table 5 (Continued) List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
219	19	*Xgwm0445-2A*	2A		-	192(188-194)	192(188-190)
220	20	*Xgwm0312-2A*	2A		-	235(199-221)	235(216-219)
221	21	*Xgwm0761-2A*	2A		-	100(82-108)	100(102-108)
222	22	*Xgwm0294-2A*	2A		-	100(83-108)	100(96-102)
223	23	*Xgwm1070-2A*	2A, 2B	2B	70	107,115?(106-120)	120(108-null)
224	24	*Xgwm0356-2A*	2A		183	183,225(218-210)	224(216-null)
225	25	*Xgwm0526-2A!*	2B	2B	141	141,151,155(137-131)	140(148-138)
226	26	*Xgwm0846-2A*	2A, 2B, 2D		90	114,124(130-116)	121(null-94)
227	27	*Xgwm0382-2A*	2A, 2B, 2D		90	114,124(130-116)	115(null-86)
228	28	*Xgwm1136-2A*	2A?/2B?		-	112(112-110)	NT (not yet)
229	29	*Xgwm0739-2A*	2A, 2B	2D!	-	154(null-116)	158(156-160)
230	1	*Xgwm1031-2B*	2B?	Locus specific	-	161(null-161)	NT (not yet)
231	2	*Xgwm1171-2B!*	7A, 5A	7A, 3B!	145	146,152,158(156-null)	149(155-null)&(146-144)
232	3	*Xgwm1128-2B*	2B		-	156(164-178)	156(161-157)
233	4	*Xgwm0322-2B*	2B		96	87,119(87-131)	NT (not yet)
234	5	*Xgwm0148-2B*	2B		-	?(145-147)	163(165-167)
235	6	*Xgwm0128-2B*	2B		-	175(187-189)	NT (not yet)
236	7	*Xgwm0374-2B*	2B		180, 190, 204	220(226-202)	213(210-192)
237	8	*Xbarc91-2B*	2B, 4D		-	122(125-131)	-
238	9	*Xgwm0912-2B*	2B, 2A		191	172,192(183-181)	179, 279(290-282)
239	10	*Xgwm0120-2B*	2B		-	138(147-152)	139(162-174)
240	11	*Xgwm1070-2B*	2B, 2A	2A	70	118(92-72)	120(112-85)
241	12	*Xgwm0526-2B*	2B	2A!	141	141,151,155(159-157)	140(148-138)
242	13	*Xgwm1027-2B*	2B		-	?(131-121)	135(134-120)
243	14	*Xgwm0619-2B*	2B		-	150(160-142)	148(158-152)
244	1	*Xgwm1099-2D*	2D		-	130(138-124)	131(123-135)
245	2	*Xgwm0886-2D*	2D		104	127(113,127,139-115,162)	125(128, 142 - 136, 164)

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Table 5 (Continued) List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
246	3	*Xgdm0035-2D*	2D		-	212(204-220)	
247	4	*Xgwm0702-2D*	2D		-	187(182-200)	151(190-173)
248	5	*Xgwm0071-2D!*	2A, 2A, 3D	2A, 2A, unlink	96, 128	102, 111, 131(null,116)	128(126-124)&(120-118)&(null-101)
249	6	*Xgwm0484-2D*	2D		-	148(167-171)	145(153-143)
250	7	*Xgwm0988-2D*	2D		-	185(159-142)	183(161-173)
251	8	*Xgwm1010-2D*	2D?		-	203(203-193)	NT (not yet)
252	9	*Xgwm0030-2D*	2D, 3A		-	209(222-234)	206(null-156)
253	10	*Xgwm0242-2D*	2D (2A, 2B)		116	140(144-152)	NT (not yet)
254	11	*Xbarc361-2D!*	2B, 5D, 6B		195	195, 227, 268, 237(228-226)	261
255	12	*Xgwm0539-2D*	2D		-	145(136-145)	147(143-157)
256	13	*Xgwm0349-2D*	2D		-	234(216-242)	230(243,null)
257	14	*Xgwm1235-2D*	2D		-	128(124-122)	129(120-142)
258	15	*Xgwm0320-2D*	2D		-	?(261-268)	>263(null-226)
259	16	*Xgwm0228-2D*	2D		-	210(204-214)	NT (not yet)
260	17	*Xgwm0739-2D!*	2A, 2B	2A	-	154(null-154)	158(156-160)&(119-115)
261	1	*Xgwm0772-1A.1*	1A	1A!	202	195(241-null)	NT (not yet)
262	2	*Xgwm0772-1A.2!*	1A	1A	202	195(null-216)	NT (not yet)
263	3	*Xgwm0395-1A*	1A, 1B (1D)	1B, 1B!, 1D	-	137,144,148(144-null)	NT (not yet)
264	4	*Xgwm0752-1A*	1A		-	140(140-136)	125(136-127)
265	5	*Xgwm1148-1A*	1A		-	134(187-177)	133(155-176)
266	6	*Xgwm0357-1A*	1A		-	124(124-122)	123(123-120)
267	7	*Xgwm0497-1A*	1A, 2A, 3D	2A, 5B!	85, 92 and many co-segr.	84,91,98,153,170(154-130)	>106(null-147)
268	8	*Xgwm1139-1A*	1A, 1B		-	236(206-222)	235(226-238)
269	9	*Xgwm0750-1A*	1A		-	219(217-221)	217(null-215)
270	1	*Xgwm1078-1B*	1B		-	145(155-143)	144(142-null)
271	2	*Xgwm0835-1B!*	1A		204	196(196-194)	NT (not yet)

Appendix 3

Table 5 (Continued) List of mapped SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
272	3	*Xgwm0762-1B*	1B		-	145(120-122)	147(130-162)
273	4	*Xgwm0011-1B*	1B		-	195(194-196)	196(202-213)
274	5	*Xgwm0018-1B*	1B		-	186(187-189)	186(188-182)
275	6	*Xgwm1100-1B*	1B		-	223(224-226)	227(225-203)
276	7	*Xgwm0413-1B*	1B		-	94(96-94)	94(91-95b)
277	8	*Xgwm0395-1B.1!*	1B (1A,1D)	1A, 1B, 1D	-	137,144,148(null-150)	NT (not yet)
278	9	*Xgwm0395-1B.2*	1B (1A,1D)	1A, 1B!, 1D	-	137,144,148(148-null)	NT (not yet)
279	10	*Xgwm0131-1B*	1B, 3B		-	131(157-153)	131(165-157)
280	11	*Xgwm0274-1B*	1B, 7B	5B!	140	167,186(186-204)	179(184-177)
281	12	*Xgwm0153-1B*	1B		-	181(181-183)	188(183-195)
282	13	*Xgwm0268-1B*	1B		-	248(204-217)	241(204-198)
283	14	*Xgwm0818-1B*	1B		-	147,163(146-163)	149(164-147)
284	15	*Xgwm0140-1B*	1B		237??	254(293-275)	251?(223-233)
285	1	*Xgwm1291-1D*	1D		-	189(162-189)	187(159-157)
286	2	*Xgwm0395-1D*	1B(1A, 1D)	1A, 1B, 1B!	-	137,44,148(null-137)	NT (not yet)
287	3	*Xgwm0458-1D*	1D		-	112(113-115)	113(115-119)
288	4	*Xgdm0019-1D*	1D, 2D		-	184(210-200)	
289	5	*Xgwm1012-1D*	1D		-	115(120-118)	113(117-115)
290	6	*Xgwm1230-1D*	1D		-	119(119-121)	114(123-112)
291	7	*Xgwm0232-1D*	1D		-	143(142-144)	141(140-144)
292	8	*Xgwm1041-1D*	1D		-	245(226-246)	243(232-null)
293	9	*Xgwm1202-1D*	1D		-	?(274-262)	269(237-208)

Appendix 3

Table 6 List of unlinked SSR loci

		SSR locus	Mapped on ITMI	Other locus	Monomorphic Peak	ChS(HTRI11712-HTRI105)	ChS(Opata-Synthetic)
1	1	*Xgwm1163-6D!*	4D		-	137(144-137)	138(136-136)
2	2	*Xbarc48-6D!*	1A, 4D, 6A, 6B		178	200(195-200)	-
3	3	*Xgwm1009-6D*	6D, 6A	6A	-	97,130(119-127)	95(140?-96)
4	4	*Xbarc273-6D*	6D		-	229(214-229)	225( )
5	5	*Xgdm0098-6D*	6D	Locus specific	-	151(null-151)	
6	1	*Xgwm0944-4D*	4D		-	107(112-108)	NT (not yet)
7	2	*Xgwm0203-4D*	4D, 4A		90	142(138-140)	NT (not yet)
8	3	*Xgwm0165-4D*	4D, 4A, 4B	4A, 4B	196 segr. But not clear	186,200,249(null,198)	199(257-261)
9	4	*Xgwm0192-4D*	4D, 4B, 4A	4A, 4B	134 segr. But not clear	130,140,189(null,136)	191(197-201)
10	1	*Xgwm0071-3B*	3D, 2A, 2A	2A, 2A, 2D!	96, 128	102,111,131(103-null)	128(null-101)
11	2	*Xgwm0155-3A*	3A		-	143(141-149)	141(143-127)
12	3	*Xgwm0161-3D*	3D		-	154(152-154)	154(154-145)
13	4	*Xgwm0480-3A*	3A		-	191(177-173)	188(172-168)
14	5	*Xgwm0533-3B*	3B	3B	-	...(null,120)	147(120-null)
15	6	*Xgwm0663-4A*	4A		-	165(238-161)	163(null-179)
16	7	*Xgwm0719-6A*	6A	6A	-	153,164,189(179-192)	186(155-162)
17	8	*Xgwm0751-3A*	3A	3B!	-	126,141(144-164)	126(129,195-142)
18	9	*Xgwm0961-2D*	2D?		-	179(163-180)	NT (not yet)
19	10	*Xgwm0999-6D*	6D (6B)		160	140(142-140)	NT (not yet)
20	11	*Xgwm1229-3A*	3A		-	149(151-155)	143

ChS- cultivar Chinese Spring, extra loci indicated by cell in yellow color, first time mapped loci by red color and extra loci of the first time mapped loci by red color in yellow cell

First column shows number of unlinked loci,

First column shows number of loci in the linkage map of the present study and in total 293 loci, red and yellow colored cells in second column indicate that these loci probably belong to chromosomes 6D and 4D.

Appendix 3

Table 7 SSR Primer pairs with some difficulties in scoring

	GWM No.	Tm	Length(bp) in ChS	Length(bp) in HTRI 11712 x HTRI 105	Mapped	Chromosome
1	GWM0169	60 °C	196	189, 200	ITMI	6A
2	GWM0334	50 °C	123	115, 121	ITMI	6A
3	GWM0638	60 °C	145	151, 143	ITMI	3A
4	GWM0721	60 °C	146	148, 119	ITMI	2D
5	GWM0767	50 °C	150	162, 166	ITMI	7B
6	GWM0815	55 °C	195	205, 215	ITMI	2D
7	GWM0892	60 °C	150	150, 146	ITMI	3D
8	GWM1025	55 °C	140	null, 145	ITMI	7B
9	GWM1047	60 °C	291	258, 268	ITMI	3D
10	GWM1108	60 °C	~>173	151, 197	ITMI	5B
11	GWM1120	50 °C	193	201, 162	ITMI	1B
12	GWM1204	60 °C	283	273, 278	ITMI	2D
13	GWM1223	60 °C	150	130, 123	ITMI	3A
14	GWM1243	60 °C	142	117, 144	ITMI	3D

ChS- cultivar Chinese Spring

Appendix 4

Co-dominant and dominant scoring:

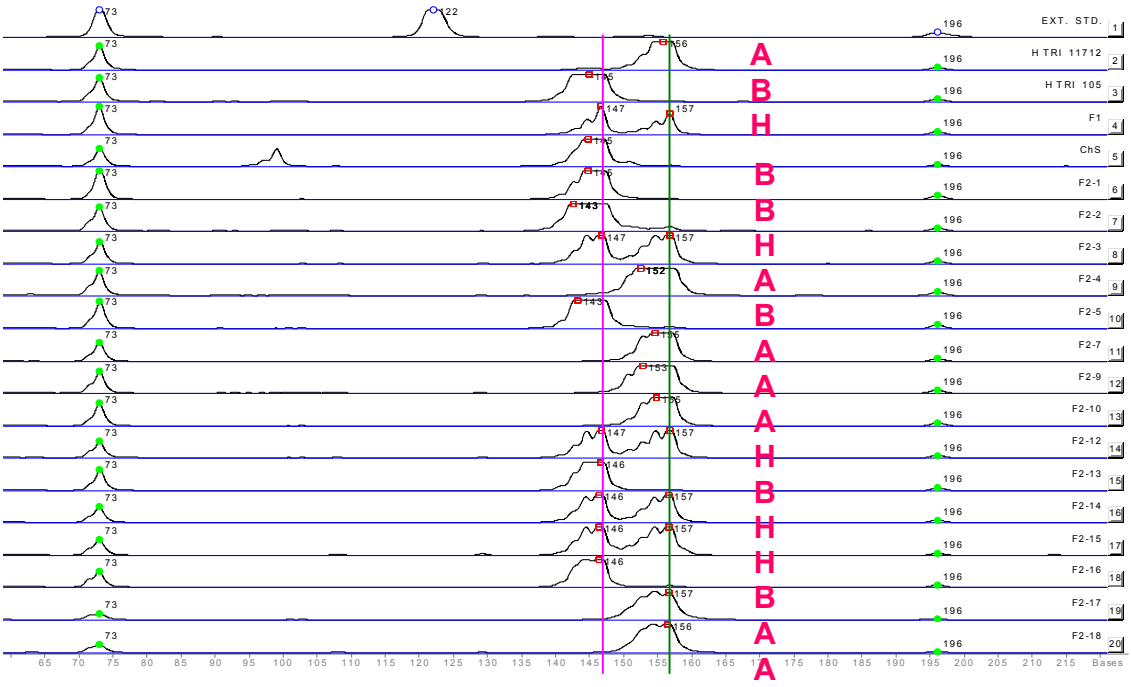


Figure1 Co-dominant scoring of alleles at a SSR locus

Alleles at a co-dominant SSR locus in F_2 population were scored as A, B, and H to mark F_2 individuals like parent A (HTRI 11712), parent B (HTRI 105), and heterozygote, respectively.

Dominant scoring were applied in the three following cases: several peaks genetate from a primer pair, at locus bearing null allele, and at individuals showing of unclear peak.
At locus bearing null allele in F_2 population, the heterozygous individuals can not be detected from one of the homozygous parent. At individuals having unclear peak it is difficulte to distinguish between the heterozygous and homozygous individuals.

Dominant SSR marker was scored as following: A and C scoring when parent A had null allele. Therefore, individuals showing null allele are scored as A. C means the individuals correspond to the parent B or they are heterozyguse. B and D scoring when parent B had null allele. Therefore, individuals showing null allele are scored as B. D means the individuals correspond to the parent A or they are heterozygous. Finally '-' was used for missing data.

In the case of several peaks from a primer pair, each peak was scored separately as dominant marker and after constructing the linkage groups, alleles which co-segregate were considered as alleles of the same loci and then their separate dominant scoring were converted to the co-dominant score (Collard et al., 2005).

Appendix 4

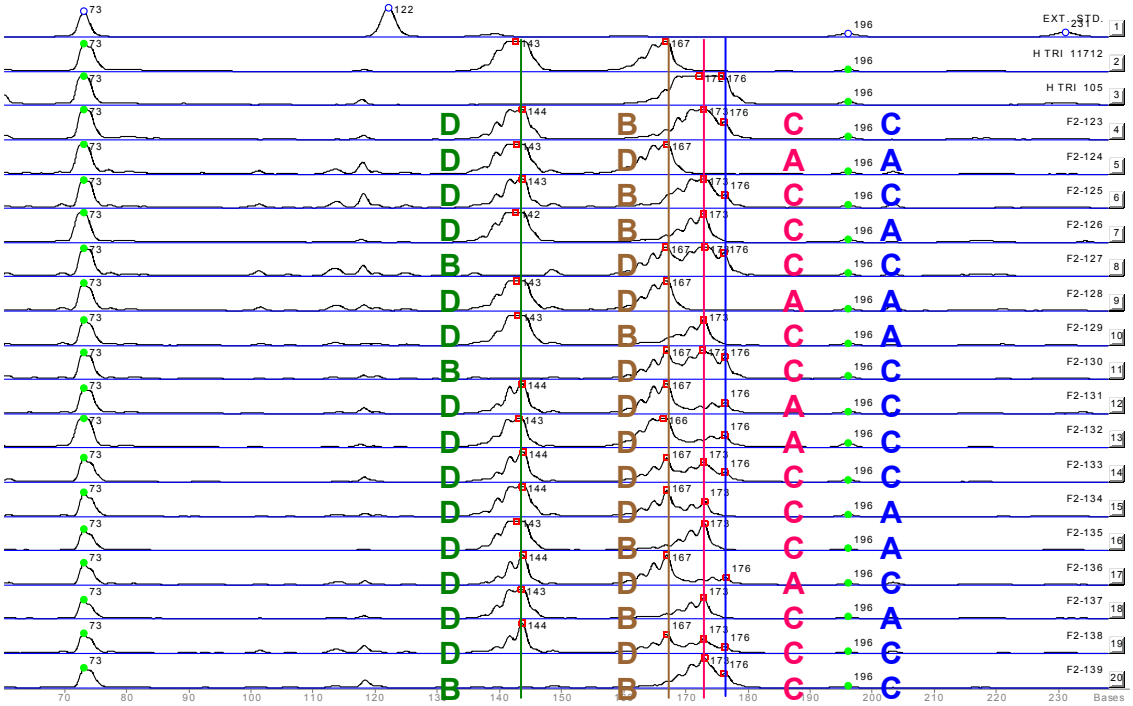


Figure 2 Dominant scoring: (A or H) = D, (B or H) = C, for the four separate SSR alleles: *Xgwm736(143,Null)*, *Xgwm736(167,Null)*, *Xgwm736(Null,173)*, and *Xgwm736(Null,176)*

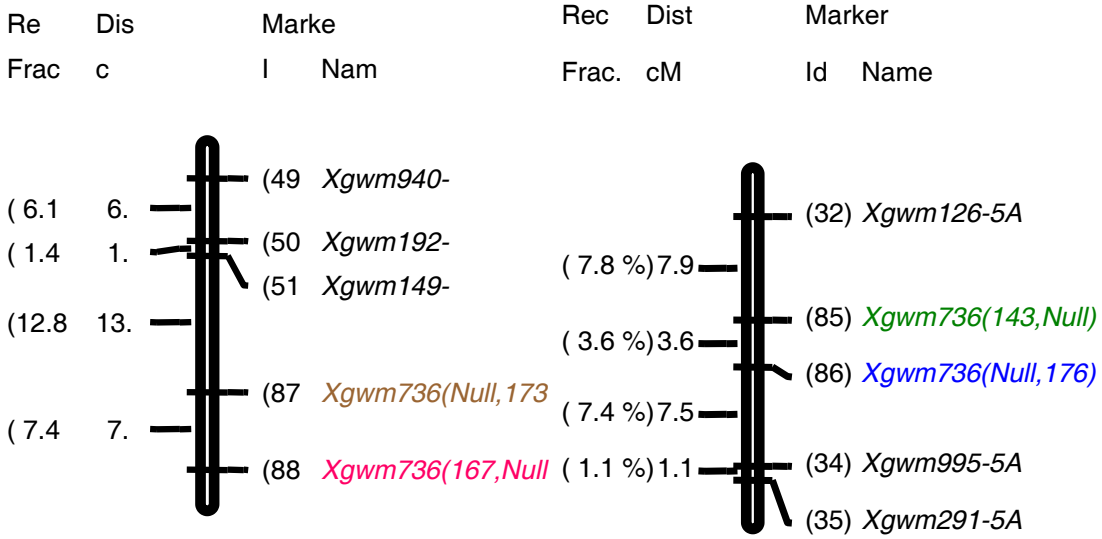


Figure 3 Co-segregation of alleles of the same locus. *Xgwm736(167,Null)* co-segregation with *Xgwm736(Null,173)* whereas *Xgwm736(143,Null)* co-segregated with *Xgwm736(Null,176)*

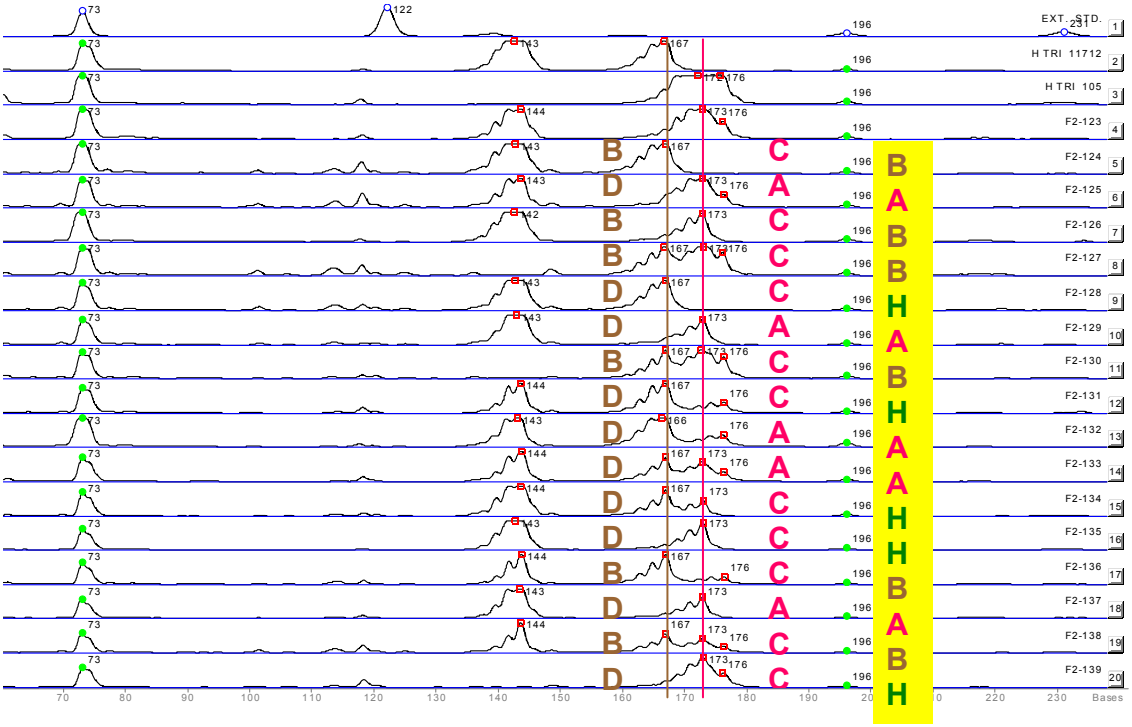


Figure 4 Conversion from doninant scoring of alleles *Xgwm736(167,Null)* and *Xgwm736(Null,173)* to co-dominant scoring as *Xgwm736(167,173)* because of their co-segregation

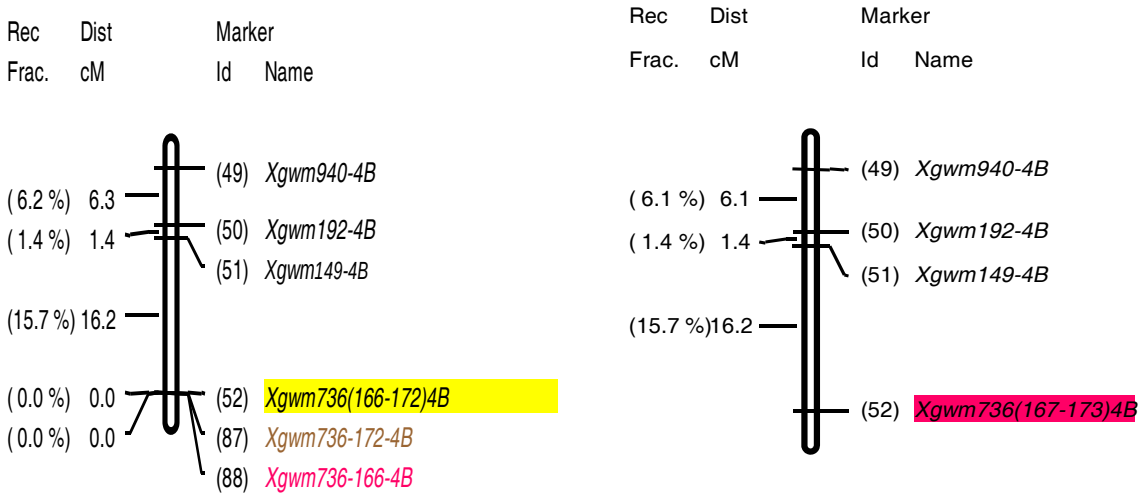


Figure 5 Co-segregation of dominant loci *Xgwm736(167,Null)* and *Xgwm736(Null,173)* with co-dominant locus as *Xgwm736(167,173)* confirmed the conversion from from doninant scoring to the co-dominant scoring. Finaly the two loci from doninant scoring were replaced by locus from co-diminant scoring.

Appendix 4

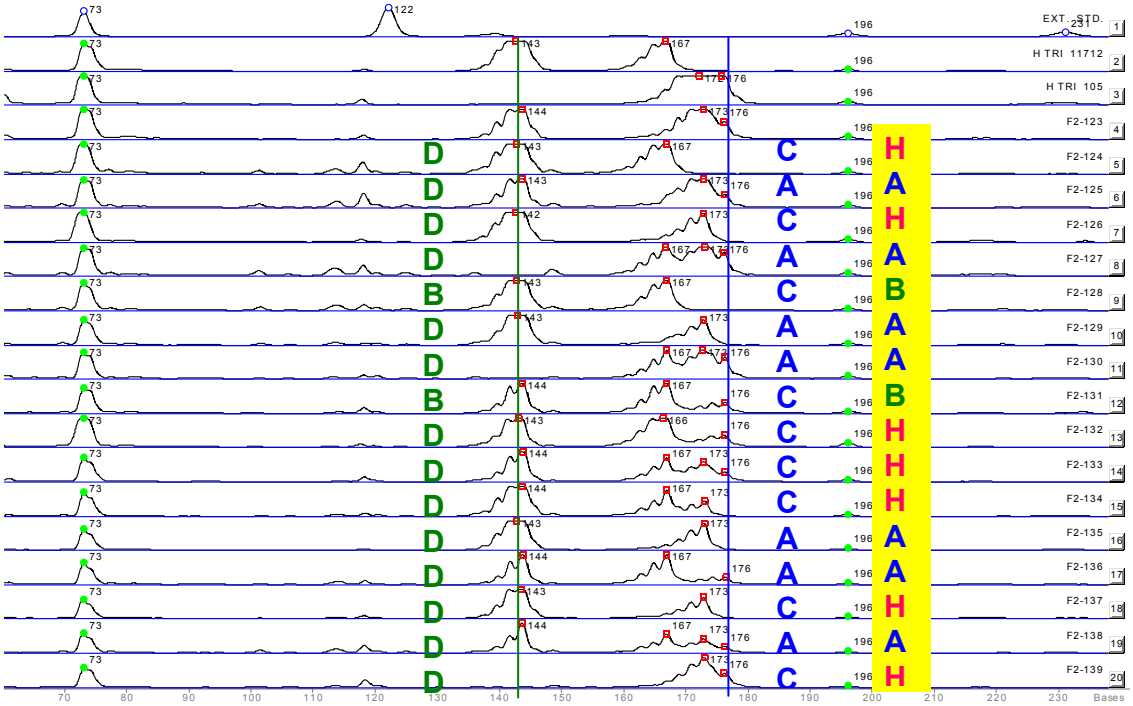


Figure 6 Conversion from doninant scoring of alleles *Xgwm736(143,Null)* and *Xgwm736(Null,176)* to co-dominant scoring as *Xgwm736(143,176)*

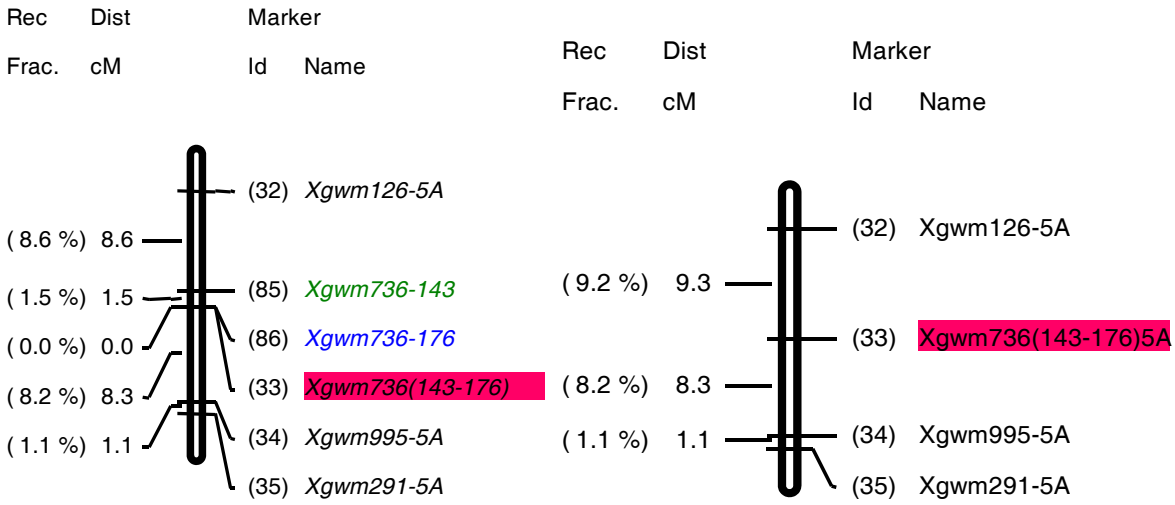


Figure 7 Co-segregation of dominant loci *Xgwm736(143,Null)* and *Xgwm736(Null,176)* with co-dominant locus as *Xgwm736(143,176)* confirmed the conversion from from doninant scoring to the co-dominant scoring. Finaly the two loci from doninant scoring were replaced by locus from co-diminant scoring.

Appendix 5

Details of linkage map:

1-Chromosome point of view

Chromosome 1A:

There were nine loci Figure 3.3, and Table 3.2, about 3.1 percent of the total mapped loci, including 3 dominant loci and with the map length of 99.1 cM. There were 18 missing data (1.4%), out of 1285 genotypic data points. Two loci, *Xgwm0772-1A.1*, and *Xgwm0497-1A* were distorted and five out these nine loci were locus specific, four loci were multiple loci. The following three loci *Xgwm0772-1A.1, Xgwm0772-1A.2!*, and *Xgwm0395-1A* were mapped for first time. Simultaneously, locus *Xgwm0772-1A.2!* was considered also as new locus by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 1B:

There were 15 loci Figure 3.3, and Table 3.2, about 5.2 percent of the total mapped loci, including 2 dominant loci and with the map length of 163.8 cM. There were 29 missing data (1.3%), out of 2145 genotypic data points. None of them were distorted and 10 out these 15 loci were locus specific, three loci were multiple loci and finally two loci were detected extra loci. The following three loci *Xgwm0835-1B*, *Xgwm0395-1B.1!*, *Xgwm0395-1B.2* were mapped for first time. Simultaneously, loci *Xgwm0835-1B,* and *Xgwm0395-1B.1!* were considered as extra loci by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007)

Chromosome 1D:

There were nine loci Figure 3.3, and Table 3.2, about 3.1 percent of the total mapped loci, including one dominant locus and with the map length of 88.9 cM. There were eight missing data (0.6%), out of 1287 genotypic data points. Only one locus, *Xgwm1202-1D,* was distorted and eight out these nine loci were locus specific, only one locus *Xgwm0395-1D* was multiple loci which was detected simultaneously as first time mapped locus.

Chromosome 2A:

There were 29 loci Figure 3.3, and Table 3.2, about 10 percent of the total mapped loci, including six dominant loci and with the map length of 179.1 cM. There were 40 missing data (0.9%), out of 4147 genotypic data points. One locus of them *Xgwm1045-2A* was distorted and 22 out these 29 loci were locus specific, seven loci were multiple loci. The

following three loci *Xgwm1244-2A, Xgwm0939-2A,* and *Xgwm1136-2A* were mapped for first time. Loci *Xgwm0630-2A,* and *Xgwm0526-2A!* were considered as extra loci.

Chromosome 2B:

There were 14 loci Figure 3.3, and Table 3.2, about 4.8 percent of the total mapped loci, including 2 dominant loci and with the map length of 167.1 cM. There were 14 missing data (0.7%), out of 2002 genotypic data points. None of the loci were distorted, 11 out these 14 loci were locus specific, and three loci were multiple loci. There were three loci *Xgwm1031-2B*, *Xgwm0322-2B*, and *Xgwm0128-2B* which were mapped for first time. Locus *Xgwm1171-2B* was considered as extra locus by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 2D:

There were 17 loci Figure 3.3, and Table 3.2, about 5.9 percent of the total mapped loci, including two dominant loci and with the map length of 192.4 cM. There were 29 missing data (1.2%), out of 2431 genotypic data points. One locus of them like *Xgwm0228-2D* was distorted and 15 out these 17 loci were locus specific, two loci were multiple loci. The following three loci *Xgwm1010-2D*, *Xgwm0242-2D*, and *Xgwm0228-2D* were mapped for first time. Loci *Xgwm0071-2D!, Xbarc361-2D*, and *Xgwm0739-2D!* were considered as extra loci by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 3A

There were 10 loci Figure 3.3, and Table 3.2, about 3.4 percent of the total mapped loci, without any dominant loci and with the map length of 57.3 cM. There were 17 missing data (1.2%), out of 1430 genotypic data points. Two of them *Xgwm0353-3A*, and *Xgwm1110-3A* were distorted and 14 out these 18 loci were locus specific, four loci were multiple loci. There were three loci *Xgwm0353-3A, Xgwm0804-3A!,* and *Xgwm0134-3A!* which were mapped for first time. Loci *Xgwm0859-3A, Xgwm0804-3A, Xgwm0133-3A!,* and *Xgwm0134-3A!* were considered as extra loci by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 3B:

There were 18 loci Figure 3.3, and Table 3.2, about 6.2 percent of the total mapped loci, including three dominant loci and with the map length of 226.6 cM. There were 23 missing data (0.9%), out of 2574 genotypic data points. Three of these were distorted and 14 out

these 18 loci were locus specific, four loci were multiple loci. There were two loci *Xgwm0144-3B*, and *Xgwm0134-3B* which were mapped for first time. Loci *Xgwm1171-3B,* and *Xgwm0751-3B!* were considered as extra loci.

Chromosome 3D

There were seven loci Figure 3.3, and Table 3.2, about 2.4 percent of the total mapped loci, including one dominant locus and with the map length of 174.3 cM. There were 10 missing data (1%), out of 1001 genotypic data points. None of them was distorted and five out these seven loci were locus specific, two loci were multiple loci. There was not any locus as for first time mapped. Locus *Xbarc125-3D.1!* was considered as extra locus by comparing with consensus map (Somers et al., 2004).

Chromosome 4A

There were 15 loci Figure 3.3, and Table 3.2, about 5.1 percent of the total mapped loci, including three dominant loci and with the map length of 175.1 cM. There were 25 missing data (1.2%), out of 2,145 genotypic data points. Two of them *Xgwm0165-4A*, and *Xgwm0192-4A* were distorted and nine out these 15 loci were locus specific, four loci were multiple loci. There were two loci *Xgwm0884-4A* and *Xgwm1169-4A* which were mapped for first time. Locus *Xgwm1258-4A* was considered as extra locus by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 4B:

There were 14 loci Figure 3.3, and Table 3.2, about 4.5 percent of the total mapped loci, including 3 dominant loci and with the map length of 46.2 cM. There were 17 missing data (0.8%), out of 2002 genotypic data points. None of them were distorted and eight out these 14 loci were locus specific, six loci were multiple loci. There was not any locus which was mapped for first time. Locus *Xgwm0940-4B.2!* was considered as extra locus by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 5A:

There were 11 loci Figure 3.3, and Table 3.2, about 3.8 percent of the total mapped loci, without dominant loci and with the map length of 77.8 cM and 22.100 cM and a gap spanning more then 50 cM. These regions which were found to be genetically independent on the current map suggesting the occurrence of a recombination hot spot in the cross between

parental lines used in the present study. There were 34 missing data (2.2%), out of 1573 genotypic data points. None of them was distorted and 10 of these loci were locus specific, only one locus was multiple loci. There was one locus *Xgwm0865-5A* which was mapped for first time. Locus *Xgwm0443-5A* was considered as extra locus.

Chromosome 5B:

There were 25 loci Figure 3.3, and Table 3.2, about 8.6 percent of the total mapped loci, with 2 dominant loci and with the map length of 187.3 cM. There were 46 missing data (1.3%), out of 3575 genotypic data points. Two of them *Xgwm0605-5B,* and *Xgwm1257-5B* were distorted and 20 of these loci were locus specific, 5 were multiple loci. There were four loci *Xgwm0197-5B, Xgwm0996-5B, Xgwm0605-5B,* and *Xgwm0118-5B* which were mapped for first time. Loci *Xgwm0274-5B*, *Xgwm0133-5B!*, *Xgwm0497-5B!*, and *Xgwm1257-5B* were considered as extra loci by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 5D:

There were 13 loci Figure 3.3, and Table 3.2, about 4.5 percent of the total mapped loci, without dominant loci and with the map length of 223.0 cM. There were 14 missing data (0.7%), out of 1859 genotypic data points. One of them *Xgwm0583-5D* was distorted and all these 12 loci were locus specific, there was no multiple loci. There was one locus *Xgwm1059-5D* which was mapped for first time.

Chromosome 6A:

There were 14 loci Figure 3.3, and Table3.2, about 4.8 percent of the total mapped loci, with two dominant loci and with the map length of 135.8 cM. There were 23 missing data (1.5%), out of 2717 genotypic data points. Two of them *Xgwm1009-6A*, and *Xgwm1210-6A* were distorted and seven of these loci were locus specific and five were as multiple loci. There were three loci *Xgwm1210-6A, Xgwm0530-6A* and *Xgwm0799-6A* which were mapped for first time. Locus *Xgwm0907-6A* was considered as extra locus by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 6B

There were 19 loci Figure 3.3, and Table 3.2, about 6.6 percent of the total mapped loci, with four dominant loci and with the map length of 70.5 cM. There were 17 missing data (0.6%), out of 2717 genotypic data points. Two of them *Xgwm0825-6B*, and *Xgwm0940-6B* were

distorted and 12 of these loci were locus specific, seven were multiple loci. There were five loci *Xgwm0244-6B*, *Xgwm0313-6B*, *Xgwm0390-6B*, *Xgwm1016-6B* and *Xgwm0058-6B* which was mapped for first time. Locus *Xgwm0608-6B,* was considered as extra locus.

Chromosome 7A:

There were 22 loci Figure 3.3, and Table 3.2, about 7.2 percent of the total mapped loci, with two dominant loci and with the map length of 186.3 cM. There were 34 missing data (1%), out of 3146 genotypic data points. One of them *Xgwm1011-7A* was distorted, 17 of these loci were locus specific, and five were multiple loci. There were three loci *Xgwm1069-7A, Xgwm0900-7A,* and *Xgwm1126-7A* which was mapped for first time. Loci *Xgwm1044-7A*, *Xgwm1011-7A*, and *Xgwm0344-7A* were considered as extra loci by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 7B:

There were 16 loci Figure3.3, and Table 3.2, about 5.5 percent of the total mapped loci, with one dominant locus and with the map length of 65.4 cM and 21.2 cM and a gap spanning more then 50 cM. These regions which were found to be genetically independent on the current map suggesting the occurrence of a recombination hot spot in the cross between parental lines used in the present study. There were 23 missing data (1%), out of 2288 genotypic data points. Three of them *Xgwm1173-7B, Xgwm0297-7B,* and *Xgwm0963-7B* were distorted and all the 16 loci were locus specific, there was no loci as multiple loci. There were three loci *Xgwm0941-7B*, and *Xgwm0393-7B* which were mapped for first time. There was not any extra locus to compare with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Chromosome 7D:

There were 16 loci Figure 3.3, and Table 3.2, about 5.5 percent of the total mapped loci, with one dominant locus and with the map length of 150.9 cM. There were 22 missing data (1%), out of 2288 genotypic data points. Seven of them *Xgwm0834-7D, Xgwm0885-7D*, *Xgwm1002-7D*, *Xbarc126-7D*, *Xgwm1242-7D*, *Xgwm1168-7D*, and *Xgwm1276-7D* were distorted and 15 were locus specific, and one locus was as multiple loci. There were two loci *Xgwm1205-7D,* and *Xgwm0740-7D*, which were mapped for the first time. Loci *Xgwm1205-7D*, *Xgwm0834-7D*, and *Xgwm0746-7D* were considered as extra loci by comparing with the ITMI map (Röder et al., 1998b; Ganal and Röder, 2007).

Appendix 5

2-Homoeologous point of view

Homoeologous group 1

With 33 mapped loci Table 3.3 bearing 11.5 percent of all mapped loci, including 27 co-dominant and six dominant loci, had a total length of 351.8 cM with three linkage groups corresponding to all the three chromosomes of this genome. The average of chromosome length and number of loci per chromosome was 117.3 cM and 11, respectively. Therefore, there was on average one locus per each 10.7 cM. From 33 mapped loci there were seven loci and three loci as first time and extra loci, respectively.

Table 3.3 Genetic linkage map, Homoeologous point of view

Linkage group	Total mapped loci	No. co-dominant loci	No. dominant loci	Map length (cM)	Marker density (cM)	No. loci first time mapped	percent of mapped loci	missing data	No. extra loci
Homoeologous group 1	33	27	6	351.8	10.7	7	11.5	55	3
Homoeologous group 2	60	50	10	538.6	9.0	9	20.8	83	6
Homoeologous group 3	35	32	3	458.2	13.1	6	12.2	50	7
Homoeologous group 4	29	23	6	221.3	7.6	2	8.7	42	2
Homoeologous group 5	49	47	2	511.4	10.3	6	17.0	94	6
Homoeologous group 6	33	27	6	206.3	6.3	8	11.5	40	2
Homoeologous group 7	54	51	3	423.8	7.8	8	18.4	79	6
Total	**293**	**257**	**36**	**2711.4**	**9.2**	**46**	**100**	**443**	**32**

Homoeologous group 2

With 60 mapped loci Table 3.3 bearing 20.8 percent of all mapped loci, including 50 co-dominant and 10 dominant loci, had a total length of 538.6 cM with three linkage groups associated with all the three chromosomes of this genome. The average of chromosome length and number of loci per chromosome was 179.4 cM and 20, respectively. Therefore, there was on average one locus per each nine cM. From 60 mapped loci there were nine loci and six loci as first time and extra loci, respectively.

Homoeologous group 3

With 35 mapped loci Table 3.3 bearing 12.2 percent of all mapped loci, including 32 co-dominant and three dominant loci, had a total length of 458.2 cM with three linkage groups associated with the three chromosomes of this genome. The average of chromosome length and number of loci per chromosome was 152.7 cM and 11.6, respectively. Therefore, there

was on average one locus per each 13.1 cM. From 35 mapped loci there were six loci and seven loci as first time and extra loci, respectively.

Homoeologous group 4

With 29 mapped loci Table 3.3 bearing 8.7 percent of all mapped loci, including 23 co-dominant and six dominant loci, had a total length of 221.3 cM with two linkage groups associated with the 4A and 4D chromosomes of this genome. The average of chromosome length and number of loci per chromosome was about 110.6 cM and 14.5, respectively. Therefore, there was on average one locus per each eight cM. From 25 mapped loci there were two first time mapped loci and two extra loci.

Homoeologous group 5

With 49 mapped loci Table 3.3 bearing 17 percent of all mapped loci, including 47 co-dominant and two dominant loci, had a total length of 511.4 cM with three linkage groups associated with the three chromosomes of this genome. The average of chromosome length and number of loci per chromosome was 170.5 cM and 16.3, respectively. Therefore, there was on average one locus per each 10.3 cM. From 49 mapped loci there were six loci and six loci as first time and extra loci, respectively.

Homoeologous group 6

With 33 mapped loci Table 3.3 bearing 11.5 percent of all mapped loci, including 27 co-dominant and six dominant loci, had a total length of 351.94 cM with three linkage groups associated with the 6A and 6B chromosomes of this genome. The average of chromosome length and number of loci per chromosome was 206.3 cM and 16.5, respectively. Therefore, there was on average one locus per each 6.3 cM. From 33 mapped loci there were eight loci and two loci as first time and extra loci, respectively.

Homoeologous group 7

With 54 mapped loci Table 3.3 bearing 18.4 percent of all mapped loci, including 51 co-dominant and three dominant loci, had a total length of 423.8 cM with three linkage groups associated with the three chromosomes of this genome. The average of chromosome length and number of loci per chromosome was 141.2 cM and 17.6, respectively. Therefore, there was on average one locus per each eight cM. From 53 mapped loci there were eight loci and six loci as first time and extra loci, respectively.

Appendix 6

Photos from the experiments

Figure 1 Segregating of the $F_{2:3}$ families for awns in greenhouse experiment 2007

Figure 2 Segregating of the $F_{2:3}$ families for spike length in the greenhouse experiment 2007

Figure 3 Segregating of the $F_{2:3}$ families for No. spikes per plant (left) and spike characters (right) in the greenhouse experiment 2007

Appendix 6

Figure 4 Greenhouse experiment 2007, grain filling period (left) and harvesting time (right)

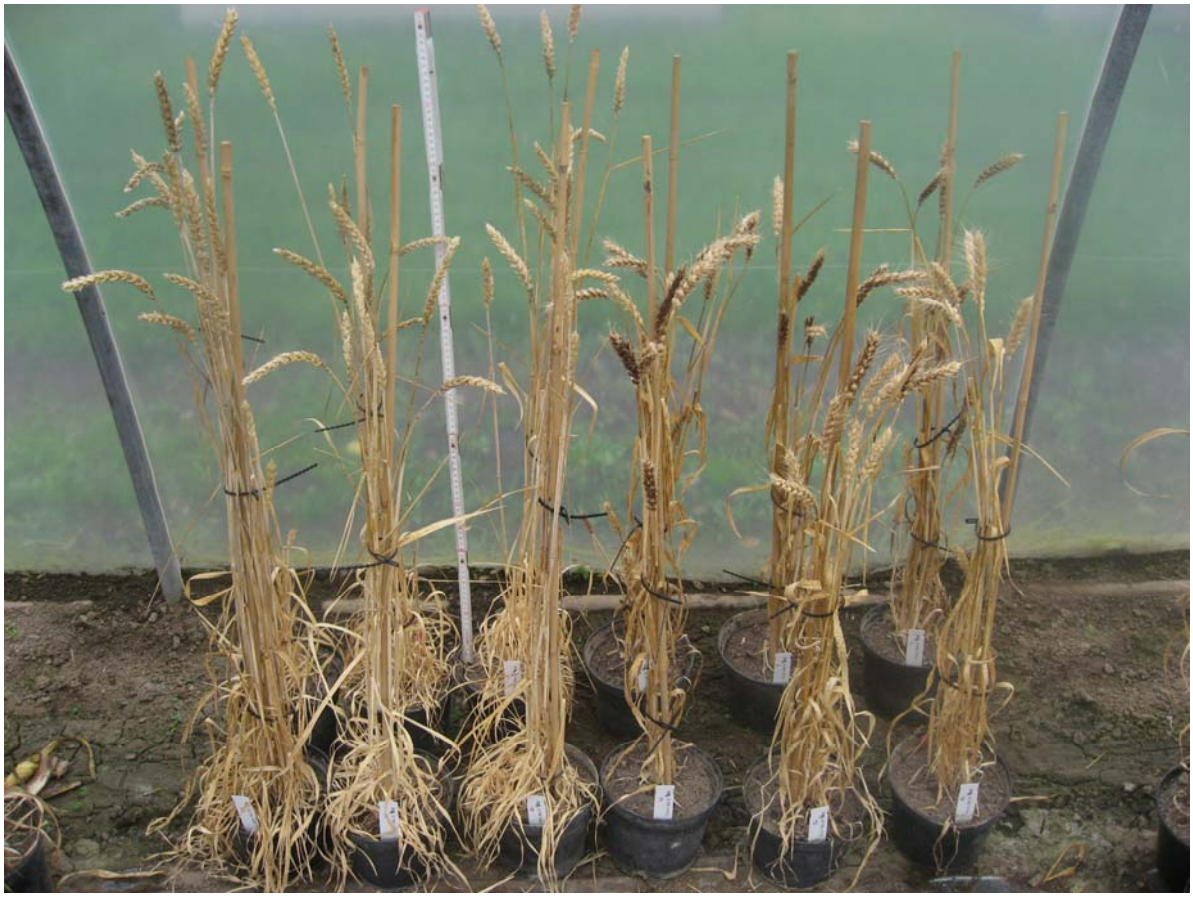


Figure 5 Parent B and parent A (the first six plants in the left and right, respectively) in the greenhouse experiment 2007 showed differences on plant height, spike color, and No. of unfertile tiller per plant

Appendix 6

Figure 6 Greenhouse experiment 2007

Figure 7 Field experiment 2005

Appendix 6

Figure 8 Data recording in selection room (above and bottom), IPK-Gatersleben, Germany

Appendix 6

Figure 9 DNA extraction

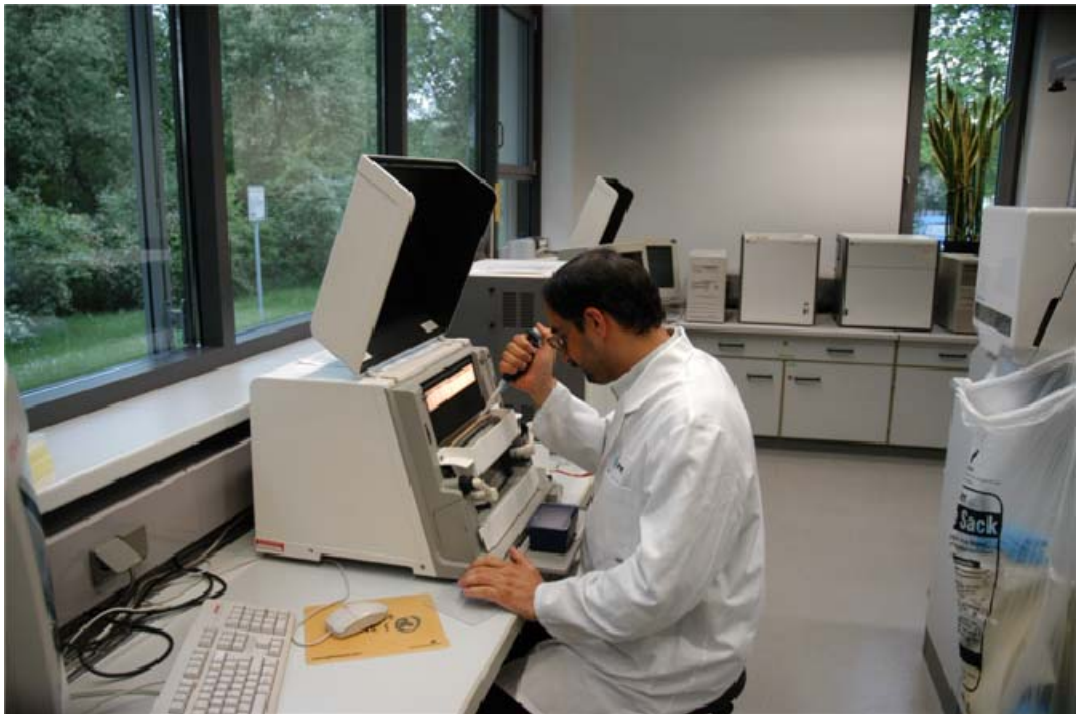


Figure 10 Electrophoresis with ALFexpress

Appendix 7

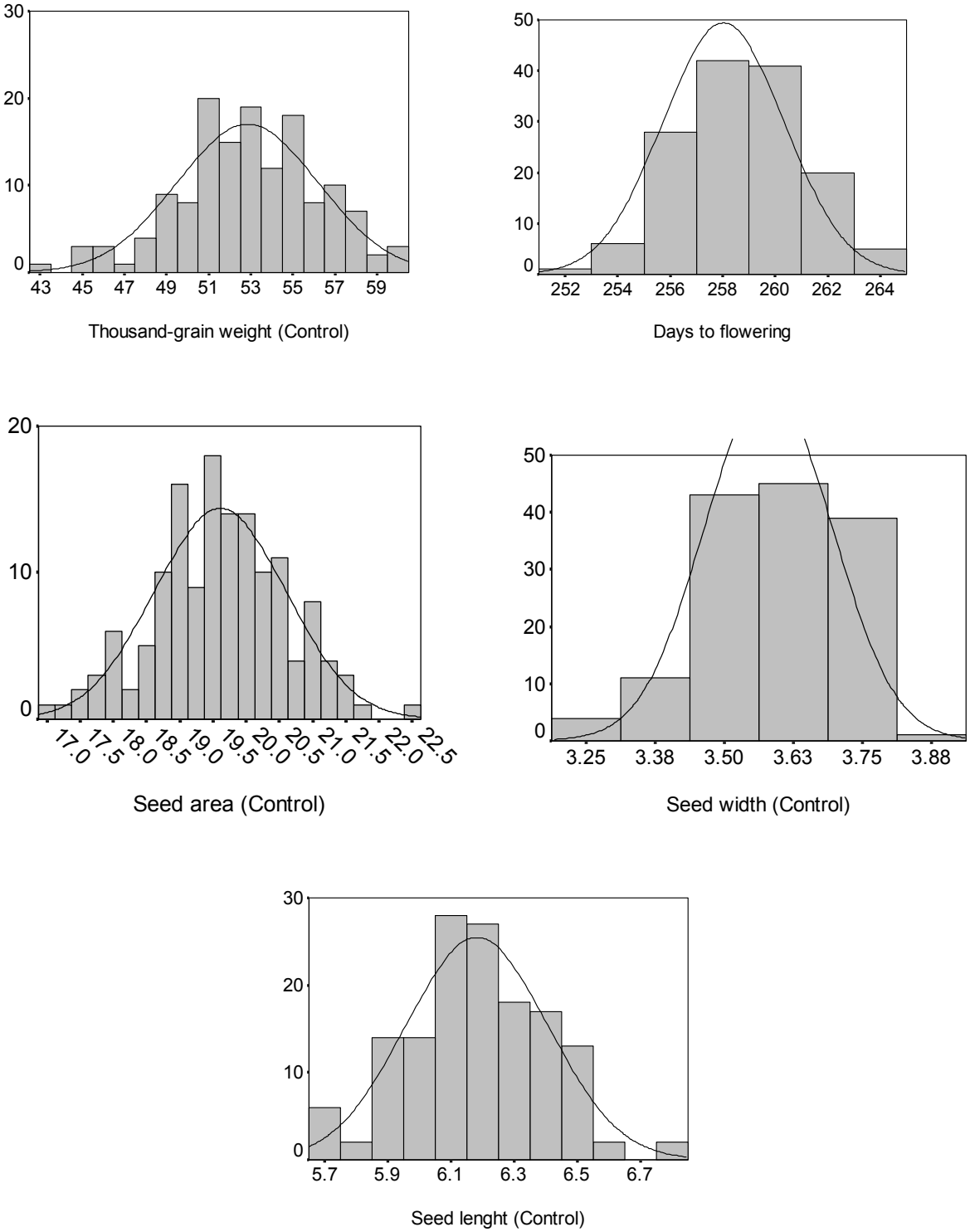


Figure 11 Histograms of the traits recorded at the field experiment under control condition in 2004

Appendix 7

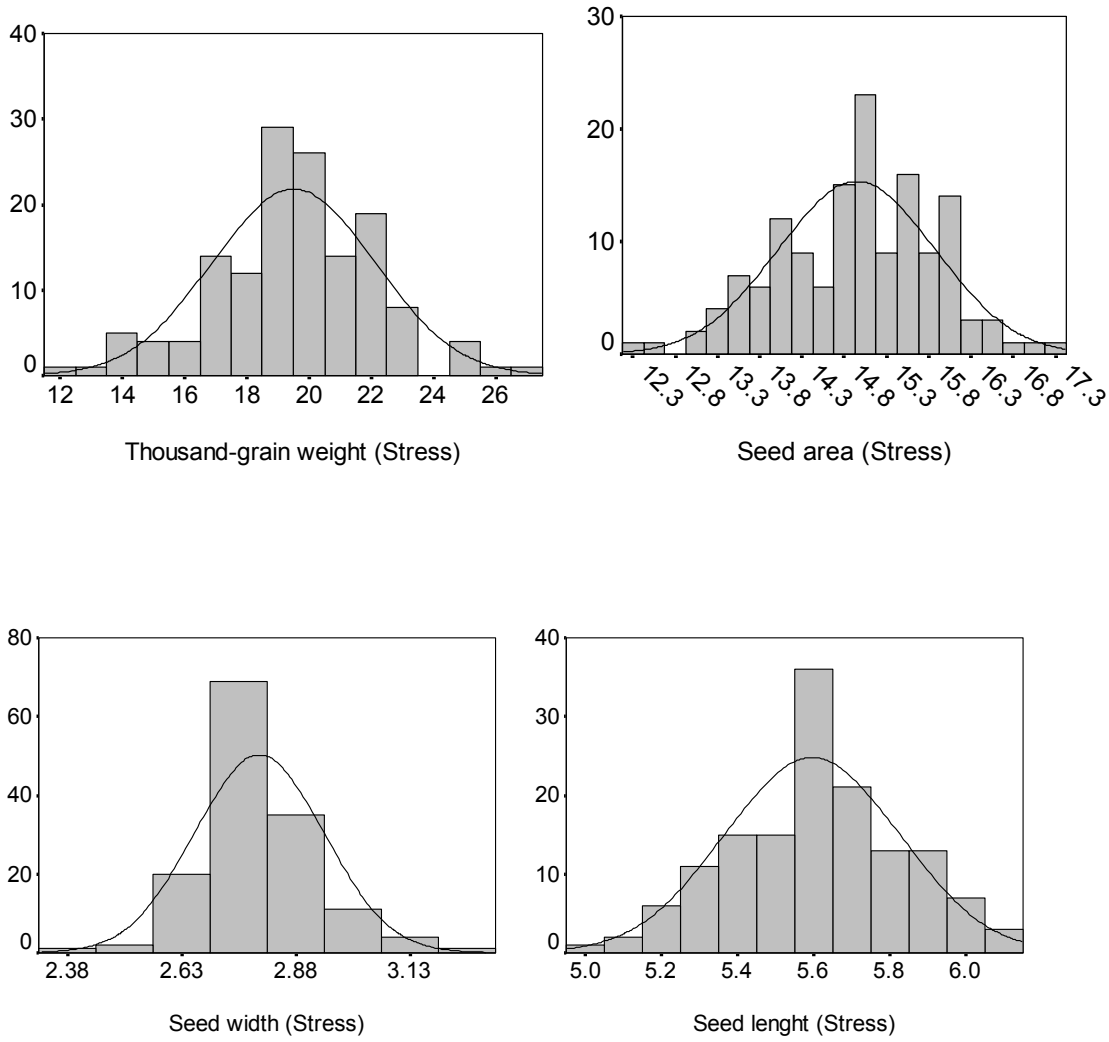


Figure 12 Histograms of the traits recorded at the field experiment under drought stress condition in 2004

Appendix 7

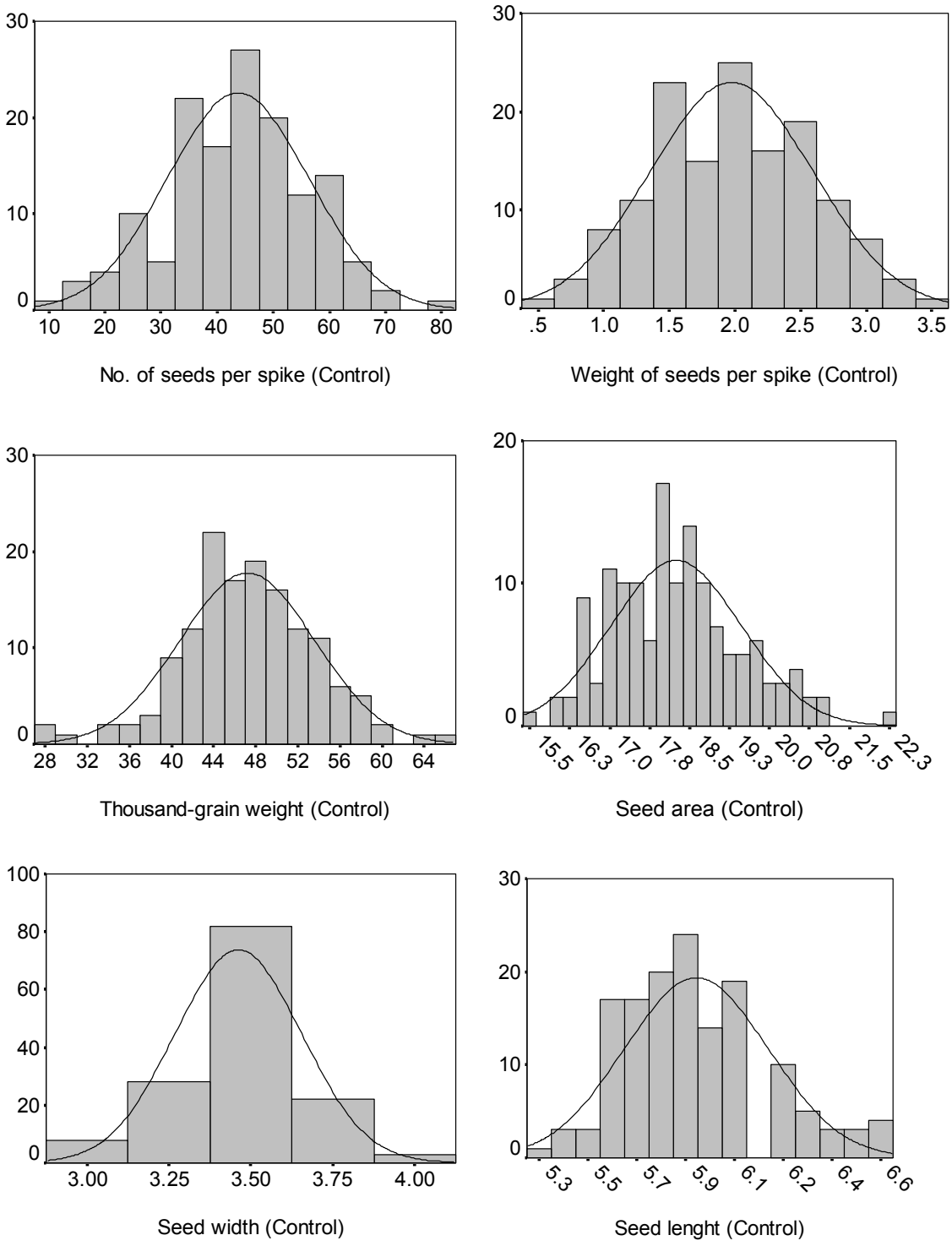


Figure 13 Histograms of the traits recorded at the greenhouse experiment under control condition in 2004

Appendix 7

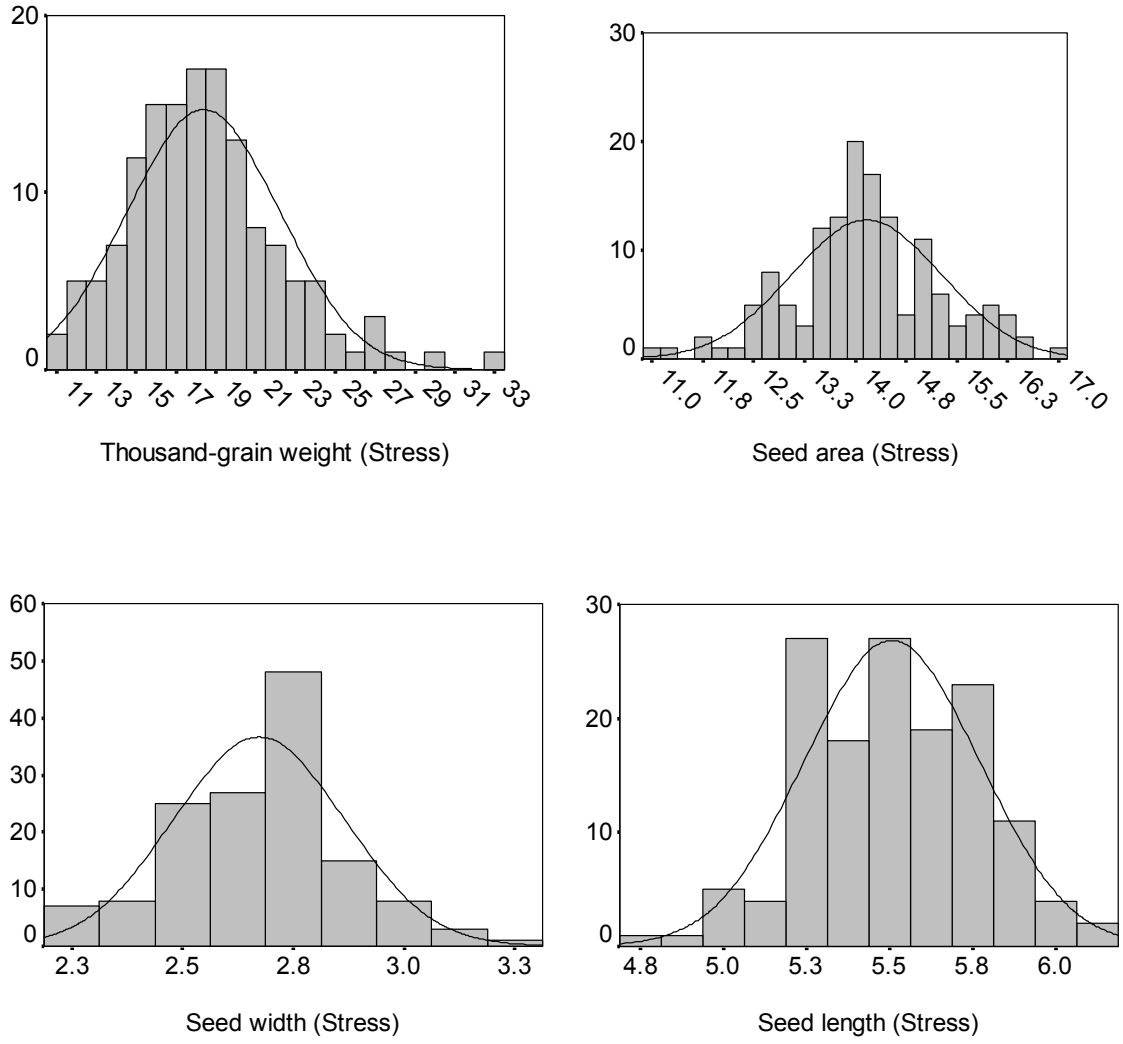


Figure 14 Histograms of the traits recorded at the greenhouse experiment under drought stress condition in 2004

Appendix 7

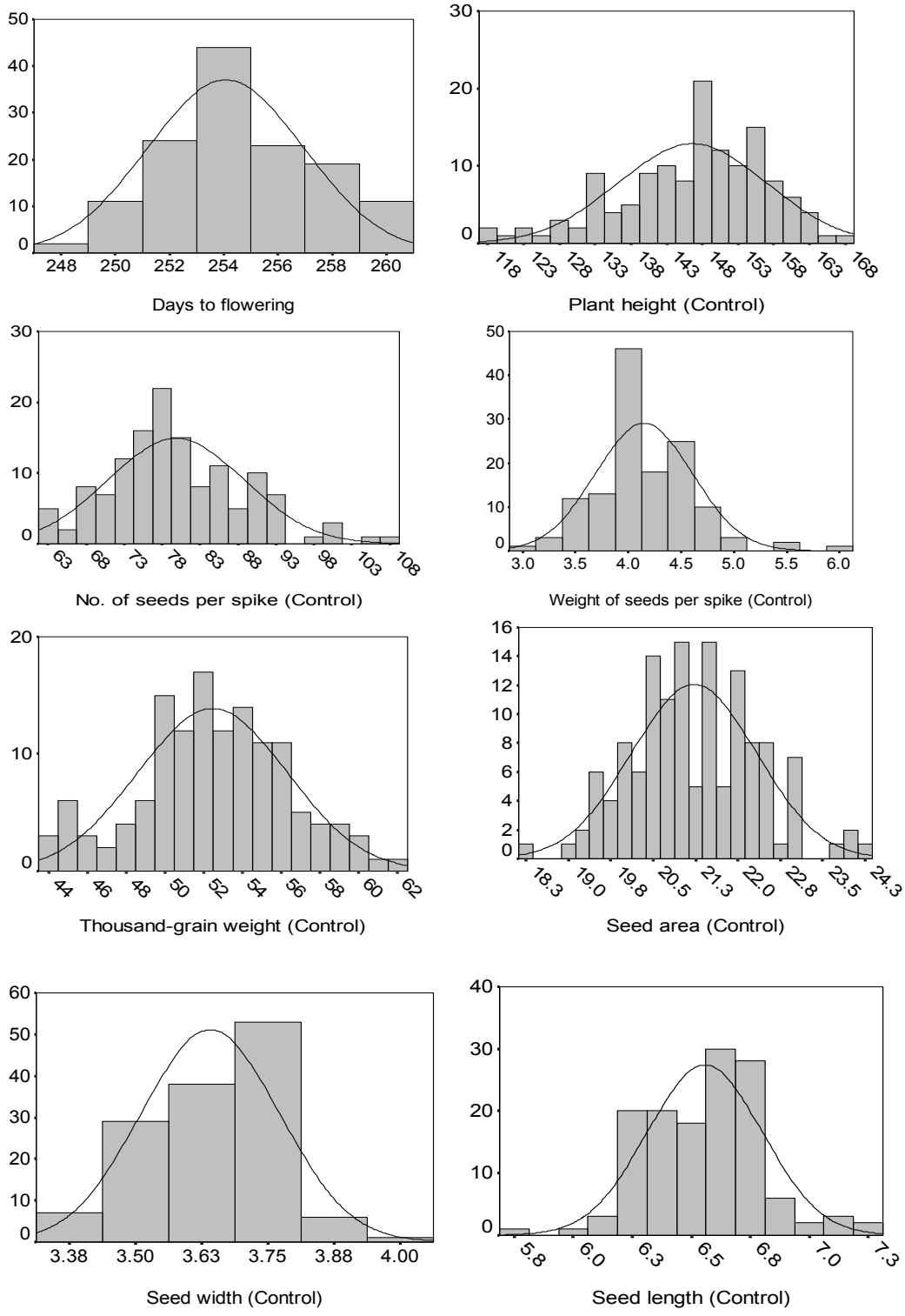


Figure 15 Histograms of the traits recorded at the field experiment under control condition in 2005

Appendix 7

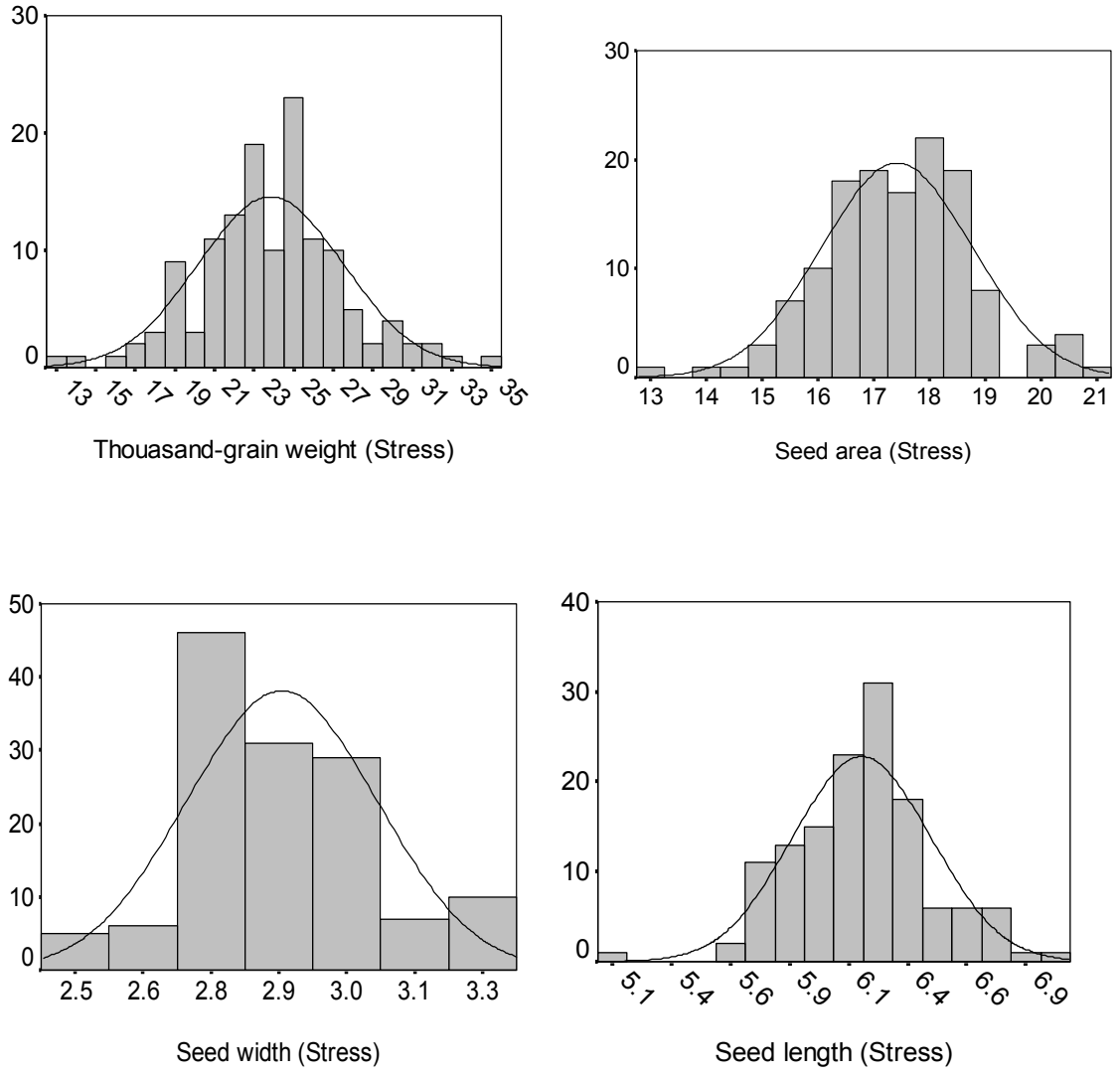


Figure 16 Histograms of the traits recorder at the field experiment under drought stress condition in 2005

Appendix 7

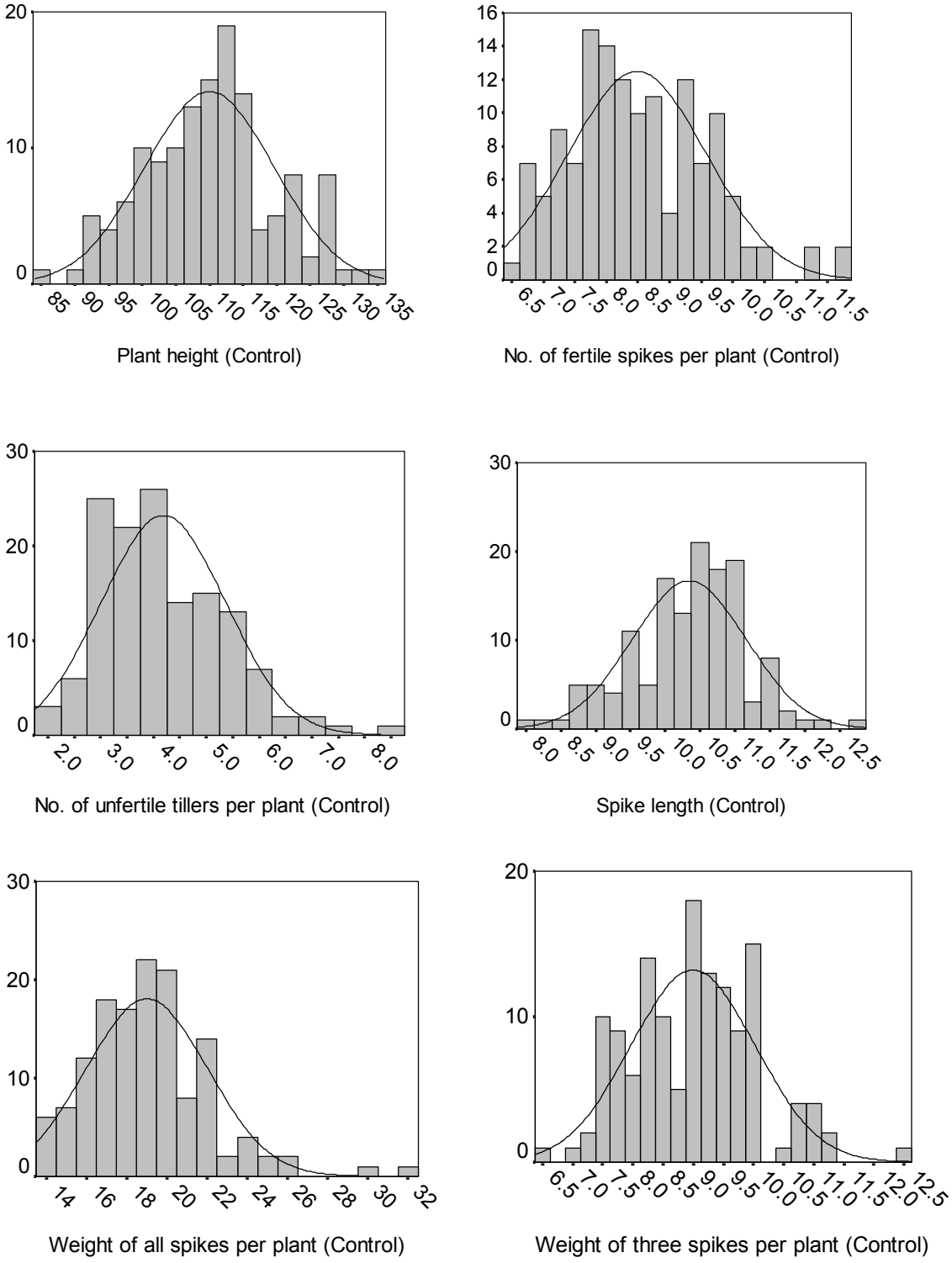


Figure 17 Histograms of the traits recorded at the greenhouse experiment under control condition in 2007

Appendix 7

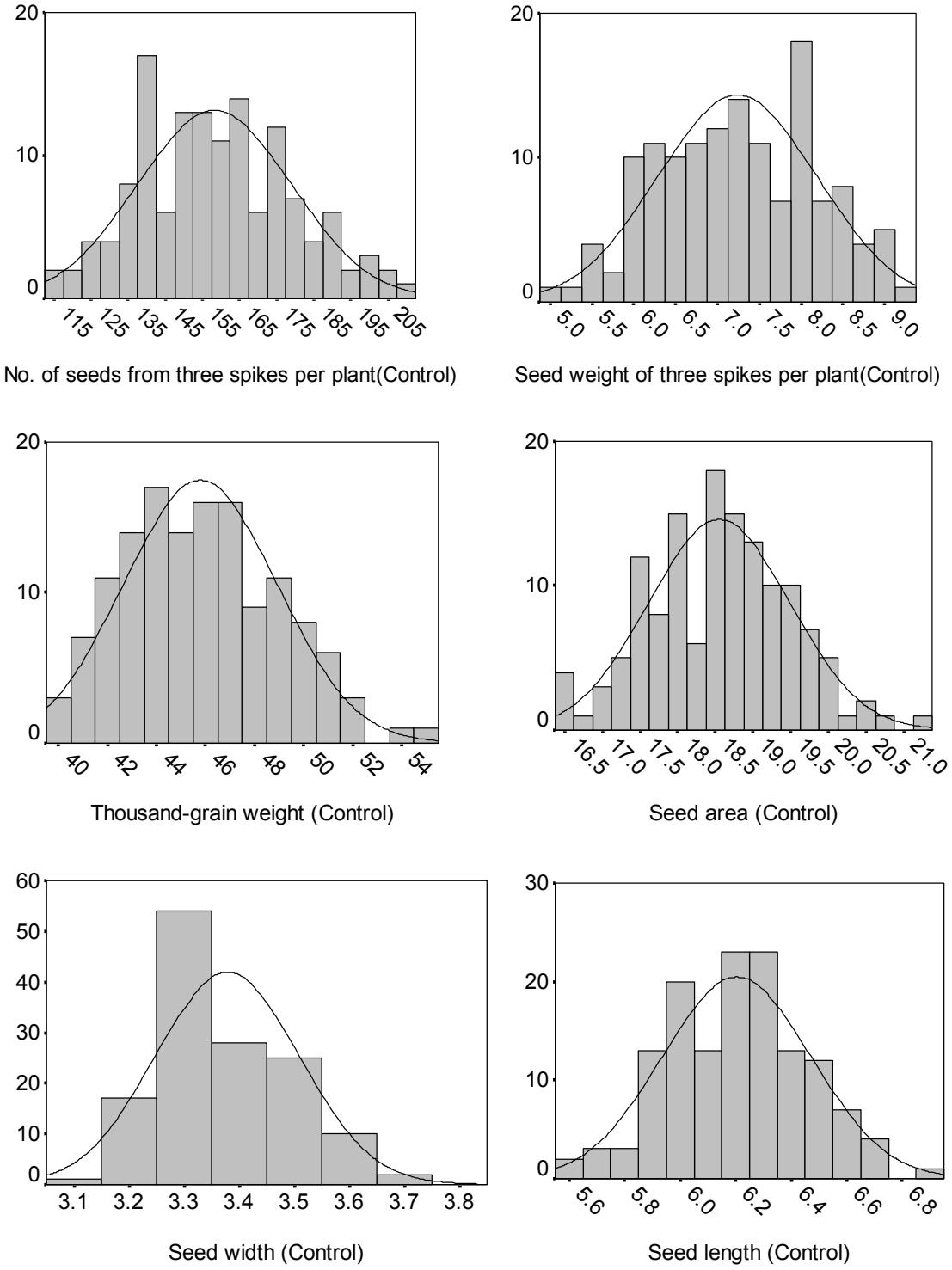


Figure 17 (Continued)

Appendix 7

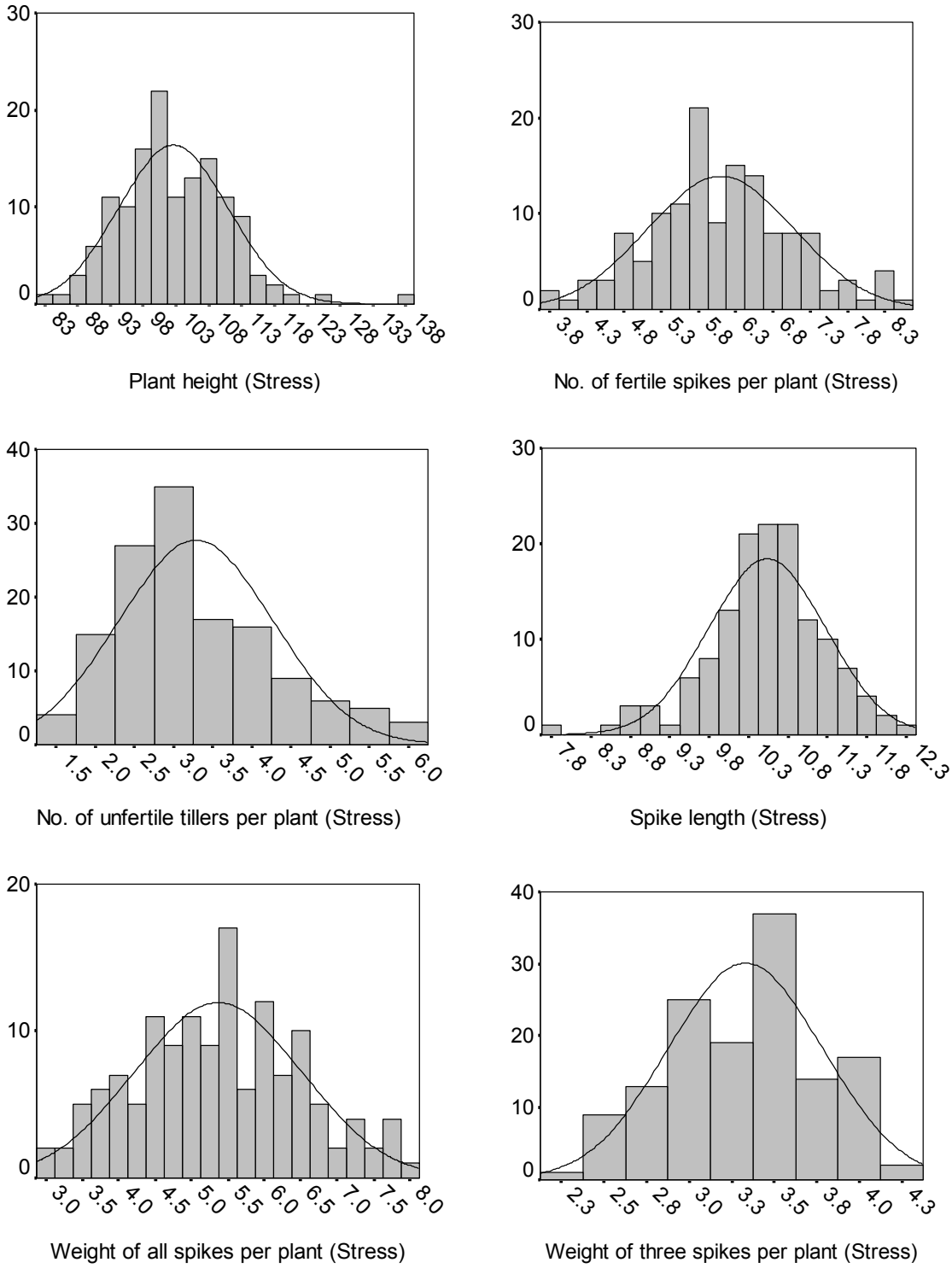


Figure 18 Histograms of the traits recorded at the greenhouse experiment in 2007 under drought stress condition.

Appendix 7

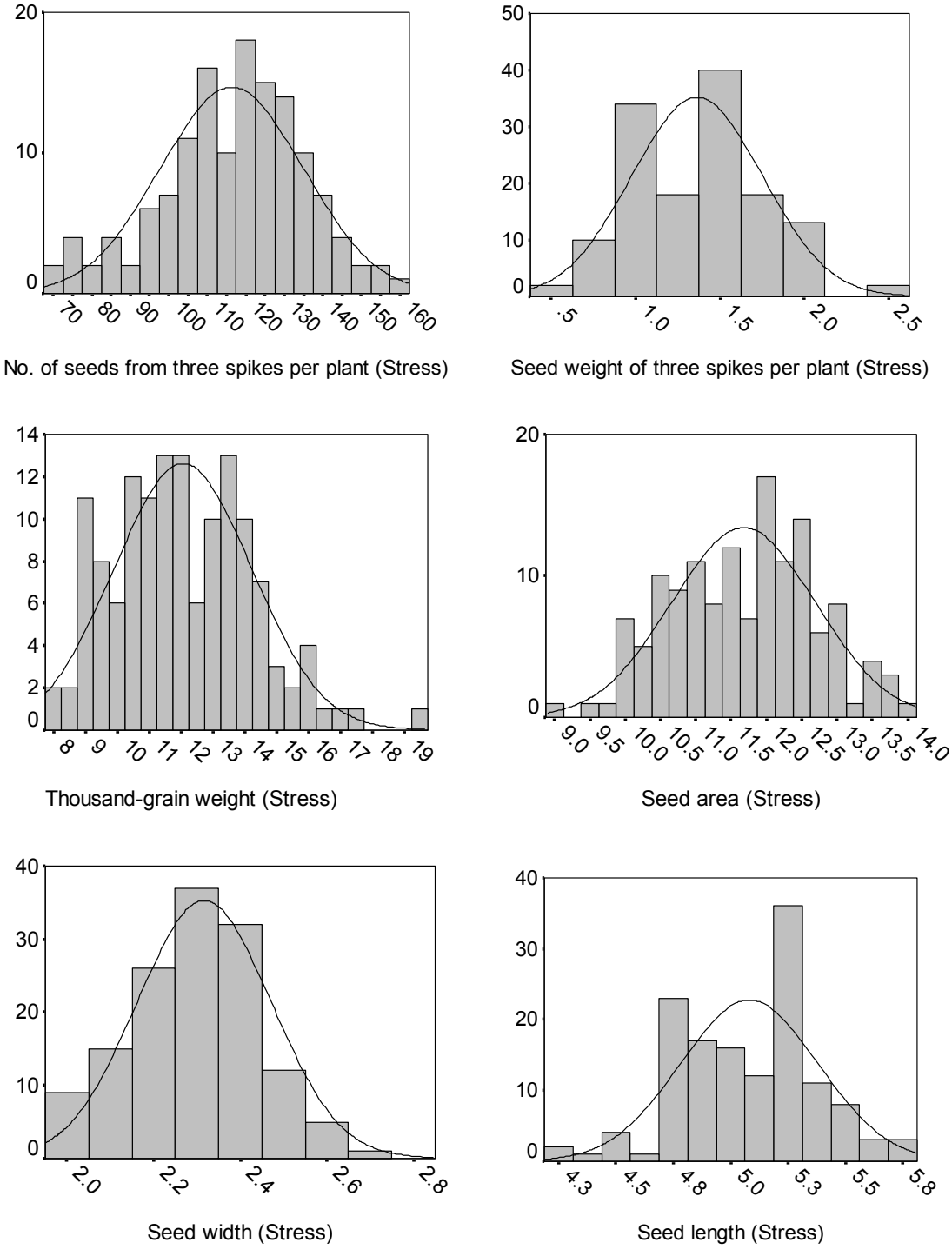


Figure 18 (Continued)

Appendix 7

Table 1 Pearson correlation between traits from greenhouse experiment in 2007

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1)Plant-height(C)	1.00	0.14	0.17	0.26	0.11	0.05	-0.02	0.05	0.13	0.14	0.09	0.07
(2)No-of-fertile-spikes-per-plant(C)	0.14	1.00	0.05	0.01	0.61	-0.04	-0.13	-0.09	0.07	-0.01	0.06	-0.08
(3)No-of-unfertile-tillers-per-plant(C)	0.17	0.05	1.00	0.19	0.01	-0.10	-0.01	-0.02	0.01	-0.01	0.00	-0.03
(4)Spike-length(C)	0.26	0.01	0.19	1.00	0.41	0.48	0.25	0.37	0.24	0.23	0.08	0.23
(5Weight-of-all-spike-per-plant(C)	0.11	**0.61**	**0.01**	0.41	1.00	0.70	0.43	0.54	0.25	0.15	0.01	0.15
(6)Weight-of-three-spikes-per-plant(C)	0.05	**-0.04**	**-0.10**	0.48	0.70	1.00	0.70	0.83	0.28	0.22	-0.04	0.30
(7)Mean of No-of-seeds-from-three-spikes-per-plant(C)	-0.02	**-0.13**	**-0.01**	0.25	0.43	0.70	1.00	0.86	-0.21	-0.21	-0.43	0.03
(8)Mean of Seed-weight-of-three-spikes-per-plant(C)	0.05	**-0.09**	**-0.02**	0.37	0.54	0.83	0.86	1.00	0.31	0.23	-0.04	0.33
(9)Thousand-grain-weight(C)	**0.13**	**0.07**	**0.01**	**0.24**	**0.25**	**0.28**	**-0.21**	**0.31**	1.00	0.85	0.73	0.57
(10)Seed-area(C)	**0.14**	**-0.01**	**-0.01**	**0.23**	**0.15**	**0.22**	**-0.21**	**0.23**	**0.85**	1.00	0.64	0.83
(11)Seed-width(C)	**0.09**	**0.06**	**0.00**	**0.08**	**0.01**	**-0.04**	**-0.43**	**-0.04**	**0.73**	**0.64**	1.00	0.15
(12)Seed-length(C)	**0.07**	**-0.08**	**-0.03**	**0.23**	**0.15**	**0.30**	**0.03**	**0.33**	**0.57**	**0.83**	**0.15**	1.00
(13)Plant-height(S)	**0.72**	0.18	0.17	0.29	0.14	0.02	-0.05	0.06	0.19	0.14	0.15	0.06
(14)No-of-fertile-spikes-per-plant(S)	-0.27	**-0.07**	-0.43	-0.16	-0.13	-0.08	-0.04	-0.13	-0.21	-0.12	-0.17	0.02
(15)No-of-unfertile-tillers-per-plant(S)	0.10	0.11	**0.38**	0.22	0.12	0.00	0.01	0.02	0.02	0.03	-0.04	0.05
(16)Spike-length(S)	0.33	0.05	0.17	**0.76**	0.32	0.34	0.18	0.28	0.20	0.23	0.11	0.20
(17)Weight-of-all-spikes-per-plant(S)	-0.33	-0.13	-0.57	-0.12	**-0.01**	0.15	0.08	0.02	-0.14	-0.08	-0.13	0.03
(18)Weight-of-three-spikes-per-plant(S)	-0.26	-0.18	-0.54	-0.05	0.08	**0.33**	0.22	0.17	-0.08	-0.04	-0.12	0.07
(19)Mean of No-of-seeds-from-three-spikes-per-plant(S)	-0.15	-0.16	-0.43	0.02	0.13	0.39	**0.38**	0.32	-0.10	-0.11	-0.19	0.02
(20)Mean of Seed-weight-of-three-spikes-per-plant(S)	-0.25	-0.18	-0.49	-0.16	-0.04	0.14	0.11	**0.06**	-0.07	-0.06	-0.09	0.01
(21)Thousand-grain-weight(S)	-0.25	-0.12	-0.40	-0.23	-0.16	-0.10	-0.16	-0.17	**-0.01**	0.03	0.03	0.04
(22)Seed-area(S)	-0.15	-0.09	-0.42	-0.22	-0.16	-0.11	-0.19	-0.15	0.07	**0.14**	0.09	0.13
(23)Seed-width(S)	-0.12	-0.03	-0.35	-0.29	-0.21	-0.26	-0.32	-0.29	0.05	0.02	**0.23**	-0.12
(24)Seed-length(S)	-0.13	-0.12	-0.40	-0.14	-0.09	0.03	-0.04	0.01	0.08	0.19	-0.06	**0.30**

C = control, S = stress, Number of individuals = 133, Blue color show correlation between seed related traits, gray color show correlation between seed related traits and other measure traits, red color show correlation of seed related traits between control and stress condition.

Appendix 7

Table 1 (Continue) Pearson correlation between traits from greenhouse experiment in 2007

	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)
(1)Plant-height(C)	0.72	-0.27	0.10	0.33	-0.33	-0.26	-0.15	-0.25	-0.25	-0.15	-0.12	-0.13
(2)No-of-fertile-spikes-per-plant(C)	0.18	-0.07	0.11	0.05	-0.13	-0.18	-0.16	-0.18	-0.12	-0.09	-0.03	-0.12
(3)No-of-unfertile-tiller-per-plant(C)	0.17	-0.43	0.38	0.17	-0.57	-0.54	-0.43	-0.49	-0.40	-0.42	-0.35	-0.40
(4)Spike-length(C)	0.29	-0.16	0.22	0.76	-0.12	-0.05	0.02	-0.16	-0.23	-0.22	-0.29	-0.14
(5)Weight-of-all-spike-per-plant(C)	0.14	-0.13	0.12	0.32	-0.01	0.08	0.13	-0.04	-0.16	-0.16	-0.21	-0.09
(6)Weight-of-three-spikes-per-plant(C)	0.02	-0.08	0.00	0.34	0.15	0.33	0.39	0.14	-0.10	-0.11	-0.26	0.03
(7)Mean of No-of-seeds-from-three-spikes-per-plant(C)	-0.05	-0.04	0.01	0.18	0.08	0.22	0.38	0.11	-0.16	-0.19	-0.32	-0.04
(8) mean of Seed-weight-of-three-spikes-per-plant(C)	0.06	-0.13	0.02	0.28	0.02	0.17	0.32	0.06	-0.17	-0.15	-0.29	0.01
(9)Thousand-grain-weight(C)	0.19	-0.21	0.02	0.20	-0.14	-0.08	-0.10	-0.07	-0.01	0.07	0.05	0.08
(10)Seed-area(C)	0.14	-0.12	0.03	0.23	-0.08	-0.04	-0.11	-0.06	0.03	0.14	0.02	0.19
(11)Seed-width(C)	0.15	-0.17	-0.04	0.11	-0.13	-0.12	-0.19	-0.09	0.03	0.09	0.23	-0.06
(12)Seed-length(C)	0.06	0.02	0.05	0.20	0.03	0.07	0.02	0.01	0.04	0.13	-0.12	0.30
(13)Plant-height(S)	1.00	-0.31	0.14	0.33	-0.39	-0.33	-0.18	-0.21	-0.18	-0.12	-0.09	-0.09
(14)No-of-fertile-spikes-per-plant(S)	-0.31	1.00	-0.46	-0.12	0.83	0.52	0.38	0.49	0.47	0.43	0.33	0.44
(15)No-of-unfertile-tiller-per-plant(S)	0.14	-0.46	1.00	0.24	-0.56	-0.50	-0.44	-0.48	-0.35	-0.33	-0.32	-0.32
(16)Spike-length(S)	0.33	-0.12	0.24	1.00	-0.10	-0.03	0.02	-0.19	-0.24	-0.18	-0.24	-0.10
(17)Weight-of-all-spikes-per-plant(S)	-0.39	**0.83**	**-0.56**	-0.10	1.00	0.87	0.65	0.77	0.67	0.62	0.49	0.62
(18)Weight-of-three-spikes-per-plant(S)	-0.33	**0.52**	**-0.50**	-0.03	0.87	1.00	0.78	0.83	0.64	0.58	0.43	0.60
(19)Mean of No-of-seeds-from-three-spikes-per-plant(S)	-0.18	**0.38**	**-0.44**	0.02	0.65	0.78	1.00	0.77	0.38	0.34	0.21	0.42
(20)Mean of Seed-weight-of-three-spikes-per-plant(S)	-0.21	**0.49**	**-0.48**	-0.19	0.77	0.83	0.77	1.00	0.85	0.77	0.62	0.76
(21)Thousand-grain-weight(S)	**-0.18**	**0.47**	**-0.35**	**-0.24**	**0.67**	**0.64**	**0.38**	**0.85**	1.00	0.92	0.81	0.85
(22)Seed-area(S)	**-0.12**	**0.43**	**-0.33**	**-0.18**	**0.62**	**0.58**	**0.34**	**0.77**	**0.92**	1.00	0.88	0.93
(23)Seed-width(S)	**-0.09**	**0.33**	**-0.32**	**-0.24**	**0.49**	**0.43**	**0.21**	**0.62**	**0.81**	**0.88**	1.00	0.67
(24)Seed-length(S)	**-0.09**	**0.44**	**-0.32**	**-0.10**	**0.62**	**0.60**	**0.42**	**0.76**	**0.85**	**0.93**	**0.67**	1.00

C = control, S = stress, Number of individuals = 133

Appendix 7

Table 2 Pearson correlation coefficient between seed related traits in pairs of experiments (The green and yellow cells show control and stress condition)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
(1)Thousand-grain-weight(F-2004-C)	1.00	0.34	0.39	0.41	0.30	0.26	0.27	0.29	0.79	0.41	0.46	0.45	0.31	0.40	0.39	0.38
(2)Thousand-grain-weight(G-2004-C)	0.34	1.00	0.29	0.13	0.17	0.29	0.30	0.25	0.39	0.82	0.27	0.18	0.22	0.35	0.32	0.32
(3)Thousand-grain-weight(F-2005-C)	0.39	0.29	1.00	0.28	0.15	0.18	0.40	0.25	0.42	0.33	0.80	0.39	0.23	0.27	0.38	0.35
(4)Thousand-grain-weight(G-2007-C)	**0.41**	**0.13**	0.28	1.00	-0.09	0.08	0.27	-0.05	0.35	0.25	0.35	0.84	-0.02	0.21	0.33	0.04
(5)Thousand-grain-weight(F-2004-S)	0.30	0.17	0.15	-0.09	1.00	0.04	0.21	0.42	0.26	0.09	0.16	-0.04	0.77	0.04	0.16	0.41
(6)Thousand-grain-weight(G-2004-S)	0.26	0.29	0.18	0.08	**0.04**	1.00	0.19	0.10	0.23	0.21	0.18	0.06	0.01	0.82	0.15	0.13
(7)Thousand-grain-weight(F-2005-S)	0.27	0.30	0.40	0.27	0.21	0.19	1.00	0.07	0.37	0.32	0.43	0.29	0.30	0.27	0.81	0.13
(8)Thousand-grain-weight(G-2007-S)	0.29	0.25	0.25	-0.05	**0.42**	0.10	0.07	1.00	0.27	0.19	0.22	0.00	0.34	0.12	0.11	0.92
(9)Seed-area(F-2004-C)	**0.79**	0.39	0.42	0.35	0.26	0.23	0.37	0.27	1.00	0.52	0.60	0.53	0.46	0.44	0.51	0.39
(10)Seed-area(G-2004-C)	0.41	**0.82**	0.33	0.25	0.09	0.21	0.32	0.19	0.52	1.00	0.46	0.40	0.28	0.42	0.44	0.28
(11)Seed-area(F-2005-C)	0.46	0.27	**0.80**	0.35	0.16	0.18	0.43	0.22	**0.60**	0.46	1.00	0.59	0.40	0.38	0.56	0.32
(12)Seed-area(G-2007-C)	0.45	0.18	0.39	**0.84**	-0.04	0.06	0.29	0.00	0.53	**0.40**	0.59	1.00	0.15	0.28	0.47	0.11
(13)Seed-area(F-2004-S)	0.31	0.22	0.23	-0.02	**0.77**	0.01	0.30	0.34	0.46	0.28	0.40	0.15	1.00	0.15	0.40	0.38
(14)Seed-area(G-2004-S)	0.40	0.35	0.27	0.21	0.04	**0.82**	0.27	0.12	0.44	0.42	0.38	0.28	**0.15**	1.00	0.35	0.21
(15)Seed-area(F-2005-S)	0.39	0.32	0.38	0.33	0.16	0.15	**0.81**	0.11	0.51	0.44	0.56	0.47	**0.40**	0.35	1.00	0.20
(16)Seed-area(G-2007-S)	0.38	0.32	0.35	0.04	0.41	0.13	0.13	**0.92**	0.39	0.28	0.32	0.11	0.38	0.21	0.20	1.00
(17)Seed-width(F-2004-C)	**0.67**	0.39	0.39	0.27	0.28	0.20	0.44	0.22	**0.73**	0.38	0.40	0.33	0.38	0.33	0.45	0.32
(18)Seed-width(G-2004-C)	0.28	**0.83**	0.35	0.14	0.14	0.32	0.43	0.16	0.31	**0.72**	0.29	0.16	0.21	0.36	0.35	0.25
(19)Seed-width(F-2005-C)	0.17	0.25	**0.75**	0.19	0.18	0.10	0.41	0.13	0.25	0.24	**0.67**	0.28	0.27	0.16	0.34	0.19
(20)Seed-width(G-2007-C)	0.25	0.18	0.33	**0.71**	0.00	0.09	0.36	-0.02	0.25	0.20	0.28	**0.64**	0.05	0.14	0.34	0.06
(21)Seed-width(F-2004-S)	0.16	0.18	0.18	-0.08	**0.73**	0.02	0.33	0.22	0.26	0.13	0.21	-0.01	**0.83**	0.07	0.31	0.24
(22)Seed-width(G-2004-S)	0.24	0.29	0.23	0.09	0.10	**0.84**	0.30	0.06	0.24	0.22	0.23	0.10	0.15	**0.83**	0.27	0.12
(23)Seed-width(F-2005-S)	0.25	0.30	0.34	0.23	0.20	0.14	**0.86**	0.10	0.34	0.30	0.39	0.28	0.35	0.24	**0.86**	0.15
(24)Seed-width(G-2007-S)	0.27	0.27	0.36	0.02	0.41	0.18	0.21	**0.81**	0.25	0.16	0.23	0.00	0.35	0.19	0.18	**0.88**
(25)Seed-length(F-2004-C)	**0.65**	0.27	0.31	0.33	0.15	0.13	0.20	0.23	**0.88**	0.48	0.58	0.56	0.40	0.38	0.42	0.34
(26)Seed-length(G-2004-C)	0.34	**0.40**	0.17	0.25	-0.02	0.01	0.05	0.10	0.46	**0.76**	0.43	0.46	0.19	0.28	0.30	0.15
(27)Seed-length(F-2005-C)	0.47	0.18	**0.52**	0.33	0.09	0.12	0.26	0.18	0.60	0.47	**0.85**	0.59	0.36	0.38	0.52	0.27
(28)Seed-length(G-2007-C)	0.41	0.10	0.25	**0.57**	-0.01	0.00	0.08	0.03	0.49	0.37	0.56	**0.83**	0.18	0.26	0.34	0.11
(19)Seed-length(F-2004-S)	0.36	0.19	0.19	0.03	**0.63**	-0.03	0.19	0.34	0.51	0.33	0.44	0.24	**0.87**	0.17	0.35	0.39
(30)Seed-length(G-2004-S)	0.42	0.28	0.23	0.28	-0.05	**0.50**	0.14	0.11	0.48	0.47	0.43	0.36	0.08	**0.80**	0.31	0.20
(31)Seed-length(F-2005-S)	0.43	0.29	0.35	0.33	0.10	0.10	**0.64**	0.11	0.57	0.50	0.61	0.54	0.36	0.36	**0.90**	0.22
(32)Seed-length(G-2007-S)	0.39	0.26	0.28	0.06	0.34	0.08	0.02	**0.85**	0.41	0.29	0.32	0.16	0.33	0.19	0.13	**0.93**

Appendix 7

Table 2 (Continued) Pearson correlation coefficient between seed related traits in pairs of experiments

	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)	(28)	(29)	(30)	(31)	(32)
(1)Thousand-grain-weight(F-2004-C)	0.67	0.28	0.17	0.25	0.16	0.24	0.25	0.27	0.65	0.34	0.47	0.41	0.36	0.42	0.43	0.39
(2)Thousand-grain-weight(G-2004-C)	0.39	0.83	0.25	0.18	0.18	0.29	0.30	0.27	0.27	0.40	0.18	0.10	0.19	0.28	0.29	0.26
(3)Thousand-grain-weight(F-2005-C)	0.39	0.35	0.75	0.33	0.18	0.23	0.34	0.36	0.31	0.17	0.52	0.25	0.19	0.23	0.35	0.28
(4)Thousand-grain-weight(G-2007-C)	0.27	0.14	0.19	0.71	-0.08	0.09	0.23	0.02	0.33	0.25	0.33	0.57	0.03	0.28	0.33	0.06
(5)Thousand-grain-weight(F-2004-S)	0.28	0.14	0.18	0.00	0.73	0.10	0.20	0.41	0.15	-0.02	0.09	-0.01	0.63	-0.05	0.10	0.34
(6)Thousand-grain-weight(G-2004-S)	0.20	0.32	0.10	0.09	0.02	0.84	0.14	0.18	0.13	0.01	0.12	0.00	-0.03	0.50	0.10	0.08
(7)Thousand-grain-weight(F-2005-S)	0.44	0.43	0.41	0.36	0.33	0.30	0.86	0.21	0.20	0.05	0.26	0.08	0.19	0.14	0.64	0.02
(8)Thousand-grain-weight(G-2007-S)	0.22	0.16	0.13	-0.02	0.22	0.06	0.10	0.81	0.23	0.10	0.18	0.03	0.34	0.11	0.11	0.85
(9)Seed-area(F-2004-C)	0.73	0.31	0.25	0.25	0.26	0.24	0.34	0.25	0.88	0.46	0.60	0.49	0.51	0.48	0.57	0.41
(10)Seed-area(G-2004-C)	0.38	0.72	0.24	0.20	0.13	0.22	0.30	0.16	0.48	0.76	0.47	0.37	0.33	0.47	0.50	0.29
(11)Seed-area(F-2005-C)	0.40	0.29	0.67	0.28	0.21	0.23	0.39	0.23	0.58	0.43	0.85	0.56	0.44	0.43	0.61	0.32
(12)Seed-area(G-2007-C)	0.33	0.16	0.28	0.64	-0.01	0.10	0.28	0.00	0.56	0.46	0.59	0.83	0.24	0.36	0.54	0.16
(13)Seed-area(F-2004-S)	0.38	0.21	0.27	0.05	0.83	0.15	0.35	0.35	0.40	0.19	0.36	0.18	0.87	0.08	0.36	0.33
(14)Seed-area(G-2004-S)	0.33	0.36	0.16	0.14	0.07	0.83	0.24	0.19	0.38	0.28	0.38	0.26	0.17	0.80	0.36	0.19
(15)Seed-area(F-2005-S)	0.45	0.35	0.34	0.34	0.31	0.27	0.86	0.18	0.42	0.30	0.52	0.34	0.35	0.31	0.90	0.13
(16)Seed-area(G-2007-S)	0.32	0.25	0.19	0.06	0.24	0.12	0.15	0.88	0.34	0.15	0.27	0.11	0.39	0.20	0.22	0.93
(17)Seed-width(F-2004-C)	1.00	0.52	0.42	0.42	0.44	0.38	0.51	0.39	0.38	0.04	0.20	0.10	0.22	0.13	0.31	0.17
(18)Seed-width(G-2004-C)	**0.52**	1.00	0.41	0.35	0.29	0.44	0.46	0.35	0.09	0.11	0.08	-0.06	0.08	0.12	0.22	0.08
(19)Seed-width(F-2005-C)	0.42	0.41	1.00	0.42	0.37	0.26	0.46	0.33	0.07	-0.03	0.23	0.06	0.10	0.01	0.19	0.03
(20)Seed-width(G-2007-C)	0.42	**0.35**	0.42	1.00	0.18	0.25	0.40	0.21	0.08	-0.06	0.05	0.14	-0.09	-0.03	0.20	-0.09
(21)Seed-width(F-2004-S)	0.44	0.29	0.37	0.18	1.00	0.24	0.44	0.37	0.08	-0.11	0.03	-0.10	0.49	-0.14	0.12	0.06
(22)Seed-width(G-2004-S)	0.38	0.44	0.26	0.25	**0.24**	1.00	0.33	0.27	0.06	-0.11	0.09	-0.06	0.01	0.35	0.12	-0.01
(23)Seed-width(F-2005-S)	0.51	0.46	0.46	0.40	**0.44**	0.33	1.00	0.29	0.12	0.00	0.20	0.03	0.15	0.05	0.58	-0.02
(24)Seed-width(G-2007-S)	0.39	0.35	0.33	0.21	0.37	0.27	0.29	1.00	0.08	-0.13	0.03	-0.14	0.22	-0.01	0.07	0.67
(25)Seed-length(F-2004-C)	**0.38**	0.09	0.07	0.08	0.08	0.06	0.12	0.08	1.00	0.62	0.73	0.68	0.58	0.57	0.61	0.47
(26)Seed-length(G-2004-C)	0.04	**0.11**	-0.03	-0.06	-0.11	-0.11	0.00	-0.13	**0.62**	1.00	0.63	0.64	0.40	0.58	0.51	0.33
(27)Seed-length(F-2005-C)	0.20	0.08	**0.23**	0.05	0.03	0.09	0.20	0.03	0.73	0.63	1.00	0.74	0.55	0.57	0.70	0.39
(28)Seed-length(G-2007-C)	0.10	-0.06	0.06	**0.14**	-0.10	-0.06	0.03	-0.14	0.68	0.64	**0.74**	1.00	0.40	0.50	0.55	0.28
(19)Seed-length(F-2004-S)	0.22	0.08	0.10	-0.09	**0.49**	0.01	0.15	0.22	0.58	0.40	0.55	0.40	1.00	0.26	0.47	0.46
(30)Seed-length(G-2004-S)	0.13	0.12	0.01	-0.03	-0.14	**0.35**	0.05	-0.01	0.57	0.58	0.57	0.50	**0.26**	1.00	0.48	0.32
(31)Seed-length(F-2005-S)	0.31	0.22	0.19	0.20	0.12	0.12	**0.58**	0.07	0.61	0.51	0.70	0.55	0.47	**0.48**	1.00	0.27
(32)Seed-length(G-2007-S)	0.17	0.08	0.03	-0.09	0.06	-0.01	-0.02	**0.67**	0.47	0.33	0.39	0.28	0.46	0.32	0.27	1.00

Appendix 7
Homogeneity of field condition at experiment in 2005

In order to check weather the $F_{2:3}$ lines were under equal field condition, following hypothesis was tested:

H_0: no difference between the blocks

H_1: difference between the blocks

10												**C**			**A**			**B**
9					**B**						**C**						**A**	
8		**C**								**A**								**B**
7	**A**						**C**						**B**					
6						**B**				**A**				**C**				
5				**C**			**B**										**A**	
4	**B**					**A**					**C**							
3			**C**						**A**						**B**			
2							**B**					**C**						**A**
1								**A**				**C**					**B**	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**

Plan for field experimental design, *Augmented* Randomized Complete Block Design (RCBD)

Analysis of variance was carried out on data from three standard cultivars and as the following table shows there was no significant difference between blocks for thousand-grain weight. So there was not enough reason to reject the null hypothesis. Therefore the null hypothesis was accepted and it means the standard cultivars were grown under situation with homogeny. No significant differences between Blocks for other characters like no. of seeds per spike, spike length, seed area, seed length, and seed width were detected, too.

Analysis of variance for thousand-grain weight under control condition.

S.O.V	**df**	**SS**	**MS**	***F* value**	***P* value**
Block	9	39.876	4.431	1.50	0.2209 ns
genotype	2	107.463	53.732	18.21	0.0001 **
error	18	53.108	2.950	------	------
total	29	200.449	-------	------	------

S.O.V = Source of variation d.f.= Degree of freedom S.S. = Sum of Square
M.S. = Mean of square ns = Not significant ** = Significant at 0.01 level

Appendix 7
SAS software program which were applied for the linear mixed model nested three-factor (a), and four-factor (b) factorial analysis of variance without replication and test for *F*-values**.**

a)

```
data khalil;
input gen year loc $ treat $ tkm seedarea seedwidt seedleng days nseed weiseed spikelen spikewei  plheight fert weiallsp unfert;
cards;
.
.(DATA)
.
proc glm;
where treat = 'C';
class gen year loc;

model tkm seedarea seedwidt seedleng days nseed  weiseed spikelen spikewei  plheight fert weiallsp unfert = gen loc gen * loc year (loc);
run;
proc glm;
where treat = 'S';
class gen year loc;

model tkm seedarea seedwidt seedleng days nseed  weiseed spikelen spikewei  plheight fert weiallsp unfert = gen loc gen * loc year (loc);
run;
```

b)

```
proc glm;
class gen year loc treat ;

model tkm seedarea seedwidt seedleng days nseed weiseed spikelen spikewei plheight fert weiallsp unfert = gen loc treat gen*loc gen*treat loc*treat gen*loc*treat year(loc) gen*year(loc) year*treat(loc);

test  h=gen       e=gen*year(loc) ;
test  h=loc       e=year(loc) ;
test  h=treat     e=treat*year(loc) ;
test  h=gen*loc   e=gen*year(loc) ;
test  h=treat*loc e=treat*year(loc) ;
run;
```

Appendix 7

Table 3 Details of the table of analysis of variance for control condition, including Source of variation (SOV), Degree of freedom (df), Expected mean square (MS), and *F* value for the nested three-factor factorial analysis of variance without replication.

Thousand-grain weight (C)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	4632.205	35.092	M1/M3	2.85 **
2	Location	1	5062.299	5062.299	M2/M4	72.91 *
3	Genotype * Location	132	1621.847	12.286	M3/M5	0.8 ns
4	Year (Location)	2	138.86	69.43	M4/M5	4.49 *
5	Error	264	4079.299	15.451		
	Total	531	15534.512			

Seed area (C)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	390.99	2.962	M1/M3	5.64 **
2	Location	1	539.627	539.627	M2/M4	5.86 ns
3	GEN* Location	132	69.327	0.525	M3/M5	0.89 ns
4	Year (Location)	2	184.303	92.151	M4/M5	155.37 **
5	Error	264	156.576	0.593		
	Total	531	1340.825			

Seed width (C)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	5.908	0.044	M1/M3	4.19 **
2	Location	1	5.063	5.063	M2/M4	13.06 ns
3	Genotype * Location	132	1.394	0.0105	M3/M5	0.79 ns
4	Year (Location)	2	0.775	0.387	M4/M5	28.99 **
5	Error	264	3.5297	0.0133		
	Total	531	16.67			

Seed length (C)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	24.453	0.185	M1/M3	8.81 **
2	Location	1	12.363	12.363	M2/M4	1.63 ns
3	Genotype * Location	132	2.829	0.021	M3/M5	1.09 ns
4	Year (Location)	2	15.145	7.572	M4/M5	385.28 **
5	Error	264	5.189	0.0196		
	Total	531	59.981			

* and ** = Significant at the $\alpha = 0.05$ and $\alpha = 0.01$ level (2-tailed), respectively. ns = not significant

Appendix 7

Table 3 (Continued)

Days to flowering (C)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	1433.037	10.856	M1/M5	4.07 **
2	Location	0	0	.		.
3	Genotype * Location	0	0	.		.
4	Year (Location)	1	1024.375	1024.375	M4/M5	384.55 **
5	Error	132	351.624	2.663		
	Total	265	2809.037			

No. seeds per spike(C)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	18029.316	136.585	M1/M3	1.98 **
2	Location	1	86226.421	86226.421	M2/M4	16.90 ns
3	Genotype * Location	132	9089.6	68.86	M3/M5	0.8 ns
4	Year (Location)	1	5101.473	5101.473	M4/M5	59.44 **
5	Error	132	11329.711	85.831		
	Total	398	129776.522			

Weight of seeds per spike (C)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	38.087	0.2885	M1/M3	1.58 **
2	Location	1	340.099	340.0994	M2/M4	28.89 ns
3	Genotype * Location	132	24.003	0.1818	M3/M5	1.04 ns
4	Year (Location)	1	11.772	11.7726	M4/M5	67.09 **
5	Error	132	23.1639	0.1754		
	Total	398	437.127			

Spike length (C)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	216.8405	1.6427	M1/M3	3.6 **
2	Location	1	1197.9708	1197.9708	M2/M3	
3	Genotype * Location	132	60.1641	0.4557		
4	Year(Location)	0	0	.		
5	Error	0	0			
	Total	265	1474.975			

Plant height (C)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	19096.624	144.6713	M1/M3	2.47 **
2	Location	1	86739.291	86739.2915	M2/M3	
3	Genotype * Location	132	7732.648	58.5806		
4	Year (Location)	0	0	.		
5	Error	0	.	.		
	Total	265	113568.564			

Appendix 7

Table 4 Details of the table of analysis of variance for stress condition, including Source of variation (SOV), Degree of freedom (df), Expected mean square (MS), and *F* value for the nested three-factor factorial analysis of variance without replication.

Thousand grain weight (S)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	1921.688	14.558	M1/M3	1.71 *
2	Location	1	6077.519	6077.519	M2/M4	2.89 ns
3	Genotype * Location	132	1118.372	8.472	M3/M5	0.97 ns
4	Year (Location)	2	4197.011	2098.505	M4/M5	241.27 **
5	Error	263	2287.48	8.697		
	Total	530	15602.072			

Seed area (S)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	306.5	2.321	M1/M3	2.34 **
2	Location	1	1203.719	1203.719	M2/M4	3.63 ns
3	Genotype * Location	132	130.634	0.989	M3/M5	1.13 ns
4	Year (Location)	2	663.208	331.604	M4/M5	379.01 **
5	Error	263	230.106	0.874		
	Total	530	2534.169			

Seed width (S)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	7.154	0.054	M1/M3	2.7 **
2	Location	1	17.15	17.15	M2/M4	3.51 ns
3	Genotype * Location	132	2.684	0.02	M3/M5	1.12 ns
4	Year (Location)	2	9.719	4.859	M4/M5	266.82 **
5	Error	263	4.79	0.0182		
	Total	530	41.499			

Seed length (S)

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	Genotype	132	21.005	0.159	M1/M3	3.24 **
2	Location	1	48.241	48.241	M2/M4	2.92 ns
3	Genotype * Location	132	6.593	0.049	M3/M5	1.08 ns
4	Year (Location)	2	33.012	16.506	M4/M5	357.95 **
5	Error	263	12.127	0.046		
	Total	530	120.98			

* and ** = Significant at the $\alpha = 0.05$ and $\alpha = 0.01$ level (2-tailed), respectively. ns = not significant

Appendix 7

Table 5 Details of combined analysis of variance

Thousand-grain weight

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	**Genotype**	132	4892.420	37.060	MS1 / MS9	2.72 **
2	**Location**	1	11242.560	11242.560	MS2 / MS8	8.33 ns
3	**Treatment**	1	259444.810	259444.810	MS3 / MS10	318.64 **
4	**Genotype x Location**	132	1436.790	10.880	MS4 / MS9	0.79 ns
5	**Genotype x Treatment**	132	1700.370	12.880	MS5 / MS11	1.22 ns
6	**Treatment x Location**	1	23.100	23.100	MS6 / MS10	0.03 ns
7	**Genotype x Location x Treatment**	132	1299.050	9.840	MS7 / MS11	0.93 ns
8	**Years (Location)**	2	2699.470	1349.730	MS8 / MS11	127.81 **
9	**Genotype x Years (Location)**	264	3597.400	13.620	MS9 / MS11	1.29 *
10	**Treatment x Years (Location)**	2	1628.420	814.210	MS10 / MS11	77.1 **
11	**Residuals**	263	2777.35	10.560		
	Total	1062	290741.784			

* and ** = Significant at the α = 0.05 and α = 0.01 level (2-tailed), respectively. ns = not significant

Seed area

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	**Genotype**	132	590.132	4.470	MS1 / MS9	5.65 **
2	**Location**	1	1684.261	1684.261	MS2 / MS8	5.75 ns
3	**Treatment**	1	6651.174	6651.174	MS3 / MS10	50.8 *
4	**Genotype x Location**	132	109.139	0.826	MS4 / MS9	1.04 ns
5	**Genotype x Treatment**	132	104.204	0.789	MS5 / MS11	1.17 ns
6	**Treatment x Location**	1	65.552	65.552	MS6 / MS10	0.50 ns
7	**Genotype x Location x Treatment**	132	91.568	0.693	MS7 / MS11	1.02 ns
8	**Years (Location)**	2	585.728	292.864	MS8 / MS11	433.23 **
9	**Genotype x Years (Location)**	264	208.903	0.791	MS9 / MS11	1.17 ns
10	**Treatment x Years (Location)**	2	261.761	130.880	MS10 / MS11	193.61 **
11	**Residuals**	263	177.801	0.676		
	Total	1062	10530.228			

* and ** = Significant at the α = 0.05 and α = 0.01 level (2-tailed), respectively. ns = not significant

Appendix 7

Table 5 (Continued) Details of combined analysis of variance

Seed width

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	**Genotype**	132	10.950	0.082	MS1 / MS9	4.82 **
2	**Location**	1	20.560	20.560	MS2 / MS8	5.31 ns
3	**Treatment**	1	193.080	193.083	MS3 / MS10	140.42 **
4	**Genotype x Location**	132	2.290	0.017	MS4 / MS9	1.00 ns
5	**Genotype x Treatment**	132	2.110	0.016	MS5 / MS11	1.10 ns
6	**Treatment x Location**	1	1.770	1.779	MS6 / MS10	1.29 ns
7	**Genotype x Location x Treatment**	132	1.790	0.013	MS7 / MS11	0.89 ns
8	**Years (Location)**	2	7.740	3.871	MS8 / MS11	266.96 **
9	**Genotype x Years (Location)**	264	4.480	0.017	MS9 / MS11	1.17 ns
10	**Treatment x Years (Location)**	2	2.750	1.375	MS10 / MS11	94.82 **
11	**Residuals**	263	3.831	0.015		
	Total	1062	251.399			

* and ** = Significant at the α = 0.05 and α = 0.01 level (2-tailed), respectively. ns = not significant

Seed length

	Source of variation	df	Sum of Squares	Mean Square	*F* test	*F* Value
1	**Genotype**	132	40.475	0.306	MS1 / MS9	8.74 **
2	**Location**	1	54.834	54.834	MS2 / MS8	3.65 ns
3	**Treatment**	1	103.909	103.909	MS3 / MS10	11.45 ns
4	**Genotype x Location**	132	5.242	0.039	MS4 / MS9	1.11 ns
5	**Genotype x Treatment**	132	4.859	0.036	MS5 / MS11	1.22 ns
6	**Treatment x Location**	1	5.877	5.877	MS6 / MS10	0.65 ns
7	**Genotype x Location x Treatment**	132	4.227	0.032	MS7 / MS11	1.06 ns
8	**Years (Location)**	2	30.005	15.002	MS8 / MS11	496.05 **
9	**Genotype x Years (Location)**	264	9.397	0.035	MS9 / MS11	1.15 ns
10	**Treatment x Years (Location)**	2	18.150	9.075	MS10 / MS11	300.07 **
11	**Residuals**	263	7.922	0.030		
	Total	1062	284.9			

* and ** = Significant at the α = 0.05 and α = 0.01 level (2-tailed), respectively. ns = not significant

Appendix 8 Table 1 Empirical LOD threshold values

Traits	QTL	LOD					
		50%	30%	25%	10%	5%	1%
Thousand-grain weight-F4C	QTgw	2.4	3.01	3.18	4.15	4.74	6.41
Thousand-grain weight-G4C	QTgw	2.39	3.01	3.18	4.14	4.75	6.47
Thousand-grain weight-F5C	QTgw	2.41	3.03	3.23	4.15	4.80	6.67
Thousand-grain weight-G7C	QTgw	2.41	3.05	3.25	4.18	4.83	6.62
Thousand-grain weight-MC	QTgw	2.41	3.06	3.25	4.16	4.79	6.53
Thousand-grain weight-FMC	QTgw	2.41	3.07	3.25	4.16	4.80	6.46
Thousand-grain weight-GMC	QTgw	2.41	3.05	3.23	4.18	4.76	6.22
Thousand-grain weight-F4S	QTgw	2.41	2.96	3.22	4.22	4.81	6.47
Thousand-grain weight-G4S	QTgw	2.43	2.99	3.20	4.30	4.91	6.47
Thousand-grain weight-F5S	QTgw	2.41	3.01	3.22	4.21	4.89	6.27
Thousand-grain weight-G7S	QTgw	2.42	3.02	3.23	4.19	4.87	6.28
Thousand-grain weight-MS	QTgw	2.41	3.02	3.23	4.21	4.88	6.36
Thousand-grain weight-FMS	QTgw	2.42	3.03	3.24	4.23	4.90	6.32
Thousand-grain weight-GMS	QTgw	2.42	3.05	3.26	4.20	4.88	6.28
Seed area-F4C	QSea	2.4	3.07	3.27	4.19	4.75	6.28
Seed area-G4C	QSea	2.41	3.06	3.26	4.15	4.74	6.32
Seed area-F5C	QSea	2.42	3.06	3.26	4.14	4.73	6.32
Seed area-G7C	QSea	2.42	3.04	3.25	4.16	4.77	6.23
Seed area-MC	QSea	2.43	3.05	3.25	4.14	4.78	6.28
Seed area-FMC	QSea	2.42	3.05	3.26	4.16	4.80	6.28
Seed area-GMC	QSea	2.43	3.06	3.26	4.16	4.82	6.33
Seed area-F4S	QSea	2.42	2.93	3.12	4.09	4.66	6.28
Seed area-G4S	QSea	2.44	3.04	3.21	4.18	4.84	6.37
Seed area-F5S	QSea	2.43	3.03	3.22	4.14	4.76	6.18
Seed area-G7S	QSea	2.44	3.05	3.24	4.16	4.77	6.27
Seed area-MS	QSea	2.44	3.07	3.25	4.17	4.80	6.23
Seed area-FMS	QSea	2.45	3.07	3.25	4.16	4.78	6.14
Seed area-GMS	QSea	2.45	3.07	3.25	4.16	4.79	6.27
Seed width-F4C	QSew	2.39	3.03	3.22	4.18	4.93	6.48
Seed width-G4C	QSew	2.45	3.06	3.25	4.18	4.96	6.76
Seed width-F5C	QSew	2.43	3.04	3.23	4.18	4.91	6.44
Seed width-G7C	QSew	2.43	3.03	3.22	4.17	4.91	6.44
Seed width-MC	QSew	2.42	3.03	3.22	4.15	4.87	6.59
Seed width-FMC	QSew	2.42	3.02	3.21	4.14	4.84	6.44
Seed width-GMC	QSew	2.43	3.02	3.22	4.15	4.87	6.59
Seed width-F4S	Qsew	2.41	3.08	3.31	4.12	4.79	6.32
Seed width-G4S	QSew	2.42	3.07	3.29	4.16	4.82	6.32
Seed width-F5S	QSew	2.45	3.07	3.27	4.16	4.77	6.30
Seed width-G7S	Qsew	2.46	3.07	3.28	4.15	4.71	6.28
Seed width-MS	QSew	2.46	3.06	3.27	4.14	4.72	6.26
Seed width-FMS	QSew	2.46	3.06	3.27	4.15	4.72	6.28
Seed width-GMS	QSew	2.46	3.06	3.27	4.15	4.74	6.25

Appendix 8 Table 1 (Continued) Empirical LOD threshold values

Traits	QTL	LOD					
		50%	30%	25%	10%	5%	1%
Thousand-grain weight-F4C	QTgw	2.4	3.01	3.18	4.15	4.74	6.41
Thousand-grain weight-G4C	QTgw	2.39	3.01	3.18	4.14	4.75	6.47
Thousand-grain weight-F5C	QTgw	2.41	3.03	3.23	4.15	4.80	6.67
Thousand-grain weight-G7C	QTgw	2.41	3.05	3.25	4.18	4.83	6.62
Thousand-grain weight-MC	QTgw	2.41	3.06	3.25	4.16	4.79	6.53
Thousand-grain weight-FMC	QTgw	2.41	3.07	3.25	4.16	4.80	6.46
Thousand-grain weight-GMC	QTgw	2.41	3.05	3.23	4.18	4.76	6.22
Thousand-grain weight-F4S	QTgw	2.41	2.96	3.22	4.22	4.81	6.47
Thousand-grain weight-G4S	QTgw	2.43	2.99	3.20	4.30	4.91	6.47
Thousand-grain weight-F5S	QTgw	2.41	3.01	3.22	4.21	4.89	6.27
Thousand-grain weight-G7S	QTgw	2.42	3.02	3.23	4.19	4.87	6.28
Thousand-grain weight-MS	QTgw	2.41	3.02	3.23	4.21	4.88	6.36
Thousand-grain weight-FMS	QTgw	2.42	3.03	3.24	4.23	4.90	6.32
Thousand-grain weight-GMS	QTgw	2.42	3.05	3.26	4.20	4.88	6.28
Seed area-F4C	QSea	2.4	3.07	3.27	4.19	4.75	6.28
Seed area-G4C	QSea	2.41	3.06	3.26	4.15	4.74	6.32
Seed area-F5C	QSea	2.42	3.06	3.26	4.14	4.73	6.32
Seed area-G7C	QSea	2.42	3.04	3.25	4.16	4.77	6.23
Seed area-MC	QSea	2.43	3.05	3.25	4.14	4.78	6.28
Seed area-FMC	QSea	2.42	3.05	3.26	4.16	4.80	6.28
Seed area-GMC	QSea	2.43	3.06	3.26	4.16	4.82	6.33
Seed area-F4S	QSea	2.42	2.93	3.12	4.09	4.66	6.28
Seed area-G4S	QSea	2.44	3.04	3.21	4.18	4.84	6.37
Seed area-F5S	QSea	2.43	3.03	3.22	4.14	4.76	6.18
Seed area-G7S	QSea	2.44	3.05	3.24	4.16	4.77	6.27
Seed area-MS	QSea	2.44	3.07	3.25	4.17	4.80	6.23
Seed area-FMS	QSea	2.45	3.07	3.25	4.16	4.78	6.14
Seed area-GMS	QSea	2.45	3.07	3.25	4.16	4.79	6.27
Seed width-F4C	QSew	2.39	3.03	3.22	4.18	4.93	6.48
Seed width-G4C	QSew	2.45	3.06	3.25	4.18	4.96	6.76
Seed width-F5C	QSew	2.43	3.04	3.23	4.18	4.91	6.44
Seed width-G7C	QSew	2.43	3.03	3.22	4.17	4.91	6.44
Seed width-MC	QSew	2.42	3.03	3.22	4.15	4.87	6.59
Seed width-FMC	QSew	2.42	3.02	3.21	4.14	4.84	6.44
Seed width-GMC	QSew	2.43	3.02	3.22	4.15	4.87	6.59
Seed width-F4S	Qsew	2.41	3.08	3.31	4.12	4.79	6.32
Seed width-G4S	QSew	2.42	3.07	3.29	4.16	4.82	6.32
Seed width-F5S	QSew	2.45	3.07	3.27	4.16	4.77	6.30
Seed width-G7S	Qsew	2.46	3.07	3.28	4.15	4.71	6.28
Seed width-MS	QSew	2.46	3.06	3.27	4.14	4.72	6.26
Seed width-FMS	QSew	2.46	3.06	3.27	4.15	4.72	6.28
Seed width-GMS	QSew	2.46	3.06	3.27	4.15	4.74	6.25

Appendix 8 Table 1 (Continued) Empirical LOD threshold values

Traits	QTL	LOD					
		50%	30%	25%	10%	5%	1%
Seed length-F4C	Qsel	2.39	3.03	3.25	4.22	5.03	6.27
Seed length-G4C	Qsel	2.42	3.05	3.24	4.16	4.90	6.45
Seed length-F5C	Qsel	2.41	3.03	3.23	4.14	4.83	6.22
Seed length-G7C	Qsel	2.42	3.03	3.23	4.13	4.79	6.22
Seed length-MC	Qsel	2.42	3.04	3.24	4.17	4.82	6.22
Seed length-FMC	Qsel	2.44	3.06	3.25	4.20	4.82	6.23
Seed length-GMC	Qsel	2.44	3.05	3.25	4.20	4.84	6.27
Seed length-F4S	Qsel	2.41	2.95	3.17	4.07	4.81	6.09
Seed length-G4S	Qsel	2.43	3.00	3.22	4.17	4.86	6.28
Seed length-F5S	Qsel	2.42	2.99	3.20	4.14	4.80	6.13
Seed length-G7S	Qsel	2.42	3.02	3.23	4.17	4.81	6.13
Seed length-MS	Qsel	2.43	3.04	3.24	4.17	4.80	6.25
Seed length-FMS	Qsel	2.44	3.05	3.25	4.15	4.78	6.26
Seed length-GMS	Qsel	2.44	3.04	3.24	4.14	4.76	6.28
Days to flowering-FC4	QDtf	2.44	3.06	3.28	4.27	4.89	6.43
Days to flowering-FC5	QDtf	2.44	3.06	3.26	4.22	4.87	6.23
Days to flowering-FMC	QDtf	2.43	3.04	3.25	4.22	4.90	6.34
No. of seeds per spike-FC5	QNsp	2.43	3.07	3.25	4.22	4.75	6.38
No. of seeds per spike-GC7	QNsp	2.43	3.05	3.24	4.22	4.81	6.4
No. of seeds per spike-MC	QNsp	2.44	3.06	3.24	4.21	4.86	6.39
No. of seeds per spike-GS7	QNsp	2.49	3.09	3.32	4.22	4.82	6.32
No. of seeds per spike-GMS	QNsp	2.46	3.08	3.31	4.2	4.78	6.32
Weight of seeds per spike-FC5	QWsp	2.49	3.13	3.31	4.19	4.84	6.35
Weight of seeds per spike-GC7	QWsp	2.46	3.1	3.3	4.2	4.89	6.54
Weight of seeds per spike-MC	QWsp	2.45	3.09	3.28	4.18	4.87	6.47
Weight of seeds per spike-GS7	QWsp	2.42	3.08	3.3	4.29	5.2	4.46
Weight of seeds per spike-MS	QWsp	2.42	3.06	3.27	4.24	5.03	6.36
Spike length-FC5	QSpl	2.46	3.1	3.3	4.3	5.01	6.6
Spike length-GC7	QSpl	2.49	3.13	3.35	4.31	4.98	6
Spike length-MC	QSpl	2.45	3.1	3.29	4.24	4.97	6.3
Spike length-GS7	QSpl	2.5	3.06	3.27	4.23	4.89	6.22
Weight of three spikes per plant-GC7	QWts	2.45	3.12	3.33	4.25	4.9	6.44
Weight of three spikes per plant-GS7	QWts	2.38	2.93	3.15	4.07	4.82	6.32
Plant height-F5C	Qphe	2.37	3.05	3.27	4.14	4.76	6.57
Plant height-GC7	Qphe	2.39	3.05	3.28	4.16	4.73	6.34
Plant height-MC	Qphe	2.45	3.09	3.3	4.2	4.76	6.41
Plant height-GS7	Qphe	2.36	2.99	3.21	4.15	4.74	6.36
No. of fertile spikes per plant-GC7	QNfs	2.49	3.1	3.27	4.2	4.96	6.03
No. of fertile spikes per plant-GS7	QNfs	2.48	3.09	3.33	4.24	4.94	6.46
Weight of all spikes per plant-GC7	Qwas	2.57	3.19	3.37	4.28	4.91	6.38
Weight of all spikes per plant-GS7	Qwas	2.49	3.11	3.27	4.29	4.81	6.47
No. of unfertile tillers per plant-GC7	QNus	2.32	2.98	3.2	4.1	4.73	6.01
No. of unfertile tillers per plant-GS7	QNus	2.45	3.01	3.2	4.08	4.84	6.49

Appendix 8 Table 2 The details information for the all detected QTLs which was arranged based on the four experiments, the overall mean, mean of fields, and mean of greenhouses. Table shows number of QTL per experiment and trait, QTL name, left and right markers of the QTL, one LOD support intervals, QTL peak, LOD score, the explained phenotypic variance (R^2 %), and additive effect. The same QTL detected for the same trait in different experiments or different conditions marked with similar colors and numbers.

QTL per experiment	QTL per trait	QTL name	Left / Right interval marker	1-LOD interval	QTL peak	LOD	Part. R^2 %	Add. effect
		Thousand-grain weight(C)						
1	1	QTgw.ipk-4B-FC4	Xgwm0888-4B / Xgwm0935-4B	0-4	0	7.51	10.6	1.538
1	1	QTgw.ipk-4B-GC4	Xgwm0888-4B / Xgwm0935-4B	0-6	0	5.05	8.9	2.589
2	2	QTgw.ipk-1B-GC4	Xgwm0835-1B / Xgwm0762-1B	18-42	28	5.93	11.7	-3.885
1	3	QTgw.ipk-7A-FC5	Xgwm0900-7A / Xgwm0276-7A	80-96	88	6.92	14.2	2.015
2	4	QTgw.ipk-4B-FC5	Xgwm0149-4B / Xgwm1084-4B	26-30	28	12.88	26.5	2.445
3	2	QTgw.ipk-1B-FC5	Xgwm0835-1B / Xgwm0762-1B	18-44	32	3.82	11.6	-1.903
1	4	QTgw.ipk-4B-GC7	Xgwm0935-4B / Xgwm0925-4B	(2-14)	10	4.50	8.7	1.369
	4	QTgw.ipk-4B-MC	Xgwm0940-4B.2 / Xgwm0155-4B	20-28	28	9.82	20	1.69
	2	QTgw.ipk-1B-MC	Xgwm0835-1B / Xgwm0762-1B	16-44	28	5.09	12.3	-1.688
	3	QTgw.ipk-7A-FMC	Xgwm0900-7A / Xgwm0276-7A	74-96	88	4.74	9.1	1.254
	4	QTgw.ipk-4B-FMC	Xgwm0149-4B / Xgwm1084-4B	26-30	28	8.46	25.1	1.897
		QTgw.ipk-1B-FMC	Xgwm0413-1B / Xgwm0395 -1B.2	76-94	78	3.02	8.3	-1.11
	1	QTgw.ipk-4B-GMC	Xgwm0888-4B / Xgwm0935-4B	(0-12)	2	4.11	11.1	1.811
		QTgw.ipk-1B-GMC	Xgwm0018-1B / Xgwm1100-1B	46-76	60	3.02	8.5	-1.731
		Thousand-grain weight(S)						
1	1	QTgw.ipk-7A-FS4	Xgwm0834-7A / Xgwm0060-7A	30-50	42	5.35	14.6	1.328
2	2	QTgw.ipk-7D-FS4	Xgwm1002-7D / Xbarc126-7D	72-82	76	6.54	8.93	-0.84
3	3	QTgw.ipk-4A-FS4	Xgwm0160-4A / Xgwm0832-4A	110-114	112	10.25	21.04	2.107
	4	QTgw.ipk-4A-FS4	Xbarc327-4A / Xbarc70-4A	124-136	128	6.03	11.93	-1.726
	5	QTgw.ipk-1B-FS4	Xgwm0818-1B / Xgwm0140-1B	158-162	162	3.91	11.82	-1.197
1	6	QTgw.ipk-4B-FS5	Xgwm1084-4B / Xgwm538-4B	26-44	36	3.62	9.2	1.6
1	1	QTgw.ipk-7A-GS7	Xgwm0834-7A / Xgwm0060-7A	28-50	42	4.17	9.1	0.904
2	2	QTgw.ipk-7D-GS7	Xgwm1220-7D / Xgwm1002-7D	60-74	68	6.32	12.7	-0.973
		QTgw.ipk-7A-MS	Xgwm1069-7A / Xgwm0834-7A	(8-16)	12	4.07	11.4	0.868
	3	QTgw.ipk-4A-MS	Xgwm0160-4A / Xgwm0832-4A	108-116	112	8.89	8.5	0.691
	4	QTgw.ipk-4B-MS	Xgwm0940-4B.2 / Xgwm0165-4B	20-28	26	6.31	9.7	0.728
		QTgw.ipk-5B-FMS	Xgwm0408-5B / Xgwm0604-5B	104-124	114	3.52	10.17	1.105
		QTgw.ipk-7A-GMS	Xgwm1069-7A / Xgwm0834-7A	(2-16)	12	3.37	8.8	0.998
		Seed area(C)						
1	1	QSea.ipk-5B-FC4	Xgwm0067-5B / Xgwm0274-5B	46-52	50	4.82	9	-0.401
2	2	QSea.ipk-4A-FC4	Xgwm0160-4A / Xgwm0832-4A	110-126	112	3.38	9.35	0.38
3	3	QSea.ipk-4B-FC4	Xgwm0940-4B.2 / Xgwm0165-4B	20-28	26	7.01	14.2	0.467
1	1	QSea.ipk-5B-GC4	Xgwm0067-5B / Xgwm0274-5B	46-52	50	2.85	9.95	-0.516
2	4	QSea.ipk-4B-GC4	Xgwm0888-4B / Xgwm0935-4B	0-8	2	5.62	10.9	0.596
3	5	QSea.ipk-1B-GC4	Xgwm0835-1B / Xgwm0762-1B	18-44	30	6.06	13.9	-0.784
1	3	QSea.ipk-4B-FC5	Xgwm0149-4B / Xgwm1084-4B	26-30	28	8.91	23.4	0.667
2	6	QSea.ipk-2A-FC5	Xgwm0249-2A / Xgwm1045-2A	84-90	86	3.40	11	-0.466
3	7	QSea.ipk-1B-FC5	Xgwm0413-1B / Xgwm0395-1B.2	76-80	78	4.16	10.1	-0.454
1	3	QSea.ipk-4B-GC7	Xgwm0940-4B.2 / Xgwm0165-4B	20-28	26	6.42	13.3	0.443
2	7	QSea.ipk-1B-GC7	Xgwm0413-1B / Xgwm0395-1B.2	76-86	78	4.47	8.2	-0.377

Field 2004, Greenhouse2004, Field 2005, Greenhouse 2007, Over all mean, Mean of fields, Mean of greenhouses

Appendix 8 Table 2 (Continued)

QTL per experiment	QTL per trait	QTL name	Left / Right marker	1-LOD interval	QTL peak	LOD	Part. R^2%	Add. effect
	1	QSea.ipk-5B-MC	Xgwm0067-5B / Xgwm0274-5B	46-52	50	3.09	11.8	-0.351
	3	QSea.ipk-4B-MC	Xgwm0940-4B.2 / Xgwm0538-4B	20-28	24	14.31	28	0.569
	6	QSea.ipk-2A-MC	Xgwm0249-2A / Xgwm1045-2A	84-90	86	5.28	12.9	-0.365
	5	QSea.ipk-1B-MC	Xgwm0835-1B / Xgwm0762-1B	22-44	32	8.17	13.9	-0.446
	3	QSea.ipk-4B-FMC	Xgwm0940-4B.2 / Xgwm0165-4B	22-28	26	16.77	22.7	0.514
	1	QSea.ipk-5B-FMC	Xgwm0067-5B / Xgwm0274-5B	48-52	50	8.86	18.1	-0.489
		QSea.ipk-5B-FMC	Xgwm0777-5B / Xgwm0408-5B	102-122	112	5.50	13.1	0.401
	6	QSea.ipk-2A-FMC	Xgwm0249-2A / Xgwm1045-2A	84-90	86	3.10	10.7	-0.348
		QSea.ipk-1B-FMC	Xgwm0018-1B / Xgwm1100-1B	44-64	54	6.65	12.3	-0.433
	1	QSea.ipk-5B-GMC	Xgwm0067-5B / Xgwm0274-5B	46-52	50	4.49	9.3	-0.348
	3	QSea.ipk-4B-GMC	Xgwm0940-4B.2 / Xgwm0165-4B	22-28	26	8.56	19.7	0.489
	6	QSea.ipk-2A-GMC	Xgwm0249-2A / Xgwm1045-2A	82-86	84	4.16	9.4	-0.342
	5	QSea.ipk-1B-GMC	Xgwm0835-1B / Xgwm0762-1B	14-36	26	6.90	14.5	-0.549
		Seed area(S)						
1	1	QSea.ipk-4A-FS4	Xgwm0160-4A / Xgwm0832-4A	110-116	112	4.60	10.86	0.405
2	2	QSea.ipk-1B-FS4	Xgwm0818-1B / Xgwm1100-1B	138-162	162	3.40	12.6	-0.498
1	3	QSea.ipk-2D-GS4	Xgwm0702-2D / Xgwm0071-2D	(8-42)	28	4.75	14.4	0.8
1	4	QSea.ipk-5B-FS5	Xgwm0408-5B / Xgwm0604-5B	102-124	114	3.29	10.6	0.573
2	5	QSea.ipk-4B-FS5	Xgwm0888-4B / Xgwm0935-4B	0-14	6	6.30	8.4	0.533
3	6	QSea.ipk-1B-FS5	Xgwm0018-1B / Xgwm1100-1B	54-72	64	6.05	9.2	-0.574
1	7	QSea.ipk-7D-FS7	Xgwm1220-7D / Xgwm1002-7D	62-74	70	5.93	14.3	-0.479
2	8	QSea.ipk-1B-GS7	Xgwm0131-1B / Xgwm0274-1B	100-124	114	3.31	7.7	-0.421
	1	QSea.ipk-4A-MS	Xgwm0160-4A / Xgwm0832-4A	110-120	114	9.73	13.74	0.354
	5	QSea.ipk-4B-MS	Xgwm0888-4B / Xgwm0935-4B	0-6	2	8.54	15.4	0.392
	6	QSea.ipk-1B-MS	Xgwm0018-1B / Xgwm1100-1B	54-74	64	4.24	12.8	-0.366
		QSea.ipk-1D-MS	Xgdm0019-1D / Xgwm1012-1D	32-52	44	6.05	9.5	0.313
		QSea.ipk-5D-FMS	Xgwm1252-5D / Xgwm1039-5D	0-30	16	4.52	9.6	-0.393
	1	QSea.ipk-4A-FMS	Xgwm1258-4A / Xgwm0160-4A	108-116	112	5.98	13.62	0.421
		QSea.ipk-4B-FMS	Xgwm0940-4B.2 / Xgwm165-4B	20-28	26	4.08	10.2	0.348
		QSea.ipk-2A-FMS	Xgwm0939-2A / Xgwm0726-2A	18-36	28	3.87	10.9	-0.364
	6	QSea.ipk-1B-FMS	Xgwm0413-1B / Xgwm0395-1B.2	76-86	78	3.39	9.9	-0.373
	5	QSea.ipk-4B-GMS	Xgwm0888-4B / Xgwm0935-4B	0-4	0	5.75	12	0.39
	8	QSea.ipk-1B-GMS	Xgwm0131-1B / Xgwm0274-1B	101-122	112	3.82	11.8	-0.463
		Seed width(C)						
1	1	QSew.ipk-5B-FC4	Xgwm0777-5B / Xgwm0408-5B	98-120	108	5.41	12.2	0.052
2	2	QSew.ipk-4B-FC4	Xgwm0888-4B / Xgwm0935-4B	0-4	0	6.17	12.2	0.057
1	2	QSew.ipk-4B-GC4	Xgwm0888-4B / Xgwm0935-4B	0-4	0	5.34	11.8	0.09
2	3	QSew.ipk-1B-GC4	Xgwm0835-1B / Xgwm0762-1B	22-44	36	5.43	11	-0.099
1	4	QSew.ipk-4B-FC5	Xgwm0149-4B / Xgwm1084-4B	26-30	28	3.86	15.7	0.069
1	5	QSew.ipk-5B-GC7	Xgwm0843-5B / Xgwm0133-5B	52-62	56	3.19	7.9	0.045
2	4	QSew.ipk-4B-GC7	Xgwm0149-4B / Xgwm1084-4B	26-30	28	4.49	14.3	0.058
	1	QSew.ipk-5B-MC	Xgwm0777-5B / Xgwm0408-5B	96-122	108	3.24	9.7	0.046
	4	QSew.ipk-4B-MC	Xgwm0149-4B / Xgwm1084-4B	26-30	28	6.38	19.5	0.061
		QSew.ipk-1B-MC	Xgwm0018-1B / Xgwm1100-1B	46-78	58	3.03	8.6	-0.046

Field 2004, Greenhouse2004, Field 2005, Greenhouse 2007, Over all mean, Mean of fields, Mean of greenhouses

Appendix 8 Table 2 (Continued)

QTL per experiment	QTL per trait	QTL name	Left / Right marker	1-LOD interval	QTL peak	LOD	Part. R^2%	Add. effect
	1	QSew.ipk-5B-FMC	Xgwm0777-5B / Xgwm0408-5B	104-122	112	5.39	14.1	0.051
	4	QSew.ipk-4B-FMC	Xgwm0149-4B / Xgwm1084-4B	26-30	28	6.62	16.2	0.051
		QSew.ipk-5B-GMC	Xbarc74-5B / Xgwm0777-5B	68-108	84	3.92	9.1	0.063
	2	QSew.ipk-4B-GMC	Xgwm0888-4B / Xgwm0935-4B	0-6	0	6.03	15.4	0.067
	3	QSew.ipk-1B-GMC	Xgwm0835-1B / Xgwm0762-1B	24-46	36	6.39	10.3	-0.061
		Seed width(S)						
1	1	QSew.ipk-4A-FS4	Xgwm0160-4A / Xgwm0832-4A	108-114	112	4.94	6.7	0.051
1	2	QSew.ipk-4A-GS4	Xgwm1093-4A / Xgwm0695-4A	0-10	2	3.19	7.7	-0.077
2	3	QSew.ipk-4B-GS4	Xgwm0888-4B / Xgwm0935-4B	(0- 6)	0	2.76	8.46	0.074
3	4	QSew.ipk-2D-GS4	Xgwm0702-2D / Xgwm0071-2D	(4-42)	28	4.53	9.6	0.105
1	5	QSew.ipk-2A-FS5	Xgwm1244-2A / Xgwm0939-2A	20-34	28	6.45	7.4	-0.067
1	6	QSew.ipk-7D-GS7	Xgwm1220-7D / Xgwm1002-7D	66-76	70	7.66	12.9	-0.07
	3	QSew.ipk-4B-MS	Xgwm0888-4B / Xgwm0935-4B	0-6	0	3.74	9.7	0.053
		QSew.ipk-5B-FMS	Xgwm0777-5B / Xgwm0408-5B	96-126	106	3.32	11.6	0.071
	2	QSew.ipk-4A-GMS	Xgwm1093-4A / Xgwm0695-4A	0-8	2	5.92	9.6	-0.059
	3	QSew.ipk-4B-GMS	Xgwm0888-4B / Xgwm0935-4B	0-6	0	3.32	11.5	0.061
		QSew.ipk-1B-GMS	Xgwm0131-1B / Xgwm0274-1B	104-122	112	6.70	10.4	-0.068
		Seed length(C)						
1	1	QSel.ipk-5B-FC4	Xgwm0067-5B / Xgwm0274-5B	46-52	50	11.62	26.9	-0.153
2	2	QSel.ipk-4A-FC4	Xgwm0832-4A / Xgwm1169-4A	110-126	118	3.01	11.58	0.094
3	3	QSel.ipk-4B-FC4	Xgwm0149-4B / Xgwm1084-4B	26-30	28	3.70	8.7	0.071
4	4	QSel.ipk-2A-FC4	Xgwm0294-2A / Xgwm1070-2A	114-138	126	5.02	12.6	-0.105
1	1	QSel.ipk-5B-GC4	Xbarc74-5B / Xgwm0777-5B	58-76	68	12.39	25.7	-0.212
2	3	QSel.ipk-4B-GC4	Xgwm0940-4B.2 / Xgwm0165-4B	20- 28	26	2.73	9.49	0.09
3	5	QSel.ipk-2A-GC4	Xgwm0071-2A.1 / Xgwm1115-2A	56-80	72	5.35	13.3	-0.133
4	6	QSel.ipk-1B-GC4	Xgwm0835-1B / Xgwm0762-1B	(4-42)	24	3.04	9.3	-0.127
1	7	QSel.ipk-5A-FC5	Xgwm1236-5A / Xgwm0126-5A	70-122	92	3.29	10	-0.117
2	1	QSel.ipk-5B-FC5	Xgwm1284-5B / Xgwm0066-5B	34-48	46	7.76	19.8	-0.125
3	8	QSel.ipk-5D-FC5	Xgwm1252-5D / Xgwm1039-5D	36-74	64	3.02	9.2	-0.091
4	3	QSel.ipk-4B-FC5	Xgwm0940-4B.2 / Xgwm0165-4B	22-28	26	5.86	12	0.083
5	5	QSel.ipk-2A-FC5	Xgwm0071-2A.1 / Xgwm1115-2A	68-80	76	11.69	19.6	-0.119
6	9	QSel.ipk-2A-FC5	Xgwm0846-2A / Xgwm1136-2A	170-172	172	4.43	8.3	-0.073
7	10	QSel.ipk-1B-FC5	Xgwm0413-1B / Xgwm0395-1B.2	76-86	78	4.26	11.8	-0.091
1	11	QSel.ipk-7B-GC7	Xgwm0297-7B / Xgwm0897-7B	56-90	62	3.68	9.2	-0.089
2	1	QSel.ipk-5B-GC7	Xgwm0274-5B / Xgwm0843-5B	50-56	52	8.50	16.2	-0.128
3	12	QSel.ipk-1A-GC7	Xgwm0395-1A / Xgwm0752-1A	(10-18)	12	4.87	10.5	-0.099
4	13	QSel.ipk-1D-GC7	Xgwm1291-1D / Xgwm0395-1D	0-4	0	3.40	12.3	0.103
	1	QSel.ipk-5B-MC	Xgwm0066-5B / Xgwm0067-5B	46-52	48	16.95	29.6	-0.15
	3	QSel.ipk-4B-MC	Xgwm0940-4B.2 / Xgwm0165-4B	22-28	26	7.92	10.8	0.074
	5	QSel.ipk-2A-MC	Xgwm0071-2A.1 / Xgwm1115-2A	64-78	76	7.68	18.9	-0.109
	12	QSel.ipk-1A-MC	Xgwm0752-1A / Xgwm1148-1A	(10-18)	14	6.62	10.4	-0.08
	10	QSel.ipk-1B-MC	Xgwm0395-1B.2 / Xgwm0131-1B	76-94	86	3.57	7.9	-0.077
	13	QSel.ipk-1D-MC	Xgwm1291-1D / Xgwm0395-1D	0-4	0	2.81	11.07	0.074
	1	QSel.ipk-5B-FMC	Xgwm0066-5B / Xgwm0067-5B	46-52	48	16.75	28.3	-0.154

Field 2004, Greenhouse2004, Field 2005, Greenhouse 2007, Over all mean, Mean of fields, Mean of greenhouses

Appendix 8 Table 2 (Continued)

QTL per experiment	QTL per trait	QTL name	Left / Right marker	1-LOD interval	QTL peak	LOD	Part. R^2%	Add. effect
	3	QSel.ipk-4B-FMC	Xgwm0149-4B / Xgwm1084-4B	26-30	28	8.26	9.3	0.074
	5	QSel.ipk-2A-FMC	Xgwm0071-2A.1 / Xgwm1115-2A	64-80	76	3.72	16	-0.105
	12	QSel.ipk-1A-FMC	Xgwm0772-1A.2 / Xgwm0395-1A	(6-14)	10	6.48	8.1	-0.072
	1	QSel.ipk-5B-GMC	Xgwm0274-5B / Xgwm0843-5B	50-56	52	11.70	23	-0.141
		QSel.ipk-3A-GMC	Xgwm0395-1D / Xgwm0458-1D	48-54	52	3.80	7.7	0.066
	5	QSel.ipk-2A-GMC	Xgwm0071-2A.1 / Xgwm1115-2A	56-78	72	5.37	10.8	-0.096
	12	QSel.ipk-1A-GMC	Xgwm0395-1A / Xgwm0752-1A	(10-18)	12	8.90	15.7	-0.109
	13	QSel.ipk-1D-GMC	Xgwm0395-1D / Xgwm0458-1D	0-8	4	4.82	10.8	0.089
		Seed length(S)						
1	1	QSel.ipk-5B-FS4	Xgwm0067-5B / Xgwm0274-5B	46-52	50	10.39	22.4	-0.152
2	2	QSel.ipk-1B-FS4	Xgwm0268-1B / Xgwm0413-1B	132-160	146	4.38	17.3	-0.164
1	1	QSel.ipk-5B-GS4	Xgwm0274-5B / Xgwm0843-5B	50-58	52	7.05	19.4	-0.159
2	3	QSel.ipk-4B-GS4	Xgwm0940-4B.2 / Xgwm0165-4B	20-28	26	2.68	8.37	0.092
3	4	QSel.ipk-2D-GS4	Xgwm0702-2D / Xgwm0071-2D	(4-40)	26	4.59	7.9	0.117
4	5	QSel.ipk-1A-GS4	Xgwm0395-1A / Xgwm0752-1A	(10-14)	12	3.77	8.6	-0.097
1	6	QSel.ipk-5A-FS5	Xgwm1236-5A / Xgwm0126-5A	68-120	80	3.06	10.5	-0.128
2	1	QSel.ipk-5B-FS5	Xgwm0066-5B / Xgwm0067-5B	44-50	48	4.61	13.9	-0.138
3	3	QSel.ipk-4B-FS5	Xgwm1084-4B / Xgwm0538-4B	26-44	34	5.08	12.9	0.126
4	7	QSel.ipk-2A-FS5	Xgwm0071-2A.1 / Xgwm1115-2A	60-80	76	5.74	14.3	-0.135
5	8	QSel.ipk-1B-FS5	Xgwm0018-1B / Xgwm1100-1B	54-76	64	5.26	11.9	-0.13
1	1	QSel.ipk-5B-GS7	Xgwm0066-5B / Xgwm0067-5B	46-52	48	4.20	11.3	-0.143
2	8	QSel.ipk-1B-GS7	Xgwm0413-1B / Xgwm0395-1B.2	76-80	78	3.11	8.9	-0.127
	1	QSel.ipk-5B-MS	Xgwm0067-5B / Xgwm0274-5B	48-52	50	16.27	24.9	-0.136
	2	QSel.ipk-1B-MS	Xgwm0268-1B / Xgwm0818-1B	138-162	152	4.51	12	-0.102
		QSel.ipk-1D-MS	Xgdm0019-1D / Xgwm1012-1D	(6-38)	20	3.09	7.9	0.082
	1	QSel.ipk-5B-FMS	Xgwm0067-5B / Xgwm0274-5B	46-52	50	12.09	15.5	-0.132
	2	QSel.ipk-1B-FMS	Xgwm0268-1B / Xgwm0818-1B	146-162	158	4.68	7.9	-0.094
	1	QSel.ipk-5B-GMS	Xgwm0067-5B / Xgwm0274-5B	46-52	50	8.39	18.5	-0.145
		Days to flowering						
1	1	QDtf.ipk-7B-FC4	Xgwm1184-7B / Xgwm0941-7B	48-54	52	4.43	10.8	0.781
2	2	QDtf.ipk-7D-FC4	Xgwm1220-7D / Xgwm1002-7D	60-78	68	4.00	24.7	1.308
3	3	QDtf.ipk-5A-FC4	Xgwm0304-5A / Xgwm0156-5A	44-70	54	4.39	15.9	1.112
4	4	QDtf.ipk-5A-FC4	Xgwm0995-5A / Xgwm0865-5A	194-198	198	7.38	11.4	0.867
5	6	QDtf.ipk-3A-FC4	Xgwm1110-3A / Xgwm0134-3A	50-54	54	4.25	12.1	-0.833
6	5	QDtf.ipk-2D-FC4	Xgwm1010-2D / Xgwm0030-2D	72-86	82	3.81	8.7	0.745
1	2	QDtf.ipk-7D-FC5	Xgwm1220-7D / Xgwm1002-7D	64-74	70	5.99	17.2	1.434
2	7	QDtf.ipk-5D-FC5	Xgdm0063-5D / Xgwm1059-5D	160-178	166	4.95	9.7	1.247
3	8	QDtf.ipk-1B-FC5	Xgwm0818-1B / Xgwm0140-1B	158-162	162	4.18	7.7	1.079
	2	QDtf.ipk-7D-FMC	Xgwm1220-7D / Xgwm1002-7D	64-74	70	10.97	18.3	1.18
	4	QDtf.ipk-5A-FMC	Xgwm0995-5A / Xgwm0865-5A	194-198	196	3.85	8.3	0.856
	6	QDtf.ipk-3A-FMC	Xgwm1110-3A / Xgwm0134-3A	50- 54	54	2.81	7.86	-0.737
	8	QDtf.ipk-1B-FMC	Xgwm0818-1B / Xgwm0140-1B	158-162	162	3.19	13.6	1.17
		No. seeds per spike(C)						
1	1	QNsp.ipk-7A-FC5	Xgwm0060-7A / Xgwm1171-7A	44-64	48	5.37	16.3	-4.99
2	2	QNsp.ipk-7D-FC5	Xgwm1002-7D / Xbarc126-7D	72-84	76	2.72	10.09	2.906

Appendix 8 Table 2 (Continued)

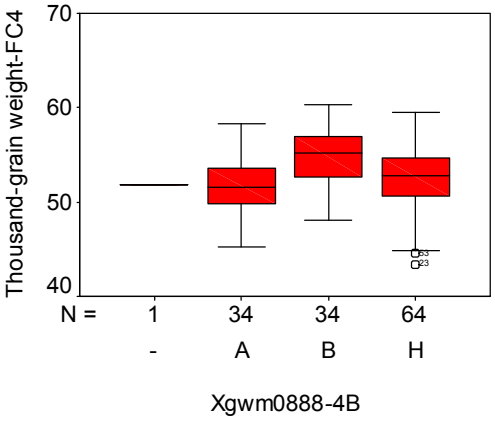
QTL per experiment	QTL per trait	QTL name	Left / Right marker	1-LOD interval	QTL peak	LOD	Part. R^2%	Add. effect
3	3	QNsp.ipk-5B-FC5	Xbarc140-5B / Xbarc142-5B	124-132	130	3.08	9.67	-3.165
4	4	QNsp.ipk-4B-FC5	Xgwm0940-4B.2 / Xgwm0165-4B	20-28	26	3.74	13.2	-4.064
5	5	QNsp.ipk-2A-FC5	Xgwm0122-2A / Xgwm0339-2A	78-86	82	2.56	7.96	-2.935
1	4	QNsp.ipk-4B-GC7	Xgwm0925-4B / Xgwm0898-4B	(12-16)	14	2.96	8.85	-2.723
2	5	QNsp.ipk-2A-GC7	Xgwm0339-2A / Xgwm0448-2A	82-86	84	3.51	9.1	-2.954
	1	QNsp.ipk-7A-MC	Xgwm0060-7A / Xgwm1171-7A	44-50	48	7.33	16.34	-3.181
	2	QNsp.ipk-7D-MC	Xgwm1002-7D / Xbarc126-7D	72-82	76	4.76	12.36	2.332
	3	QNsp.ipk-5B-MC	Xbarc140-5B / Xbarc142-5B	126-132	130	6.55	15.31	-2.885
	4	QNsp.ipk-4B-MC	Xgwm0925-4B / Xgwm0898-4B	(12-16)	14	4.71	15.2	-2.952
	5	QNsp.ipk-2A-MC	Xgwm0122-2A / Xgwm0339-2A	78-86	82	4.41	11.4	-2.565
		No. seeds per spike(S)						
1	1	QNsp.ipk-5A-GS7	Xgwm0156-5A / Xgwm1236-5A	54-72	64	6.55	12.9	-3.096
2	2	QNsp.ipk-5B-GS7	Xbarc140-5B / Xbarc142-5B	126-132	130	3.58	14.1	-2.874
3	3	QNsp.ipk-2A-GS7	Xgwm1115-2A / Xgwm0122-2A	76-84	78	6.31	12.2	-2.874
		Weight of seeds per spike(C)						
1	1	QWsp.ipk-2A-FC5	Xgwm0122-2A / Xgwm0339-2A	78-86	82	4.58	10.1	-0.211
1	2	QWsp.ipk-7D-GC7	Xgwm1002-7D / Xbarc126-7D	70-88	74	3.79	8.5	0.119
2	3	QWsp.ipk-5B-GC7	Xgwm0790-5B / Xgwm1016-5B	138-156	150	3.01	7.61	-0.115
3	1	QWsp.ipk-2A-GC7	Xgwm1045-2A / Xgwm0445-2A	84- 104	94	2.60	10.66	-0.15
	1	QWsp.ipk-2A-MC	Xgwm0071-2A.1 / Xgwm1115-2A	64-80	76	4.55	13.3	-0.173
		Weight of seeds per spike(S)						
1	2	QWsp.ipk-7A-GS7	Xgwm0060-7A / Xgwm1171-7A	44-50	48	3.41	11.8	0.077
2	3	QWsp.ipk-7D-GS7	Xgwm1220-7D / Xgwm1002-7D	60-74	66	5.81	21.7	-0.105
3	4	QWsp.ipk-5A-GS7	Xgwm0156-5A / Xgwm1236-5A	52-74	66	5.19	15.6	-0.102
4	5	QWsp.ipk-5B-GS7	Xbarc142-5B / Xgwm1246-5B	128-136	132	2.98	8.63	-0.058
5	6	QWsp.ipk-2A-GS7	Xgwm0122-2A / Xgwm0339-2A	78-86	82	3.50	13.6	-0.085
6	7	QWsp.ipk-2B-GS7	Xgwm1171-2B / Xgwm1128-2B	(2-26)	10	3.50	7.9	-0.072
7	8	QWsp.ipk-2D-GS7	Xgwm0071-2D / Xgwm0484-2D	40-56	48	3.78	13.3	-0.092
		Spike length(C)						
1	1	QSpl.ipk-7D-FC5	Xbarc352-7D / Xgwm1220-7D	50-70	56	6.75	12.7	0.575
2	2	QSpl.ipk-5B-FC5	Xgwm0067-5B / Xgwm0274-5B	46-52	50	8.30	15.1	-0.629
3	3	QSpl.ipk-3A-FC5	Xgwm0005-3A / Xgwm0804-3A	24-34	32	5.86	11.3	-0.497
1	1	QSpl.ipk-7D-GC7	Xbarc352-7D / Xgwm1220-7D	38- 62	52	2.87	10.73	0.343
2	2	QSpl.ipk-5B-GC7	Xgwm0274-5B / Xgwm0843-5B	50-58	52	5.44	7.5	-0.295
3	4	QSpl.ipk-4A-GC7	Xgwm1091-4A / Xgwm0192-4A	(8-26)	14	4.31	8.3	0.304
4	5	QSpl.ipk-2D-GC7	Xgwm0071-2D / Xgwm0484-2D	40-52	42	5.99	15.4	0.484
		QSpl.ipk-7D-MC	Xbarc352-7D / Xgwm1220-7D	46-62	54	6.74	13.4	0.452
	2	QSpl.ipk-5B-MC	Xgwm0066-5B / Xgwm0067-5B	46-52	48	9.13	13.9	-0.449
	3	QSpl.ipk-3A-MC	Xgwm0353-3A / Xgwm0005-3A	24-34	28	3.95	12.4	-0.377
		Spike length(S)						
1	1	QSpl.ipk-7D-GS7	Xbarc352-7D / Xgwm1220-7D	48-66	54	6.44	12.7	0.384
2	2	QSpl.ipk-3A-GS7	Xgwm0353-3A / Xgwm0005-3A	18-30	26	9.13	13.5	-0.337
		Weight of 3 spikes per plant(C)						
1	1	QWts.ipk-4A-GC7	Xgwm1093-4A / Xgwm0695-4A	0-6	0	3.55	8	0.388

Appendix 8 Table 2 (Continued)

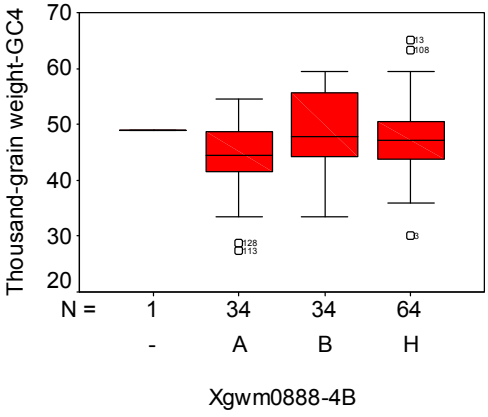
QTL per experiment	QTL per trait	QTL name	Left / Right marker	1-LOD interval	QTL peak	LOD	Part. R^2%	Add. effect
2	2	QWts.ipk-2A-GC7	Xgwm0339-2A / Xgwm0448-2A	82-86	84	3.21	9.8	-0.423
		Weight of 3 spikes per plant(S)						
1	1	QWts.ipk-7D-GS7	Xgwm1220-7D / Xgwm1002-7D	54-72	66	3.83	14.7	-0.203
2	2	QWts.ipk-5A-GS7	Xgwm0156-5A / Xgwm1236-5A	64-84	70	7.32	21.6	-0.305
3	3	QWts.ipk-2A-GS7	Xgwm0122-2A / Xgwm0339-2A	78-84	82	4.79	18.6	-0.249
4	4	QWts.ipk-2D-GS7	Xgwm0071-2D / Xgwm0484-2D	40-56	46	3.46	12.1	-0.215
		Plant height(C)						
1	1	QPhe.ipk-7B-FC5	Xgwm0897-7B / Xgwm0783-7B	58-100	64	4.79	7.91	3.412
2	2	QPhe.ipk-7D-FC5	Xgwm1002-7D / Xbarc126-7D	70-84	74	3.73	20.25	5.253
3	3	QPhe.ipk-5B-FC5	Xbarc74-5B / Xgwm0777-5B	58-74	62	9.14	15.95	-5.221
4	4	QPhe.ipk-4A-FC5	Xgwm0832-4A / Xgwm1169-4A	110-122	114	2.67	8.7	-3.247
5	5	QPhe.ipk-4B-FC5	Xgwm1084-4B / Xgwm0538-4B	26-42	32	4.60	11.12	3.942
1	2	QPhe.ipk-7D-GC7	Xbarc352-7D / Xgwm1220-7D	48-70	54	6.35	18.7	5.626
2	4	QPhe.ipk-4A-GC7	Xgwm0832-4A / Xgwm1169-4A	110-122	114	3.82	9.11	-3.462
3	5	QPhe.ipk-4B-GC7	Xgwm0940-4B.1 / Xgwm0165-4B	14-18	16	3.04	9.1	3.465
4	6	QPhe.ipk-2B-GC7	Xgwm0526-2B / Xgwm1027-2B	134-164	154	4.61	14.9	4.947
	1	QPhe.ipk-7B-MC	Xgwm0573-7B / Xgwm1184-7B	44-54	48	4.56	10.16	3.044
	3	QPhe.ipk-5B-MC	Xbarc74-5B / Xgwm0777-5B	56-70	62	7.64	17.29	-4.305
	5	QPhe.ipk-4A-MC	Xgwm0832-4A / Xgwm1169-4A	110-120	114	5.13	12.52	-3.581
	4	QPhe.ipk-4B-MC	Xgwm1084-4B / Xgwm0538-4B	26-44	34	6.17	18.5	4.518
		Plant height(S)						
1	1	QPhe.ipk-7D-GS7	Xgwm1220-7D / Xgwm1002-7D	50-72	64	4.57	12.08	3.408
2	2	QPhe.ipk-5B-GS7	Xgwm0133-5B / Xbarc74-5B	56-74	60	4.84	9.75	-3.213
3	3	QPhe.ipk-4A-GS7	Xgwm0160-4A / Xgwm0832-4A	130-144	140	5.60	14.24	-4.279
4	4	QPhe.ipk-2B-GS7	Xgwm0526-2B / Xgwm1027-2B	148-166	156	7.29	17.55	4.722
		No. of fertile spikes per plant(C)						
1	1	QNfs.ipk-7A-GC7	Xgwm0631-7A / Xgwm0900-7A	70-78	74	4.41	9.7	-0.497
		No. of fertile spikes per plant(S)						
1	1	QNfs.ipk-7D-GS7	Xgwm1220-7D / Xgwm1002-7D	54-70	62	7.44	16.3	-0.537
		Weight of all spikes per plant(C)						
1	1	QWas.ipk-7A-GC7	Xgwm1171-7A / Xgwm890-7A	46-56	50	6.95	8.7	-1.29
		Weight of all spikes per plant(S)						
1	1	QWas.ipk-7D-GS7	Xgwm1220-7D / Xgwm1002-7D	56-70	64	8.12	19.4	-0.652
2	2	QWas.ipk-5A-GS7	Xgwm0156-5A / Xgwm1236-5A	62-108	72	5.58	16.3	-0.686
		No. of unfertile tillers per plant(C)						
1	1	QNut.ipk-7B-GC7	Xgwm0297-7B / Xgwm0897-7B	56-100	60	3.15	9.5	0.417
2	2	QNut.ipk-7D-GC7	Xgwm1220-7D / Xgwm1002-7D	60-76	68	5.77	20	0.633
3	3	QNut.ipk-2B-GC7	Xgwm1171-2B / Xgwm1128-2B	0-18	10	3.36	13.1	0.616
4	4	QNut.ipk-1B-GC7	Xgwm0818-1B / Xgwm0140-1B	158-162	162	4.90	12	0.532
		No. of unfertile tillers per plant(S)						
1	1	QNut.ipk-5A-GS7	Xgwm0156-5A / Xgwm1236-5A	50-74	66	5.95	10.7	0.521
2	1	QNut.ipk-7D-GS7	Xgwm1220-7D / Xgwm1002-7D	46-72	66	2.54	8.75	0.383

Field 2004, Greenhouse2004, Field 2005, Greenhouse 2007, Over all mean, Mean of fields, Mean of greenhouses

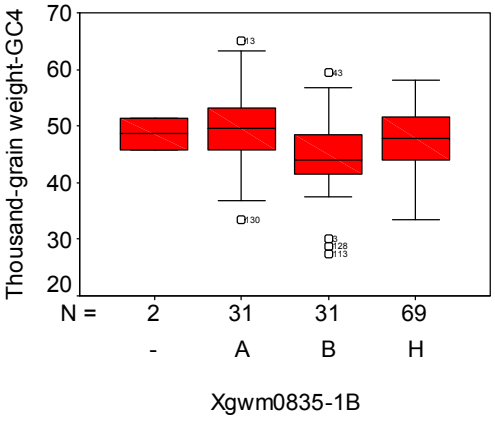
Appendix 8



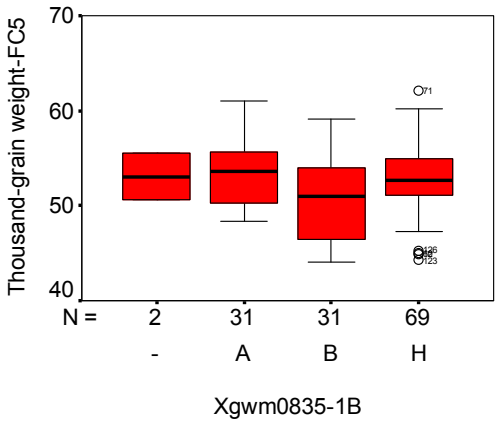
a) Boxplots for *QTgw.ipk-4B-FC4*



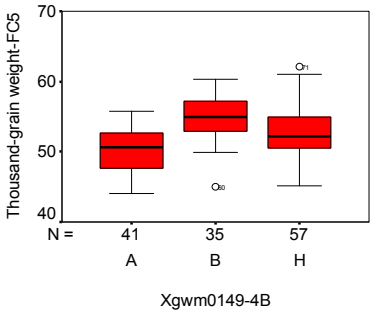
b) Boxplots for *QTgw.ipk-4B-GC4*



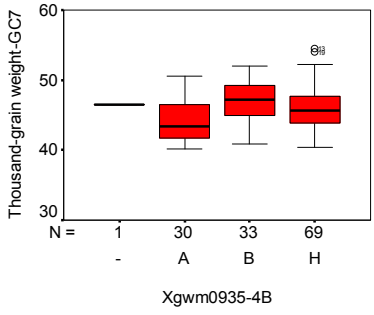
c) Boxplots for *QTgw.ipk-1B-GC4*



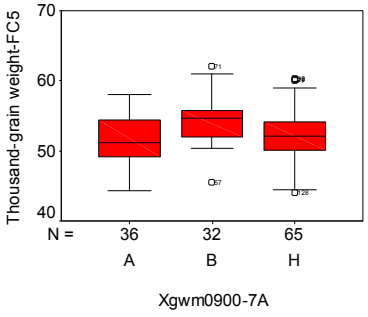
d) Boxplots for *QTgw.ipk-1B-FC5*



e) Boxplots for *QTgw.ipk-4B-FC5*



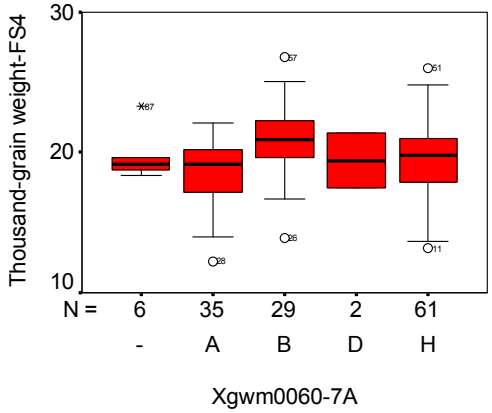
f) Boxplots for *QTgw.ipk-4B-GC7*



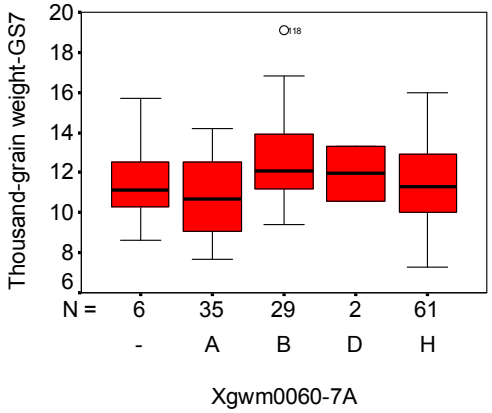
g) Boxplots for *QTgw.ipk-7A-FC5*

Figure 1 a, b, c, d, e, f, and g Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **thousand-grain weight** under control condition.

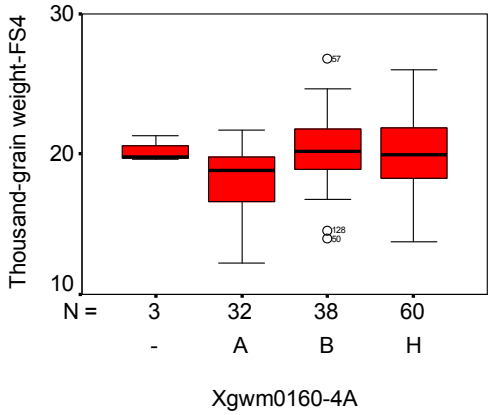
Appendix 8



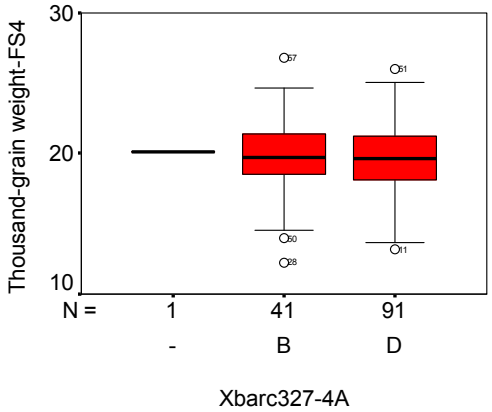
a) Boxplots for *QTgw.ipk-7A-FS4*



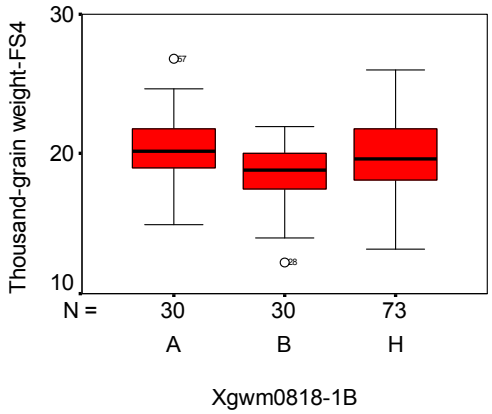
b) Boxplots for *QTgw.ipk-7A-GS7*



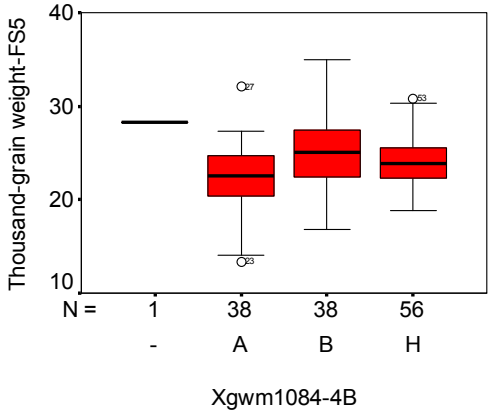
c) Boxplots for *QTgw.ipk-4A-FS4*



d) Boxplots for *QTgw.ipk-4A-FS5*



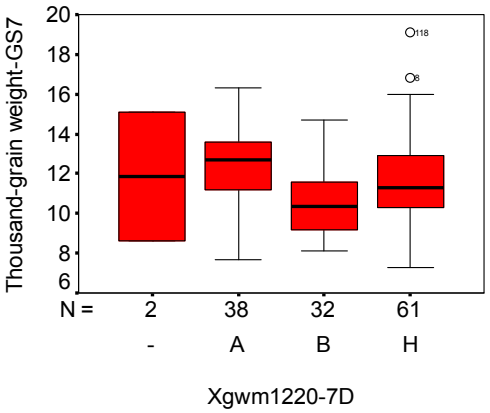
e) Boxplots for *QTgw.ipk-1B-FS4*



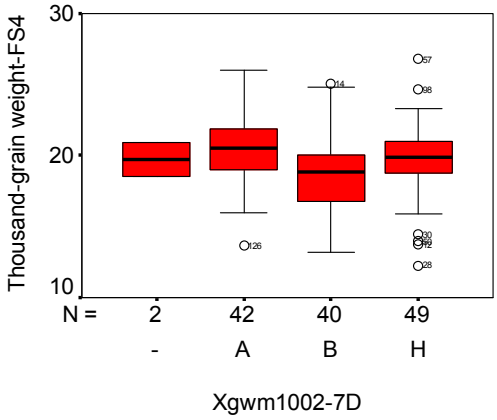
f) Boxplots for *QTgw.ipk-4A-FS5*

Figure 2 a, b, c, d, e, f, g, and h Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **thousand-grain weight** under stress condition.

Appendix 8

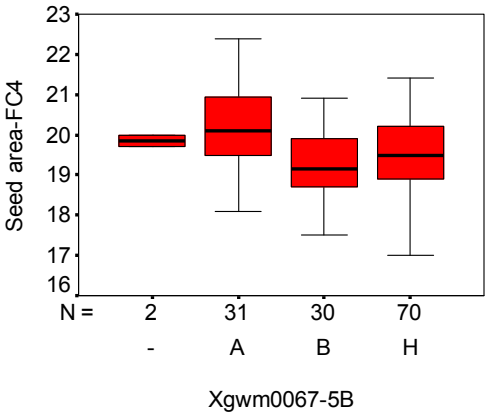


g) Boxplots for *QTgw.ipk-7D-GS7*

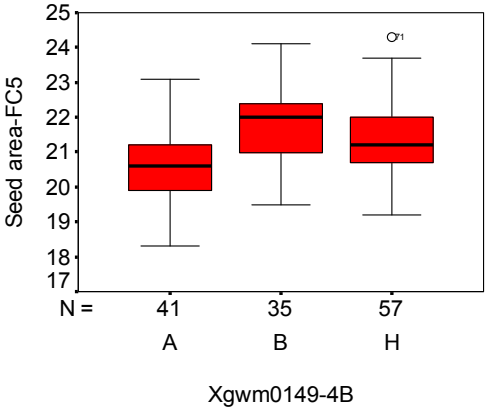


h) Boxplots for *QTgw.ipk-7D-FS4*

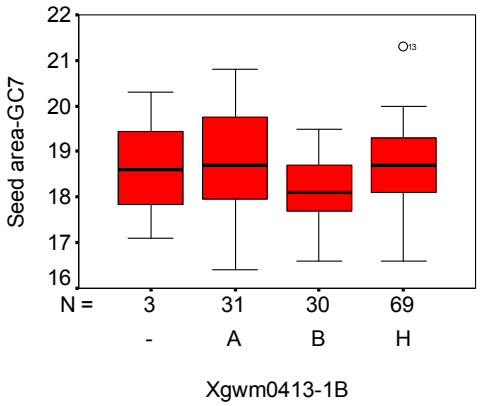
Figure 2 (continued)



a) Boxplots for *QSea.ipk-5B-FC4*



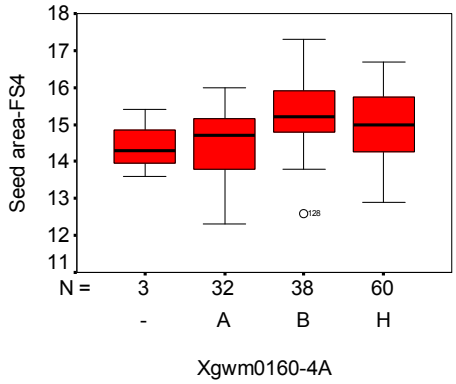
b) Boxplots for *QSea.ipk-4B-FC5*



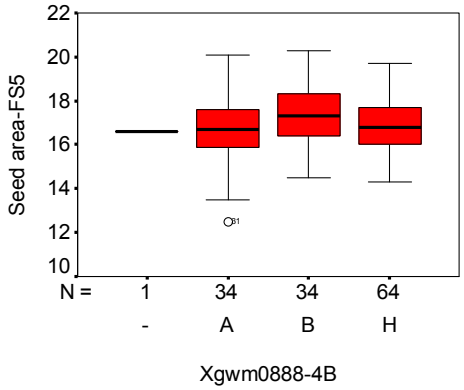
c) Boxplots for *QSea.ipk-1B-GC7*

Figure 3 a, b, and c Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **seed area** under control condition.

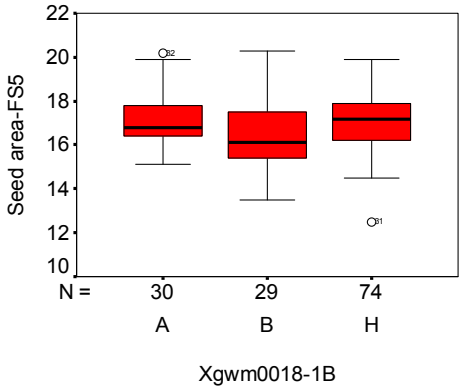
Appendix 8



a) Boxplots for *QSea.ipk-4A-FS4*

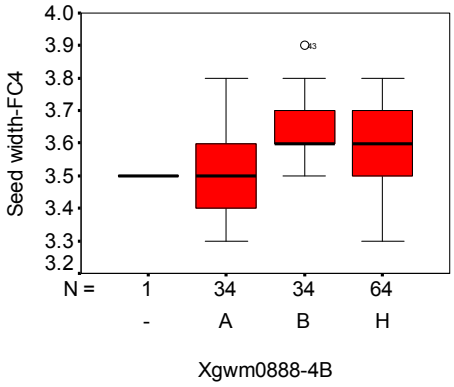


b) Boxplots for *QSea.ipk-4B-FS5*

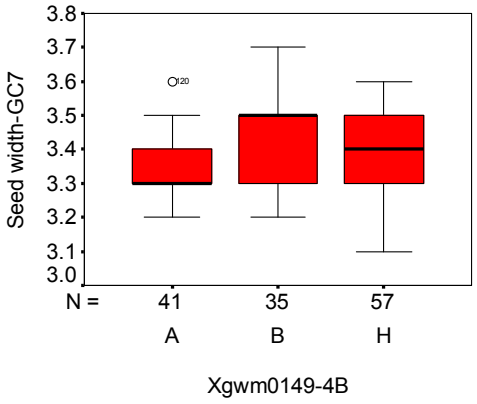


c) Boxplots for *QSea.ipk-1B-FS5*

Figure 4 a, b, and c Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **seed area** under stress condition.



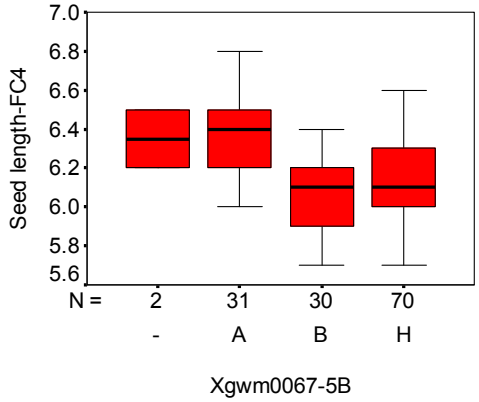
a) Boxplots for *QSew.ipk-4B-FC4*



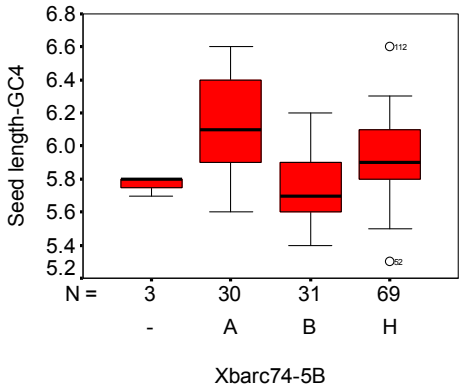
b) Boxplots for *QSew.ipk-4B-GC7*

Figure 5 a and b Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **seed width** under control condition.

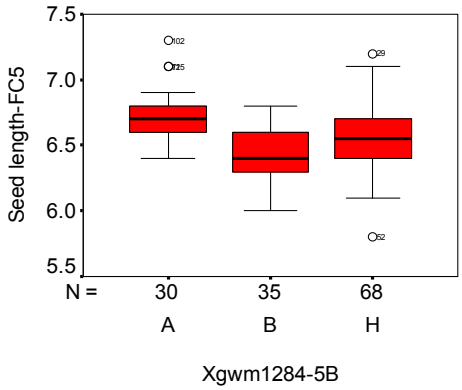
Appendix 8



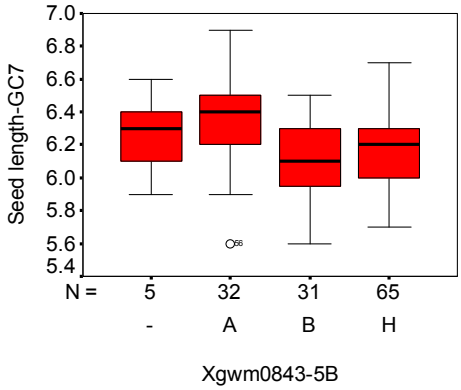
a) Boxplots for *QSel.ipk-5B-FC4*



b) Boxplots for *QSel.ipk-5B-GC4*

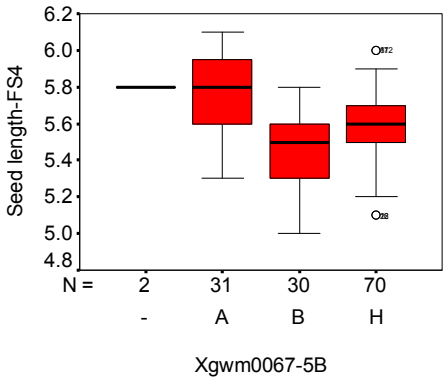


a) Boxplots for *QSel.ipk-5B-FC5*

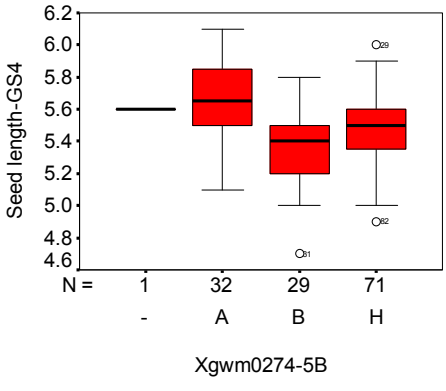


b) Boxplots for *QSel.ipk-5B-GC7*

Figure 6 a, b, c, and d Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **seed length** under control condition.



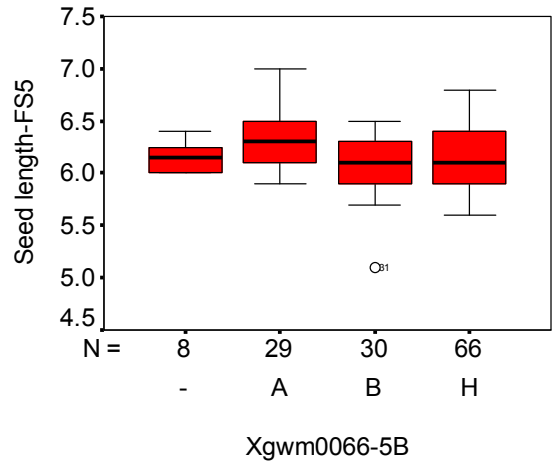
a) Boxplots for *QSel.ipk-5B-FS4*



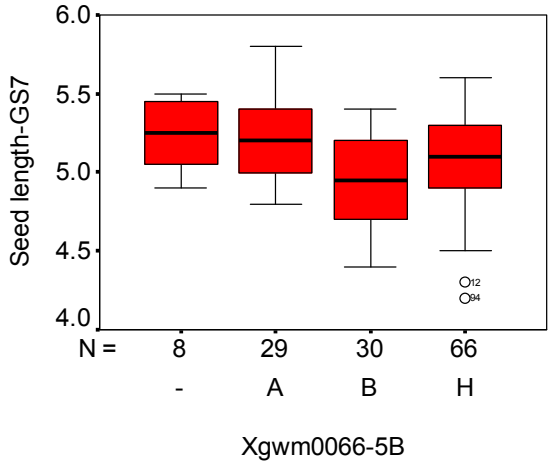
b) Boxplots for *QSel.ipk-5B-GS4*

Figure 7 a, b, c, d, e, f, g, and h Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **seed length** under stress condition.

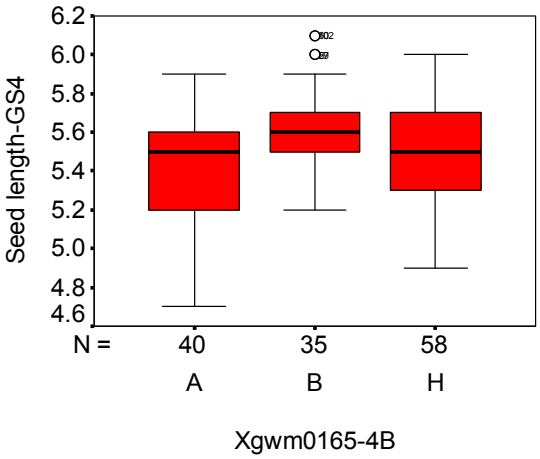
Appendix 8



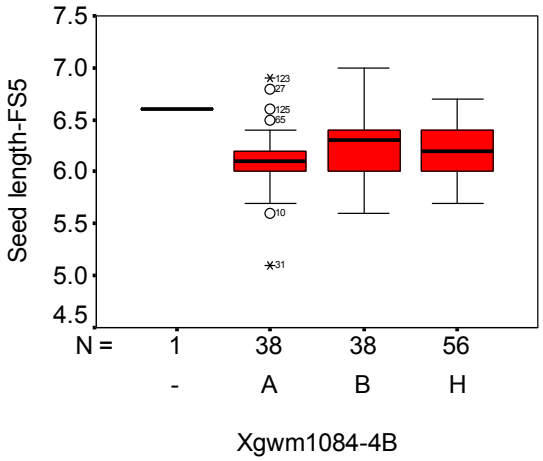
c) Boxplots for *QSel.ipk-5B-FS5*



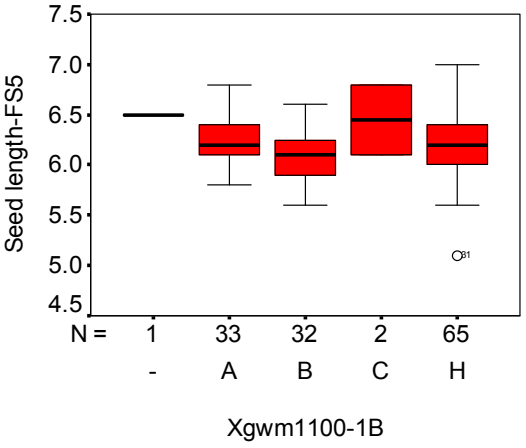
d) Boxplots for *QSel.ipk-5B-GS7*



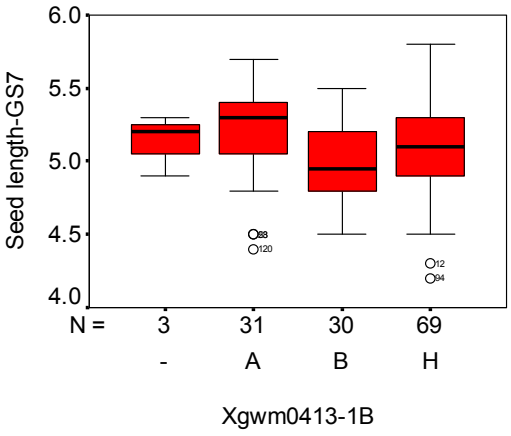
e) Boxplots for *QSel.ipk-5B-FS5*



f) Boxplots for *QSel.ipk-5B-GS7*



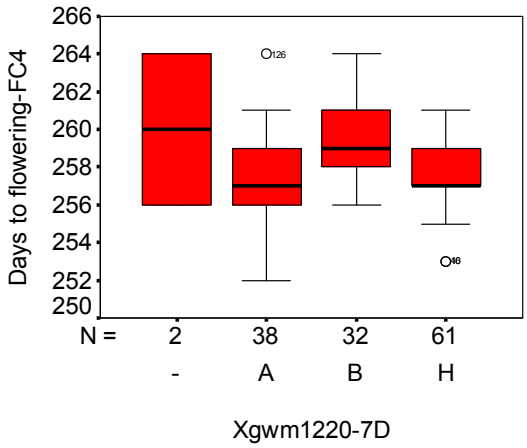
g) Boxplots for *QSel.ipk-5B-FS5*



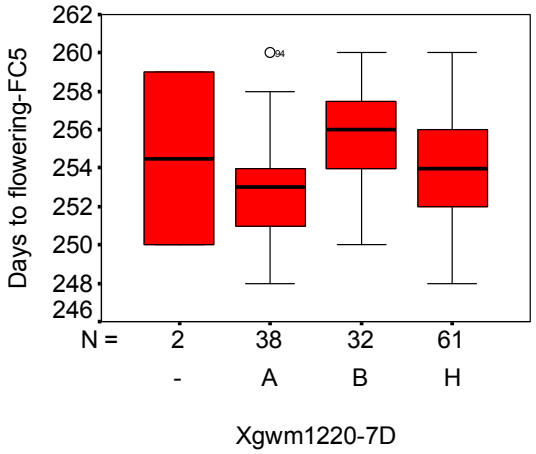
h) Boxplots for *QSel.ipk-5B-GS7*

Figure 7 (continued)

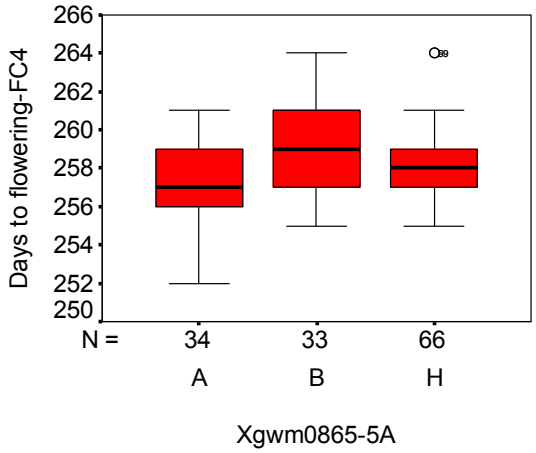
Appendix 8



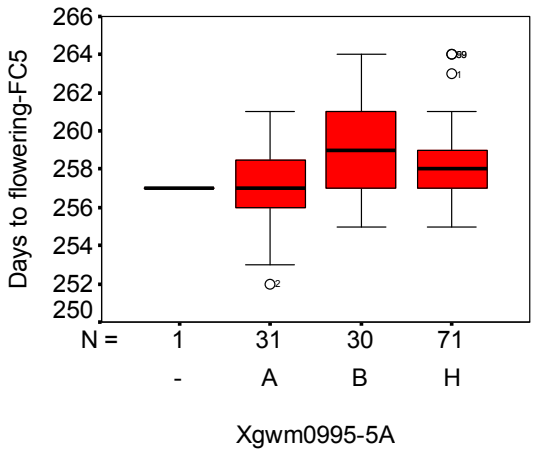
a) Boxplots for *QDtf.ipk-7D-FC4*



b) Boxplots for *QDtf.ipk-7D-FC5*



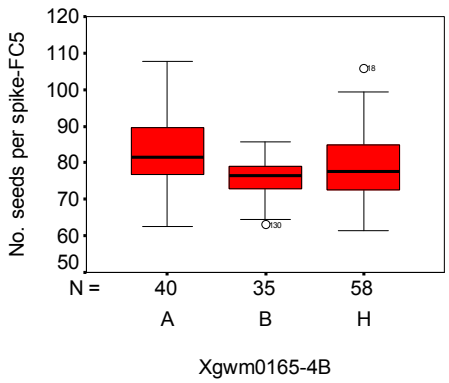
c) Boxplots for *QDtf.ipk-5A-FC4*



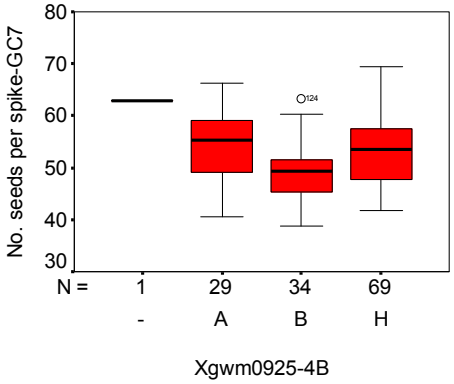
d) Boxplots for *QDtf.ipk-5A-FC5*

Figure 8 a, b, c, and d Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **days to flowering** under control condition.

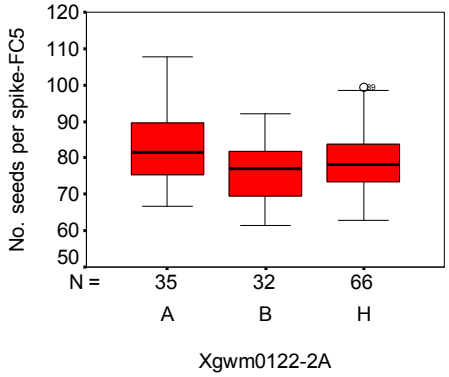
Appendix 8



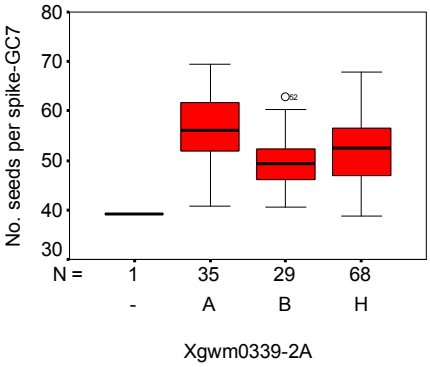
a) Boxplots for *QNsp.ipk-4B-FC5*



b) Boxplots for *QNsp.ipk-4B-GC7*

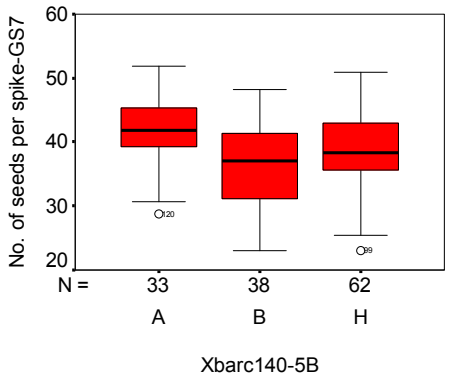


c) Boxplots for *QNsp.ipk-2A-FC5*

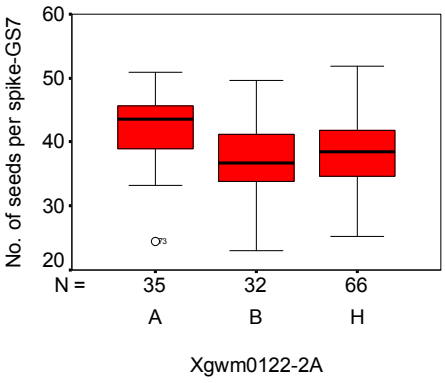


d) Boxplots for *QNsp.ipk-2A-GC7*

Figure 9 a, b, c, and d Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **number of seeds per spike** under control condition.



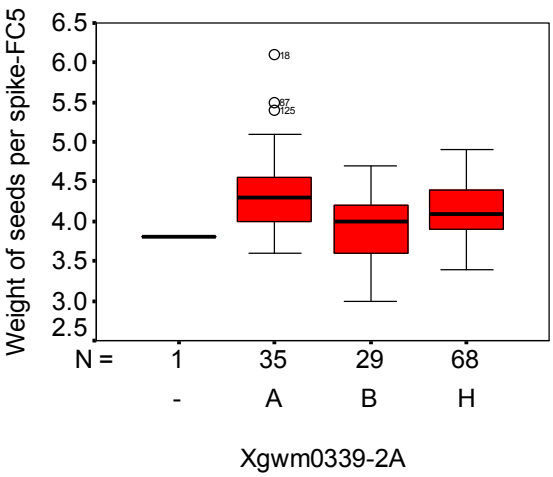
c) Boxplots for *QNsp.ipk-5B-GS7*



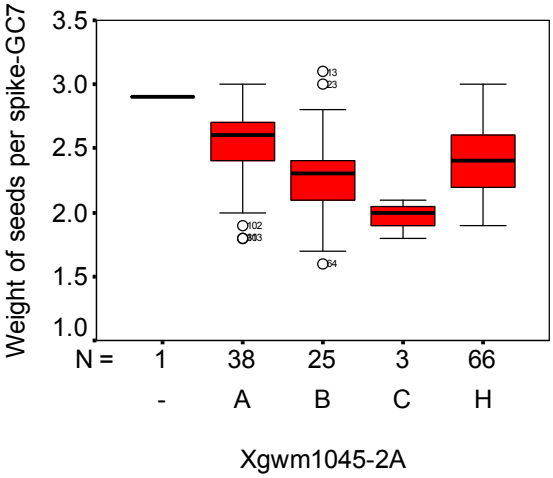
d) Boxplots for *QNsp.ipk-2A-GS7*

Figure 10 a, and b Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **number of seeds per spike** under stress condition.

Appendix 8

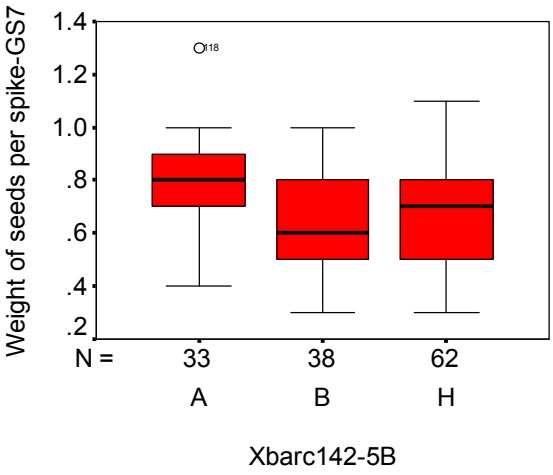


a) Boxplots for *QWsp.ipk-2A-FC5*

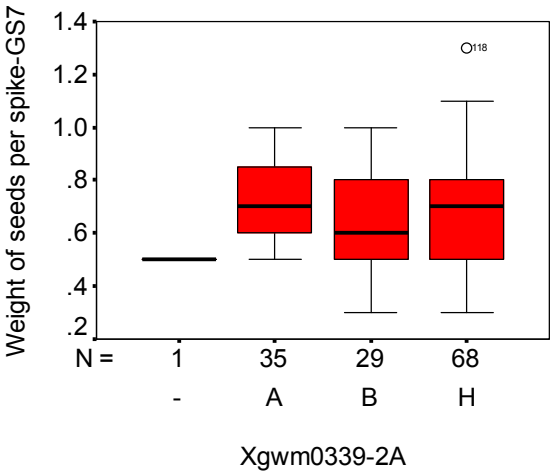


d) Boxplots for *QWsp.ipk-2A-GC7*

Figure 11 a, and b Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **weight of seeds per spike under** control condition.



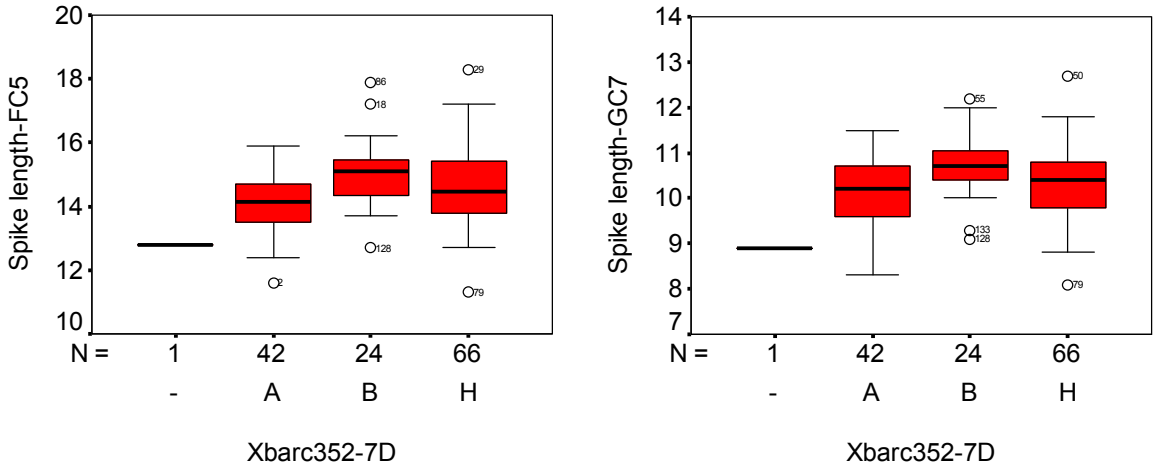
a) Boxplots for *QWsp.ipk-5B-GS7*



b) Boxplots for *QWsp.ipk-2A-GS7*

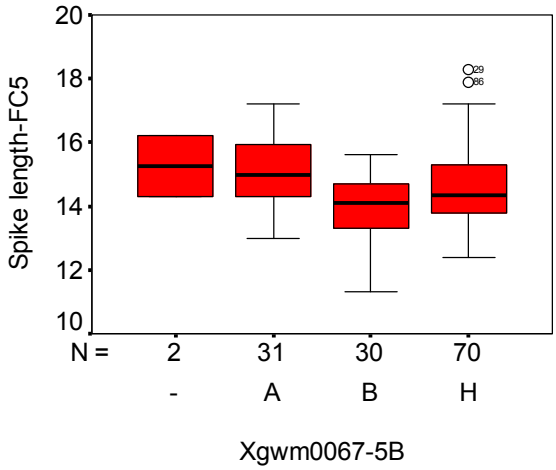
Figure 12 a, and b Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **weight of seeds per spike** under stress condition.

Appendix 8

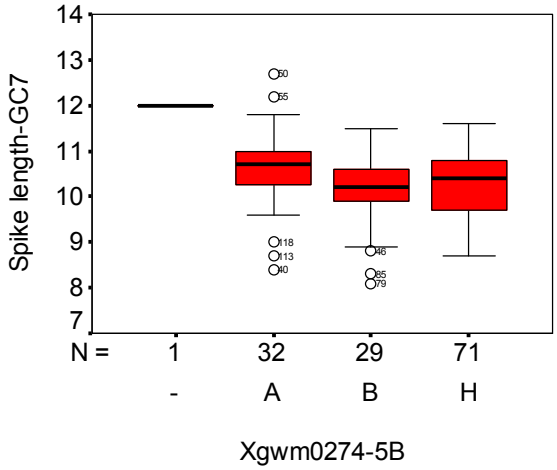


a) Boxplots for *QSpl.ipk-7D-FC5*

b) Boxplots for *QSpl.ipk-7D-GC7*



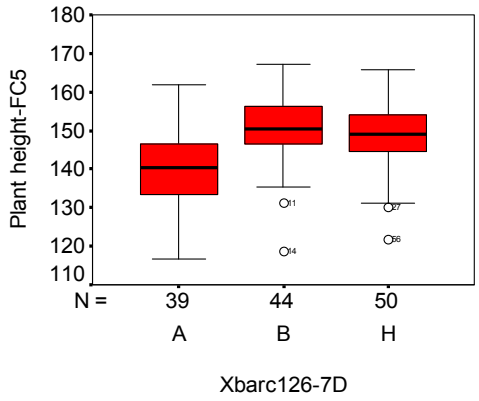
c) Boxplots for *QSpl.ipk-5B-FC5*



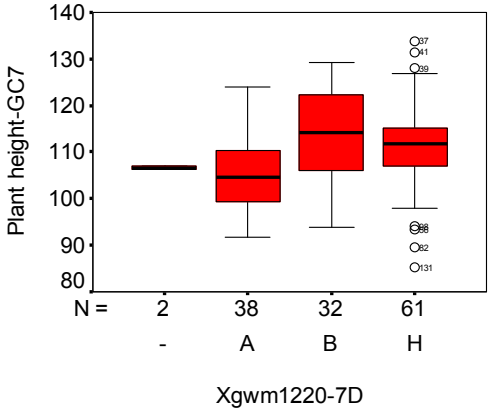
d) Boxplots for *QSpl.ipk-5B-GC7*

Figure 13 a, b, c and d Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **spike length** under control condition.

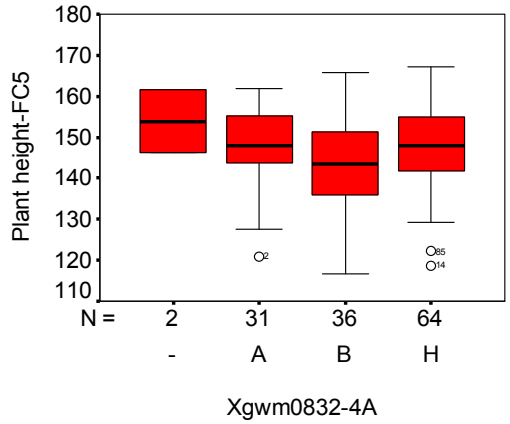
Appendix 8



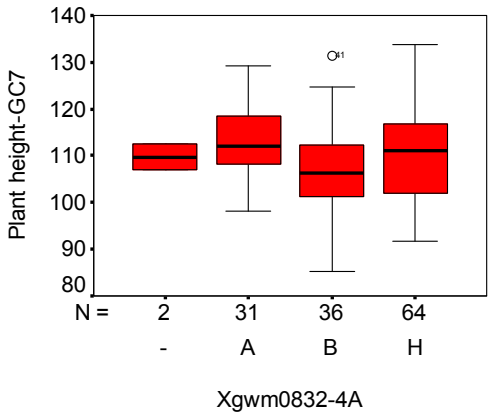
a) Boxplots for *QPhe.ipk-7D-FC5*



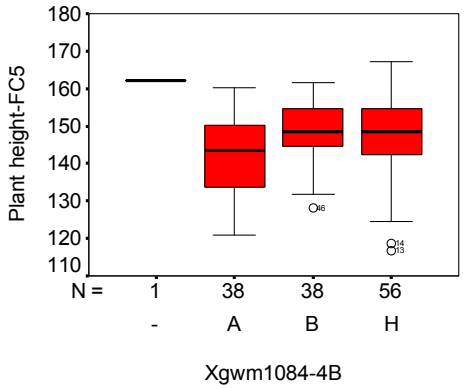
b) Boxplots for *QPhe.ipk-7D-GC7*



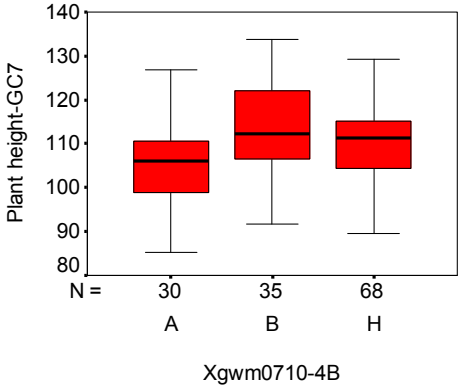
c) Boxplots for *QPhe.ipk-4A-FC5*



d) Boxplots for *QPhe.ipk-4A-GC7*



e) Boxplots for *QPhe.ipk-4B-FC5*



f) Boxplots for *QPhe.ipk-4B-GC7*

Figure 14 a, b, c, d, e and f Boxplots using left or right marker of the identified QTL to verify the efficiency of the markers to discriminate the $F_{2:3}$ families for the trait **plant height** under control condition.

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Khalil Zaynali Nezhad

26 April 2010, IPK-Gatersleben, Germany

Curriculum Vitae

Personal details
First name: Khalil
Last name: Zaynali Nezhad
Nationality: Iranian
Data of Birth: 9 February 1972 Tonekabon, Iran

E-mail: khalil@ipk-gatersleben.de & khalil1381@yahoo.com

PhD study:
PhD enrolled since April 2005 at Martin-Luther-University Halle-Wittenberg, Germany.
Research project at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany.

PhD research title:
Genetic linkage map construction and identification of Quantitative Trait Loci (QTLs) determining post-anthesis drought tolerance and other agronomic traits in bread wheat

University education:
MSc in plant breeding 2000, Isfahan University of Technology, Isfahan, Iran.
BSc in agronomy & plant breeding 1997. Tehran University, Tehran, Iran.

Presentations in meetings:

(Poster Presentations)
1. Zaynali Nezhad K, Lohwasser U, Röder MS, and Börner A. Preliminary results from studies of post anthesis drought tolerance in wheat (Triticum aestivum L.). German Society for Plant Breeding 15-16 November 2005, IPK-gatersleben, Germany. (Poster)

2. Zaynali Nezhad K, Lohwasser U, Röder MS, and Börner A.Towards molecular mapping of QTLs determining post anthesis drought tolerance in wheat (Triticum aestivum L.) German Society for Plant Breeding 14-16 March 2006, Freising- Weihenstephan, Germany (poster)

3. Zaynali Nezhad K, Lohwasser U, Khlestkina E, Röder MS, and Börner A. Assessment of post-anthesis drought tolerance on bread wheat (Triticum aestivum L.). "German Society for Plant Breeding"4-5 October 2007, Martin-Luther-University in Halle (Poster)

4. Zaynali Nezhad K, Lohwasser U, Röder MS, and Börner A. Mapping of Agronomic Important Traits in Bread Wheat (*Triticum aestivum* L.)Molecular Mapping & Marker Assisted Selection in Plants. February 3-6 2008, Vienna, Austria (Poster)

(Oral presentations)
1. Zaynali Nezhad K, Study on genetic diversity of some of Iranian rice germplasm based on morphological treats. 24-28 August 2000, Babolsar, Iran.

2. Zaynali Nezhad K, Lohwasser U, Röder MS, and Börner A. Phenotyping and genotyping of a new wheat mapping population with respect to post anthesis drought tolerance. Second PhD-Student Conference. May 30th - 1st June, 2006 IPK-gatersleben, Germany.

3. Zaynali Nezhad K, Lohwasser U, Röder MS, and Börner A. Post-anthesis drought tolerance assessment on Iranian bread wheat. Plant Science Student Conference, June, 5-8, 2007, Leibniz Institute of Plant Biochemistry in Halle/Saale

4. Zaynali Nezhad K, Lohwasser U, Röder MS, and Börner A. Linkage construction and mapping of agronomic important traits in bread wheat. Plant Science Student Conference, July 1st-4th, 2008, IPK-gatersleben, Germany

Courses and workshop participated:

1. WUEMED training course on Integrated Approaches to Improve Drought Tolerance in Crops, June 5-10, 2006 held at University of Bologna, Italy

2. Training course on Genetic Mapping QTL Analysis in Plants – Statistical Methods and Software Applications. February 12-15, 2007, Martin-Luther-University Halle-Wittenberg, Germany.

3. Training course on Map Construction and QTL Analysis in Plants, September 24-28, 2007, University of Copenhagen, Faculty of Life Sciences. Department of life Sciences. Denmark.

4. TILLING training workshop: theory and practice, April 1st -3rd, 2008, Institute for Plant Breeding, Christian-Albechts-University of Kiel, Germany.

5. Methods in population Genomics, 2nd to 5th February 2009, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany.

6. Training course on “Genetic mapping and QTL analysis in experimental populations-Theory and computing. February 09-12, 2009, Martin-Luther-University Halle-Wittenberg, Germany.

Publications

1- BÖRNER, A., DOBROVOLSKAYA, O., KHLESTKINA, E., LOHWASSER, U., NAVAKODE, S., RÖDER, M. S., SCHUBERT, V., WEIDNER, A., ZAYNALI NEZHAD, K. (2006): Leaf Rust Resistance / Drought Stress / Aluminium Tolerance / Pre-Harvest Sprouting – Dormancy / Duplicate Identification in germplasm collections / Leaf Pubescence Genes / Glume Colour and Pubescence / Purple Grain Colour. Ann. Wheat Newsletter 52, 24-27

2- Zaynali Nezhad K, Lohwasser U, Röder MS, and Börner A. 2006. Primary results from studies of post anthesis drought tolerance in wheat (*Triticum aestivum* L.). Vortr. Pflanzenzucht. 70, 90-92**.**

3- A. Börner, N. Iqbal, E.K. Khlestkina, S. Landjeva, U. Lohwasser, S. Navakode, K. Neumann, E.G. Pestsova, M.S. Röder, M.R. Simon, A. Weidner and K. Zaynali Nezhad.(2007) *Rht* dwarfing genes specific markers/Stripe rust adult-plant resistance/Leaf rust resistance originated from *Ae. Markgrafii* /Detection of Septoria tritici blotch resistance genes employing wheat *Ae. Tauschii* introgressions/Osmotic stress response in wheat seedlings/Salt tolerance/Aluminum tolerance/ Preharvest sprouting/dormancy/A novel gene for grain weight *gwl* and a novel *Rht* locus on chromosom arm 7DS. Ann. Wheat Newsletter 53, 20-25

4- Zaynali Nezhad K, Lohwasser U, Khlestkina E, Röder MS, and Börner A. 2007, Assessment of post-anthesis drought tolerance on bread wheat (*Triticum aestivum* L.) Vortr. Pflanzenzucht. 72, 219-222**.**

5- A. Börner, O. Dobrovolskaya, E.K. Khlestkina, S. Landjeva, U. Lohwasser, P. Martinek, S. Navakode, M.S. Röder, A. Voylokov, A. Weidner, K. Zaynali Nezhad.(2008) Spike morphology genes / Flowering time and protein content on chromosome 7B / Post anthesis drought tolerance /Osmotic stress response in *Rht* wheat seedlings / Anthocyanin pigmentation / Disease resistance originated form Ae. markgrafii / Aluminium tolerance / Seed longevity / Pre-Harvest Sprouting / Dormancy. Ann. Wheat Newsletter 54, 46-50

6-. A. Börner, E.K. Khlestkina, B. Kobiljski, U. Kumar, S. Landjeva, U. Lohwasser, M. Nagel, S. Navakode, M.A. Rehman Arif, M.S. Röder, A. Weidner, L.Q. Xia, and K. Zaynali Nezhad (2009) Molecular linkage map of durum wheat / Molecular linkage map of bread wheat / Stable, across-environment QTL./ Anthocyanin pigmentation genes on homoeologous group-7 chromosomes/ Anthocyanin pigmentation in durum wheat / Glume coloration / Preharvest sprouting / dormancy / Leaf rust and powdery mildew resistance derived from *Aegilops markgrafii* / Septoria tritici blotch resistance from *Triticum aestivum* subsp. *Spelta* / Seed longevity / Mapping the trait for seed vigor in the D genome / Spot blotch resistance / Stay-green trait / Viviparous-1 gene associated with preharvest sprouting tolerance in European wheat cultivars. Ann. Wheat Newsletter 55, 53-58

ERKLÄRUNG

Hiermit erkläre ich, dass mit dieser wissenschaftlichen Arbeit noch keine vergeblichen Promotionsversuche unternommen wurden.

Die eingereichte Dissertation mit dem Thema: “Genetic linkage map construction and identification of Quantitative Trait Loci (QTLs) determining post-anthesis drought tolerance and other agronomic traits in bread wheat” habe ich selbständig und nur unter Verwendung der angegeben Literatur und Hilfsmittel angefertigt.

Des weiteren erkläre ich, dass keine Strafverfahren gegen mich anhängig sind.

Halle/Saale, den 05.05.2010

Khalil Zaynali Nezhad