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Improving crop varieties of spring barley for drought and heat tolerance with AB-QTL-analysis

Inaugural-Dissertation
zur
Erlangung des Grades
Doktor der Agrarwissenschaften
(Dr. agr.)

der
Hohen Landwirtschaftlichen Fakultät
der Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt am 25.03.2004

von

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Tag der mündlichen Prüfung: 27. 05.2004

Gedruckt bei:

Improving crop varieties of spring barley for drought and heat tolerance with AB-QTL-analysis

In the years 2002-2003, 323 BC_2DH individuals of double haploid (DH) spring barley population were genotyped with SSRs markers. The BC_2DH lines were evaluated in greenhouse trials for drought and heat tolerance. Altogether 13 parameters for the determination of drought tolerance and 12 parameters for the investigation of heat tolerance were examined. There were two treatments for the drought experiment, 50% field capacity (FC) level for drought stress and at 100% FC level for the control. We used two treatments for the heat experiment (normal climate and in greenhouse). The traits measured were: relative leaf water content, osmotic adjustment, heading date, number of spikes per plant, number of kernels per spike, number of leaves per main tiller, flag leaf area, first leaf area, second leaf area, carbon isotope discrimination (for the drought experiment), yield, biomass and harvest index. The traits were compared to determine the presence of alleles from the wild barley parent by means of the AB-QTL-analysis. The 97 mapped SSRs covered 1013 cM of the barley genome; the mean SSR density is equal to 11.1 cM. Polymorphic SSRs revealed 54 putative QTLs in two groups. The first had 20 putative QTLs for the drought experiment and the second 34 putative QTLs for the heat experiment. Altogether, 30 (55.5%) favorable allele effects of the *Hsp* alleles were detected for both drought and heat experiment. 14 (70.0%) favorable effects were detected for drought tolerance. These traits, osmotic adjustment, yield, biomass, relative leaf water content, carbon isotope discrimination, number of leaves per main tiller and flag leaf area were controlled by 7, 3, 3, 3, 2, 1 and 1 QTL respectively, in the drought experiment. Most of the favorable *Hsp* alleles were located on chromosomes 1H, 5H and 7H (2, 8 and 3 respectively). Under drought stress first leaf area was positively and strongly correlated with flag leaf area. Positive correlations were expressed by second leaf area with flag leaf area and first leaf area. Yield was positively correlated with harvest index, number of spikes per plant and number of kernels per spike. Biomass showed correlations with number of spikes per plant, number of leaves per main tiller, flag leaf area, first leaf area, second leaf area and yield. 16 (47.0%) favorable effects of the *Hsp* alleles were detected for heat tolerance. Flag leaf area, osmotic adjustment, yield, harvest index, biomass, first leaf area, relative leaf water content, number of spikes per plant and heading date were controlled by 8, 7, 4, 4, 3, 3, 2, 2 and 1 QTL respectively, in heat experiment. Most of the QTLs were located on chromosomes 3H and 4H (3, and 5 respectively). Correlations of heading date with osmotic adjustment, and number of leaves per main tiller were strongly positive. Strong positive correlations were expressed by second leaf area with flag leaf area and first leaf area. Yield was positively and strongly correlated with harvest index.

Verbesserung der Trockenheits- und Hitzetoleranz von Sommergersten-Linien mit Hilfe der AB-QTL-Analyse

Während eines Versuches in den Jahren 2002 und 2003 wurde eine Sommergersten- BC_2DH- Population, die 323 BC_2DH- Einzellinien umfasste, mit 97 polymorphen SSR-Markern genotypisiert. Parallel wurden die BC_2DH-Linien in Gewächshausversuchen auf ihre Trockenheits- und Hitzetoleranz hin phänotypisch untersucht. Hierzu wurden im Trockenstressversuch 13 Merkmale und im Hitzestressversuch 12 Merkmale erhoben. Im Trockenstressversuch wurden zwei Behandlungen unterschieden: (1) Boden mit 50% Feldkapazität (FC) (zur Erzeugung von Trockenstress), (2) Boden mit 100% Feldkapazität (FC). Auch im Hitzestressversuch gab es zwei unterschiedliche Behandlungen: (1) Normales Klima, (2) Gewächshausklima. Die Linien wurden auf folgende Merkmale phänotypisch untersucht: relativer Wassergehalt des Blattes, osmotischer Druck, Zeitpunkt des Ährenschiebens, Anzahl der Ähren pro Pflanze, Anzahl der Körner pro Ähre, Anzahl der Blätter pro Trieb, Blattflächenindex des Fahnenblattes, Blattflächenindex des ersten Blattes, Blattflächenindex des zweiten Blattes, Ertrag, Biomasse und Harvest Index. Im Trockenstressversuch wurde zusätzlich das Merkmal Karbonisotopunterscheidung erhoben. Die Merkmalsdaten wurden mit dem Vorhandensein der Allele des Wildgerstenelternteils mittels der AB-QTL-Analyse verglichen. Die 97 genotypisierten SSRs decken 1013 cM des Gerstengenoms ab, wobei die mittlere SSR-Dichte 11,1 cM betrug. Die Karte Scarlett*ISR42-8 enthält vier Lücken mit einem Markerabstand von mehr als 30 cM, wobei die Lücken auf den Chromosomen 3H, 5H und 6H lokalisiert sind. Ingesamt wurden 54 putative QTLs detektiert, wobei 20 putative QTLs im Trockenstressversuch und 34 putative QTLs im Hitzestressversuch gefunden wurden. Insgesamt wurden 30 (55,5%) vorteilhafte QTL-Effekte des Wildformallels (*Hsp*- Allel) in beiden Versuch ermittelt. Für Trockentoleranz wurden 14 (70,0%) vorteilhaften QTL-Effekte des *Hsp*- Allels festgestellt. Hierbei wurden für die Merkmale Ertrag, Biomasse und relativer Wassergehalt jeweils drei QTLs, für die Merkmale Anzahl der Blätter pro Trieb und Blattflächenindex des Fahnenblattes je ein QTL und für das Merkmal osmotischer Druck sieben QTLs gefunden. Für das Merkmal Karbonisotopunterscheidung wurden zwei QTLs lokalisiert. Die meisten der vorteilhaften QTLs waren auf den Chromosomen 1H, 5H und 7H lokalisiert (2, 8 bzw. 3 QTLs). Unter Trockenstress war der Blattflächenindex des ersten Blattes positiv mit dem Blattflächenindex des Fahnenblattes und dem Blattflächenindex des zweiten Blattes korreliert. Das Merkmal Ertrag zeigte positive Korrelationen mit dem Harvest Index, der Anzahl der Ähren pro Pflanze und der Anzahl der Körner pro Ähre. Die Biomasse korrelierte mit der Anzahl der Ähren pro Pflanze, der Anzahl der Blätter pro Trieb, dem Blattflächenindex des Fahnenblattes, dem Blattflächenindex des ersten Blattes, dem Blattflächenindex des zweiten Blattes und dem Ertrag. Für Hitzetoleranz wurden 16 (47,0%) vorteilhafte QTL-Effekte des *Hsp*- Allels ermittelt. Dabei wurden für die Merkmale Blattflächenindex des ersten Blattes, relativer Wassergehalt des Blattes und Anzahl der Ähren pro Pflanze jeweils zwei QTLs lokalisiert. Für den Harvest Index und die Biomasse wurden je drei QTLs gefunden, wohingegen für das Merkmal Zeitpunkt des Ährenschiebens nur ein QTL ermittelt wurde. Für die drei Merkmale Blattflächenindex des Fahnenblattes, osmotischer Druck und Ertrag wurden acht, sieben bzw. vier QTLs gefunden. Die meisten der vorteilhaften QTLs waren auf den Chromosomen 3H und 4H lokalisiert (je 3 QTLs). Eine Korrelation konnte zwischen dem Zeitpunkt des Ährenschiebens und den Merkmalen osmotischer Druck und Anzahl der Blätter pro Trieb gemessen werden. Positiv korreliert waren außerdem der Blattflächenindex des zweiten Blattes mit Blattflächenindex des Fahnenblattes und des ersten Blattes. Der Ertrag zeigte einen positiven Zusammenhang mit dem Harvest Index.

Table Listing

Figure Listing

1. Introduction

Drought and heat represent a major problem for agriculture in arid and semiarid areas. By classical plant breeding supported by new biotechnological methods, new varieties, which are sufficient for the special growth requirements in hot and dry regions, can be bred. These drought and heat-tolerant varieties can produce increased yields, not only in semiarid zones, but also in temperate areas with temporary drought and heat occurrence. In semiarid areas, water unavailability is frequently happened. Molecular breeding methods can enable the cultivation of drought-tolerant varieties, with water saving capacity. The presence of drought and heat resistant varieties in Third World countries reduces frequent harvest failures and eliminates the need of grain import. These varieties represent an important thus economic advantage for countries of semiarid zones.

Using the AB-QTL analysis strategy as devised by Tanksley and Nelson (1996), favorable alleles from wild barley can be transferred for the improvement of heat and drought tolerance in barley cultivars. Among four German barley cultivars, 12 traits for heat and drought tolerance are examined in order to determine traits, which show significant deviations for drought and heat stressed plants.

Recurrent backcrossing was made between a wild barley parent (ISR 42-8) from Middle East and the German cultivar Scarlett as the recurrent parent. The idea seems reliable to identify the QTLs from highly tolerant wild relatives and simultaneous by to intrigues those alleles into elite cultivars.

The population of 323 BC_2DH lines was genotyped with 97 SSR-markers. Altogether 13 traits for the determination of drought tolerance and 12 traits for heat tolerance were examined over two years. In a statistical analysis, the genotypic and the phenotypic data were correlated to detect and localize alleles from the wild barley, which have an influence on the expression of the examined quantitative traits. Subsequently, lines were compared with QTL alleles of the wild barley and with QTL alleles from the barley cultivar, in order to discover favorable alleles from the wild barley.

Drought is the major cause of crop yield reduction in the world today. Breeding crops with improved drought tolerance is one approach to alleviate this problem. However, progress towards this goal has been slow because of the complexity of the trait and its quantitative inheritance. Barley is an excellent crop for studies on both the inheritance and physiology of this trait.

In an experiment during (2002 - 2003), spring barley double haploid (BC_2DH) populations were developed. The population including 323 individuals was genotyped with 97 polymorphic markers. The BC_2DH lines were evaluated in greenhouse trials for drought and heat traits. At the

end of the two-year experiment, the phenotypic and genotypic data were statistically analyzed. At the experiment, favorable alleles of wild species from the AB-QTL analysis were detected, for the important trait related to tolerance for drought and heat.

Aims of the study

The major objective of this research work was to improve the level of drought and heat tolerance in barley BC_2DH lines to be stable for production in drought prone environments of the Mediterranean region. Application of the AB-QTL strategy in barley is important for improvement of drought and heat tolerance in barley. This could be achieved through identification and simultaneous transfer of the exotic QTL alleles, which have the potential to improve yield-related traits.

The specific aims were:

- To study the QTL effects of *Hsp* alleles for marker*drought treatments interaction in BC_2DH lines.
- To study the QTL effects of Hsp alleles for marker*heat treatments interaction in BC2DH lines.
- To find favorable Hsp alleles associated with the improvement of drought and heat related traits for use in marker-assisted-selection in barley breeding.

2. Review of Literature

Most of drought and heat traits in crops are quantitative in nature. They are controlled by polygenes, displaying interactions among genes and with drought treatments as well as, heat treatments. These make their genetic inheritance complicated and difficult to be understood. The procedures for finding and locating the quantitative trait loci (QTL) and analyzing their magnitude of genetic effects and interactions with drought treatments as well as, heat treatments, are called QTL. This bridges the gap between continuous phenotypic variation and the inherited mechanisms by dissecting genetic variation into individual loci (Phillipa 1998). QTL finding might open up new possibilities for marker based selection in plant breeding. Basically, the procedures of QTL finding involve construction of linkage map and searching for a relationship between drought treatments, heat treatments and markers (Zhao 2002).

Background: Access to and control of water resources are becoming the most important. Today 450 million people are subjected to severe water shortage and in 2025; this number may be about 2.7 billion (or 1/3 of the world population). Some are advocating an increase of farm water use by 15 to 20% for sustaining food security and alleviating rural poverty. Environmentalists claim, however, that water resources should drop by 10% in the coming 25 years to be able to protect natural water resources (in rivers, lakes and wetlands). There are distinct options for managing water resources. Irrigation was the traditional approach for dealing with water shortages but now as water resources are scarce other solutions are sought. For example, plant breeders are working in the development of crops better adapted to drought-prone environments or in plants with increase water-use efficiency. Research suggests that relatively high productivity may be accomplished even in unfavorable environments if selection for adaptation to these environments occurs in targeted crops. Nevertheless, selection for tolerance in stress environments often leads to low yielding genotypes when grown in non-stress environments (Ortiz *et al* 2001).

Many observers have pointed out the dangers of future food shortages and famine due to impending global water shortages. Already, one-third of the world's population faces water shortages, and this proportion is expected to rise to two-thirds by the year 2025 report by (Annan, 2000). Competition between urban and rural areas, for water increased the demand for water due to rising living standards. In addition, changes in annual precipitation and rainfall patterns as a result of environmental change indicate that water demand and supply are in the process of a major change.

In the past, when water was insufficient for agricultural production, irrigation systems based on the construction of dams and canals had been put in place. However, the number of areas where new irrigation infrastructure is economically viable is becoming limited. Concerns have also increased about the negative impacts on the environment. New approaches are especially needed for water-limited semi-arid and arid environments, as well as in other environments with unreliable rainfall and uncertain water availability for agriculture.

For these reasons, the development of drought-resistant and stress-tolerant crops coupled with small-scale but effective technologies to make efficient use of limited water resources on a regional basis are needed. Ecological approaches, breeding, and transgenic improvements can provide crop resources to boast the resource-efficient technologies. These technologies include farm and watershed-based water collection and storage, improved agronomic practices that use soil water more efficiently, and water-saving crop production techniques. Such technologies are adapted to both the environmental conditions and the production practices of farmers in the area for which they are developed. The development of such technologies and establishment of stable and sustainable agricultural production systems, and ultimately living environments, are essential to maintain a world environment in balance.

2.1 Morphological differences between cultivars and wild barley

Taxonomy and origin: Cultivated barley, *Hordeum vulgare* L., belongs to the tribe *Triticeae* in the grass family, *Poaceae*. The *Poaceae* is the largest family of monocotyledonous plants. The *Hordeum* L. comprises 32 species (Bothmer *et al*. 1991). It has been suggested that *H. vulgare*, together with *H. bulbosum* L., should be separated into a genus of its own, but this view has not been widely accepted (Bothmer 1992). The progenitor of barley is considered to be a subspecies of cultivated barley: *H. vulgare* ssp. *spontaneum* (C. Koch) Tell. Both cultivated and wild barley have winter and summer annual forms. Barley can be divided into two-rowed and six-rowed types according to spike morphology; intermediate types also exist. In two-rowed barley the lateral spikelets are female sterile, while in six-rowed barley all spikelets are fertile (Briggs 1978).

The most widely accepted hypothesis on the origin of cultivated barley defines the Fertile Crescent as its center of origin (Harlan 1976), but a hypothesis of multicentric origin has also been proposed (Molina-Cano *et al*. 1999). Data from cpDNA analysis suggests that barley has been taken into cultivation more than once, but that only very few domestication events have occurred (Zohary 1969, Neale *et al.* 1988)

Barley is a diploid (2n = 14) and predominantly self-pollinated crop. Consequently, its variation is structured in true breeding lines. Hundreds of modern varieties and thousands of land races are known. All cultivars have non-brittle ears, the spike stay intact after ripening and are harvested and threshed by humans. This is in sharp contrast with wild barleys, in which ears always brittle. Non-brittleness in cultivated barley is governed by a mutation in either one of two tightly linked `brittle´ genes (Bt1, Bt2). The brittle wild-type allele in each locus is dominant, whereas, the non-brittle alleles are recessive. Many cultivars are homozygous for both recessive mutations. Others carry only one mutation (Takahashi 1964, 1972). The Non-brittle mutation survived only under domestication.

Wild ancestry: The wild ancestor of the cultivated barley is well known. The crop shows close affinities to a group of wild and weedy barley forms which are traditionally grouped in *Hordeum spontaneous* C. Koch, but which are, in fact, the wild race or subspecies of the cultivated crop. The correct name for this wild is therefore *H. vulgare* L. ssp. s*pontaneum* (C. Koch), Tell. These are annual, brittle, two- rowed, diploid (2n = 14), predominantly self-pollinated barley forms and the only wild *Hordeum* stock that is cross compatible and fully interceptive with the cultivated barley, *vulgare* x *spontaneum* hybrids show normal chromosome pairing in meiosis. Also morphologically, the similarity between wild *spontaneous* and cultivated two-rowed *distichal* varieties is rather striking. They differ mainly in their modes of seed dispersal. *Spontaneous* ears are brittle and maturity disarticulates into individual arrow-like triplets. These are highly specialized devices, which ensure the survival of the plant under wild conditions. Under cultivation this specialization broke down and non-brittle mutants were automatically selected for in the man-made system of sowing, reaping and threshing (Harlan and Zohary 1966; Zohary 1969).

The close genetic affinities between the cultivated crop and wild *spontaneum* barleys are indicated also by spontaneous hybridizations that occur sporadically when wild and cultivated forms grow side by side. Some of such hybridization products, combining brittle ears and fertile lateral spikelets, were in the past erroneously regarded as genuinely wild types and even given a specific rank (*H. agriocrithon* Åberg). Extensive isozyme, seed storage proteins, and DNA tests have already been carried out in barley (Nevo 1992). The results confirm the close relationships between the wild and cultivated entities grouped in the *H. vulgare* complex. They also clearly show that genetic diversity in s*pontaneum* wild population is much wider than that present in the cultivated gene pool.

Hordeum v*ulgare* ssp. s*pontaneum* is spread over the East-Mediterranean basin and West Asia, penetrating as far as Turkmenia, Afghanistan, Ladakh, and Tibet. Wild barley occupies primary habitats and man-made habitats. Its center lies of origin in the ´fertile crescent´, starting

from Israel and Jordan in the Southwest, stretching North towards South Turkey and bending southeast Iraqi of Kurdistan and Southwest Iran. In this area, wild *spontaneum* barley is continuously and massively distributed. It constitutes an important annual component of open herbaceous formations, and it is particularly common in the summer-dry deciduous oak park-forest, East, North, and West of the Syrian Desert and the Euphrates basin, and on the slopes facing the Jordan Rift Valley. From here, *H. vulgare* ssp. *spontaneum* spills over the drier steppes and semi-desert.

In the Near Eastern countries, wild barley also occupies a whole array of secondary habitats, i.e. opened-up Mediterranean marquis, abandoned fields, and roadsides. It also infests cereal cultivation and fruit tree plantations (Harlan and Zohary 1966). Further was west, in the Aegean region, the Mediterranean shore of Egypt and Cyrenaica and further East in Northeast Iran, Central Asia and Afghanistan. Wild *spontaneum* barley rarely builds large stands and seems to be completely restricted to segetal habitats, ruins, or to sites which have been drastically churned by human activity. In general, wild barley does not tolerate extreme cold and it is only occasionally found above 1500 m. It is almost completely absent from the elevated continental plateaux of Turkey and Iran. On the other hand, it is somewhat more drought resistant than the wild wheat and penetrates relatively deep into the warm steppes and deserts, Zohary and Hopf, (1993)

2.2 Economic of Barley cultivars

Cultivated barley, *Hordeum vulgare* L., is one of the main cereals of the belt of Mediterranean agriculture and a founder crop of old world Neolithic food production. All over the area barley is a universal companion of wheat, but in comparison with the latter it is regarded as an inferior staple and the poor people's bread. But barley is used to drier conditions, poorer soils and some salinity. Because of these qualities, it has been the principal grain produced in numerous areas and an important element of the human diet. Barley is also the main cereal used for beer fermentation in the old world. The preparation of this beverage seems to be a very old tradition (Darby *et al*. 1977; Hopf 1976; Samuel 1996.) The crop was, and still is an important feed supplement for domestic animals.

The annual world production of barley amounts to 10,927,970 tones (FAO, 2002). After maize, rice and wheat, barley ranks as the fourth most important crop in the world.

The average barley yield in Germany progressed in the last 20 years from 43 dt/ha to approx. 59 dt/ha. In 2000, approx 12 million tons of barley was harvested, with 9 million tons used as a feed. A tenth of the barley world production, mainly summer barley, is used for production of malt for beer

and whisky. The smallest a proportion serves directly for human nutrition in the form of barley (Zacharias 2001).

2.3 Barley breeding

Breeding new barley varieties is based on creating new allele combinations and subsequent testing and selection of the desirable phenotypes during the selfing generations. Heritable variation is created mainly by controlled crosses between adapted high yielding cultivars and breeding lines. Although variety breeding is based on elite germplasm, specific traits may be introgressed from wild barley and landraces in backcrossing programs (Nevo 1992). *Spontaneum* mutations, as well as mutations induced by radiation or chemical treatments, have also been used (Briggs 1978). Recently, genetic diversity has been added to the tools for creating new variation in barley (Ritala *et al*. 1994, Wan and Lemaux 1994). The early generations following crossing are highly heterozygous, making reliable selection difficult until an acceptable level of homozygosity is reached. A short cut to homozygosity can be achieved in barley by producing doubled haploid lines either from immature pollen grains by anther or microspore culture, or through interspecific crosses between barley and *H. bulbosum* with subsequent chromosome elimination (Pickering and Devaux 1992). Both methods are used in commercial barley breeding programs and several doubled haploid varieties have been released.

2.4 What is the importance of drought stress?

Barley crop is considered important cereal crop not only in Germany and Egypt but also all over world. As barley is feeding mankind, there is an increasing interest in barley world-wide. Barley is the important crop in Germany and Egypt covering nearly 1,970,335 and 33,007 ha, produced 10927970 and 100797 tones, respectively (F.A.O statistic production year book 2002). Barley production in Egypt can be increased by extending the presently cultivated land to places with areatic water availability in winter or season fluctuation in rainfall such as North and west Egypt. All over the world, heat and water are clearly among the most important factors affecting plant survival and function. Plant growth and yield are directly controlled by water supply. So, water deficit and changes in the environmental conditions may reduce growth and impair metabolic processes (Hsiao, 1973). Root growth is an important component of the adaptation of rice to drought-prone environments (Price *et al*. 1997). The response of plant to stresses depends on it is genetic potential to adaptation to duration and intensity of drought and heat. Heat or drought resistance in crops could be attributed to either avoiding or tolerating drought. Avoiding drought could be achieved by reducing water loss and /or maintaining water uptake. Tolerance to drought

could be attained through a mechanism that enhances plant ability to withstand low water potential, (Clarke, *et al*. 1984). Crop plant adapt to drought by either avoiding or tolerating cell dehydration (Turner, 1986). Drought avoidance involved rapid morphological development, leaf rolling, leaf shading, reduced leaf area, and increased stomata and cuticular resistance (Morgan, 1984; Turner, 1986). Plants tolerate drought by maintaining sufficient cell turgor. Lowering of the osmotic potential of cells by accumulating solutes was considered due to osmotic adjustment if the build-up compounds were not merely the result of tissue dehydration (Bray 1993). Osmotic adjustment enable water uptake to continue under increasing drought in many crop species and, in some cases, it was associated with maintenance of growth and stable yield under drought conditions (Gunasekera and Berkowitz, 1992). Drought and high temperature usually occur simultaneously, but their effects on plant development are often studied separately. The level of the other stress might alter crop responses to one stress. For instance, high temperature might interact with osmotic adjustment in plants in several ways; it might interact with osmotic adjustment directly by increasing the rate of evaporation (Gates, 1968) or by interfering with the production and utilization of solutes involved in osmotic adjustment (Li *et al*. 1993). Effects that are would alter production of solutes for osmotic adjustment to drought.

Previous studies on heat and drought stresses in crops demonstrated that crop genotypes reacted differently either to high temperature or to drought. In several crops, such as spring wheat (Mustafa *et al*. 1996) and faba bean (link *et al*. 1999); significant relationships between some morphological and physiological characteristics and drought stress have been reported. Thus, morphological and physiological studies of barley genotypes may be used in the breeding program. Reports indicate that drought could significantly increase sugar beet leaf diffusive resistance and thus decrease leaf photosynthesis (Clover *et al*. 1999). It was reported that differences in stomatal diffusive resistance might be seen between genotypes of some crops such as maize and durum wheat (Ray and Sinclair, 1997; Clarke and Clarke, 1996). Drought and heat tolerance tests that were developed for sorghum were adapted to and evaluated in field grown wheat (Blum and Ebercon 1981). In rice, the occurrence of drought at the booting stage is the most damaging event to grain yield because it drastically increases sterility (Kobata *et al*. 1994).

Genotypic differences in proline accumulation have been reported for various different plants such as barley, sorghum and rice (Blum and Ebercon 1976). Although Hanson *et al*. (1977) reported that plant proline accumulating potential should not be utilized as a positive index in screening drought resistance cereals. Physiological response was for barley genotypes to drought stress in order to determine if certain physiological characteristics can be used as a screening tool to select drought

resistance genotypes. The final yield was more reduced when drought was imposed at pollination and flowering stages than vegetative or pod filling stages (Pimentel *et al*. 1999). An only limited view of the genotypic variability of the underground organs; in addition, knowledge was deficits in the relations with the yield formation (Schwarz *et al*. 1989). Genotypic differences in root traits may be responsible for differences in yield especially under unfavorable growing conditions (Schwarz *et al*. 1991).

2.5 Why is heat stress important?

High temperature is a major stress factor limiting crop productivity (Fokar *et al* 1998). Breeding efforts by a number of national wheat breeding programs has resulted in the release of germplasm adapted to warm growing environments, such as in Egypt and Sudan (AbdElShafi and Ageeb, 1994), India (Tandon, 1994), and Uruguay (Pedretti and Kohli, 1991). Photo-assimilation is more likely to be yield limiting under heat stress than in temperate environments, especially as stress typically intensifies during grain filling, when demand for assimilates is greatest. This is borne out by the observation that under stress, total aboveground biomass typically shows a stronger association with yield than with partitioning, harvest index. The situation is usually reversed under temperate conditions. Hence traits affecting radiation use efficiency (such as ground cover, stay green, and photosynthetic rate) could be expected to be important under heat stress. Although early ground cover seems to be important in an agronomic context (Badaruddin *et al*. 1999), variation in this trait among genotypes does not seem to be associated with heat tolerance. Physiological evidence indicates that loss of chlorophyll during grain filling is associated with reduced yield in the field (Reynolds *et al*. 1994). High temperature stress (>35°C) during the grain filling period has the potential to modify grain quality (Blumenthal *et al*. 1995).

Respiration costs are higher as temperature increases, leading eventually to carbon starvation because assimilation cannot keep pace with respiratory losses (Levitt, 1980). However this apparently wasteful process would seem unavoidable, at least in current germplasm, as evidenced by positive associations observed between dark respiration at high temperatures and heat tolerance of sorghum lines (Gerik and Eastin, 1985). On the other hand, high rates of dark respiration in grains may be severely detrimental to yield (Wardlaw *et al*. 1980).

Heat shock proteins are synthesized at very high rates under high temperature stress and are thought to have a protective role under stress; nevertheless, their role in determining genetic differences in heat tolerance has not been established. Chlorophyll fluorescence may be a more promising screening trait, given that associations between heat tolerance and lower fluorescence signals have been reported in a number of crops (Moffat *et al*. 1990).

When growth resources are limited by heat stress, the size of plant organs such as leaves, tillers, and spikes are reduced (Fischer, 1984). The apparent sensitivity of metabolic processes to heat stress in the field (Reynolds *et al*. 1998), coupled with the reduced length of life cycle at high temperature (Midmore *et al*. 1984), explains why grain yield is strongly associated with total plant biomass in hot environments. These interactions make crop management practices critical to sustaining wheat yields in warm environments.

Heat stress reduced both the grain growth duration and the grain growth rate (Viswanathan and Renu 2001). In many parts of the Asian subcontinent, crop damage due to heat stress under late planting conditions has become an important factor limiting wheat yields as a result of the rice-wheat cropping system, (Aslam *et al*. 1989). A growing demand for food due to global warming will in the future push crops further into heat stress environments.

Heat stress reduces grain weight and quality (Ciaffi *et al*. 1995). It reduces the grain growth duration (Ishag and Mohamed 1996) and grain growth rate (Tashiro and Wardlaw 1990). Starch synthesis is highly sensitive to high temperature stress due to the susceptibility of the soluble starch synthesis in developing kernels of wheat (Denyer *et al*. 1994). Protein synthesis is less heat sensitive than starch accumulation (Bhullar and Jenner 1985). However, even short periods of very high temperature (35-40 °C) during development can have a negative effect on grain quality (Ciaffi *et al*. 1995). The steady expansion of the environmental range encompassed by temperate cereals since their domestication 5,000-100,000 years ago has meant that both temperature extremes and water availability have become important factors limiting the production of these cereals in many parts of the world. An added complication in the projected rise in both global mean temperature and frequency of periods of very high temperature (heat shock), as part of the greenhouse climate change, which may further increase the pressure of heat stress in many temperate cereal growing regions (Conroy *et al*. 1994)

High temperature late in the development of the crop are a feature of many of the wheat growing areas in US and maximum day temperatures above 32°C during the last 15 days of kernel filling, is associated with reduced quality. Thompson (1975) made the observation that the importance of high temperature during kernel filling was reinforced by series of time-of-planting. High temperature during grain filling can considerably reduce yield. At high temperature, photosynthesis declined (Paulsen, 1994), dark respiration and photorespiration increased (Lawlor, 1979). Heat stress caused a reduction in mean yield of the random inbred line population by 47% as compared with normal winter growing conditions (non-stress) (Blum *et al*. 2001). The cause for death after lethal heat shock is not well understood. A shift from low to intermediate temperature causes the induction of heat-shock proteins in most organisms (Davidson *et al.* 1996). Although, the

importance of temperatures greater than 32°C, coverage was also given to altered performance due to warming in the moderate temperature range from of 15-32°C during grain filling, recognizing that these two heat ranges may produce distinct reactions (Wardlaw and Wrigley 1994). The heat-shock responses of barley (*Hordeum vulgare* L. cv Himalaya) aleurone layers incubated with or without gibberellic acid (GA3) were compared. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that heat shock blocked the synthesis and secretion of secretary proteins from GA3-treated layers but not untreated layers. Heat shock modestly increased the degree of fatty acid saturation in untreated aleuronic layers. The same trend was noted in fatty acids isolated from ER membranes purified by continuous sucrose density centrifugation. Increased fatty acid saturation may help sustain ER membrane function in heat-shocked aleuronic layers incubated in the absence of GA3 (Grindstaff *et al*. 1996). Cells must survive challenges from the environment with regard to heat, UV radiation and heavy metals as well as tolerate the endogenous generation of reactive oxygen intermediates during respiration (Raitt *et al* 2000). Activation of heat shock factor binding and inducible heat shock protein expression enables cells to resist various stress forms (Schett *et al*. 1999). However, there were no major differences between heat-tolerant variants and non-tolerant variants in the time or temperature required to induce the heat shock response (Park *et al*. 1996).

Evidence suggests that the small chloroplast heat-shock protein is involved in plant thermo tolerance but its site of action is unknown. Functional disruption of this heat-shock protein using anti-heat-shock protein antibodies or addition of purified heat-shock protein to chloroplasts indicated that (a) this heat-shock protein protects thermolabile photosystem II and, consequently, whole-chain electron transport during heat stress; and (b) this heat-shock protein completely accounted for heat acclimation of electron transport in pre-heat-stressed plants. Therefore, this heat-shock protein is a major adaptation to acute heat stress in plants (Heckathorn *et al* 1998). There is increasing evidence for considerable interlinking between the responses to heat stress and oxidative stress (Panchuk *et al*. 2002). Grain sterility and specific forms of morphological and cellular damage depend on the stage of development of grain at the time of transfer (Tashiro and Wardlaw 1990). Temperature (27/22°C) (50% shade) during spike development can reduce the response of the developing grain to high temperature (30/25°C) following anthesis (Wardlaw 1994 and Wardlaw *et al*. 1995). Temperature stress during kernel development affects maize grain growth and yield stability (Cheikh and Jones 1994)

Short periods of high temperature have been shown to reduce grain weight and baking quality in wheat, but little is known about their effects on barley. The high temperature (maximum 40°C for 6 h day^{-1}) and drought treatments were maintained for 5 or 10 days. Drought reduced

individual grain weight much more (ca 20%) than high temperature (ca 5%) (Savin and Nicolas 1996)

2.6 Osmotic adjustment

Drought is an important abiotic factor affecting the yield and yield stability of food cereals of the Mediterranean basin. This stress acts simultaneously on many traits, leading to a decrease in yield. Drought tolerance could therefore, be studied by identifying the traits which have a significant impact on yield, and genetic factors controlling them (Teulat *et al* 2001). Tolerance to drought stress is difficult to characterize and quantify, and there has been relatively little progress in improving drought tolerance in cereals. Among the many physiological characteristics proposed as drought tolerance traits, osmotic adjustment is one of the few that has been associated with increased yield under drought stress (Morgen *et al*. 1986). Measurement made at full turgor may allow this distinction, osmotic adjustment depending only on the amount of solute molecules. Osmotic adjustment is defined as the difference between the osmotic potential at maximal turgor (Wilson *et al* 1979) of the stressed and the unstressed plants. The evaluation of osmotic adjustment requires a comparison between well-watered plants and plants under a defined water stress. However, the definition of well-watered plants also differs according to authors (Basnayake *et al*. 1993). The degree of osmotic adjustment increased as the soil water content decreased (Kuang *et al*. 1990).

Barley could serve as a simple genetic model as it is known to be well adapted to several abiotic stresses, especially to water deficit (Ceccarelli 1987). The maintenance of relative water content and a high osmotic adjustment are known to contribute to increase yield and yield stability under drought in cereals (Clarke and McCiag 1982). Osmotic adjustment is defined as a decrease of osmotic potential within cells, due to an active solute accumulation after water-potential reduction in response to water stress (Blum, 1988). Osmotic adjustment could arise from an increase in the amount of solutes by active solute accumulation or a decrease in the water content on a dry weight basis (Wilson *et al*. 1980). The decrease in osmotic potential leads to maintenance of cell turgor, and, more generally, turgor-dependent processes, suggesting that osmotic adjustment is a good physiological trait to be considered in breeding for drought tolerance. The solutes, which accumulate during osmotic adjustment, include inorganic cations, organic acids, free amino acids and carbohydrates (Turner and Jones 1980). The main solutes accumulated during osmotic adjustment in barley are water-soluble carbohydrates (Lewicki 1993).

Plants resort to many adaptive strategies in response to abiotic environmental stresses such as high salt, dehydration, cold, heat, and excessive osmotic pressure. These adaptive mechanisms include changes in morphological and developmental patterns as well as physiological and biochemical processes (McCue and Hanson, 1990). Among them, the accumulation of compatible solutes according to the metabolic responses has drawn much attention. Some stress-responsive genes encoding proteins for compatible solute synthesis have been cloned and expressed in transgenic plants (Bartels and Nelson, 1994). The compatible solutes may be classified into two categories: one is nitrogen-containing compounds such as proline and other amino acids, quaternary ammonium compounds and polyamines, and the other is hydroxy compounds, such as sucrose, polyhydric alcohols, and oligosaccharides (McCue and Hanson, 1990). Significant differences existed between wild desert barley and cultivated barley in resistance to a uniform root water deficit. These differences appeared to be primarily related to their differing genetic abilities of osmotic adjustment under drought conditions. The findings suggest that further genetic mapping and marker-assisted transfer of the osmotic-adjustment genes in the wild progenitor could improve resistance of cultivated barley grown in water-limited environments (Lu *et al*. 1999). The accumulation of solutes varies with the variation in adverse conditions and plant species, or even plant varieties. In general, a plant cell suspension culture is considered a relatively homogeneous population of cells. Much research has used cultured cells as a model system to study the cellular responses under various abiotic stresses, even to distinguish the difference between the short-term response and long-term adaptation involving physiological characters.

2.7 Carbon isotope discrimination

There are two naturally occurring stable isotopes of carbon ^{12}C and ^{13}C. Most of the carbon is ^{12}C (98.9%), with 1.1% being ^{13}C. This isotope is unevenly distributed among and within different compounds, and this isotopic distribution can reveal information about the physical, chemical, and metabolic processes involved in carbon transformation. The overall abundance of ^{13}C relative to ^{12}C in plant tissue is commonly less than in the carbon of atmospheric carbon dioxide. This indicates that carbon isotope discrimination occurs in the incorporation of CO_2 into plant biomass. Because the isotopes are stable, the information inherent in the ratio of abundances of carbon isotope discrimination, presented by convention as $^{13}C/\ ^{12}C$, is invariant as long as carbon is not lost (Farquhar *et al*. 1989). Theoretical and empirical studies have demonstrated that carbon isotope discrimination is highly correlated with plant water use efficiency. Carbon isotope discrimination provides an integrated measure of water-use efficiency, samples are easily collected, and processed,

and large numbers of samples may be collected from diverse environments. Moreover, in woody plants, carbon isotope discrimination can be determined on annual ring samples, providing a historical report of plant responses to environmental conditions (Cregg and Zhang 2001). In several crops including cereals, carbon isotope discrimination (CID) has been associated with drought tolerance in terms of water-use efficiency and yield stability in drought-prone environments (Teulat *et al*. 2002).

The Mediterranean basin is one of the regions where drought leads to substantial yield reductions (Loss and Siddique 1994). Drought tolerance and yield stability is therefore an important aim for breeders in these regions. As an alternative, a multitude of morph-physiological characters have been suggested as indicators for increasing grain yield under drought conditions. Amongst these, transpiration efficiency (TE: the ratio of dry matter produced to water transpired) is considered as an important drought-adaptive trait in cereals. Carbon isotope discrimination (CID) provides an integrated measurement of TE of C3 crop species (Farquhar and Richards 1984). During photosynthesis, plants discriminate against the heavy isotope of carbon (^{13}C). And, as a result, in several C3 species including wheat and barley, CID is positive correlated with the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration (Ci/Ca) and negatively correlated with TE (Farquhar and Richards 1984; Johnson and Bassett 1991). Thus, a high Ci/Ca leads to a higher and a lower TE (Farquhar and Richards 1984). The major advantage of using CID in selection is its high habitability, which is primarily due to small genotype x environment interactions in dryland areas (Richards *et al*. 1999; Merah *et al*. 2001b). CID has been found to be positive correlated with grain yield in cereals within and across contrasting environments (Acevedo 1993; Araus *et al*. 1997; Voltas *et al*. 1998; Merah *et al*. 2001a, b; Teulat *et al*. 2001b). Although the accession which part of the plant to use for CID measurements is still being debated, for cereals grown under Mediterranean conditions, the grain is considered most appropriate (Voltas *et al*. 1998; Merah *et al*. 2001b). Measuring CID by mass spectrometry remains expensive. As a result, a number of alternative criteria for CID have been suggested including stomatal conductance (Rebetzke *et al*. 2001), leaf structural traits such as dry weight per unit leaf area (Araus *et al*. 1997; Merah *et al*. 2001a) and as content (Araus *et al*. 1997; Voltas *et al*. 1998; Merah *et al*. 2001a). Overall these have been shown to be less-effective measures. CID is therefore a good example of a trait, which could be efficiently, tracked by molecular markers through the identification of quantitative trait loci (QTLs). Markers diagnostic of individual QTLs represent an important surrogate for physiological trait measurements (Price and Courtois 1999), and may ultimately improve selection efficiency through marker-assisted breeding.

There is currently limited insight into the genetic control of TE and CID. Matin *et al*. (1989) found that 70% of the genetic variation for CID in populations derived from a cultivated and a wild tomato was associated with three RFLP loci, mapped on three different chromosomes. In soybean, several QTLs for CID were identified under favorable plant growth conditions (Mian *et al*. 1996). Surprisingly, the identification of QTLs involved in CID variation under drought conditions is undocumented in cereals.

2.8 Systematic decency of the barley BC_2DH lines

Doubled haploid 323 lines of a backcross population between wild barley (ISR42-8) from the Middle East and German barley cultivars (Scarlett) were examined for their tolerance to drought and heat. The German Scarlettt cultivar is a variety with high yield good quality characteristics was crossed with a wild barley accession from the Middle East. The resulting backcross population with Scarlettt as recurrent parent carries average 87, 5% of the barley cultivars genotype and 12.5% of the wild barley genotype. Since the wild barley originates from a semiarid area.

2.9 Doubled haploids population (BC_2DH)

Doubled haploids are commonly used in many plant species in recent years, which are amenable to anther or microspore culture (usually from F1 plants), followed by chromosome doubling. Because the plant has two identical homologues, the amount of recombination information is exactly equivalent to a backcross. However, BC_2DH individuals are completely homozygous, and can be self-pollinated to produce large numbers of progenies, which are all genetically identical. This permit replicated testing of phenotypes, and also facilitates distribution of identical BC_2DH populations to many different researchers. Thus, a BC_2DH population can also be called a permanent population. Major drawbacks of BC_2DH populations are firstly, it is impossible to estimate effects and types of epistasis; secondly, the rates of pollen or microspores successfully turned into BC_2DH plants vary between genotypes, which may cause segregation distortion and false linkage between some marker loci (Zhao 2002).

2.10 The role of plant physiology in plant breeding for drought tolerance

Plant mechanisms that enable plants to become better adapted to water-scarce environments are widely, but most of them are not yet well understood. Among the most important are root architecture, leaf morphology, physiological characters such as osmotic adjustment or proline accumulation, partitioning of total biomass (as determined by dry matter or harvest index), timing for plant development (e.g. earliness), or others associated to the plant reproductive biology. Some

of these characteristics are specific while others are common for many species. Some reports indicate a significant association between crop tolerance to heat and respective adaptation to drought-prone environments in the warm tropics (Ortiz *et al*. 2001).

2.11 Use of backcross populations for QTL analysis

The reason for the production of a DH population for a QTL analysis is to induce the recombination of genes and alleles in the descendants to those created from variability. The alleles are distributing in equal parts to the two homozygosis class genotypes.

A DH population specified so far is however not suitable for the identification of positive alleles from wild forms with the goal to increase and improve of quantitative characteristics. After two recurrent of backcrossing of a wild species with an elite variety, the wild form portion of the entire genome is on the average decreased to12, 5% the restriction of the wild alleles genome portion in each individual line of the backcross population quantitative traits like increased yield or improve quality can be better seized, since unwanted wild alleles and epistatic effects are reduced. The idea is based on the fact that favorable QTL alleles of the wild form barley can be identified and transferred in elite barleys to stabilize the drought and heat tolerance. First successful experiments on applications of the QTL analysis were reported on tomato (Tanksley *et al*. 1996; Fulton *et al*. 1997a, 2000; Bernacchi *et al*. 1998), rice (Xiao *et al*. 1996, 1998), barley (Pillen *et al.* 2003; 2004) and wheat (Huang 2003). In, a self-pollinating diploid crop likes barley, variation evolved primarily by mutation and selection. Since the middle of the last century more or less pure lines in the form of land-races have been collected and crossed (Horvath *et al*. 2001).

2.12 Application of simple sequence repeats (SSRs) marker

Barley is one of the most important crop species in the world and has been subject to considerable genetic study. It is a diploid (2n = 2x = 14) largely self-pollinating species with a large genome of 5.3 x 10^9bp/1C (Bennett and Smith 1976). The development of SSR markers for barley has followed a common pattern with the first few derived from sequences held in public databases (Saghai-Maroof *et al*. 1994; Becker and Heun 1995). This has been followed by screening small insert genomic libraries for SSRs motifs (Struss and Plieske 1998). The limited progress indicates that SSR isolation and characterization from plants is not trivial, and that effective strategies need to be devised which increase the efficiency of the SSR discovery and development phase (Ostrander *et al*. 1992; Edwards *et al*. 1996).

The ubiquity of SSRs in eukaryotic genomes and their usefulness as genetic markers is well established. In mammalian systems, SSRs are the primary assay for detecting molecular

polymorphism and well-developed SSR linkage maps are available for a number of species (Sverdlov *et al*. 1998). A high level of SSR in formativeness has also been revealed for plant species (Milbourne *et al.* 1997) and this has prompted the initiation of SSR discovery programmers for all major crops (Milbourne *et al.* 1998). However, there exist a number of limitations associated with SSR discovery and application in plants, including a lack of DNA sequence information in databases, a perceived low abundance of SSRs, differences in the most common types of repeats and the problem of rapid forward and back mutation rates making assumptions of 'allelic identity' based on repeat number difficult to confirm.

In humans, it has been estimated that, on average, one SSR occurs every 6 kb (Beckmann and Weber 1992). Dinucleotide repeats are most frequent, with CA/GT repeats estimated to occur every 30 to 60 kb (Stallings *et al.* 1991). In plants, analysis of DNA sequence database entries for all possible motifs has revealed a frequency of one SSR every 29kb (Lagercrantz *et al.* 1993) to one every 50kb (Morgante and Olivieri 1993). AT/TA repeats comprise the majority of the database-derived plant SSRs. Because of the relatively low number of plant DNA sequences and the bias towards coding regions, SSR frequency has also been assessed by oligonucleotide hybridization. Such analyses have suggested figures of one SSR every 80 kb in rice (Panaud *et al.* 1996) and one every 65 kb in pine (Echt and Maymarquardt 1997). Generally lower estimates have been obtained in studies using only dinucleotide repeats (Roeder *et al.* 1995) with CA/GT and CT/GA repeats approximately an order of magnitude less frequent in plants than in animals (i.e. one every 250 - 750kbp).

To overcome this problem of abundance, plant geneticists have suggested screening large numbers of clones (Roeder *et al.* 1995) or develop selective SSR enrichment techniques (Edwards *et al.* 1996; Milbourne *et al.* 1998). These were generally successful and resulted in the development of significant collections of SSRs (Roeder *et al.* 1998).

2.13 Mapping quantitative trait loci

2.13.1 Quantitative traits

The Advanced Backcross Quantitative Trait Locus (AB-QTL) strategy (Tanksley and Nelson 1996) was proposed as a new molecular breeding method based on QTL mapping, that can integrate the processes of QTL analysis and variety development while exploiting the full potential of genetic variation available in unadapted germplasm for the improvement of quantitative traits. This study intends to apply the AB-QTL strategy, to the simultaneous detection and introgression of favorable barley wild species genes of quantitative traits.

Characters exhibiting continuous variation are termed quantitative traits. Continuous variation is caused by two factors: simultaneous segregation of many genes affecting the trait and/or

environment influencing the expression of the trait (Falconer and Mackay 1996). In crop plants most traits of economical importance, including yield, heading date, height and many quality traits, are quantitative by inherited. The unknown genes affecting these traits are commonly referred to as quantitative trait loci (QTL). Biometrical approaches have traditionally been used for studying quantitative traits and the statistical quantitative genetic model assuming essentially infinitely many genes with tiny effects works well for many applied purposes, such as plant breeding. The details of the genetic basis of quantitative traits however remained unclear until genetic maps based on DNA markers were marked.

2.13.2 Method of QTL mapping

Association of morphological markers with quantitative traits in plants was observed early on (Sax 1923) and the first steps towards mapping of QTLs or polygenes were taken based on the scarce markers available (Thoday 1961). Currently, complete genetically maps exist for many crop species and algorithms have been developed for QTL mapping in a wide range of pedigrees and experimental designs including F2, backcross, recombinant inbred, doubled haploid and many other designs (Paterson 1995). All share the basic principle of testing association between marker genotypes and quantitative phenotypes.

The simplest methods were based on single marker analysis, where the difference between the phenotypic means of the marker classes are compared using F-statistics, t-tests, linear regression or nonparametric tests (Sax 1923, Edwards *et al*. 1987, Soller and Brody 1976). A major shortcoming of single marker analysis is that it cannot distinguish between tight linkage to a QTL with small effect and loose linkage to a QTL with large effect (Lander and Botstein 1989).

The significance thresholds used for reclaiming a QTL are of major importance. Because QTL mapping involves many analyses of independent genetic markers throughout the genome, there are many opportunities for false-positive results. The appropriate threshold for controlling the type I error rate depends on the size of the genome and on the density of markers genotyped: a LOD threshold of 2.4 was considered adequate in simple interval mapping (SIM) for a genome of 1100 cM covered with markers every 20 cM (Lander and Botstein 1989). This threshold was deduced from an assumed distribution for the test statistics, but the true distribution may deviate from the assumed distribution due to random distribution of the markers on the map (Tinker and Mather 1995a). Alternate methods are based on resembling: permutation involves shuffling the phenotypes so that the effects of the parameters are lost and the distribution of test statistics under the null hypothesis can be derived from repeated permutations (Churchill and Doerge 1994).

The power of finding a QTL can be increased by decreasing the variation caused by the environment as well as by the background genome. Environmental variation can be decreased by repeated phenotype measurements or by using progeny testing for phenotype measures (Lander and Botstein 1989). The power of QTL detection also depends on the type and numbers of progeny studied. Based on computer simulation studies, progeny sizes from a few hundreds to a thousand have been suggested to detect QTLs of minor effect. In practical barley studies, doubled haploid population of 100-200 lines have been used frequently for mapping purposes. The density of the marker map is not as important as the progeny size: a map with 50 cM marker spacing is adequate for detection of QTLs. A denser map helps to locate the QTLs more precisely (Darvasi *et al*. 1993).

Recent advances in QTL mapping procedures include analysis of QTL x environment interaction (Tinker and Mather 1995a, b, Jansen *et al*. 1995, Korol *et al*. 1998), a nonparametric approach to map QTLs (Kruglyak and Lander 1995), Bayesian mapping of QTLs (Satagopan *et al*. 1996, Sillanpaeae and Arjas 1998) and methods for differentiating pleiotropy from close linkage (Lebreton *et al*. 1998).

2.14 Method of QTL calculation

The basic principle of using genetic markers to study quantitative trait loci (QTL) is well established (Sax 1923, Lander and Botstein1989; Jansen 1993; Zeng1994). Sax (1923) first used pattern and pigment markers in beans by investigating the segregation ratio of F2 progeny of different crosses. Thoday (1961) proposed the idea of using two markers to bracket a region for detecting QTL. The basic idea of Sax and Thoday for detecting the association of a QTL with a marker rests on the comparisons of trait means of different marker (chromosomal segment) classes. These methods, such as *t*-test and simple and multiple regressions, directly analyze markers.

A further AB-QTL study, which used *L. hirsutum* as the donor species, revealed 25 favorable wild species QTL alleles out of 121 detected QTLs (Bernacchi *et al*. 1998a) Again, the authors detected wild species alleles which increased yield by 15 %. The most recent AB-QTL study in tomato was published by (Fulton *et al*. 2000). In rice yield QTL effect on chromosome 1 was validated in a second cross using the same *Oryza rufipogon* donor accession (Moncada *et al*. 2001).

2.15 Marker assisted selection

In breeding autogamous species lines are developed from crossing schemes including two parents. In a backcross programmer a few traits would be transferred from a donor to a recipient. In line

development, however, good characteristics from all parents should be combined in a single line (Weber and Wricke 1994). Information on mapped QTLs can be used to design mating that maximize the probability of pyramiding most, if not all, favorable QTL alleles in a single genotype (Dudley 1993). For traits with significant interactions between QTLs emphasis should be placed on identification of the best multi-locus allelic combinations instead of simply collecting many alleles with positive effects (Zhu *et al*. 1999).

The relative efficacies of marker assisted selection and traditional selection for improving quantitative traits have been considered in several simulation studies, as reviewed by Lee (1995), the efficiency of marker assisted selection is enhanced and may be more efficient than traditional selection under the following circumstances: 1) the trait under selection has low heritability; 2) a tight linkage is parent between the trait an the marker (<5cM); 3) in earlier generations of selection prior to fixation of alleles at or near marker loci; 4) large sample sizes for mapping and selecting QTL are used to improve estimates of QTL alleles. Markers very closely linked to the target genes or even located in the gene can greatly enhance the use of marker-assisted selection in advanced generations, where the linkage disequilibrium becomes smaller. The accurate chromosomal locations of QTLs, as well as the magnitude of QTL effects, should be verified prior to their use in an applied breeding program. In barley, the effect of four yield QTLs was verified using a set of BC_2DH lines different from the lines used for mapping (Romagosa *et al*. 1999). In that study, selections based on marker genotypes, or combined information from markers and phenotype, were at least as efficient as phenotypic selection alone, but qualitative QTL x E interactions decreased the efficiency of marker-assisted selection for some of the QTLs. In the same barley lines, effects of only one of the two major QTL regions for several malting quality traits were verified, the effects of the other region were lost probably due to inaccurate location of the QTL (Han *et al*. 1997).

Simultaneous selection for multiple traits complicates the use of marker-assisted selection in breeding. Information on several markers needs to be combined when selection is made. One method is to determine the marker genotype of each line being tested and sum the significant additive effects of each marker locus to an index value (Dudley 1997). A large number of plants have to be scored in order to find the desired marker combination in the progeny, which may render the selection procedure costly (Graner 1996).

2.16 Advanced backcross quantitative trait (AB-QTL) strategy

The advanced backcross quantitative trait (AB-QTL) strategy was introduced by Tanksley and Nelson (1996). The authors integrated the mapping of favorable QTL alleles and the introgression of these alleles into one process. In order to achieve this goal, they utilized exotic germplasm as the genetic donor for the improvement of quantitative traits and conducted the marker and phenotype analysis in advanced backcross generations like BC_2. It is expected that through the introgression of new exotic QTL alleles, the AB-QTL strategy will contribute to an increased level of genetic diversity in our modern crop varieties.

To date, several reports on the application of the AB-QTL strategy are available for tomato and rice. In all cases, favorable exotic QTL alleles for important agronomic traits have been identified. For instance, fruit yield could be improved in tomato through the introgression of wild-species alleles from *Lycopersicon pimpinellifolium* and *L. peruvianum* by 17% and 34%, respectively (Tanksley *et al*. 1996; Fulton *et al.* 1997). A further AB-QTL study, which used *L. hirsutum* as the donor species, revealed 25 favorable wild-species QTL alleles out of 121 detected QTLs (Bernacchi *et al.* 1998a). Again, the authors detected wild-species alleles which increased yield by 15%. A recent AB-QTL study in tomato was reported by Fulton *et al.* (2000). As in other tomato wild species, the authors could localize favorable exotic QTL alleles from *L. parviflorum* which, for instance, increased yield by 27%. Similar results could be found in AB-QTL studies in rice. Here, two wild-species QTL alleles have been associated with an increase of yield by 17% and 18% on rice chromosomes 1 and 11, respectively (Xiao *et al*. 1996, 1998). Subsequently, the yield QTL effect on chromosome 1 was validated in a second cross using the same *Oryza rufipogon* donor accession (Moncada *et al.* 2001). Recently, reports appeared on the first AB-QTL analyses in maize (Ho *et al*. 2002), wheat (Huang *et al*. 2003) and barley (Pillen *et al*. 2003; 2004). In most instances, significant improvements in yield and yield components could be associated with exotic donor segments. The effects were dramatic in tomato and rice, where yield increased up to 34% and 18%, respectively. The effects of exotic QTL alleles on yield were less pronounced in maize, wheat and barley but still reached levels of 11%, 15% and 7%, respectively.

The favorable wild-species QTL alleles are useful as a breeding resource after they have been fixed in nearly isogenic lines (QTL-NILs) and after the superior performance of a QTL-NIL has been confirmed in comparison to the recurrent elite line. Bernacchi *et al*. (1998b) have already validated the effects of exotic tomato QTLs in QTL-NILs. In field evaluations at five locations worldwide, 22 QTL-NILs out of 25 tested (88%) exhibited phenotypic improvement compared to

the recurrent parent, as had been predicted in the previous AB-QTL analysis. For instance, a QTL-NIL possessing an exotic QTL allele for a 15% yield increase did, indeed, outperform the control line by 12%. These reports clearly illustrate that the AB-QTL strategy is a powerful tool for the improvement of quantitative agronomic traits in elite varieties.

3. Materials and Methods

The present study was carried out during the period of 2001-2003 at Poppelsdorf Experimental Station, Department of Crop Science and Plant Breeding, Faculty of Agriculture, Rheinische Friedrich-Wilhelms-University Bonn.

Four experiments were used to study the performance of genotypes of barley for heat and drought tolerance. The experiments were arranged in a split-plot design with heat or drought assigned to main plot treatments and genotypes or BC_2DH lines to sub-plot treatments.

In 2001 Thuringia, Scarlett, Harry and Apex were evaluated for morphological, physiological, and agronomical traits in a green house trial using a randomized complete block design with three replications and four treatments for drought and heat tolerance.

In 2002 and 2003 two experiments (drought tolerance and heat tolerance) the population parents (Scarlett and ISR42-8) were evaluated for morphological, physiological, and agronomical traits in a green house trial using a randomized complete block design with three replications, four treatments and two years

In 2002 and 2003 two separate experiments (of drought tolerance and heat tolerance) were conducted with 323 BC_2DH lines to evaluate morphological, physiological, and agronomical traits inside the green house trial using two treatments for two years.

Recording of phenotype data

Growth habit:

Scarlett showed a slow growth and development, hence has a medium stature. The shoot growth is good, due to the dense tillering, somewhat weaker seed strength is to be selected. A Scarlett high inventory density, a long, upright standing ear and middle TGW.

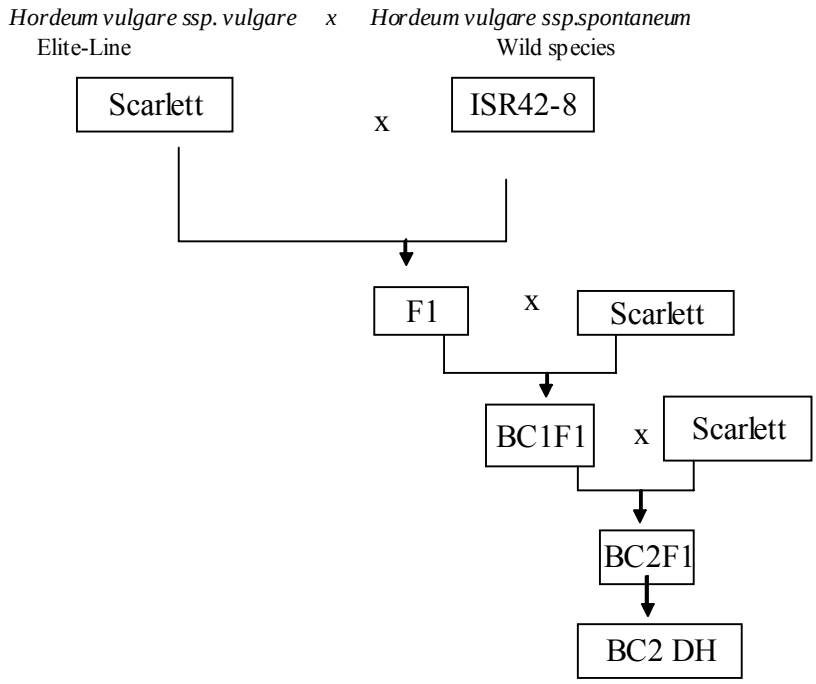
The F_1 was backcrossed twice with Scarlett by Dr. K. Pillen and friendly subjected to a double haploid procedure in order to develop a set of 323 BC_2DH.

Table 1: Pedigree description of European spring barley cultivars and Wild barley *(H. vulgare ssp. spontaneum)* genotypes

Variety	Breeder	Type	Pedigree/ Source
Apex	v.Lochow/ Cebeco	S2	Aramir*F1(Ceb.6721*(Julia(Volla*L100)))
Harry	Svalöf Weibull	S2	Arls M*Tellus
Scarlett	Saatzucht Breun	S2	Amazone Br.St.2730e*Kym
Thuringia	SZ Schöndorf	S2	(Steffi*Gerlinde)*(243/4*Salome)
ISR42-8	Prof. G.Fischbeck	S2	Israel, Eastern Lower Galilee

S2: spring form in two rows

This pedigree for cultivars and wild genotypes was taken according to Pillen (2002).



Plant material

Scarlett was crossed onto ISR42-8 and then backcrossed with Scarlett, the observed Scarlett population (323 BC_2DH lines). Doubled haploid lines of a backcross population between a wild barley accession from the Middle East (ISR 42-8) and a German barley cultivar were examined on their tolerance in relation to drought and heat. Scarlett is a high yielding German cultivar, where as high quality characteristics. Scarlett was crossed with the wild accession ISR 42-8.

Location

The experiments were carried out in the green house during the years 2001-2003 at the Poppelsdorf Experimental Station, Department of Crop Science and Plant Breeding, Faculty of Agriculture, Rheinischen Friedrich-Wilhelms-University Bonn.

3.1 Measurement of phenotypic data

3.1.1 Experimental evaluation of Thuringia, Scarlett, Harry and Apex barley genotypes for drought tolerance

The soil water holding characteristics were determined through the devolvement of soil absorption and thereafter the quantity of daily water supply was determined. Four levels of water treatment (irrigation) were used (35%, 50%, 65% and 100% field capacity (FC)), in case of irrigation studies Four treatments (35%, 50%, 65% and 100% FC), were tested in four different drought stress (see Table 2) in order to evaluate a drought stress regime, which could be used to test the BC2DH lines for their drought tolerance. The day/night regime was exposed14/10 hour light in the green house (Morgan 1980). The remaining water content was determined by weighting the pots every day until the weight became constant.

Four genotypes (Thuringia, Scarlett, Harry and Apex) in 3 replicates and with 4 treatments (35% FC, 50% FC, 65% FC and 100 % FC) were selected for drought experiment. The water stress was imposed at 4-leaf stage by stopping the irrigation. The relative soil moisture content was 14% of the FC for the stressed-plants and 100% FC for irrigated plants (pots were weighed and watered daily) (This *et al* 2000; Teulat *et al*. 2001). After the second leaf reached up to the first true leaf length, the drought treatment via water withholding was started, and it was maintained 8 days without watering when the sand water content was about 50% field capacity (Guoxiong *et al*. 2002).

Barley seeds were sown in plastic pots of 28-cm-diameter and 22 cm in length, with nine holes pierced at the bottom for drainage. Plastic pots contained a mixture of loamy soil, sand and peat moss (3:1:1 v/v) respectively. The parents were germinated in green house without temperature and humidity control. High –pressure sodium lamps supplemented natural sunlight by a 14-h photoperiod and 10-h dark period.

3.1.2 Experimental evaluation of 323 BC_2DH lines for drought tolerance

323 BC_2DH lines and two drought treatments (50% and 100% FC) a cross two years were observed for drought tolerance. On the other hand, two parents (Scarlett and ISR 42-8), 18 replicates and 4 treatments (25%, 50%, 75% and 100% FC) a cross two years were tested.

Table 2: Drought treatment for parents and BC_2DH lines

Treatments	Field capacity	Start of treatment
1-Stress	50% of field capacity	After one month from planting.
2-Control	100 % of field capacity	After one month from planting.
3-Parents	25%, 50%, 75%, 100 % of field capacity	After one month from planting.

Barley seeds were sown in 14-cm-diameter and 12 cm in length, with four holes pierced at the bottom for drainage plastic pots containing a mixture of clay /loam soil, sand and peat moss (3:1:1 v/v) and germinated in greenhouse set at greenhouse temperature. High –pressure sodium lamps supplemented natural sunlight a 14-h photoperiod. Humidity was uncontrolled.

3.1.3 Experimental evaluation of four barley genotypes for heat tolerance

Four genotypes (Thuringia, Scarlett, Harry and Apex), were tested in three different heat regimes (see Table 3) in order to evaluate a heat stress regime, which could be used to test the BC_2DH lines for their heat tolerance. Our method for heat stress is similar with the method used by Blum *et al*. (1994); Stone and Nicolas (1996).

A Hydro-Thermograph (ADOLF THIES GMBH & CO.KG Goettingen) was used to measurement the temperature and humidity in the greenhouse.

Table 3: Temperature treatment in and outside the green house

Treatments	Temperature
Heat stress	In greenhouse season 2001 Maximum temperature between 26-48.5°C. Minimum temperature between 14-25°C.
Heat stress + drought stress	In greenhouse + 65% field capacity
Control	In normal weather season 2001 Maximum temperature between 6-34.6 °C Minimum temperature between -2.3- 18 °C (Out greenhouse)

3.1.4 Heat experiment for 323 BC_2DH lines

The 323 BC_2DH lines were tested for heat tolerance for two years. The control was planted outside the green house under field condition. The lines were grown under high temperature conditions inside green house.

323 genotypes, two heat treatments across two years were observed for heat tolerance. On the other hand, two parents (Scarlett and ISR 42-8), 18 replicate and two heat treatments a cross two years were made only for parents

Table 4: Treatment of BC2DH lines for heat stress

Treatments	Temperature
Heat stress	**In greenhouse season 2002** Maximum temperature from 19 to 52 °C. Minimum temperature from 15 to 27 °C. **In greenhouse season 2003** Maximum temperature from 19 to 45 °C. Minimum temperature from 10 to 24°C.
Control	**Out of greenhouse in season 2002** Maximum temperature from 4,8 to 36,9 °C Minimum temperature from -2,8 to 19,8 °C **Out of greenhouse in season 2003** Maximum temperature from -1.7 to 38.7°C Minimum temperature from -8.1 to 20.6 °C

3.1.5 Fertilization

The seedling of the four barley genotypes of the drought and heat tolerance experiment were fertilized with a solution of 4 g of Ammonium sulfate fertilizer containing 21 % N and 24 % S, and NPK fertilizer 12-12-17-2, containing 12 % N, 12 P_2O_5%, 17 % K_2O and 2 % Mg; (1: 2 v/v) for three time. The BC_2DH lines seedlings were fertilized with a liquid fertilizer, containing 7 % N, 3% P_2O_5, and 6% K_2O for one time every two weeks.

Table 5: Traits abbreviation for studied drought and heat stress parameters

Trait	Abbreviation	[A]Value for drought experiment	[A]Value for heat experiment
Relative Leaf water content	RWC	+	+
Number of tillers per plant	TILL	+	+
Number of spikes per plant	SPK	+	+
Number of kernels per spike	KER	+	+
Plant height	PH	-	-
Chlorophyll content	CHL	+	+
Osmotic adjustment	OA	+	+
Days until heading	HEA	-	-
Number of leaves of main tiller	LEA	+	+
Flag leaf area	FLA	-	+
First leaf area	ARE1	-	+
Second leaf area	ARE2	-	+
Carbon isotope discrimination	CID	-	not tested
Yield	YLD	+	+
Biomass	MAS	+	+
Harvest index	HI	+	+

[A]The value of the trait should be increased (+) or reduced (–) with respect to the breeding goal.

3.1.6 Data collection and sample harvesting

Measurement of traits for four genotypes and BC_2DH lines were measured for the falling traits drought and heat tolerance:

Number of tillers per plant: average number of tillers per plant carried from six plants.

Number of spikes per plant: number of tillers with fertile spike observed from six plants.

Number of kernels per spike: number of kernels measured as an average of 6 spikes sample.

Relative leaf water content

Relative leaf water content was measured different field capacity levels according to (Matin *et al*. 1989; Ali *et al*. 1999). The relative water content of the leaf tissues was calculated as follows: RWC (%) =(FW- DW) x 100 /(TW-DW), on the last fully expanded leaf according to (Barrs and Weatherly 1962), where FW is leaf fresh weight, TW the turgid weight obtained after 24 h floating

on distilled water at room temperature under dim light. Dry weight (DW) was measured after the samples had been dried for 24 h at 80 °C.

Osmotic adjustment

For evaluation of leaf osmotic values, the penultimate leaf was cut, wrapped in plastic foil, frozen in liquid nitrogen. Then 500 µl sterile water was added and material was homogenize with ultraturrax. Then the material was incubated 1.5 hours in the refrigerator at 4 °C, centrifuged at 13000 U/min for 3 minutes and finally stored at – 20°C until measurement. A sample of 50µl was taken and measured by Osomat 300 (gonotec, Berlin) with sterile water as standard. Osmotic adjustment was calculated according to (Wilson *et al*. 1979 and Ludlow *et al*. 1983).

Chlorophyll content

Chlorophyll-Photometer SPAD-502 (Fa. Minolta) was used to measure chlorophyll content. We measured chlorophyll content in fresh leaves in the first part of leaf, medium part of leaf and last part of leaf as an average of a three leaves.

Days until heading

Number of days observed from sowing until the upper most spikes appeared beyond the auricles of the flag leaf sheath (50% heading on plants basis)

Plant height (cm)

The distance from the base of the culm to the tip of the spike of the main culm

Yield (g)

It was recorded as the grain weight from six plants for four barely genotypes for from two plants for BC_2DH lines.

Biomass (g)

The above ground dry matter was produced by a crop during the growing season of six plants for four barely genotypes or for two plants for BC_2DH lines (excluding roots).

Harvest Index

It carried from the ratio between grain yield and biomass

Leaf area index (LAI)

Leaf length (cm) x width (cm) x 0.75 was observed according to (Jatimliansky *et al*. 1984).

Carbon isotope discrimination ($^{13}C_{12}$ ratio)

Carbon isotope discrimination (CID) was measured on a bulk of flag leaf from several plants of each BC_2DH lines ground into a fine powder and dried for 48 h at 80 °C. The carbon isotope composition was determined using an isotope mass spectrometer (20-20 European Scientific, UK).

$CID^{13}C$ (%) = [($^{13}C/^{12}C$) sample/ ($^{13}C/^{12}C$) reference-1] x 1000. The carbon isotope discrimination values were obtained from CIDa and CIDp according to the formula (Farquhar and Richards *1984*): CID (%) = (CIDa - CIDp)/ (1+CIDp), where a and p refer to air and plant.

3.2 Execution of genotypic data

3.2.1 Extraction of barley DNA

This method was described by Saghai-Maroof (1984). Briefly, young expanded leaves were collected from each plant and kept in (-80°C) freezing. Leaf tissue from each plant of the BC_2DH lines were used for DNA extraction. 15ml Sorbitol-Buffer was used and 0.075g Sodium-disulphite and was added to the leave samples and homogenized with ultraturrax. The filtrate was token into a new tube. The filtrate was centrifuged at 5000 U/min and 4°C for 15 minutes. The pellet was resuspended in 2.5ml Sorbitol and 0.0125g Sodium-disulphite. 2.5ml lysis buffer and 1ml Laurylsarkosin was added. The suspension was incubated in a water bath under continuous gentle rocking at 60°C for 30-60 minutes (150 U/min). 6ml chloroform/isoamyl alcohol was added and gently but thoroughly mixed for 10 minutes. The suspension was centrifuged at 5000 U/min and 4°C for 30 minutes. 4.5 ml of the aqueous phase were transferred with a pipette into a new sterile tube. 4.5ml of cold isopropyl alcohol was added and gently mix to precipitate the nucleic acids. The solution was incubated at 4°C for 60 minutes or over night. There upon centrifuged at 5000 U/min, 4°C, for 30 minutes. The supernatant was discarded isopropyl. 2 ml ethanol (70%) was added and centrifuged briefly at 5000 U/min for 4 minutes at 4°C. The supernatant was decanted and the pellet was in air-dried for 10 minutes at 60°C. The DNA pellet was finally dissolved in 50-1000µl ddH2O (depending on DNA quantity) at 4° C over night. Then DNA solution was centrifuged of 2000 U/min for 5min and the DNA was transferred in deep well plates and stored at –20° C.

3.2.2 Agarose gel electrophoresis procedure

Agarose gel electrophoresis separates DNA fragments according to their size. Typically, a DNA molecule is digested with restriction enzymes, and the agarose gel electrophoresis is used as a diagnostic tool to visualize the fragments. An electric current is used to move the DNA molecules across an agarose gel, which is a polysaccharide matrix that functions as a sort of sieve to help "catch" the molecules as they are transported by the electric current. This technique has lots of applications. Generally speaking you can determine the size of DNA fragments. In addition to its usefulness in research techniques, agarose gel electrophoresis is a common forensic technique and

is used in DNA fingerprinting. Unknown DNA samples are typically run on the same gel with a "DNA ladder." A DNA-ladder is a sample of known-fragments DNA. After electrophoresis you can compare the unknown fragments to the DNA ladder fragments and determine the approximate size of the unknown DNA bands by how they match up to the known bands of the ladder.

To pour a gel, agarose powder is mixed with 0.5 x TBE buffer to the desired concentration, and then heated in a microwave oven until completely melted. Most commonly, ethidium bromide (final concentration 0.5 ug/ml) is added to the gel at this point to facilitate visualization of DNA after electrophoresis. After cooling the solution to about 60°C, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature. After the gel has solidified, the comb is removed. The gel, still in its plastic tray, is inserted horizontally into the electrophoresis chamber and just covered with buffer. Samples containing DNA mixed with loading buffer are then pipeted into the sample wells, the lid and power leads are placed on the apparatus, and a current is applied. You can confirm that a current is flowing by observing bubbles coming off the electrodes. DNA will migrate towards the anode.

The DNA migration in the gel can be judged by visually monitoring migration of the blue tracking dyes.

DNA fragments are visualized by staining with ethidium bromide**.** This fluorescent dye intercalates between bases of DNA. It is often incorporated into the gel so that staining occurs during electrophoresis, but the gel can also be stained after electrophoresis by soaking in a dilute solution of ethidium bromide. To visualize DNA, the gel is placed on an ultraviolet transilluminator. Be aware that DNA will diffuse within the gel over time, and examination or photography should take place shortly after cessation of electrophoresis.

1x Tris-acetate-EDTA-buffer (TAE)-Solution	200 ml
1 % Agarose	2 g

5x Tris-borate-EDTA-buffer (TBE)-Solution, pH 8.3

0.45 M	Tris	275.56 g
0.45 M	Boric oxide	139.12 g
10 mM	Ethylenediaminetetraacetate (EDTA)	18.61 g
	H_2O high purity	ad 5 l

Adjust to pH 8.3 with NaOH at room temperature

Sorbitol – Solution

350 mM	Sorbitol	127.5 g
100 mM	Tris	24.2 g
5 mM	EDTA	3.36 g
	H_2O high purity	ad 2 l

nucleic lysis -Solution

200 mM	Tris	121.14 g
50 mM	EDTA	93.06 g
2 M	NaCl	584.4 g
2 %	CTAB	100 g
	H_2O high purity	ad 5 l

Adjust to pH 7, 5 with HCl

5 % Laurylsarkosin 25 mM $MgCl_2$

Laurylsarkosin	25 g	25 mM	$MgCl_2$	0.254 g
H_2O high purity	ad 500 ml		H_2O high purity	ad 50 ml

3.2.3 SSR-Marker analysis

Plant material: for all 323 BC_2DH lines, DNA was extracted from 3-week old leaf material using the Cetyltrimethylammoniumbromide (CTAB) method (Saghai-Maroof *et al*. 1984).

Polymerase chain reaction (PCR) and fragment analysis

PCR reactions were performed in a total volume of 25µl and consisted of 50 ng genomic DNA, 2.5µl 10x PCR buffer, 0.05 µl *Taq* (*Thermus aquaticus*) polymerase (Promega 5 unites/µl), 0.25 µl(10µm) of forward and reverse primers, 2.5 µl dNTP (2mM) and 2.5 µl $MgCl_2$ (25mM). The optimized PCR conditions varied and have been given a letter code for each primer. The following prefixes of SSR names indicate the published sources from which the primer sequences were taken: HVM, Liu *et al*. (*1996*); Bmac, Bmag, Ebmag and Ebmac, Ramsay *et al*. (*2000*); Hv, Becker and Heun (*1995*) and Pillen *et al*. (*2000*). A suffix with the chromosomal identifier in brackets was added to each SSR name as a simple reference. Linkage distances between SSR markers were inferred from Ramsay *et al*. (*2000*) and Pillen *et al*. (*2000).*

Table 6: Reactants for Polymerize chain reaction (PCR) for SSR markers

PCR-React.	µL
Template DNA	5µl
H2O (high purity)	11.95µl
dNTP*	2,5µl
MgCl2	2.5µl
10x Puffer	2.5µl
Forward-primer	0.25µl
Reverse-primer	0.25µl
Taq-Polymerase	0.05µl

* 2'-Desoxynukleotid (dNTP)

Table 7: Procedure for Polymerize chain reaction

Denaturing °C Min.		Annealing °C Min.		Extension/polymers °C Min.		Number of cycles	Notes
94	3	-		-			Hot start
94	1	64-55	0.5	72	1	10	SSR (A)
94	1	55	1	72	1	30	SSR (A)
-		-		72	5		
94	8	-		-			Denaturing for sequences
-		4	∞	-			Cold

Stop-mix

95% formamide 47, 5 ml

0. 05% Xylencyanol 25 mg

10Mm NaOH, 10 M 50 µl

H_2O high purity ad. 50 ml

For the Electrophoresis injection was every PCR –add. With 10 µl micro Stop-Mix was heated at 95 °C for 3 min. for denaturing.

3.2.4 Gel electrophoresis

DNA fragments are separated in a horizontal electrophoresis system using a polyacrylamide-based vinyl polymer Gels were prepared as follows:

Electrophoresis was carried out in TBE buffer for 45 minutes for warm. 1 L of 5 x TBE buffer was added. 1 µL of the loading buffer and 5 µL of the final DNA were injected, Load this sample into the gel and conduct electrophoresis at 2600 Volt, 25 Amper and 90 Watt. The DNA was visualized on gel transfer illuminator for 90 minutes. Stop the electrophoresis when the front of the dye migrates blue was in the bottom of the gel.

3.2.5 Silver Staining for DNA visualization

Gels were silver stained using a modified procedure. Gently shake the gel in glacial acetic acid for 20 min at room temp. Rinse the gel in sterile water three times for about 2 min each. Immerse the gel in silver staining solution (2 g silver nitrate and 1.6 L water) for 30 min. Pour out the silver stain solution, and wash the gel quickly with sterile water. Immerse the gel in an 40 g sodium carbonate, 2.4 ml formaldehyde, and 320 µl sodium thiosulfate in 1.6 L water) until optimal image intensity is obtained. Stop the developing process by immersing the gel in glacial acetic acid. Airs dry the gel and back it with a Gel Band plastic film.

Fixer (10 % Acetic acid)

160 ml	Acetic acid
ad 1600 ml	H_2O high purity

Color solution

2 g	Silver nitrate
ad 1600 ml	H_2O high purity

Acidifications

48 g	Na_2CO_3 (water free)
2,4 ml	Formaldehyde (37 %)
320 µl	Na-Thiosulfat
ad 1600 ml	H_2O high purity

Before use on 10 °C Cold. Formaldehyde and Na-Thiosulfat we were gave short time for acidification

Marker

A) BC_2DH population: the BC_2DH population Scarlett*ISR42-8 was developed by PD Dr. Klaus Pillen and colleagues. The initial cross Scarlett x ISR42--8 was backcrossed twice and thereafter in vitro propagated by production of doubled haploids.

B) Genotyping: the 323 BC_2DH individuals were genotyped with 97 markers. The maternal or paternal inheritance of a chromosome segment was identified by means of SSR analysis on a Li-Cor 4200S automated sequencer. The SSR data were collected and provided by Mrs. Maria von Korff and Mr. Huajun Wang.

C) SSR map: The SSR-map was provided by Mrs. Maria von Korff. The SSRs were integrated into a consensus map using mapping information from Ramsay *et al*. (2000, = Lina x *H. spontaneum* cross), Kleinhofs *et al*. (1993, = Steptoe x Morex cross), Graner *et al*. (1991, = Igri x Franka cross) and von Korff *et al*. (personal communication, = Scarlett x ISR42-8 cross).

3.3 Statistical analysis of data

Statistic evaluation for experiment data arranged in 2 parts:

- The evaluation of phenotype data was conducted by means of variance and correlation analysis.
- QTL were detected by means of three factorial (drought or heat treatment, marker and year) ANOVA of the BC2DH population.

3.3.1 Variance analysis and coefficient of correlation for drought and heat treatments

The data were calculated using the SAS software (SAS Institute *1999*). Three factors can use the quick and easy ANOVA to analyze the variation and correlation coefficient explained by those factors (analysis of variance, or ANOVA).

Experiments Analysis of variance of the attempt data the execution of the more-factorial analysis of variance served the question whether significant differences between the individual factor levels of the worked on characteristics are present. The analysis of variance became under SAS 6, 12 (company: SAS of institutes Inc., USA) with procedure GLM (General linear Model) accomplished.

3.4 Detection of putative QTLs

The QTL detection from BC_2DH genotype and phenotype data were conducted using the procedure GLM (General Linear Model) from the SAS software (SAS Institute *1999*). The model used to detect QTLs included the effects marker genotype (M), drought treatment (D), or heat treatment (H) and M*D or M*H interaction. A mixed model with the marker and the drought or heat treatment was chosen as fixed effects and year as a random effect. Following Stuber *et al*.

1992; Xiao 1998; and Pillen *et al*. 2003, the presence of a stable QTL in the vicinity of a marker locus was accepted, if the marker main effect was significant at $P < 0.01$. Adjacent marker effects (distance <20cM) are considered as one putative QTL. The presence of a drought or heat treatment-dependent QTL was accepted, if the M*D or M*H interaction was significant at $P < 0.01$.

The relative performance of the homozygous (*H. v*. ssp. *spontaneum*, is hereafter abbreviated with *Hsp*) *Hsp* genotype (RP [*Hsp*]) as a measure of the improvement of a trait by replacing both (*Hordeum v*. L. *distichon*, hereafter abbreviated with *Hvd*) *Hvd* elite alleles with the exotic *Hsp* alleles was calculated as follows:

For each trait, aa and AA are the least square means calculated across all BC_2DH lines of the homozygous *Hvd* and the homozygous *Hsp* genotypes, respectively.

RP [Genotype] = $\frac{(Ms - Mv) * 100}{Mv}$ in % effect of the *Hsp* alleles a cross both environments.

RP [T*M T1] = $\frac{(MsT1 - MvT1) * 100}{MvT1}$ in % was effects of the *Hsp* alleles for control treatments

RP [T*M T2] = $\frac{(MsT2 - MvT2) * 100}{MvT2}$ in % was effects of the *Hsp* alleles for drought or heat stress.

Mv = trait value of homozygote of *Hvd* genotypes.

Ms = trait value of homozygote of *Hsp* genotypes.

T1 = Control treatment

T2 = Stress treatment for drought or heat

Favorable QTL: Ms < Mv for example days until heading.

Ms > Mv for example grain yield (Table 5).

The goal from our studies are detection favorable QTL, because the favorable QTL improve all traits and this the goal for breeder.

4. Result

4.1 Drought tolerance

Phenotypic characters

We have in this study 11 quantitative (tillers per plant, number of spikes per plant, number of kernels per spike, relative leaf water content, osmotic adjustment, chlorophyll content, days to heading, plant height, yield, biomass and harvest index) traits for evaluation of barley (Thuringia, Scarlett, Harry, and Apex) genotypes, then found non-significant for the interaction among genotypes and drought treatments for tillers, but for chlorophyll content non-significant the interaction among genotypes. In the study for (Scarlett and ISR42-8) parents and BC_2 DH population we have tillers per plant, plant height, and chlorophyll content not studied, but we have (number of kes per plant, number of kernels per spike, relative leaf water content, osmotic adjustment, days to heading, yield, biomass and harvest index) and other traits more like carbon isotope discrimination, flag leaf area, first lea area and second leaf area, because related for drought study.

4.1.1 Evaluation of four barley genotypes

1) Number of tillers per plant

Analysis of variance among replications and the interaction among genotypes and drought treatments showed non-significant. Whereas, there were highly significant effects for genotypes and drought treatments (see Table 8). The Harry and Thurnigia ranges from 10.42 to 15.25 tillers per plant respectively (see Table 9). Mean for drought treatments ranged from 5.83 tillers for 35% field capacity (FC) to 16.0 tillers for 100% FC (see Table 10).

2-Number of spikes per plant

The replication was not significant. The interaction among genotypes was highly significant. The difference among genotypes, and drought treatments were highly significant, there are showed in Table 8 and Figure 1). The genotypes Scarlett and Harry ranged from 4.99 to 9.0 spikes per plant respectively (see Table 9). Mean for drought treatments ranged from 5.69 spikes per plant for 35% FC to 8.51 spikes per plant for 100% FC (see Table 10).

Table 8: Analysis of variance for drought treatment in Thuringia, Scarlett, Harry, and Apex genotypes

Trait		Replications	Drought treatments	Genotypes	G x D	Error
	DF:	2	3	3	9	29
TILL	Ms	3.04	16.87	51.28	7.74	4.78
	F	0.64	3.53**	10.72**	1.62	
SPK	Ms	7.65	258.41	558.46	45.73	14.87
	F	0.51	17.38**	37.38**	3.08**	
KER	Ms	2.52	141.82	27310	18.43	10.58
	F	0.24	13.41**	25.81**	1.74	
RWC	Ms	122.31	499.34	123.51	64.48	78.70
	F	1.55	6.34**	1.57	0.82	
OA	Ms	0.0000014	0.0114	0.0088	0.00043	0.000066
	F	0.02	172.5**	133.4**	6.48**	
CHL	Ms	18.74	38.45	32.25	27.82	11.93
	F	1.57	3.22*	2.7	2.33*	
HEA	Ms	3.81	317.47	1248.31	7.92	24.57
	F	0.16	12.92**	50.81**	0.32	
PH	Ms	3.69	514.40	449.99	35.59	24.08
	F	0.15	21.36**	18.68**	1.48	
YLF	Ms	3.75	99.97	93.45	15.88	1.53
	F	2.45	65.22**	60.97**	10.36**	
MASS	Ms	6.68	1565.12	113.88	51.19	15.38
	F	0.43	101.79**	7.41**	3.33**	
HI	Ms	52.311	301.76	1955.12	100.78	33.04
	F	1.58	9.13**	59.18**	3.05*	

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

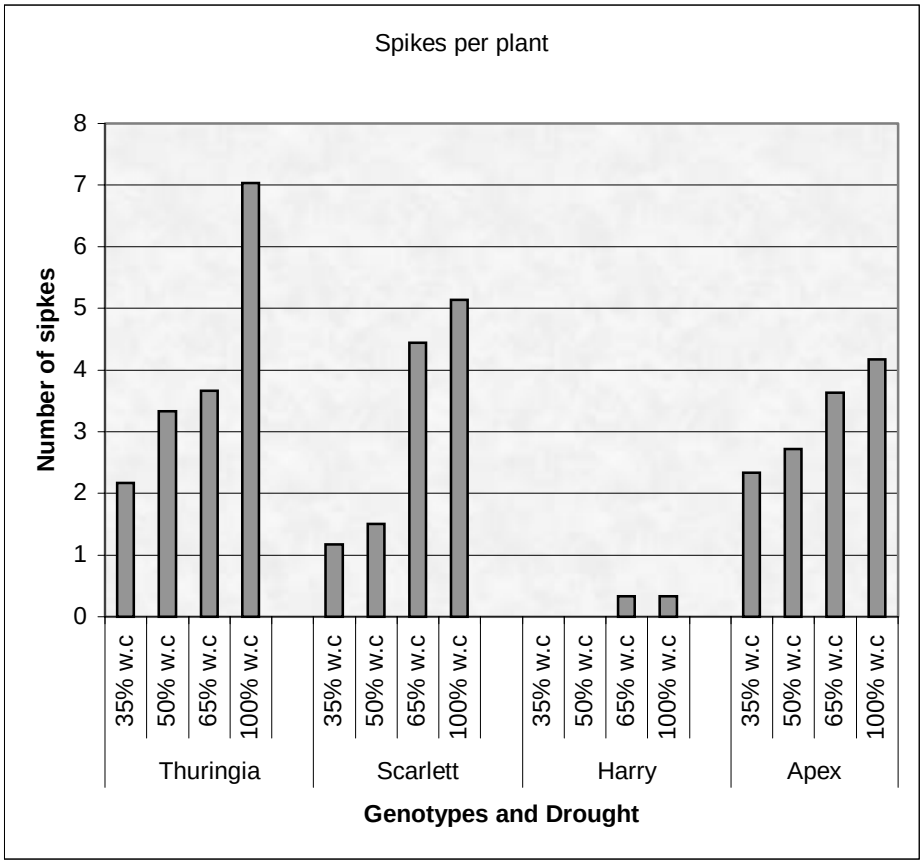


Figure 1: Effect of drought on the number of spikes per plant of Thuringia, Scarlett, Harry, and Apex genotypes.

Figure 1 shows little differences among 35%, 50%, and 65% FC for Thuringia, but high differences between 100% FC and other treatments. Scarlett obtained little differences between (35% and 50%), (65% and 100%), on other hand revealed high differences between (35% and 65%, 35% and 100%), (50% and 65%, 50% and 100%) for spikes per plant. Harry genotype was very susceptible for 35% and 50% FC treatments were no-spike and for 65% and 100% treatments nearly no spikes. The different was little among all treatments for Apex. Were little different between Scarlett and Apex, on other hand high different between Harry and other genotypes.

3) Number of kernels per spike

The analysis of variance was non-significant for replications, and interaction among genotypes and drought treatments, but was highly significant for genotypes and drought treatments (see Table 8). The average number of kernels per spike ranged from 1.92 for Harry to 12.27 kernels for Scarlett (see Table 9). Mean for drought treatments ranged from 4.87 kernels per spike for 35% FC to 12.67 kernels per spike for 50% FC (see Table 10).

4) Relative leaf water content

The analysis of variance revealed non-significant for replications, genotypes and the interaction between genotypes and drought treatments, but a highly significant effect for drought

treatments (Table 8). The relative leaf water content ranged from 74.58% for Thuringia to 82.01% for Harry (see Table 9). Mean for drought treatments ranged from 72.72% for 35% FC to 87.28% for 100% FC (see Table 10).

5) Osmotic adjustment

The analysis of variance revealed highly significant effects genotypes, drought treatments and the interaction among genotypes and drought treatments, but no effect for replications in Table 8 and Figure 2. The value of osmotic adjustment for four genotypes ranged from 0.078 for Harry to 0.143 for Thuringia (see Table 9). Mean for drought treatments ranged from 0.079 for 100% FC to 0.147 for 35% FC (see Table 10).

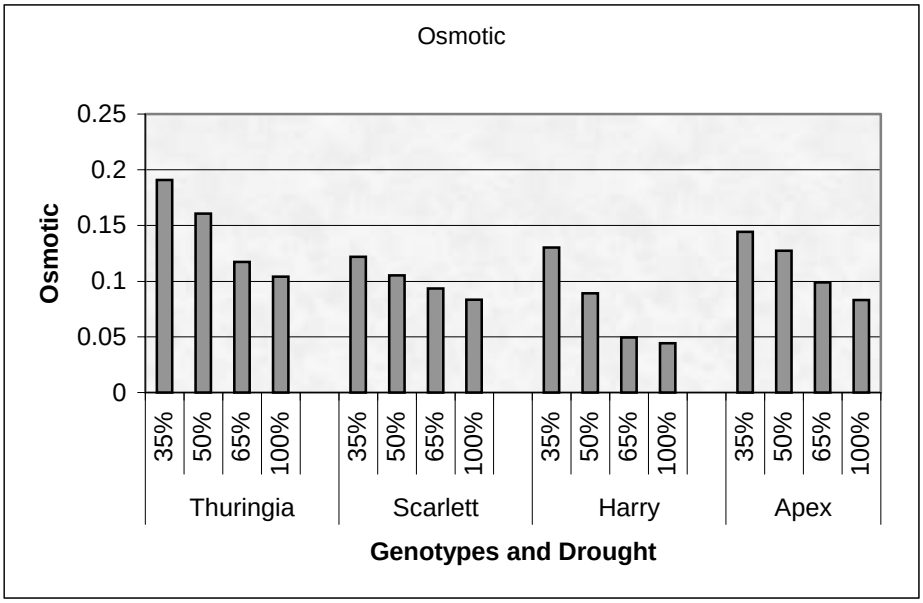


Figure 2: Osmotic adjustment of Thuringia, Scarlett, Harry, and Apex genotypes for drought experiment.

Figure 2 shows, Thuringia obtained little different between (35% and 50%), (65% and 100%), on other hand revealed high different between (35% and 65%, 35% and 100%), (50% and 65%, 50% and 100%) for osmotic adjustment. Were little different among all treatments Scarlett. Harry obtained little different between (35% and 50%), (65% and 100%), on other hand revealed high different between (35% and 65%, 35% and 100%), (50% and 65%, 50% and 100%) for osmotic adjustment. The different were moderate among all treatments for Apex. General was moderate different between all genotypes.

6) Chlorophyll content

The variation among replications and genotypes were non-significant, but the effects for drought treatments and the interaction between genotypes and drought treatments were significant (Table 8 and Figure 3). The average chlorophyll content for the genotypes ranged from 50.49 for

Apex to 54.48 for Scarlett (see Table 9). Mean for drought treatments ranged from 49.95 for 35% FC to 53.91% chlorophyll content for 100% FC (see Table 10).

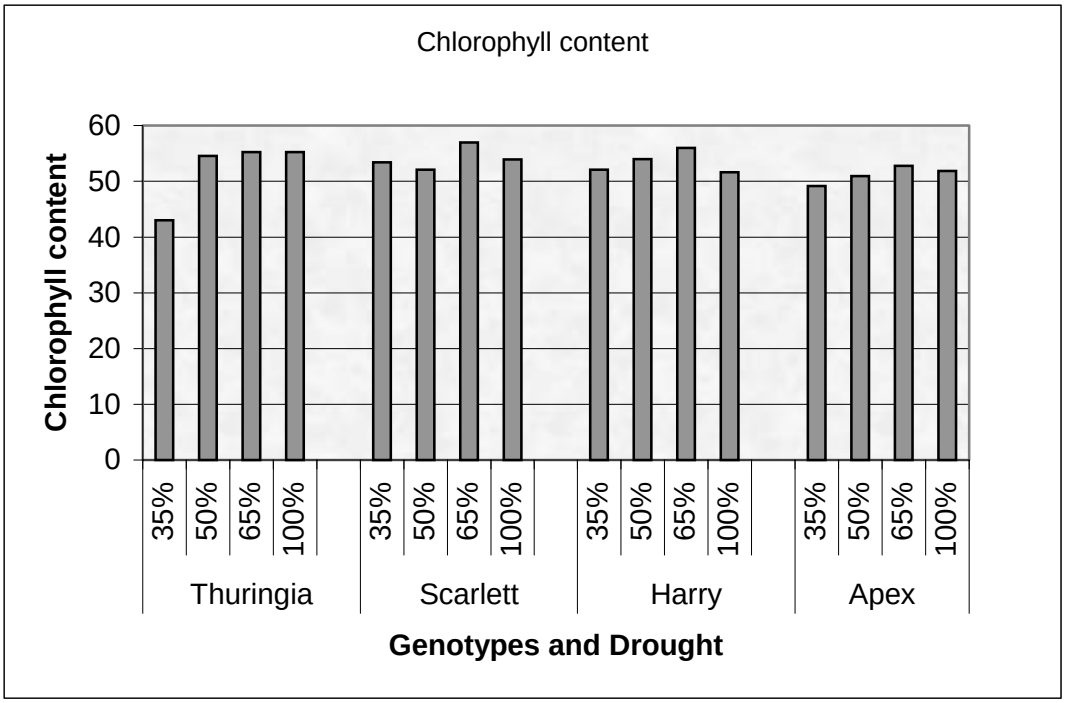


Figure 3: Chlorophyll content of Thuringia, Scarlett, Harry, and Apex genotypes for drought experiment.

Figure 3 shows the different were little for all treatments and genotypes except 35% treatment in Thuringia has few Chlorophyll content.

7) Days until heading

The variation among replications and the interaction between genotypes and drought treatments were non-significant, but the difference among genotypes and drought treatments were highly significant in Table 8. The average of days to heading ranged from 64.83 days for Apex to 87.17 days for Harry (see Table 9). Mean for drought treatments ranged from 68.58 days for 35% FC to 80.50 days plant for 100% FC (Table 10).

8) Plant height

The analysis of variance among replications and the interaction between genotypes and drought treatments were non-significant, but the difference among genotypes and drought treatments were highly significant (Table 8). The average plant height among genotypes ranged from 41.71 cm for Harry to 56.33 cm for Scarlett (see Table 9). Mean for drought treatments ranged from 40.81 cm for 35% FC to 55.61 cm for 100% FC (see Table 10).

9) Yield

The analysis of variance among replications was non-significant, but the difference among genotypes, drought treatments and the interaction between genotypes and drought treatments were highly significant (Table 8 and Figure 4). The grain yield ranged from 0.14 g for Harry to 6.43 g for Apex (see Table 9). Mean for drought treatments ranged from 1.51 g for 35% FC to 8.13 g for 100% FC (see Table 10).

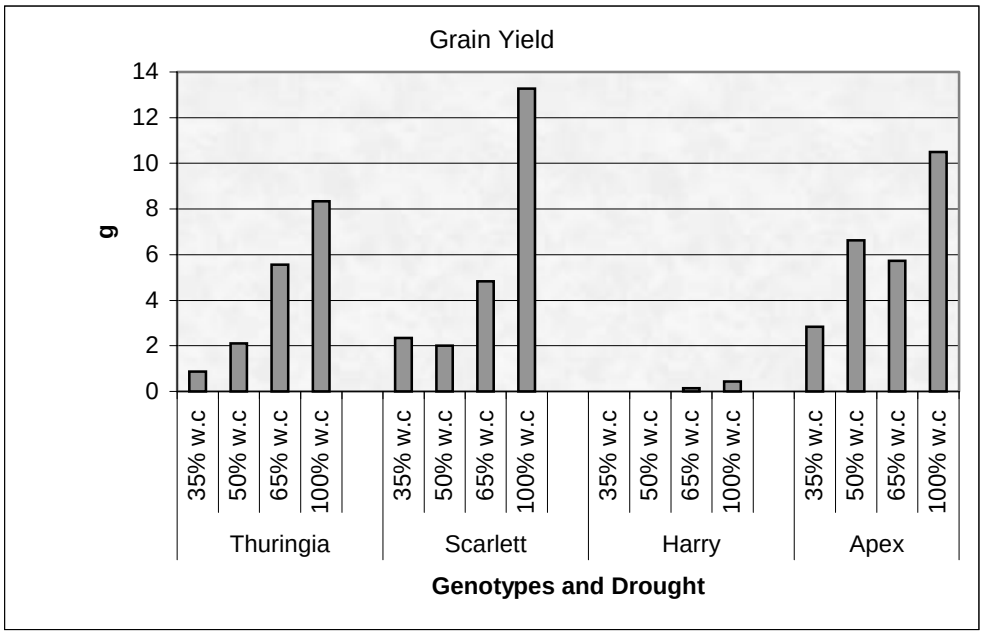


Figure 4: Yield of Thuringia, Scarlett, Harry, and Apex genotypes for drought experiment.

Figure 4 shows, Thuringia obtained little different between (35% and 50%), (65% and 100%), on other hand revealed high different between (35% and 65%, 35% and 100%), (50% and 65%, 50% and 100%) for yield. Were little different among (35%, 50% and 65%), but high different between 100% and other treatments Scarlett. Harry genotype was very susceptible for 35% and 50% FC treatments were no-yield and for 65% and 100% treatments almost no yield. The different was little between 50% and 65% treatments, but high different between 35% and 100% treatments for Apex. General was high different between all genotypes.

10) Biomass

The analysis of variance among replications was non-significant, while the effects of genotypes, drought treatments and the interaction between genotypes and drought treatments were highly significant (Table 8 and Figure 4). The result found average for biomass ranged from 20.68 g for Apex to 27.36 g for Harry (see Table 9). Mean for drought treatments ranged from 11.08 g for 35% FC to 38.88 g for 100% FC (see Table 10).

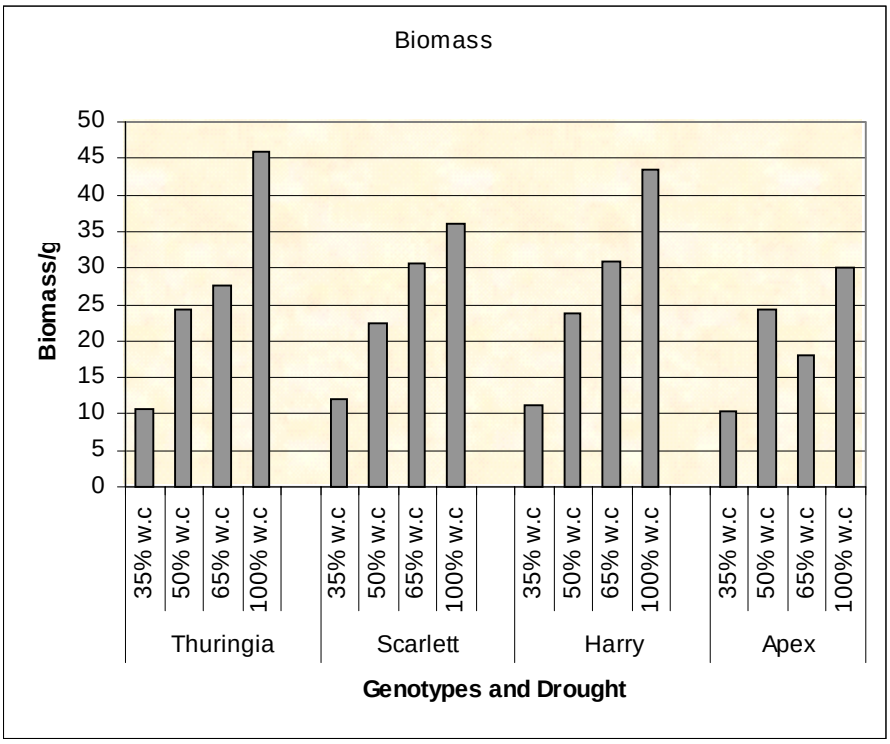


Figure 5: Biomass of Thuringia, Scarlett, Harry, and Apex genotypes for drought experiment.

Figure 5 shows, Thuringia obtained little different between (50% and 65%), on other hand revealed high different between (35% and 65%, 35% and 50%, 35% and 100%), (50% and 100%, 65% and 100%) for Biomass. Were moderate differenced among all treatments for Scarlett. Harry obtained high different among all treatments. The different were moderate among all treatments except 35% treatment for Apex. General was high different between all genotypes.

11) Harvest index

The variation among replications was non-significant, but was highly significant among genotypes, drought treatments and the interaction among genotypes and drought treatments (Table 8 and Figure 6). The average harvest index ranged from 0.35% for Harry to 30.83% for Apex (see Table 9). Mean for drought treatments ranged from 11.31% for 50% FC to 22.79% for 100% FC (see Table 10).

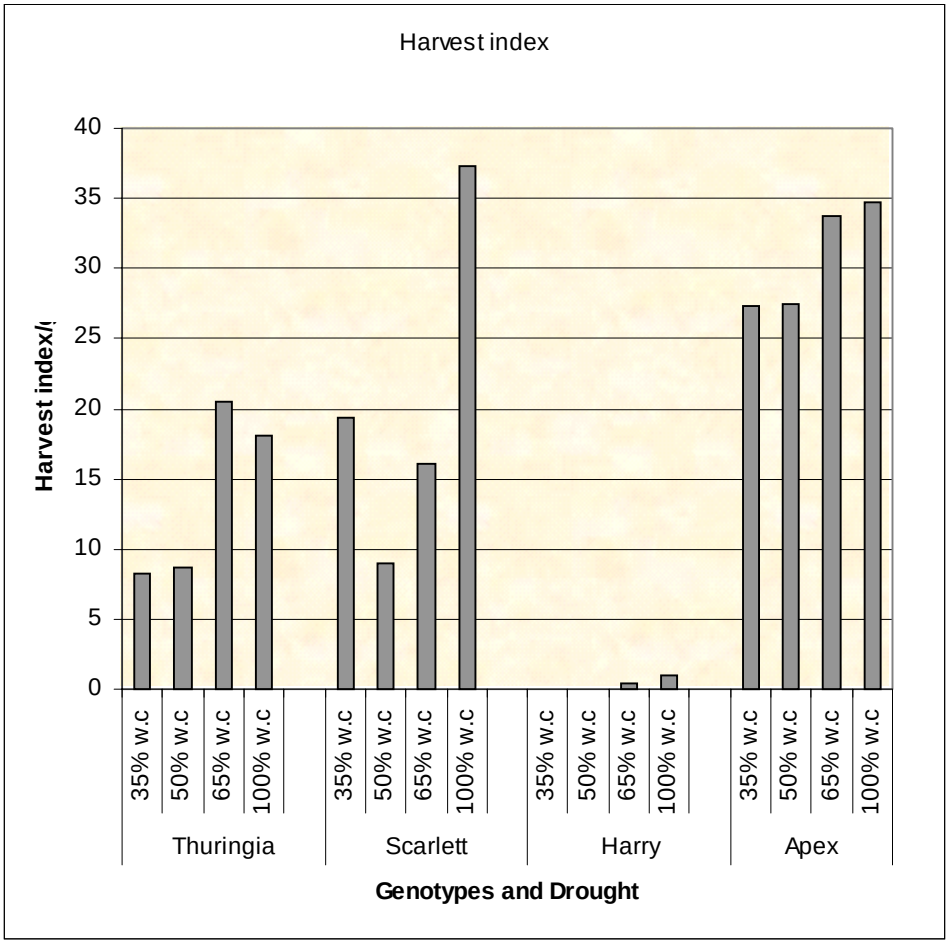


Figure 6: Harvest index of Thuringia, Scarlett, Harry, and Apex genotypes for drought experiment.

The above figure shows that, Thuringia obtained small difference between (35% and 50%), (65% and 100%), on other hand, it revealed high difference between (35% and 65%, 35% and 100%), (50% and 65%, 50% and 100% for harvest index. Whereas, small difference among (35%, 50% and 65%), and high difference between 100% and other treatments for Scarlett were recorded. Harry genotype was very susceptible for 35% and 50% FC treatments where no-harvest index and for 65% and 100% treatments nearly no harvest index. The different was little for all treatments for Apex. General was high different between all genotypes.

Table 9: Means of traits for Thuringia, Scarlett, Harry, and Apex genotypes with Ryan-Gabriel-Welsch Multiple Range Test in drought experiment

Trait	Thuringia	Scarlett	Harry	Apex
Tillers per plant	15.25^{A}	11.00^{B}	$1\ 0.42^{B}$	14.42^{AB}
Spikes per plant	8.34^{A}	$4.99A^{B}$	9.00^{A}	5.26^{B}
Kernels per spike	7.56^{B}	12.27^{A}	1.92^{C}	11.65^{A}
Relative leaf water content	74.58^{A}	79.26^{A}	82.01^{A}	80.73^{A}
Osmotic adjustment	0.143^{A}	0.101^{C}	0.078^{D}	0.114^{B}
Chlorophyll content	52.31^{AB}	54.48^{A}	$52.\ 74^{AB}$	50.49^{B}
Days to heading	68.00^{C}	78.50^{B}	87.17^{A}	64.83^{C}
Plant height	49.79^{B}	56.33^{A}	41.71^{C}	46.66^{BC}
Grain yield	4.22^{B}	5.61^{A}	0.14^{C}	6.43^{A}
Biomass	27.06^{A}	25.27^{A}	27.36^{A}	20.68^{B}
Harvest index %	13.88^{C}	20.48^{B}	0.35^{D}	30.83^{A}

Mean values with different superscript letters are significantly different at $P \leq 0.05$.

Table 10: Mean value of traits of heat treatments with Ryan-Gabriel-Welsch Multiple Range Test for drought experiment

Traits	35% FC	50% FC	65% FC	100% FC
N0.Tillers per plant	5.83^{C}	9.08^{B}	11.17^{B}	16.00^{A}
No. Spikes per plant	5.69^{B}	8.51^{A}	6.92^{AB}	6.47^{AB}
No. Kernels per spike	4.87^{C}	6.45^{BC}	9.40^{B}	12.67^{A}
Relative leaf water content	72.72^{B}	75.18^{B}	80.84^{AB}	87.28^{A}
Osmotic adjustment	0.147^{A}	0.121^{B}	0.0898^{C}	0.0789^{D}
Chlorophyll content	49.95^{B}	53.91^{A}	53.54^{A}	52.63^{AB}
Days to heading	68.58^{C}	72.67^{BC}	76.75^{AB}	80.50^{A}
Plant height	40.81^{B}	46.01^{B}	52.01^{A}	55.61^{A}
Grain yield	1.51^{C}	2.68^{C}	4.07^{B}	8.13^{A}
Biomass	11.08^{C}	23.66^{B}	26.76^{B}	38.88^{A}
Harvest index %	13.74^{BC}	11.31^{C}	17.69^{AB}	22.79^{A}

Mean values with different superscript letters are significantly different at $P \leq 0.05$.

4.1.2 Drought result for parents Scarlett and ISR42-8

Relative leaf water content: the differences were significant among drought treatments and parents for relative leaf water content. There were significant result of interaction between drought treatments and years, the interaction between drought treatments and parents and interaction among drought treatments, years and parents for relative leaf water content, but was non-significant for the interaction between years and parents (Table 11). **Number of spikes per plant: v**ariation among drought treatments, parents, interaction between drought treatments and years as well as, the interaction between drought treatments and parents and the interaction between years and parents were significant. It was non-significant between years, and interaction among drought treatments, years and parents (Table 11). **Number of kernels per spike:** it was highly significant among drought treatments, parents, years, the interaction between drought treatments and parents, the interaction between years and parents and interaction between drought treatments and years. It was non-significant for among the interaction drought treatments, years and parents (Table 11). **Osmotic adjustment:** variation among drought treatments, parents, years and the interaction between drought treatments and parents were highly significant. It was non-significant for interaction between drought treatments and years, the interaction between years and parents and interaction among drought treatments, years and parents (Table 11). **Days until heading:** the result showed highly significant among drought treatments, parents, years, interaction between drought treatments and parents, the interaction between years and parents as well as, interaction between drought treatments, years and parents. On other hand, was non-significantly for the interaction between drought treatments and years (Table 11). **Number of leaves per main tiller:** variation significant for number of leavers per tiller between years, parents, and interaction between drought treatments, years and parents. It was non-significantly for drought treatments, the interaction between drought treatments and parents, interaction between drought treatments and years, the interaction between years and parents (Table 11). **Yield:** the result indicated highly significant among drought treatments, parents. It was non-significant between years. Whereas, were highly significant for all interactions in yield (Table 11). **Biomass:** the result revealed highly significant among drought treatments, parents, years whilst, were highly significant for all interactions in biomass (Table 11).
Harvest index: the value among drought treatments and interaction between drought treatments, years and parents were non-significantly. It was significant for parents, years, interaction between drought treatments and parents, the interaction between drought treatments and years and the interaction between years and parents (Table 11). **Flag leaf area:** variation among drought treatments, years, the interaction between years and parents, the interaction between drought

treatments and years were highly significant, as well as, interaction among drought treatments, years and parents. It was non-significantly for parents, the interaction between drought treatments and parents (Table 11). **First leaf area:** it was non-significantly between years, parents, the interaction between years and parents, while was significantly between drought treatments, interaction between drought treatments and years, the interaction between drought treatments and parents and as well as, interaction among drought treatments, years and parents (Table 11). **Second leaf area:** the variation among drought treatments, years and the interaction between drought treatments and years, the interaction between drought treatments and parents as well as, interaction among drought treatments, years and parents were significant, whereas was non-significantly for parents and the interaction between years and parents (Table 11). **Carbon isotope discrimination:** the result revealed highly significant between drought treatments, parents, and years. Whilst, were highly significant for all interactions (Table 11).

Table 11: Analysis of variance of Scarlett and ISR42-8 for drought tolerance

Trait		Drought (D)	Year (Y)	Parents (P)	D*Y	D* P	Y* P	D*Y* P	Error
	DF:	3	1	1	3	3	1	3	191
RWC	M S	5913.63	2957.61	564.96	2432.65	518.07	105.61	393.30	100.5
	F	58.82***	29.42***	5.62*	24.20***	5.15**	1.05	3.91**	
SPK	MS	22.27	4.87	25.24	44.84	8.08	189.09	3.11	1.28
	F	17.37***	3.80	19.69***	34.75***	6.30***	147.45***	2.43	
KER	MS	832.93	685.53	5566.37	764.66	127.17	1669.5947	20.51	11.18
	F	74.47***	61.29***	497.67***	68.37***	11.37***	149.27***	1.83	
OA	MS	0.022	0.0065	0.0073	0.00049	0.0061	0.00035	0.00016	0.00068
	F	31.97***	9.55**	10.72**	0.71	8.91***	0.52	0.24	
HEA	MS	324.39	604.52	7411.35	4.24	33.41	84.85	72.79	2.71
	F	119.75***	223.17***	2735.99***	1.57	12.33***	31.32***	26.87***	
LEA	MS	0.27	51.03	10.36	0.39	0.12	0.01	1.01	0.38
	F	0.7	134.47***	27.30***	1.03	0.33	0.03	2.67*	
YLD	MS	89.44	0.69	756.57	35.39	63.01	11.36	4.34	0.88
	F	101.49***	0.79	858.44***	40.16***	71.49***	12.89***	4.92**	
MASS	MS	737.40	200.44	1293.14	87.29	68.69	37.59	118.29	3.60
	F	204.73***	55.65***	359.02***	24.24***	19.07***	10.44**	32.84***	
HI	MS	76.54	845.63	810.04	583.95	297.01	2569.71	146.72	106.3
	F	0.72	7.95**	214.51***	5.49**	2.79*	24.17***	1.38	
FLA	MS	141.24	382.52	0.35	25.42	37.14	0.17	27.48	2.74
	F	51.5***	139.52***	0.13	9.27***	13.91***	0.06	10.03***	
ARE1	MS	327.25	7.94	0.95	14.74	89.72	5.79	96.79	4.88
	F	67.01***	1.63	0.19	3.02*	18.37***	1.19	19.82***	
ARE2	MS	419.36	472.30	20.88	35.67	132.59	0.0025	156.94	8.42
	F	49.79***	56.07***	2.48	4.23**	15.74***	0.00	18.63***	
CID	MS	56.46	48.11	10.99	29.13	4.59	6.9	4.86	0.78
	F	72.24***	61.55***	14.07***	37.27***	5.87***	8.84**	6.22***	

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively

Table 12: T test (LSD) for average mean values of parents Scarlett and ISR42-8 for 13 quantitative traits.

Trait	Scarlett	ISR42-8
Relative leaf water content	68.23^{A}	65.23^{B}
Number of spikes per plant	5.64^{A}	4.09^{B}
Number of kernels per spike	24.31^{A}	10.74^{B}
Osmotic	0.075^{B}	0.087^{A}
Heading	86.84^{A}	73.35^{B}
Number of leaves per main tiller	5.18^{A}	4.71^{B}
Yield	5.42^{A}	1.08^{B}
Biomass	13.43^{A}	8.36^{B}
Harvest index	40.59^{A}	14.98^{B}
Flag leaf area	5.44^{A}	5.31^{A}
First leaf area	11.91^{A}	11.59^{A}
Second leaf area	16.21^{A}	15.57^{A}
Carbon isotope discrimination	-27.92^{A}	-28.37^{B}

Mean values with different superscript letters are significantly different at $P \leq 0.05$.

Table 12 shows LSD between mean values of parents Scarlett and ISR42-8 was significant when different litters. All traits were significant difference for parents except flag leaf area, first leaf area and second leaf area.

Table 13: Mean value of traits for drought treatments with Student-Newman-Keuls Test (SNK) Test in drought experiment.

Trait	100% FC	75% FC	50% FC	25% FC
Relative leaf water content	78.29^{A}	63.17^{C}	67.96^{B}	57.66^{D}
Number of spikes per plant	5.17^{A}	5.09^{A}	4.82^{AB}	4.40^{B}
Number of kernels per spike	20.22^{A}	19.61^{A}	16.18^{B}	14.27^{B}
Osmotic adjustment	0.059^{D}	0.085^{C}	0.07^{B}	0.109^{A}
Heading days	82.98^{A}	81.73^{B}	78.56^{C}	77.27^{D}
Number of leaves tiller	4.85^{A}	4.92^{A}	4.98^{A}	5.04^{A}
Yield	4.62^{A}	3.62^{B}	2.62^{C}	2.19^{D}
Biomass	15.62^{A}	11.52^{B}	9.08^{C}	7.42^{D}
Harvest index	26.99^{A}	29.19^{A}	27.21^{A}	28.03^{A}
Flag leaf area	8.23^{A}	5.37^{B}	4.48^{C}	3.42^{D}
First leaf area	15.60^{A}	12.18^{B}	10.44^{C}	8.79^{D}
Second leaf area	19.82^{A}	17.10^{B}	14.69^{C}	11.96^{D}
Carbon isotope discrimination	-29.42^{C}	$-28{:}33^{B}$	-27.9^{B}	$-26{:}91^{A}$

Mean values with different superscript letters are significantly different at $P \leq 0.05$.

Table 13: shows LSD between mean values of parents Scarlett and ISR42-8 was significant when different litters. All traits were significant difference among drought treatments except number of leaves per main tiller and harvest index.

4.1.3 Drought results for BC_2DH lines (AB-DH lines Scarlett*ISR42-8 population)

Relative leaf water content: the value effects of drought treatments, BC_2DH lines, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines and the interaction among drought treatments, years and BC_2DH lines were highly significant. The effect of years and the interaction between drought treatments and years was non-significantly (Table 14). **Number of spikes per plant:** variation between drought treatments, BC_2DH lines, years, the interaction between drought treatments and years, the interaction between years and BC_2DH lines, interaction among treatments, years and BC_2DH lines were significant, while was non-significant for between the interaction between drought treatments and BC_2DH lines (Table 14).

Table 14: Analysis of variance of traits of BC_2DH lines, years and drought treatments in drought experiment

Trait		Drought (D)	Years (Y)	lines (DH)	D*Y	D*DH	Y*DH	D*Y*DH	Error
	DF:	1	1	318	1	313	309	304	80
RWC	MS	21014.17	285.07	243.06	18.43	239.57	227.1	220.38	77.78
	F	270.19***	3.67	3.13***	0.24	3.08***	2.93***	2.83***	
SPK	MS	45.86	982.91	3.12	7.68	1.52	3.18	1.62	1.13
	F	40.47***	867.40***	2.75***	6.78*	1.34	2.80***	1.62*	
KER	MS	26677.92	1734.66	107.29	2739.22	45.98	55.96	48.96	10.76
	F	2478.67***	161.17***	9.97***	254.50***	4.27***	5.20***	4.55***	
OA	MS	0.063	0.033	0.0011	0.0000075	0.00074	0.00084	0.00085	0.00029
	F	216.17***	113.49***	4.05***	0.03	2.53***	2.88***	2.92***	
HEA	MS	8539.81	745.63	233.68	964.51	30.04	195.90	26.69	2.78
	F	3071.26***	268.16***	84.04***	346.88***	10.80***	70.45***	9.60***	
LEA	MS	2.63	450.44	0.67	7.69	0.55	0.75	0.44	0.36
	F	7.26**	1242.59***	1.84***	21.22***	1.52*	2.08***	1.21	
YLD	MS	1943.97	713.13	5.09	82.08	2.22	2.75	1.48	0.91
	F	2132.72***	782.72***	5.58***	90.05***	2.43***	3.02***	1.62**	
MASS	MS	15853.28	21018.96	17.43	124.25	12.79	12.99	11.04	2.90
	F	5457.75***	7236.12***	6.00***	42.77***	4.40***	4.47***	3.80***	
HI	MS	4010.41	49217.68	305.74	4222.37	161.58	215.74	171.41	99.71
	F	40.22***	493.63***	3.07***	42.35***	1.62**	2.16***	1.72**	
FLA	MS	1009.61	3450.13	20.66	325.39	9.72	15.03	9.86	3.38
	F	299.12***	1022.17***	6.12***	96.40***	2.88***	4.45***	2.92***	
ARE1	MS	4321.87	4178.86	31.65	1399.09	13.76	19.99	15.77	6.04
	F	1047.12***	692.12***	5.24***	231.72***	2.28***	3.31***	2.61***	
ARE2	MS	6192.49	15766.34	94.26	231.7	59.08	79.31	53.63	10.16
	F	609.60***	1552.08***	9.28***	22.81***	5.82***	7.81***	5.28***	
CID	MS	849.93	8.91	4.9	40.05	2.76	5.84	2.85	1.18
	F	719.21***	7.55**	4.15***	33.89***	2.34***	4.95***	2.41***	

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

Number of kernels per spike: the result showed highly significant between drought treatments, BC_2DH lines, years, the interaction between drought treatments and years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines and the interaction among treatments, years and BC_2DH lines (Table 14). **Osmotic adjustment:** the value revealed highly significant between drought treatments, BC_2DH lines, years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines and the interaction between treatments, years and BC_2DH lines, whereas was non-significantly for the interaction between drought treatments and years (Table 14). **Days until heading:** the analysis of variance indicated highly significant results of days until heading between drought treatments, among BC_2DH lines and years. All interactions were also highly significant for days until heading (Table 14). **Number of leaves per main tiller:** variation was significant for number of leavers per tiller between drought treatments, years, for BC_2DH lines, interaction drought treatments and years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines, whereas was non-significantly for interaction among drought treatments, years and BC_2DH lines (Table 14). **Yield:** the result indicated highly significant for yield between drought treatments, years, BC_2DH lines, the interaction between drought treatments and years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among drought treatments, years and BC_2DH lines (Table 14). **Biomass:** variation was highly significant for biomass between drought treatments, years, BC_2DH lines, the interaction between drought treatments and years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among drought treatments, years and BC_2DH lines (Table 14). **Harvest index:** the variation was found highly significant for harvest index between drought treatments, years, BC_2DH lines, the interaction between drought treatments and years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among drought treatments, years and BC_2DH lines (Table 14). **Flag leaf area:** the result revealed highly significant for flag leaf area between drought treatments, years, BC_2DH lines, the interaction between drought treatments and years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among drought treatments, years and BC_2DH lines (Table 14). **First leaf area:** variation were significant for first leaf area between drought treatments, years, BC_2DH lines, and the interaction between drought treatments and years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among drought treatments, years and BC_2DH lines (Table 14). **Second leaf area:** result obtained highly significant for second leaf area

between drought treatments, years, BC_2DH lines, the interaction between drought treatments and years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among drought treatments, years and BC_2DH lines (Table 14). **Carbon isotope discrimination:** the result indicated highly significant for carbon isotope discrimination between drought treatments, years, BC_2DH lines, the interaction between drought treatments and years, the interaction between drought treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among drought treatments, years and BC_2DH lines (Table 14).

Table 15: Pearson's correlation coefficients (r) between 13 quantitative traits[1] for drought tolerance

	SPK	**KER**	**OA**	**HEA**	**LEA**	**FLA**	**ARE1**	**ARE2**	**CID**	**YLD**	**MAS**	**HI**
RWC	-0.01	0.11***	-0.03	0.02	0.02	0.09***	0.11***	0.054	-0.12***	0.12***	0.12***	-0.03***
SPK		-0.00	-0.02	0.02	-0.27***	0.05*	0.10***	0.18***	-0.05*	0.51***	0.49***	-0.01
KER			-0.06*	0.24***	-0.02	0.13***	0.27***	0.18***	-0.24***	0.68***	0.47***	0.20***
OA				-0.05*	-0.09***	0.01	-0.02	0.00	0.04	-0.10***	-0.03	-0.11***
HEA					0.04	-0.09***	0.08**	0.11***	-0.07**	0.27***	0.15***	0.17***
LEA						-0.21***	-0.18***	-0.22***	0.01	-0.16***	-0.35***	0.22***
FLA							0.69***	0.38***	-0.08**	0.17***	0.34***	-0.26***
ARE1								0.52***	-0.21***	0.34***	0.47***	-0.23***
ARE2									-0.16***	0.30***	0.41***	-0.13***
CID										-0.27***	-0.29***	0.10***
YLD											0.76***	0.18***
MAS												-0.35***

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

(1) Abbreviation for traits Table 5.

Correlation coefficient among 13 traits for all BC_2DH population

The correlation result for 13 traits is shown in Table 15. We have three levels for correlation <0.2 was weak, from ≥0.2 to <0.5 was moderate, and more than ≥0.5 was strong. **Relative leaf water content** was weak correlated with number of kernels per spike, (P≤0.001), flag leaf area (P≤0.001), first leaf area (P≤0.001), yield (P≤0.001), biomass (P≤0.001) and harvest index (P≤0.001). Positive and strong correlations were revealed for **number of spikes per plant** with yield (P≤0.001), while moderate correlations were obtained for SPK with number of leaves per main tiller (P≤0.001**)** and biomass (P≤0.001), whilst it weak correlation were obtained with flag leaf area (P≤0.05), first leaf area (P≤0.001), and second leaf area (P≤0.001)**. Number of kernels per spike** was revealed strongly correlation with yield (P≤0.001), while it moderate correlations were obtained for kernels per spike with days until heading (P≤0.001), first leaf area (P≤0.001), biomass (P≤0.001), and harvest index (P≤0.001), whereas it weak correlation was obtained with osmotic adjustment (P≤0.05), relative leaf water content (P≤0.001), flag leaf area (P≤0.001) and second leaf area (P≤0.001). **Osmotic adjustment** was associated weak with number of kernels per spike (P≤0.05), days until heading (P≤0.05), and number of leaves per main tiller (P≤0.001), yield (P≤0.001) and harvest index (P≤0.001). Correlations were positive and moderate for **days until heading** with number of kernels per spike (P≤0.001) and yield (P≤0.001), whereas it was weak correlation osmotic adjustment (P≤0.05), flag leaf area (P≤0.001), and first leaf area (P≤0.01) second leaf area (P≤0.001), biomass (P≤0.001) and harvest index (P≤0.001). **Number of leaves per main tiller** was moderate with number of spikes per plant (P≤0.001), flag leaf area (P≤0.001), second leaf area (P≤0.001), biomass (P≤0.001), and harvest index (P≤0.001), whereas it was negatively and weak with osmotic adjustment (P≤0.001), first leaf area (P≤0.001) and yield (P≤0.001). **Flag leaf area** positive and strongly correlated with first leaf area (P≤0.001), whilst it was correlated moderate with number of leaf for tiller (P≤0.001), second leaf area (P≤0.001), biomass (P≤0.001) and harvest index (P≤0.001), however it was wear correlated with relative leaf water content (P≤0.001), number of spikes per plant (P≤0.05), number of kernels per spike (P≤0.001), and days until heading (P≤0.001), and yield (P≤0.001). However, **first leaf area** was positive and strongly with flag leaf area (P≤0.001) and second leaf area (P≤0.001), while it was moderate correlation with number of kernel per plant (P≤0.001), yield (P≤0.001), biomass (P≤0.05), and harvest index (P≤0.001), whilst it was weak correlation with relative leaf water content (P≤0.001), number of spikes per plant (P≤0.001), days until heading (P≤0.01) and number of leaves per main tiller (P≤0.001). Positive and strong correlations were expressed by **second leaf area** with first leaf area (P≤0.001), whereas it was moderate correlated with number of leaves per main tiller (P≤0.001), flag leaf area (P≤0.001), yield (P≤0.001) and biomass (P≤0.001), while was weak

correlation with number of spikes per plant (P≤0.001), number of kernels per spike (P≤0.001), days until heading (P≤0.001) and harvest index (P≤0.001) were detected. **Carbon isotope discrimination** was moderate correlated with number of kernels per plant (P≤0.001), first leaf area (P≤0.001), yield (P≤0.001) and biomass (P≤0.001), furthermore it was weak correlated with relative leaf water content (P≤0.001), number of spikes per plant (P≤0.05), days until heading (P≤0.01), flag leaf area (P≤0.01), second leaf area (P≤0.001) and harvest index (P≤0.001). **Yield** was positive and strongly correlated with number of spikes per plant (P≤0.001), number of kernels per spike (P≤0.001), and biomass (P≤0.001), whereas it was moderate correlation with days until heading (P≤0.001), first leaf area (P≤0.001), and second leaf area (P≤0.001), while it was weakly correlations with relative leaf water content (P≤0.001), osmotic adjustment (P≤0.001), number of leaves per plant (P≤0.001) flag leaf area (P≤0.001) and harvest index (P≤0.001). However, **biomass** was strongly and positively correlation with yield (P≤0.001), while it was moderate correlations with number of spikes per plant (P≤0.001), number of kernels per plant (P≤0.001), number of leaves per main tiller (P≤0.001), flag leaf area (P≤0.001), first leaf area (P≤0.001), second leaf area (P≤0.001) and harvest index (P≤0.001), furthermore it was weak and positive correlated with relative leaf water content (P≤0.001), and days until heading (P≤0.001). **Harvest index** was moderate correlated with number of kernels per spike (P≤0.001), number of leaves per main tiller P≤0.001), flag leaf area (P≤0.001), first leaf area (P≤0.001) and biomass (P≤0.05), while it was weak correlations with relative leaf water content (P≤0.001), osmotic adjustment (P≤0.001), days until heading (P≤0.001), second leaf area (P≤0.001), and yield (P≤0.001).

Table 16: Pearson's correlation coefficients (r) between 13 quantitative traits[1] under drought stress

	SPK	**KER**	**OA**	**HEA**	**LEA**	**FLA**	**ARE1**	**ARE2**	**CID**	**YLD**	**MAS**	**HI**
RWC	-0.09*	0.085	0.11**	-0.00	-0.02	0.06	0.04	0.07	-0.00	-0.00	-0.00	-0.01
SPK		0.02	-0.02	-0.02	-0.29***	0.14***	0.24***	0.26***	-0.03	0.62***	0.53***	0.05
KER			0.07	0.06	-0.22***	0.13***	0.27***	0.18***	-0.01	0.68***	0.47***	0.20***
OA				-0.01	-0.08*	0.12**	0.10**	0.09*	-0.07	-0.00	0.11**	-0.15***
HEA					0.01	-0.05	-0.00	0.08*	0.26***	0.06	-0.04	0.21***
LEA						-0.37***	-0.35***	-0.44***	0.1*	-0.40***	-0.55***	0.23***
FLA							0.76***	0.65***	-0.12**	0.39***	0.55***	-0.28***
ARE1								0.78***	-0.13***	0.43***	0.56***	-0.25***
ARE2									-0.06	0.48***	0.63***	-0.25***
CID										-004	-0.15**	0.17***
YLD											0.72***	0.14***
MAS												-0.45***

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

(1) Abbreviation for traits Table 5.

Correlation among 13 traits under for BC_2DH population drought stress

Results of correlation studies of among 13 traits are shown in Table16. We have three levels for correlation <0.2 was weak, from ≥0.2 to <0.5 was moderate, and more than ≥0.5 was strong. **Relative leaf water content** was resulted weak correlations with number of spikes per plant (P<0.05) and osmotic adjustment (P<0.01). Strongly and positive correlations were revealed for **number of spikes per plant** with yield (P<0.001), and biomass (P<0.001), as well as it was moderate with number of leaves per main tiller (P<0.001**),** first leaf area (P<0.001), second leaf area (P<0.001), while it was a weak correlated with relative leaf water content (P<0.05) and flag leaf area (P<0.001)**. Number of kernels per spike** was revealed strong correlation with yield (P≤0.001), whereas moderate correlations with number of leaves per main tiller (P≤0.001), first leaf area (P≤0.001), biomass (P≤0.001), and harvest index (P≤0.001), altogether it was weak correlated with flag leaf area (P<0.001) and second leaf area (P≤0.001). **Osmotic adjustment** was associated weakly with relative leaf water content (P≤0.01), number of leaves per main tiller (P≤0.05), flag leaf area (P≤0.01), first leaf area (P≤0.01), second leaf area (P≤0.05), biomass (P≤0.01), and harvest index (P≤0.001). Correlations were positive and moderate for **days until heading** with harvest index (P≤0.001), whereas it was positive and weak correlation with second leaf area (P≤0.05). **Number of leaves per main tiller** was moderate with number of spikes per plant (P≤0.001), number kernels per plant (P≤0.001), flag leaf area (P≤0.001), first leaf area (P≤0.001), second leaf area (P≤0.001), yield (P≤0.001) and harvest index (P≤0.001), whereas it was negatively and strongly correlated biomass (P≤0.001), but was negatively and weak with osmotic adjustment (P≤0.05). **Flag leaf area** strong and positive correlated with first leaf area (P≤0.001), second leaf area (P≤0.001), and biomass (P≤0.001), whilst it is moderate correlated with number of leaves for tiller (P≤0.001), yield (P≤0.001), and harvest index (P≤0.001), in addition it was weak correlated with number of spikes per plant (P≤0.001), number of kernels per spike (P≤0.001) and osmotic adjustment (P≤0.01). However, **first leaf area** was strongly and positively correlated with flag leaf area (P≤0.001), second leaf area (P≤0.001), and biomass (P≤0.05), while it was moderate correlation with and number of spikes per plant (P≤0.001), number of kernels per plant (P≤0.001), number of leaves per main tiller (P≤0.001), yield (P≤0.001) and harvest index (P≤0.001), while was weak correlated with osmotic adjustment (P≤0.01). Strong and positive correlations were expressed by **second leaf area** with number of flag leaf area (P≤0.001), first leaf area (P≤0.001) and biomass (P≤0.001), while it was moderate correlations with number of spikes per plant (P≤0.001), leaves per tiller (P≤0.01), yield (P≤0.001) and harvest index (P≤0.001), however was weak correlation with number of kernels per spike (P≤0.001), osmotic adjustment (P≤0.05) and days until heading (P≤0.001). **Carbon isotope discrimination** was moderate correlated with days until heading

(P≤0.01), furthermore it was weak correlated with number of leaves per main tiller (P≤0.05), flag leaf area (P≤0.01), first leaf area (P≤0.001), biomass (P≤0.001), and harvest index (P≤0.001). **Yield** was strongly and positively correlated with number of spikes per plant (P≤0.001), with number of kernels per spike (P≤0.001), and biomass (P≤0.001), whereas was moderate correlation with number of leaves per main tiller (P≤0.001) flag leaf area (P≤0.001), first leaf area (P≤0.001), and second leaf area (P≤0.001), whereas a weak correlated with harvest index (P≤0.001). However, **biomass** was strongly correlated with number of spikes per plant (P≤0.001), number of leaves per main tiller (P≤0.001) flag leaf area (P≤0.001), first leaf area (P≤0.001), second leaf area (P≤0.001), yield (P≤0.001), and whereas it was moderate correlation with number of kernels per spike (P≤0.001), harvest index (P≤0.001). **Harvest index** was moderate with number of kernels per spike (P≤0.001), days until heading (P≤0.001), number of leaves per main tiller (P≤0.05) flag leaf area (P≤0.001), first leaf area (P≤0.001), second leaf area (P≤0.001) and biomass (P≤0.001), whilst it was weak correlated with osmotic adjustment (P≤0.001) and yield (P≤0.001).

Result of marker analysis

4.1.4 Identification of Microsatellite markers in the Scarlett backcross population

Ninety-seven SSR markers detected polymorphisms in the BC_2DH population. The distribution of the 97 mapped SSRs is show in Figure 7. They were distributed over all seven barley chromosomes. The 323 BC_2DH lines were successfully genotyped with 97 SSRs. The chromosomal location of the SSRs were inferred from Ramsay *et al*. (*2000*), Pillen *et al*. (*2000*, *2003*), from linkage analysis in a reference BC_2DH population from the Scarlett and ISR42-8 cross. All 97 mapped SSRs cover 1013 cM of the barley genome; the mean SSR density is equal to 11.1 cM (see Table 17). The linkage map for sugar beet covered 789 cM and 1057.3 cM equivalent to an average genetic spacing of 6.8 cM and 6.0 cM per marker respectively (Pillen *et al*. 1992; 1993). The first SSR map for barley includes 299 SSRs and covers 1173 cM (Ramsay *et al* 2000), while the SSRs map of Pillen *et al*. (2003) contains 67 mapped SSRs and covers 852 cM of the barley genome. The Scarlett*ISR42-8 map includes four gaps with a marker distance of more than 30 cM, four gaps are located on chromosomes 3H, 5H and 6H (Table 18, Figure 7).

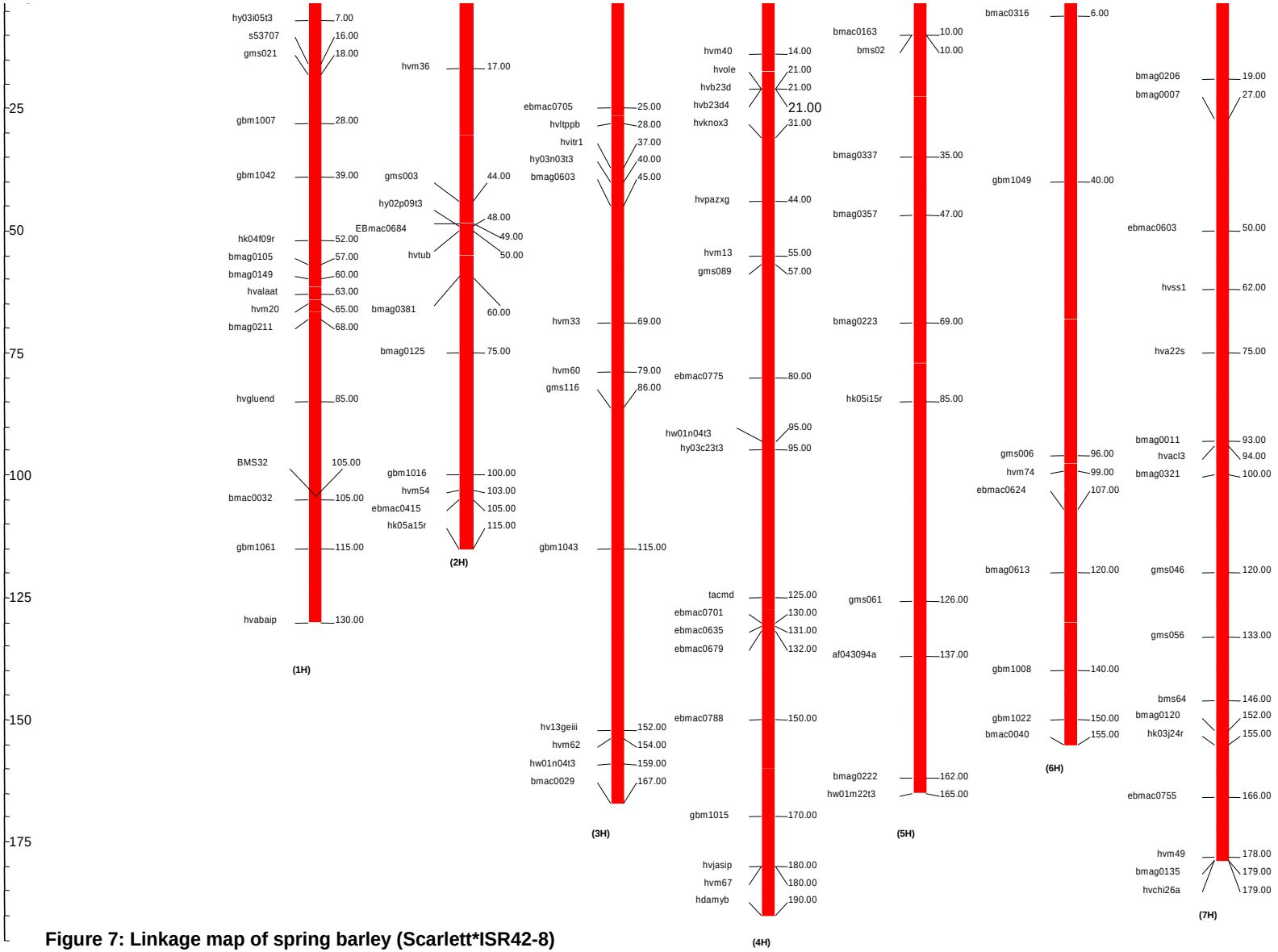


Figure 7: Linkage map of spring barley (Scarlett*ISR42-8)

Linkage map of spring barley (Scarlett*ISR42-8) based on 323 BC2DH lines from the cross of Scarlett*ISR42-8. Marker loci on the left side of each linkage group were used for linkage map construction. Distances are in Kosambi centiMorgen (cM) units on the right side of the map.

Table 17: Number of markers and genome coverage putative QTLs for drought and heat tolerance

Chromosome	Number of mapped markers	Genome coverage (cM)	Marker density (cM pro map marker)	Number of Gaps (>30cM)	Number of putative QTLs	Number of drought experiment QTLs	Number of heat experiment QTLs
1H	17	123	7.2	0	8	4	4
2H	10	98	9.8	0	9	0	9
3H	13	142	10.9	1	5	1	4
4H	20	176	8.8	0	10	2	8
5H	11	165	15.0	1	11	8	3
6H	9	149	16.7	2	5	2	3
7H	17	160	9.4	0	6	3	3
Total	97	1013	-	4	54	20	34
Mean	13.8	144.7	11.1	0.57	7.7	2.8	4.7

4.1.2.1 Results of the AB-QTL-analysis in the backcross population

The 97 polymorphic SSRs revealed 54 putative QTLs from 78 regions in two groups. The first 20 putative QTLs found for drought treatments; and the second 34 putative QTLs found for heat treatments. Altogether, 30 (55.5%) favorable QTL effects were detected for both drought and heat experiment (see Table 29 and 30). At these loci, the homozygous ISR42-8 (*H. v*. ssp. *spontaneum*, is thereafter abbreviated with *Hsp*). Genotype was associated with an improvement of the trait compared to the homozygous (*Hordeum vulgare* L. *distichon*, hereafter abbreviated with *Hvd*) *had* genotype as shown in (Figure 8, 13 and Table 18).

4.1.5 Detection of QTLs for drought tolerance.

Single-point marker analysis by means of a three-factorial ANOVA rather than interval mapping was preferred for QTL analysis. Ninety-seven markers, 20 putative QTLs were detected. Eight regions for the marker main effect and 25 regions for the M*D interaction were significant at $P < 0.01$ (Figure 8 and Table 18). In two cases, both effects (marker main effect and M*D interaction) were significant.

Altogether, 14 (70.0%) favorable QTL effects were detected (see Table 29). At these loci, the homozygous *Hsp* genotype was associated with an improvement of the trait compared to the homozygous *Hvd* genotype (Figure. 8 and Table 18). The putative QTLs were unevenly distributed over the chromosomes (Figure. 8). While four QTLs were located on chromosome 1H, one QTLs were located on chromosome 3H, two QTLs were located on chromosome 4H, eight QTLs were located on chromosome 5H, two QTLs were located on chromosome 6H, three QTLs were located on chromosome 7H, and zero QTLs were detected on chromosomes 2H. Most of the favorable QTLs were located on chromosomes 1H, 5H and 7H (2, 8 and 3 respectively). The distribution of putative QTLs among the 18 genotyped SSR markers was also irregular. Marker Bmag0357 $_{[5H]}$ showed putative QTL effects on three traits (LEA, MAS and YLD), Marker Bmac0316 $_{[6H]}$ obtained putative QTL effects on two traits (OA and YLD) and Marker HW01M22T3 $_{[5H]}$ revealed putative QTL effects on two traits (MAS and OA). The detected putative QTLs are represented for the traits in the Table 18.

Figure 8: Linkage map of QTL in spring barley (Scarlett*ISR42-8) for drought tolerance.

Linkage map contain 20 QTLs for drought experiment. The short and long arms are from top to bottom respectively. Map contains 20 putative QTLs with 20 favorable *Hsp* alleles detected from the BC_2DH cross Scarlett x ISR42-8. Putative QTLs which revealed either a significant ($P < 0.01$) marker main effect or M*D interaction are written to the right of the SSR locus. Adjacent marker effects (distance study <20cM) are considered as one putative QTL. A vertical for represent markers were showing a significant QTL effect within a vicinity of 20 cM. The abbreviations of the quantitative traits follow Table 5.

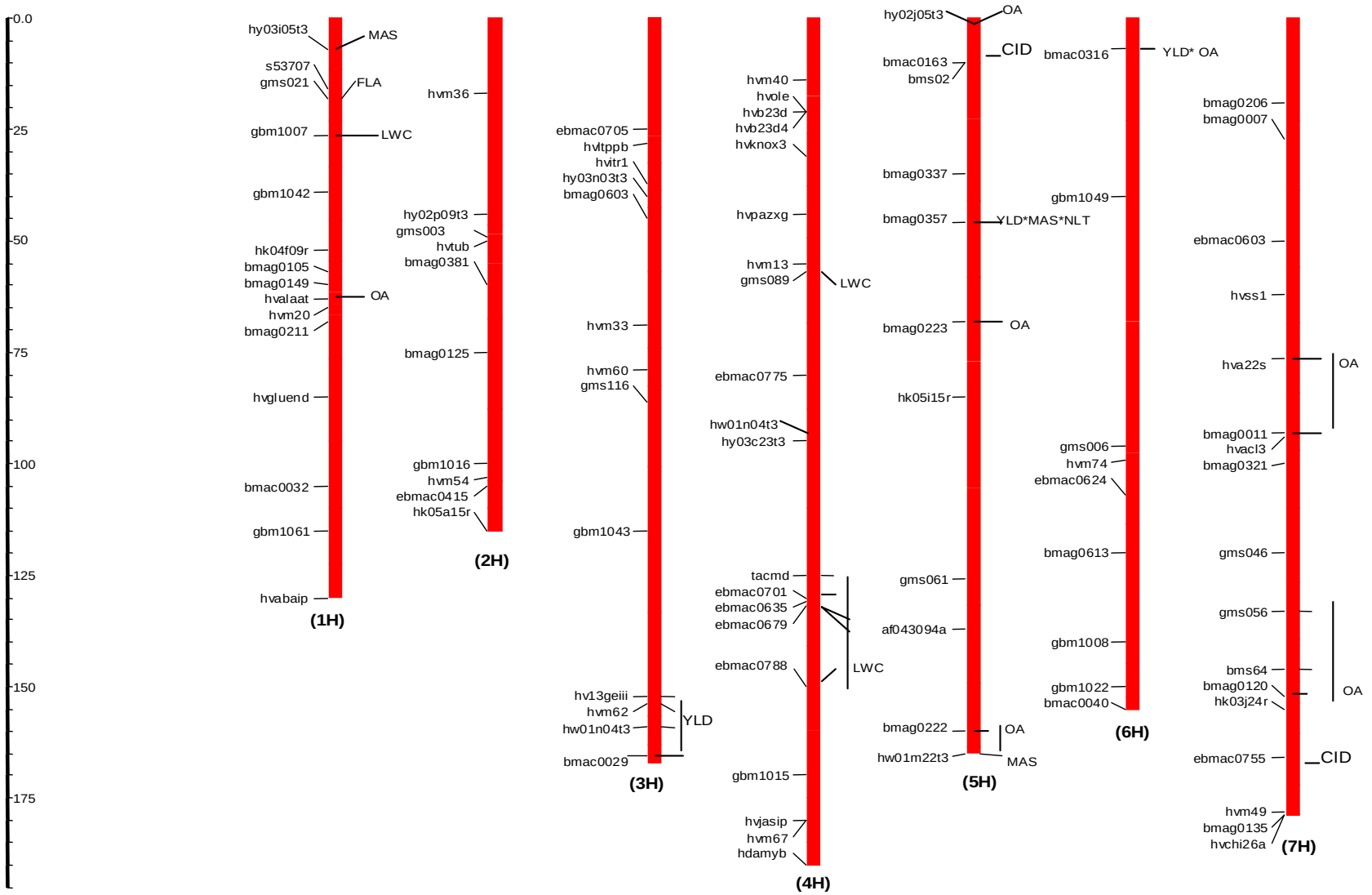


Figure 8: Linkage map of QTL in spring barley (Scarlett*ISR42-8) for drought tolerance

Table 18 : List of 20 putative QTLs detected from the BC_2DH cross Scarlett x ISR42-8[A] for drought tolerance.

[A]Trait	Marker	[B]Ch	[C]Position (cM)	[D]Effect	[E]Sig.	[F]RP Wild allele effect	[G]RP Wild allele effect for control	[H]RP Wild allele effect for drought stress	QTLs
FLA	GMS021	1H	18	M	**	-16.4	-13.6	-20.1	1
LEA	Bmag0357	5H	47	M*D	**	2.2	-1.6	6.1	1
MASS	HY03I05T3	1H	7	M*D	**	1.2	3	-2.1	1
	Bmag0357	5H	47	M*D	**	8.9	13.1	1.7	2
	HW01M22T3	5H	165	M*D	**	-7.8	-15.5	6.3	3
OA	HVALAAT	1H	63	M	***	-11.2	-8.6	-13.3	1
	HY02J05T3	5H	0	M	***	11.5	7.2	15.2	2
	Bmag0223	5H	69	M	***	8.1	7	9	3
	Bmag0222	5H	162	M*D	**	19.7	2.6	34	4
	HW01M22T3	5H	165	M*D	**	22.3	1.6	39.5	4
	Bmac0316	6H	6	M	***	-11.2	-2.5	-18	5
	HVA22S	7H	75	M*D	**	8.4	-0.9	16.2	6
	Bmag0011	7H	93	M	***	8.8	1.2	15.3	6
	GMS056	7H	133	M + M*D	***	14.8	4.7	23	7
	BMS64	7H	146	M + M*D	***	14.3	1.4	25	7
	Bmag0120	7H	152	M*D	***	17.7	3.6	29.8	7
RWC	GBM1007	1H	28	M*D	**	-4.3	3.4	-12.8	1
	GMS089	4H	57	M*D	**	-0.8	-4.8	3.9	2
	TACMD	4H	125	M*D	**	-0.8	4	-6.1	3
	EBmac0701	4H	130	M*D	**	-0.3	4	-5	3
	EBmac0635	4H	131	M*D	**	-0.8	3.4	-5.5	3
	EBmac0679	4H	132	M*D	**	-0.9	3.6	-5.8	3
	EBmac0788	4H	150	M*D	**	-0.7	3	-4.7	3
YLD	HV13GEIII	3H	152	M*D	**	10	14.5	2	1
	HVM62	3H	154	M*D	***	10.8	16.3	0.9	1
	HW01N04T3	3H	159	M*D	**	9.9	14.2	2.1	1
	Bmac0029	3H	167	M*D	**	18.4	21.4	13.1	1
	Bmag0357	5H	47	M*D	**	8.9	14.5	-1.2	2
	Bmac0316	6H	6	M*D	**	4.4	11	-7.4	3
CID	Bmac0163	5H	10	M*D	**	-0.9	1.2	-3.2	1
	EBmac0755	7H	166	M*D	**	-1.3	-3.1	0.6	2

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

[A]The quantitative traits are defined in Table 5.

[B]Chromosomal assignment of SSRs

[C]Chromosomal position of SSRs deduced from Ramsay *et al*. (2000) and Pillen *et al*. (2000).

[D]Effect A QTL was assumed within the vicinity of a marker locus if the marker main effect or the M*D interaction was significant in the three-factorial ANOVA at $P < 0.01$

[E]Level of significance of the marker main effect and the M*D interaction, respectively, with: P < 0.01, ***P < 0.001.

[F]RP [Genotype] = (Ms—Mv)*100/Mv in % in % effect of the Hsp alleles a cross both environments..

[G]RP [T*M T1] = (MsT1—MvT1)*100/MvT1 in % was favorable effects of the Hsp alleles for control treatments.

[H]RP [T*M T2] = (MsT2—MvT2)*100/MvT2 in % was effects of the Hsp alleles for drought stress.

[I]QTLs number of QTLs for every trait.

M + M*D their marker plus interaction between marker and drought treatment.

Relative leaf water content (RWC)

Three putative QTLs for Relative leaf water content were located on chromosomes 1H and 4H. All seven loci exhibited significant M*D interactions. On chromosome 4H was found TACMD highly significant than other marker EBMAC0701, EBMAC0635, EBMAC0679, and EBMAC0788. At three loci, the presence of the *Hsp* allele led to a reduction in relative leaf water content of up to 4.3% at GBM1007 [1H]. The *Hsp* alleles showed positive effects for control treatment except GMS089 [4H]. The *Hsp* increased relative leaf water content in the control treatment with 4.0% at Ebmac0701 [4H] and TACMD [4H], while the *H*sp allele decreased the RWC in the control treatment by 4.8% at GBM089 [4H]. On the other hand, the *Hsp* allele decreased the RWC in the drought stress with maximum of 12.8% at GBM1007 [1H], while the *Hsp* allele increased the RWC in the drought stress up to 3.9% at GMS089 [4H] (see Table 18).

Osmotic adjustment (OA)

A total of 7 putative QTLs have effect on osmotic adjustment were located on chromosomes 1H, 5H, 6H and 7H. Seven loci exhibited a significant marker main effect, the other 6 loci showed a significant D*M interaction. Three regions at Bmag0222 [5H], HW01M22T3 [5H] and Bmag0222 [5H] were found on chromosome 5H like one QTL, but were found Bmag0222 [5H] highly significant. On chromosome 7H, were found HVA22S highly significant than Bmag0011, while GMS056 was found highly significant than BMS64 and Bmag0120. However, five favorable *Hsp* alleles effect

was detected, these loci improved osmotic adjustment to a maximum value 22.3% at HW01M22T3 [5H]. On other hand, two *Hsp* allele decreased OA up to 11.2% at both Bamg0120 [7H], HVALAAT [1H]]. The *Hsp* allele four loci lifted OA in control treatment up to maximum 7.2% HY02J05T3 [5H], while, three loci *Hsp* allele decreased OA in control treatment for osmotic adjustment up to 8.6% at HVALAAT [1H]]. Two wild allele loci decreased OA in drought stress to maximum 18.0% Bmag0316 [7H], whilst five *Hsp* alleles showed increasing OA for drought stress up to 39.5% at HW01M22T3 [5H] (Table 18).

Number of leaves per main tiller (LEA)

Only one putative QTL for number of leaves per main tiller was located on chromosome 5H. It showed a significant M*D interaction at $P < 0.01$. The *Hsp* allele has favorable effect increasing the LEA by 2.2% at Bmag0357 [5H]. The *Hsp* allele resulted decrease LEA control treatment with 1.6%. Whereas, *Hsp* allele lifted LEA under drought stress by 6.1% (see Table 18).

Flag leaf area (FLA)

One putative QTL was located for flag leaf area on chromosome 1H. QTL was detected, exhibited a significant marker main effect. Favorable *Hsp* allele effect was detected at GMS021 [1H] reduce FLA by 16.4%. The *Hsp* allele one locus showed a decrease in FLA in control treatment 13.6% at GMS021 [1H]. Drought stress obtained negative effect FLA in one locus of *Hsp* allele value found 20.1% at GMS021 [1H] (see Table 18).

Yield (YLD)

Three putative QTLs for yield were located on chromosomes 3H, 5H and 6H. Six loci showed an M*D interaction were significant at the linked loci for their four loci located on chromosome 3H and one QTL, HV13GEIII [3H] and HVM62 [3H] highly significant than Bmac0029 [3H] and HW01N04T3 [3H]. Three *Hsp* alleles have favorable effects were detected, was improved positive effects for yield to maximum 18.4% at Bmac0029 [3H]. Control treatment resulted positive effects for yield at three loci of the *Hsp* alleles up to 21.4 % at Bmac0029 [3H]. Result showed positive effects for yield at one locus of the *Hsp* alleles under drought stress up to 13.1% at Bmac0029 [3H],

whereas, drought stress obtained negative effects at two loci of *Hsp* alleles in 1.2% and 7.4% at Bmac0316 [6H] and Bmac0357 [5H] respectively (see Table 18).

Biomass (MAS)

Three QTLs were located for biomass trait on chromosomes 1H and 5H. All QTLs were showed as significant M*D interactions. The negative effect of the *Hsp* allele at one locus resulted in a 7.8% reduction of the above ground biomass at HW01M22T3 [5H] and favorable effects of the *Hsp* alleles detected for biomass, positive effects, 1.2% and 8.9% were found at tow loci both at HY03I05T3[1H] and Bmag0357[5H] respectively. Control treatments increased biomass at two loci *Hsp* allele a maximum 13.1% at Bmag0357 [5H], whereas other locus reduced biomass control treatments up to 15.5% at HW01M22T3 [5H]. Drought stress decreased biomass at all two loci *Hsp* allele a maximum 6.3% HW01M22T3 [5H], whereas other locus reduced biomass under drought stress up to 2.1% at HY03I05T3 [1H] (Table 18).

Carbon isotope discrimination (CID)

Two putative QTLs for carbon isotope discrimination were located on chromosomes 5H and 7H. Two loci showed an M*D interaction were significant. Two *Hsp* alleles, which have favorable effects, were detected. They improved negative effects for carbon isotope discrimination up to 0.9% and 1.3% at both Bmac0163 [5H] and Ebmac0755 [7H]. Control treatment resulted positive effects for carbon isotope discrimination at one locus of the *Hsp* allele up to 1.2 % at Bmac0163 [5H], while was negative effects for CID discrimination at one locus the *Hsp* allele up to 3.1% at EBmac0755 [7H]. The *Hsp* allele obtained negative effect at one locus for CID under drought stress in 0.6% EBmac0755 [7H], while it was positive effect for CID at one locus of *Hsp* allele in 3.2% Bmac0163 [5H] (see Table 18).

4.2 Heat results

Morphological characters

In this study 11 quantitative traits (tillers per plant, number of spikes per plant, number of kernels per spike, relative leaf water content, osmotic adjustment, chlorophyll content, days to heading, yield, biomass and harvest index) traits were investigated for evaluation of barley (Thuringia, Scarlett, Harry, and Apex) genotypes. The number of tillers was found to be non-significant between genotypes as well as the interaction among genotypes and drought treatments, but chlorophyll content non-significant for the interaction among genotypes. In the study for (Scarlett and ISR42-8) parents and BC_2 DH population, we have tillers per plant, plant height, and chlorophyll content not studied, but we have (number spikes per plant, number of kernels per spike, relative leaf water content, osmotic adjustment, days to heading, yield, biomass and harvest index) and other traits more like flag leaf area, first lea area and second leaf area, because related for drought study.

4.2.1 Evaluation of four barley genotypes

1) Number of tillers per plant

The analysis of variance for replicates, genotypes and the interaction for genotypes and heat treatments were non-significant, but highly significant for heat treatments (Table 19). Result showed that the mean number of tillers ranged from 11.89 tillers for Harry to 26.78 tillers for Apex (Table 20).). Mean for heat treatments ranged from 12.50 tillers for heat treatment + 65% FC to 33.08 tillers for control (see Table 21).

2) Number of spikes per plant

Variation for replications and the interaction among genotypes and heat treatments were non-significant, but highly significant ($P<0.001$) among genotypes, and heat treatments (Table 19). The average number of spikes per plant ranged from 2.01 for Harry genotype to 3.09 spikes for Apex (see (Table 20). Mean for heat treatments ranged from 0.89 spikes for heat treatment + 65% FC to 4.23 spikes for control (see Table 21).

Table 19: Analysis of variance traits for Thuringia, Scarlett, Harry, and Apex genotypes for heat experiment.

Trait		Replications	Heat treatments	Genotypes	G x H	Error
	DF:	2	2	3	6	22
TILL	MS	4.98	34.85	4.54	8.42	5.66
	F	0.88	6.16**	0.80	1.49	
SPK	MS	16.33	1271.08	472.62	14.88	49.06
	F	0.33	25.91***	9.63**	0.30	
KER	MS	22.82	802.84	71.84	12.83	21.44
	F	1.06	37.44***	3.35*	0.60	
RWC	MS	27.81	1482.57	886.73	262.62	29.21
	F	0.95	50.76***	30.36***	8.99***	
OA	MS	0.000044	0.0051	0.001	0.00025	0.00003
	F	1.43	164.78***	32.48***	8.10***	
CHL	MS	1.12	301.82	49.81	4.66	6.85
	F	0.16	44.09***	7.28**	0.68	
HEA	MS	2.27	1.09	273.04	21.92	4.67
	F	0.49	0.23	58.43**	4.69***	
PH	MS	0.94	254.39	240.64	17.70	4.10
	F	0.23	62.05***	58.69***	4.32**	
YLD	MS	6.38	2148.13	157.35	10.88	8.99
	F	0.71	238.88***	17.50***	1.21	
MASS	MS	146.69	2031.90	116.33	112.09	107.17
	F	1.37	18.96***	1.09	1.05	
HI	MS	36.67	5133.75	840.71	57.47	27.39
	F	1.34	187.42***	30.69***	2.10	

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively

3) Number of kernels per spike

ANOVA revealed that replications and interaction between genotypes and heat treatments were not significant for kernels per spike. Genotypes and heat treatments, however, were highly significant, ((Table 19). Average number of kernels per spike ranged from 10.51 for Harry to 16.90 kernels per spike for Apex (see Table 20). Mean for heat treatments ranged from 6.40 kernels for heat treatment + 65% FC to 22.74 kernels for control (see Table 21).

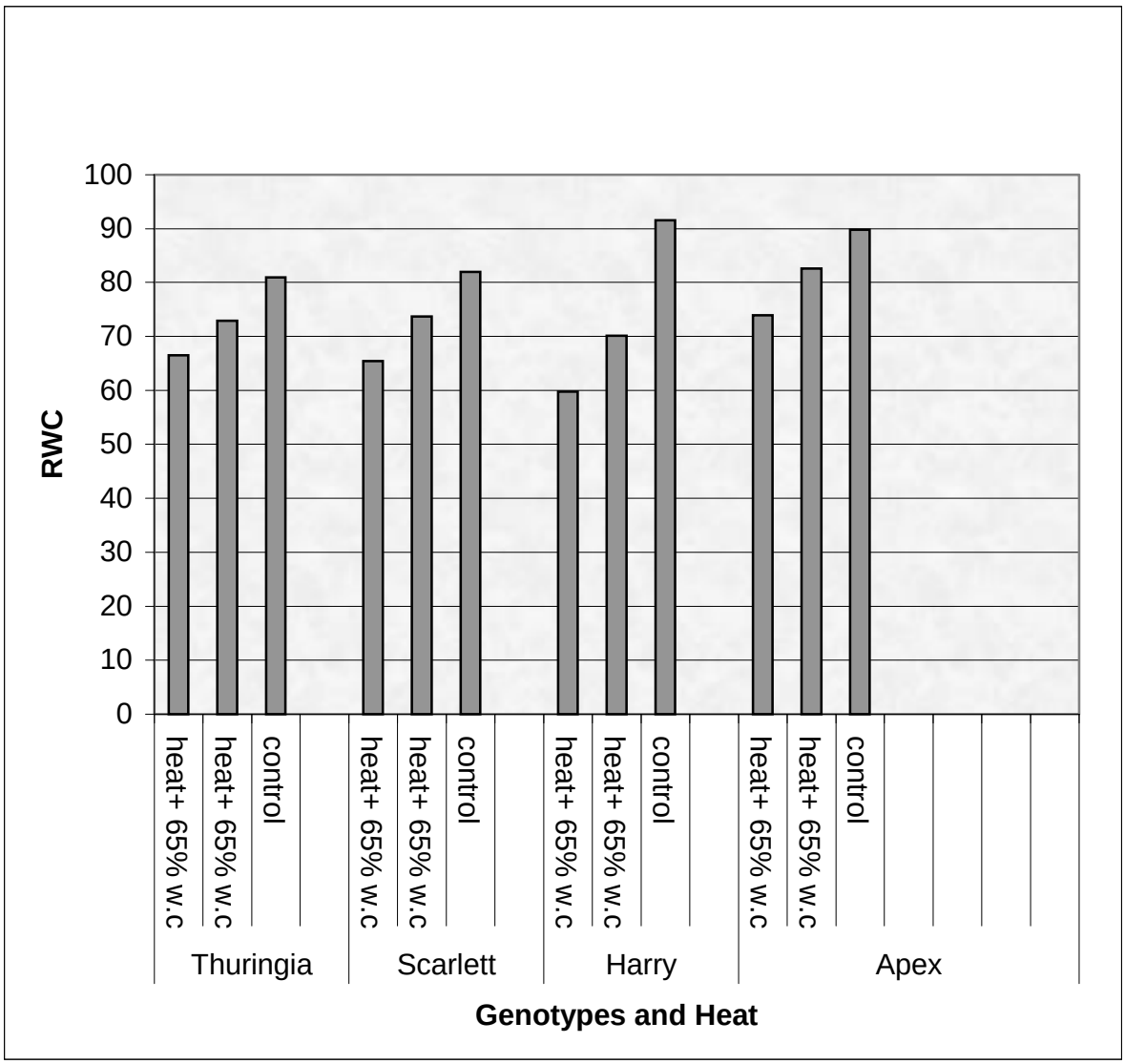


Figure 7: Relative leaf water content of Thuringia, Scarlett, Harry, and Apex genotypes for heat experiment.

Figure 9 shows that a difference was weak for all treatments and genotypes except for the heat treatment + 65% FC which resulted in low RWC for all genotypes.

4) Relative leaf water content

Variation was highly significant for the genotypes, heat treatments, and the interaction of genotypes and heat treatments, but was non-significant for the replicates (Table 19 and Figure 9). Result obtained for relative leaf water content revealed that average RWC ranged from 39.99 in for Apex to 63.45 for Thuringia (see Table 20). Mean for heat treatments ranged from 40.32 for heat treatment + 65% FC to 62.55 for control (see Table 21).

5) Osmotic adjustment

The variation among replications was non-significant, but highly significant among the genotypes, heat treatments, and the interaction of genotypes and heat treatments in (Table 19 and Figure 10). It was found that the average for osmotic adjustment ranged from 0.084 for Scarlett to 0.107 for Apex (see Table 20). Mean for heat treatments ranged from 0.071 for control to 0.112 for heat treatment + 65% FC (see Table 21).

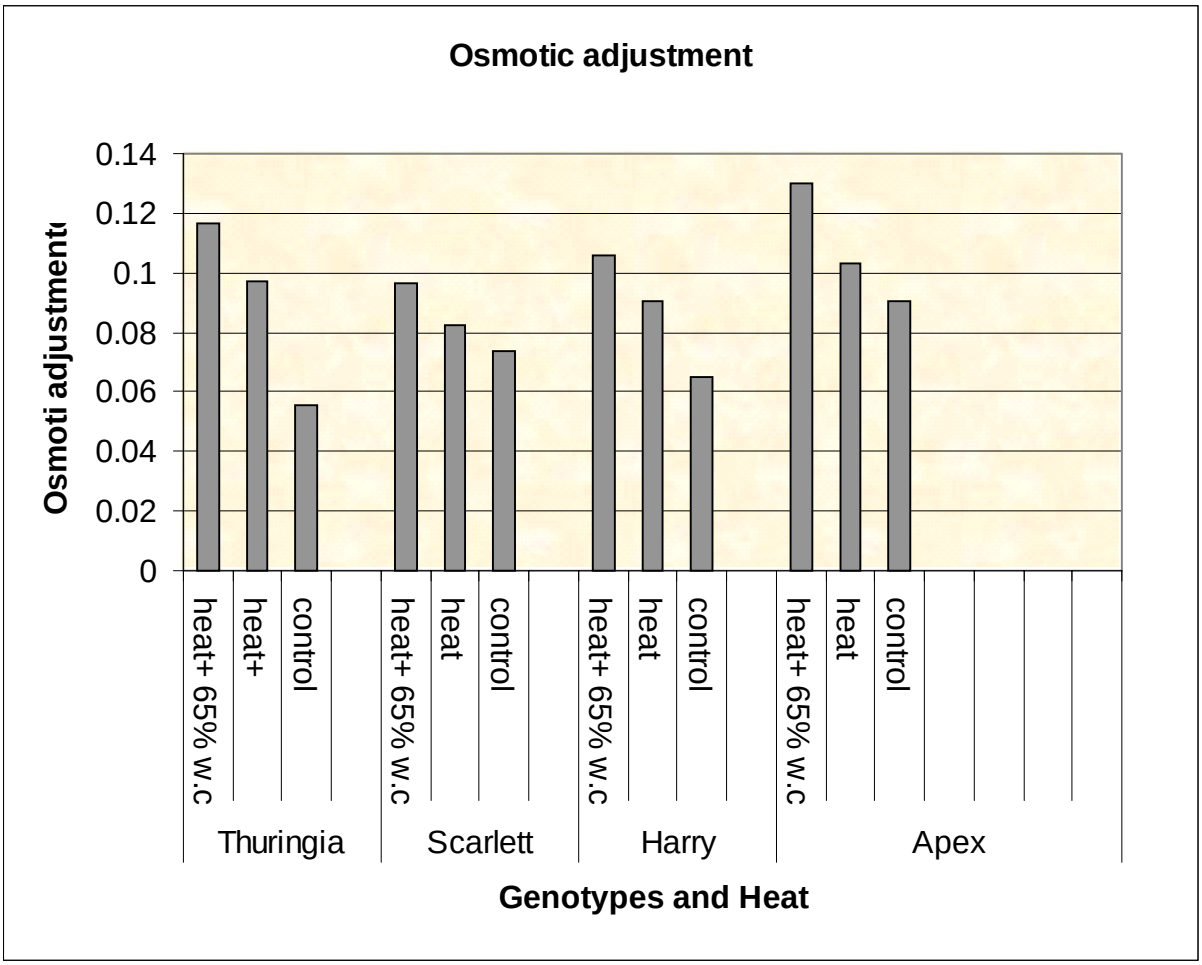


Figure 8: Osmotic adjustment of Thuringia, Scarlett, Harry, and Apex genotypes for heat experiment.

Figure 10 shows considerable differences for all treatments, but moderate differences among all genotypes.

6) Chlorophyll content

The variation among replications and the interaction between genotypes and heat treatments were non- significant, but among genotypes and heat treatments highly significant (Table 19). Table 20 shows that average the chlorophyll content for the genotypes ranged from 49.98 for Harry and Apex to 55.04 for Scarlett (see Table 20). Means for heat treatments were ranged from 46.82 for chlorophyll content for heat treatment + 65% FC to 56.81 of chlorophyll content for control treatment (see Table 21).

7) Days until heading

Variation among replicates and heat treatments was non-significant, but highly significant among genotypes and highly significant interaction of genotypes and heat treatments (Table 19 and Figure11). The average number of days to heading ranged from 56.00 days for Thuringia to latest 69.67 days for Harry (see Table 20). Means for heat treatments ranged from 60.78 days for heat treatment + 65% FC to 61.42 days for heat treatment (see Table 21).

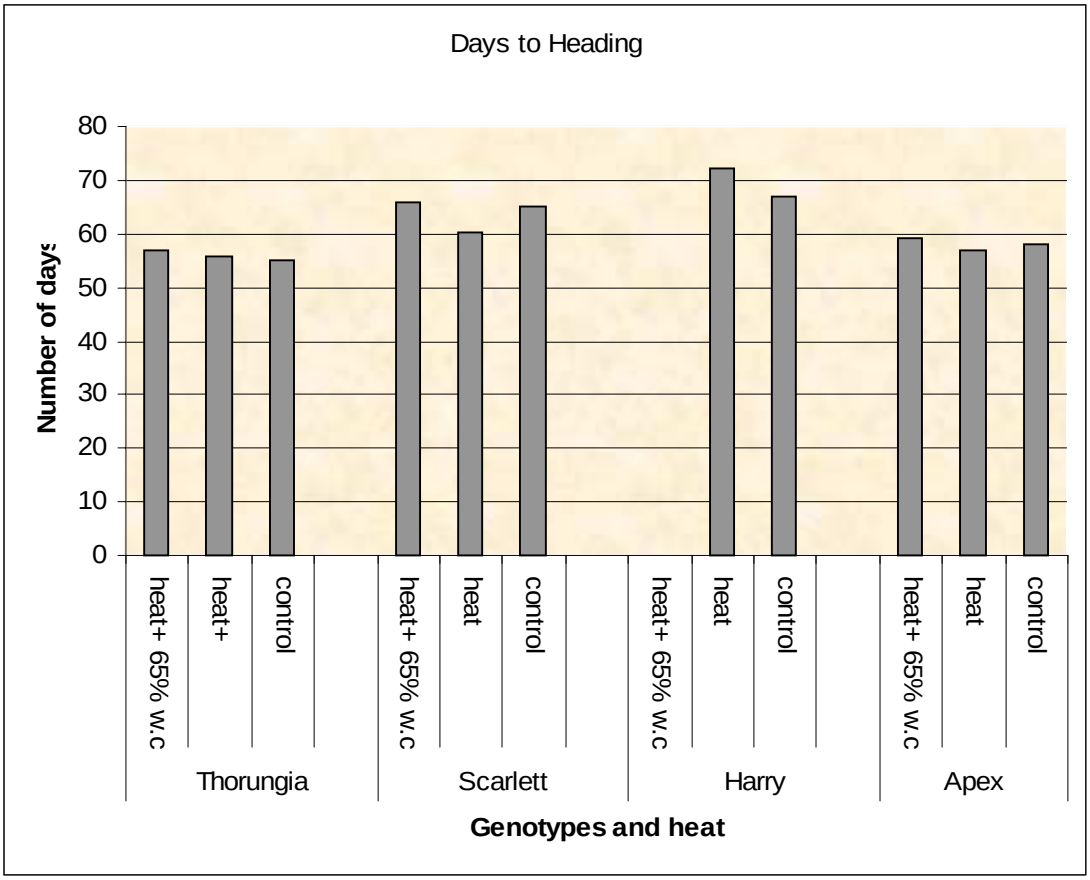


Figure 9: Days until heading of Thuringia, Scarlett, Harry, and Apex genotypes for the heat experiment.

Figure11 shows that differences were small treatments except for the heat treatment + 65% FC in Harry were susceptible the plant not arrived to heading. The variation among all genotypes was moderate for days to heading.

8) Plant height

Variation among the replicates was non-significant, but genotypes, heat treatments and the interaction of genotypes and heat treatments were highly significant (Table 19 and Figure12). As above, the mean of plant height for the genotypes ranged from 48.74 cm for Apex as shortest genotype to 59.87 cm for Scarlett as tallest genotype (see Table 20). Mean height for heat treatments ranged from 48.03 cm for heat treatment + 65% FC to 56.81 cm for control (see Table 21).

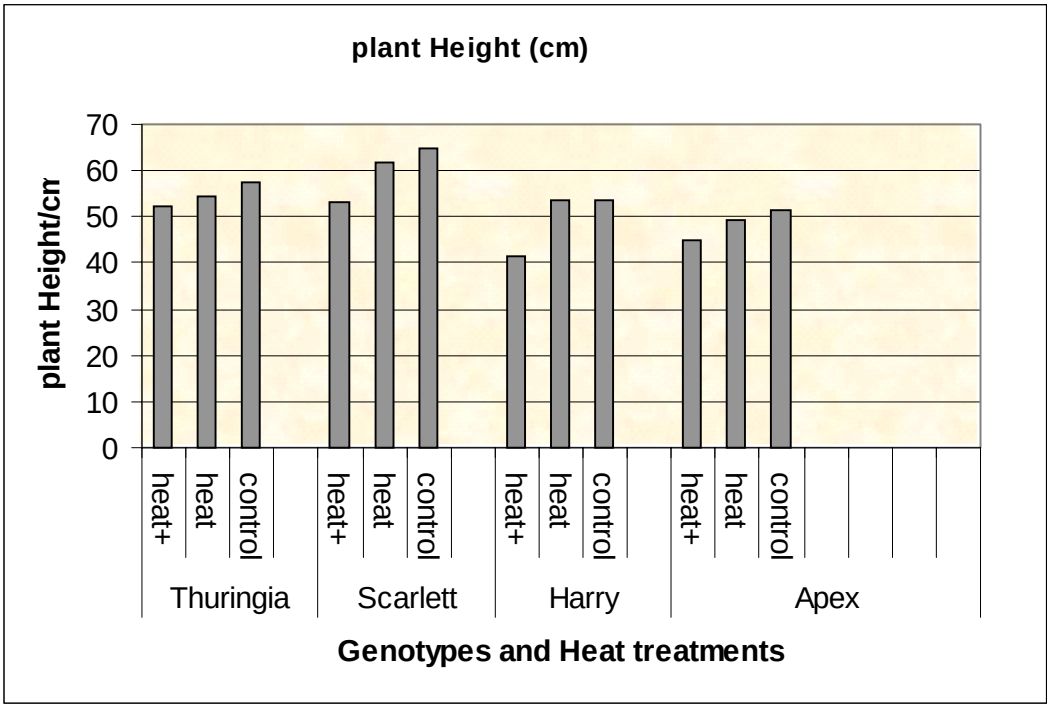


Figure 10: Plant height of Thuringia, Scarlett, Harry, and Apex genotypes for the heat experiment.

Figure12 shows the differences were small for all treatments and genotypes except for the heat + 65% FC treatment in Harry and Apex which resulted in a considerable reduction of plant height. The height differences of genotypes were moderate for plant height.

9) Yield

Variation among the replicates and the interaction of genotypes and heat treatments were non-significant, but between genotypes and heat treatments were highly significant (Table 19).

The lower yield (9.71) was revealed for Harry, whereas the highest yield (19.33 g) was found by Apex (see Table 20). Mean for heat treatments ranged from 3.68 g for heat treatment + 65% FC to 30.08 g for control (see Table 21).

10) Biomass

Analysis of variance among replications, genotypes and the interaction among genotypes and heat treatments were non-significant, but were highly significant among heat treatments (Table 19). The average for biomass of the genotypes ranged from 39.94 g for Thuringia to 48.43 g for Apex (see Table 20). The mean for heat treatments ranged from 30.42 g for heat treatment + 65% FC to 56.44 g for control (see Table 21).

11) Harvest index

The analysis of variance among replications and the interaction between genotypes and heat treatments were non-significant, but were highly significant among heat treatments and genotypes (Table 19). The average of harvest index for the genotypes ranged from 17.61 % for Harry lowest genotype to 38.51% for Apex highest genotype (see Table 20). Mean for heat treatments ranged from 12.11% for heat treatment + 65% FC to 53.38% for control (see Table 21).

Table 20: Mean value of traits for Thuringia, Scarlett, Harry, and Apex genotypes with Ryan-Gabriel-Welsch Multiple Range Test for heat experiment.

Trait	Thuringia	Scarlett	Harry	Apex
Tillers per plant	26.22^{A}	26.11^{A}	11.89^{B}	26.78^{A}
No. spikes per plant	2.83^{A}	2.56^{A}	2.01^{A}	3.09^{A}
No. kernels per spike	13.75^{AB}	16.10^{A}	10.51^{B}	16.90^{A}
Relative leaf water content	63.45^{A}	54.58^{B}	48.21^{C}	39.99^{D}
Osmotic adjustment	0.089^{B}	0.084^{B}	0.087^{B}	0.1077^{A}
Chlorophyll content	50.30^{B}	55.04^{A}	49.98^{B}	49.98^{B}
Days until heading	56.00^{C}	63.79^{B}	69.67^{A}	58.11^{C}
Plant height	54.73^{B}	59.87^{A}	49.52^{C}	48.74^{C}
Grain yield	15.91^{A}	17.53^{A}	9.71^{B}	19.33^{A}
Biomass	39.94^{A}	43.10^{A}	42.21^{A}	48.43^{A}
Harvest index	35.26^{A}	36.48^{A}	17.61^{B}	38.51^{A}

Mean values with different superscript letters are significantly different at $P \leq 0.05$.

Table 21: Mean value of traits of heat treatments with Ryan-Gabriel-Welsch Multiple Range Test for heat experiment.

Trait	Heat+65% FC	Heat in greenhouse	Control out greenhouse
Tillers per plant	12.50^{C}	22.67^{B}	33.08^{A}
No. Spikes per plant	0.89^{B}	1.98^{B}	4.23^{A}
No. Kernel per spike	6.40^{C}	13.87^{B}	22.74^{A}
Relative leaf water content	40.32^{C}	51.80^{B}	62.55^{A}
Osmotic adjustment	0.112^{A}	0.093^{B}	0.071^{C}
Chlorophyll content	46.82^{C}	51.08^{B}	56.81^{A}
Days to heading	60.78^{A}	61.42^{A}	61.25^{A}
Plant height	48.03^{C}	54.79^{B}	56.83^{A}
Yield	3.68^{C}	13.10^{B}	30.08^{A}
Biomass	30.42^{C}	43.41^{B}	56.44^{A}
Harvest index	12.11^{C}	30.40^{B}	53.38^{A}

Mean values with different superscript letters are significantly different at $P \leq 0.05$.

4.2.2 Heat results for population parents Scarlett and ISR42-8.

Relative leaf water content: the analysis of variance among heat treatments and parents were highly significant for relative leaf water content, it was non-significant between the years, interaction between heat treatments and years, the interaction between heat treatments and parents, The interaction between years and parents, as well as, interaction among heat treatments, years and parents (Table 22). **Number of spikes per plant:** variation among heat treatments, parents, years, interaction between heat treatments and years as well as, the interaction between heat treatments and parents were significant. It was non-significant for the interaction between years and parents as well as, interaction between heat treatments, years and parents (Table 22). **Number of kernels per spike:** it was highly significant among heat treatments, parents, years, the interaction between heat treatments and parents, the interaction between years and parents as well as, interaction between heat treatments, years and parents. It was non-significant for interaction between heat treatments and years (Table 22). **Osmotic adjustment:** variation among heat treatments, parents, years and the interaction between heat treatments and parents were significant, it was non-significant for interaction between heat treatments and years, the interaction between years and parents and interaction among heat treatments, years and parents were highly significant (Table 22). **Days until**

heading: the result was highly significant for heat treatments, parents, years, interaction between heat treatments and years, the interaction between years and parents as well as, interaction among heat treatments, years and parents. It was significant for the interaction between heat treatments and parents (Table 22). **Number of leaves per main tiller:** the variation was significant for number of leavers per tiller between years and parents. It was non-significant for heat treatments, the interaction between heat treatments and parents, interaction between heat treatments and years, the interaction between years and parents as well as, interaction between heat treatments, years and parents (Table 22). **Yield:** the result indicated highly significant among heat treatments, parents, years, interaction between heat treatments and years as well as, interaction between heat treatments and parents. It was non-significant for the interaction between years and parents as well as, interaction between heat treatments, years and parents (Table 22). **Biomass:** the result revealed highly significant for heat treatments, parents, years, interaction between heat treatments and parents, the interaction between years and parents as well as, interaction between heat treatments, years and parents. It was non-significant for the interaction between heat treatments and years (Table 22). **Harvest index:** the variation of heat treatments, interaction heat treatments and parents, the interaction between heat treatments and years, as well as, interaction between heat treatments, years and parents were non-significantly. It was significant for parents, years, the interaction between years and parents (Table 22). **Flag leaf area**: variation among heat treatments, years, interaction heat treatments and parents, the interaction between heat treatments and years were highly significant. It was non- significant for parents, the interaction between years and parents, as well as, interaction among heat treatments, years and parents (Table 22). **First leaf area**: it was non-significantly among heat treatments, parents, years, interaction between heat treatments and years, the interaction between heat treatments and parents, the interaction between years and parents as well as, interaction among heat treatments, years and parents (Table 22). **Second leaf area**: the variation between heat treatments, parents and interaction between heat treatments and years were significant. Whereas, was non-significantly for years, the interaction between heat treatments and parents, the interaction between years and parents as well as, interaction for heat treatments, years and parents (Table 22).

Table 22: Analysis of variance of traits for population parents (Scarlett and ISR42-8) for heat tolerance.

Trait		Heat (H)	Year (Y)	Parents (P)	H*Y	H* P	Y* P	H*Y* P	Error
	DF:	1	1	1	1	1	1	1	80
RWC	MS	5179.518	45.505	3135.613	5.235	335.833	580.031	44.888	95.491
	F	54.24***	0.48	22.36***	0.05	3.52	0.06	0.47	
SPK	MS	122.778	37.430	77.778	11.505	3.940	1.278	1.394	0.575
	F	312.27***	6.5*	135.10***	19.99***	6.84*	2.22	2.42	
KER	MS	597.354	3330.727	4293.242	0.565	131.670	387.067	1776.13	7.201
	F	82.94***	462.48***	596.12***	0.08	18.28***	53.74***	246.6***	
OA	MS	0.0156	0.0019	0.021	0.00003	0.0018	0.00007	0.00011	0.00007
	F	217.83***	26.88***	293.2***	0.46	25.36***	1.04	1.58	
HEA	MS	1037.557	6651.840	2715.578	4677.57	10.669	193.76	60.669	1.886
	F	5558.35***	3563.49***	1454.77***	2505.8***	5.72*	103.80***	32.50***	
LEA	MS	0.363	9.091	5.818	0.363	0.010	1.454	0.010	0.531
	F	0.69	17.13***	10.97*	0.69	0.02	2.74	0.02	
YLD	MS	189.005	34.379	212.446	7.320	105.989	1.855	0.203	0.773
	F	244.50***	44.47***	274.82***	9.47*	137.11***	2.4	0.26	
MASS	MS	1788.293	25.361	353.496	3.099	161.555	103.351	64.561	2.123
	F	842.29***	11.95**	166.5***	1.46	76.09***	48.68***	30.41***	
HI	MS	0.369	1004.386	6615.186	102.189	184.385	749.048	107.242	103.5
	F	0.00	9.70*	63.91***	0.99	1.78	7.24*	1.04	
FLA	MS	168.898	54.637	1.4540	122.471	17.979	0.006	2.184	4.037
	F	41.84***	13.53**	0.36	30.33***	4.45*	0.00	.054	
ARE1	MS	197.568	56.872	64.353	0.364	247.821	58.645	224.812	235.9
	F	0.84	0.24	0.24	0.00	1.05	0.25	0.95	
ARE2	MS	738.519	2201740	65.288	220.897	20.800	28.004	7.634	11.89
	F	62.11***	0.02	5.49*	18.58***	1.75	2.36	0.64	

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

Table 23: T Tests (LSD) for parents (Scarlett and ISR42-8) for heat experiment

Traits	Scarlett	ISR42-8
Relative leaf water content	86.38^{A}	71.51^{B}
No. Spikes per plant	4.56^{A}	3.68^{B}
No. Kernels per spike	21.11^{A}	6.46^{B}
Osmotic adjustment	0.046^{B}	0.1064^{A}
Heading date	68.41^{A}	56.45^{B}
Number of leaves per main tiller	5.29^{A}	4.84^{B}
Flag leaf area	5.92^{A}	6.27^{A}
First leaf area	15.73^{A}	12.17^{A}
Second leaf area	16.87^{B}	13.71^{A}
Yield	4.54^{A}	0.75^{B}
Biomass	11.21^{A}	7.8^{B}
Harvest index	40.06^{A}	12.77^{B}

Mean values with different superscript letters are significantly different at $P \leq 0.05$.

Table 23 shows LSD for parents Scarlett and ISR42-8 of heat experiment, different significant between Scarlett and ISR42-8 for all traits except flag leaf area and first leaf area.

Table 24: T Tests (LSD) between mean values of heat treatments for 12 quantitative traits

Trait	Control (Outside green house)	Heat treatment (Inside green house)
Relative leaf water content	83.518^{A}	63.224^{B}
No. spikes per plant	6.3295^{A}	2.6705^{B}
No. kernels per spike	17.233^{A}	10.346^{B}
Osmotic adjustment	0.05100^{B}	0.086614^{A}
Days until heading	82.523^{A}	42.341^{B}
Number of leaves per main tiller	4.9318^{A}	5.2045^{A}
Flag leaf area	8.8689^{A}	3.3307^{B}
First leaf area	15.845^{A}	12.066^{A}
Second leaf area	20.3570^{A}	10.2320^{B}
Yield	4.3098^{A}	0.9859^{B}
Biomass	15.5018^{A}	3.5043^{B}
Harvest index	25.608^{A}	27.218^{A}

Mean values with different superscript letters are significantly different at $P \leq 0.05$.

Table 24 shows LSD for control and heat stress of the heat experiment, different significances between control and heat stress for all traits except number of leaves per main tiller, first leaf area and harvest index.

4.2.3 Heat result for BC_2DH lines (Scarlett*ISR42-8 population)

Table 25: Analysis of variance in traits for BC_2DH for heat tolerance.

Trait		Heat (H)	Year (Y)	lines (DH)	H*Y	H*DH	Y*DH	H*Y*DH	Error
	DF:	1	1	318	1	313	309	304	80
RWC	MS	1641.89	384.915	193.742	265.439	219.382	183.155	159.981	108.054
	F	15.19**	3.56	1.79*	2.46	2.03***	1.70*	1.48*	
SPK	MS	2960.480	260.473	1.593	160.511	1.756	1.848	1.273	1.057
	F	2800.36***	246.39***	1.51*	151.83***	1.66*	1.75*	1.20	
KER	MS	12512.218	16686.667	70.261	5616.155	35.750	48.601	52.821	7.411
	F	1688.17***	2251.40***	9.48***	757.74***	4.82***	6.56***	7.13***	
OA	MS	0.002	0.095	0.001	0.207	0.001	0.001	0.001	0.0001
	F	12.65**	648.40***	4.87***	1415.55***	5.37***	3.43***	4.49***	
HEA	MS	204272.807	28032.342	166.88	12456.481	56.091	95.087	85.08	1.886
	F	109432.0***	15017.3***	89.40***	6673.12***	30.05***	50.94***	45.87***	
LEA	MS	8.120	415.287	0.702	5.451	0.524	0.711	0.531	0.531
	F	15,31**	782,74***	1,32	10,27*	0,99	1,34	1,00	
YLD	MS	6.118.397	188.589	5.399	65.219	4.746	5.270	5.751	0.773
	F	7914.82***	243.96***	6.98***	84.37***	6.14***	6.82***	7.44***	
MASS	MS	47691.921	5299.560	14.400	3678.265	12.082	11.623	10.188	2.123
	F	22462.9***	2496.09***	6.78***	1732.46***	5.69***	5.47***	4.80***	
HI	MS	7831.613	563.863	224.009	13675.641	225.992	185.398	230.872	103.509
	F	760.62***	5.45*	2.16***	132.12***	2.18***	1.79*	2.23***	
FLA	MS	1583.887	515.816	16.322	209.942	15.071	13.616	14.680	4.037
	F	392.33***	127.77***	4.04***	52.00***	3.73***	3.37***	3.64***	
ARE1	MS	9175.435	118.781	32.962	1255.061	20.597	23.683	23.632	235.927
	F	38.89***	0.50	0.14	5.32*	0.09	0.10	0.10	
ARE2	MS	9368.265	612.452	46.095	6085.611	30.637	29.480	34.944	11.891
	F	787.84***	51.51***	3.88***	511.78***	2.58***	2.48***	2.94***	

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

Relative leaf water content: the variation among heat treatments, BC_2DH lines, years, the interaction between heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines and the interaction among heat treatments, years and BC_2DH lines were significant, whereas it was non-significant for years and the interaction between heat treatments and years (Table 25). **Number of spikes per plant:** variation among heat treatments, BC_2DH lines, years, the interaction between heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines were significant. While it was non-significant for interaction among heat treatments, years and BC_2DH lines (Table 25). **Number of kernels per spike:** the result showed highly significant among heat treatments, BC_2DH lines, years, the interaction between heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines, as well as, the interaction among heat treatments, years and BC_2DH lines (Table 25). **Osmotic adjustment:** the variation was highly significant for heat treatments, BC_2DH lines, years, the interaction between heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines and the interaction among heat treatments, years and BC_2DH lines (Table 25). **Days until heading:** the analysis of variance for heat treatments, BC_2DH lines, years, the interaction between heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines and the interaction among heat treatments, years and BC_2DH lines was highly significant (Table 25). **Number of leaves per main tiller:** variation was significant for number of leaves per main tiller of heat treatments and years interaction heat treatments and years, whereas was non-significantly for BC_2DH lines, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among heat treatments, years and BC_2DH lines (Table 25). **Yield:** result indicated highly significant for yield among heat treatments, years, BC_2DH lines, the interaction among heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among heat treatments, years and BC_2DH lines (Table 25). **Biomass**: variation was highly significant for biomass among heat treatments, years, BC_2DH lines, the interaction between heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among heat treatments, years and BC_2DH lines (Table 25). **Harvest index:** the result found high significant difference for harvest index among heat treatments, BC_2DH lines, the interaction for heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as interaction among heat treatments, years and BC_2DH lines. However, the difference was significant among years (Table 25). **Flag leaf area:**

the result revealed highly significant for flag leaf area among heat treatments, years, BC_2DH lines, the interaction among heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines as well as, interaction among heat treatments, years and BC_2DH lines (Table 25). **First leaf area:** variation was significant for first leaf area among heat treatments and the interaction among heat treatments and years. The analysis of variance was non-significant among years, BC_2DH lines, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines, as well as, interaction between heat treatments, years and BC_2DH lines (Table 25). **Second leaf area:** result obtained highly significant for second leaf area among heat treatments, years, BC_2DH lines, the interaction among heat treatments and years, the interaction between heat treatments and BC_2DH lines, the interaction between years and BC_2DH lines, as well as, interaction among heat treatments, years and BC_2DH lines (Table 25).

Correlation for 12 traits for heat treatments and BC_2DH lines

Results of correlation studies of 12 traits are shown in Table 26. Three levels of correlation were established $\leq$0.2 was weak, from >0.2 to $\leq$0.5 was moderate, and more than >0.5 was strong. **Relative leaf water content** showed a weak correlation with number of spikes per plant ($P\leq0.01$), osmotic adjustment ($P\leq0.05$), biomass ($P\leq0.05$) and harvest index ($P\leq0.05$). Positive and strong correlations were obtained for **number of spikes per plant** with days until heading ($P\leq0.001$), yield ($P\leq0.001$), and biomass ($P\leq0.001$), while it moderate correlations were revealed for spike with flag leaf area ($P\leq0.001$), first leaf area ($P\leq0.05$) second leaf area ($P\leq0.01$), and harvest index ($P\leq0.05$), whereas it was weak correlated with relative leaf water content ($P\leq0.01$), number of kernels per spike ($P\leq0.001$), osmotic adjustment ($P\leq0.05$), and number of leaves per main tiller ($P\leq0.001$). **Number of kernels per spike** was revealed positive and moderate correlation with days until heading ($P\leq0.001$), number of leaves per main tiller ($P\leq0.001$), yield ($P\leq0.001$), biomass ($P\leq0.001$), and harvest index ($P\leq0.001$), while it showed a weak correlation with relative leaf water content ($P\leq0.01$), number of spikes per plant ($P\leq0.001$), flag leaf area ($P\leq0.05$), and second leaf area ($P\leq0.001$). **Osmotic adjustment** had a medium association with heading date ($P\leq0.001$), and number of leaves per main tiller ($P\leq0.001$), whilst a weak correlated with relative leaf water content ($P\leq0.05$), number of spikes per plant ($P\leq0.05$), second leaf area ($P<0.001$). Correlations were positive and strong for **days until heading** with number of spikes per plant ($P\leq0.001$), yield ($P\leq0.001$) and biomass ($P\leq0.001$), while showed a medium correlation with number of kernels per spike ($P\leq0.001$), osmotic adjustment ($P\leq0.001$), first leaf area ($P\leq0.001$), second leaf area ($P\leq0.001$), and harvest index ($P\leq0.001$). There was a weak correlation with number of leaves per

main tiller (P≤0.05), and flag leaf area (P≤0.001). **Number of leaves per main tiller** revealed a positive and moderate correlation with number of kernels per spike (P≤0.001), and osmotic adjustment (P≤0.001), whereas the correlation was weak for number of spikes per plant (P≤0.001), days until heading (P≤0.05) and flag leaf area (P≤0.05), second leaf area (P≤0.05), yield (P≤0.001) and biomass (P≤0.001). **Flag leaf area** positive and strongly correlated with first leaf area (P≤0.001), and second leaf area (P≤0.001), moderate correlated with number of spikes per plant (P≤0.001), and biomass (P≤0.001), a weak correlated with number of kernels per spike (P≤0.05), heading date (P≤0.001), number of leaves per main tiller (P≤0.05), and yield (P≤0.001). However, this **first leaf area** was positive and strongly correlated with flag leaf area (P≤0.001), and second leaf area (P≤0.001), while showed a moderate correlation with number of spikes per plant (P≤0.001), heading date (P≤0.001), yield (P≤0.001) and biomass (P≤0.001). The correlation was weak for number of kernels per spike (P≤0.001) and harvest index (P≤0.001). Positive and strong correlations were exhibited by **second leaf area** with flag leaf area (P≤0.001), and first leaf area (P≤0.001), whereas the correlated was medium for number of spikes per plant (P≤0.01), days until heading (P≤0.001), yield (P≤0.001) and biomass (P≤0.001), a weak correlated with osmotic adjustment (P≤0.001), number of leaves per main tiller (P≤0.05), and harvest index (P≤0.001). **Yield** was positive and strongly correlated with number of spikes per plant (P≤0.001), heading date (P≤0.001), biomass (P≤0.001) and harvest index (P≤0.001), however the correlation was moderate for number of kernels per spike (P≤0.001), first leaf area (P≤0.001), and second leaf area (P≤0.001), whilst a weak correlated with number of leaves per main tiller (P≤0.001) and flag leaf area (P≤0.001). However, **biomass** was positive and strongly correlated with number of spikes per plant (P≤0.001), days until heading (P≤0.001) and yield (P≤0.001), whilst was positive and moderate correlations with number of kernels per spike (P≤0.001), number of leaves per main tiller (P≤0.001), flag leaf area (P≤0.001), first leaf area (P≤0.05), second leaf area (P≤0.001) and harvest index (P≤0.001), whilst the correlation was a wear with relative leaf water content (P≤0.05). **Harvest index** was weak correlated with relative leaf water content (P≤0.001), first leaf area (P≤0.001) and second leaf area (P≤0.001), while the correlation was moderate with number of spikes per plant (P≤0.05), number of kernels per spike (P≤0.001) heading date (P≤0.001) and biomass (P≤0.001), while the correlation was positive and strongly with yield (P≤0.001).

Table 26: Pearson's correlation coefficients (r) between 12 quantitative traits[1] for heat tolerance

	SPK	KER	OA	HEA	LEA	FLA	Are1	Are2	YLD	MAS	HI
RWC	-0.07**	0.01	-0.06*	0.04	-0.05	0.05	-0.03	-0.02	0.04	0.06*	-0.06*
SPK		0.12***	0.06*	0.63***	-0.16***	0.22***	0.31***	0.40***	0.71***	0.81***	0.38***
KER			0.00	0.48***	0.26****	-0.05*	0.13***	0.05	0.38***	0.25***	0.40***
OA				0.22***	0.22***	-0.03	0.04	0.13***	0.04	0.021	-0.09
HEA					0.14***	0.18***	0.35***	0.43***	0.61***	0.67***	0.32***
LEA						-0.07**	-0.01	-0.05*	-0.12***	-0.21***	-0.05
FLA							0.58***	0.51***	0.18***	-0.21***	-0.05
Are1								0.62***	0.29***	0.36***	0.14***
Are2									0.39***	0.49***	0.14***
YLD										0.77***	0.69***
MAS											0.27***

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

1) Abbreviation for traits Table 5.

Table 27 : Pearson's correlation coefficients (r) between 13 quantitative traits[1] under heat stress

	SPK	KER	OA	HEA	LEA	FLA	Are1	Are2	YLD	MAS	HI
RWC	-0.03	-0.02	-0.02	0.03	0.03	0.01	-0.00	0.01	-0.05	0.00	-0.08*
SPK		-0.19***	0.02	-0.15***	-0.08*	-0.12**	0.04	-0.09*	0.38***	0.13**	0.30***
KER			0.11**	0.33***	0.17***	0.00	0.04	0.116**	0.44***	0.15***	0.27***
OA				0.56***	0.46***	-0.03	0.06	0.19***	-0.28***	-0.04	-0.30***
HEA					0.50***	0.05	0.10**	0.24***	-0.23***	0.01	-0.32***
LEA						-0.01	0.08*	0.18***	-0.25***	-0.05	-0.25***
FLA							0.49***	0.58***	-0.04	0.05	-0.07
Are1								0.51***	-0.01	0.03	-0.06
Are2									-0.13***	0.01	-0.14***
YLD										0.37***	0.72***
MAS											-0.21***

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

1) Abbreviation for traits Table 5.

Correlation of 12 traits under heat stress in BC_2DH population

Results of correlation studies of 12 traits are shown in Table 27. There are three levels of correlation <0.2 was weak, from ≥0.2 to ≤0.5 was moderate, and more than ≥0.5 was strong. **Relative leaf water content** was resulted in negative and weak correlation with harvest index (P≤0.05). Moderate correlations was revealed for **number of spikes per plant** with yield (P≤0.001), and harvest index (P≤0.001), correlation was calculated with number of kernels per spike (P≤0.001), days until heading (P≤0.001), number of leaves per main tiller (P≤0.05**)** flag leaf area (P≤0.01), second leaf area (P≤0.05) and biomass (P≤0.01). **Number of kernels per spike** was revealed moderate correlations with yield (P≤0.001), days until heading (P≤0.001) and harvest index (P≤0.001), whereas it was wear correlations with number of spikes per plant (P≤0.001), osmotic adjustment (P≤0.01), number of leaves per main tiller (P≤0.001), second leaf area (P≤0.01), and biomass (P≤0.001). **Osmotic adjustment** was strongly and positive associated with days until heading (P≤0.001), while it was associated moderate number of leaves per main tiller (P≤0.001), yield (P≤0.001) and harvest index (P≤0.001), the other hand, it was associated positive and weak with number of kernels per spike (P≤0.001), second leaf area (P≤0.001). Correlations were strong for **days until heading** with osmotic adjustment (P≤0.001), and number of leaves per main tiller (P≤0.001), whereas it were moderate correlation for number of kernels per spike (P≤0.001), second leaf area (P≤0.001), yield (P≤0.001) and harvest index (P≤0.001), it was wearily correlated with number of spikes per plant (P≤0.001) and first leaf area (P≤0.01). **Number of leaves per main tiller** was strong by correlated with hading days (P≤0.001), whereas correlation was moderate with osmotic adjustment (P≤0.001), yield (P≤0.001) and harvest index (P≤0.001), while it weak by correlated with number of spikes per plant (P≤0.05), number kernels per plant (P≤0.001), (P≤0.001), first leaf area (P≤0.05, and second leaf area (P≤0.001). **Flag leaf area** was positive and strongly correlated with second leaf area (P≤0.001), whilst positively and moderate correlated with first leaf area (P≤0.001), while a weak number of spikes per plant (P≤0.01). However, **first leaf area** was positive and strong correlated with second leaf area (P≤0.001), while it was positive and moderate correlated with flag leaf area (P≤0.001), however positive and weak correlation with heading date (P≤0.01), and number of leaves per main tiller (P≤0.05). Positive and strong correlations were expressed by **second leaf area** with flag leaf area (P≤0.001), first leaf area (P≤0.001), while the correlation was moderate with days until heading (P≤0.001), while it was weakly correlated with number of spikes per plant (P≤0.05), number of kernels per spike (P≤0.01), osmotic adjustment (P≤0.001), number of leaves per main tiller (P≤0.001), yield (P≤0.001) and harvest index (P≤0.001). **Yield** was positive and strongly correlated with harvest index (P≤0.001), whereas it showed a moderate correlation with number of spikes per plant (P≤0.001), number of kernels per spike (P≤0.001), osmotic adjustment (P≤0.001), days until heading (P≤0.001), number of leaves per plant (P≤0.001), and biomass (P≤0.001), while the correlation was wear with second leaf area (P≤0.001). However, **biomass** showed positive and moderate correlations with yield (P≤0.001), and harvest index (P≤0.001), while the correlation with

number of spikes (P≤0.001), number of kernels per plant (P≤0.001) was wear. **Harvest index** was negatively and strongly correlated with yield (P≤0.001), while the correlation with number of number of spikes per plant (P≤0.001), kernels per spike (P≤0.001), osmotic adjustment (P≤0.001), days until heading (P≤0.001), number of leaves per main tiller (P≤0.001), and biomass (P≤0.001) was medium. The correlation with relative leaf water content (P≤0.05), second leaf area (P≤0.001) was weak.

4.2.4 QTL detection for heat experiment

Ninety-seven polymorphic markers detected 34 putative QTLs were detected from 45 regions. For 4 regions, marker main effect and at 41 regions the M*H interaction were significant at $P \leq 0.01$ (Figure. 13 and Table 28). 16 (47.0%) favorable QTL effects were detected (see Table 30). At these loci, the homozygous *Hsp* genotype was associated with an improvement of the trait compared to the homozygous *Hvd* genotype (Figure 13. and Table 28). The putative QTLs were unevenly distributed over the chromosomes (Figure 13). 8 and 9 QTLs were located on chromosomes 4H and 2H, respectively. Most of the favorable QTLs were located on chromosomes 3H and 4H (3, and 5 respectively). No favorable QTLs were detected on chromosome 1H. At the marker GMS003 $_{[2H]}$ has putative QTLs effects for three traits (MAS, YLD and HI). HV13GEIII $_{[3H]}$ were found a putative QTLs effects for four traits (OSM, FLA, MAS and YLD). HVM62 $_{[3H]}$ marker was detected for putative QTLs on four traits (OSM, FLA, MAS and YLD). HW01N04T3 $_{[2H]}$ showed putative QTLs effects on three traits (OSM, FLA and YLD). HY02P09T3 $_{[1H]}$ obtained putative QTLs effects on three traits (ARE1, FLA and OSM). The detected putative QTLs are represented for each trait is shown in Table 28.

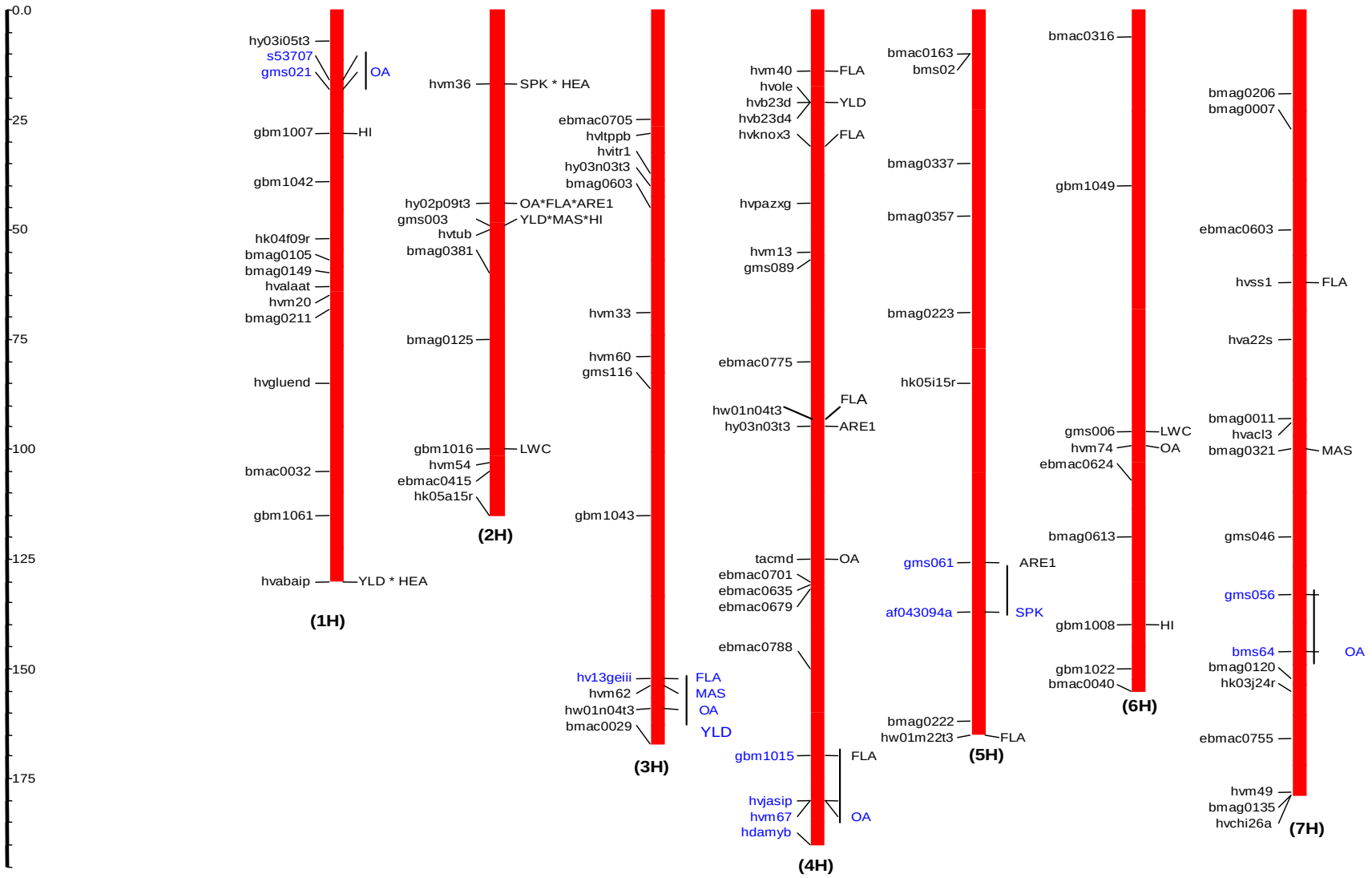


Figure 13: Linkage map of spring barley (Scarlett*ISR42-8) for heat tolerance

Figure 13: Linkage map of spring barley for heat tolerance (Scarlett*ISR42-8)

Linkage map containing 34 putative QTLs for heat experiment. The short arms of the chromosomes represented at the top. The linkage map for spring barley contains 34putative QTLs with16 favorable *Hsp* alleles detected in the BC_2DH population Scarlett x ISR42-8. Putative QTLs which revealed either, a significant ($P \leq 0.01$) marker main effect or M*H interaction are written to the right of the SSR locus. Adjacent markers effects (distance≤20cM) are considered as one putative QTL. A vertical line represents markers showing a significant QTL, which show an effect within a vicinity of 20 cM. The abbreviations of the quantitative traits follow Table 5.

Table 28: **List of 34 putative QTLs detected from the BC_2DH cross Scarlett x ISR42-8[A] for heat experiment.**

[A]Trait	Marker	[B]Ch	[C]Position (cM)	[D]Effect	[E]Sig.	[F]RP Wild allele effect	[G]RP Wild allele effect for control	[H]RP Wild allele effect for drought stress	[I]QTLs
MASS	GMS003	2H	48	M*H	***	-9.89	-10.62	-6.57	1
	HV13GEIII	3H	152	M*H	**	8.25	10.36	-0.98	2
	HVM62	3H	154	M*H	**	7.85	10.01	-1.57	2
	Bmag0321	7H	100	M	***	-5.55	-5.38	-6.27	3
HI	GBM1007	1HS	25	M*H	**	-1.88	13.17	-30.14	1
	HVM36	2H	17	M*H	**	9.04	-1.74	30.84	2
	GMS003	2H	48	M*H	**	3.16	-4.63	19.02	3
	GBM1008	6HL	140	M*H	***	1.67	14.57	-21.4	4
HEA	HVABAIP	1HL	130	M*H	**	2.67	4.62	-0.19	1
ARE1	HY02P09T3	2H	44	M*H	**	-13.62	-3.2	-28.7	1
	HY03N03T3	4H	95	M*H	**	17.66	23.81	8.59	2
	GMS061	5H	126	M*H	**	-6.27	-19.11	13.16	3
FLA	HY02P09T3	2H	44	M*H	***	-8.54	8.77	-31.41	1
	HV13GEIII	3H	152	M*H	***	15.42	-1.11	39.72	2
	HVM62	3H	154	M*H	**	19.6	-0.05	35.97	2
	HVM40	4H	14	M*H	***	-0.46	14.17	-19.89	3
	HVKNOX3	4H	35	M*H	**	5.16	14.96	-7.81	4
	HW01N04T3	4H	95	M*H	**	16.93	3.52	36.67	5
	GBM1015	4HL	152	M*H	***	-1.96	12.29	-20.13	6
	HW01M22T3	5H	165	M*H	**	5.16	25.37	-22.21	7
	HVSS1	7H	62	M*H	**	3.76	-27.32	47.86	8

[A]Trait	Marker	[B]Ch	[C]Position (cM)	[D]Effect	[E]Sig.	[F]RP Wild allele effect	[G]RP Wild allele effect for control	[H]RP Wild allele effect for drought stress	QTLs
OA	GMS021	1H	16	M*H	**	-6.44	-18.67	5.75	1
	S53707	1H	19	M*H	**	-6.22	-19.36	6.7	1
	HY02P09T3	2H	44	M	***	-11.61	-11.79	-11.44	2
	HV13GEIII	3H	152	M*H	***	-0.55	15.43	-15.32	3
	HVM62	3H	154	M*H	***	-1.68	13	-15.2	3
	HW01N04T3	3H	159	M*H	***	-3.64	9.09	-15.38	3
	TACMD	4H	135	M*H	***	-0.84	-10.75	9.21	4
	GBM1015	4HL	160	M*H	***	1	-8.11	10.26	5
	HVJASIP	4H	180	M*H	***	3.4	-3.89	10.79	5
	HVM67	4H	180	M*H	**	3.1	-4.6	10.91	5
	HVM74	6H	102	M*H	**	4.1	13.11	-4.35	6
	GMS056	7H	133	M	***	6.93	4.24	9.56	7
	BMS64	7H	146	M	***	8.71	2.96	14.38	7
SPK	HVM36	2H	17	M*H	**	-3.46	-7.5	11.8	1
	GMS061	5H	126	M*H	***	18.94	27.01	-9.96	2
	AF043094A	5H	137	M*H	**	11.51	14.51	0.69	2
RWC	GBM1016	2HL	100	M*H	**	-1.09	6.51	-8.4	1
	GMS006	6H	96	M*H	**	-3.53	0.05	-6.95	2
YLD	HVABAIP	1HL	130	M*H	**	-12.93	-14.05	-3.22	1
	GMS003	2H	48	M*H	**	-12.9	-15.05	6.3	2
	HV13GEIII	3H	152	M*H	***	19.32	21.74	-0.39	3
	HVM62	3H	154	M*H	***	20.77	23.15	1.56	3
	HW01N04T3	3H	159	M*H	***	20.75	22.77	4.32	3
	HVB23D	4H	21	M*H	**	19.11	21.37	0.24	4

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

[A]The quantitative traits are defined in Table 5.

[B]Chromosomal assignment of SSRs

[C]Chromosomal position of SSRs deduced from Ramsay *et al*. (2000), Pillen *et al*. (2003).

[D]Effect a QTL was assumed within the vicinity of a marker locus if the marker main effect or the M*D interaction was significant in the three-factorial ANOVA at $P \leq 0.01$.

[E]Level of significance of the marker main effect and the M* H interaction, respectively, with: P < 0.01, ***P < 0.001.

[F]RP [Genotype] = (Ms—Mv)*100/Mv in % in % effect of the Hsp alleles a cross both environments..

[G]RP [T*M T1] = (MsT1—MvT1)*100/MvT1 in % was effects of the Hsp alleles for control treatments.

[H]RP [T*M T2] = (MsT2—MvT2)*100/MvT2 in % was effects of the Hsp alleles for heat stress.

[I]QTLs number of QTLs for every trait.

Relative leaf water content (RWC)

Two putative QTLs for Relative leaf water content were located on chromosomes 2H and 6H. Three loci exhibited significant M*H interaction. The presence of the *Hsp* allele at two loci led to a reduction in the RWC with maximum 3.5% (GBM1016 [2H]). The *Hsp* alleles at two loci were positive effects for control treatment, the *Hsp* increased RWC in control treatment with 6.5%, while the *Hsp* allele at two loci decreased the RWC heat treatment up to 8.4% at GBM1016 [2H] (Table 28).

Number of spikes per plant (SPK)

Two QTL were detected for number of spikes per plant and located on chromosomes 2H and 5H. Markers AF043094A [5H], GMS061 [5H] and HVM36 [2H] showed a significant M*H interaction. Two markers were compared AF043094A and GMS061. AF043094A was highly significant. One favorable *Hsp* allele detected for number of spikes per plant positive effect of 18.9% increase in the number of spikes per plant at markers GMS061 [5H]. The other QTL showed negative effect and the *Hsp* allele was associated with a 3.5% decrease of SPK at HVM36 [2H]. The *Hsp* allele caused an increase SPK in control treatment up to 27.0% at GMS061 [5H], the other hand it caused a decrease SPK in the control treatment of 7.5% at HVM36 [2H]. The *Hsp* allele caused an increase in heat stress 11.8% HVM36 [2H], while caused a decrease in heat stress up to 9.9% at GMS061 [5H] (Table 28).

Osmotic adjustment (OA)

A total of 7 putative QTLs were located for osmotic adjustment and were showed on all barley chromosomes except for 5H. While three loci exhibited a significant marker main effect, the other 11 loci showed a significant M*H interaction. We have compared markers S53707 and GMS021 on chromosome. However, 1H, S53707 was highly significant. It was found on chromosome 3H HV13GEIII highly significant than HVM62 and HW01N04T3, on other hand was found GBM1015 marker on chromosome 4H highly significant than JVJASIP, HVM74, and TACMD, as well as on chromosome 7H was found BMS64 highly significant than GMS056. Four loci with the *Hsp* allele decreased OA maximum 11.6% at HY02P09T3 [2H]. On other hand, 3 favorable effects of the *Hsp* alleles detected for OA improved up to 8.7% at BMS64 [7H]. The *Hsp* allele at 4 loci decreased OA in control treatment led to a 19.4% S53707 [1H]. On other hand the *Hsp* allele of 3 loci lifted OA in control treatment up to 15.4% at HV13GEIII [3H]. Three wild allele loci

decreased OA in heat stress maximum 15.4% HY02P09T3 [2H], whilst four loci *Hsp* allele showed increasing heat stress for OA up to 14.4% at BMS64 [7H] (Table 28).

Days until heading (HEA)

Only one putative QTL for days until heading was located one chromosome. In this case, showed a significant M*H interaction at $P \leq 0.01$. In addition, the loci HVABAIP [1H] exhibited a significant M*H interaction, which was located on chromosome 1H. At this locus, the *Hsp* allele increased days until heading 2.7% at HVABAIP [1H]. The *Hsp* allele obtained increase days until heading control treatment with 4.6%, whereas it reduced heading time by 0.2% under heat stress (Table 28).

Flag leaf area (FLA)

Eight putative QTLs were located for flag leaf area was on all barley chromosomes except for 1H and 6H. On chromosome 3H, HV13GEIII was highly significant than HVM62, but on 4H marker HVKNOX3 highly significant than HVM40. All QTLs were detected as significant M*H interactions and exhibited at five favorable *Hsp* alleles effect detected for flag leaf area lifted improving at HVM62 [4H] by a maximum 19.6%, where three loci resulted decreasing FLA up to 8.5% at HY02P09T3 [2H]. The *Hsp* allele at six loci showed increasing FLA in control treatment resulted in maximum 25.4% at HW01M22T3 [8H], whereas two loci resulted in decreasing FLA up to 27.3% at HVSS1 [7H]. Heat stress obtained negative effect FLA in five loci *Hsp* allele maximum value found 31.4% at HY02P9T3 [2H], however, the *Hsp* alleles at three loci had positive effect increasing FLA under heat stress for flag leaf area up to 47.9% at HVSS1[7H] (Table 28).

First leaf area (ARE1)

Three putative QTLs were located for the first leaf area on chromosomes 1H, 4H and 5H. Both QTLs were detected significant marker main effects and M*H interactions. Due to the *Hsp* alleles at two loci were showed negative effects in control treatment for ARE1 3.2% and 19.1% at HY02P09T3 [2H], GMS061 [5H], respectively. Whilst, the *Hsp* allele at one locus positive effect was detected of ARE1 under control treatment up to 23.8 % at HY03N03T3 [4H]. Favorable effect of the *Hsp* allele detected first leaf area; positive effect (17.7 %) was detected by one locus HY03NO3T3 [4H]. Furthermore, two loci *Hsp* alleles heat stress was showed positive effects 8.6% and 13.2% at HY03N03T3 [4H], GMS061 [5H], respectively. While, negative effect (28.7 %) was detected at one locus HY02P09T3 [2H], under heat stress (Table 28).

Yield (YLD)

Altogether, 4 putative QTLs for yield were located on four chromosomes 1H, 2H, 3H and 4H. Six loci showed a significant M*H interaction. The interaction were significant at the linked loci for their three loci located on chromosome 3H like one QTL, but HV13GEIII [3H], highly significant than HVM62 [3H] and HW01N04T3 [3H]. Two favorable *Hsp* alleles effect detected for improved yield exhibited positive effects with maximum 20.8% at HVM62[3H]. In contrast, two loci *Hsp* alleles obtained negative effects for yield at HVABAIP [1H], GMS003 [2H] both by 12.9%. Control treatment resulted positive effects for yield at two loci *Hsp* alleles up to 23.1 % at HVM62 [3H]. While, *Hsp* alleles at two loci revealed negative effects in control treatment for yield in 14.0% and 15.1% at HVABAIP [1H], GMS003 [2H] respectively. Result showed positive effects for yield at three loci *Hsp* alleles up to 6.3% at GMS003 [2H] whereas; *Hsp* alleles at one locus obtained negative effects heat stress in 3.2% HVABAIP [1H] respectively (Table 28).

Biomass (MAS)

Three QTLs were located for biomass trait on chromosomes 2H, 3H and 7H. All QTLs were detected as significant M*H interactions and marker, favorable effects of the *Hsp* alleles, detected for biomass. On chromosome 3H, HV13GEIII was highly significant than HVM62. Markers Bmag0321 [7H] exhibited a significant main effect and an M× H interactions for GMS003 [2H], HV13GEIII [3H] and HVM62 [3H]. One favorable *Hsp* allele effect detected for biomass lifted improving a maximum 8.3% at HV13GEIII [3H], while the other two loci wild *Hsp* allele decreased biomass maximum 9.9 at GMS003 [2H]. The *Hsp* allele at two loci increased biomass in control treatments a maximum 10.4% at HV13GEIII [3H], whereas other *Hsp* alleles at two loci reduced biomass control treatments up to 10.6% at GMS003 [2H]. The *Hsp* alleles decreased at four loci under heat stress a maximum 6.6% at GMS003 [2H] (Table 28).

Harvest index (HI)

Three putative QTLs were located for harvest index on chromosomes 1H, 2H and 6H. All loci exhibited a significant M*H interaction at GBM1007 [1H], GBM1008 [6H], GMS003 [2H] and HVM36 [2H]. The presence of the *Hsp* allele resulted one locus negative in a harvest index decrease of up 1.9% (GBM1007 [1H]), while, three favorable *Hsp* alleles effect detected for harvest index obtained positive improved at three loci with a maximum 9.0% at HVM36 [2H]. Two loci *Hsp* allele increased harvest index in control treatments up to 14.6% at GBM1008 [6H], whereas other the *Hsp* alleles at two loci decreased harvest index up to 4.6% at GMS003 [2H]. Heat stress lifted HI in from two loci *Hsp* allele a maximum 30.8% at HVM36 [2H], whilst other two loci *Hsp* allele decreased HI in heat stress by 30.1% at GBM1007 [1H] (Table 28).

5. Discussion

Comparative methods for drought and heat

Environmental stresses come in many forms, yet the most prevalent stresses have a common effect on plant water status. The availability of water for its biological roles as solvent transport moderate, as electron donor in the Hill reaction, and as evaporative coolant is often impaired by environmental conditions. Although plant species vary in their sensitivity and response to the decrease in water potential caused by drought, low temperature, or high salinity, it may be assumed that all plants have encoded capability for stress perception, signaling and response (Bohnert *et al*. 1995).

In the season (2001) 4 German barley cultivars were examined under four treatments (35%, 50%, 65% and 100% FC) for drought treatments and three treatments for heat experiment normal climate, heat stress and heat stress plus 65% FC in the greenhouse. Twelve traits for heat and drought tolerance were examined in order to determine traits, for which the lines show significant variation under drought and heat stress and to determine the parameters of the treatments for drought experiment. Results of the first season showed small or in plant response no differences between 35% and 50% FC, 50% and 65% FC as well as 65% and 100% treatments. On the other hand, high differences between 35% and 65% as well as between 50% and 100% (Table 10). Two treatments 50% and 100% for drought experiment were selected, because of significant differences between the two treatments and reduce the work that would result from load with respect to daily weighing of the pots under four treatments. The drought or heat stress after one month was applied from planting for our study. Our methods for stress are in agreement with other Methods, the water stress was imposed at the 4-leaf stage by stopping the irrigation, the relative soil moisture content was 14% of the field capacity (FC) for the stressed-plants and 100% FC for irrigated plants Pots were weighed and watered daily (This *et al*. 2000; Teulat *et al*. 2001). After the second true leaf reached up to the first true leaf length, the drought treatment via water withholding was started, and it was maintained 8 days without watering when the sand water content was about 50% FC (Guoxiong *et al*. 2002). Different irrigation levels were studied in nine wild populations of *Lycopersicon chilense*, transferred to a common environment and grown under three soil water conditions: (80 % FC), (40 % FC) and (20 % FC) (Maldonado *et al*. 2003). Three treatments, normal climate, heat stress and heat stress plus 65% FC were included in the heat experiment in the season 2001 (Table 3). In this study results revealed high differences among the three treatments for heat experiment first season (Table 21). Due to insufficiency of experimental place only one treatment for 323 lines was carried out in the greenhouse, whereas the control was placed outside in the normal climate. Our methodology is similar to that proposed by Blum *et al*.

(1994) who used heat stress (35/25°C) or non-stress (25/15°C) conditions after anthesis in growth chamber. According to Stone and Nicolas (1996) heat-treated plants were moved at night to a naturally-lit glasshouse in which the night temperature of 19°C was maintained 11h and peak of 40°C was kept for 6h. High-temperature stress 40°C for heat regime for three days (Blumenthal *et al*. 1995). Similar methodology temperature was of day/night 20/15°C for control and 35/30°C for heat stress (Xu and Huang 2001). Early heat shock 35-40°C 18h of temperature was for five days during grain filling (Corbellini *et al*. 1997).

In the experiment carry out during (2002 to 2003), the DH population was evaluated in replicated greenhouse trials for drought and heat traits. Altogether 13 traits for the determination of the drought tolerance and 12 traits for the investigation of the heat tolerance were examined. Two treatments for drought experiment were used: 50% FC for drought stress and 100% FC for control. Two treatments for heat experiment were used: normal climate and heat stress in greenhouse. The data was obtained for the studied characters under two test environments (drought and heat). The DH lines were very contrasting in their characters as they were measurement on the basis of their performance under control and drought stress conditions, normal climate and heat stress across two years.

The goal of the present work was to detect putative QTL where the *Hsp* genotype (Accession ISR 42-8), which leads to an improvement of quantitative characteristics of the population. AB-QTL analysis strategy after Tanksley and Nelson 1996 was applied in order to transfer favorable alleles from wild barley for the improvement of heat and drought tolerance into elite barley cultivars.

5.1 Morphological traits

In this study, 11 quantitative traits (tillers per plant, number spikes per plant, number of kernels per spike, relative leaf water content, osmotic adjustment, chlorophyll content, days to heading, plant height, yield, biomass and harvest index) were evaluated for barley genotypes (Thuringia, Scarlett, Harry, and Apex). The interaction among genotypes and drought treatments was for tillers as non-significant, but was for chlorophyll content, non-significant interaction among genotypes was observed. For this reason in the study for (Scarlett and ISR42-8) parents and BC_2 DH population, tillers per plant, plant height, and chlorophyll content were not studied, but we have (number spikes per plant, number kernels per spike, relative leaf water content, osmotic adjustment, days to heading, yield, biomass and harvest index) and other traits like carbon isotope discrimination, flag leaf area, first lea area and second leaf area, which are related to drought were studied.

Selection of the investigated traits, reasons and justification

Number of spikes per plant is related to yield, thus it will be affected by drought or heat stress. When growth resources are limited by heat stress, the size of plant organs such as leaves, tillers, and spikes are reduced (Fischer, 1984). Temperature (27/22°C) (50% shade) during spike development can reduce the response of the developing grain to high temperature (30/25°C) following anthesis (Wardlaw 1994). Temperature stress during kernel development affects maize grain growth and yield stability (Cheikh and Jones 1994). Number of kernels per spike (KER) was related with yield; KER will be affected by drought or heat stress. Starch synthesis is highly sensitive to high temperature stress due to the susceptibility of the soluble starch synthesis in developing kernels of wheat (Denyer *et al*. 1994). High temperature late in the development of the crop are a feature of many of the wheat growing areas in US and maximum day temperatures above 32°C during the last 15 days of kernel filling, is associated with reduced quality. Thompson (1975) made the observation that the importance of high temperature during kernel filling was reinforced by series of time-of-planting.

Relative leaf water content (RWC) identifies that can be used in cereal breeding programs for selecting drought tolerant individuals. The RWC was previously demonstrated to be a relevant screening tool of drought-tolerance in cereals, as well as a good indicator of plant water-status (Teulat *et al*. 2003). During the drought stress, relative growth rates were more reduced (Costa Franca *et al*. 2000). The parental genotypes of these cross also differed by at least two other traits – leaf size and the relative water content (Altinkut *et al*. 2001). The maintenance of relative water content and a high osmotic adjustment are known to contribute to increased yield and yield stability under drought in cereals (Clarke and McCiag 1982). Osmotic adjustment could arise from an increase in the amount of solutes by active solutes accumulation or a decrease in the water content on a dry weight basis (Wilson *et al*. 1980). Osmotic adjustment has been found to be one of the most effective physiological mechanisms underlying plant resistance to water deficit. Osmotic adjustment, as a process of active accumulation of compatible osmolytes in plant cells exposed to water deficit, may enable (1) a continuation of leaf elongation, though at reduced rates (Turner 1986); (2) stomatal and photosynthetic adjustment (Morgan 1984); (3) maintained root development and soil moisture extraction (Morgan and Condon 1986); (4) delayed leaf senescence and better dry matter accumulation and yield production for crops in stressful environments (Blum 1988).

Theoretical and empirical studies have demonstrated that carbon isotope discrimination is highly correlated with plant water use efficiency. Carbon isotope discrimination provides an integrated measure of water-use efficiency, samples are easily collected, and processed, and large numbers of samples may be collected in diverse environments. Moreover, in woody plants, carbon isotope discrimination can be determined on annual ring samples, providing a historical analysis of plants response to environmental conditions (Cregg and Zhang 2001). In several crops including cereals, carbon isotope discrimination (CID) has been associated with drought tolerance in terms of water-use efficiency and yield stability in drought-prone environments (Teulat *et al*. 2002).

Flag leaf area, first leaf area and second leaf area are important traits for drought and heat tolerance. For drought if the leaf area is a large then more water is lost by transpiration. So it is better if the leaf area is small. For heat stress after optimal heat was decreased leaf area, it is better with a large leaf area (see Table 5). When growth resources are limited by heat stress, the size of plant organs such as leaves, tillers, and spikes are reduced (Fischer, 1984). Leaf area index of a canopy is an important variable in models for predicting crop growth and yields, quantifying crop–weed competition, or modeling heat, energy and water exchanges in the plant–soil–atmosphere continuum. Empirical data have shown that nitrogen is an important factor-affecting crop at early stages (Zhong, 1999). Appropriate quantification of leaf area index (LAI) is important for accurate prediction of photosynthetic productivity by crop growth models. Estimation of LAI requires accurate modeling of leaf senescence (Yin *et al*. 2000). Irrigated versus non-irrigated treatment were significant influenced leaf areas of all leaves developed on the different nodal position of ryegrass plant. The same effect of the water treatment was observed on leaf length and width. The change in leaf length was found the major cause in change of leaf area development (Mohammad *et al*. 1999).

Yield is very important trait, but has high effect with environment conditions. Temperature (27/22°C) (50% shade) during spike development can reduce the response of the developing grain to high temperature (30/25°C) following anthesis (Wardlaw 1994). Water deficit during meiosis in pollen mother cells of wheat induces male sterility, which can reduce grain set by 40 to 50% (Dorian *et al*. 1996). Morphological and physiological traits discussed so far all contribute to greater yields through increases in total biomass. At maturity a high harvest index is desirable to achieve high yields. Determinant of harvest index is independent on drought. Determinant of harvest index is drought dependent and depends largely on water availability during grain filling, but also on other factors such as pre-anthesis partitioning between structural and soluble carbohydrates (Richards *et al*. 2002

5.1.1 Evaluation of four barley genotypes for drought tolerance

The analysis of variance for number of tillers per plant and number of spikes per plant for the genotypes and drought treatments were highly significant (Table 8). Our study shows differences among treatments; in which both number of tillers and spikes decreased under drought or heat stress. The results are in agreement with that onus obtained by Fischer (1984). For number of kernels per spike the interaction among genotypes was highly significant. The difference among genotypes and drought treatments were highly significant (see Table 8 and Figure 1). These results are similar those obtained by Wiegand and Cuellar (1981). The analysis of variance for relative leaf water content was highly significant among drought treatments (Table 8). The same results were obtained by Costa Franca *et al*. (2000) and Altinkut *et al*. (2001). The analysis of variance for osmotic adjustment of genotypes, drought treatments and the interaction among genotypes and drought treatments were highly significant in our study (Table 8 and Figure 2). The results are in agreement with Lu and Tamar (1999), who studied the differences between wild barley and modern cultivars in resistance to a uniform water deficit.

In this study, the variation in chlorophyll content among drought treatments and the interaction between genotypes and drought treatments were significant different (Table 8 and Figure 3). Similar results were obtained by Havaux and Tardy (1999), the Syrian barley landrace Tadmor is adapted to semi-arid environments and characterized by reducing chlorophyll content (ca-25% on a leaf area basis) compared to improved barley genotypes, such as the European variety Plaisant. Drought is a multi-dimensional stress, which causes various physiological and biochemical effects on plants. Such effects may include reduction in cell division and thus retardation of cellular growth, decrease in photosynthesis, closure of stomata and change in the amount of chlorophyll (Turner, 1986).

In the present study differences for days to heading among genotypes and drought treatments were highly significant (Table 8). The average days of heading were for genotypes between 64.83 days for Apex to 87.17 days for Harry (Table 14). The analysis of variance for heading date between genotypes and drought treatments were highly significant (Table 13). The results are agreed with that obtained Ahmed *et al*. (2000), who reported mean heading date over two years from 27.3 to 55.8 days. Plant height was significantly correlated with the heading date.

The analysis for the data showed differences in yield among genotypes. Drought treatments and the interaction between genotypes and drought treatments were highly significant (Table 8 and Figure 4). Similar results were obtained by Sinclair and Muchow (2001), the analysis of putative plant traits to increase crop yields under water-limited conditions was undertaken as an approach particularly relevant to growers for increasing water use efficiency. Consequently, a number of

traits for improving crop performance under limited water conditions were tested in a simulation of growth and yield in maize and sorghum.

The variation of biomass among genotypes, drought treatments and the interaction between genotypes and drought treatments were highly significant (Table 8 and Figure 5). The same results were reported by Simane *et al*. (1993), who showed that yield and straw varied among cultivars and was reduced under drought stress. The association between yield in drought stressed environments and yield in non drought-stressed environments was interpreted to reflect genotypic high yield potential, mainly by way of high biomass development (Ginkel *et al*. 1998). Variation for harvest index was highly significant among genotypes, drought treatments and the interaction among genotypes and drought treatments, (Table 8 and Figure 6). Same finding were obtained by (Richards *et al*. 2002).

5.1.2 Drought results for BC_2DH lines.

The analysis of variance of BC_2DH lines for 13 quantitative traits for the drought experiment was highly significant for all parameters. The variation between years of all quantitative traits was highly significant for all parameters except relative leaf water content. The analysis of variance between drought treatments of all quantitative traits was highly significant for all parameters. The interaction between drought treatments and years was significant for all parameters except relative leaf water content and osmotic adjustment. The interaction between drought treatments and BC_2DH lines was significant for all parameters except number of spikes per plant. The interaction between years and BC_2DH lines was highly significant for all parameters. The analysis of variance for the interaction between BC_2DH lines, years, and BC_2DH lines of all quantitative traits was significant for all parameters except number of leaves per main tiller (Table 14).

5.1.3 Evaluation of four barley genotypes for heat tolerance

The conditions encountered by plants during extended periods of drought, accompanied by brief exposures to heat shock typically occur between midday to late afternoon (Merquiol *et al*. 2001). He subjected tobacco plants to drought stress until they reached relative water content of 65% to 70%. Plants were then exposed to a heat shock treatment and sampled. As control, they used well-watered plants, drought-stressed plants that were not subjected to heat shock, and well-watered plants that were subjected to heat shock (heat shock). All plants were analyzed and sampled at the same time. Recovery tests indicated that plants subjected to a combination of drought stress and heat shock could recover within a few days upon watering and changing of temperature to 23°C. The conditions used were not lethal to plants (Rizhsky *et al*. 2002). The cause for death after lethal heat

shock is not well understood. A shift from low to intermediate temperature causes the induction of heat-shock proteins in most organisms (Davidson *et al*. 1996). The response of spring wheat to heat stress has been determined in several hot wheat growing environments worldwide on different types of germplasm. Physiological data has been collected to identify potential traits to assist in the empirical breeding for heat tolerance (Reynolds 1998).

The analysis of variance for number of tillers per plant was highly significant among heat treatments (Table 21). Variation for number of spikes per plant was highly significant among genotypes and heat treatments (Table 21). Similar results were reported by (Fischer, 1984 and Xu and Huang 2001).

The analysis of variance for number of kernels per spike revealed highly significant difference for kernels per spike among genotypes and heat treatments (Table 19). The results are accordance to those obtained by Fischer, (1984) and Wiegand and Cuellar (19981). High temperature during reproductive development after kernel development reduces yield quality in wheat (Banowetz *et al*. 1999). Heat treatment was exposed to very high temperature (40/19°C day/night) for periods or 1-10 days duration. As little as 1 day of heat treatment reduced kernel mass by 14% in the heat-sensitive variety, but only by 5% in the heat tolerant variety (Stone and Nicolas 1998).

Variation for relative leaf water content was highly significant among the genotypes, heat treatments, and the interaction among genotypes and heat treatments (Table 19 and Figure 9). Similar results were reported by (Wilson *et al*. 1980 and Clarke and McCiag 1982).

The variation for osmotic adjustment was non-significant among replications, but highly significant among genotypes, heat treatments, and the interaction between genotypes and heat treatments (Table 19 and Figure 10). Our results were accordance with those obtained by (Turner *et al*. 1986). He observed differences in osmotic adjustment among rice cultivars, but no differences among treatments. Drought and high temperature usually occur simultaneously, but their effects on plant development are often studied separately. The level of the other stress might alter crop responses to one stress. For instance, high temperature might interact with osmotic adjustment in plants in several ways; it might interact with osmotic adjustment directly by increasing the rate of evaporation (Gates, 1968) or by interfering with the production and utilization of solutes involved in osmotic adjustment (Li *et al*. 1993).

The variation for chlorophyll content among genotypes and heat treatments was highly significant (Table 19). Similar results were reported by Reynolds *et al*. (1994). Physiological evidence indicates that loss of chlorophyll during grain filling is associated with reduced yield in the field. Chlorophyll fluorescence may be more promising as a screening trait, given that associations between heat tolerance and lower fluorescence signals have been reported in a number of crops (Moffat *et al*. 1990).

The Variation for days to heading was highly significant among genotypes and the interaction between genotypes and heat treatment (Table 19 and Figure 11). Results are in agreement with (Teulat *et al*. 2002).

Variation for plant height among genotypes, heat treatments and the interaction among genotypes and heat treatments was highly significant (Table 19 and Figure 12). Our result is in agreement with those obtained by Ahmed *et al*. (2000).

Variation for yield among genotypes and heat treatments was highly significant (Table 19). Similar results were reported by Condon et al. (2002). Grain sterility and specific forms of morphological and cellular damage depend on the stage of development of grain at the time of transfer (Tashiro and Wardlaw 1990). High temperature during reproductive development after kernel development reduces yield quality in wheat (Banowetz *et al*. 1999). Heat stress during grain filling is a major constraint to wheat (*Triticum aestivum* L.) yield. Significant variation was seen among cultivars in the reduction of grain weight per ear, kernel number, and single kernel weight under heat stress. Differences in grain weight per ear among cultivars were ascribed to variation in the reduction in both kernel number and kernel weight under heat stress (Fokar *et al*. 1998). Temperatures above 27°C, in a growth cabinet, have resulted in floral sterility and yield loss in *Brassica napus* (Morrison and Stewart 2002). Heat stress caused a reduction in main yield of the random inbred line population by 47% as compared with normal winter growing conditions (non-stress) (Blum *et al*. 2001). Crop damage due to heat stress under late planting conditions has become an important factor limiting wheat yields (Aslam *et al*. 1989). When heat shock came late in grain filling and yield were not negatively affected but a `dough weakening´ effect, which may reduce the commercial value of the production, is to be expected (Corbellini *et al* 1997). Short period of very high temperature (>35°C) are common in many of the world's wheat growing areas and can be a significant factor in reducing yield and quality (Stone and Nicolas 1995b).

Analysis of variance among heat treatments was highly significant for biomass. The variance was also, highly significant among heat treatments and genotypes for harvest index (Table 19). The same finding was reported by Badaruddin *et al*. (1999), Photo-assimilation is more likely to be yield limiting under heat stress than in temperate environments, especially as stress typically intensifies during grain filling, when demand for assimilates is greatest. This is borne out by the observation that under stress, total aboveground biomass typically shows a stronger association with yield than with partitioning and harvest index. The situation is usually reversed under temperate conditions. Hence traits affecting radiation use efficiency (such as ground cover, stay green, and photosynthetic rate) could be expected to be important under heat stress. Although early ground cover seems also important in an agronomic context, variation in this trait among genotypes does not seem to be associated with heat tolerance. Physiological evidence indicates that loss of chlorophyll during grain filling is associated with reduced yield in the field (Reynolds *et al*. 1994).

5.1.4 Heat results for BC_2DH lines

The analysis of variance for BC_2DH lines of 12 quantitative traits for heat experiment was significant for all parameters except number of leaves per main tiller and first leaf area. The variation between years of all quantitative traits was significant for all parameters except relative leaf water content and number of leaves per main tiller. The analysis of variance between heat treatments of all quantitative traits was highly significant for all parameters. The interaction between heat treatments and years was significant for all parameters except relative leaf water content. The interaction between heat treatments and BC_2DH lines was significant for all parameters except number of leaves per main tiller and first leaf area. The interaction between years and BC_2DH lines was highly significant for all parameters except number of leaves per main tiller and first leaf area. The analysis of variance of the interaction between BC_2DH lines, years, and BC_2DH lines of all quantitative traits for heat experiment was significant for all parameters except number of spikes per plant, number of leaves per main tiller and first leaf area (see Table 25**)**. Similar results were found by (Wilson *et al*. 1980; Fischer, 1984; Reynolds *et al*. 1994; Stone and Nicolas 1998; Morgan and Condon 1986; Simane *et al*. 1993; Dorion *et al*. 1996; Ahmed *et al*. 2000; Altinkut *et al*. 2001; Fokar *et al*. 1998 and Teulat *et al*. 2002).

5.2 Discussion of QTL analysis

5.2.1 Discussion the AB-QTL-analysis in the BC_2DH population

Classical QTL analysis was conducted in early, balanced generations like doubled haploids. The AB-QTL analysis was based on a BC_2DH population. This change was necessary since we used an exotic cross with the barley progenitor *Hordeum Spontenum* (*Hsp)* as the donor of potential favorable QTL alleles. However, it is still open if the identified favorable QTL alleles from *Hsp* are indeed unmatched in the elite gene pool of barley. Results for *Hordeum* have been reported by Powell and Russell (*2000*). Based on this findings, it is likely that at least a portion of the identified favorable QTL alleles from *Hsp* are new alleles, so far not present in the barley elite gene pool (Pillen *et al*. 2003). Several software programs which are based on these methods have been written for detection of QTLs, e.g. MAPMARKER/QTL (Lander and Botstein *1989*), QTL-CARTOGRAPHER (Basten *et al*. *1994*), MQTL (Tinker and Mather *1995*) and PLAB-QTL (Utz and Melchinger *1996*). Unfortunately, these programs are focused on the analysis of balanced populations, which are used in classical QTL analysis. For unbalanced populations, which are used in AB-QTL studies, the program QGENE was written (Nelson *1997*). QGENE operates with single marker regression as well as simple interval mapping for QTL detection. Our AB-QTL study, in two separate drought or heat experiments were conducted and since we wanted to include the M*D interaction or the M*H interaction as a measure of the environment stability of a QTL effect, we preferred to use a 3-factorial ANOVA with the marker genotype, the drought or heat treatment and the year as factors. By including the year in the statistical model, we expected to reduce the residual variance of the experiment. A 3-factorial model allowed us to differentiate between a QTL significant as a marker main effect, which is considered to be stable across the tested drought or heat tolerance, and a QTL significant as a M*D interaction or M* H interaction where the effect is considered to depend on a particular drought and heat treatment.

5.2.2 AB-QTL Analysis in barley

The goals of the AB-QTL analysis are the identification and simultaneous transfer of those exotic QTL alleles, which have the potential to improve drought or heat tolerance. Within the Scarlett*ISR42-8 population, a total of 28 favorable *Hsp* alleles (53.8%) were identified among 52 localized QTLs (see Table 29 and 30). These favorable *Hsp* alleles were detected for six of the 13 traits for drought experiment and nine of the 12 traits for heat tolerance investigated. The QTLs consistent across drought stress for biomass on chromosome 1H and 5H were found separately in

drought treatments, exhibited significant M*D interaction. By contrast, one QTL detected in drought for flag leaf area on chromosomes 1H at GMS021, detected as significant marker main effect. This was also the case the QTL for number of leaves per main tiller for which identified on chromosome 5H, which exhibited a significant M*D interaction. The QTLs identified were QTLs interacting with drought on chromosomes 1H (HVALAAT), 5H (HY02J05T3; Bmag0223; Bmag0222; and HW01M22T3), 6H (Bmac0316), and 7H (HVA22S; Bmag0011; GMS056; BMS64; and Bmag0120) for osmotic adjustment, on chromosomes 1H (GMB1007) and 4H (GMS089; TACMD; Ebmac0701; Ebmac0635; Ebmac0679; and Ebmac0788) for relative leaf water content, and yield obtained on chromosome 3H (HV13GEIII; HVM62; HW01N04T3; and Bmac0029) 5H (Bmag0537), and 6H (Bmac0316) (Tables 26). The results are in agreement with those obtained by (Tinker *et al*. 1996; Xiao *et al*. *1998*; Bernacchi *et al*. *1998a*; Hemamalini *et al*. 2000 and Pillen *et al*. 2003).

Table 29: List of 14 favorable QTL alleles detected from the BC_2DH cross Scarlett x ISR42-8[A] for drought tolerance

[A]Trait	Marker	[B]Ch	[C]Value	[D]RP Wild allele effect	[F]Favorable QTL alleles
FLA	GMS021	1H	-	-16.4	1
LEA	Bmag0357	5H	+	2.2	1
MASS	HY03I05T3	1H	+	1.2	1
	Bmag0357	5H	+	8.9	2
OA	HY02J05T3	5H	+	11.5	1
	Bmag0223	5H	+	8.1	2
	Bmag0222	5H	+	19.7	3
	HW01M22T3	5H	+	22.3	3
	HVA22S	7H	+	8.4	4
	Bmag0011	7H	+	8.8	4
	GMS056	7H	+	14.8	5
	BMS64	7H	+	14.3	5
	Bmag0120	7H	+	17.7	5
YLD	HV13GEIII	3H	+	10	1
	HVM62	3H	+	10.8	1
	HW01N04T3	3H	+	9.9	1
	Bmac0029	3H	+	18.4	1
	Bmag0357	5H	+	8.9	2
	Bmac0316	6H	+	4.4	3
CID	Bmac0163	5H	-	-0.9	1
	EBmac0755	7H	-	-1.3	2

[A]The quantitative traits are defined in Table 5.

[B]Chromosomal assignment of SSRs.

[C]The value of the trait should be increased (+) or reduced (–) with respect to the breeding goal.

[D]RP [Genotype] = (Ms--Mv)*100/Mv in % in % effect of the Hsp alleles a cross both environments.

[F]Favorable *QTL* alleles

Table 30: **List of 16 favorable QTL alleles detected from the BC_2DH cross Scarlett x ISR42-8[A] for heat experiment.**

[A]Trait	Marker	[B]Ch	[C]Value	[D]RP Wild allele effect	[F]Favorable *QTL* alleles
MASS	HV13GEIII	3H	+	8.25	1
	HVM62	3H	+	7.85	1
HI	HVM36	2H	+	9.04	1
	GMS003	2H	+	3.16	2
	GBM1008	6HL	+	1.67	3
ARE1	HY03N03T3	4H	+	17.66	1
FLA	HVM62	3H	+	19.6	1
	HVKNOX3	4H	+	5.16	2
	HW01N04T3	4H	+	16.93	3
	HW01M22T3	5H	+	5.16	4
	HVSS1	7H	+	3.76	5
OA	GBM1015	4HL	+	1	1
	HVJASIP	4H	+	3.4	1
	HVM67	4H	+	3.1	1
	HVM74	6H	+	4.1	2
	GMS056	7H	+	6.93	3
	BMS64	7H	+	8.71	3
	GMS061	5H	+	18.94	1
SPK	AF043094A	5H	+	11.51	1
	HV13GEIII	3H	+	19.32	1
YLD	HVM62	3H	+	20.77	1
	HW01N04T3	3H	+	20.75	1
	HVB23D	4H	+	19.11	2

Osmotic adjustment (OA)

Studies showed a total of 14 putative QTLs from 26 regions were found for osmotic adjustment on all barley chromosomes (see Table 18, 28 and Figure 8, 13). Recently, the region of rice chromosome 2 was also identified as involved in osmotic adjustment (Zhang *et al*. *2001*). 22 QTLs were leading to total regions 32 (Teulat *et al*. 1998; 2001a). Controlling traits were related to osmotic adjustment in the barley genetic background studied. It is necessary to identify the most consistent and important of these QTLs, in terms of improving drought or heat tolerance, based on the whole analysis. In a genetic study the traits are measured under standardized and often simplified conditions (e.g. given soil moisture, growth stage), and from this point of view it is difficult to give a physiological meaning to a QTL (This and Teulat-Merah 1999). Thus, to be relevant to plant improvement, the traits employed and the QTLs identified must be assessed according to their physiological effect on reducing yield losses under drought. Preliminary information can be obtained from a genetic evaluation: they could come: (a) from a correlative approach (correlation between traits) conducted on a large population, (b) from a comparison of results at several soils water status levels (here in our study 50% and 100% FC) or standardized at 100% relative leaf water content by calculation. It was suggested a possible contribution of water-soluble carbohydrates accumulated during osmotic adjustment in the crosses. However, even this hypothesis is in accordance with pervious results obtained from the parental genotypes (Teulat *et al*. 1997b, 2000 and al. 2001a) and from observation made by (Lewicki 1993). He was suggested that was the solutes mostly accumulated during osmotic adjustment in barley, the role of this QTL in controlling solute content contributing to osmotic adjustment remains to be proven. Osmotic adjustment under 50% FC was weak correlated with relative leaf water content (r = 0.11**), flag leaf area (r = 0.12**), first leaf area (r = 0.10**), biomass (r =0.11**), and harvest index (r = -0.15***) for drought tolerance Table 16**.** Osmotic adjustment in heat stress was weak correlated with number of kernels per spike (r = 0.11**) and second leaf area (r = 0.19**), it was associated moderate with number of leaves per main tiller (r =0.467***), yield (r = -0.286***) and harvest index (r = -0.3025***). On other hand, it was correlated positively and strongly with days until heading (r = 0.568***) for heat tolerance Table 27. These results are in agreement with those reported by Teulat *et al*. (2001a). For drought tolerance QTLs were identified on chromosomes 1H (HVALAAT), 5H (HY02J05T3; Bmag0223; Bmag0222; and HW01M22T3), 6H (Bmac0316), and 7H (HVA22S; Bmag0011; GMS056; BMS64; and Bmag0120) for OA. For heat tolerance located on chromosome 1H (GMS021; S53707), chromosome 2H (HY02P09T3), chromosome 3H (HV13GEIII; HVM62; and HW01N04T3) 4H (HVJASIP; HVM67; TACMD; and GMB1015), chromosome 6H (HVM74) and chromosome 7H (GMS056; and BMS64). For drought tolerance a

total of 7 putative QTLs from 13 regions having effects on osmotic adjustment were located on chromosomes 1H, 5H, 6H and 7H. Five favorable QTL effects were detected of *Hsp* alleles, which improved osmotic adjustment with a maximum 22.3% at HW01N04T3 [5H] and an average value 13.9%. Two *Hsp* alleles decreased osmotic adjustment an average by 11.2%. The *Hsp* allele increased osmotic adjustment at four loci for control treatment with by an average 4.0% and by a maximum of 8.6% at HVALAAT [1H]. At 3 *Hsp* loci alleles decreased osmotic adjustment in the control treatment by an average of 4.0%. Two *Hsp* alleles as loci decreased osmotic adjustment under drought stress by average 15.7%. At five QTLs the *Hsp* alleles showed increase in osmotic adjustment under drought stress with an average of 19.7% and with a maximum of 39.5% at HW01M22T3 [5H] (Table 18). In the present study for osmotic adjustment, the effects of *Hsp* alleles are weak to moderate for drought tolerance. Similar results were obtained in maize and barley where four and thirteen QTLs related to invertase activity and hexode content were identified when under control or under water stress conditions. Other QTLs were effective under one of the latter conditions (Pelleschi *et al*. 1999 and Teulat *et al*. 2001a). The trait was considered to be interesting when the allele effect at a QTL was in favor of a stronger relative water content under stress; the maintenance of relative water content, together with high osmotic adjustment capacity, being in favor of turgor maintenance and contributing to yield stability under drought conditions in cereals (Clarke and McCaig 1982; Blum 1988; Schonfeld *et al*. 1988; Matin *et al*. 1989). The intrinsic ability to accumulate solutes has also a physiological significance for drought tolerance. This capacity was detected for the susceptible parental genotype Er/Apm (Teulat *et al*. 1997b, 1998, and 2001a. In Teulat *et al*. (1998), the 7H region was emphasized because it controlled the variation of relative water content and water stress at 14% FC in barley and common to the major QTL found by Lilley *et al*. (1996) for osmotic adjustment70% relative water content in homoeologous portion of rice chromosome 8 (Teulat *et al*. 1998; This and Teulat-Merah 1999). Zhang *et al*. (1999) Presented a figure where the gene (Morgan and Tan 1996) that could be involved in osmotic adjustment in wheat, seemed to be collinear to Lilley´s QTL for OA. The gene is linked to the xpsr 119 marker and the region could correspond to a portion of rice chromosome 6. Indeed, the small arm of *Triticeae* chromosome group 7 could correspond to rice chromosome 6 and 8. In the present study for heat tolerance a total of 7 putative QTLs from 13 regions were located for osmotic adjustment, which was on all barley chromosomes except for 5H. Four of the *Hsp* alleles decreased OA with an average of 4.4%. On the other hand, three favorable effects of three *Hsp* alleles improved osmotic adjustment up to 8.7% at BMS64 [7H] and an average by 4.5%. The *Hsp* allele of three loci increased osmotic adjustment in the control treatment up to 15.4% at HV13GEIII [3H] with an average of 11.04%. On the other hand, the *Hsp* allele of four loci decreased osmotic adjustment in control

treatment by average 9.6%. Three wild alleles decreased osmotic adjustment in heat stress the by an average 12.3%, whilst eight loci *Hsp* allele showed an increase osmotic adjustment under heat stress by a maximum of 14.4% at BMS64$_{[7H]}$ with an average of 7.9% (Table 28). In the study for osmotic adjustment under heat tolerance found moderate effects for the *Hsp* alleles. A QTL found for leaf osmotic potential variation in rice chromosome 3 (Lilley *et al*. 1996) was mapped in the homologous portion of barley chromosome 5H. The region 2H is also interesting for several traits like osmotic adjustment and other traits in barley (Teulat *et al*. 2001a). Regions were found as directly involved in osmotic adjustment there are (4H, 6H and 5H) (Teulat *et al*. 2001a). Seven putative QTLs for OA regions found in the present study of drought tolerance detected five favorable alleles effect. These loci improved osmotic adjustment to a maximum value 22.3% at HW01M22T3 $_{[5H].}$ However, at seven putative QTLs for OA of heat tolerance three favorable effects of *Hsp* alleles were detected as M*H interaction on chromosomes 4H, 6H and 7H. Recently, mapping single genes or/and quantitative trait loci (QTLs) for osmotic adjustment has been conducted in wheat (Morgan and Tan 1996), rice (Lilley *et al*. 1996) and barley (Teulat *et al*. 1998).

Relative leaf water content (RWC)

In this study a total of 5 putative QTLs from 9 regions were located for relative leaf water content on all barley chromosomes (see Table 18, 28 and Figure 8, 13). The QTL results obtained underlined that several putative genomic regions contribute to the total variation of relative leaf water content (Teulat *et al*. 2002). The first results obtained with a barley population grown under controlled conditions at two different soil-moisture contents have also revealed several loci involved (Teulat *et al*. *2001a*). This is also in agreement with the results from Schonfeld *et al*. (*1988*), who have shown that the phenotypic distribution of relative leaf water content in F2 indicated that the trait was quantitatively inherited and not controlled by one or two genes in wheat. In rice, Courtois *et al*. (*2000*) identified 11 QTLs grouped on nine genomic regions for relative leaf water content measured in two different environments, and Price *et al*. (*2002*) identified eight QTLs for relative leaf water content measured in three different environments. Among the nine genomic areas identified in the present study, two presented QTL*environment interaction (on the long arms of chromosomes 7H and 1H) and four were detected for only one of the environments studied. In contrast, three QTLs presented main effects across five environments and could be considered as stable regions controlling relative leaf water content (chromosomes 2H, 4H and 6H). In the present study from greenhouse experiments, drought tolerance for all alleles were positive effects for RWC in control treatment except at GMS089 $_{[4H]}$ marker. The *Hsp* alleles at two loci increased relative leaf water content in control treatment with average of 3.6%. The *H*sp alleles decreased relative leaf

water content in the control treatment up to 4.8%. The *Hsp* alleles decreased relative leaf water content under drought stress by an average of 6.65%, while it was increased at one locus under drought stress up to 3.9% at GMS089 [4H] (Table 18). In our study of drought tolerance for relative leaf water content the *Hsp* allele effect were weak. Two QTLs were controlled RWC under drought stress were mapped on chromosomes 1H and 6H (Teulat *et al*. 1997). QTL controlling relative leaf water content for the trial was mapped in the same area. The most-consistent example is the genomic region on the long arm of chromosome 6H (Teulat *et al* 2002). It was previously identified as controlling leaf osmotic potential and osmotic potential at full turgor, with osmotic adjustment as well as relative leaf water content measured under water-deficit conditions (Teulat *et al*. *1998*, *2001a*). Similarly Price *et al*. (*2002*) identified a QTL for relative leaf water content on rice chromosome 8 that was co-localized with a QTL for osmotic adjustment identified in another population (Lilley *et al 1996*). This region is homoeologous to a barley region near CDO673 where a QTL for relative leaf water content was identified in stressed conditions (Teulat *et al*. *1998*). The osmotic adjustment capacity allows cell-turgor maintenance and turgor-dependent processes (Turner and Jones *1980*). In addition, relative leaf water content is an indicator of the cell volume. These traits are involved directly or indirectly in plant water and turgor status. The genomic region of the barley chromosome 6H was again identified as controlling relative leaf water content measured in Mediterranean field conditions. In addition, this is probably the most stable and confident QTL obtained across the field environments studied. For the most-stable regions, the nearest molecular markers could be identified and used to improve breeding efficiency, as a selection criterion for the trait (Teulat *et al*. 2002). In present study for heat tolerance two putative QTLs for relative leaf water content were located on chromosomes 2H and 6H. At these loci, the presence of the *Hsp* allele led to a reduction in relative leaf water content in average 2.3%. The *Hsp* allele increased RWC in the control treatment with maximum 6.9% at GMB 1015 [2H] and with average 3.3% (Table 28 and Figure 13). The molecular genetics approach could also help our understanding of the process of drought-tolerance through genetic interaction between traits or co-locations of QTLs with gene sequences (Teulat *et al*. *2002*). It is now known that the grass genomes contain gene-rich compartments (Sandhu and Gill *2002*). This has an effect on recombination that was shown to be high in gene-rich barley regions (Kunzel *et al*. *2000*). This also shows the difficulty to identify the genes that are really involved in an individual-trait phenotypic variation. However, the co-locations of QTLs controlling water-status and/or turgor with sequences corresponding to dehydrin *(dhn)* genes on the same portion of chromosome 6H, was a great indication of the possible role of these genes in the variation of plant water-status under drought (Teulat *et al*. 2002). In present study *Hsp* allele increased under drought stress relative leaf water

content with average 3.9%, while *Hsp* alleles decreased relative water content under heat stress with average 7.6%. The latter region of chromosome 6H was previously proved to contain a cluster of *dhn* genes including the barley *dhn4* and *dhn5* (Campbell and Close *1997*), whereas the wheat *wsp23* sequence corresponds to an analog of barley and maize dehydrin protein, and to rice and wheat RAB proteins expressed under water stress (Joshi *et al*. *1992*). Another chromosomal region contains a QTL for relative leaf water content and a dehydrin locus *dhn1* on chromosome 5H. The dehydrins are water-soluble lipid-associated proteins that accumulate in response to dehydration, low temperature, osmotic stress, or during seed maturation (Close *et al*. *1989*). Several QTLs controlling tolerance traits, and particularly freezing tolerance, have already been identified close to dehydrin genes (Campbell and Close *1997*). These authors have underlined that the recurring physiological and genetic correlations constitute mounting evidence that dehydrin genes may be key genetic determinants of stress tolerance in a number of species, particularly freezing and drought-tolerance. The first example was for a QTL for winter-hardiness overlapping with a cluster of *dhn* genes, including *dhn1* on barley chromosome 5H associated with a cold- specific induction of a member of this dehydrin family (Pan *et al*. *1994*; Van Zee *et al*. *1995*). Recently Koag *et al*. (*2003*) have shown the binding of maize DHN1 to lipid vesicles, suggesting membrane stabilization under stress conditions. The link between cell volume/turgor maintenance and the properties of these proteins seems possible but must be proved. The positional cloning of the main QTL and the allelic variation study of *dhn* genes in a collection of barley genetic resources differing for their drought-tolerance response could elucidate if the *dhn* genes are involved in plant water-status and drought-tolerance variation. Ismail *et al*. (*1999*) have conducted this type of experiment on *Vigna unguiculata* plantlets. They have demonstrated the co-segregation of a dehydrin gene with chilling tolerance, and the usefulness of the normal protein compared to a dehydrin mutant-allele in this phenomenon. For drought tolerance relative leaf water content was weakly correlated under drought stress with number of spikes per plant (r = -0. 09*) and osmotic adjustment(r= -0.11**) see Table 16. There was a weakly correlation under heat stress with harvest index (r = - 0.08*) Table 27. Similar some finding was obtained by (Teulat *et al*. 1997).

Drought-tolerance evaluation and QTL value for breeding purposes

Most of the drought-tolerance traits are quantitative. These are difficult to measure on a large number of plants. The difficulty increases when the traits are evaluated under field condition (Teulat *et al* 2002). Indeed the genetic part of the phenotypic variation is often hidden due to the abiotic or biotic source of variability acting on the trait (disease attack, risk of inappropriate rainfalls), involving difficulty of trial management and relevant measurement time. In addition, the

trait must be measured instantaneously on all the plants, which is nearly impossible. In parallel, it is now commonly accepted that the use of the molecular-genetics approach and of molecular markers could help to improve the selection efficiency. For all those reasons, QTLs for traits evaluating plant water-status and/or osmotic adjustment were previously investigated with barley lines grown under controlled conditions (Teulat *et al*. *1998*, *2001a, 2002*). However, Price and Courtois (*1999*) have underlined that locating QTLs for drought resistance mechanisms, by the use of controlled greenhouse or growth chamber experiments combined with field evaluations under relevant conditions, should allow us to identify QTLs of value for breeding. In the previous experiment conducted under controlled conditions, 13 chromosomal regions were identified as controlling traits related to plant water-status and/or osmotic adjustment with the same genetic background (Teulat *et al*. *2001a*). Considering the difficulty of quantitative trait evaluation under field conditions, the measurements were restricted to relative water-content. However, to assess the variation in the trait and in the QTLs across different drought situations, the trait was measured under several Mediterranean field conditions to verity, the QTLs previously identified from the experiment conducted under controlled conditions. The compilation of the data from the two sets of experiments allowed us to identify common from both types of experiments, confirming the interest of the strategy undertaken. These regions seemed to be relevant targets for breeding purposes. The one on the long arm of chromosome 6H was also shown to control thousand-grain-weight across several Mediterranean environments (Teulat *et al*. *2001b*), reinforcing its interest; A QTL for a drought-tolerance mechanism, or a criterion being of little value, cannot be shown to improve or stabilize yield under stress conditions or if it causes a substantial reduction of yield under ideal conditions (Price and Courtois *1999*). In maize, encouraging results of molecular-assisted-selection under drought conditions were obtained (Ribaut *et al*. *1999*). The *Hsp* allele in the present study was improved these traits; FLA, LEA, MASS, OA, YLD and CID with maximum up to 16.4%, 2.2%, 8.9%, 22.3%, 18.4% and 1.3%, respectively, under drought stress. In addition, the Hsp allele was improved these traits; MAS, HI, HEA, ARE1, FLA, OA, SPK and YLD with maximum up to 8.3%, 9%, 2.7%, 13.6%, 11.6%, 18.9% and 20.7%, respectively, under heat stress.

Considering the drought-tolerant genotypes in terms of yield stability, Teulat *et al*. (*1997a*) have presented higher relative leaf water content values compared to Er/Apm at different soil-moisture contents during an imposed water deficit. The large differences observed for relative leaf water content in the RIL population could be due to differences in solute accumulation and osmotic adjustment, the two traits characterizing the population and the two parental lines studied (Teulat *et al*. *1997a*; *2001a*). The use of adjusted entry means, generated by fixing the block within the

environment effect, has improved the power of QTL detection. This also underlined the need of relevant experimental designs in this type of strategy (Teulat *et al*. *2002*).

Yield (YLD)

In this study a total of 7 putative QTLs from 12 regions were located for yield on all barley chromosomes except chromosome 7H (see Table 18, 28 and Figure 8, 13). Our results are in agreement with those obtained by Kandemir *et al*. (2000) and Hittalmani *et al*. (2002); three previously were identified grain yield QTL on chromosomes 1H, 2H and 3H, one QTL was identified for grain yield per plant respectively. The crop yield is a complex trait can be considered to be the result of many dynamic processes during crop ontogeny (Yin *et al*. 1999). For drought tolerance yield was positively and strongly correlated under drought stress with number of spikes per plant (r =0.62***), number of kernels per spike (0.68***), and biomass (r =0.72***), while it was moderately correlated with number of leaves per main tiller (r = -0.40***), flag leaf area (r =0.39***), first leaf area (r =0.43***), second leaf area (r =0.48***), and whereas it was weakly correlation with harvest index (r =0.14***) see (Table 16). Yield was positive and strongly correlated under heat stress only with harvest index (r = 0.72***), whereas it was moderately correlated with number of spikes per plant (r = 0.38), number of kernels per spike (r = 0.44***), number of leaves per main tiller (-0.26***) osmotic adjustment (r = - 0.29***), days until heading (r = - 0.23***) and biomass (r = 0.37***), yield was weakly correlated with second leaf area (r = - 0.13***) (Table 27). The results agree with those obtained by Fokar *et al*. (1998), Pillen *et al*. (2003). The results indicated that drought stress influenced genotypes for yield in the AB-QTL analysis (Table 18). Alleles from the wild barley ISR42-8 were associated with a positive effect on yield for three QTLs detected for yield. The three QTLs for yield increase are located on chromosomes 3H at (HV13GEIII; HVM62; HW01N04T3; and Bmac0029), 5H (Bmag0537), and 6H (Bmac0316) respectively (Table 18 and Figure 8). For heat tolerance four QTLs for yield increase were mapped on chromosomes 1H (HVABAIP), 2H(GMS003), 3H (HV13GEIII; HVM62; and HW01N04T3), and 4H (HVB23D) (Tables 28 and Figure 13). Similar results were found by (Tinker *et al*. 1996). Five QTLs were detected in barley for plant grain weight on chromosomes 2H, 5H and 7H (Bezant *et al*. 1997b). The strength of the trait improvement can be taken as a further measure of the efficiency of the QTL detection. In all AB-QTL analyses published so far for tomato and rice, the total yield could be raised due to the presence of at least one favorable exotic allele. The yield increases amounted to maximal values of 18% in rice (Xiao *et al*. *1996*; Xiao *et al*. *1998*),

of 17%, 34%, 15% and 27%, respectively, in four tomato studies (Tanksley *et al*. *1996*; Fulton *et al*. *1997*, *2000*; Bernacchi *et al*. *1998a*) and 7.7% in barley (Pillen *et al*. 2003). In our study for barley the maximum yield increase was associated with the exotic *Hsp* allele at three loci. The increase of yield at locus Bmac0029 $_{[3H]}$ could be detected in drought experiment and ranged from 4.4% to 18.4% with an average of 10.4%. The control treatment resulted in positive effects at three loci of *Hsp* alleles with a maximum up to 21.4 % at Bmac0029 $_{[3H]}$ and an average of 15.3 %. Under drought stress result showed positive effects for yield from the *Hsp* allele with a maximum of 13.1% at Bmac0029 $_{[3H]}$ and an average of 4.5%. Whereas, the *Hsp* allele at 2 loci resulted in negative effects for yield under drought stress in average for 4.3% (see Table 18). In the present study for heat tolerance in barley yield increase with the exotic *Hsp* allele at two loci the maximum up to 20.8% was associated with the exotic *Hsp* allele at HVM62 $_{[3H]}$. The control treatment resulted in positive effects for yield at two *Hsp* alleles of up to 23.1 % at HVM62 $_{[3H]}$. The control treatment negative effects in yield were revealed by *Hsp* alleles at 2 loci in average of 14.6%. Our results showed positive effects for yield from *Hsp* alleles at three up to 6.3% at GMS003 $_{[2H]}$ with average of 3.1%. Heat stress obtained negative effects for yield from *Hsp* alleles at two loci in average 1.8% (Table 28). The effects of *Hsp* allele of present study showed for drought and heat tolerance weak to strong effects. Classical QTLs for grain yield have been reported in overlapping BIN groups at marker Xpsb37 (L) (Bezant *et al*. *1997a*) and in marker intervals ABG472-ABG366 (Tinker *et al*. *1996*) and ABG472-ABG397 (Hayes *et al*. *1993*). In addition, Ellis *et al*. (*2002*) also reported a QTL for grain yield in the region between HVM68 and HVM67 on chromosome 4H where GMS89 $_{[4H]}$ is placed. Moderate conformity between the QTLs identified in our AB-QTL analysis and in classical QTL analysis can be regarded as a confirmation that most QTL effects from the exotic donor *Hsp* are unique. Thus, these QTLs can be exploited for improving and broadening the genetic basis of the barely elite gene pool, Pillen *et al*. (2003); nevertheless, it should be noted that there is also little conformity present between classical QTL studies. Thomas *et al* (*1995*) reported considerable differences in QTL identification between the Scottish cross Blenheim × E224/3 and the North American crosses Steptoe × Morex and Harrington × TR306. Likewise, Mather *et al*. (*1997*) reported that, when comparing the two aforementioned North American crosses, they found more differences than confirmations of QTL positions. Although the favorable allele effects of the *Hsp* donor accession ISR42-8 are less pronounced than the effects from exotic donors in previous AB-QTL analysis. By means of marker-assisted BC_2DH lines, we generate, BC_2DH-lines, which harbor the yield increasing *Hsp* alleles around the SSR loci HVM62 $_{[3H]}$, Bmag0357 $_{[5H]}$ and Bmac0316 $_{[6H]}$. The BC_2DH lines will be exploited for the validation of the original favorable *Hsp* allele effect and, as pure introgression lines, can be utilized for further breeding cycles. Thus, the

BC_2DH lines can be utilized for high-resolution mapping of the region of interest, ultimately leading to a map-based cloning of the QTL factor. The results are in agreement with those obtained by (Pillen *et al*. 2003). Both strategies have already been carried out in tomato. For example, Bernacchi *et al*. (1998b), Monforte and Tanksley (2000) and Monforte *et al*. (2001) produced detailed high-resolution maps of introgressed exotic tomato segments based on older AB-QTL analysis and validated the detected exotic effects in refined QTL- BC_2DH lines. Furthermore, the production of a high-resolution map has already led to the first cloning of a QTL factor (Alpert and Tanksley 1996; Frary *et al*. 2000).

Number of spikes per plant (SPK)

Two QTL were detected from three regions for number of spikes per plant on chromosomes 2H and 5H. The *Hsp* allele has a moderate effect for the NPK. The *Hsp* allele has positive effect of improved SPK 18.9% at GMS061 [5H]. The *Hsp* allele was associated with a 3.5% decrease of SPK at HVM36 [2H]. The *Hsp* allele caused an increase SPK in the control treatment of 27.0% at GMS061 [5H]. On the other hand, the *Hsp* allele caused a decrease SPK in the control treatment of 7.5% at HVM36 [2H. The *Hsp* allele caused an increase SPK under heat stress of 11.8% at HVM36 [2H], while it caused a decrease SPK under heat stress a 9.9% at GMS061 [5H] (Table 28). On contrast no QTL was detected for ear (Pillen *et al*. 2003). Only one QTL was detected for spike density on chromosome 3H (Kandemir *et al*. 2000). Moderately correlations under heat stress were revealed for number of spikes per plant with yield (r =0.38**), and harvest index (r =0.30***), as well as a weak association with number of kernels per spike (r = -0.19***), days until heading (r = -0.15***), number of leaves per main tiller (r = -0.8***)** flag leaf area (r = -0.13**), second leaf area (r = -0.09*) and biomass (r =0.13**). The results are in agreement with this obtained by (Pillen *et al*. 2003).

Flag leaf area (FLA)

One putative QTL for drought tolerance was located for flag leaf area on chromosome 1H marker GMS021. Moderate to strong effects were seen for the *Hsp* allele for flag leaf area in both, drought and heat experiments. Favorable allele effect was detected, reduced flag leaf area by 16.4%. The *Hsp* allele showed a decrease for flag leaf area in the control treatment and drought stress 13.6% and 20.1% respectively (see Table 18). Eight putative QTLs for heat tolerance were located for flag leaf area on all barley chromosomes except 1H and 6H. Five favorable allele effects of the *Hsp* alleles were detected for flag leaf area and improved at (HVSS1; HW01M22T3; HVKNOX3; HV13GEIII; HW01N04T3 and HVM62) by 3.7%, 5.2% 5.2%, 15.4% 16.9% and 19.6% respectively with an average 11.0%. Three loci resulted in a decrease by an average of 3.7%. The

Hsp allele at six loci showed an increase in flag leaf area in control treatment by an average 13.2%. Whereas two loci resulted in a decrease of flag leaf area by an average 9.6%. Heat stress reveled in negative effect for flag leaf area at five loci for the *Hsp* alleles with an average value of 20.3%. The *Hsp* alleles at three loci have positive effect increasing flag leaf area in heat stress up to 35.9%, 36.7%, 39.7% and 47.9% at (HVM62; HW01M22T3; HV13GEIII; HVSS1) respectively with an average 40.1% (Table 28). In our study an increase for leaf area related to heat stress after optimal heat, because after optimal heat all growth in plant reduced. When growth resources are limited by heat stress, the size of plant organs such as leaves, tillers, and spikes are reduced (Fischer, 1984). Similar finding was obtained by (Grindlay 1997; Yin *et al*. 1999); two QTLs were detected on chromosome 2H and 3H. Pleiotropy has been observed between number of tiller and panicle size and leaf area in sorghum (Pereira and Lee 1995). One locus on chromosome 4H showed significant associations with leaf area (Blauth *et al*. 1998). Flag leaf area under drought stress strongly correlated with first leaf area (r = 0.76***), second leaf area (r = 0.65***), and biomass (r = 0.55***), whilst moderate correlated with number of leaves per main tiller (r = - 0.37***), yield (r = 0.39***) and harvest index (r = - 0.28***). Altogether, it was weakly correlated with number of spikes per plant (r = 0.14***), number of kernels per spike (r = 0.13***), osmotic adjustment (r = 0.12**) and carbon isotope discrimination (r = -0.12**) (Table 16). Flag leaf area under heat stress was positively and strongly correlated with second leaf area (r = 0.58***), whilst it was negatively and moderately correlated with first leaf area (r = 0.49***). In addition a weak correlation with number of spikes per plant (r = - 0.12**) was observed (Table 27). In contrast no significant correlations were noticed for leaf area (Blauth *et al*. 1998).

First leaf area (ARE1)

Three QTLs were found for first leaf area. One favorable effect of the *Hsp* allele detected for first leaf area in heat tolerance, with a positive effect (17.7 %) of the *Hsp* allele detected at one locus (HY03C23T3) on chromosomes 4H. Furthermore, due to the *Hsp* allele at one locus control treatment positive effects was showed 23.8% at HY02P09T3 $_{[2H]}$. Positive effects (8.6 % and 13.2%) were detected by two favorable loci HY03N03T3 $_{[4H],}$ and GMS061 $_{[5H],}$ under heat stress (Table 28). In our study an increase for leaf area related to heat stress after optimal heat all growth in plants reduced. When growth resources are limited by heat stress, the size of plant organs such as leaves, tillers, and spikes are reduced (Fischer, 1984). In the present study for first leaf area the *Hsp* allele was moderate to strong effect on first leaf area. Byrne *et al*. (1997) have two QTLs affecting leaf area were suggested the presence of a single gene. Differences have been observed between tiller number, panicle size and leaf area in sorghum (Pereira and Lee 1995). One locus on chromosome 4H showed significant associations with leaf area (Blauth *et al*. 1998). However, first

leaf area under heat stress was positive and strongly correlated with second leaf area (r = 0.58***), while was positively and moderate correlation with flag leaf area leaf (r = 0.49***), however positive and weak correlation with number of leaves per main tiller (r = 0.08*), and days until heading (r = 0.10**) Table 27. On contrast no significant correlations were observed for leaf area (Blauth *et al*. 1998).

Heading date (HEA)

Only one putative QTL for days until heading was located on chromosome 1H at marker HVABAIP. The *Hsp* allele increased time to in heading by 2.7% at HVABAIP $_{[1H]}$. The *Hsp* allele resulted in an increase in time to heading in the control treatment of 4.6%. The favorable allele reduced time to heading under heat stress by 0.2%. A reduction in time to heading helps plants to escape from heat stress (Table 28). Lin *et al*. (1998) have five putative QTLs controlling heading date were detected on chromosomes 2H, 3H, 4H, 6H and 7H. Two previously detected QTLs on barley *Ppd* and *Sh2* loci on chromosomes 2H and 7H (Karsai *et al*. 1997). The effect of *Hsp* allele is weak to moderate for time to heading under heat tolerance. Two QTLs were detected for heading date on chromosome 2H (Kicherer *et al*. 2000). Twenty-two putative QTLs for days until heading were located on five chromosomes (Pillen *et al*. 2003). In our study one putative QTL found in the Scarlett*ISR24-8 cross QTL for days until heading was found with (HVABAIP) $_{[1H]}$. The first QTL is the putative QTL for heading date associated with HVM67 $_{[4H]}$ on BIN 13. A classical QTL for heading date was also detected in the same or in overlapping BIN groups at locus Bmy1 (Hackett *et al*. *1992*) and in marker intervals ABG397-ksuH11 and ABG397-Bmy1 (Hayes *et al*. 1993, *1996*). The second QTL was again detected for heading date but associated with *HvPRP1B* $_{[7H]}$ on BIN 12. This QTL was recovered at marker BCD512A (Laurie *et al*. *1995*) and in marker interval MWG539-MWG929 (Backes *et al*. *1995*). The third QTL was found for heading date associated with HVM6 $_{[5H]}$ on BIN 15. This QTL was also detectable in the marker interval MWG650-MWG002 (Backes *et al*. *1995*). For heading date four QTLs were mapped on emmer wheat (Peng *et al*. 2003). The resistance gene on 6H is located in the same region as a QTL for post-heading duration in the Rolfi x Botnia cross. A cluster of QTLs affecting yield, heading date and several malting quality traits has been recognized at the centromeric region of 6H in several barley crosses (Hayes *et al*. 1996). The putative linkage of this tolerance gene to QTLs for important characters may hinder its use in breeding. Correlations were strongly under heat stress for days until heading with osmotic adjustment (r = 0.56***) and number of leaves per main tiller (r = 0.50***), whereas there was a moderate correlation with number of kernels per spike (r = 0.33***) and second leaf area (r = 0.24***), yield (-0.23***) and harvest index (r = - 0.33), in addition was weakly

correlated with number of spikes per plant (r = - 0.15***), and first leaf area (r = 0.10*) (Table 27). Similar results were found by (Kicherer *et al*. 2000; Pillen *et al*. 2003).

Number of leaves per main tiller (LEA)

Only one putative QTL for number of leaves per main tiller for drought tolerance was located on chromosome 5H (Bmag0357). The *Hsp* allele has in an increase in effect for the number of leaves per main tiller 2.2% at Bmag0357 [5H]. The *Hsp* allele resulted decreasing number of leaves per main tiller in the control treatment of 1.6%.The *Hsp* allele increased the number of leaves per main tiller under drought stress by 6.1% (see Table 18). In the present study the *Hsp* allele was weak effect for number of leaves per main tiller. Four QTLs controlling number of leaves per main tiller under drought stress were found on chromosomes 1H, 5H, 6H and 7H (Teulat *et al*. 1997). Number of leaves per main tiller was moderately under drought stress correlated with number of spikes per plant (r = - 0.29***), number of kernels per plant(r = - 0.22***), flag leaf area (-0.38***), first leaf area (r = - 0.35***), second leaf area (-0.44***), yield (-0.40***) and harvest index (r = 0.23***), whereas it was negatively and strongly correlated with biomass (r = - 0.55***), but it was negatively and weak with osmotic adjustment (r = - 0.08*) and carbon isotope discrimination (r = 0.1*) (Table 16). Significant positive correlations were noted between numbers of leaves on the main tiller (Teulat *et al* 1997); the results are in agreement with the present results.

Biomass (MAS)

Three QTLs were found for biomass in drought tolerance on chromosomes 1H and 5H. The negative effect of the *Hsp* allele resulted in a 7.8% reduction of the biomass at HW01M22T3 $_{[5H]}$. However, favorable effects of the *Hsp* alleles were detected for biomass. They caused an increase in biomass of 1.2% and 8.9% at HY03I05T3 $_{[1H]}$ and Bmag035 $_{[5H]}$ respectively. Therefore, the *Hsp* alleles increased on biomass an average 5.1%. The *Hsp* alleles increased biomass in the control treatment and drought stress by a maximum of 13.1% at Bmag035 $_{[5H]}$ and a maximum of 6.3% at HW01M22T3 $_{[5H]}$ with an average of 9.6% and 4.0% respectively (Table 18). Three QTLs were located for biomass for heat tolerance on chromosomes 2H, 3H and 7H. One favorable effect of the *Hsp* alleles were detected for biomass and increased biomass by a maximum of 8.25% at HV13GEIII $_{[3H]}$, while for the other two loci the *Hsp* allele decreased biomass by on average 7.7% for both Bmag0321 $_{[7H]}$ and GMS003 $_{[2H]}$. Under control treatments biomass was increased by the *Hsp* alleles up to 10.0% at HVM62 $_{[3H]}$ and 10.4% at HV13GEIII $_{[3H]}$. The other two loci *Hsp* reduced biomass under control treatments by an average 8% at GMS003 $_{[2H}$ and Bmad0321 $_{[7H]}$. Heat stress decreased biomass at three *Hsp* alleles by an average 3.8% (Table 27). Similar finding

was obtained by (Li *et al*. 2001). One QTL was detected for biomass at "*denso* "locus (Yin *et al*. 1999). One QTL was detected for biomass at marker H*v*A22S $_{[7H]}$ (Pillen *et al*. 2003). However, biomass was positive and strongly correlated under drought stress with number of spikes per plant (r = 0.53***), number of leaves per main tiller (r = - 0.55***) flag leaf area (r = 0.55***), first leaf area (r = 0.57***), second leaf area (r = 0.63***), and yield (r = 0.723***), while it was moderately correlated with number of kernels per plant (r = 0.476***), carbon isotope discrimination (r = - 0.15**) and harvest index (r = - 0.46***). Biomass was weakly correlated with osmotic adjustment (r = 0.108**) (Table 16). However, biomass was positively and moderately correlated under heat stress with yield (r = 0.37***), while it was weakly correlated for biomass with number of spikes per plant, number of kernels per plant (r = 0.15**), and harvest index(r = - 0.22***) (Table 27). Similar result was reported by (Pillen *et al*. 2003).

Harvest index (HI)

Four putative QTLs were located for harvest index on chromosomes 1H, 2H and 6H. Favorable effects of the *Hsp* alleles detected for harvest index resulted in improved biomass at three loci with a 1.7%, 3.2% and 9.0% at GMB1008$_{[6H]}$, GMS003$_{[2H]}$ and HVM36 $_{[2H]}$ with an average 4.6%. Two loci *Hsp* allele increased harvest index in the control treatment by 13.17% and 14.6% at GBM1007 $_{[1H]}$ and GBM1008 $_{[6H]}$ with average 13.4%. Under heat stress harvest index was increased from two *Hsp* alleles by 19.1% and 30.8% at GMS003 $_{[2H]}$ and HVM36 $_{[2H]}$ respectively by an average 24.9% (Table 28). In the present study for harvest index is moderate to high effects from the *Hsp* alleles under heat stress. Two putative QTLs were located for harvest index obtained by (Pillen *et al*. 1998; 2003). A total of 8 QTLs were detected for harvest index (Okogbenin and Fregene 2001). Harvest index under heat stress was strongly correlated with yield (r = 0.72***), while it was moderately correlated with number of spikes per plant (r = 0.30***), osmotic adjustment (r = - 0.30), days until heading (r = - 0.33***), number of kernels per spike (r = 0.27***), number of leaves per main tiller (r = - 0.25***), and biomass (r = - 0.21), whereas it was weakly correlated with relative leaf water content (r = - 0.08*) and second leaf area (r = - 0.14***) (Table 27). Similar finding was obtained by (Pillen *et al*. 2003).

Carbon isotope discrimination (CID)

Two putative QTLs for carbon isotope discrimination were located on chromosomes 5H and 7H. Two loci showed an M*D interaction were significant. Two *Hsp* alleles having favorable effects were detected, they improved negative effects for carbon isotope discrimination of 0.9% and 1.3% at Bmac0163 $_{[5H]}$ and Ebmac0755 $_{[7H]}$. In the control treatment the *Hsp* allele at one locus were

positive effects for CID of up to 1.2 % at Bmac0163 [5H], while it had negative effect for CID at one locus of 3.1% at EBmac0755 [7H]. Drought stress resulted in a positive effect from the *Hsp* allele at one locus of 0.6% EBmac0755 [7H], while it had a negative effect for CID at one locus of *Hsp* allele of 3.2% at Bmac0163 [5H] (Table 18). Our results are in agreement with (Robinson *et al*. 2000); wild barley germplasm has been tested for physiological traits associated with abiotic stress tolerance. Biomass changes under experimentally imposed stress, measurements included shoot stable isotope discrimination ($CID^{13}C$), % C. The abundance of carbon isotope discrimination has been used as a screening tool to assess barley genotypes for their responses to abiotic stress (Handley *et al*. 1997). Ten QTLs were identified: one was specific to one environment, two presented interaction with the environment, six presented main effects across three or two environments and one presented both effects. Heading date did not contribute to the environment (E) and G x E effects acting on CID. Seasonal rainfall and the ratio of rainfall to evapotranspiration made large contributions to the environmental effect, but their influence on G x E was weaker. Eight QTLs for CID co-located with QTLs for physiological traits related to plant water status and/or osmotic adjustment, and/or for agronomic traits previously measured on the same population. Some perspectives in terms of characterizing drought tolerance are evoked (Teulat *et al*. 2002). Present results for carbon isotope discrimination under drought stress was moderate correlation with days until heading (r = 26***), furthermore it was weak correlated with number of leaves per main tiller (r = 0.1*), flag leaf area (r = -0.12**), first leaf area (r = -0.13***), biomass (r = -0.15***), and harvest index (r = 0.17***). Our results are in agreement with those obtained by (Fischer *et al* 1998).

In this study, we report on the first AB-QTL project which utilizes spring barley as a model. Our goal was: (1) to localize QTLs for the expression of quantitative traits in spring barley. The 97 polymorphic SSRs revealed 54 putative QTLs from 78 regions in two groups. The 20 putative QTLs were detected for drought treatments; and the 34 putative QTLs found for the heat treatments.

(2) To identify favorable QTL alleles from the wild barley donor which improve the respective traits. On average, 30 (55.5%) favorable *Hsp* allele effects were detected for improvement of both drought and heat tolerance in the tested lines. 14 (70.0%) favorable *Hsp* alleles effects for drought tolerance and 16 (47.0%) favorable effects of the *Hsp* alleles for heat tolerance (see Table 29 and 30). Theses results are pertaining to better improvement in drought tolerance than heat.

6. Summary

In the season (2001) the performance of four German barley cultivars was examined under drought and heat stress. The four cultivars were grown under four treatments (35%, 50%, 65% and 100% FC) for drought experiment, under heat experiment three treatments (normal climate, heat stress and heat stress plus 65% FC) in the greenhouse, 12 traits for heat and drought tolerance were examined, in order to determine traits, which show significant deviations for drought and heat stress in the plants and to determine which treatments for the drought experiment is applause of analyze stress response in the BC_2DH population. Results showed little or no differences between 35% and 50% FC, 50% and 65% FC as well as 65% and 100% treatments. On the other hand, high differences between 35% and 65% as well as between 50% and 100% (Table 10). The heat experiment the cultivars were tested under three treatments; normal climate, heat stress and heat stress plus 65% FC. The results revealed high differences among the three treatments for heat experiment (Table 21).

In the years 2002-2003, 323 individuals of BC_2DH population derived from a cross between a cultivar variety (Scarlett) and wild variety (ISR42-8) were genotyped with 97 DNA markers. The BC_2DH lines were evaluated in greenhouse trials for drought and heat. Altogether 13 parameters for the determination of the drought tolerance and 12 parameters for the investigation of the heat tolerance were examined. There were two treatments for drought experiment; 50% FC level for drought stress and at 100% FC level for control. Two treatments were used for the heat experiment (normal climate and in greenhouse) and the traits measured were: relative leaf water content, osmotic adjustment, heading date, number of spikes per plant, number of kernels per spike, number of leaves per main tiller, flag leaf area, first leaf area, second leaf area, carbon isotope discrimination (for drought experiment), yield, biomass and harvest index. Single-point marker analysis by means of a three-factorial ANOVA rather than an interval mapping was preferred for QTL analysis. A QTL analysis was calculated with 3-factorial ANOVA, with marker main effect, drought or heat treatment and year. The model used to detect QTLs included the effects of marker genotype (M), drought treatment (D), or heat treatment (H), M*D interaction or M*H interaction. Under the assumption of a mixed model with the marker as a fixed effect, the drought treatment or heat treatment was as a fixed effect and year as a random effect. The genotype and phenotype data were subjected to analysis in GLM procedure of SAS software (SAS institute, 1999). The 323 BC_2DH lines were successfully genotyped polymorphic with 97 SSRs. All 97 mapped SSRs cover 1013 cM of the barley genome; the mean SSR density is equal to 11.1 cM (Table 17). The Scarlett*ISR42-8 map includes four gaps with a marker distance of more than 30 cM, the gaps are located on chromosomes 3H, 5H and 6H (Table 17, Figure 7).

The 97 polymorphic SSRs revealed 54 putative QTLs from 78 regions in two groups. The 20 putative QTLs were detected for drought treatments; and the 34 putative QTLs found for the heat treatments. Altogether, 30 (55.5%) favorable *Hsp* allele effects were detected in both, the drought and the heat experiment (see Table 29 and 30), genotype was associated with an improvement of the trait compared to the homozygous (*Hordeum vulgare* L. *distichon*, hereafter abbreviated with *Hvd*) had genotype as shown in Figure 8, 13 and Table 18).

20 putative QTLs were detected for drought experiment. Eight regions showed a marker main effect and 25 regions an M*D interaction (Figure 8 and Table 18). In two cases, both effects (marker main effect and M*D interaction) were significant. Altogether, 14 (70. %) favorable *Hsp* allele effects were detected (see Table 29). The putative QTLs were unevenly distributed over the chromosomes (Figure 8). Four QTLs were located on chromosome 1H, one QTL was located on chromosome 3H, two QTLs were located on chromosome 4H, eight QTLs were located on chromosome 5H, two QTLs were located on chromosome 6H, three QTLs were located on chromosome 7H, and zero QTLs were detected on chromosomes 2H. Most of the favorable *Hsp* alleles were located on chromosomes 1H, 5H and 7H (2, 8 and 3 respectively). The distribution of putative QTLs among the 97 genotyped SSR markers was also irregular. Marker Bmag0357 $_{[5H]}$ showed putative QTL effects on three traits (LEA, MAS and YLD), Marker Bmac0316 $_{[6H]}$ obtained putative QTL effects on two traits (OA and YLD) and Marker HW01M22T3 $_{[5H]}$ revealed putative QTL effects on two traits (MAS and OA). The detected putative QTLs are represented for the traits in the Table 18.

Ninety seven polymorphic markers detected 34 putative QTLs were detected from 45 regions for heat experiment. Four marker main effect at 41 an M*H interaction were significant at $P \leq 0.01$ (Figure 13 and Table 28). 16 (47.0%) favorable QTL effects were detected (see Table 30). The putative QTLs were unevenly distributed over the chromosomes (Figure 13). 8 and 9 QTLs were located on chromosomes 4H and 2H, respectively. Most of the favorable Hsp alleles were located on chromosomes 3H and 4H (3, and 5 respectively). No favorable were detected on chromosome 1H. At the marker GMS003 $_{[2H]}$ was found putative QTLs effects for three traits (MAS, YLD and HI). HV13GEIII $_{[3H]}$ was found putative QTLs effects for four traits (OSM, FLA, MAS and YLD). HVM62 $_{[3H]}$ was detected putative QTLs effects on four traits (OSM, FLA, MAS and YLD). HW01N04T3 $_{[2H]}$ showed putative QTLs effects on three traits (OSM, FLA and YLD). HY02P09T3 $_{[1H]}$ obtained putative QTLs effects on three traits (ARE1, FLA and OSM). The detected putative QTLs are represented for each trait is shown in Table 28.

These traits were osmotic adjustment, yield, biomass, relative leaf water content; numbers of leaves per main tiller and flag leaf area were controlled with 7, 3, 3, 3, 1 and 1 QTL, respectively, in the drought experiment. Under drought stress first leaf area was positively and strongly correlated with flag leaf area (r = 0.77). Positive correlations were expressed by second leaf area with flag leaf area (r= 0.66) and first leaf area (r = 0.78). Yield was positively correlated with harvest index (r = 0.72), number of spikes per plant (r = 0.63) and number of kernels per spike (r = 0.69). Biomass showed positively correlations with number of spikes per plant (r = 0.53), number of leaves per main tiller (r = -0.55), flag leaf area (r= 0.55), first leaf area (r = 0.56) second leaf area (r = 0.63) and yield (r = 0.72). The 16 (47.0%) favorable effects were detected for heat tolerance. Flag leaf area, osmotic adjustment, yield, harvest index, biomass, first leaf area, relative leaf water content, number of spikes per plant and heading date were controlled with 8, 7, 4, 4, 3, 3, 2, 2 and 1 QTL respectively, in heat experiment. Correlations for days until heading was with osmotic adjustment (r = 0.57), and number of leaves per main tiller (r = 0.51). Positive correlations were expressed by second leaf area with flag leaf area (r = 0.59) and first leaf area (r = 0.51). Yield was positive and strongly correlated with harvest index (r = 0.73).

Drought results for BC_2DH lines (AB-DH lines Scarlett*ISR42-8 population)

The analysis of variance of BC_2DH lines for the drought experiment was highly significant for all parameters. The variation between years was highly significant for all parameters except relative leaf water content. The analysis of variance between drought treatments was highly significant for all parameters. The interaction between drought treatments and years was significant for all parameters except relative leaf water content and osmotic adjustment. The interaction between drought treatments and BC_2DH lines was significant for all parameters except number of spikes per plant. The interaction between years and BC_2DH lines was highly significant for all parameters. The analysis of variance for the interaction between BC_2DH lines, years, and drought treatments was significant for all parameters except number of leaves per main tiller (see Table 14).

QTLs for drought tolerance

Three putative QTLs for Relative leaf water content were found. All *Hsp* alleles showed positive effects for control treatment except $GMS089_{[4H]}$. The *Hsp* increased relative leaf water content in the control treatment of 4.0% at both $Ebmac0701_{[4H]}$ and $TACMD_{[4H]}$. On the other hand, the *Hsp* allele increased the RWC under the drought stress up to 3.9% at ($GMS089_{[4H]}$ (Table 18). A total of 7 putative QTLs have effect on osmotic adjustment were found. However, five favorable

Hsp allele effects were detected, these *Hsp* allele improved osmotic adjustment with a maximum value of 22.3% at HW01M22T3 [5H]. The *Hsp* allele at four loci increased osmotic adjustment in the control treatment up to maximum of 7.2% HY02J05T3 [5H]. Five *Hsp* alleles showed an increase in osmotic adjustment under drought stress up to 39.5% at HW01M22T3 [5H] (see Table 18). Only one putative QTL for number of leaves per main tiller was located on chromosome 5H. The *Hsp* allele effect increased the number of leaves per main tiller by 2.2% at Bmag0357 [5H]. The *Hsp* allele resulted decrease control treatment of 1.6%. Whereas *Hsp* allele lifted the number of leaves per main tiller of 6.1% under drought stress (Table 18). One putative QTL was located for flag leaf on chromosome 1H. Favorable *Hsp* allele effect was reduced flag leaf area by 16.4% at GMS021 [1H]. The *Hsp* allele showed a decrease in flag leaf area in the control treatment 13.6% at GMS021 [1H]. The *Hsp* allele obtained negative effect of value found 20.1% at GMS021 [1H] under drought stress (Table 18). Three putative QTLs for yield were found. Three favorable *Hsp* alleles were detected positive effects, was improved yield to maximum of 18.4% at Bmac0029 [3H]. The *Hsp* alleles at three loci improved yield in Control treatment up to 21.4 % at Bmac0029 [3H]. Result showed positive effects from the *Hsp* alleles at two loci up to 13.1% at Bmac0029 [3H] under drought stress (Table 18). Three QTLs were located for biomass. Effects of favorable *Hsp* alleles were detected for biomass, positive effects were found at 1.2% and 8.9% at two loci both at HY03I05T3[1H] and Bmag035[5H], respectively. Biomass was increased in control treatments at two loci *Hsp* allele a maximum of 13.1% at Bmag0357 [5H]. The *Hsp* allele increased biomass at two loci a maximum 6.3% HW01M22T3 [5H] under drought stresses (Table 18). Two putative QTLs for carbon isotope discrimination were located on chromosomes 5H and 7H. Two *Hsp* alleles having favorable effects were detected, they improved negative effects for carbon isotope discrimination of 0.9% and 1.3% at Bmac0163 [5H] and Ebmac0755 [7H]. In the control treatment the *Hsp* allele at one locus was negative effect for CID of 3.1% at EBmac0755 [7H]. Drought stress resulted in negative effect for CID at one locus of *Hsp* allele of 3.2% at Bmac0163 [5H] (Table 18).

Heat results for BC_2DH lines (AB-DH lines Scarlett*ISR42-8 population)

The analysis of variance for BC_2DH lines of heat experiment was significant for all parameters except number of leaves per main tiller and first leaf area. The variation between years was significant for all parameters except relative leaf water content and number of leaves per main tiller. The analysis of variance between heat treatments was highly significant for all parameters. The interaction between heat treatments and years was significant for all parameters except relative leaf water content. The interaction between heat treatments and BC_2DH lines was significant for all

parameters except number of leaves per main tiller and first leaf area. The interaction between years and BC_2DH lines was highly significant for all parameters except number of leaves per main tiller and first leaf area. The analysis of variance of the interaction between BC_2DH lines, years, and heat treatments for heat experiment revealed a significant variation for all parameters except number of spikes per plant, number of leaves per main tiller and first leaf area (see Table 25).

QTLs for heat tolerance

Two putative QTLs for relative leaf water content were found. The *Hsp* alleles were positive effects, the *Hsp* at two loci increased RWC in control treatment with 6.5 % (GBM1016 [2H]) (Table 28). Two QTL were detected for number of spikes per plant. Two favorable *Hsp* alleles detected for number of spikes per plant had positive effect of 18.9% of increase the number of spikes per plant at GMS061 [5H]. The *Hsp* allele caused an increase in control treatment 27.0% at GMS061 [5H]. The *Hsp* allele caused an increase under heat stress 11.8% at HVM36 [2H] (Table 28). A total of 7 putative QTLs were located for osmotic adjustment. Favorable effects of the *Hsp* alleles for OA were observed for 3 alleles on chromosomes 4H, 6H and 7H. Four loci with the *Hsp* allele decreased OA a maximum of 11.6% at HY02P09T3 [2H]. On the other hand, 3 favorable effects of the *Hsp* alleles detected for OA improved up to 8.7% at BMS64 [7H]. The *Hsp* allele of 3 loci lifted OA in control treatment up to 15.4% at HV13GEIII [3H]. Four loci *Hsp* allele showed increasing under heat stress for OA up to 14.4% at BMS64 [7H] (Table 28). Only one putative QTL for days until heading was found. The *Hsp* allele increased heading date 2.7% at HVABAIP [1H]. The obtained *Hsp* allele increased heading date in control treatment with 4.6%, whereas, the *Hsp* allele reduced heading time by 0.2% under heat stress (Table 28).

Eight putative QTLs putative were located for flag leaf area. Five favorable effects of the *Hsp* alleles detected for flag leaf area lifted improve at HVM36 [4H] by a maximum of 19.6%. The *Hsp* allele at four loci showed increase FLA in control treatment maximum of 25.4% at HW01M22T3 [5H]. The *Hsp* alleles at three loci showed a positive effect in increase of flag leaf area up to 47.9% at HVSS1 [7H] under heat stress (Table 28). Three putative QTLs were located for the first leaf area. Positive effect (23.8 %) was detected by one locus HY03N03T3 [4H] lifted ARE1 in control treatment. Favorable effect of the *Hsp* allele was detected for first leaf area, positive effect (17.7 %) was detected by one locus HY03C23T3 [4H]. Two loci *Hsp* alleles were showed positive effects 8.6% and 13.2% at HY03N03T3 [4H], GMS061 [5H], respectively under heat stress (Table 28). Altogether, 4 putative QTLs for yield were found. Two favorable effects of the *Hsp* alleles detected

for yield improved positive effects with maximum of 20.8% at HVM62 [3H]. The *Hsp* alleles resulted positive effects at two loci for yield in control treatment up to 23.1 % at HVM62 [3H]. Result showed positive effects *Hsp* alleles at three loci up to 6.3% at GMS003 [2H] (Table 28). Three QTLs were located for biomass. One favorable effect of the *Hsp* alleles detected for biomass lifted improve a maximum of 8.3% at HV13GEIII [3H]. The *Hsp* allele at one locus in control treatments increased biomass a maximum of 10.4% at HV13GEIII [3H. The *Hsp* allele decreased at three loci a maximum of 6.6% at GMS003 [2H] under heat stress (Table 28). Four putative QTLs were located for harvest index. Favorable effects of the *Hsp* alleles detected for harvest index obtained positive improved at three loci with a maximum of 9.0% at HVM36 [2H]. The *Hsp* allele at two loci increased harvest index in control treatments up to 14.6% at GBM1008 [6H]. The *Hsp* allele lifted harvest index at two loci a maximum of 30.8% at HVM36 [2H] under heat stress (Table 28).

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Acknowledgments

I would like to express my deep and sincere gratitude to Prof. Dr. Jens Léon for suggesting this subject, his kindness, admirable supervision, direct guidance and fruitful and interesting discussions throughout this work. He assisted in all matters, provided solutions to different problems and always gave input even at times of personal anxieties. Prof. Dr. Léon supported and helped me from beginning till the end of the study.

Deepest gratitude is due to Prof. Dr. M. Janssens who never hesitated to accept the role of co-referee Prof. Dr. M. Janssens has been very helpful. His tireless efforts to help in reordering the list of reference are highly acknowledged.

My deep thanks to PD Dr. Klaus Pillen. He has given me the BC_2DH population Scarlett*ISR42-8 which was developed by him and colleagues. He has helped me in SAS analysis and read my thesis in advance. His keenness and valuable discussions have been very helpful.

My deepest thanks to Mrs. Maria von Korff, Mr. Huajun Wang, Ms. Merle Noschinski and Mr. Carsten Golletz for genotyping the BC_2DH population Scarlett*ISR42-8.

I am grateful and deeply indebted to the staff and members of the institute for their generous encouragement and kind help during my stay in the institute.

I would like to express my deep thanks to the Egyptian government for providing me with a PhD scholarship and the Department of Crop Science and Plant Breeding (Institut fuer Pflanzenbau, Professur fuer Speziellen Pflanzenbau und Pflanzenzuechtung) University of Bonn for support of this work. I would also like to acknowledge the support of the German Plant Genome initiative (GABI) for financing the genotyping of the BC_2DH population.

My deep thanks to Prof Dr. A. M. Damarany Dean of Faculty, Assoc. Prof. G.A.R. El-Sherbeny Head of Agronomy Dep. and the staff members of the Department of Agronomy, Sohag Faculty of Agriculture, South Valley University, Sohag, Egypt for conducting of one-season field trials at the Experimental Station of the Faculty.

I thank Dr. Thomas Gebbing (Institut fuer Pflanzenbau, Universitaet Bonn) for carbon isotope discrimination measurement with Delta –S Isotope ratio mass spectrometer.

I thank Prof. Dr. H. Schnabl and Dr. R. Boehm (Institut fuer Molekulare Physiologie und Biotechnologie der Pflanzen der Universitaet Bonn) for osmotic adjustment measurement with OSMOMAT300.
My special thanks to my mother, father, wife (Omaima) and sons (Mohamed and Ali) for lovely smiles that can relieve any kind of tiredness.

To all of you, thank you very much.

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