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QTL Analysis for Drought Tolerance Related to Root and Shoot Traits in Barley (*Hordeum vulgare* L.)

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Zussamenfassung

Die Verbesserung der Trockentoleranz von Kulturgerste durch die Identifizierung positiver QTL-Allele von Wildgersten (*H. vulgare* ssp. *spontaneum* C. Koch) ist ein großes Ziel in der Gerstenzüchtung. Daher waren die übergeordneten Ziele der geplanten Studie: 1) Variation in Sproß, Wurzel und physiologischen Eigenschaften von BC_2DH-Linien unter kontrollierten und trockengestressten Bedingungen zu beurteilen. 2) Lokalisierung und Charakterisierung QTLs bezüglich Trockentoleranz . Die Kartierung wurde unter Verwendung von phänotypischen Daten aus drei Versuchsjahren und 371 DNA-Marker durchgeführt. Die phänotypischen Daten wurden unter kontrollierten und trockenstressbedingten Bedingungen durchgeführt. Die Varianzkomponentenanalyse zeigt ein breites Spektrum an Variabilität für die Mehrheit der untersuchten Merkmale. Insgesamt konnten 79 putative QTLs für 15 untersuchte Merkmale unter 5565 Marker x Merkmal Kombination in der Gerstenpopulation S42 nachgewiesen werden. Diese könne in 55 QTLs für Sproßlmerkmale, 15 QTLs für Wurzelmerkmale und 9 QTLS für physiologische Merkmale unterteilt werden. Insgesamt 27 QTLs zeigten positive Effekte aufgrund der Anwesenheit von exotischen Allelen. Die meisten der vermutlichen QTLs wurden auf den Chromosomen 1H, 2H, 4H und 5H lokalisiert. Zum Beispiel hatten zwei QTLs (QWS.S42.1H und QWS.S42.4H) positive Effekte durch exotische Allele bezüglich verminderte Welke um 17%. Die SSR-Marker GMS2 (2H), HvNAM2(2H) und M1o(4H) sind assoziiert mit QTls bezüglich Anzahl Triebe/Pflanze und Anzahl Ähren/Pflanze und die Introgression des Wildgerstenalleles ermöglicht die Erhöhung beider Merkmale in der S42 Population. Für das Merkmal Wurzellänge und das Vernalisationsgen $VrnHi_{[5H]}$ ergaben sich Signifikanzen mit dem QTL (*QRL.S42.5H*). Die Anwesenheit des exotischen Allels an diesem Markerlocus bewirkte eine Zunahme des Wurzelwachstums um 9,17% unter Trockenstress Die Anwesenheit des exotischen Allels für Marker MGB338 auf Chromosom 5H führte zu erhöhten Prolingehalten in den *Hsp*-tragenden BC_2DH-Linien um 53%. Die Mehrheit der epistatischen Effekte, die in dieser Studie nachgewiesen wurden, hatten positive Auswirkungen auf den phänotypischen Wert. Interessanterweise reagierten die exotischen Allele nur positiv bei trockengestressten Bedingungen, welches auf Trockenstress induzierbare Gene schließen lässt. Die Studie unterstreicht die Bedeutung von exotischen Allelen im Zusammenhang mit Trockenstress. Anschließend kann ein kombinatorischer Ansatz für die Selektion auf exotische Allele für die negativen Auswirkungen des Trockenstresses angewendet werden.

Abstract

Enhancement of drought tolerance of cultivated barley via identifying the potential and beneficial QTL alleles of wild species (*H. vulgare* ssp. *spontaneum* C. Koch) is a great target in barley breeding. Therefore, the overall objectives of the proposed study were: 1) to assess variations in shoot, root and physiological traits of BC_2DH lines under control and drought stress conditions. 2) to localize and characterize the QTLs underlying drought tolerance related to shoot, root and physiological traits. Mapping was conducted using a combination of phenotypic data of three investigated years and 371 DNA markers. This investigation was done under control and drought stress conditions. Components of variance revealed a wide range of variability for majority of the investigated traits. In total, 79 putative QTLs for 15 studied traits were detected among 5,565 marker by trait combinations in the population S42 under study. They can be divided into 55 QTLs for shoot traits, 15 QTLs for root traits and 9 QTLs for physiological traits. Overall 27 (34.1 %) QTLs showed favorable effects derived from the presence of exotic alleles. Most of putative QTLs were located on chromosomes 1H, 2H, 4H and 5H. For instance, two QTLs (*QWS.S42.1H* and *QWS.S42.4H*) had favorable effects due to the presence of the exotic alleles (*Hsp*) that were responsible for decreasing plant wilting score by 17%. The SSR markers GMS3 $_{[2H]}$, HvNAM2 $_{[2H]}$ and M1o $_{[4H]}$ were associated with QTLs are likely to be dominating number of tillers/plant and number of spikes/plant and the introgressions from wild barley may increase both traits in S42 population. Also for root length, the vernalisation gene VrnH1$_{[5H]}$ was associated significantly with the QTL (*QRL.S42.5H*). The presence of exotic alleles at this marker locus led to increase root length by 9.17 % under drought conditions. For proline accumulation, the superior performance of exotic allele at marker locus MGB338 on chromosome 5H suggests a transgression effect of the exotic alleles and led to increase proline content in the BC_2DH lines carrying *Hsp* alleles by 53% under drought conditions. The majority of the digenic epistatic interaction pairs which were detected in current study had favorable effects in enrich the phenotypic values of the studied traits. Interesting, these exotic QTL alleles responded favorably under drought conditions only that indicates the possibility of underlying a novel drought inducible gene. This study has highlighted the role of the exotic alleles for the detection of favorable leads for drought tolerance. Subsequently, a combinatory approach for the selection of favorable exotics alleles can be employed to develop a better shield against the adverse effects of drought.

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Foreword

Abiotic stresses curtail production and lower the quality and nutritional value of the grain in cereal crops world-wide. Among all abiotic stresses, drought is the most important from the economic standpoint. Drought tolerance in plants is one of the most interesting phenomena in all of biology (Wood 2007). Crop yield losses due to drought stress are considerable.

Barley (*Hordeum vulgare* ssp. *vulgare* L.) is one of the important crops worldwide and provides an excellent system for genome mapping and genetic studies, due to (1) its diploid nature, (2) low chromosome number (2n=14), (3) relatively large chromosomes (6-8 μm), (4) high degree of self fertility, and (5) ease of hybridization (Sreenivasulu *et al.* 2008, Hussain *et al.* 2006). Barley genotypes, in particular landraces and wild species, represent an important source of variation for adaptive traits that may contribute to increase yield and yield stability under drought conditions, and that could be introgressed into improved varieties. Producing more drought-tolerant of barley as well as the other crops would be the most economical approach to improve agricultural productivity and to reduce agricultural use of fresh water resources in arid areas (Jenks and Hasegawa 2005). As a result, identifying and understanding the genetics basis of drought tolerance mechanisms in crops is fundamental to enable breeders and molecular biologists to develop new varieties with more drought tolerant characters (Zhang *et al.* 2001).

Genetically, drought stress tolerance is a quantitatively inherited trait, controlled by several genetic loci (QTL). Furthermore, crop performance under drought conditions is a highly complex phenomenon because of unpredictable factors in the environments and the interaction with other abiotic and biotic factors (Reynolds *et al*. 2006). Tolerance to drought involves a complex of mechanisms working in combination to avoid or tolerate water deficits (Diab 2004). Adaptive mechanisms involve different root and shoot characteristics that allow plants to maintain high internal water status when available water is less than the evaporative demand (Zhang *et al*. 1999, Farooq *et al.* 2009). In addition, it has been reported that the physiological traits such as relative water content, proline accumulation and osmotic adjustment are considered to be associated with plant adaptability to drought-prone environments. (Ludlow and Muchow 1990, Diab *et al.* 2004, and Cattivelli *et al.* 2008, Farooq *et al.* 2009). Knowledge and understanding of drought tolerance related traits are

important for further understanding drought tolerance mechanisms of influences water and nutrient uptake, maintenance of the whole plant.

The advent of molecular markers, genomic technologies and statistical methods has revolutionized the genetic analysis of crop plants and provide valuable tools to identify chromosomal regions influencing tolerance to drought stress. This led to an increasing understanding the processes underlying plant responses to drought from the molecular through the whole plant level (Chaves *et al.* 2003 and Bradford *et al.* 2005). Marker technologies and saturated marker maps allow the location of genomic regions or quantitative trait loci (QTL) with significant effects on drought tolerance or yield stability under adverse environmental conditions. QTL mapping is a very popular and powerful tool to assign specific positions to genes contributing to traits related to drought. QTL mapping has been used widely for nearly two decades during which molecular markers have become available in conjunction with interval mapping methods (Lander *et al*. 1986). QTL mapping is a first step towards unraveling the molecular basis of drought resistance, i.e., by map-based cloning (Frary *et al.* 2000). QTL analysis can be performed to statistically analyze the association between markers and traits of interest. This identifies regions of the chromosomes that influence these traits. QTL maps have been made for traits thought to be involved in drought tolerance in many species including rice, barley, and wheat (Zhang *et al.* 2001; Teulat *et al*, 2001; Teulat *et al.* 2003; Quarrie *et al.* 2005). Cattivelli *et al.* (2008) reviewed progress of breeding for drought tolerance and suggested that markers tightly linked to traits conferring drought tolerance could improve breeding efficiency. The identification of these QTLs with linked markers allows the breeders to use marker-assisted selection as a complementary tool instead of traditional selection. Numerous QTL mapping studies examining drought tolerance is complex and is comprised of contributions from multiple loci (Diab *et al.* 2004, Siangliw *et al*. 2007).

Another interesting point is the expression of the quantitative phenotype that can be controlled through genotype, environment and genotype by environment interaction effects. Furthermore, genotype effects can be attributed to major genes, quantitative trait loci (QTL) and gene by gene interactions, which are also termed epistatic interactions. In addition, markers showing repeatable interactions with different environments and treatments that can give insight into the genetics of adaptation to drought stressed environments (von Korff *et al*. 2008, 2010). The improved coverage of the barley genetic map with DNA markers will

facilitate the mapping of genes and QTLs which are of economic importance in barley, and support studies of genetic diversity, pedigree analysis and the display of graphical genotypes.

In the present study, we used 301 lines of a BC_2DH population carrying wild barley (*H. vulgare* ssp. *spontaneum* C. Koch.) introgression alleles in order to identify the beneficial exotic alleles which are important for the expression of the drought related traits.

1 Introductory Review

Cereals, including wheat, rice, maize, sorghum, barley, rye, oats and millets constitute the staple food of the world since their domestication approximately 10,000 years ago. They are the most important cultivated plants for food production and acreage, providing more than 75% of human food needs (FAO, 2009). Most likely, they will remain as a major food source in the foreseeable future. Therefore, any constraints on cereal production directly impact world food security. Barley (*Hordeum vulgare* ssp. *vulgare* L.) is one of the seven internationally grown cereal grains, currently ranking fourth in world production behind maize, rice, and wheat and ahead of sorghum, oats, and rye (FAO 2009). A doubled haploid population of barley was used in this study, therefore we will focus on this crop in the present literature review.

1.1 Barley crop

The importance of barley (*Hordeum vulgare* ssp. *vulgare* L.) as a crop plant has prompted widespread genetic research onto this species. In the following parts, more information about barley crop is reviewed.

1.1.1 World barley production and utilization

Barley is a short season, early maturing grain with a high yield potential, and may be found on the fringes of agriculture in widely varying environments (Harlan 1976). World barley production in 2009 was approximately 155.1 million metric tons (MMT) produced on 54.13 million hectares (MH). Europe had the largest growing area of barley, harvesting 27.8 MH and producing 95.9 MMT in 2009, which was 61.8% of the total world barley production. It is grown for animal feed, human food, and malt. However, in developing countries, most barley is grown in marginal environments, often on the fringes of deserts and steppes or at high elevations in the tropics, receiving modest or no inputs. This partly explains

why yields there are nearly half of those in developed countries. Although barley is considered to be one of oldest cultivated cereal grains and was used extensively as a food in the past. Barley use as food in the European Community was even less (0.3%) than in the United States. The largest use for barley as a food was in Morocco (61%), Ethiopia (79%), China (62%), and India (73%) (Kent and Evers 1994).

1.1.2 Taxonomic position and origin of barley

Linnaeus was the first to provide a botanical description of barley in his Species Plantarium in 1753 (Bothmer and Jacobsen 1985). Barley is a grass belonging to the family Poaceae, the tribe *Triticeae* and the genus *Hordeum*. There are 32 species, for a total of 45 taxa in the genus *Hordeum* that are separated into four sections (Bothmer 1992). The four sections proposed by Bothmer are as follows: *Hordeum*, *Anisolepis*, *Critesion*, and *Stenostachys*. The division of the genus into sections puts plants into groups that have similar morphological characteristics, life forms, similarities in ecology, and geographical area of origin. The basic chromosome number of x = 7 is represented across the 45 taxa as diploid (2n = 2x = 14), tetraploid (2n = 4x = 28), and hexaploid (2n = 6x = 42). Six species are listed in the section *Hordeum; H. bulbosum, H. murinum* ssp. *glaucum, H. murinum* ssp. *leporinum, H. murinum* ssp. *murinum* L., *H. vulgare* ssp. *vulgare*, and *H. vulgare* ssp. *spontaneum*. The genomes of *H. vulgare ssp. vulgare* (cultivated barley) and *H. vulgare ssp. spontaneum* (wild barley) are identical and interfertile (Fedak 1992).

The position of barley within the Poaceae (grass family) is of interest from the evolutionary viewpoint but also reveals the important relationship with other members of the Triticeae tribe, rye (*Secale cereale*) and wheat (*Triticum* spp.). Taxonomic classification of barley not only reveals these relationships but also allows the identification of barley types and varieties from the morphological characteristics of the plant and grain.

Regarding to the origin of barley, the species *Hordeum vulgare* ssp. *spontaneum* C. Koch is still found in abundance in many parts of Asia and North Africa (Zohary and Hopf 2000; Nevo 1992). The theory that barley was first domesticated in the Fertile Crescent in the Near East, which spans present-day Israel, northern Syria, southern Turkey, eastern Iraq, and western Iran (Harlan 1978), has been widely accepted but not without controversy. A noted Russian agronomist, N. I. Vavilov proposed that barley originated in two separate centers: one in the mountains of Ethiopia and the second in eastern Asia bordering to the north on present-day Tibet and Nepal and south into India in the subcontinent (Vavilov 1926). Abundant

evidence as reviewed by Molina-Cano *et al.* (2002) indicates that the East Asian and Indian wild forms of barley are distinctly different from the Near Eastern forms in morphological and biochemical characteristics but have the brittle rachis characteristic of *H. vulgare* ssp. *spontaneum* C. Koch. This evidence strongly suggests that domestication of wild barley occurred in both the Near and Far East, although domestication in the latter may have occurred considerably more recently (Xu 1982). Although Harlan (1978) felt very strongly in favor of the Fertile Crescent as the true center of the origin of cultivated barley, evidence gathered and presented over the past 20 years suggests a hypothesis for a multicentric origin for barley (Molina-Cano *et al.* 2002).

1.1.3 The wild progenitor of barley

Most evidence indicates that the immediate ancestor of cultivated barley (*Hordeum vulgare* ssp. *vulgare* L.) is the two-rowed wild barley *H. vulgare* ssp. *spontaneum* C.Koch (Harlan and Zohary, 1966). It was first discovered in Turkey by the German botanist Carl Koch, and described by him as a separate species, *H. vulgare* ssp. *spontaneum* C. Koch. The centre of distribution for *H. vulgare* ssp. *spontaneum* C. Koch lies in the Middle East. The natural distribution includes the eastern Mediterranean area with eastern Greece and Turkey, the Cyrenaica area of Libya and Egypt and the taxon extends eastwards to Afghanistan, Turkmenia and Baluchistan in West Pakistan (Giles and Bothmer 1985; Zohary and Hopf 1993).

H. vulgare ssp. *spontaneum* C. Koch is an annual plant with a short life cycle, diploid with only seven pairs of chromosomes, and mostly inbreeding annual, and has large ecological amplitude which grows in a wide range of habitats in the eastern Mediterranean and in Southwest Asia (Zohary 1969). The genetic diversity of *H. vulgare* ssp. *spontaneum* C. Koch has been identified by many markers, including isozyme polymorphisms (Liu *et al.* 2002), RFLP-markers (Saghai-Maroof *et al.* 1984) RAPD-markers (Dawson *et al.* 1993), SSR-markers (Saghai Maroof *et al.* 1994; Matus and Hayes 2002), AFLP-markers (Pakniyat *et al.* 1997; Turpeinen *et al.* 2003), and SNP-markers (Kanazin *et al.* 2002), respectively. *H. vulgare* ssp. *spontaneum* C. Koch possesses more variation than cultivated barley, and many alleles are associated with specific environments (Forster *et al.* 2000).

Some major differences between *H. vulgare* ssp. *vulgare* L.and *H. vulgare* ssp. *spontaneum* C. Koch are the tough rachis of cultivated barley as opposed to the brittle rachis of wild barley; the wild traits include long and tough bristles on rachis segments and on the

rachilla as well as a tough (non-brittle) awn. The kernels are often shrunken, not plump, as in cultivated barley. Additionally, *H. vulgare* ssp. *vulgare* L. may have two- or six-rowed spikes, whereas the spikes of *H. vulgare* ssp. *spontaneum* C. Koch are mostly two rowed and often shorter than *H. vulgare* ssp. *spontaneum* C. Koch of the same area. *Ssp. spontaneum* is usually more open-flowering and hence has a higher frequency of cross-pollination than the cultivated form. Outbreeding of up to 10% has been reported (Brown *et al.* 1978; Nevo, 1992). Because of the genomes of *H. vulgare ssp. vulgare* (cultivated barley) and *H. vulgare ssp. spontaneum* (wild barley) are identical and interfertile (Fedak 1992). Wild barley is the only wild *Hordeum* species that can produce fully fertile hybrids when crossed with cultivated barley.

1.1.4 Contribution of wild barley to crop improvement

Due to limited genetic variation among modern crops, efficient use of the genetic variation available in unadapted or wild relatives of modern cultivars is therefore essential to the continued improvement of cereal varieties (Tanksley and McCounch 1997). The wild populations that adapt to drought environments are expected to have genes or QTL alleles for drought tolerance (Nevo and Chen 2010). These alleles could be cloned and transferred to increase crop tolerance (Araus *et al.* 2003).

The wide ecological range of wild barley (*H. vulgare* ssp. *spontaneum* C. Koch) differs in water availability, temperature, soil type, altitude and vegetation, generating a high potential for adaptive genetic diversity against abiotic and biotic stresses (Suprunova *et al.* 2007). These adaptive genetic diversities indicate the potential of wild barley as a source for salt- and drought-resistant alleles for breeding purposes. Cultivated barley contains, on average, 40% of *H. vulgare* ssp. *spontaneum* alleles (Ellis *et al*. 2000). Because *H. vulgare* ssp. *spontaneum* C. Koch and cultivated barley are inter-fertile, *H. vulgare* ssp. *spontaneum* can be used to increase the genetic diversity of cultivated barley by crosses. The adaptation of wild barley to drought and salinity environments has accumulated rich adaptive genetic diversities for drought and salt tolerance in wild barley, which is an excellent genetic resource for crop improvement.

Genes in H. spontaneum for drought tolerance Hsdr4.

A novel gene, *Hsdr4* (*H. spontaneum dehydration responsive*), is identified by its differential expression between tolerant and sensitive genotypes in control and stress conditions (Suprunova *et al.* 2007). *Hsdr4* is mapped on the long arm of chromosome 3H between markers EBmac541 and EBmag705 (Suprunova *et al.* 2007), within a region harboring a QTL

for osmotic potential (OP) and a QTL that affects the relative water content (RWC) (Diab *et al.* 2004). The higher expression level of *Hsdr4* under dehydration stress in tolerant rather than sensitive genotypes and its co-localization with drought tolerance QTLs suggests that *Hsdr4* could be a viable candidate gene for drought tolerance.

Differential expression of dehydrin genes in wild barley, H. spontaneum, associated with tolerance to water deficit.

Dehydrins (*DHNs*, LEA D-11) are water-soluble vesicle-associated proteins involved in adaptive responses of plants to dehydration-related environmental stress such as drought, low temperature and salinity (Close *et al.* 2000). A number of alleles of *Dhn4* from *H. vulgare* ssp. *vulgare* L. and its progenitor *H.vulgare* ssp. *spontaneum* C. Koch, have been sequenced to examine allelic variation in Dhn4. The association of differential expression of dehydrin genes (Dhn 1, 3, 5, 6 and 9) with drought tolerance is found in wild barley (Suprunova *et al.* 2004).

1.1.5 The cytology and genetics of barley genome

Barley (*H. vulgare* ssp. *vulgare* L.) is not only an important crop worldwide but also an excellent system for genome mapping and genome-based analyses (Costa *et al.* 2001), because its chromosomes are homoeologous to cultivated wheat and rye, respectively (Hori *et al.* 2003). The nuclear DNA content often varies somewhat among different cultivars (Bennett 1985). The nuclear genome size of barley (*H. vulgare* ssp. *vulgare* L.) is approximately 4.9×10^9 bp/1C (Arumuganathan and Earle 1991), a bit smaller than 5.3×10^9 bp/1C (Bennett and Smith, 1976). Approximately 10-20 % of the barley genome is tandemly arranged repeated sequences while 50-60 % is repeated sequences interspersed among one another or among unique nucleotide sequences (Rimpau *et al.* 1980). Current estimates of gene number in higher plants vary between 25 000 and 43 000 (Miklos and Rubin 1996). In barley, a gene density of one gene per 123-212 kb can be expected if genes are distributed equidistantly (Panstruga *et al.* 1998). However, grass genomes seem to contain regions that are highly enriched in genes with very little or no repetitive DNA (Feuillet and Keller 1999).

Barley is a diploid (2n = 2x = 14), self-pollinated species. Seven barley chromosomes were identified and labeled based on their sizes and characteristics (Burnham and Hagberg 1956). Since the barley chromosomes have the same DNA content as those in other members of the *Triticeae*, and the gene loci in barley are largely collinear with the loci in other members of the *Triticeae*, with few ancestral translocations involving whole chromosome

segments. The chromosomes 1 to 7 of barley (*H. vulgare* ssp. *vulgare* L.) were redesignated as chromosomes 7H, 2H, 3H, 4H, 1H, 6H, and 5H respectively (Singh and Tsuchiya 1982; Linde-Laursen 1997).

1.2 Abiotic stresses:

Stress may be defined as any factor that causes reduction of yield when it is present or absent (Tollenaar and Wu, 1999). Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment. It is estimated that less than 10% of the world's arable lands may be free of major environmental stresses (Dudal 1976), Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Boyer 1982; Bray *et al.* 2000). Drought and salinity are becoming particularly widespread in many regions and may cause serious salinization of more than 50% of all arable lands by the year 2050 (Wang *et al.* 2003).

1.2.1 Drought stress: a serious threat

Drought stress is one of the prime abiotic stresses in the world and up to 45% of the world agricultural lands are subject to continuous or frequent drought, wherein 38% of the world human population resides (Bot *et al.* 2000). Drought stress is being one of the major causes for crop loss worldwide including that of barley (Jana and Wilen 2005), and the agricultural regions that affected by drought can experience yield loss up to 50% or more (Wang *et al.* 2003 and Jenks and Hasegawa 2005). In the UN, drought is the most serious environmental stress affecting agricultural production by 40.8 % among the most causes of the crop loses (Boyer 1982). It is a serious problem not only in arid and semi-arid environments but also in middle Europe, where the rainfall varies from year to year (Rapacz *et al.* 2010).

Drought is a meteorological term and is commonly defined as a period without significant rainfall. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation (Abdul-Jaleel *et al.* 2009).

1.2.2 Barley and the drought tolerance

In general, wild species have great variability and are potential sources of novel genetic variation for crop improvement. The characterization of genetic variability in wild species and the development of tools to introduce it into cultivated crops are important plant-breeding goals Hernández *et al.* (2002). Wild barley is one of the important wild species that represents an important genetic resource for cultivated barley which has a narrowed gene pool due to intensive breeding. Therefore, it is imperative to study the genetics of different traits in wild barley, if it is to be used for cultivar improvement (Vanhala 2004).

Barley (*H. vulgare* ssp. *vulgare* L.) is widely grown in the arid and semiarid regions of the Mediterranean for forage purposes and as a grain crop (Al-Karaki 2001), and characterized by being relatively high drought tolerance, where it can grow with lesser soil moisture (Mishra *et al.* 2000). Numerous physiological changes occur in barley in response to drought stress, including a reduction in water potential and photosynthetic rate, and an increase in stomata conductance (Sanchez *et al.* 2002, Jones *et al.* 2003).

1.2.3 Drought tolerance mechanisms

Plants respond and adapt to survive under drought stress by the induction of various morphological, biochemical and physiological responses. Drought tolerance is defined as the ability to grow, flower and display economic yield under suboptimal water supply (Farooq *et al.* 2009). In the following part, mechanisms of drought tolerance at different levels are presented.

1.2.3.1 Morphological mechanisms

An account of various morphological mechanisms operative under drought conditions is given below.

Drought Escape

Escape from drought is attained through a shortened life cycle that allows plants that reproduce before the environment becomes dry. Flowering time is an important trait related to drought adaptation, where a short life cycle can lead to drought escape (Araus *et al.* 2002). Drought escape occurs when phenological development is successfully matched with periods of soil moisture availability, where the growing season is shorter and terminal drought stress predominates (Araus *et al.* 2002). Time of flowering is a major trait of a crop adaptation to the environment, particularly when the growing season is restricted by terminal drought and

high temperatures. Developing short-duration varieties has been an effective strategy for minimizing yield loss from terminal drought, as early maturity helps the crop to avoid the period of environmental stress (Kumar and Abbo 2001).

Drought avoidance

Drought avoidance is the ability of plants to maintain relatively high tissue water potential by reducing water loss from plants, due to stomatal control of transpiration loses. Also to maintain water uptake through an extensive and prolific root system (Turner *et al.* 2001; Kavar *et al.* 2007). Glaucousness or waxy bloom on leaves helps with maintenance of high tissue water potential and therefore considered as a desirable trait for drought tolerance (Richards *et al.* 1986; Ludlow and Muchow 1990). The root characters such as biomass, length, density and depth are the main drought avoidance traits that contribute to final yield under terminal drought environments (Subbarao *et al.* 1995; Turner *et al.* 2001). A deep and thick root system is helpful for extracting water from considerable depths (Kavar *et al.* 2007).

Phenotypic flexibility

Plant growth is greatly affected by water deficit. At a morphological level, the shoot and root are the most affected and both are the key components of plant adaptation to drought. Plants generally limit the number and area of leaves in response to drought stress just to cut down the water budget at the cost of yield loss (Schuppler *et al.* 1998). Hairy leaves have reduced leaf temperatures and transpiration (Sandquist and Ehleringer, 2003). This trait increases the light reflectance and minimizes water loss under high temperature and radiation stress by increasing the boundary layer resistance to water vapor movement away from the leaf surface.

Roots characteristics are the key plant organ for adaptation to drought, and the only source to acquire water from soil. Root growth, its density, proliferation and size are key responses of plants to drought stress (Kavar *et al.* 2007). The possession of a deep and thick root system allowed access to water deep in the soil, which was considered important in determining drought resistance in upland rice (Kavar *et al.* 2007). Evidence suggests that it is quality, i.e. the distribution and structure but not quantity of roots that determines the most efficient strategy for extracting water during the crop-growing season (Farooq *et al.* 2009).

1.2.3.2 Physiological mechanisms

Osmotic adjustment, osmoprotection, antioxidation and a scavenging defense system have been the most important bases responsible for drought tolerance. Various physiological mechanisms have been suggested as described below.

Plant water conservation

It has been identified that among various mechanisms, osmotic adjustment, abscisic acid and induction of dehydrins may confer tolerance against drought injuries by maintaining high tissue water potential (Turner *et al.* 2001). With the accumulation of solutes, the osmotic potential of the cell is lowered, which attracts water into the cell and helps with turgor maintenance. Osmotic adjustment helps to maintain the cell water balance with the active accumulation of solutes in the cytoplasm, thereby minimizing the harmful effects of drought (Morgan 1990). Osmotic adjustment is an important trait in delaying dehydrative damage in water-limited environments by continued maintenance of cell turgor and physiological processes (Taiz and Zeiger 2006).

Plant growth regulators

Plant growth regulators or phytohormones, are substances that influence physiological processes of plants at very low concentrations (Morgan 1990), and play vital roles in drought tolerance of plants. Under drought, endogenous contents of auxins, gibberellins and cytokinin usually decrease, while those of abscisic acid and ethylene increase (Nilsen and Orcutte 1996). Abscisic acid is a growth inhibitor and produced under a wide variety of environmental stresses, including drought. All plants respond to drought and many other stresses by accumulating abscisic acid. It has been proposed that abscisic acid and cytokinin have opposite roles in drought stress. Increase in abscisic acid and decline in cytokinins levels favor stomatal closure and limit water loss through transpiration under water stress (Morgan 1990). When plants wilt, abscisic acid levels typically rise as a result of increased synthesis (Taylor 1991). Increased abscisic acid concentration leads to many changes in development, physiology and growth. Abscisic acid alters the relative growth rates of various plant parts such as increase in the root-to-shoot dry weight ratio, inhibition of leaf area development and production of prolific and deeper roots (Sharp *et al.* 1994).

Over production of the compatible solutes

One of the most common stress tolerance strategies in plants is the overproduction of different types of compatible organic solutes (Serraj and Sinclair 2002). Compatible solutes are low-molecular-weight; highly soluble compounds that are usually nontoxic even at high cytosolic concentrations. Osmotic adjustment is a mechanism to maintain water relations under osmotic stress. It involves the accumulation of a range of osmotically active molecules/ions including soluble sugars, sugar alcohols, proline, glycinebetaine, organic acids, calcium, potassium, chloride ions, etc. Under water deficit and as a result of solute accumulation, the osmotic potential of the cell is lowered, which attracts water into the cell and helps with the maintenance of turgor.

Proline is one amongst the most important cytosolutes and its free accumulation is a widespread response of higher plants, algae, animals and bacteria to low water potential (Zhu 2002; Wahid and Close 2007). Its synthesis in leaves at low water potential is caused by a combination of increased biosynthesis and slow oxidation in mitochondria. Despite some controversy, many physiological roles have been assigned to free proline including stabilization of macromolecules, a sink for excess reductant and a store of carbon and nitrogen for use after relief of water deficit (Zhu 2002). Proline contents were increased under drought stress in pea cultivars (Alexieva *et al.* 2001). Drought-tolerant petunia (*Petunia hybrida*) varieties were reported to accumulate free proline under drought that acted as an osmoprotectant and induced drought tolerance (Yamada *et al.* 2005).

1.2.4 Effects of drought stress

On morphological characteristics

Drought affects both elongation and expansion of cells (Anjum *et al.* 2003a; Bhatt and Srinivasa Rao 2005; Kusaka *et al.* 2005; Shao *et al.* 2008). Among the crops, rice as a submerged crop that probably more susceptible to drought stress than most other plant species. In soybean, the stem length was decreased under water deficit conditions (Specht *et al.* 2001). The plant height was reduced up to 25% in water stressed citrus seedlings (Wu *et al.* 2008). Stem length was significantly affected under water stress in potato (Heuer and Nadler 1995), *Vigna unguiculata* (Manivannan *et al.* 2007a) and soybean (Zhang *et al.* 2004). Water stress greatly suppresses cell expansion and cell growth due to the low turgor pressure. Osmotic regulation can enable the maintenance of cell turgor for survival or to assist plant

growth under severe drought conditions in pearl millet (Shao *et al.* 2008). The reduction in plant height was associated with a decline in the cell enlargement and more leaf senescence in *A. esculentus* under water stress (Bhatt and Srinivasa Rao, 2005).

Development of optimal leaf area is important to photosynthesis and dry matter yield. Water deficit stress mostly reduced leaf growth and in turn the leaf areas in many species of plant like Populus (Wullschleger *et al.* 2005), soybean (Zhang *et al.* 2004) and many other species (Farooq *et al.* 2009). The leaf growth was more sensitive to water stress in wheat than in maize (Sacks *et al.* 1997); *Vigna unguiculata* (Manivannan *et al.* 2007a) and sunflower (Manivannan *et al.* 2007b & 2008).

Production of ramified root system under drought is important to above ground dry mass and the plant species or varieties of a species show great differences in the production of roots. The development of root system increases the water uptake and maintains requisite osmotic pressure through higher proline levels in *Phoenix dactylifera* (Djibril *et al.* 2005). An increased root growth due to water stress was reported in sunflower (Tahir *et al.* 2002) and *Catharanthus roseus* (Jaleel *et al.* 2008a & c). The root dry weight was decreased under mild and severe water stress in *Populus* species (Wullschleger *et al.* 2005). An increase in root to shoot ratio under drought conditions was related to ABA content of roots and shoots (Sharp and LeNoble, 2002; Manivannan *et al.* 2007b).

Greater plant fresh and dry weights under water limited conditions are desirable characters. A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Farooq *et al.* 2009). Plant productivity under drought stress is strongly related to the processes of dry matter partitioning and temporal biomass distribution (Kage *et al.* 2004). Mild water stress affected the shoot dry weight, while shoot dry weight was greater than root dry weight loss under severe stress in sugar beet genotypes (Mohammadian *et al.* 2005). Reduced biomass was seen in water stressed soybean (Specht *et al.* 2001), *Poncirus trifoliatae* seedlings (Wu *et al.* 2008), common bean and green gram (Webber *et al.* 2006) and *Petroselinum crispum* (Petropoulos *et al.* 2008).

On yield and related traits

Fetching greater harvestable yield is the ultimate purpose of growing crops. The crop species show great differences for final harvestable yield under drought stress. The yield components like grain number and grain size were decreased under pre-anthesis drought stress treatment in wheat (Edward and Wright 2008). In some studies on maize, drought stress

greatly reduced the grain yield which was dependent on the level of defoliation due to water stress during early reproductive growth (Kamara *et al.* 2003; Monneveux *et al.* 2006). Water stress reduces seed yield in soybean usually as a result of fewer pods and seeds per unit area (Specht *et al.* 2001). In water stressed soybean the seed yield was far below when compared to well-watered control plants (Specht *et al.* 2001). Water stress reduced the head diameter, 100- achene weight and yield per plant in sunflower. There was a negative correlation of head diameter with fresh root and shoot weight, while a positive one between dry shoot weight and achene yield per plant under water stress (Tahir and Mehid 2001). Water stress for longer than 12 days at grain filling and flowering stage of sunflower (grown in sandy loam soil) was the most damaging in reducing the achene yield in sunflower (Mozaffari *et al.* 1996; Reddy *et al.* 2004), seed yield in common bean and green gram (Webber *et al.* 2006), maize (Monneveux *et al.* 2006) and *Petroselinum crispum* (Petropoulos *et al.* 2008).

1.3 Molecular genomics

1.3.1 Quantitative traits and QTL mapping

Quantitative characters have been a major area of study in genetics for over a century, as they are a common feature of natural variation in population of all eukaryotes including crop plants. Traits 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, earliness, height and many quality traits, drought and some forms of disease resistance are controlled by many genes and are known as quantitative traits (also ‘polygenic,’ ‘multifactorial’ or ‘complex’ traits). QTL (Quantitative Trait Loci), a term first coined by Geldermann (1975). The regions within genomes that contain genes associated with a particular quantitative trait are known as QTLs. Conceptually, a QTL can be a single gene, or it may be a cluster of linked genes that affect trait. The procedures for finding and locating the quantitative trait loci (QTLs) and analyzing their magnitude of genetic effects and interactions with environment are called QTL mapping. In the past 20 years there has been a remarkable increase in the use of QTL mapping as a tool to uncover the genetic control of traits. Studies of QTL mapping have been reported in most crop plants for divers traits including yield, quality, disease and insect resistance, abiotic stress tolerance, and environmental adaptation.

QTL mapping requires the construction of a linkage map using a cross between phenotypically divergent accessions. In the offspring of such a cross, association between a trait and marker alleles arises from linkage between marker loci and trait. By identifying these associations, the method allows the location of genomic regions on a marker linkage map that most likely contain genes involved in the trait. The results of QTL mapping provide the most likely position of the QTL, together with an estimate of the allele substitution effects (the additive effect) and so called 'supportive intervals' that roughly correspond to confidence intervals for the QTL map positions. The vast majority of molecular marker research in quantitative traits has been devoted to mapping QTL. These experiments basically have the following major objectives: To identify the regions of the genome that affect the trait of interest and to analyze the effect of the QTL on the trait.

1.3.2 Doubled haploids as a mapping population

Mapping populations consist of individuals of one species or in some cases they derive from crosses among related species where the parents differ in the traits to be studied. Most QTL analysis in plants involved populations derived from pure lines and several approaches have been developed to associate QTL with molecular markers in such populations (Kearsey and Pooni 1996).

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 recombinational information is exactly equivalent to a backcross. However, DH individuals are completely homozygous, and can be self-pollinated to produce large numbers of progeny which are all genetically identical. This permits replicated testing of phenotypes and also facilitates distribution of identical DH populations to many different researchers. Thus, a DH population can also be called a permanent population. A major drawback of DH population is firstly, it is not possible to estimate dominance effects and related types of epistasis; secondly, the rates of pollens or microspores successfully turned into DH plants may vary with genotypes, thus causing segregation distortion and false linkage between some marker loci.

1.3.3 Molecular markers

Since the discovery of the primary structure of DNA, it has been characterized in a number of species. Many molecular marker detection systems that have the ability to

distinguish variation present in genomic DNA sequences have been developed for genetic analysis. Molecular markers are now widely used to track loci and genome regions in several crop-breeding programmes, as molecular markers tightly linked with a large number of agronomic and disease resistance traits in major crop species (Phillips and Vasil 2001, Jain *et al.* 2002, Gupta and Varshney 2004). These molecular markers include: (i) hybridization-based markers such as restriction fragment length polymorphism (RFLP), (ii) PCR-based markers: random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellite or simple sequence repeat (SSR), and (iii) sequence-based markers: single nucleotide polymorphism (SNP).

SSR-markers

Simple sequence repeat (SSR) (Tautz 1989) also called microsatellite, is one of the most important categories of molecular markers. It comprises the core marker system of the PCR based molecular markers and is widely used for DNA fingerprinting, genetic mapping, MAS and studies of genetic diversity and population genetics (Hearne *et al.* 1992; Zietkiewicz *et al.* 1994). SSRs are stretches of DNA consisting of tandemly repeated short units of 1-6 basepairs in length, and are codominantly inherited (Johansson *et al.* 1992). Such motifs are abundant and highly polymorphic in the genome of eukaryotes (Tóth *et al.* 2000). Microsatellites can be found anywhere in the genome, both in protein-coding and noncoding regions. The conserved sequences in the flanking regions of simple sequence repeats can be designed as a pair of specific primers to detect the DNA length polymorphism via the polymerase chain reaction (Litt and Luty 1989; Weber and May 1989). A high level of polymorphism is to be expected because of the proposed mechanism responsible for generating SSR allelic diversity by replication slippage (Tautz *et al.* 1986). The SSR markers can be identified by sequencing microsatellite-containing clones isolated from small-insert genomic DNA libraries via hybridization with synthetic oligonucleotide probes, a method which is time-consuming and relatively expensive. A low cost way of SSRs development is screening of sequences in the public database.

The most frequently found repetitive motifs of mono-, di-, tri-, or tetranucleotide units are (A)n, (GA)n, (TAT)n and (GATA)n in plants (de Vienne *et al.* 2003). The most abundant dimeric microsatellite in several well-known mammals is the AC repeat (Beckmann and Weber 1992), while in many plant species they are AT or GA repeat (Wang *et al.* 1994). More than 75% of the barley genome comprises repetitive DNA sequences (Flavell *et al.*

1977). It is estimated that the barley genome contains one GA repeat every 330kb and one GT repeat every 620kb (Liu *et al.* 1996b), which is in agreement to the findings that GA repeats occur in barley at a higher frequency than GT repeats by Struss and Plieske (1998). Similar results were obtained with other important crops, such as wheat (Plaschke *et al.* 1995; Röder *et al.* 1995), rice (Wu and Tanksley 1993), and maize (Gupta and Varshney 2000). Among trinucleotide repeats in barley, (CCG)n, (AGG)n and (AGC)n repeats are the most-frequent motifs while (ACGT)n and (ACAT)n in tetrameric microsatellites (Thiel *et al.* 2003).

The discovery of microsatellites has significantly increased the marker density of linkage maps for some mammals, human (Engelstein *et al.* 1993; Dib *et al.* 1996) and mouse (Dietrich *et al.* 1996). Molecular linkage maps in many model plants and crops were improved rapidly by the addition of SSR markers, such as in Arabidopsis (Bell and Ecker 1994), rice (McCouch *et al.* 1997), wheat (Röder *et al.* 1998) and maize (Senior and Heun 1993). The informative value of microsatellite markers for genetic studies and as a powerful tool for barley breeding was confirmed in several studies (Maroof *et al.* 1994; Becker and Heun 1995; Liu *et al.* 1996b; Struss and Plieske 1998). Among several important DNA marker systems, SSR markers showed the highest polymorphism, followed by RFLPs, RAPDs and AFLPs (Russell *et al.* 1997). A second-generation linkage map of barley using only PCR-based microsatellite markers was constructed (Ramsay *et al.* 2000). Besides microsatellites derived from genomic clones, also ESTs were exploited for the development of PCR-based SSR-markers (Thiel *et al.* 2003; Pillen *et al.* 2000; Holton *et al.* 2002).

DArT Markers

The Diversity Arrays Technology (DArT) is one of the recently developed molecular techniques and a hybridisation based high-throughput (Jaccoud *et al.* 2001). To date, the performance of the method was validated in several species including cereals such as barley (*H. vulgare* ssp. *vulgare* L.) (Wenzl *et al.* 2004), wheat (*Triticum aestivum* L.) (Akbari *et al.* 2006) and sorghum (*Sorghum bicolor* (L.) Moench) (Mace *et al.* 2008). The current list of species for which DArT arrays are available commercially as service is at http://www.diversityarrays.com (Bolibok-Brągoszewska *et al.* 2009). The key attraction of technology platform is the promise of high throughput capability. Studies such as (Wenzl *et al.* 2004 and Xia *et al.* 2005) report simultaneous analysis of hundreds of markers at once with the added advantage of much lower cost per marker than other technologies like SNPs and microsatellites (Huttner *et al.* 2005).

The pattern of hybridisation for a genotype provides a unique genetic fingerprint that is especially useful for quantitative trait analysis. For quantitative trait analysis, DArT has many potential applications. So far, DArT marker patterns have been principally applied to the assessment of genetic variability in a group of organisms such as the assessment of cassava diversity by Xia *et al.* (2005) and barley diversity by Wenzl *et al.* (2004). As these studies illustrate, the most accurate diversity analysis require proportional amounts of clones from all individuals tested to be present on the array. DArT is especially suited to QTL mapping (Wittenberg *et al.* 2005) and can be used to construct medium-density linkage maps relatively quickly. Wenzl *et al.* (2004) gave an example of such a map showing how the standard techniques of map construction using linkage disequilibrium can be applied using DArT markers.

DArT markers can be used to track phenotypic traits in breeding like other molecular markers, and the high throughput and low cost nature of the technology makes DArT more affordable for marker assisted selection. Multiple loci can be involved in the selection process but using an array means all loci simultaneously. Such markers can then be tracked though an introgression or crossing program, and used to supplement phenotyping to reduce potential miss-identification of a trait due to environmental effects (Lande and Thompson 1990), as per any other marker-aided selection tool. Even though DArT can be applied in the absence of sequence information, individual DArT markers are sequence-ready and can be used in the development of probe-based markers for further research (Kilian 2004).

The advantages of DArT marker technique:

Using DArT Markers in genetic diversity and mapping study has been many advantages as follow:

1. It does not need prior sequence information for the species to be studied; this makes the method applicable to all species regardless of how much DNA sequence information is available for that species.
2. It is high throughput, quick and highly reproducible method.
3. It is cost effective, with an estimated cost per data point tenfold lower than SSR markers (Xia *et al.* 2005).
4. The genetic scope of analysis is defined by the user and easily expandable.
5. It is not covered by exclusive patent rights, but on the contrary open-source (i.e., it is designed for open use and shared improvement).

The limitations of DArT marker technique:

1. DArT is a microarray-based technique that involves several steps, including preparation of genomic representation for the target species, cloning, management and analysis. The latter requires dedicated software's such as DArTsoft and DArTdb. The establishment of DArT system, therefore, is highly likely to demand an extensive investment both in laboratory facility and skilled manpower.
2. DArT assays for the presence (or amount) of a specific DNA fragment in a presentation. Hence, DArT markers are primarily dominant (present or absent) or differences in intensity, which limits its value in some applications.
3. The technology has been used in few species primarily by the team that developed it (who has setup a quite economical commercial service for some species); only a single independently group has so far successfully established the methodology to *Eucalyptus grandis* in South Africa (Lezar *et al.* 2004).

1.3.4 Statistical Methods for QTL Mapping

Undoubtedly, the development of statistical methods has played an important role for the detection of the association between DNA markers and quantitative characters. The first report of an association between a morphological marker and a quantitative trait was reported by Sax (1923).

QTL mapping programs can be roughly classified into different groups according to the number of markers or genetic models and analytical approaches applied (Liu 1998; Hoeschele *et al.* 1997). According to the number of markers, single-QTL models and multiple-locus models can be classified (Liu 1998). According to the analytical technology, the methods can be grouped into one-way ANOVA or simple t-test, simple linear regression, multiple linear regression, nonlinear regression, log-linear regression, likelihood functions, MCMC (Markoff Chain Monte Carlo), mixed linear models, and Bayesian approach (Wang *et al.* 1999b).

Briefly, the statistical analyses of associations between phenotype and genotype in a population to detect QTLs include single-marker mapping (Luo and Kearsey 1989), interval mapping (Lander and Botstein 1989), and composite interval mapping (CIM) (Zeng 1994), plus multiple traits mapping (Jiang and Zeng 1995; Ronin *et al.* 1995) as follow:

Single-marker tests

The simplest method for QTL mapping is single-marker mapping, including t-test, and analysis of variance (ANOVA) and simple linear regression, which assess the segregation of a phenotype with respect to a marker genotype (Soller 1976). According to this principle progeny classified by marker genotype and compare phenotypic mean between classes (t-test or ANOVA). A significant difference indicates that a marker is linked to a QTL. The difference between the phenotypic means provides an estimate of the QTL effect. This approach can indicate which markers linked to potential QTLs are significantly associated with the quantitative trait investigated. In short, QTL location is indicated only by looking at which markers give the greatest differences between genotype group averages. Depending on the density of markers, the apparent QTL effect at a given marker may be smaller than the true QTL effect as a result of recombination between the marker and the QTL. The advantage of this method is a simple procedure that can be accomplished by a standard statistical analysis software package, such as SAS and Minitab. In contrast, the main weakness of single-marker tests is the failure to provide an accurate estimate of QTL location or recombination frequency between the marker and the QTL because the evaluation of individual markers is independently, and without reference to their position or order (Doerge 2002).

Simple interval mapping (SIM)

Interval mapping is probably the most familiar method of QTL analysis. The introduction of interval mapping offered a new strategy to discern weak effects from genetic distance between marker locus and putative QTL using the power of a complete genetic map. The interval that is defined by ordered pairs of markers are searched in increments, and statistical methods are used to test whether a QTL is likely to be present at the location within the intervals or not. The principle behind interval mapping is to test a model for the presence of a QTL at many positions between two mapped marker loci. The model is fit, and its goodness is tested using the method of maximum likelihood. If it is assumed that a QTL is located between two markers, the 2-locus marker genotypes contain mixtures of QTL genotypes each. Maximum likelihood involves searching for QTL parameters that give the best approximation for quantitative trait distributions that are observed for each marker class. Models are evaluated by computing the likelihood of the observed distributions with and without fitting a QTL effect. The LOD (logarithm of the odds) score is the log of the ratio

between the null hypothesis (no QTL) and the alternative hypothesis (QTL at the testing position). Large LOD scores correspond to greater evidence for the presence of a QTL. The best estimate of the location of the QTLs is given by the chromosomal location that corresponds to the highest significant likelihood ratio. The LOD score is calculated at each position of the genome. In the case of many missing genotypes and large gaps on the map, the missing data are replaced by probabilities estimated from the nearest flanking markers (Broman 2001). Until now, many software packages based on interval mapping were developed for QTL mapping, such as MAPMAKER/QTL (Lincoln *et al.* 1992) and QGene (Nelson 1997). In comparison to single marker mapping, the benefits of these programs are a curve available across the genetic map indicating the evidence of QTL location and which allows the inference of QTLs to positions or gaps between two markers in order to make proper analysis for incomplete marker genotype data. Meanwhile, analysis can be used for testing the presence of genotyping errors (Lincoln *et al.* 1992).

Composite interval mapping (CIM)

There are two problems with single interval mapping (SIM) method as a result from single QTL model mentioned above. One is that the effects of additional QTL will contribute to sampling variance. The other is that combined effects of two linked QTLs will cause biased estimates. The ideal solution would be to fit a model that contains the effects of all QTL.

However, the tremendous number of potential QTL and their interactions will lead to innumerable statistical models and heavy computational demands as using statistical approaches to locate multiple QTL. To deal with the this problem, several key papers were published (Jansen and Stam 1994; Zeng 1994). The approach of composite interval mapping assesses the probability that an interval between two markers is associated with a QTL that affects the trait of interest, and is as well controlling for the effects of other background markers on the trait. In theory, CIM gives more power and precision than SIM because the effects of other QTL are not present as residual variance. Furthermore, CIM can remove the bias that would normally be caused by QTL that are linked to the position being tested. The key problem with CIM concerns the choice of suitable background markers to serve as covariates.

1.3.5 Advanced backcross-QTL analysis

With the development of the molecular marker technologies and plant breeding methods, Tanksley and Nelson (1996a) developed a strategy, which allows a targeted transfer of favorable exotic alleles into elite breeding material. Through this approach, specific exotic alleles derived from the exotic donor are tagged with molecular markers and tested for association with agronomic traits. In parallel, these QTL alleles will be transferred into near-isogenic lines (NILs) by means of marker associated selection breeding. Therefore, unlike the conventional QTL mapping methods, AB-QTL analysis can accelerate the process of marker based breeding because the end products of analysis are close to NILs carrying favorable alleles. Since the first report in tomato (Tanksley *et al.* 1996b), AB-QTL analysis has been successfully applied in many crops to detect and transfer valuable QTLs from unadapted germplasm into elite breeding lines.

In barley, several studies have employed the AB-QTL strategy to introgress exotic barley alleles into barley cultivars and examine agronomic performance, quality and disease resistance (Pillen *et al.* 2003, 2004; Matus *et al.* 2003; Talamé *et al.* 2004; Forster *et al.* 2004; Li *et al.* 2005, 2006; Hori *et al.* 2005; von Korff *et al.* 2004, 2005, 2006; Yun *et al.* 2006. Whereas Pillen *et al.* (2003, 2004), Talamé *et al.* (2004), Forster *et al.* (2004), Li *et al.* (2005, 2006), von Korff *et al.* (2005, 2006) and Yun *et al.* (2006) concentrated on the analysis of phenotypic data from extensive field or greenhouse trials, Matus *et al.* (2003), Hori *et al.* (2004) and von Korff *et al.* (2004) focused more on the development of advanced backcross populations and detailed characterization of the genetic structures of these new genetic resources.

1.3.6 QTL x environment interaction

Genotype by environment (QE) interaction is a common phenomenon for quantitative traits. QE interaction has been demonstrated by classical genetics studies and has been of great concern for plant breeding programs (Falconer 1960; Lin *et al.*1986; Westcott 1986). QTL mapping offer the opportunity to trace genotype by environment interactions between individual QTLs and environments. Reports about inconsistency in detection of QTLs across different environments are numerous. In contrast, Stuber *et al.* (1992) and Schön *et al.* (1994) reported that QTL detection was relatively consistent across diverse environments. The difference in observations may be a function of the traits studied and may also be a function of the methods of identifying genotype by environment interaction. In most previous mapping

reports, possible QTL x environment interactions were analyzed by comparing the QTLs detected separately in each environment or using the mean value of all environments. It was suggested that a QTL detected in one environment but not in another might indicate QTL x environment interaction. However, even in the absence of true QTL x environment interaction, a QTL can be detected in one environment but not in another, because the chance of simultaneous detection in both environments is naturally small (Jansen *et al.* 1995). On the other hand, consistency in detection of QTLs at different environments may not conclusively indicate the absence of QTL x environment interaction. Recently, some methods have been proposed for dealing with QE interactions (Jansen *et al.* 1995; Romagosa *et al.*1996; Wang *et al.* 1999; Piepho 2000).

1.3.7 Marker-Assisted Selection

DNA markers are reliable selection tools because they are stable and are relatively easy to score in laboratory. Marker assisted selection (MAS) is an indirect selection method based on markers linked with the target gene affecting the desirable trait. With marker-assisted backcrossing, genes, such as qualitative and quantitative resistance genes, can be transferred rapidly from wild progenitors to advanced breeding lines, and several resistance genes can be pyramided into a single line. Applying MAS requires, first, segregation for both the marker and the target gene and, second, close linkage between a marker and the target gene. Effective use of marker-based selection or marker-assisted introgression should significantly decrease the amount of time required by plant breeders to develop new cultivars. For MAS to be effective, the marker and trait should be as tightly linked as possible to minimize recombinations between the marker and the gene of interest. Selection based on molecular markers is particularly useful in the introgression of specific traits into existing cultivars through repeated backcrossing. In addition to selecting for the markers of interest from the donor parent, a breeder can also select for recovery of recurrent parent alleles elsewhere in the genome to hasten recovery of the recurrent genome (Arús and Moreno-González 1993), especially if there are known markers for specific traits in the recurrent parent. However, phenotypic selection is also essential to recovery of the desired characteristics of the recurrent parent, and should not be overlooked. The most applications of DNA markers in marker-assisted selection include genetic distance analysis, variety identification, identification of markers tightly linked to specific genes, and marker-assisted backcrossing.

1.3.8 Identifying QTLs for agronomical and physiological traits in different BC population of barley

Wild barley has often been considered a promising resource for the improvement of agronomic and quality traits as well as stress tolerance. For example Ellis *et al.* (2000) postulated that exotic barley being adapted to a wide range of environments offers the prospect of a goldmine of untapped genetic reserves. Nevo *et al.* (1992) demonstrated that wild barley harbours considerably more genetic variation than the cultivated species and that many exotic alleles are associated with adaptation to specific environments with different abiotic stress conditions.

In barley, von Korff *et al.* (2004) developed two BC_2DH populations 'S42' from 'Scarlett x ISR42-8' (301 lines) and 'T42' from 'Thuringia x ISR42-8' (84 lines). Pillen *et al.* (2003, 2004) conducted the first thorough analysis of the agronomic performance of exotic barley germplasm. They genotyped two BC_2F_2 populations Apex x ISR101-23 (136 lines) and 'Harry x ISR101-23' (164 lines) with 45 and 50 SSRs, respectively. They field-tested them for agronomic traits and malting quality parameters in two consecutive years and at three different locations in Germany. The performance of the exotic germplasm of a selected set of 123 DH lines under drought conditions was analyzed by Talamé *et al.* (2004). Forster *et al.* (2004) studied the DH lines for agronomic traits and conducted a QTL analysis with 54 polymorphic AFLP markers and 59 SSRs. Li *et al.* (2005) performed an AB-QTL analysis in 181 selected BC_3DH lines derived from the spring barley cultivar, Brenda, and the exotic accession, HS213. von Korff *et al.* (2005, 2006) phenotyped 301 BC_2DH lines of the population 'S42' for agronomic performance and disease resistance in two consecutive years and at four different locations in Germany.

The exotic donors used in these studies were originated from Israel (Pillen *et al.* 2003, 2004; Li *et al.* 2005, 2006; Matus *et al.* 2003; von Korff *et al.* 2004), Greece (Talamé *et al.* 2004), and the Caspian Sea region (Hori *et al.* 2005). Their selection was primarily based on per se performance, origin, and passport data. QTL analyses, however, have shown that the phenotype of a plant is only a modest predictor of its genetic potential, especially with respect to quantitative traits (Tanksley *et al.* 1996). Accordingly, von Korff (2005) selected the donors based on agronomic performance of backcross progeny derived from crosses between ten barley cultivars and ten *H. vulgare ssp. spontaneum* accessions, rather than on per se performance of the wild barley accessions.

Pillen *et al.* (2003) found that at 34% out of all QTLs detected, the exotic allele improved agronomic performance. Similarly, von Korff *et al.* (2006) detected favorable exotic alleles at 36% of all QTL in the BC_2DH population 'S42'. Pillen *et al.* (2003) and von Korff *et al.* (2006) reported that the maximum average yield increase associated with an exotic QTL allele resulted in an average yield improvement of 7.7% and 7.1 %. Pillen *et al.* (2003) explained weak favorable effect of exotic alleles on yield compared to the strong effect of exotic alleles in tomato 34% (Fulton *et al.* 1997) and rice 18% (Xiao *et al.* 1998) with different breeding systems.

The AB-populations also show a large variation for plant height in barley. Talamé *et al.* (2004) found a maximum variation in plant height between 88 and 144 cm in Morocco. von Korff *et al.* (2006) reported an average plant height in the population 'S42' across eight environments of between 63 cm and 110 cm. Major plant height QTL were located on 2H (Pillen *et al.* 2004; von Korff *et al.* 2006; Li *et al.* 2006), 3H (Talamé *et al.* 2004; von Korff *et al.* 2006), 4H (Pillen *et al.* 2003, 2004; Talamé *et al.* 2004; von Korff *et al.* 2006) and 5H, (Talamé *et al.* 2004; Li *et al.* 2005; von Korff *et al.* 2006; Li *et al.* 2006). Corresponding candidate genes are the semi-dwarf genes *sdw3* (Gottwald *et al.* 2004), *sdw1* and *ari-e* and the flowering loci *Ppd-H1* and *Vrn-H2*. At the majority of QTL-loci, the exotic allele increased plant height, in particular at QTL close to the candidate genes *sdw1* and *ari-e.GP*, but at the QTL on 2HS and 4HL the exotic allele consistently reduced plant height.

Under drought conditions, heading is negatively and plant height positively correlated with yield, indicating that tall early heading genotypes present a good yielding capacity under water limiting conditions. Indeed, the strongest favorable effects of the exotic germplasm on yield were found under drought conditions in Tunisia and Morocco (Talamé *et al.* 2004). The same authors, however, observed that the exotic alleles with a delay in flowering time showed a favorable effect on yield, indicating that the favorable effect on yield under conditions of limited water was not due to drought escape but to an increase in yield potential. Similarly, in the AB-population 'S42' the exotic introgression on 4HL showed a favorable effect on yield under drought conditions, although this QTL-allele postponed flowering.

In AB-QTL studies, major QTL loci often showed pleiotropic effects on a number of different traits and resulted in a strong clustering of QTL in particular on 2HS, 3HL, 4HL, and 5HL. The exotic introgression at the semi-dwarf locus *sdw1*, for example, affected, next to flowering time, plant height, yield and thousand grain weight (Pillen *et al.* 2003; Li *et al.*

2005; von Korff *et al.* 2006; Li *et al.* 2006). Similarly, QTL close to the *Vrn-H2* locus influenced heading data, plant height, and yield (Pillen *et al.* 2003; Talamé *et al.* 2004; von Korff *et al.* 2006). Although the donor and recipient germplasm differed between the cited AB-QTL studies, the exotic alleles exhibited predominantly the same qualitative effect at these major QTL for heading date, plant height, and yield. The exotic alleles are thus often similar in their effects and clearly different from the elite alleles. Wild barley thus harbors novel genetic variability for these key loci.

The AB-QTL strategy allows the selection of major genes/alleles from the exotic gene pool with the most beneficial pleiotropic effects, especially in stress environments, and introduces these into breeding programs while eliminating negative alleles such as brittleness. Eshed and Zamir (1994) demonstrated that introgression lines in tomato are a powerful tool for map-based cloning (Frary *et al.* 2000) and the discovery of gene function by transcriptome and metabolome analysis (Schauer *et al.* 2006).

In barley, advanced backcross populations enable the fast generation of such introgression lines as demonstrated by von Korff *et al.* (2004) and Hori *et al.* (2005). von Korff *et al.* (2004) selected from each of the BC_2DH populations 'S42' and 'T42' and Hori *et al.* (2005) from the BC_3F_1 population Haruna Nijo x H602, a minimal set with 49, 43, and 19 introgression lines, respectively, which cover a large percentage of the exotic genome in overlapping exotic segments. Further backcrossing and establishment of nearly isogenic lines generate a valuable resource for validating the effects of exotic QTL-alleles, introducing them into elite cultivars, map-based cloning of verified QTL, and ultimately for the study of gene function.

1.4 Objectives

Considering to the facts about barley and drought, sufficient literatures have discussed the genetic analysis of yield and its components under drought conditions. On other hand, despit of that, the agronomical, root and physiological characteristics are known to be important in improving drought tolerance in barley. In addition, there is limited knowledge on the inheritance of these traits, in particular studying the effect of QTL by treatments as well as epistatic interactions. Therefore, the overall objectives of the proposed study were:

1. to assess variations in shoot, root and physiological traits of BC_2DH lines under control and drought stress conditions.
2. to perform the AB-QTL analysis with REML forward selection approach in order to detect the QTLs influencing the interested traits.
3. to identify, localize and characterize the QTLs underlying drought tolerance related to shoot, root and physiological traits.
4. to dissect the QTLs with additive main effects, QTL by treatment interaction effects and digenic epistatic effects which responsible for drought tolerance related to shoot, root and physiological traits.
5. to enhancement the drought tolerance of cultivated barley via identifying the potential and favorable QTL alleles of wild species (*H. vulgare* ssp. *spontaneum* C. Koch) related to drought tolerance.

2 Materials and methods

Advanced backcross QTL analysis has been successfully applied in detecting and transferring QTLs from unadapted germplasm into elite breeding lines for various plant species. A double haploid population of 301 lines was used for this study, and derived from a cross between an exotic accession of *H. vulgare ssp. spontaneum* C. Koch (ISR42-8) and German spring barley cultivar ‘’Scarlett’’ (*H. vulgare* ssp. *vulgare*). In this part, the development of the mapping population, phenotypic evaluation, molecular characterisation, phenotypic data measurements and models of the statistical analysis of the phenotypic data and quantitative trait loci (QTLs) are described.

2.1 Population development

The development of the BC_2DH population was conducted according to the advanced backcross strategy of Tanksley and Nelson (1996) and has been described in Figure 1. An exotic accession of *H. vulgare* ssp. *spontaneum* from Israel (ISR42-8) was crossed with a German spring barley cultivar ‘’Scarlett’’ (*H. vulgare* ssp. *vulgare* L.). The German spring barley cultivar Scarlett was selected as high-yielding and high quality characteristics variety and obtained from the breeders Saatzucht Josef Breun GdbR and Saaten-Zentrum Schndorf. The wild barley accession, ISR42-8, from Eastern Lower Galilee, Israel, was provided by Prof. G. Fischbeck, Weihenstephan.

The recurrent parent, Scarlett, was used as the female and the donor as the male parent to generate the F1 generation. A single F1 plant (maternal) was backcrossed to Scarlett (paternal). From this cross 12 BC_1F_1 plants were backcrossed a second time with Scarlett. BC_1F_1 plants have been subjected to anther culture (in the lab of the Saaten-Union Resistenzlabor, Leopoldshöhe, Germany). The BC_2DH population (S42) contains 301 BC_2DH lines and designated as S42.

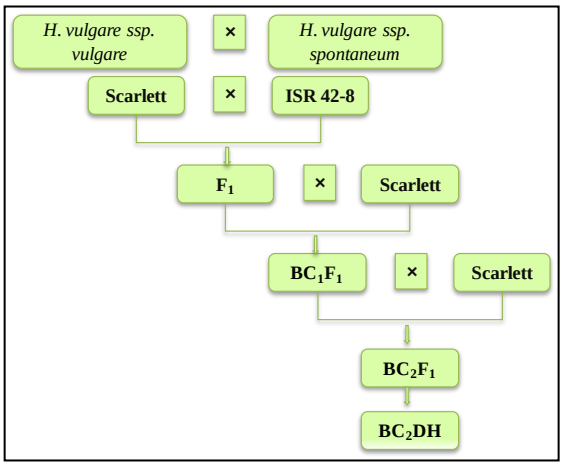


Figure 1 Development of the S42 population

2.2 Phenotypic evaluation

In this part, the location and design of the experiment, the agricultural practices and control and drought treatments are described.

Location and design of the experiment

The experiments were conducted in plastic tunnels during the summer seasons 2007-2008 and 2009 at the poppelsdorf experimental station, dept. of Crop Science and Plant Breeding, Faculty of Agriculture, Bonn University. The experiments were arranged in a split-plot design with one-replications, the treatments (drought and control) assigned to main plots and BC_2DH lines were assigned randomly to sub-plots as described in Figure 2.

Figure 2 this scheme illustrates the design and location of the experiment, since the experiments were arranged in split plot design, where the treatments (drought and control) assigned to main plots and BC_2DH lines were assigned randomly to sub-plots.

Agricultural practices

A total of 12 kernels from each of BC_2DH lines and their parents were sown in two rows in plastic pots of 22 x 22 cm with 25 cm depth, with 4 holes pierced at the bottom for drainage. The soil of the experiment contained a mixture of top soil, silica sand, milled lava and peat dust (Terrasoil®, Cordel & Sohn, Germany). The sowing dates were 13th and 1st of April in the summer seasons 2007 and 2008, respectively, and 27th of March in season 2009. The plants were fertilized three times per season with 250 ml of NPK liquid fertilizer containing 7 % N, 3% P_2O_5 and 6% K_2O. The plants were sprayed against fungi and insects as recommended for barley cultivation.

Treatments

Depending on weather and transpiration conditions as well as the status of soil moisture and in irrigation treatment the plants were watered with up to 660 ml water per pot a

day. Water supply was carried out with drip irrigation by watering each pot three times a day. Soil moisture and weather data were measured by sensors from Decagon Dev., USA.

Picture 1 Twelve kernels from each BC_2DH lines and the parents were sown in plastic pots containing mixture soil

Picture 2 Location and design of the experiment. The experiments were arranged in split plot design and conducted in plastic tunnels at INRES institute

Picture 3 Process of washing roots from the soils in order to measure root characteristics

Picture 4 Soil moisture and weather data were measured by sensors from Decagon Dev., USA.

The aim of water management in the control treatment was to hold the soil moisture near to field capacity (plant available water content AWC 100%). After 40 days of vegetative growth in the drought treatment a gradual reduction of water supply was carried out to reach the maximum drought stress threshold near wilting point (AWC near 0%). The desired drought stress level has been achieved in the duration of 21 days.

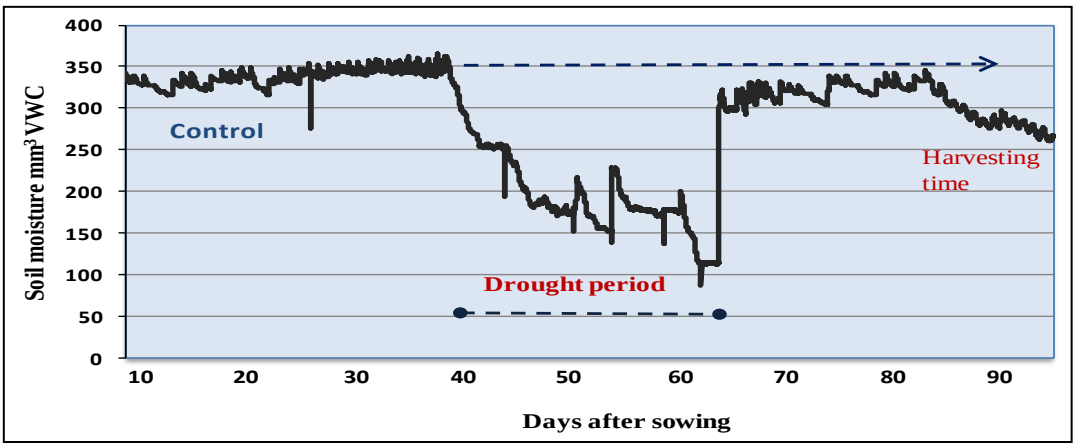


Figure 3 In drought treatment, the gradual reduction of water content (from AWC 100%) has started after 40 days of vegetative growth in order to reach the maximum drought stress threshold near wilting point (AWC near 0%)

2.3 Molecular characterisation

The population S42 was developed by Prof. Dr. Klaus Pillen and Prof. Dr. Jens Lèon. This population has been genotyped with simple sequence repeats (SSRs), diversity array technology (DArT) and gene-specific marker systems. A linkage map of 371 genetic markers has been established that contains 106 SSRs, 255 DArT and 10 gene-specific DNA markers.

2.3.1 DNA extraction and genotyping with SSR and specific markers

The DNA of BC_2DH population has been extracted and genotyped with 106 SSR markers by von Korff *et al*. (2004). Additional ten flowering time candidate genes from the photoperiod and vernalisation pathways have been added and described by von Korff *et al*. (2004) and Wang *et al*. (2010). At each locus, a homozygous elite barley genotype (*Hv*) and a homozygous exotic barley genotype (*Hsp*) have been scored.

2.3.2 DNA extraction and genotyping with DArT markers

DNA extraction

Out of 301 BC_2DH lines and the two parents, the DNA of 231 accessions was extracted from leaves of four leaves old seedlings grown in a greenhouse. The DNA was extracted using "Kit" procedure according to DNeasy Plant Handbook 07/2006 (QIAGEN). The DNA of other accessions ''70 accessions'' and the two parents (Scarlett and ISR 42-8) was extracted using CTAB method according to Tanksley á la Paul and modified by J.Carling. **http://www.diversityarrays.com**

Purification of Total DNA from Lyophilized Plant Tissue (DNeasy 96 Protocol)

The Procedure

1. Place sample material (10 mg lyophilized tissue) into each tube in 2 collection microtube racks. Add one tungsten carbide bead to each collection microtube. Seal the microtubes with the caps provided. Cool the racks of collection microtubes in liquid nitrogen. Ensure that the microtubes remain tightly closed.
2. Place a clear cover (saved from step 1) over each rack of collection microtubes, and knock the racks upside down against the bench 5 times to ensure that all tungsten carbide beads can move freely within the microtubes. Ensure that no liquid nitrogen remains, but do not allow the leaf material to thaw. Remove the clear cover.
3. Sandwich each rack of collection microtubes between adapter plates and fix into TissueLyser clamps as described in the TissueLyser User Manual. Work quickly so that the plant material does not thaw. Grind the samples for 1 min at 20 Hz.
4. Remove and disassemble the plate sandwiches, noting the orientation of the racks of collection microtubes during the first round of disruption. Ensure that the collection microtubes are tightly closed.
5. Cool the racks of collection microtubes again in liquid nitrogen. Place a clear cover over each rack of collection microtubes and knock the racks upside down against the bench 5 times to ensure that all tungsten carbide beads can move freely within the microtubes. Ensure that no liquid nitrogen remains, but do not allow the leaf material to thaw. Remove the clear cover.
6. Ensure that the collection microtubes are tightly closed. Reassemble the plate sandwiches so that the collection microtubes nearest the. Reinsert the plate sandwiches into the TissueLyser. Work quickly so that the plant material does not thaw.
7. Grind the samples for another 1 min at 20 Hz. Remove the plate sandwiches from the TissueLyser and remove the adapter plates from each rack of collection microtubes. Knock the racks against the bench 5 times to ensure that no tissue powder remains in the caps. Keep the samples frozen until working lysis solution is added.
8. Combine Buffer AP1, RNase A, and Reagent DX according to the table below to make a working lysis solution. Carefully remove the caps from the collection microtubes. Immediately pipet 400 µl working lysis solution into each collection microtube.
9. Seal the microtubes with new caps (provided); ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover over each rack of collection

microtubes, and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.

10. Remove and discard caps. Add 130 µl Buffer AP2 to each collection microtube. Close the microtubes carefully with new caps (provided); ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover over each rack of collection microtubes, and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.
11. Incubate the racks of collection microtubes for 10 min at –20°C. Centrifuge the racks of collection microtubes for 5 min at 6000 rpm. Remove and discard the caps. Carefully transfer 400 µl of each supernatant to new racks of collection microtubes (provided), ensuring that the new tubes are in the correct orientation. Add 1.5 volumes (typically 600 µl) of Buffer AP3/E to each sample.
12. Close the collection microtubes with new caps (provided); ensure that the tubes are properly sealed to prevent leakage during shaking. Place a clear cover over each rack of collection microtubes and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.
13. Place two DNeasy 96 plates on top of S-Blocks (provided). Mark the DNeasy 96 plates for later sample identification. Remove and discard the caps from the collection microtubes. Carefully transfer 1 ml of each sample to each well of the DNeasy 96 plates. Seal each DNeasy 96 plate with an AirPore Tape Sheet (provided). Centrifuge for 4 min at 6000 rpm.
14. Remove the tape. Carefully add 800 µl Buffer AW to each sample. Centrifuge for 15 min at 6000 rpm to dry the DNeasy membranes. To elute the DNA, place each DNeasy 96 plate in the correct orientation on a new rack of Elution Microtubes RS (provided), add 100 µl Buffer AE to each sample, and seal the DNeasy 96 plates with new AirPore Tape Sheets (provided). Incubate for 1 min at room temperature (15–25°C). Centrifuge for 2 min at 6000 rpm. Repeat step 26 with another 100 µl Buffer AE.

DNeasy 96 Plant Kit (6)

Number of preps	6 x 96
DNeasy 96 Plates	6
S-Blocks	2
Collection Microtubes, 1.2 ml (racked)	12 x 96
Collection Microtube Caps	4 x (120 x 8)
Elution Microtubes RS (racked) and caps	6 x 96
AirPore Tape Sheets	5 + 25
Buffer AP1	2 x 140 ml
Buffer AP2	90 ml
Buffer AP3/E (concentrate)	125 ml
Buffer AW (concentrate)‡	2 x 81 ml
Buffer AE	128 ml
RNase A (100 mg/ml)	2 x 440 µl
Reagent DX	1 ml
96-Well-Plate Registers	6

2.3.3 The DNA extraction according to CTAB method for DArT genotyping.

The DNA of other accessions ‘’70 accessions’’ and the two parents (Scarlett and ISR 42-8) was extracted using CTAB method according to Tanksley á la Paul and modified by J.Carling as follow:

Protocol for 2 ml Eppendorf tubes:

1. Aliquot 1 ml of freshly prepared preheated to 65ºC, well mixed “fresh buffer solution” and place tubes to the 65ºC incubator or water bath, (3, 4 days old “fresh buffer solution” works fine),
2. Grind required amount (same across all samples) of plant material in mortar and pestle under liquid nitrogen to fine powder,
3. Suspend powder in 1 ml “fresh buffer solution” kept at 65ºC (make sure there are no clumps, vortex if necessary),
4. Incubate at 65ºC for 1 h (can extend for another 30 min), invert tubes in every 20 minutes or incubate with gentle shaking,
5. Cool down for 5 min and add 1 ml of chloroform : isoamyl alcohol (24 : 1) mixture,
6. Mix well for 30 min,
7. Spin 20 min, 10000 x g, RT,
8. Transfer water phase to fresh tube, add same volume of ice cold isopropanol and invert tube ~ 10 times, nucleic acids should become visible,
9. Spin 30 min, 10000 x g, RT,
10. Discard supernatant, wash pellet with 2 ml 70 % EtOH,

11. Discard EtOH, dry pellet and dissolve in 250 µl of 1 x TE (10 mM TrisHCl pH 8.0, 1 mM EDTA pH 8.0),
12. Check DNA quality and quantity on 0.8 % agarose gel. (If RNA quantity is several folds less than DNA, RNase treatment is not necessary for DArT applications).

Buffer stock solutions

Extraction buffer stock	**To make 500 ml:**
0.35 M sorbitol	31.9 g sorbitol
0.1 M TrisHCl pH 8.0	50 ml 1M TrisHCl pH 8.0
5 mM EDTA pH 8.0	5 mM EDTA pH 8.0
	fill up to 500 ml MiliQ H2O

Lysis buffer stock	**To make 500 ml:**
0.2 M Tris HCl pH 8.0	100 ml 1M Tri HCl pH 8.0
0.05 M EDTA pH 8.0	50 ml 0.5 M EDTA pH 8.0
2M NaCl	200 ml 5 M NaCl
2% CTAB	10 g CTAB
	fill up to 500 ml with MilliQ H20

SARCOSYL STOCK 5% (w/v)

Fresh buffer working soultions:

0.5 % (w/v) sodiumdisulfite (= sodium metabisulfite) 2 % (w/v) PVP-40 (K29-32) Sigma Dissolve in required volume of extraction buffer stock; add same volume of lysis buffer stock and 0.4 volume of extraction (=lysis) buffer stock of sarcosyl stock.

2.3.4 DArT Markers analysis

The produced DNA of the population was sent to Diversity Arrays Technology P/L - Triticarte P/L, 1 (http://www.triticarte.com.au/default.html) Wilf Crane Crescent, Yarralumla ACT 600, AUSTRALIA (Wenzl *et al.* 2004). For DArT marker analysis, the genotyping with 255 DArT markers has been done by hybridization based markers. The chromosomal positions of the DArT markers are according to Wenzl *et al.* (2006). Their technology involves reducing the complexity of the sample by cutting with restriction enzymes and annealing adaptors. Then fragments are amplified from the adaptors. The fragments are labelled and hybridized to a microarray of variable fragments representing the diversity within the species. DArT markers are biallelic dominant markers. Each marker was scored for each sample as 0 (absent) and 1 (present); they represent exotic (*Hsp*) and elite (*Hv*) alleles respectively. By using DArT, SSR and specific genes positions, the linkage map has been drawn by using MapChart ver.2.2 (Voorrips 2002).

2.4 Phenotypic data measurements

Fifteen shoot, root and physiological traits related to drought tolerance were investigated in this study.

1) **Plant height (PH):** was measured at harvest maturity in centimetre from soil surface to the top of the spike excluding the awns.
2) **Wilting Score (WS):** Visual rating (from 0 up to 9), was scored at the end of the drought period, where 0 with no symptoms of stress effect and 9 with all plants apparently dried. (de Datta *et al.* 1988).
3) **Number of Tillers / plant (TILS):** was measured at harvest maturity as an average of number of tillers of six plants.
4) **Number of Spikes / plant (SPS):** was measured at harvest maturity as an average of number of spikes of six plants.
5) **Shoot dry weight / plant (SDW):** after harvesting, six plants from each pot were dried in the oven at 80 ^{0}C for 48 hours, and the average of shoot dry weight per plant was calculated and scored in gram.
6) **Number of kernels / spike (KERS):** as an average of number of kernels of all spikes of the plant.
7) **Grain yield / plant (GY):** as an average of kernels weight of six plants and scored in gram.
8) **Thousand Kernel weight (TKW):** was calculated from the grain yield of the plant and number of kernels per plant as follow: (1000*GY)/KERS).
9) **Harvest index (HI):** was obtained as ratio of grain weight to total aboveground oven-dried weight (grain yield + straw yield) * 100.
10) **Root Length (RL):** was measured as length of the twelve roots of twelve plants and scored in centimetre.
11) **Root Dry Weight (RDW):** the obtained roots were dried in the oven at 80 ^{0}C for 72 hours, and the dry weight of roots was determined and scored in gram.
12) **Root Shoot Ratio (RSR):** was calculated as a ratio between root dry weight (RDW) and shoot dry weight (SDW).
13) **Relative water content (RWC):**

    Relative water content was determined according to Barrs and Weatherly (1962). The upper two fully developed and expanded leaves of the main stem of two plants were

cut, and collected at midday to determine fresh weight (FW). Leaf blades were then placed with their cut end pointing down into a Falcon tube containing about 50 ml of distilled water for 4 h at room temperature. After soaking, leaves were quickly and carefully blotted dry with tissue paper prior to determine of turgid weight (TW). For dry weight (DW) determination, samples were oven-dried at 80 0C for 24 h. RWC was calculated according to the following equation:

RWC % = [(fresh weight – dry weight)/ (turgid weight- dry weight)] x 100**.**

14) **Proline Content (PC**): PC has been measured by colorimetric procedure of Bates *et al.* (1973). For this, free proline content was extracted from the upper fully expanded leaves of the main stem and the first biggest tiller at the end of drought period. The leaves were cut and wrapped in plastic foil, then frozen in liquid nitrogen, freeze-dried (Lyophilizer Leybold Heraeus Lyovec G12) and grinded in a mill maschine (Retsch MM 2000) into a fine powder. A total of 30 mg of leaf tissue was homogenized in 3% (w/v) sulphosalicylic acid in 2-mL microfuge tubes. Tubes were vortexed for 15 s to suspend tissues a total of three times and centrifuged at 14,000 RPM for 10 min (Heraeus Centrifuge Biofuge 28RS); 500-µL aliquots were removed for proline quantification, and test tubes were adjusted to 1 mL using sulphosalicylic acid. Next, 1.0 mL acid ninhydrin (1.25 g ninhydrin in 30 mL glacial acetic acid, 20 mL 6m- 85% H3PO4) and 1.0 mL glacial acetic acid were added. Tubes were vortexed for 15 s and the resulting mixture was heated at 100°C for 1 hour in a water bath. The reaction was stopped after incubation by placing the tubes in an ice bath. The tubes were removed from the bath and 2 mL of toluene was added to each tube. The tubes were then vortexed for 20 s, and 5 min was allowed for phase separation. The absorbance of fraction with toluene aspired from liquid phase was read at 520 nm in a Spectrophotometer using toluene as a blank. Proline concentration (µmol proline/ml) was determined using L-proline (Sigma P-0380) as a standard and calculated on a dry-weight basis (µmol proline/g DW) as follow:

Proline (µmol proline / g DW) = ((µg proline/ml x4 x10) / (0.03 x 115.1).

15) **Osmotic Potential (OP):** for determination of OP, the upper fully developed leaf of the main stem and the first biggest tiller were cut and wrapped in plastic foil, immediately frozen in liquid nitrogen. For the analysis 500 µl sterile water were added to 10 – 30 mg of the sample all was homogenized with a BOHR machine, and

incubated in refrigerator at 4^0C for 1 hour and centrifuged at 13000 U/min (in Biofuge Pico maschin) for 10 min and finally stored at -20 ^{0}C until the measurement. 15 µl from each sample were taken and measured was measured using a freezing-point micro-osmometer 'Osmomat 300' (Gonotec, Berlin) with sterile water as a standard.

Table 1 List of the 15 investigated traits in this study under control and stress conditions as well as the breeding goal under stress conditions.

	Trait	Abbr.	Unit	Seasons	Breeding goal(*)
	Shoot traits				
1	Plant height	PH	cm	2007-09	-
2	Wilting Score	WS	Score (0-9)	2007-09	-
3	No. of Tillers	TILS	Tillers/plant	2007-09	+
4	No. of Spikes	SPS	Spikes/plant	2007-09	+
5	Shoot dry weight	SDW	g SDW/plant	2007-09	+
6	No. of kernels	KERS	Kernels/spike	2007-09	+
7	Grain yield	GY	g Grain/plant	2007-09	+
8	Thousand Kernel weight	TKW	g	2007-09	+
9	Harvest index	HI	ratio %	2007-09	+
	Root traits				
10	Root Length	RL	Cm	2007-09	+
11	Root Dry Weight	RDW	g RDW/plant	2007-09	+
12	Root Shoot Ratio	RSR	ratio %	2007-09	+
	Physiological traits				
13	Relative water content	RWC	%	2007-09	+
14	Proline Content	PC	µmol proline / g DW	2007-09	+
15	Osmotic Potential	OP	Osmol/kg	2008-09	-

(*) According to the breeding purposes of barley under drought conditions, the value of the trait should be improved (+) or debased (-).

2.5 Analysis of variance of phenotypic data

To detect the differences and variation among doubled haploid (DH) lines under both treatments over years, ANOVA of BC_2DH lines was performed with the Statistical Analysis System SAS (SAS Institute, ver. 9.2 2008), using PROC MIXED procedure, restricted maximum likelihood (REML) method, as follow:

$$X_{ijklm}=\mu+L_i+T_j+L_i*T_j+Y_k+T_j*Y_k+L_i*Y_k+B_l(T_j*Y_k)+\varepsilon_{m(ijkl)}$$

Where, X_{ijklm} is the phenotypic observation of the trait under study, μ is the general mean, L_i is the fixed effect of the *i*-th BC_2DH lines, T_j is the fixed effects of the *j*-th treatment, L_i*T_j is the random effect of the interaction of the *i*th BC_2DH lines and *j*th of the

treatments, Y_k is the fixed effect of the *k*-th of years, T_j*Y_k is the fixed effect of the *j*-th of the treatment and *k*-th year, L_i*Y_k is the fixed effect of the *i*-th BC_2DH lines and *k*-th year, $B_l(T_j*Y_k)$ is the random effect *l*- th of the blocks nested in *j*-th of treatment and *k*-th year and $\varepsilon_{m(ijkl)}$ is residual $\varepsilon_{m(ijk)}$ of X_{ijklm}.

In relation to the two parents Scarlett and ISR 42-8, the significant differences between means of the two parents were calculated with PROC GLM procedure (SAS Institute, ver. 9.2 2008) using a Tukey-Kramer adjustment for multiple comparisons.

2.6 Phenotypic correlation of investigated traits

The phenotypic correlations between trait performances were computed using the correlation procedure (PROC CORR), the LS-means of the investigated traits across BC_2-DH lines across years and separately for each treatment were used for the calculation of the Pearson correlation coefficients (r).

2.7 QTL and Epistasis analysis

In the following, description of QTL and digenic epistatic effects detection models:

2.7.1 QTLs detection

According to Bauer *et al.* (2009), the forward selection strategy is very effective to detect QTLs influencing the interested traits. We used a multiple QTL model iteratively extended and reduced by forward selection and backward elimination, respectively, using the PROC MIXED procedure in SAS software (SAS version 9.2, SAS, 2008). In each round of the forward selection process, the selection of the most significant and informative marker was added as a fixed factor (QTL) into the model according to the F value with the probability of false discovery rate (FDR ≤ 0.05) and then all remaining markers were scanned with the respective model containing the previously found QTLs. The process of the following iterations of this model was continued until no more additional QTL could be detected. The detection of QTL for studied traits was carried out using the following mixed hierarchical model in the MIXED procedure as starting point of forward selection process:

$$X_{ijklmn}=\mu+M_i+L_j(M_i)+T_k+L_j*T_k+M_i*T_k+Y_l+T_k*Y_l+B_m(T_k*Y_l)+\varepsilon_{n(ijklm)},$$

where the total of phenotypic value was sum of general mean μ, fixed effect M_i of the i-th marker genotype, random effect $L_j(M_i)$ of the *j*-th DH line nested in the *i*-th marker genotype, fixed effect T_k of the *k*-th treatment, fixed interaction effect L_j*T_k of the *j*-th DH line and the

k-th treatment, fixed interaction effect M_i*T_k of the $_i$-th marker genotype and the *k*-th treatment, fixed effect Y_l of the *l*-th year, fixed interaction effect T_k*Y_l of the *k*-th treatment and *l*-th year, random effect $B_m(T_k*Y_l)$ of *m*-th block nested in treatment and years, residue $\varepsilon_{n(\text{ijklm})}$ of X_{ijklmn}. P values from F-tests were adjusted genome-wide across all single marker tests using the false discovery rate (FDR). The significant marker main effects as well as marker × treatment interaction with $P_{FDR} \leq 0.05$ were accepted as putative QTLs for the next iteration, however, the final model was:

$$X_{ijklmn}=\mu+\sum QTL+M_i+L_j(M_i)+T_k+L_j*T_k+M_i*T_k+Y_l+T_k*Y_l+B_m(T_k*Y_l)+\varepsilon_{n(ijklm)},$$

where ∑QTL represents the detected QTLs from the forward/backward selection process.

2.7.2 Digenic epistatic effects

The digenic epistatic interactions between all DArT and SSR marker pairs were tested with SAS procedure MIXED (SAS ver. 9.2, SAS Institute, 2008) using the following mixed hierarchical model:

$$X_{ijklmno}=\mu+\sum QTL+M1_i+M2_j+M1_i*M2_j+L_k(M1_i*M2_j)+T_l+L_j*T_k+Y_m+T_l*Y_m+B_n(T_l*Y_m)+\varepsilon_{o\ (ijklmn)},$$

Here $M1_i$ and $M2_j$ are the fixed effects of the *i*-th marker and *j*-th marker (*M2*). $M1_i*M2_j$ is the fixed interaction effect of the *i*-th *M1* genotype with *j*-th *M2* genotype, $L_k(M1_i*M2_j)$ is the random effect of the *k*-th BC_2DH line nested in the *i*-th *M1* and *j*-th *M2* marker genotype interaction.

2.7.3 Calculation of relative performance of the exotic parent ($RP_{[Hsp]}$)

To evaluate the performance of the homozygous exotic genotype (ISR 42-8) under drought conditions, the relative performance RP [*Hsp*] was computed by

$$RP_{[Hsp]}=(([\ Hsp]-[\ Hv])/[\ Hv])*100,$$

where [*Hsp*] represents LS-means of the homozygous exotic genotype and [*Hv*] LS-means of the elite genotype.

According to the relative performance of the exotic genotype (ISR 42-8), if it improves or debases the trait under drought conditions as well as matching with the breeding goals of drought tolerance at a given marker locus, the marker main effects as well as their interaction with the treatments were characterized as favorable or unfavorable QTL.

2.7.4 Calculation of the coefficient of determination (R^2)

In order to explain the strength of the marker main effect (R^2_M) and the marker-treatment interaction (R^2_{M*T}), the coefficient of determination was calculated to each as follow:

$$R^2_M = SS_M / SS_L, \qquad R^2_{M*T} = SS_{M*T} / SS_{L*T}$$

Where, SS_M, SS_{M*T} and SS_{L*T} represent the sum of squares of the marker main effect, the marker-treatment interaction and doubled haploid lines-treatment interaction, respectively.

3 Results

Since the developing of the advanced backcross quantitative trait locus (AB-QTL) mapping approach by Tanksley and Nelson (1996a) which allows a targeted transfer of favorable exotic alleles into elite breeding material, several studies have applied this strategy on different crops. In this study, the main aim was to identify the effects of exotic QTL alleles on drought tolerance related traits which were introgressed from exotic accessions into BC_2DH lines of the population S42 which derived from crossing between a German elite cultivar of *H. vulgare* ssp. *vulgare* 'Scarlett' with an exotic accession of *H. vulgare* ssp. *spontaneum* 'ISR42-8'. The population has been evaluated in a plastic tunnel for 15 traits under control and drought stress conditions in three successive summer seasons (2007, 2008 and 2009). A total of 15 quantitative traits were investigated for drought tolerance. The investigated traits, abbreviations, units, tested seasons and breeding goals are described in Table (1). The population was genotyped with 106 SSRs, 255 DArT and 10 gene-specific DNA markers in order to perform QTL analysis. In this chapter, the evaluation of the performance of the doubled haploid lines as well as their parents and the main effect and the interactions of the QTLs were described.

3.1 Analysis of variance of the parents

The elite parent 'Scarlett' and the exotic genotype 'ISR 42-8' were evaluated for 15 traits under control and drought across three years. The significant differences between means of the two parents were calculated with PROC GLM procedure (SAS Institute, ver. 9.2 2008) using a Tukey-Kramer adjustment for multiple comparisons. ANOVA revealed high significant differences between Scarlett and ISR 42-8 in the majority of the investigated traits except SPS and OP. Table 2 shows the analysis of variance and summary statistics of the two parents across both control and drought conditions. In the following part, results of ANOVA for the parents can be grouped into three sections.

Shoot traits

For PH, the wild accession 'ISR 42-8' was taller than the elite cultivar 'Scarlett' under both treatments. Drought led to decrease plant height of the two parents. The parents, Scarlett and ISR42-8 showed significant variations in term of wilting score (WS) under drought and control conditions. Scarlett showed a mean wilting score 3.6 under control conditions that

increase up to 6 under drought. The drought tolerant parent, ISR42-8 has displayed a moderate increase of WS under drought as compared to control. The wild accession produced more tillers and less spikes under both treatments than Scarlett. For SDW, both parents produced approximately the same quantity of shoot dry weight under drought conditions. Due to the strong correlation between yield and its attributes and under both treatments, the elite parent 'Scarlett' was yielded more grain/plant, produced more kernels/spike, had higher thousand grain weight and had higher percentage of harvest index than the wild accession 'ISR 42-8'.

Root traits

High significant difference was identified between both parents for root characteristics. The wild parent was superior in all of root characteristics which investigated in this study. The wild accession had longer root length (by 91%), higher root dry weight (by 175%) and has explained high percentage of root/shoot ratio than the elite parent 'Scarlett' under both treatment.

Physiological traits

High significant difference was identified between both parents for RWC. The wild accession 'ISR 42-8' had high relative content (75.08 %) under drought conditions, while the elite parent 'Scarlett' had moderate percentage of water content (60.08 %). Significant variation has been observed in proline accumulation between both parents, since the PC of Scarlett was increased from 0.92 µmol/gDW (control) to 9 µmol/gDW under drought conditions. ISR42-8 responded to a slight variation in PC under drought conditions as compared to control. A cross comparison of both parents showed a remarkable increase of PC in Scarlett that synthesize 9 µmol/gDW of proline than 1.4 µmol/gDW in ISR42-8 under drought. There was no much different between elite parent and the wild accession for osmotic potential, however Scarlett showed little increase in OP under control (0.23 osmol/kg).

3.2 Evaluation of the population S42 with compared to the parents

The population S42 which consists of 301 BC_2DH lines was tested for tolerance to drought. Analysis of variance revealed high significant variation among BC_2DH lines and treatments in most of investigated traits. For detailed description, results ANOVA of the investigated traits in S42 population are shown in (Table 3) and discussed separately for each trait. Frequency

distribution and summary statistics of the investigated traits of the population S42 are shown in figures.

3.2.1 Shoot traits

Plant height (PH)

ANOVA of S42 population for PH revealed highly significant differences among accessions, treatments, years as well as the interaction accession by year and year by treatment, while the interaction 'accession by treatment' was not significant (Table 3). The population has influenced by drought stress, the plants were shorter under drought treatment compared to control. Under control, the height of the plants ranged from 41 to 126 cm with an average of 69.18 cm, while it ranged from 42 to 109 cm with an average of 67.01 cm under drought (Figure 4). Comparing PH of BC_2DH lines to the parents under drought conditions, 147 lines were shorter than the elite parent 'Scarlett' while there was no line exceeded the plant height of the exotic parent 'ISR 42-8'.

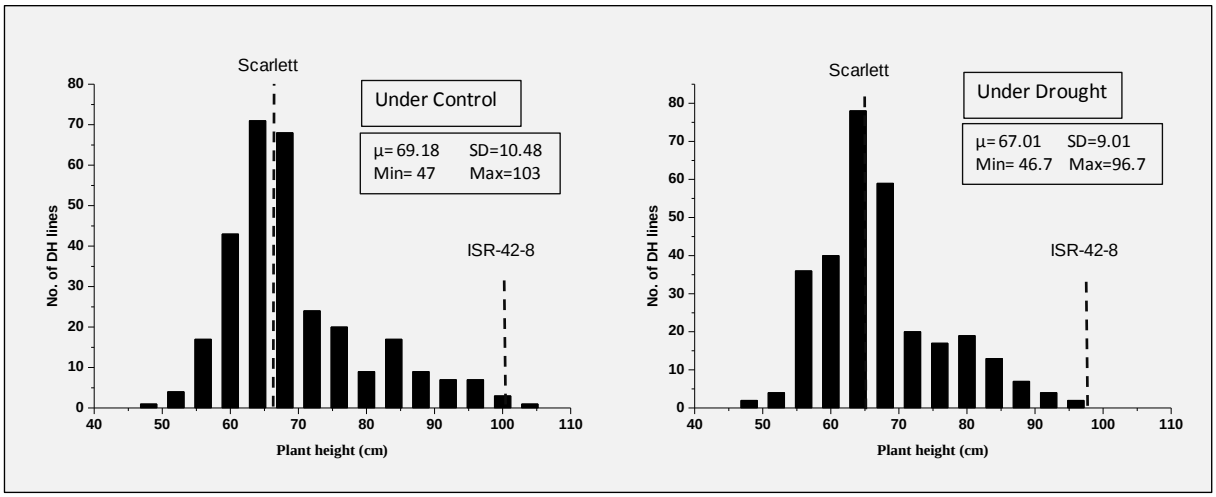


Figure 4 Frequency distribution of PH under control and drought conditions with compared to the parents. The classes of PH are shown on X-axis while the number of doubled haploid lines is presented on Y-axis.

Wilting Score (WS)

The population S42 showed a significant variation in leaf wilting and showed a mean wilting score of 5.07 (moderate drought susceptible) under drought conditions (Figure 5). Fifteen BC_2DH lines presented wilting scores ranged between 3 and 4 as drought resistant lines to drought. A maximum number of 230 BC_2DH lines showed moderately susceptible response to drought treatment and scored a range between 4 and 6. While fifty and six BC_2DH lines posed susceptible and highly susceptible wilting scores of 6 and 8, respectively (Figure 5).

Table 2 Means and simple statistics of the two parents across both control and drought conditions.

Trait	LS-Mean		Tukey	Mean				Minimum				Maxumum				Standard deviation				Standard error			
				Control		Drought		Control		Drought		Control		Drought		Control		Drought		Control		Drought	
	SCA	ISR	Sign	SCA	ISR	SCA	ISR	SCA	ISR	SCA	ISR	SCA	ISR	SCA	ISR	SCA	ISR	SCA	ISR	SCA	ISR	SCA	ISR
PH	66,0	99,8	**	67,0	101,7	65,0	98,0	61,0	84,0	61,0	95,0	75,0	121,0	68,0	103,0	7,2	18,6	3,6	4,4	4,2	10,7	2,1	2,5
WS	4,8	2,8	**	3,7	2,0	6,0	3,7	3,0	1,0	5,0	3,0	4,0	3,0	7,0	4,0	0,6	1,0	1,0	0,6	0,3	0,6	0,6	0,3
TILS	2,5	5,0	**	2,4	5,5	2,6	4,5	1,3	3,3	1,5	3,0	3,2	7,5	3,3	7,3	0,9	2,1	0,9	2,5	0,5	1,2	0,5	1,4
SPS	2,3	1,6	ns	2,4	1,9	2,2	1,3	1,3	1,2	1,5	0,8	3,2	2,7	3,2	1,8	0,9	0,8	0,9	0,5	0,5	0,4	0,5	0,3
SDW	3,7	4,4	*	4,3	5,7	3,1	3,1	3,0	3,4	2,2	2,4	5,1	7,4	4,2	4,3	1,1	2,1	1,0	1,0	0,7	1,2	0,6	0,6
GY	2,0	0,4	**	2,4	0,4	1,6	0,3	1,5	0,4	1,1	0,2	3,0	0,5	2,1	0,4	0,8	0,0	0,5	0,1	0,5	0,0	0,3	0,0
KERS	17,4	10,8	**	18,8	9,9	15,9	9,3	18,5	3,6	14,6	6,6	19	13,7	17,9	13,4	0,24	5,4	1,7	3,6	0,1	3,1	1,0	2,1
TKW	50,6	24,4	***	54,8	30,3	46,3	28,3	50,5	20,5	37,2	20,4	58,5	44,3	51,1	40,7	4,0	12,4	7,8	10,8	3,3	7,1	4,5	6,3
HI	53,2	9,4	***	55,5	8,5	50,9	10,3	49,8	5,8	50,6	5,3	61,2	12,4	51,3	13,8	5,7	3,5	0,4	4,5	3,3	2,0	0,2	2,6
RL	19,8	40,3	**	20,3	43,7	19,3	37,0	15,0	30,0	10,0	27,0	29,0	51,0	34,0	42,0	7,6	11,8	12,9	8,7	4,4	6,8	7,4	5,0
RDW	2,2	7,3	**	1,9	7,8	2,4	6,7	1,4	5,3	0,7	5,7	2,2	12,1	4,3	7,9	0,4	3,7	1,8	1,1	0,3	2,1	1,0	0,7
RSR	5,6	16,3	**	4,0	13,2	7,2	19,4	2,4	7,2	1,5	18,5	6,3	17,5	11,9	19,9	2,0	5,4	5,3	0,8	1,2	3,1	3,1	0,4
RWC	74,1	81,2	**	88,1	87,3	60,1	75,1	83,3	82,9	30,1	61,6	92,6	91,4	75,9	91,6	4,6	4,2	26,0	15,2	2,7	2,4	15,0	8,8
PC	5,0	0,8	**	0,9	0,2	9,0	1,4	0,5	0,1	0,6	0,1	1,8	0,2	23,7	3,8	0,8	0,1	12,7	2,1	0,4	0,0	7,3	1,2
OP	0,20	0,17	ns	0,23	0,16	0,17	0,18	0,18	0,12	0,15	0,18	0,28	0,21	0,19	0,19	0,07	0,06	0,03	0,01	0,05	0,05	0,02	0,01

The Lsmeans of the two parents Scarlett (SCA) and ISR 42-8 (ISR) were calculated as an average of the phenotypic data for each trait across 2007-08 and 09 and for each treatment separately except trait OP were calculated only from two years 2008 and 2009.

Trait: PH (Plant Height), WS (Wilting Score), TILS (No. of Tillers/plant), SPS (No. of Spikes/plant), SDW (Soot Dry Weight/plant), GY (Grain Yield/plant), KERS (No. of Kernels/spike), TKW (Thousand kernel weight), HI (Harvest Index), RL (Root Length), RDW (Root Dry Weight), RSR (Root Shoot Ratio), RWC (Relative Water Content), PC (Proline Content) and OP (Osmotic Potential).

Sign.: Significance were determined with the Tukey-Kramer test (*** P = 0.0001, ** P = 0.001,*P = 0.05, n.s. not significant).

Table 3 Analysis of variance of the population S42 for all studied traits across all environments

S.V.	DF	F values and significance of shoot traits									Root traits			Physiological traits		
		PH	WS	TILS	SPS	SDW	GY	KERS	TKW	HI	RL	RDW	RSR	RWC	PC	OP [1)]
Lines	300	13,8 ***	5,2 ***	5,9 ***	5,92 ***	2,9 ***	3,2 ***	6,9***	4,7***	7,9 ***	1,83 ***	2,7 ***	2,2 [ns]	1,8 ***	2,12 [*]	0,89 [ns]
Treat	1	14,5 **	312 ***	31,6 **	40,1 **	259***	219***	70,1***	115,4***	11,8 **	66,3 ***	3,3 [ns]	25,9 **	162***	34,2 ***	0,52[ns]
Year	2	15,1 **	3,93 *	827***	618***	253***	196***	13,4***	50,5***	11,3 **	714***	70,8 ***	80,8 [ns]	25,9 ***	11,2 **	15,04 [ns]
Lines*Treat	300	1,0 [ns]	1,26 *	1,25 *	1,16 [ns]	1,2 *	1,18 [ns]	1,1ns	1,2*	1,5 ***	1,21 *	1,2 *	1,17 [ns]	1,19 *	1,09 [*]	0,91 [ns]
Lines *Year	600	1,3 **	1,74 ***	1,7 ***	1,4***	1,32 **	1,32 **	1,32**	1,6***	1,4 ***	1,25 **	1,18 *	1,21 [ns]	1,11 [ns]	1,06 [ns]	1,05 [ns]
Treat.*Year	2	34,6 ***	18,4 ***	8,2 **	18,6 **	38,8***	35,4 ***	14,6***	35,3***	11,1 **	105***	11,7 **	34,1 ***	44,9 ***	10,3 **	0,1 [ns]

Where Lines represents the population S42 which contains 301 BC_2DH lines, Treat denote for the treatments (Control and drought), Year, the experiments were carried out over three years (2007,08 and 09), and their interactions.

Trait: PH (Plant Height), WS (Wilting Score), TILS (No. of Tillers/plant), SPS (No. of Spikes/plant), SDW (Soot Dry Weight/plant), GY (Grain Yield/plant), KERS (No. of Kernels/spike), TKW (thousand kernel weight), HI (Harvest Index), RL (Root Length), RDW (Root Dry Weight), RSR (Root Shoot Ratio), RWC (Relative Water Content), PC (Proline Content) and OP (Osmotic Potential).

*, **, ***: Significant at 0.05, 0.01 and 0.001 levels, respectively. ns: not significant.

[1)] the analysis of variance of osmotic potential (OP) was calculated only from two years 2008 and 2009.

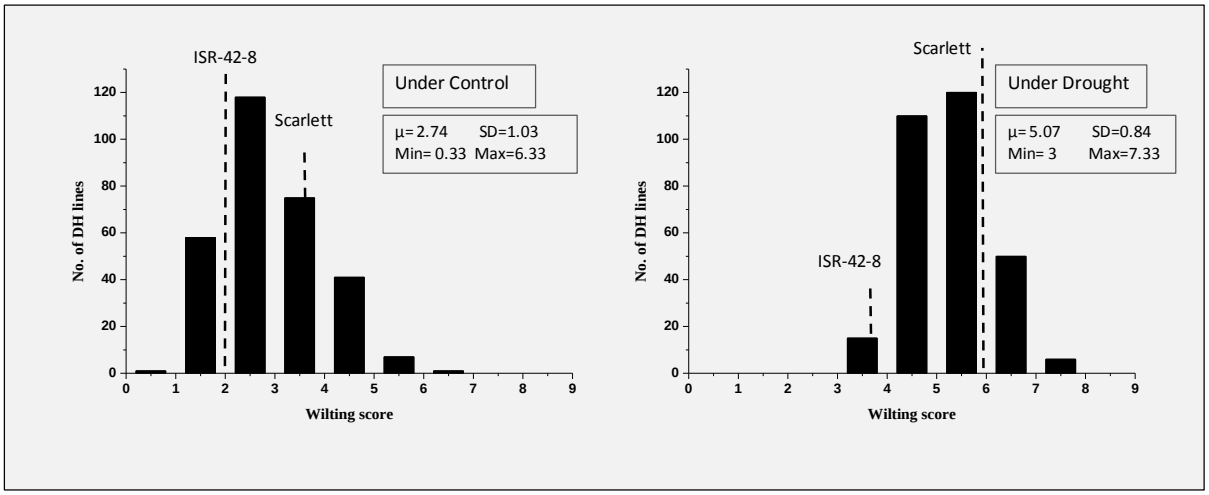


Figure 5 Frequency distribution of WS under control and drought conditions with compared to the parents. The classes of WS are shown on X-axis while the number of doubled haploid lines is presented on Y-axis.

Number of tillers/plant (TILS)

Highly significant differences were observed among accessions, treatments, years and all types of interactions (Table 3). No. of tillers/plant ranged from 1.35 to 5.33 with an average of 2.75 tillers/plant under control, while it ranged from 1.77 to 4.5 with an average of 2.37 tillers/plant under drought conditions (Figure 6). Under drought conditions, as an average over years, two BC_2DH lines produced tillers with an average of 4.5 tillers/plant equally with the adaptive parent. A maximum number of 137 BC_2DH lines were produced more tillers/plant than the elite parent under drought.

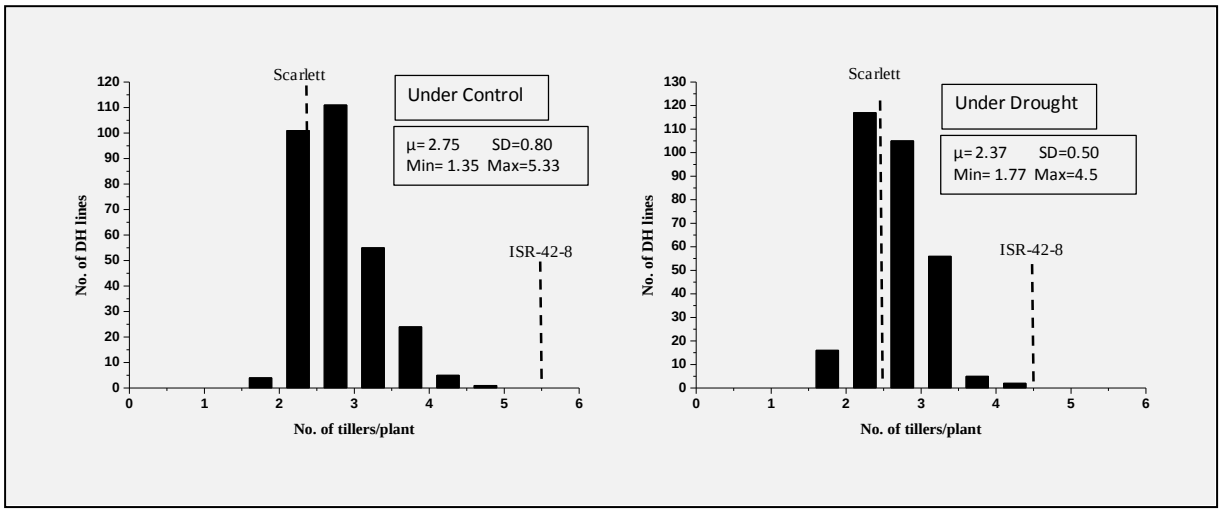


Figure 6 Frequency distribution of TILS under control and drought conditions with compared to the parents. The classes of TILS are shown on X-axis while the number of doubled haploid lines is presented on Y-axis.

Number of spikes/plant (SPS)

High significant differences were detected for all source of variances except the interaction between accessions and treatments was not significant (Table 3). Under drought conditions, a total of 159 BC_2DH lines were produced more spikes/plant than the elite parent. Among them, twenty three BC_2DH lines yielded more than three spikes per plant indicating that these lines were adapted well under drought stress (Figure 7).

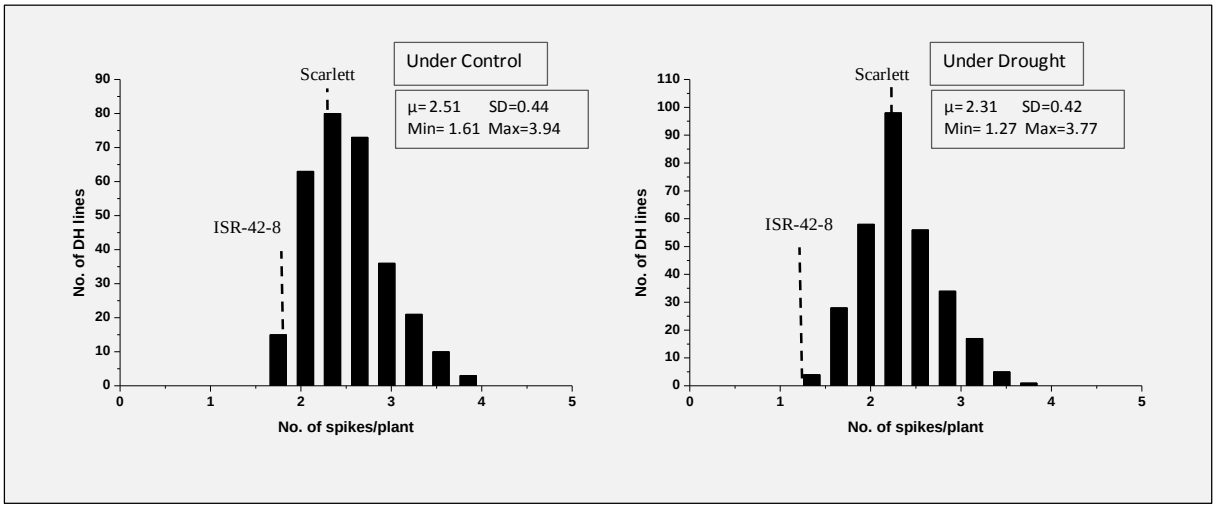


Figure 7 Frequency distribution of SPS under control and drought conditions with compared to the parents. The classes of SPS are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

Shoot dry weight (SDW)

For population S42, the BC_2DH lines were revealed highly significant differences under both treatments with general average of 3.77 g SDW/plant (Figure 8). The mean of SDW under control was 4.17 g/plant and decreased to 3.17 g SDW/plant under drought conditions. A maximum 48 of BC_2DH lines yielded shoot dry weight more than both parents under drought stress (Figure 8).

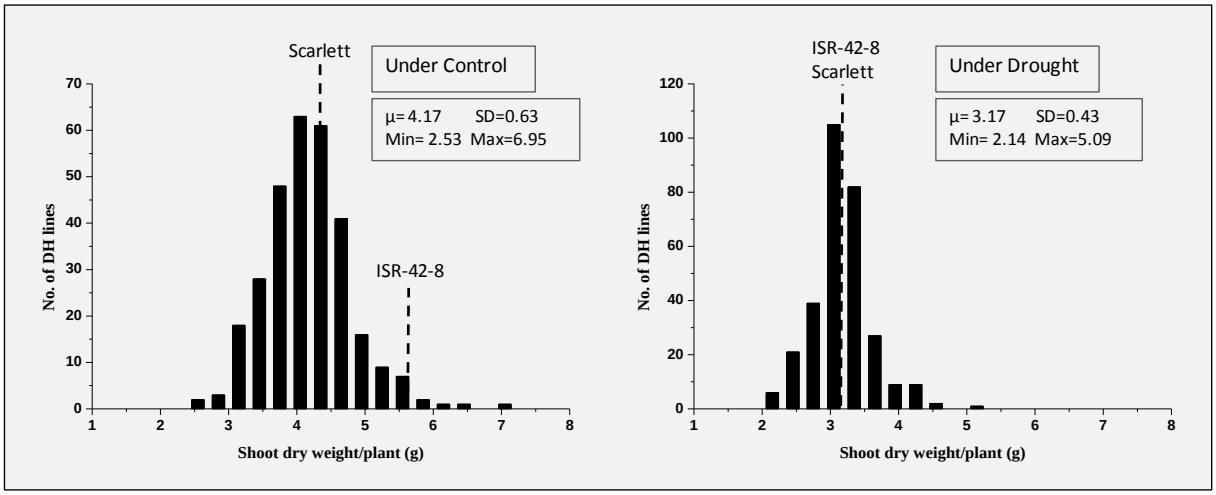


Figure 8 Frequency distribution of SDW under control and drought conditions with compared to the parents. The classes of SDW are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

Grain Yield/plant (GY)

The analysis of variance of the population for GY was revealed highly significant differences among accessions, treatments, years, accessions x year interaction and year x treatment interaction, while it was not significant for accessions x treatment interaction (Table 3). As an average over years, the grain yield/plant ranged from 0.89 to 3.37 g under control with an average of 2.14 g, while it ranged from 0.62 to 2.28 g with an average of 1.56 g (Table 4 and Figure 8). A total of 131 BC_2DH lines yielded more than Scarlett under drought conditions (Figure 9).

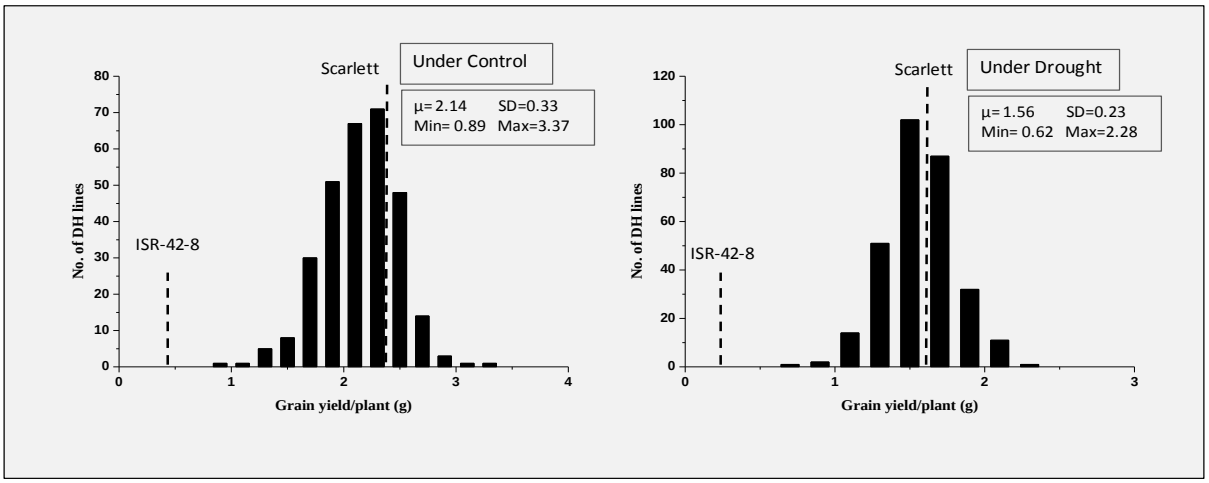


Figure 9 Frequency distribution of GY under control and drought conditions with compared to the parents. The classes of GY are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

Number of kernels/spike (KERS)

For the population, the same trend of GY has been observed for KERS because of the strong correlation between them. Highly significant differences were detected for KERS in relation to most of the source of variance including that accession, treatments, years, the interaction between accessions and years and the interaction between years and treatment, while the interaction between accessions and treatment was not significant (Table 3). The no. of kernels/spike ranged from 10.4 to 22.1 under control with an average of 16.4 kernels/spike, compared with KERS under drought conditions where it ranged from 9.25 to 26.3 with an average of 15.1 kernels/plant (Figure 10). A total of 113 BC_2DH lines yielded more kernels/spike than the elite parent.

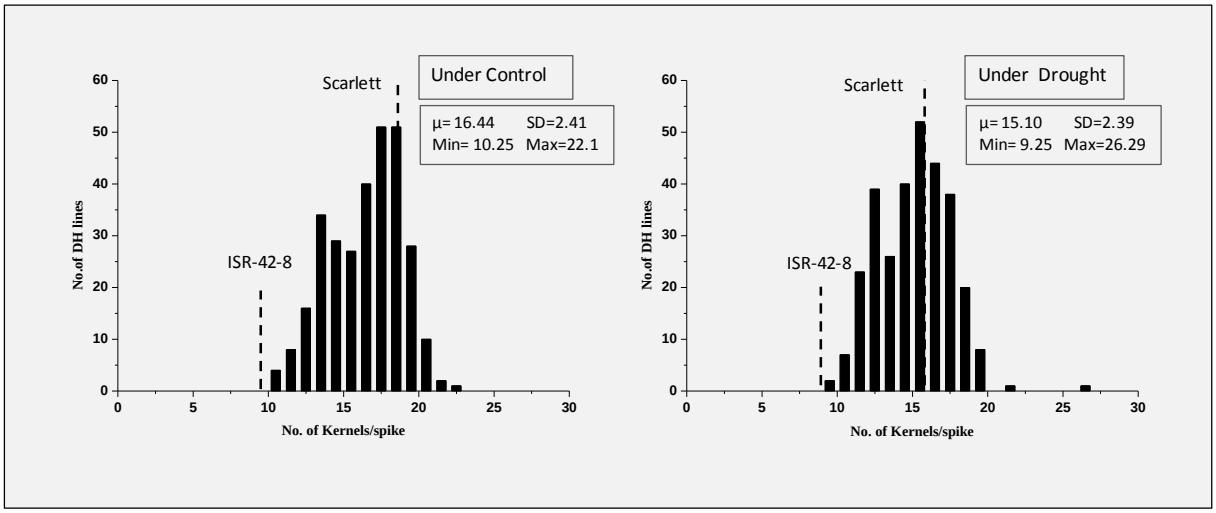


Figure 10 Frequency distribution of KERS under control and drought conditions with compared to the parents. The classes of KERS are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

Thousand grain weight (TGW)

The analysis of variance of the population for TGW was revealed highly significant differences among all types of source of variance. (Table 3). As an average over years, the weight of thousand grains ranged from 42.1 to 67.4 g under control with an average of 52.6 g, while it ranged from 37.3 to 55.2 g with an average of 47.3 g (Table 4 and Figure 11). A total of 157 BC_2DH lines had a higher weight of thousand grains than Scarlett under drought conditions (Figure 11).

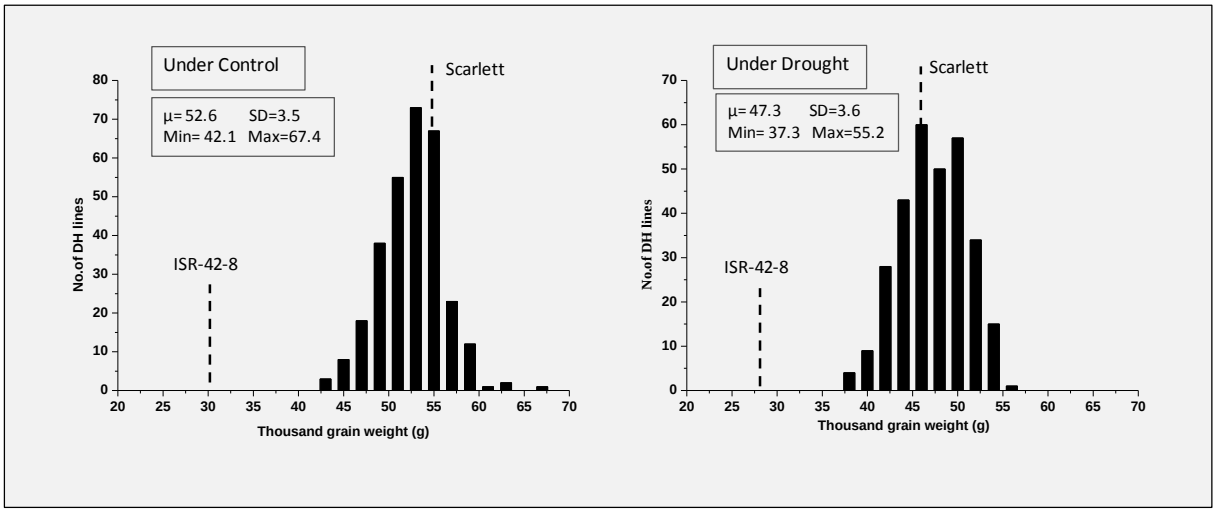


Figure 11 Frequency distribution of TKW under control and drought conditions with compared to the parents. The classes of TKW are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

Harvest Index (HI)

For the population, the analysis of variance was revealed highly significant differences among accessions, treatments, years and their interactions (Table 3). The harvest index of the population has been decreased under drought conditions. Since, the percentage of harvest index ranged from 26.65 to 66.22 % with an average of 51.24 % under control, while it ranged from 28.04 to 63.30 % with an average of 49.50 % under drought stress conditions (Figure 12). A maximum 127 of BC_2DH lines had higher percentage of harvest index than the Scarlett.

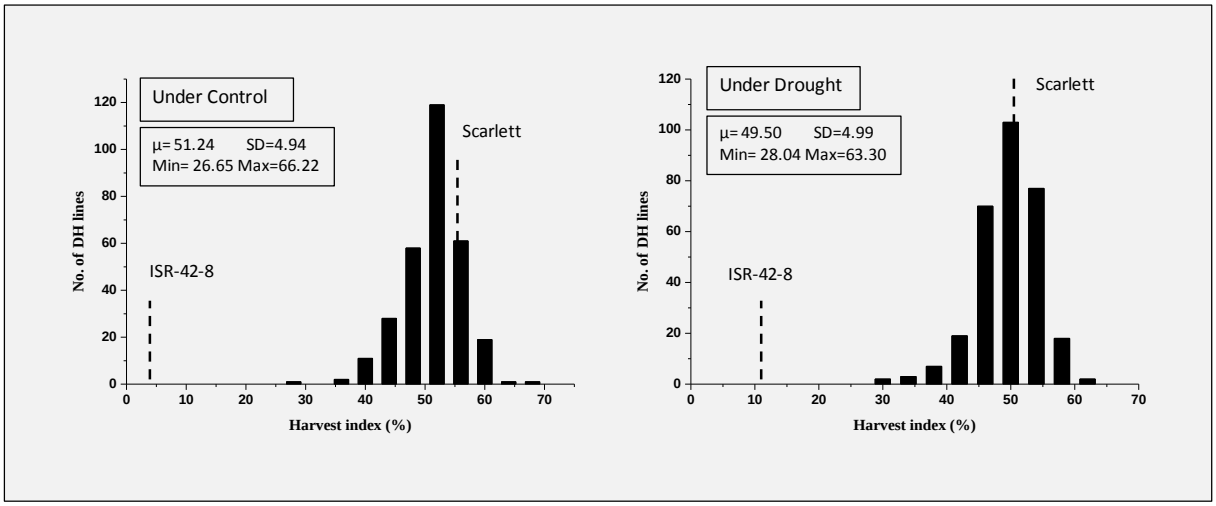


Figure 12 Frequency distribution of HI under control and drought conditions with compared to the parents. The classes of HI are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

3.2.2 Root traits

Root length (RL)

Highly significant differences were identified for root length in all sources of variance (Table 3). Root length was longer under drought stress conditions with an average of 22.96 cm and ranged from 10.66 to 42.00 cm compared with the root length under control, which ranged from 13.00 to 32.00 cm with an average of 20.53 cm. Three BC_2DH lines showed superior increase in root length (more than 38 cm long) under drought conditions than the wild accession (Figure 13).

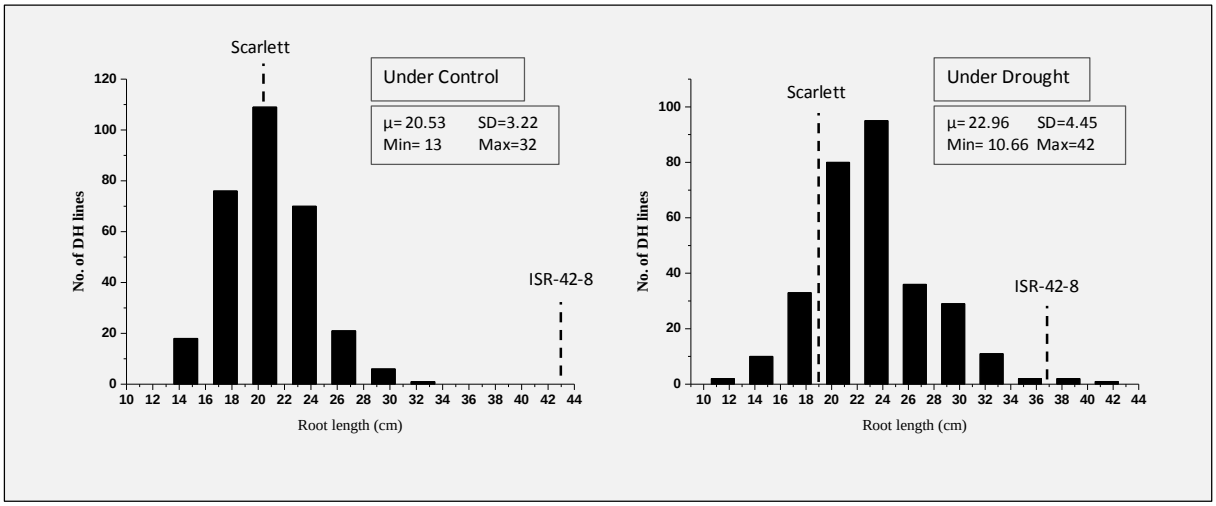


Figure 13 Frequency distribution of RL under control and drought conditions with compared to the parents. The classes of RL are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

Root dry weight (RDW)

The differences of RDW among accessions as well as years were highly significant, while they were not significant between treatments. On the other hand, the interactions between accessions with treatments, accession with years and years with treatments were high significant (Table 3). The mean of RDW under control (2.11 g) was higher than under drought conditions (1.93 g). No BC_2DH lines were observed to be higher root dry weight than exotic parent (Figure 14).

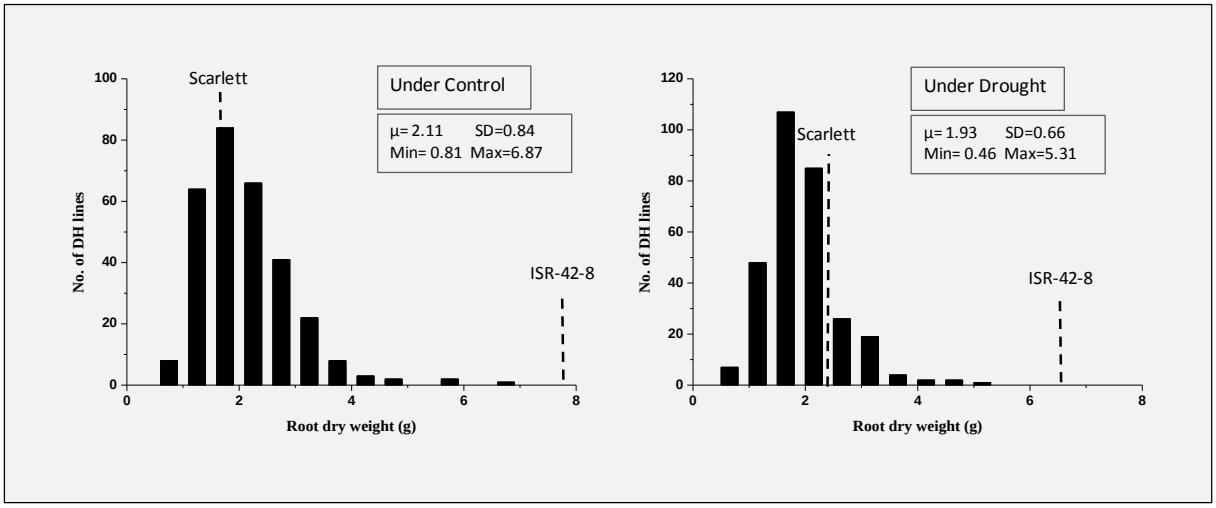


Figure 14 Frequency distribution of RDW under control and drought conditions with compared to the parents. The classes of RDW are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

Root shoot ratio (RSR)

The analysis of variance of RSR was revealed high significant differences only among the accessions and for the interaction between years and treatment (Table 3). The dry weight of the roots under control ranged from 1.58 to 15.56 g with an average 4.47 g, while it ranged from 1.87 to 12.31 g with an average of 5.57 g under drought conditions. No BC_2DH lines were observed to be higher root shoot ratio than exotic parent (Figure 15).

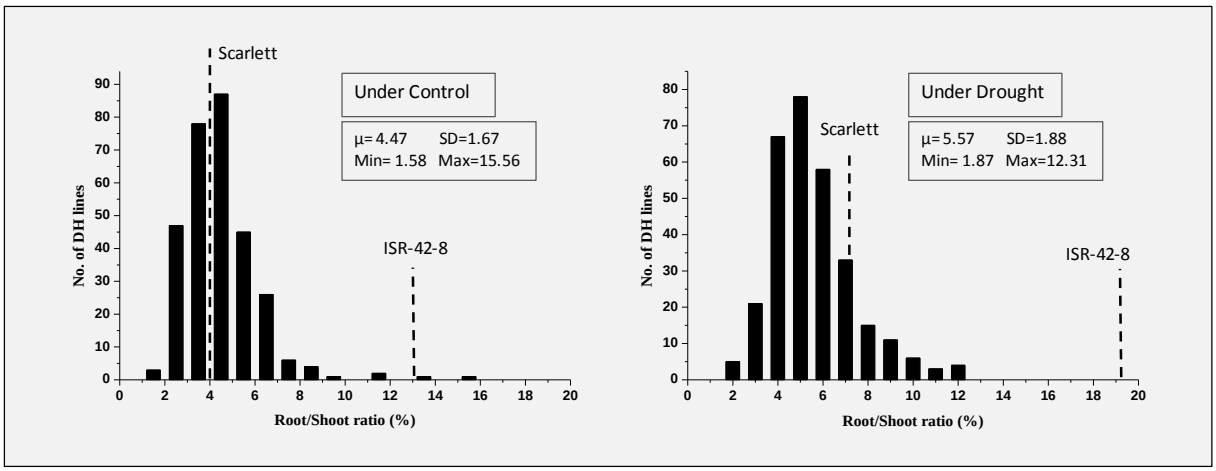


Figure 15 Frequency distribution of RSR under control and drought conditions with compared to the parents. The classes of RSR are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

3.2.3 Physiological traits

Relative water content (RWC)

For the population, highly significant differences for RWC were detected among accessions, treatments, years and their interactions except the interaction between accessions and years was not significant (Table 3). The accessions have affected by drought stress and content of water in leaves has been reduced, where the mean of RWC under control was 83.39 % and ranged from 55.8 to 92.8 %, while the mean of RWC under drought conditions was 52.61 % and ranged from 11.24 to 85.5 %. A total of eight BC_2DH lines showed superior increase in RWC than the wild accession under drought conditions (Figure 16).

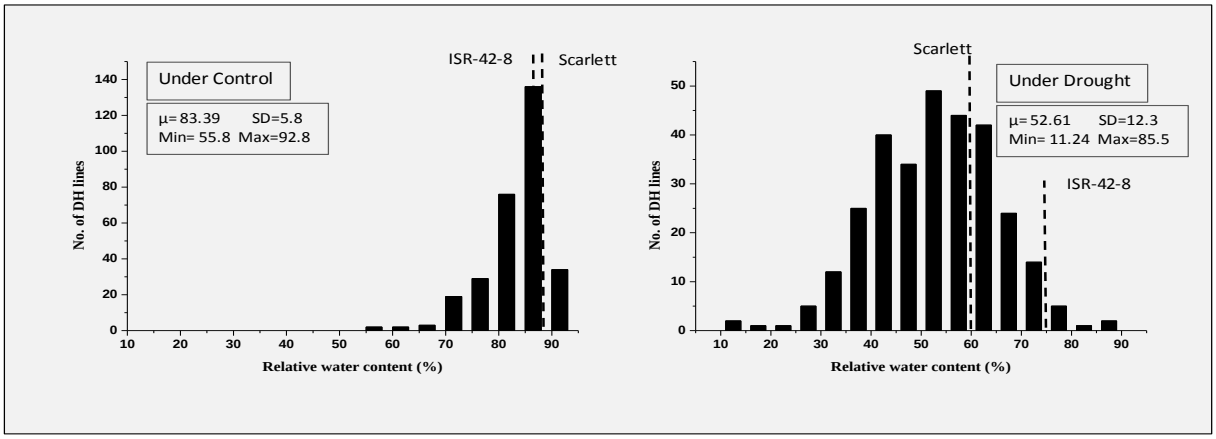


Figure 16 Frequency distribution of RWC under control and drought conditions with compared to the parents. The classes of RWC are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

Proline content (PC)

In response to proline accumulation, significant variation has been identified for the population S42 under both treatments. The variation between control and drought treatments was highly significantly. Drought stress led to increase PC in population S42 which showed a range of PC values from 0.42 to 23.33 µmol/gDW with an average of 5.9 µmol/g DW. A total of 87 BC_2DH lines showed higher values of PC than Scarlett under drought conditions. On average, a nine fold increase of PC (5.9 µmol/gDW) under drought conditions has been found as compared PC (0.67 µmol/gDW) under control (Figure 17).

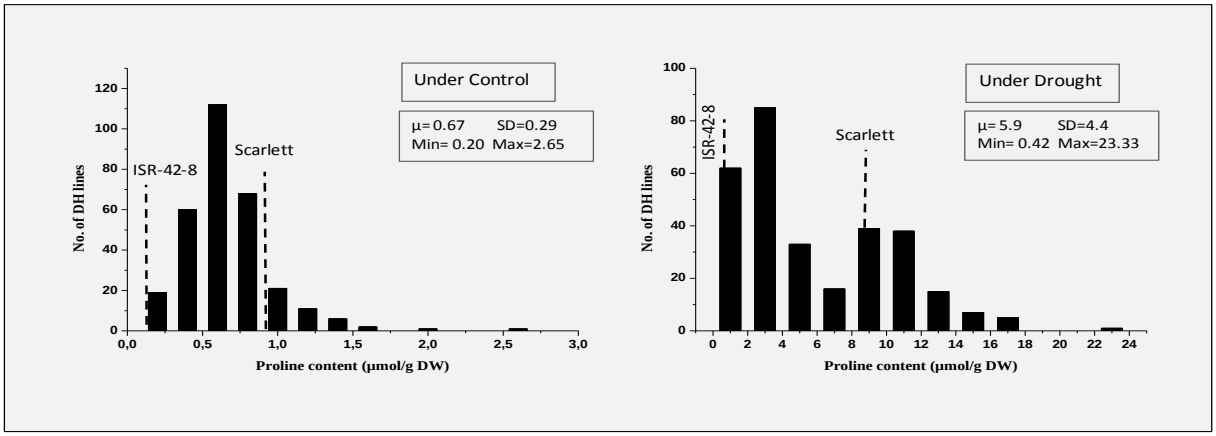
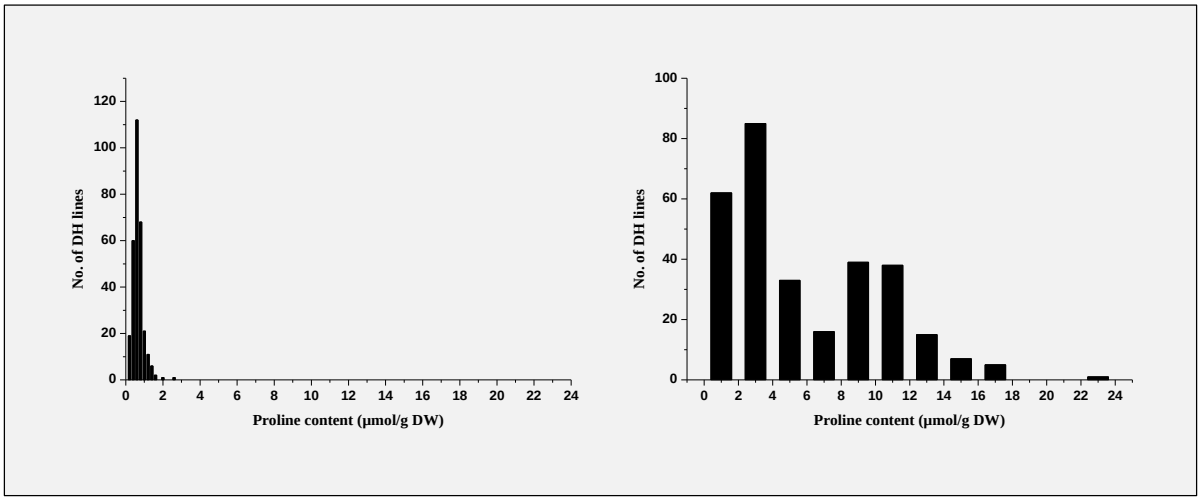


Figure 17 Frequency distribution of PC under control and drought conditions with compared to the parents. The classes of PC are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

In the following figure, we can see the real difference between the accumulation of proline under control and drought treatments. Proline accumulation has been increased many folds under drought conditions compared with control.



This figure shows content of proline under control and drought conditions with same scale.

Osmotic potential (OP)

The means of OP under both treatments almost was the same but it was little bit higher under drought conditions (Figure 18). A total of 157 BC_2DH lines showed higher values of OP than ISR 42-8 under drought conditions.

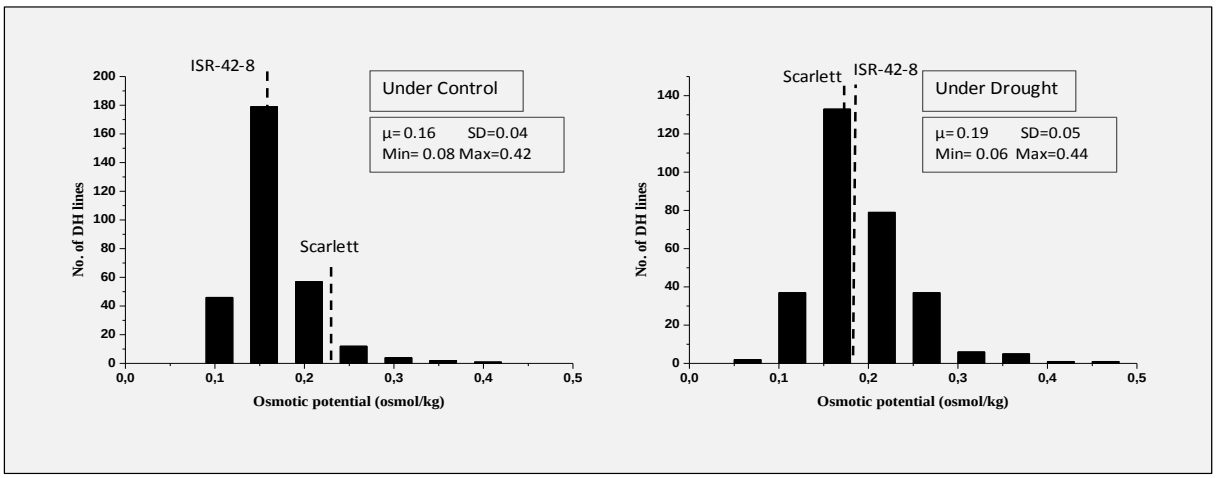


Figure 18 Frequency distribution of OP under control and drought conditions with compared to the parents. The classes of OP are shown on X-axis while the number of doubled haploid lines is presented on Y-axis

3.3 Phenotypic correlation among investigated traits

Mutual correlation of selected traits have been presented in Table (4), which were computed using the LS-mean of a trait for all accessions averaged across tested years and separately for control and drought stress treatments. A total of 70 significant correlation coefficients were determined for each treatment. Strong positive correlations were found between GY and with TILS, SPS, SDW, KERS and HI under both treatments, where the

correlation coefficients were 0.76, 0.81, 0.92, 0.31 and 0.47 under control, and were 0.59, 0.67, 0.84, 0.21 and 0.39 for TILS, SPS, SDW, KERS and HI under drought respectively. For the correlation between GY and root and physiological traits ranged between weak and relatively strong, where the correlation between GY and with all of RL, RDW, RSR and RWC was negative, and with all of PC and OP was positive under drought conditions, while under control, it was positive and highly significantly with all of RL, RDW, RWC and PC, while with all of RSR and OP it was negative and highly significant and non significant respectively. For TKW, correlation coefficients of different trends and significance have been observed, since negative and highly significant correlation between TGW and most of shoot traits were found under drought conditions, while it was positive and highly significant with SDW and GY under control. Moderate positive and highly significant correlation between TGW and each of RL, RDW, RSR and RWC has been observed under both treatments. Strong, positive and highly significant correlations were detected among root traits RL, RDW and RSR under both treatments, where the r values were 0.45, 0.29 and 0.81 under control, and were 0.68, 0.62 and 0.93 under drought conditions respectively. Negative correlations were detected among the physiological traits RWC, PC and OP and ranged from weak and strong correlation under both treatments, and it has been observed that RWC was correlated negatively with PC under control and drought conditions ,where r values were - 0.18* and - 0.62*** respectively.

Table 4 Correlation coefficients (r) according to Pearson in S42, computed between 14 investigated traits under control (left) and drought stress (right) conditions across three years.

Trait	PH	WS	TILS	SPS	SDW	GY	KERS	TKW	HI	RL	RDW	RSR	RWC	PC	OP
PH		0,23***	-0,14***	-0,12***	0,21***	0,021^{ns}	0,20***	0,01^{ns}	-0,37***	-0,09**	-0,08*	-0,14***	-0,06 ^{ns}	-0,01 ^{ns}	-0,004
WS	0,26***		0,05 ^{ns}	0,06*	-0,03 ^{ns}	-0,11***	-0,01^{ns}	-0,35**	-0,21***	-0,12***	-0,22***	-0,19***	-0,37***	0,15***	0,02 ^{ns}
TILS	-0,01 ^{ns}	-0,36***		0,91***	0,66***	0,59***	-0,36***	-0,48***	0,01 ^{ns}	0,01 ^{ns}	-0,01 ^{ns}	-0,17***	-0,55***	0,40***	0,05 ^{ns}
SPS	-0,04 ^{ns}	-0,33***	0,93***		0,68***	0,67***	-0,35***	-0,55***	0,10**	-0,13***	-0,15***	-0,30***	-0,59***	0,40***	0,08*
SDW	0,25***	-0,39***	0,71***	0,72***		0,84***	0,13***	-0,22***	-0,12***	-0,06*	-0,05 ^{ns}	-0,31***	-0,38***	0,28***	0,07 ^{ns}
GY	0,08**	-0,38***	0,76***	0,81***	0,92***		0,21***	-0,12***	0,39***	-0,01 ^{ns}	-0,02 ^{ns}	-0,27***	-0,30***	0,21***	0,07 ^{ns}
KERS	0,16***	-0,07^{ns}	-0,1***	-0,21***	0,31***	0,31***		-0,01ns	0,10**	-0,16***	-0,14***	-0,19***	0,10**	-0,06^{ns}	0,06^{ns}
TKW	0,11**	-0,20***	0,03^{ns}	0,03^{ns}	0,31***	0,27***	-0,01^{ns}		0.22***	0,39***	0,40***	0,38***	0,65***	-0,44***	-0,16^{ns}
HI	-0,35***	-0,11***	0,36***	0,47***	0,12***	0,47***	0,09**	-0,01ns		0,13***	0,06*	0,06*	0,11***	-0,10**	-0,01 ^{ns}
RL	0,24***	-0,02 ^{ns}	0,18***	0,12***	0,28***	0,21***	0,03^{ns}	0,28***	-0,06*		0,68***	0,62***	0,13***	0,02 ^{ns}	-0,09*
RDW	0,12***	-0,27***	0,26***	0,17***	0,38***	0,28***	0,08*	0,28***	-0,09**	0,45***		0,93***	0,20***	-0,01 ^{ns}	-0,11**
RSR	-0,03 ^{ns}	-0,09**	-0,11**	-0,17***	-0,13***	-0,18***	-,09**	0,09*	-0,15***	0,29***	0,81***		0,26***	-0,07*	-0,11**
RWC	-0,10**	-0,43***	0,18***	0,17***	0,27***	0,26***	0,14***	0,15***	0,05 ^{ns}	0,02 ^{ns}	0,16***	0,04 ^{ns}		-0,62***	-0,08*
PC	0,17***	0,22***	0,14***	0,13***	0,17***	0,16***	-0,06ns	0,20***	0,06*	0,33***	0,16***	0,01 ^{ns}	-0,18***		0,01 ^{ns}
OP	-0,03 ^{ns}	-0,01 ^{ns}	0,03 ^{ns}	0,02 ^{ns}	-0,02 ^{ns}	-0,01 ^{ns}	0,02ns	-0,06ns	0,01 ^{ns}	-0,07 ^{ns}	-0,06 ^{ns}	-0,08*	-0,01 ^{ns}	-0,06 ^{ns}	

Pearson correlation coefficients (r) were calculated by averaging the Lsmeans of a trait performance for each treatment separately, under control (left) and under drought stress conditions (right). The significance thresholds for r values were (***) P = 0.001, (**) P = 0.01, (*) P = 0.05. The phenotypic correlations were computed using the correlation procedure (PROC CORR, SAS institute 9.2 2008). PH (plant height), WS (wilting score), TILS (No. of tillers/plant), SPS (No. of spikes/plant), SDW (Shoot dry weight/plant), GY (Grain yield/plant), KERS (No. of Kernels/spike), Thousand kernel weight (TKW), HI (Harvest index), RL (Root length), RDW (Root dry weight), RSR (Root shoot ratio), RWC (Relative water content), PC (Proline content) and OP (Osmotic potential).

3.4 Genotyping of the population S42 (BC_2DH)

The population S42 was successfully genotyped with 371 polymorphic markers, 255 DArT, 106 SSR and 10 gene specific markers (Table 5 and Figure 16). The genotyping with DArT markers was done in Diversity Arrays Technology institute, AUSTRALIA, for the marker analysis with their hybridization based markers. The chromosomal positions of the DArT markers are according to Wenzl *et al.* (2006). Linkage distances between SSR and gene specific markers were taken from von Korff *et al.* (2004) and Wang *et al.* (2010) respectively. The genotyped markers were distributed over all seven chromosomes and covered 1154.31 cM of the barley genome in this population with an average of 164.90 cM (Table 5). The average distance between markers was 3.20 cM. However, the chromosome 7H had largest number of markers (67 markers), while the chromosome 4H had the smallest number (40 markers) of markers, the distribution of DArT markers ranged from 20 to 47 with an average of 36.43, while the distribution of SSR markers ranged from 11 to 20 with an average of 16.57. Only two gaps (> 20 cM) were observed on chromosomes 2H and 3H. 21 gaps (> 10 cM) were observed in this population and distributed on all chromosomes with an average 3 gaps per chromosome except chromosome 7H had no gaps exceeded 10 cM (Table 5).

Table 5 Number of genotyped DArT and SSR markers in the population S42.

Chrom.	No. of marker	DArT	SSR	Length	average (cM)	Gaps (> 10 cM)
1H	56	37	19	162,00	2,89	4
2H	58	40	18	163,34	2,82	3
3H	62	47	15	181,32	2,92	3
4H	40	20	20	148,58	3,71	4
5H	43	30	13	186,98	4,35	4
6H	45	34	11	147,09	3,27	3
7H	67	47	20	165,00	2,46	0
Total	371	255	116	1154,31	22,43	21
Average	53	36,43	16,57	164,90	3,20	3

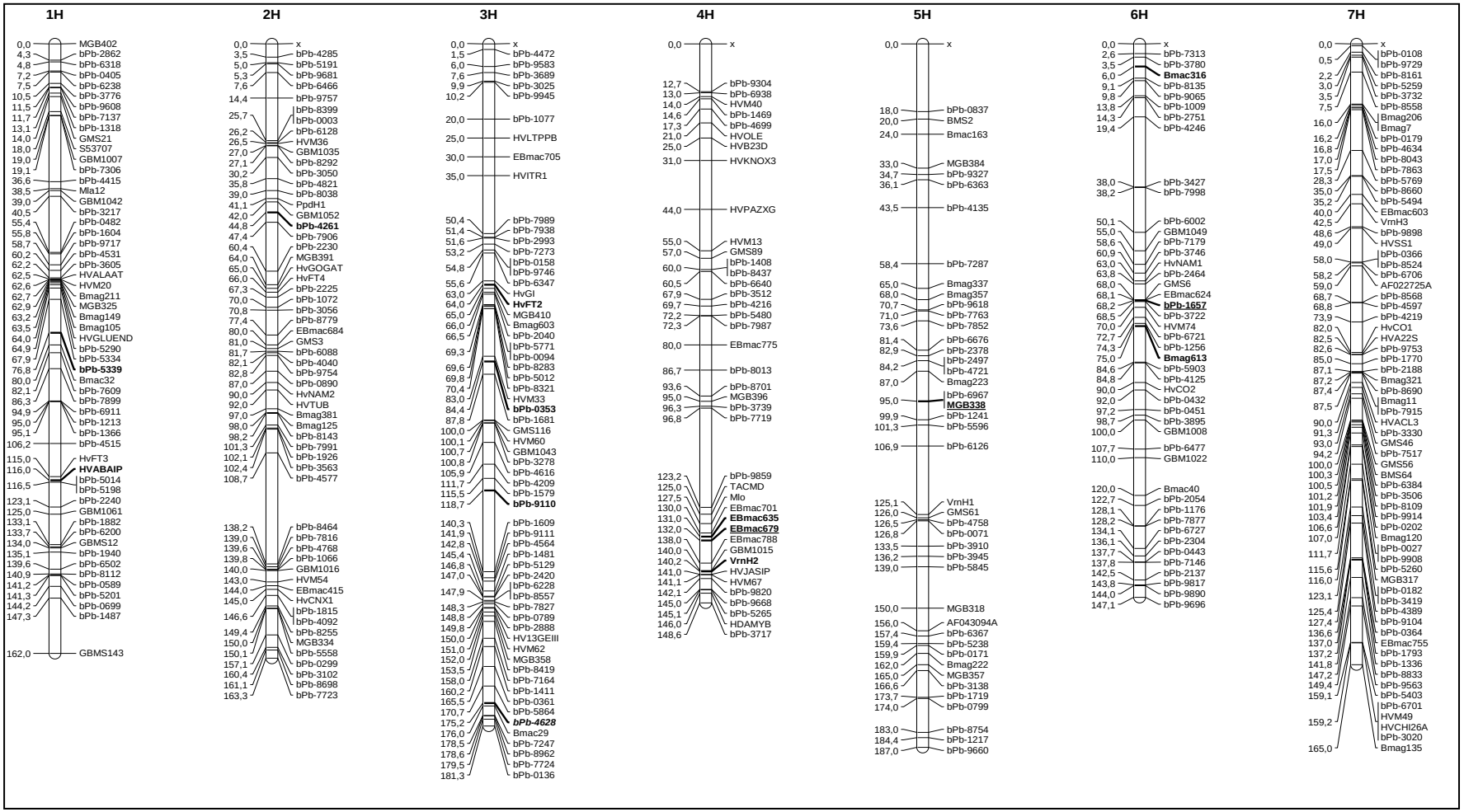


Figure 19 Molecular linkage map of barley derived from the Scarlett x ISR 42-8 population, contains 371 genetic markers. The 106 SSRs and 10 gene specific markers order is based on von Korff *et al.* (2004) and Wang *et al.* (2010). The DArT markers which prefixed by 'bPb' were genotyped according to Diversity Arrays Technology institute, Australia.

3.5 Detection of QTLs

In this study, the QTL effects were divided into two groups. The first group contains favorable QTL effects, where the marker main effect or marker×treatment (M×T) interaction effect of the *Hsp* genotype improves the trait in regard to the breeding goals under control and drought stress conditions. The second group contains unfavorable QTL effects, where the marker main effect or M×T interaction effect of the *Hsp* genotype reduces the trait in regard to the breeding goals under control and drought stress conditions (to see breeding goals, see Table 1). In total, 79 putative QTLs for all studied traits were detected among 5,565 marker × trait combinations which revealed 55 QTLs for shoot traits, 15 QTLs for root traits and 9 QTLs for physiological traits. Among of 79 putative QTLs, 72 QTLs were significant as marker main effects, 4 QTLs were significant as marker×treatment interaction effects and 3 QTLs had both effects. Overall 27 (34.1 %) QTLs showed favorable effects derived from the presence of exotic alleles. Out of 55 QTLs only 17 (30.9 %) QTLs for shoot traits were identified with favorable effects of the exotic alleles, nine (60 %) QTLs out of fifteen showed favorable effects for root traits and two (22.2 %) QTL out of nine showed favorable effect of the exotic alleles for physiological traits.

3.5.1 Detection of QTLs for shoot traits in the population S42

Altogether, 55 putative QTLs were detected for nine shoot traits (PH, WS, TILS, SPS, SDW, GY, KERS, TGW and HI) in S42 (Table 6 and Figure 17). Among these loci, 17 (30.9 %) QTLs for shoot traits were identified with favorable effects of the exotic alleles. Most of putative QTLs were located on chromosomes 1H, 2H, 4H and 5H by one, seven, eight and one QTL respectively. However, most of favorable effects of the *Hsp* alleles were detected on chromosomes 2H and 4H. In the following, the detected QTLs are described for each trait.

Plant height (PH)

Six putative QTLs for PH were mapped on chromosomes 1H, 2H, 3H and 4H (Figure 17). All loci exhibited significant marker main effects. According to the relative performance of the exotic allele ($Rp_{[aa]}$), the alleles of three QTLs (*QPH.S42.2H, QPH.S42.4H.a and QPH.S42.4H.b*) were exhibited a favorable performance of reducing PH by 10.91, 7.98 and 7.81 %, indicating by negative additive effects score were -2.60, -1.42 and -1.23 cm, respectively. These QTLs explained 12.96, 5.93 and 7.03 % of the genetic variance

respectively. The other QTLs (*QPH.S42.1H, QPH.S42.3H.a* and *QPH.S42.3H.b*) were exhibited increase in PH ranged between 1.69 and 28.12 %. Noteworthy, during the process of forward / backward selection for plant height, the marker locus bPb-9110 showed the highest F-value (533.27) along with iteration. This linked marker revealed a huge proportion of explained genetic variance (R^2g 59.16%) as marker main effect and exhibited high positive additive effect (8.22 cm) (Table 6).

Wilting score (WS)

Four QTLs were detected for WS and distributed on chromosomes 1H, 2H, 3H and 4H (Figure 19). All QTLs exhibited significant marker main effects. Two favorable QTL (*QWS.S42.1H* and *QWS.S42.4H*) effects were influenced by the presence of exotic alleles. At these loci, the favorable exotic alleles were responsible for almost 17% decrease in WS. These exotic alleles explain 11.96% and 9.41% of the genetic variance respectively. Negative additive effects were detected for these two QTLs with scores - 0.256 and - 0.180, respectively. In contrast, the exotic alleles at QTLs, *QWS.S42.2H* and *QWS.S42.3H* were associated to an enhancement of WS as compared to elite alleles. It means elite alleles appeared to be desirable for WS as compared to exotic alleles. An exotic allele at QTL, *QWS.S42.2H* posed 21.84% variation in WS and accounted for 5.63% of the R^2. Likewise, the relative performance of exotic allele at *QWS.S42.3H* was 34.87% inferior in comparison to respective elite allele. This QTL allele showed the highest F-value (211.38) along with iteration and presented a huge proportion of explained genetic variance 33.92% (Table 6).

Number of tillers/plant (TILS)

Five QTLs were associated significantly with TILS as marker main effects, and located on chromosomes 2H, 4H and 6H (Figure 19). Relative performances of the exotic genotype ranged between -10.90% and 24.66%. These loci showed crossover interactions. Four QTLs (*QTILS.S42.2H.a, QTILS.S42.2H.b, QTILS.S42.4H.a* and *QTILS.S42.4H.b*) exhibited favorable performance of exotic alleles and revealed an increasing of TILS. It is worth mentioning that, during the process of forward/backward selection for TILS, the marker locus GMS3 showed the highest F-value (324.63) along with iteration. This linked marker revealed a huge proportion of explained genetic variance (R^2g 39.86%) as marker main effect and exhibited high positive additive effect (0.27) (Figure 18). The result of the additive

effects of those QTLs indicates that the exotic alleles appeared to be desirable for TILS as compared to elite alleles. On other hand, the QTL *QTILS.S42.6H* showed decreasing in TILS by 10.90% and explained 8.83% of genetic variance (Table 6).

Number of spikes/plant (SPS)

Seven QTLs were detected for SPS and located on chromosomes 2H, 3H, 4H and 6H (Figure 19). All these QTLs showed significant marker main effects. The relative performances of the exotic genotype ranged between -19.89% and 25.68%. Among these, four QTLs showed favorable performance of the exotic genotype alleles and revealed an increasing of SPS. The QTLs (*QSPS.S42.2H.a* and *QSPS.S42.2Hb2*) explained 40.95 and 34.80% of the genetic variance respectively. The alleles for SPS were contributed from the parent 'ISR 42-8' and led to increase number of spikes/plant. As in the case of TILS, the same marker locus GMS3 showed the highest F-value (297.97) along with process of forward selection and revealed a huge proportion of explained genetic variance (40.95%). On other hand, the QTLs (QSPS.S42.3H, QSPS.S42.6H.a and QSPS.S42.6H.b) showed decreasing in SPS by percentage up to 19.89% and explained up to 11.71% of the genetic variance.

Shoot dry weight (SDW)

Five QTLs were associated for SDW and distributed on chromosomes 2H, 5H and 6H (Figure 20). Four QTLs exhibited significant marker main effects, and one QTL showed significant marker×treatment interaction effect. Only one QTL, at *QSDW.S42.5H* revealed favorable alleles to increase SDW, and the exotic alleles explained by 3.64% of the genetic variance with favorably increased SDW by 11% (Table 7). The other four QTLs showed negative effects of the exotic alleles and led to the reduction of SDW by 21.92% and explained up to 14.88% of the genetic variance.

Table 6 Localization of 79 QTLs for 15 studied traits as marker main and interactions effects ($P_{FDR} \leq 0.05$), as well as coefficient of determination R^2 (%) and relative performance Rp[aa] of *Hsp*.

Trait	Marker	Type	Chrom	Pos.	Effect	F value	Sign.	P_{FDR}	*Ls-Hv DT*	*LS-Hsp DT*	Diff.*Hsp*	Rp[aa]	Add.*	R^2 (%)	*QTL Effect*	*QTLs*
Shoot traits																
PH	bPb-3605	DArT	1H	62,23	M	13,97	*	< 0,05	66,83	68,73	1,90	1.69	2,321	0,17	+	*QPH.S42.1H*
	GMS3	SSR	2H	81,00	M	42,68	***	< 0,01	69,16	61,53	-7,63	-10.91	-2,603	12,96	-	*QPH.S42.2H*
	GBM1043	SSR	3H	100,70	M	12,51	*	< 0,05	65,86	71,20	5,34	9.18	1,435	7,85	+	*QPH.S42.3H.a*
	bPb-9110	DArT	3H	118,72	M	533,27	***	< 0,01	63,65	80,60	16,96	28.12	8,223	59,16	+	*QPH.S42.3H.b*
	EBmac635	SSR	4H	131	M	15,25	*	< 0,05	68,10	62,99	-5,11	-7.98	-1,419	5,93	-	*QPH.S42.4H.a*
	HDAMYB	SSR	4H	146,00	M	11,58	*	< 0,05	68,44	63,36	-5,08	-7.81	-1,229	7,03	-	*QPH.S42.4H.b*
WS	HVABAIP	SSR	1H	116,00	M	43,40	***	< 0,01	5,22	4,54	-0,68	-17.29	-0,256	11,96	-	*QWS.S42.1H*
	bPb-4261	DArT	2H	44,79	M	28,36	***	< 0,01	5,02	5,87	0,85	21.84	0,432	5,63	+	*QWS.S42.2H*
	bPb-9110	DArT	3H	118,72	M	211,38	***	< 0,01	4,86	5,93	1,07	34.87	0,477	33,92	+	*QWS.S42.3H*
	VrnH2	SSR	4H	140,20	M	21,52	**	< 0,01	5,24	4,61	-0,64	-16.69	-0,180	9,41	-	*QWS.S42.4H*
TILS	GMS3	SSR	2H	81,00	M	324,63	***	< 0,01	2,44	2,98	0,54	24.66	0,273	39,86	+	*QTILS.S42.2H.a*
	HvNAM2	SSR	2H	90,00	M	13,84	*	< 0,05	2,43	2,93	0,51	22.20	0,092	35,99	+	*QTILS.S42.2H.b*
	Mlo	SSR	4H	127,50	M	22,63	**	< 0,01	2,49	2,87	0,38	16.27	0,101	15,18	+	*QTILS.S42.4H.a*
	VrnH2	SSR	4H	140,20	M	45,05	***	< 0,01	2,49	2,82	0,33	14.25	0,083	14,27	+	*QTILS.S42.4H.b*
	bPb-5903	DArT	6H	84,64	M	34,61	***	< 0,01	2,65	2,36	-0,29	-10.90	-0,111	8,83	-	*QTILS.S42.6H*
SPS	GMS3	SSR	2H	81,00	M	297,97	***	< 0,01	2,17	2,72	0,55	25.68	0,247	40,95	+	*QSPS.S42.2H.a*
	HvNAM2	SSR	2H	90,00	M	12,56	*	< 0,05	2,16	2,67	0,51	22.82	0,087	34,80	+	*QSPS.S42.2H.b*
	Bmag603	SSR	3H	66,00	M	13,10	*	< 0,05	2,36	1,85	-0,51	-19.89	-0,112	9,94	-	*QSPS.S42.3H*
	Mlo	SSR	4H	127,50	M	49,51	***	< 0,01	2,23	2,63	0,39	17.70	0,082	17,53	+	*QSPS.S42.4H.a*
	GBM1015	SSR	4H	140,00	M	19,45	**	< 0,01	2,20	2,62	0,42	17.08	0,095	18,75	+	*QSPS.S42.4H.b*
	Bmag613	SSR	6H	75,00	M	62,96	***	< 0,01	2,40	2,04	-0,36	-12.70	-0,081	11,71	-	*QSPS.S42.6H.a*
	bPb-0432	DArT	6H	91,99	M	24,75	***	< 0,01	2,37	2,12	-0,25	-10.89	-0,100	7,26	-	*QSPS.S42.6H.b*
SDW	PpdH1	SSR	2H	41,10	M	51,84	***	< 0,01	3,21	2,50	-0,70	-21.92	-0,36	14,88	-	*QSDW.S42.2H.a*
	GMS3	SSR	2H	81,00	M	14,85	*	< 0,05	3,25	2,99	-0,26	-6.63	-0,10	5,82	-	*QSDW.S42.2H.b*
	bPb-8143	DArT	2H	98,21	M*T	18,42	**	< 0,01	3,18	3,09	-0,09	-6.44	-0,12	4,73	-	*QSDW.S42.2H.c*
	bPb-0071	DArT	5H	126,77	M	27,21	***	< 0,01	3,16	3,54	0,38	11.00	0,27	3,64	+	*QSDW.S42.5H*
	EBmac624	SSR	6H	68,10	M	28,76	***	< 0,01	3,23	2,96	-0,28	-8.53	-0,14	6,50	-	*QSDW.S42.6H*

Table (6) Continued.

Trait	Marker	Type	Chrom	Pos.	Effect	F value	Sign.	P_{FDR}	*Ls-Hv DT*	*LS-Hsp DT*	Diff.*Hsp*	Rp[aa]	Add.*	R^2 (%)	*QTL Effect*	*QTLs*
Shoot traits																
GY	bPb-4261	DArT	2H	44,79	M	22,71	***	< 0,01	1,58	1,37	-0,21	-13.30	-0,12	5,87	-	*QGY.S42.2H.a*
	bPb-8143	DArT	2H	98,21	M	17,32	**	< 0,01	1,59	1,47	-0,12	-8.96	-0,11	7,60	-	*QGY.S42.2H.b*
	bPb-7989	DArT	3H	50,43	M	47,24	***	< 0,01	1,58	1,41	-0,16	-13.57	-0,06	7,22	-	*QGY.S42.3H.a*
	Bmag603	SSR	3H	66,00	M	25,32	***	< 0,01	1,59	1,29	-0,30	-17.90	-0,09	12,57	-	*QGY.S42.3H.b*
	bPb-9110	DArT	3H	118,72	M	68,60	***	< 0,01	1,61	1,41	-0,19	-12.51	-0,09	14,34	-	*QGY.S42.3H.c*
	GMS6	SSR	6H	68,00	M	65,67	***	< 0,01	1,61	1,39	-0,22	-12.38	-0,10	13,17	-	*QGY.S42.6H*
KERS	PpdH1	SSR	2H	41,1	M	41,2	***	< 0,01	15,24	11,94	-3,30	-22,97	-1,29	11,28	-	*QKER.S42.2H.a*
	bPb-8779	DArT	2H	77,4	M	408,3	***	< 0,01	16,05	12,59	-3,46	-21,42	-1,32	48,99	-	*QKER.S42.2H.b*
	HvNAM2	SSR	2H	90,0	M	31,5	***	< 0,01	16,07	12,91	-3,16	-18,11	-0,66	37,96	-	*QKER.S42.2H.c*
	bPb-7938	DArT	3H	51,4	M	23,6	***	< 0,01	15,17	14,54	-0,63	-4,03	-0,73	0,60	-	*QKER.S42.3H*
	Mlo	SSR	4H	127,5	M	11,2	*	< 0,05	15,41	13,91	-1,50	-9,12	-0,33	6,96	-	*QKER.S42.4H*
	Bmac40	SSR	6H	120,0	M	11,9	*	< 0,01	14,84	15,95	1,12	6,44	0,32	3,50	+	*QKER.S42.6H*
TGW	HvFT3	SSR	1H	115,0	M	19,6	***	< 0,01	46,81	49,33	2,52	4,56	0,92	7,40	+	*QTGW.S42.1H*
	bPb-4209	DArT	3H	111,7	M	25,6	***	< 0,01	47,59	45,89	-1,70	-4,14	-0,74	5,85	-	*QTGW.S42.3H*
	EBmac635	SSR	4H	131,0	M	56,5	***	< 0,01	47,24	47,50	0,25	0,18	0,04	1,35	-	*QTGW.S42.4H.a*
	HVM67	SSR	4H	141,1	M	15,0	**	< 0,01	47,82	45,56	-2,26	-4,53	-1,46	9,13	-	*QTGW.S42.4H.b*
	HvNAM1	SSR	6H	63,0	M	39,9	***	< 0,01	47,46	46,88	-0,59	-1,63	-0,60	1,64	-	*QTGW.S42.6H.a*
	bPb-6721	DArT	6H	72,7	M*T	11,8	**	< 0,01	47,47	46,86	-0,61	-1,70	-0,68	1,89	-	*QTGW.S42.6H.b*
	BMS64	SSR	7H	100,3	M	17,9	**	< 0,01	47,76	45,35	-2,41	-5,14	-1,30	9,61	-	*QTGW.S42.7H*
HI	HVALAAT	SSR	1H	62,50	M	15,95	**	< 0,01	49,74	47,55	-2,19	-5.26	-1,10	3,11	-	*QHI.S42.1H*
	PpdH1	SSR	2H	41,10	M	25,25	***	< 0,01	49,32	53,79	4,47	8.73	2,06	3,81	+	*QHI.S42.2H*
	bPb-7989	DArT	3H	50,43	M	27,80	***	< 0,01	50,00	43,69	-6,31	-13.19	-2,34	14,71	-	*QHI.S42.3H.a*
	HvGI	SSR	3H	63,00	M	203,87	***	< 0,01	50,28	42,00	-8,29	-16.79	-0,30	22,98	-	*QHI.S42.3H.b*
	bPb-9110	DArT	3H	118,72	M	133,48	***	< 0,01	50,70	44,88	-5,82	-10.69	-2,33	23,15	-	*QHI.S42.3H.c*
	EBmac701	SSR	4H	130,00	M	26,82	***	< 0,01	48,97	51,53	2,56	6.48	1,61	7,78	+	*QHI.S42.4H*
	MGB384	SSR	5H	33,00	M	28,55	***	< 0,01	49,89	46,62	-3,26	-6.89	-0,63	6,97	-	*QHI.S42.5H.a*
	GMS61	SSR	5H	126,00	M	29,18	***	< 0,01	49,82	42,94	-6,88	-10.95	-2,50	5,82	-	*QHI.S42.5H.b*
	HvCO2	SSR	6H	90,00	M	24,43	***	< 0,01	50,35	47,26	-3,09	-6.30	-0,42	8,36	-	*QHI.S42.6H*

Table (6) Continued.

Trait	Marker	Type	Chrom	Pos.	Effect	F value	Sign.	P_{FDR}	*Ls-Hv DT*	*LS-Hsp DT*	Diff.*Hsp*	Rp[aa]	Add.*	R^2 (%)	*QTL Effect*	*QTLs*
Root traits																
RL	PpdH1	SSR	2H	41,10	M	17,59	**	< 0,01	23,16	18,67	-4,49	-15.09	-1,83	6,13	-	*QRL.S42.2H*
	bPb-9110	DArT	3H	118,72	M	18,07	**	< 0,01	23,45	21,08	-2,37	-7.69	-0,95	5,52	-	*QRL.S42.3H*
	VrnH1	SSR	5H	125,10	M	13,29	*	< 0,05	22,83	25,14	2,32	9.17	1,35	1,73	+	*QRL.S42.5H*
RDW	GBM1042	SSR	1H	39,00	M	16,12	**	< 0,01	1,90	2,21	0,31	28.89	0,21	6,50	+	*QRDW.S42.1H.a*
	bPb-2240	DArT	1H	123,09	M	26,12	***	< 0,01	1,85	2,18	0,33	21.39	0,20	7,85	+	*QRDW.S42.1H.b*
	bPb-4261	DArT	2H	44,79	M	24,37	***	< 0,01	1,97	1,36	-0,62	-34.07	-0,35	6,54	-	*QRDW.S42.2H*
	bPb-9110	DArT	3H	118,72	M	31,55	***	< 0,01	2,02	1,63	-0,39	-21.40	-0,21	7,88	-	*QRDW.S42.3H*
	EBmac635	SSR	4H	131	M	11,32	***	< 0,01	1,98	1,80	-0,18	-13.03	-0,14	3,41	-	*QRDW.S42.4H*
	bPb-0071	DArT	5H	126,77	M	30,58	***	< 0,01	1,90	2,46	0,56	29.16	0,36	4,21	+	*QRDW.S42.5H*
	VrnH3	SSR	7H	42,50	M	35,74	***	< 0,01	1,91	2,61	0,70	41.88	0,43	6,91	+	*QRDW.S42.7H*
RSR	GBM1042	SSR	1H	39,00	M	21,22	***	< 0,01	5,48	6,37	0,89	25.96	0,55	6,27	+	*QRSR.S42.1H.a*
	bPb-2240	DArT	1H	123,09	M	29,06	***	< 0,01	5,32	6,32	1,00	20.13	0,42	8,26	+	*QRSR.S42.1H.b*
	bPb-9110	DArT	3H	118,72	M	17,62	**	< 0,01	5,78	4,77	-1,01	-18.95	-0,42	7,35	-	*QRSR.S42.3H*
	bPb-0071	DArT	5H	126,77	M	12,94	*	< 0,05	5,50	6,59	1,09	22.67	0,57	3,16	+	*QRSR.S42.5H*
	VrnH3	SSR	7H	42,50	M	28,13	***	< 0,01	5,50	7,20	1,70	37.21	0,97	6,60	+	*QRSR.S42.7H*
Physiological traits																
RWC	GBM1052	SSR	2H	42,00	M	30,40	***	< 0,01	59,80	47,72	-12,07	-14.07	-3,30	11,50	-	*QRWC.S42.2H.a*
	EBmac684	SSR	2H	80,0	M	29,33	***	< 0,01	60,84	55,74	-5,10	-4.08	-1,74	5,70	-	*QRWC.S42.2H.b*
	HvNAM2	SSR	2H	90,00	M*T	10,95	**	< 0,01	60,71	55,68	-5,02	-3.44	-1,24	14,96	-	*QRWC.S42.2H.c*
	bPb-9110	DArT	3H	118,72	M	50,41	***	< 0,01	60,26	55,24	-5,02	-7.51	-2,61	5,60	-	*QRWC.S42.3H*
PC	bPb-4628	DArT	3H	175,24	M, M*T	23,61	***	< 0,01	6,50	3,45	-3,05	-42.54	-0,77	6,13	-	*QPC.S42.3H*
	EBmac635	SSR	4H	131-132	M, M*T	15,51	*	< 0,05	6,29	4,42	-1,87	-27.20	-0,47	4,19	-	*QPC.S42.4H*
	MGB338	SSR	5H	95,00	M*T	13,95	***	< 0,01	5,63	9,09	3,47	53.75	0,89	4,07	+	*QPC.S42.5H*
	Bmag613	SSR	6H	68-75	M, M*T	20,48	**	< 0,01	6,29	4,46	-1,84	-26.49	-0,44	3,94	-	*QPC.S42.6H*
OP	HVM67	SSR	4H	141,10	M	16,68	*	< 0,05	0,20	0,18	-0,02	-9.94	-0,01	6,75	-	*QOP.S42.4H*

Table (6). Continued.

Trait: **PH** (Plant Height), **WS** (Wilting Score), **TILS** (No. of Tillers/plant), **SPS** (No. of Spikes/plant), **SDW** (Soot Dry Weight/plant), **GY** (Grain Yield/plant), **KERS** (No. of Kernels/spike), **TGW** (Thousand grain weight), **HI** (Harvest Index), **RL** (Root Length), **RDW** (Root Dry Weight), **RSR** (Root Shoot Ratio), **RWC** (Relative Water Content), **PC** (Proline Content) and **OP** (Osmotic Potential).

Chrom.: Chromosomal location of SSR markers were derived from Von Korff *et al.* (2004), while chromosomal locations of DArT markers were derived from Diversity Array Technology Institute, Australia.

Pos.: Position of SSR markers in cM on chromosome derived from Von Korff *et al.* (2004), while Position of DArT markers were derived from Diversity Array Technology Institute, Australia.

Effect: A significant marker×trait association was specified with marker main effect (M) or marker×environment interaction effect (M×E).

F-val.: F-value was computed using the Proc mixed procedure (REML).

Sign.: Level of significance computed using the Proc mixed procedure (REML). of the significant marker×trait associations for marker main effect (M) or marker×treatmentt interaction effect (M×T), (**) P = 0.001, (*) P = 0.01.

P_{FDR}: The portability of false discovery rate was computed by proc mixed procedure.

LS-*Hv*: LS-means of trait values for the German spring barley cultivar ''Scarlett'' (*H. vulgare* ssp. *vulgare*) under drought conditions for BC2DH accessions carrying the cultivar genotype (Scarlett) at the given marker locus.

LS-*Hsp*: LS-means of trait values for the exotic accession of *H. vulgare ssp. spontaneum* (ISR42-8) under drought conditions for BC2DH accessions carrying the exotic genotype (Scarlett) at the given marker locus.

RP [aa]: Relative performance of exotic genotype (ISR 42-8) at a given marker locus across all tested environments computed using the Proc mixed procedure (REML).. Relative performance was computed as ([aa] - [AA])×100 / [AA], where [AA] or [aa] were LS-means of BC2DH lines carrying the cultivar genotype (Scarlett) or the exotic genotype (ISR 42-8) at the given marker locus.

Add. The additive value is half the difference between the phenotypic values of the two homozygous parents. A positive value indicates that the allele increasing the trait value originates from ISR 42-8.

R^2 (%): Proportion of the genetic variance computed using the Proc mixed procedure (REML)., which was explained the marker main effect (M) or the marker×treatement interaction effect (M×T).

QTL effect: Relative performance of exotic genotype (ISR 42-8) at a given marker locus under drought conditions computed using the proc mixed procedure specified a favorable QTL effect (+) with a improved effect from the exotic genotype (ISR 42-8) and not favorable QTL effect (-) with a reduced effect from the exotic genotype (ISR 42-8).

QTL: A significant marker×trait association was specified as QTL, if marker main effect (M) or marker×treatment interaction effect (M×T), was significant with FDR = 0.05 in the Proc mixed procedure.

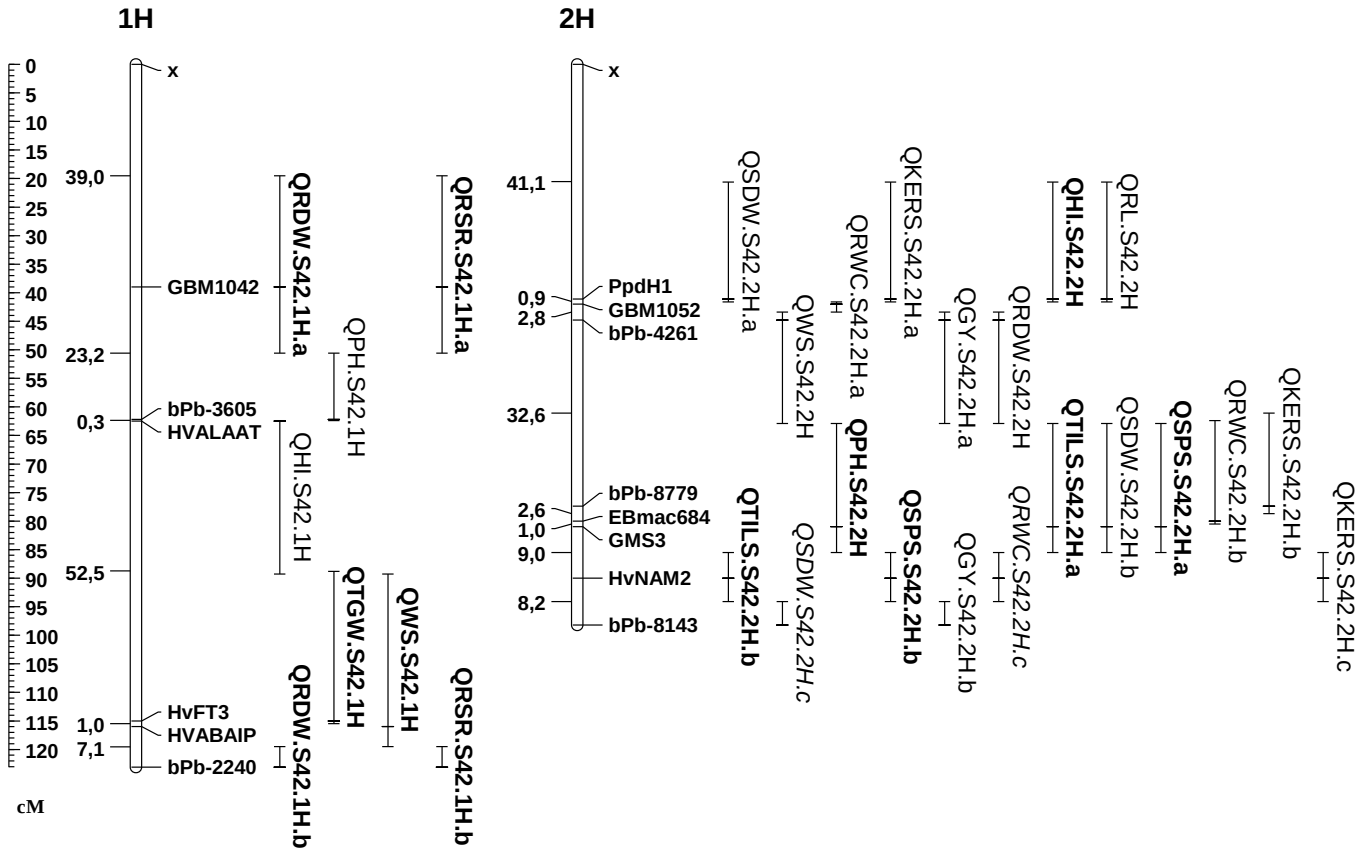


Figure 20 Localization of 79 putative QTLs ($P_{FDR} \leq 0.05$) detected for shoot, root and physiological traits including 27 favorable QTLs**.**

This linkage map was drawn using MapChart ver.2.2 (Voorrips 2002). The ruler (in cM) was on the left. Mapped markers were indicated on the right and their corresponding genetic intervals (cM) were indicated on the left of the chromosomes. Non italic QTLs were marker main effects and italic QTLs were marker×treatment interaction effects., while QTLs marked with an asterisk had both marker main and marker x treatment interaction. Bold QTLs were specified as favorable QTLs where the exotic genotype (ISR 42-8) improved the trait performance in regard to the breeding goals

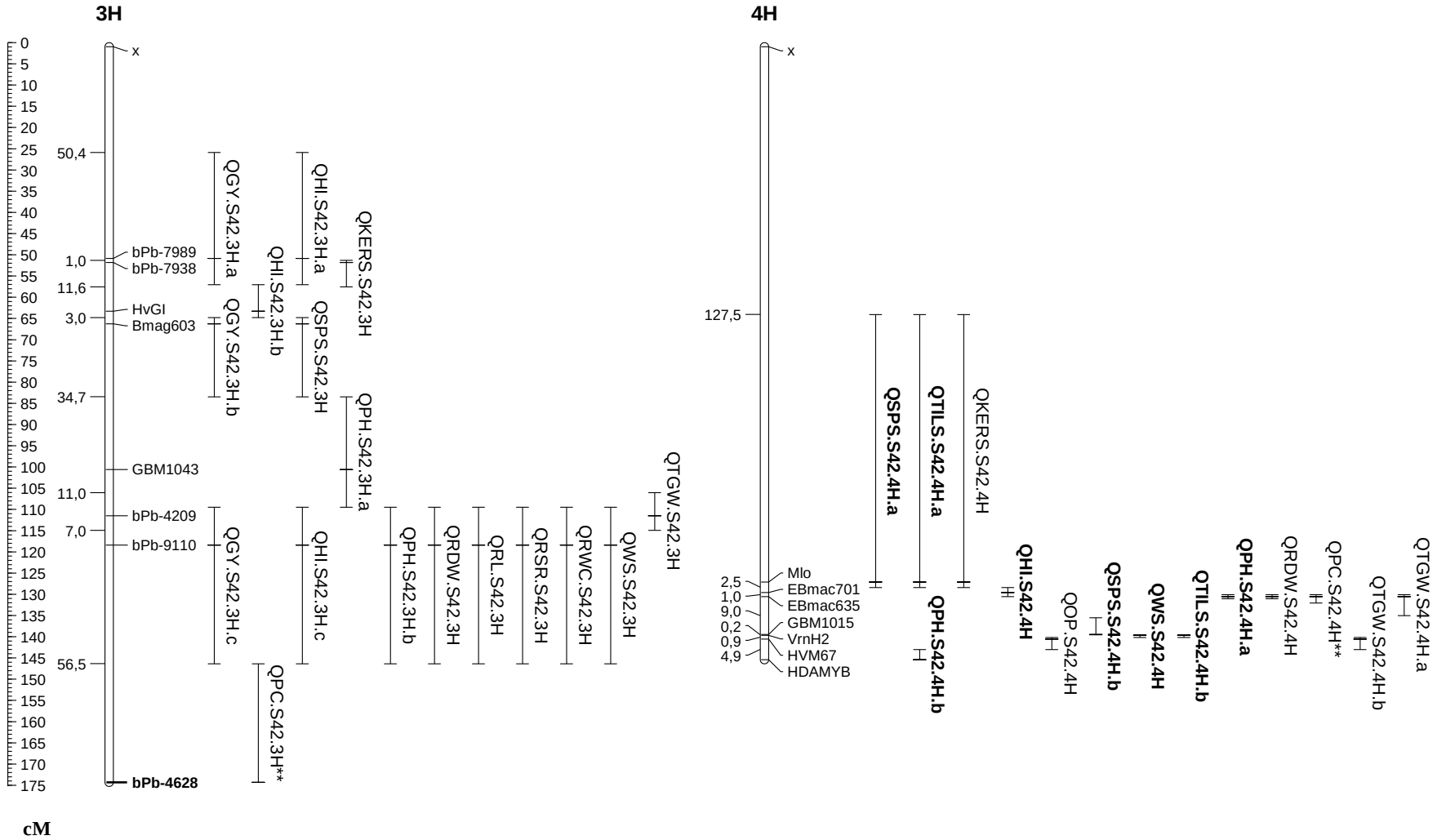


Figure 20 Continued.

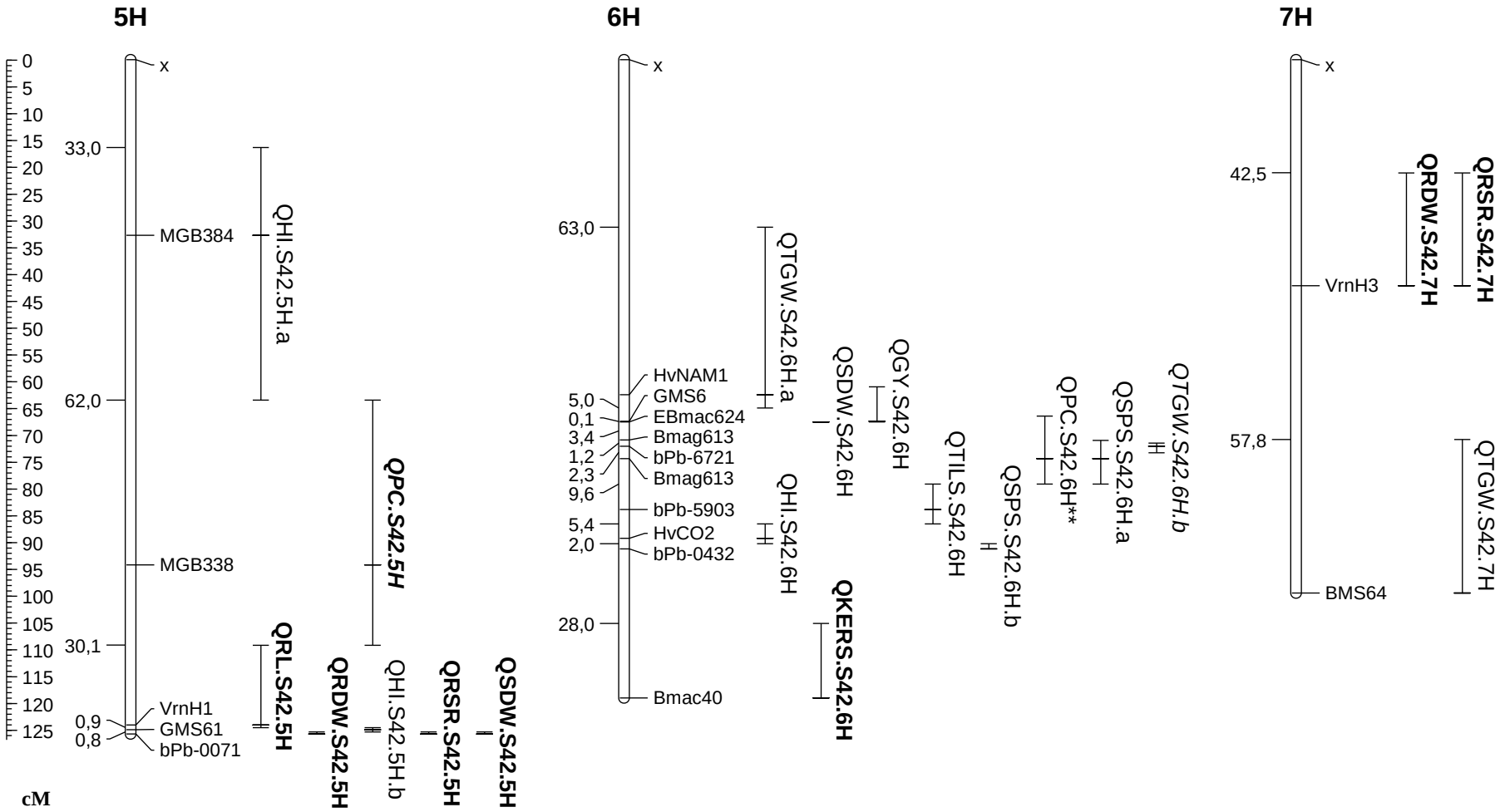


Figure 20 Continued.

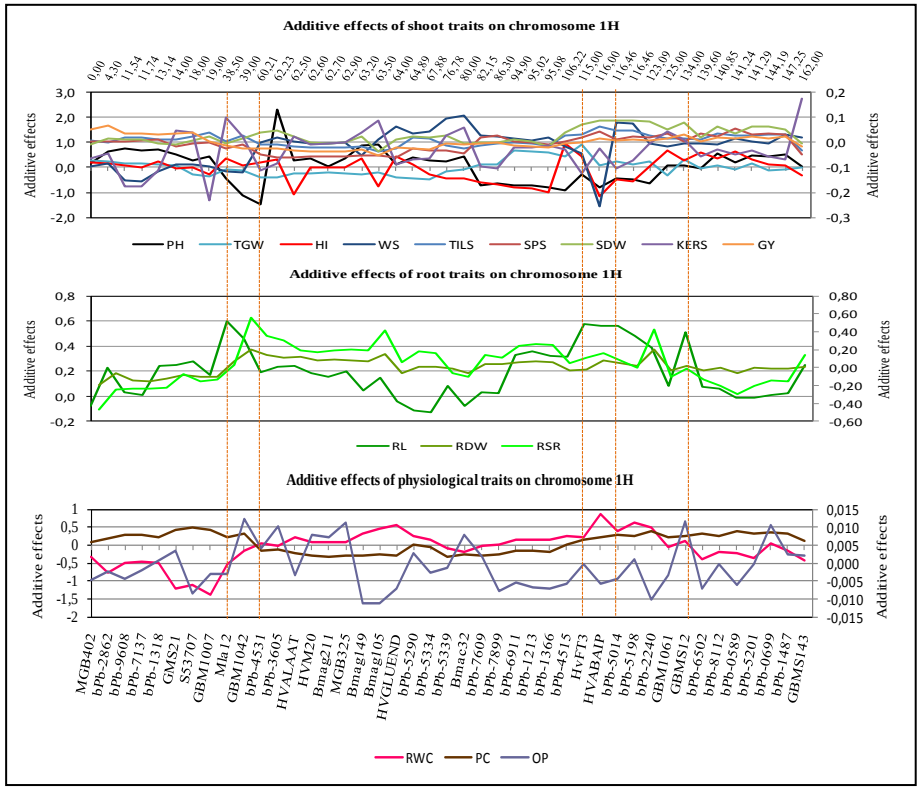


Figure 21 Additive effects on chromosome 1H

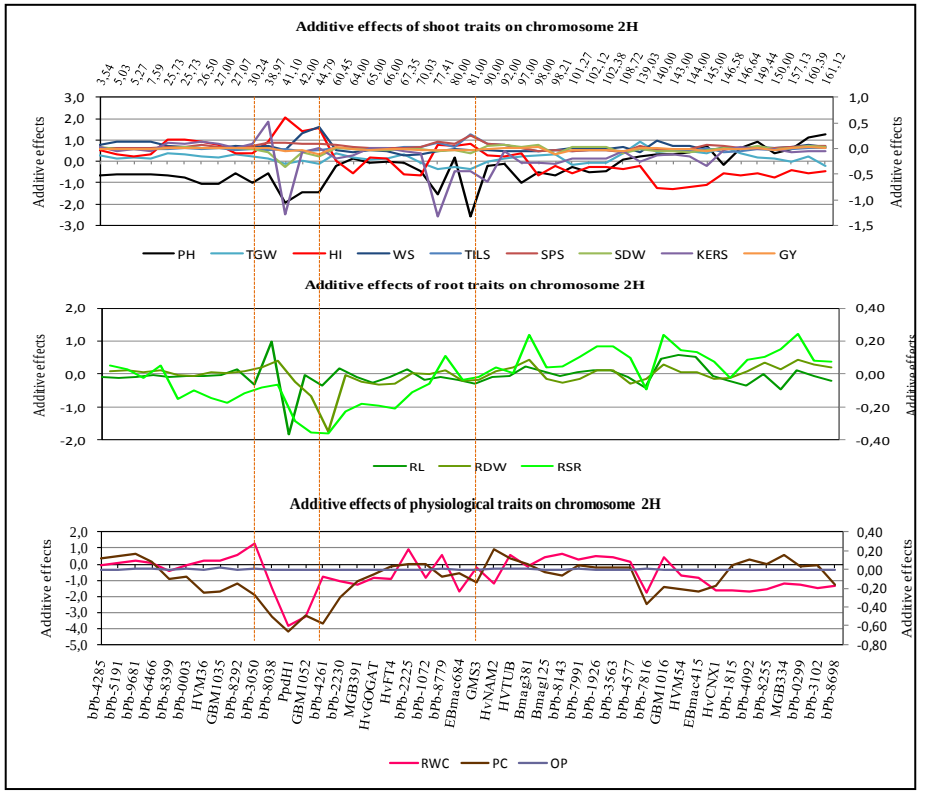


Figure 22 Additive effects on chromosome 2H

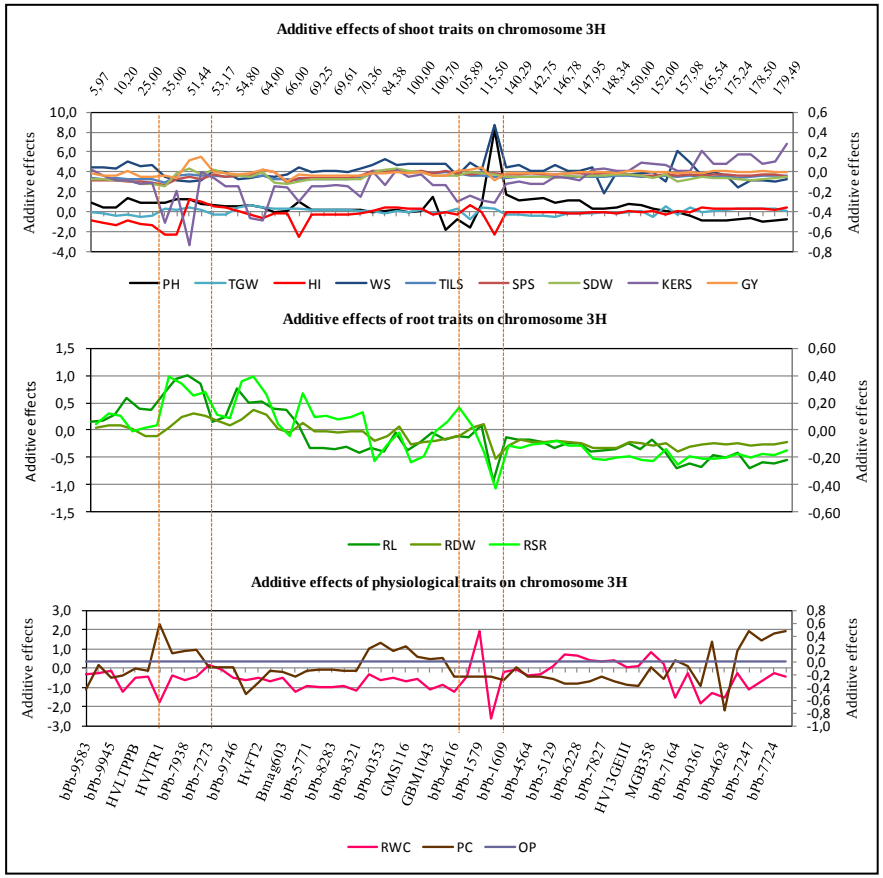


Figure 23 Additive effects on chromosome 3H

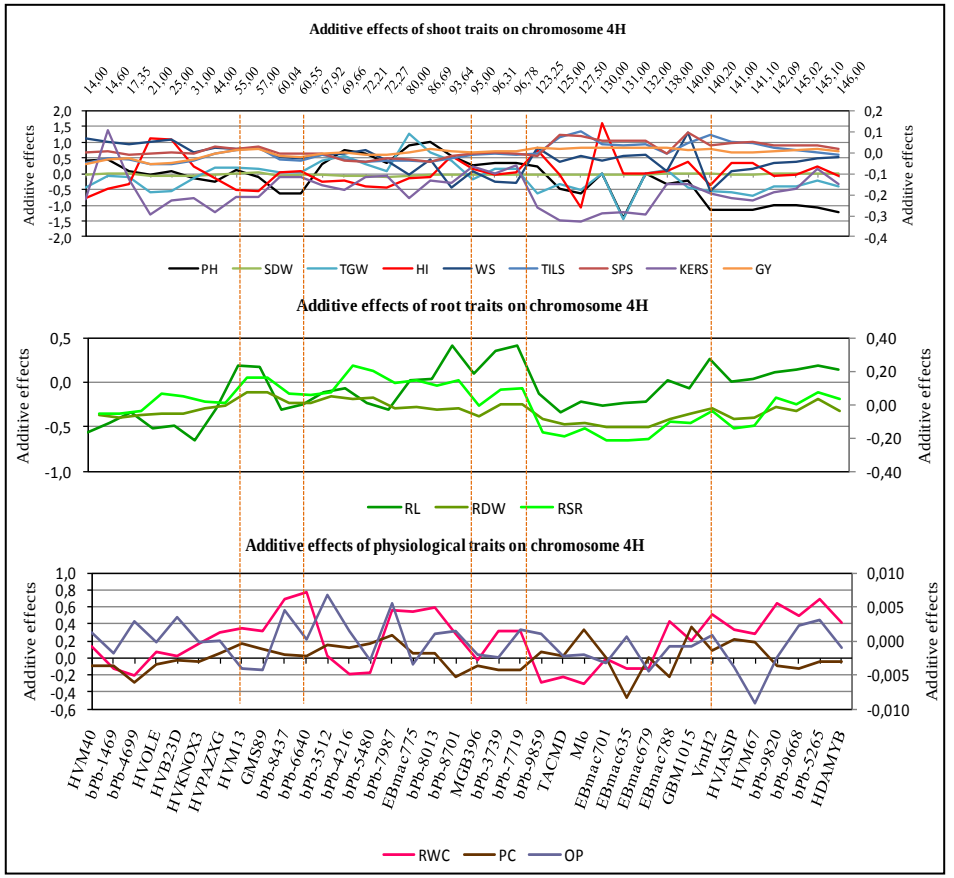


Figure 24 Additive effects on chromosome 4H

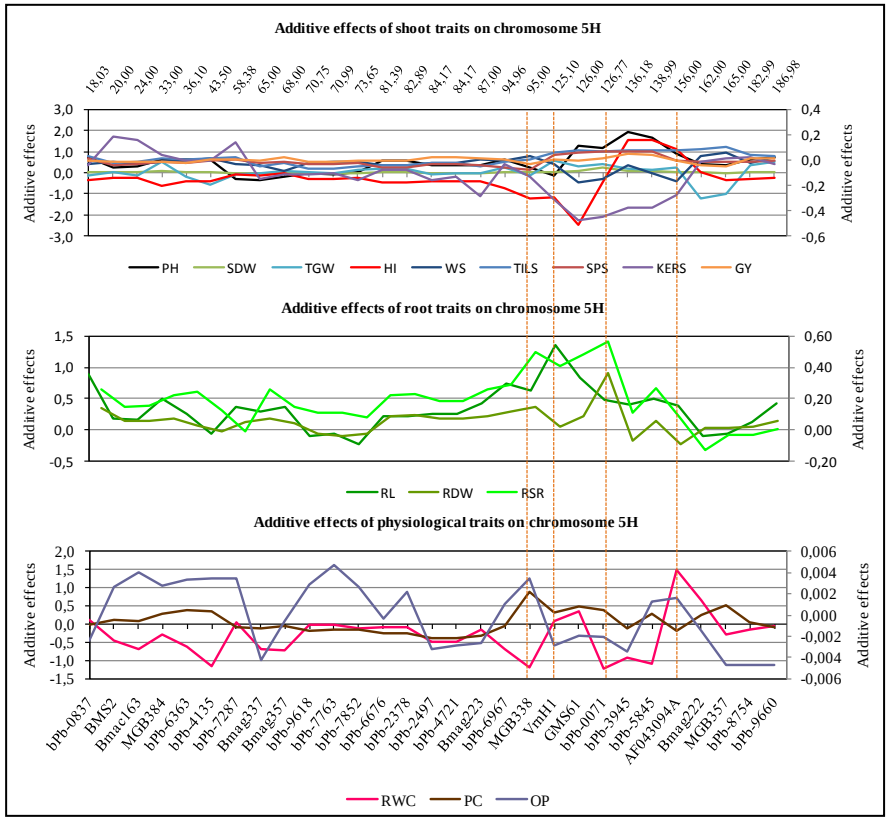


Figure 25 Additive effects on chromosome 5H

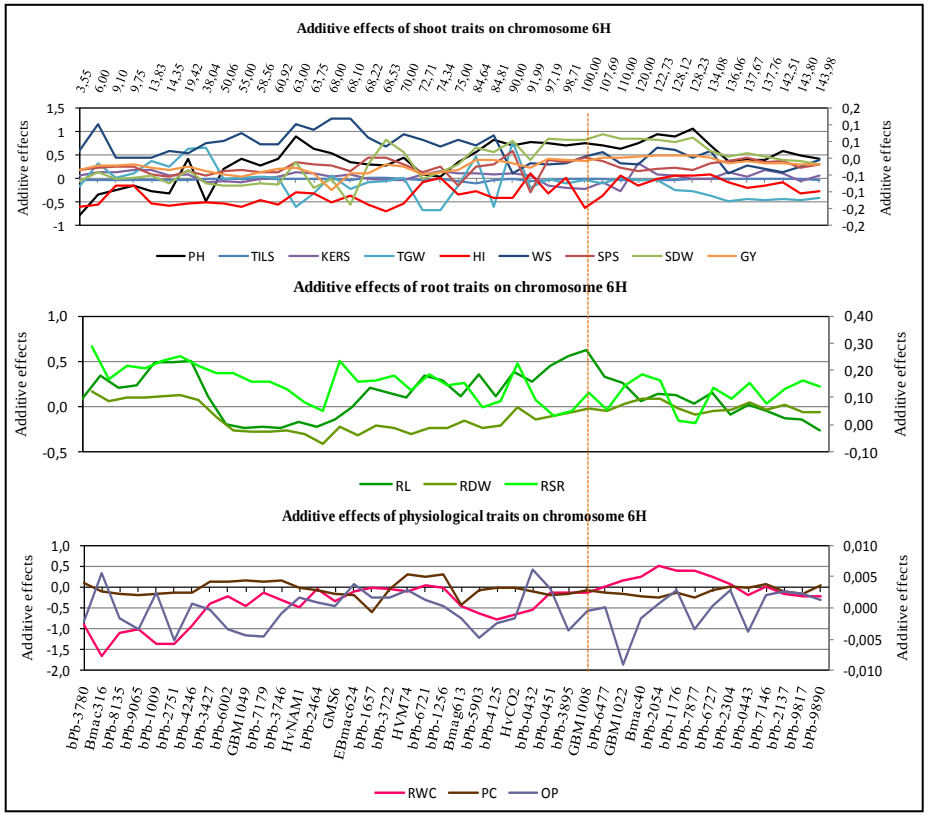


Figure 26 Additive effects on chromosome 6H

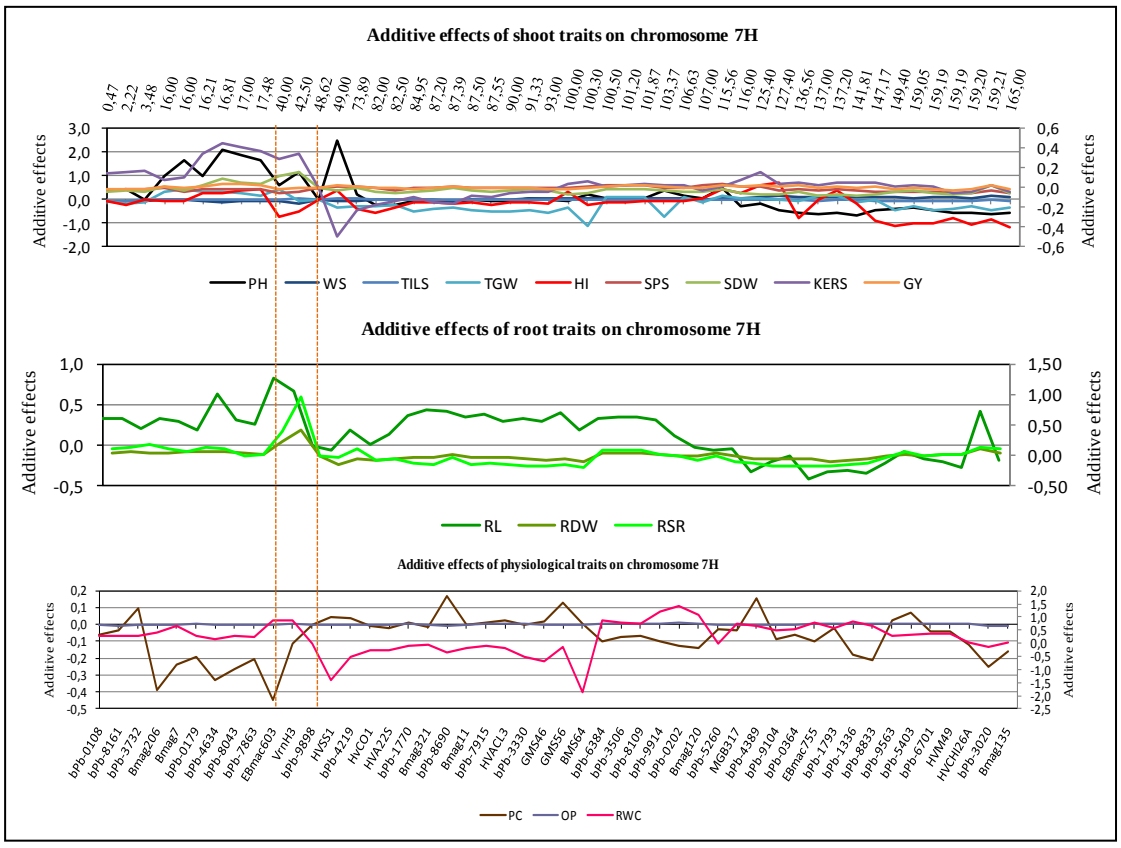


Figure 27 Additive effects on chromosome 7H

Grain yield/plant (GY)

Six QTLs were identified for GY and located on chromosomes 2H, 3H and 6H (Figure 20). All QTLs revealed significant marker main effects and showed unfavorable effect with an explained genetic variance up to 14.34%. The relative performances of the exotic genotype led to reducing GY with range between -17.90% and -8.96%. This trait was negatively influenced by 'ISR 42-8' alleles indicated that by the negative additive scores (Table 6). In contrast, the elite alleles at these QTLs were associated to an enhancement of GY as compared to exotic alleles. It means elite alleles appeared to be desirable for GY as compared to exotic alleles.

Number of kernels/spike (KERS)

Six QTLs were detected for KERS and distributed on chromosomes 2H, 3H, 4H and 6H (Figure 20). All QTLs showed significant marker main effects, five of them exhibited unfavorable effects with an explained variance ranged from 0.60 up to 48.99%. These QTLs were responsible of the reduction of KERS with values ranged between -22.97 and -4.03%.

Only at one QTL, *QKERS.S42.6H* the exotic genotype showed a favorable increasing of KERS by 6.44% and explained 3.50% of the genetic variance. As in the SDW case, this trait was influenced negatively by 'ISR 42-8' alleles indicating that by the negative additive values.

Thousand grain weight (TGW)

Seven QTLs were associated for TGW and distributed on chromosomes 1H, 3H, 4H, 6H and 7H (Figure 20). Six QTLs exhibited significant marker main effects, and one QTL showed significant marker×treatment interaction effect. Only one QTL, at *QTGW.S42.1H* revealed a favorable increase in TGW, and the exotic alleles explained by 7.40% of the genetic variance with favorably increased TGW by 4.56% (Table 6). The other six QTLs showed negative effects of the exotic alleles and led to the reduction of TGW by 5.47% and explained up to 9.61% of the genetic variance.

Harvest index (HI)

Nine QTLs were associated significantly with HI and mapped on all chromosomes of Barley except the chromosome 7H (Figure 20). All QTLs showed significant marker main effects. The *QTLs, QHI.S42.2H* and *QHI.S42.4H* explained 3.81 and 7.78% of the genetic variance. Both QTLs showed favorable performance of the exotic genotype and revealed an increasing of HI with values 8.73 and 6.48% respectively. On other hand, seven QTLs showed a significant reduction in harvest index that ranged between -16.79 and 5.26% due to the presence of the exotic genotype alleles. They also explained a genetic variance up to 23.15%. Noteworthy, during the process of forward / backward selection for harvest index, the marker loci HvGI and bPb-9110 showed the highest F-value (203.87 and 133.48) along with iteration respectively. This linked markers revealed a huge proportion of explained genetic variance R^2g 22.98 and 23.15% as marker main effect respectively (Table 6).

3.5.2 Detection of QTLs for root traits in the population S42

A total of fifteen putative QTLs were associated for three root traits (RL, RDW and RSR) in S42 (Table 6 and Figure 20). Among these loci, nine (60 %) QTLs for root traits were identified with favorable effects and located on chromosomes 1H, 5H and 7H (Figure 20). However, most of favorable effects of the *Hsp* alleles were detected on chromosomes 1H and 5H. In the following, the detected QTLs are described for each trait.

Root Length (RL)

Three QTLs were detected for RL and distributed on chromosomes 2H, 3H and 5H (Figure 20). The three QTLs exhibited significant marker main effects. Two QTLs at, *QRL.S42.2H* and *QRL.S42.3H* explained 6.13 and 5.52% of the genetic variance, and showed unfavorable performance of the exotic genotype and revealed a shortening of RL with values 15.09 and 7.69% respectively. Only one QTL, at locus *QRL.S42.5H* the exotic genotype had a positive additive effect (score 1.35) and showed a favorable increasing of RL by 9.17% as well as explained 1.73% of the genetic variance (Table 6). It means that exotic alleles appeared to be desirable for RL as compared to the elite alleles.

Root dry weight (RDW)

Seven QTLs were associated significantly with RDW and located on chromosomes 1H, 2H, 3H, 4H, 5H and 7H (Figure 20). These QTLs showed significant marker main effects. Four QTLs *QRDW.S42.1H.a, QRDW.S42.1H.b, QRDW.S42.5H* and *QRDW.S42.7H* had positive additive effects and explained 6.50, 7.85, 4.21 and 6.91% of the genetic variance. They showed favorable performance of the exotic genotype and revealed an increasing of RDW with values ranged between 21.39 and 41.88%. While the other three loci showed unfavorable performance of the exotic alleles on RDW (Table 6).

Root shoot ratio (RSR)

Five QTLs were associated significantly with RSR and distributed on chromosomes 1H, 3H, 5H and 7H (Figure 20). The five loci exhibited significant marker main effect. At four QTLs, the exotic genotype showed favorable performance and revealed an increasing of RSR with values ranged between 20.13 and 3721 %. The strongest effect was identified at the QTL, *QRSR.S42.1H.b* and that explained 8.26% of the genetic variance. Only one QTL, at locus *QRSR.S42.3H,* the exotic genotype had a negative additive effect (score 0.42) and showed unfavorable performance of RSR by -18.95% as well as explained 7.35% of the genetic variance (Table 6).

3.5.3 Detection of QTLs for physiological traits in the population S42

Altogether, 9 putative QTLs were detected for three physiological traits (RWC, PC and OP) in S42 (Table 6 and Figure 20). Among these loci, only two (22.22 %) QTLs showed

favorable effect for physiological traits and located on chromosome 5H. The detected QTLs are described below.

Relative water content (RWC)

Four QTLs were detected for RWC and mapped on chromosomes 2H, 3H and 4H (Figure 20). Three QTLs exhibited significant marker main effects, while one QTL exhibited significant marker x trait interaction. All QTLs showed unfavorable effect with an explained genetic variance up to 14.96%. The relative performances of the exotic genotype led to reducing RWC with values ranged between -14.07% and -3.44%. This trait was negatively influenced by 'ISR 42-8' alleles (Table 6). In contrast, the elite alleles at these QTLs were associated to an enhancement of RWC as compared to exotic alleles. It means elite alleles appeared to be desirable for RWC as compared to exotic alleles.

Proline content (PC)

QTL analysis revealed four QTLs for PC that have been localized to chromosomes 3H, 4H, 5H and 6H (Table 6 and Figure 20). The strongest QTL effect, *QPC.S42.5H* is detected on chromosome 5H where an exotic allele accounts for 53.75% increase in PC as well as the highest positive additive effect (0.89). The linked marker to this exotic allele shows maker by treatment (M*T) interaction effect only and explains 4.07% of the R^2. The remaining three QTLs, *QPC.S42.3H, QPC.S42.4H* and *QPC.S42.6H* have shown a decreasing trend of PC due to the preeminence of exotic alleles that range from 26.49% to 42.54%. The inferior performance of exotic alleles at these loci was linked to the superior of performance of elite alleles. At QTL, *QPC.S42.3H* the elite allele showed 42.54% superior performance with respect to its counter exotic allele. This locus explains major part of the R^2 (6.13%). Similarly, the exotic alleles at QTLs, *QPC.S42.4H* and *QPC.S42.6H* have been accounted for 26.49% and 27.20% decrease in PC when compared to their respective elite alleles, respectively.

Osmotic potential (OP)

Only one QTL (QOP.S42.4H.a) was identified for OP and located on chromosome 4H at positions 141.1 cM. The QTL at QOP.S42.4H exhibited significant marker main effects. The QTL exhibited favorable effect with an explained genetic variance of 6.75% and was

responsible of reducing OP with value of 9.94%. This trait was negatively influenced by *Hsp* alleles (Table 6). The exotic alleles at this QTL were associated to an enhancement of OP as compared to elite alleles. It means exotic alleles appeared to be desirable for OP as compared to elite alleles.

3.6 Detection of common QTLs for shoot, root and physiological traits

In present investigation, detection of putative QTLs for each trait are listed in Table 2 and their map positions are shown in Figure 20. A total of 79 putative QTLs were identified, ranging from one to nine QTLs for each trait. 16 common QTLs have been found to be governing different traits and covered the whole genome of S42 population except chromosome 6H. The highest number of the common QTLs was found on 2H (five QTLs) followed by chromosome 4H (4 QTLs).

Common QTLs for shoot traits

Because of the strong correlation between yield and its attributes, different common QTLs have been identified to be influencing different shoot traits. For instance, on chromosome 2H five common QTLs were identified at marker loci PpdH1 (41.1 cM), bPb-4261 (44.79 cM), GMS3 (81 cM), HvNAM2 (90 cM) and bPb-8143 (98.21 cM). The alleles of exotic genotype at most of these loci led to decrease grain yield and its components and increased leaf wilting (Figure 29). Only at marker locus GMS3 (81 cM) the exotic alleles was responsible of increasing TILS and SPS. The same case has been observed on chromosome 3H, since three common QTLs were found to be influencing shoot traits (Figure 30). These QTLs were at marker loci bPb-7989 (50.43 cM) Bmag603 (66 cM) and bPb-9110 (118.72 cM). Again the alleles of exotic genotype led to decrease the agronomical and shoot traits such as GY, HI, SPS, TGW and KERS, while they increased PH and WS. On chromosome 4H, four common QTLs at marker loci Mlo (127.5 cM), EBmac635 (131 cM), VrnH2 (140.2 cM) and HVM67 (141.1 cM) were governing shoot traits (Figure 31). Only traits TILS and SPS have been affected positively by the presence of the exotic alleles indicating the favorable effects from the exotic alleles on these traits (Figure 31).

Common QTLs for root traits

Moderate to strong, positive and highly significant correlation coefficients were observed among root traits RL, RDW and RSR under control and drought conditions. Five common QTLs were found on chromosomes 1H (39 and 123.09 cM), 3H(118.72 cM), 5H (126.77 cM) and 7H (42.5 cM). At these loci, the exotic alleles were found to be desirable in increasing root traits in particular RDW and RSR (Figures 28 and 33). While at marker locus bPb-9110 (118.72 cM) the exotic alleles had unfavorable effects on root traits (Figure 30).

Common QTLs for physiological traits

No common QTLs have been detected for physiological traits and this indicates the dependent inheritance of these traits. However, these traits were involved in the previous common QTLs which detected for shoot and root traits.

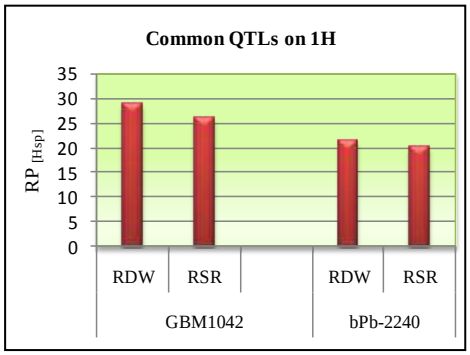


Figure 28 Two common QTLs were detected on 1H influencing RDW and RSR

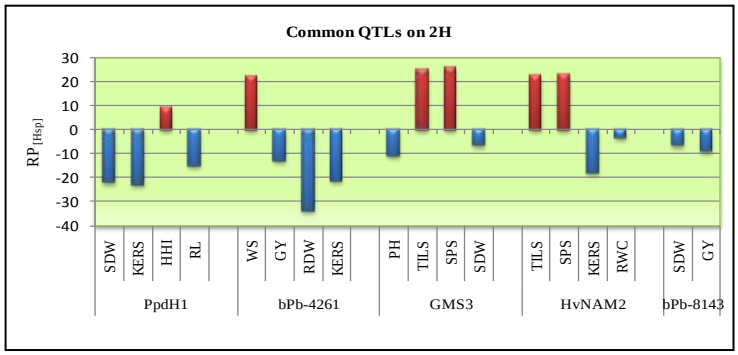


Figure 29 Five common QTLs were detected on 2H influencing several traits.

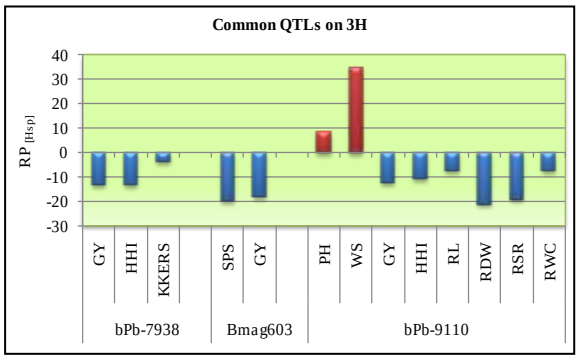


Figure 30 Three common QTLs were detected on 3H influencing several traits

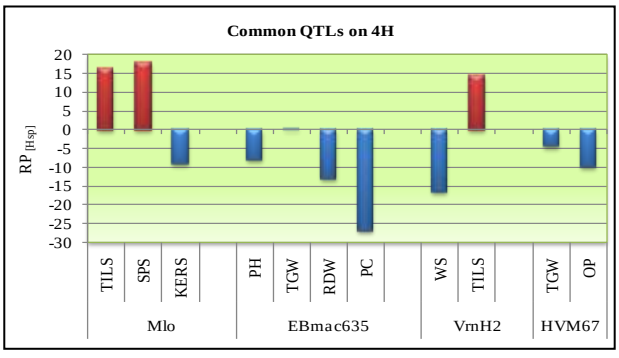


Figure 31 Four common QTLs were detected on 4H influencing traits

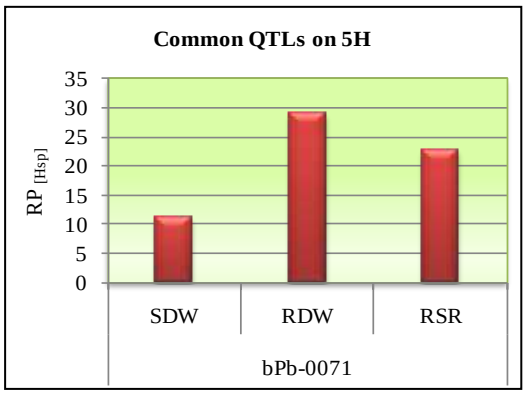


Figure 32 One common QTL was detected on 5H influencing SDW, RDW and RSR

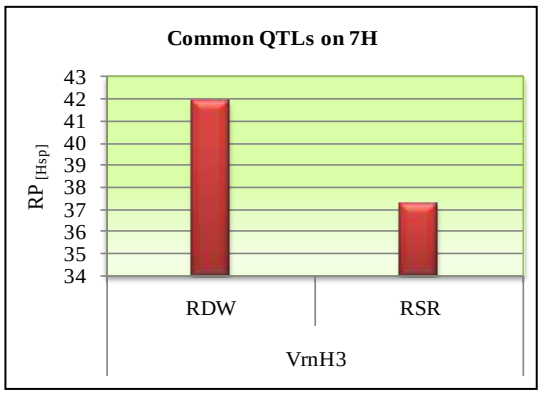


Figure 33 One common QTL was detected on 7H influencing RDW

3.7 Comparison of the additive effects of the putative QTLs

Several chromosomal regions or intervals have been identified to be acted additively and governed several traits. The signs of the additive effects reflected the correlations between traits. Three different regions were detected along chromosome 1H, the exotic alleles at these loci exhibited desirable additive effects on shoot, root and physiological traits. At intervals Mla12 - bPb-4531 (38.50-60.21 cM), the exotic genotype alleles revealed desirable effects on PH, WS, KERS, RL, RDW, RSR, PC and OP, since the exotic alleles were responsible of the enhancement of these traits. At marker intervals HvFT3 – bPb-5014 (115-116 cM), the exotic alleles showed favorable additive effects on WS, SDW, TILS, SPS, GY, RL, RDW, RSR, RWC and PC. At marker locus GBMS12 (134 cM), the exotic alleles showed favorable effects on PH, SDW, RL and OP (Figure 21). In contrast, exotic alleles at these regions were responsible for the reduction of the remaining traits.

Two regions were detected on chromosome 2H, at location interval bPb-3050 – bPb-4261 (30.24-44.79 cM), in this interval the exotic alleles exhibited desirable additive effects on traits PH, HI and RL, while they showed undesirable additive effects on most of the traits in particular root and physiological traits. Another region at marker locus $GMS3_{[2H]}$ (81 cM), the exotic alleles showed undesirable additive effects on majority of the traits and showed a favorable additive effects on PH (Figure 22).

Two regions were observed on chromosome 3H and showed notable additive effects on the shoot, root and physiological traits. The first region was at interval HVITR1-bPb-7273 (35-53.17 cM), the alleles of the exotic genotype were responsible for the enhancement of GY, RL, RSR and PC while they were responsible for the reduction of the other traits at the

same region. Another region on 3H at interval bPb-4616 – bPb-1609 (105.89-140.29 cM), the exotic alleles led to reduce most of studied traits with exception of PH and WS (Figure 23).

Several regions were identified on chromosome 4H and showed different trends of the additivity. At marker interval HVM13-bPb-6640 (55-60.55 cM), the exotic alleles showed slight increase in shoot and root traits and notable increase in RWC and OP. the same trend has been observed at region MGB396-bPb7719 (95-96.78 cM), with remarkable increasing in RL due to the presence of the exotic alleles. At marker locus VrnH2 (140.20 cM), the presence of the exotic alleles were responsible for the enhancement of TILS, RL and RWC with a desirable association with each of PH and WS (Figure 24).

Three regions with additive effects were detected on chromosome 5H. At marker locus MGB338 (95 cM) the exotic alleles were responsible for increasing RSR, PC and OP, while they reduced the remaining traits. Exotic alleles at marker interval VrnH1-bPb-0071 (125.10-126.77 cM) led to reduce each of HI, KERS and WS while they increased RL, RDW and RSR. At marker locus AF043094A (156 cM) the exotic alleles led to increase RWC with reduction of the other traits (Figure 25).

There is no notable region on chromosome 6H can show remarkable increase in studied traits with exception of slight increasing in RL at marker locus GBM1022 (100 cM) (Figure 26). A remarkable region has been observed on chromosome 7H. The exotic alleles showed positive additive effects and led to increase root traits and some of shoot traits such as KERS and PH, while they reduced the physiological traits (Figure 27).

3.8 Epistatic effects

Epistatic effects are statistically defined as interactions between effects of alleles from two or more genetic loci (Fisher 1918). Interactions, however are simply deviations from additivity in a general linear model; as such they are often treated as statistical errors. Cockerham (1954) showed that epistatic effects can be partitioned into various epistatic components, e.g.,$A\times A$ and $A\times D$ effects, etc. Epistasis is now considered as an important source of genetic variation for quantitative traits. Because different components involve interactions of different numbers and different types of alleles, some components are more important than others. Especially, the *additive* × *additive* component ($A\times A$) is shown to be heritable (Goodnight 1988) and thus much attention has been paid to the study of $A\times A$ effects in response to selection and evolution (Goodnight 2000; Jannink 2003).

3.8.1 Estimation of *additive* × *additive* interactions

Altogether 33 pairs of epistatic QTLs as *additive* × *additive* effects were detected for nine studied traits related to drought tolerance in S42 population. Among them, eleven pairs displayed QTL by marker interaction and twenty two displayed marker by marker interaction (Table 7 and Figure 44).

3.8.1.1 Epistatic effects for shoot traits

A total of 19 pairs of epistatic QTLs were identified for PH, WS, TILS, SDW, KERS and HI with 8, 2, 1, 3, 3 and 2 pairs of epistatic effects respectively (Table 7 and Figure 44). Among these loci, 13 pairs of epistatic QTLs were identified with favorable effects. Four pairs were displayed QTL × marker epistatic interaction. In the following, the detected epistatic QTLs pairs are described for each trait.

Plant height (PH)

In present study, we identified eight pairs of epistatic QTLs were associated significantly with plant height (PH), and mapped on the whole genome of the S42 population except chromosome 5H. Among these loci, four pairs of epistatic effects reduced the plant height up to -18.63 cm. The most favorable pair of epistatic QTLs for reducing plant height was (*HvGI*bPb-1366*) and located on chromosomes 3H (63 cM) and 1H (95.08 cM) and had the highest F value and accounted for 2.30% of genetic variation (Figure 34). The BC_2DH lines carrying the *Hsp*/*Hsp* genotype at these loci were on average 10.92 cm shorter than lines with the allelic combination *Hv*/*Hv*, comparing with other allelic combinations of these loci, we found that, the BC_2DH lines carrying the *Hsp*/*Hv* genotype or *Hv*/*Hsp* increased PH significantly at three loci while decreased PH at one locus but this decreasing was non-significant. While for the other four pairs of the QTLs , they increased the plant height (PH) up to 13,57 cm. The most favorable pair of epistatic QTLs for increasing plant height was (*bPb-5339* * *MGB396*) and increased PH by 5,71 cm and located on chromosomes 1H (76.78 cM) and 4H (95 cM) and had the highest F value and accounted for 1.47% of genetic variation. The BC_2DH lines carrying the *Hsp*/*Hsp* genotype at these loci were on average 9.84 cm taller than lines with the allelic combination *Hv*/*Hv*, comparing with other allelic combinations of these loci, we found that, the BC_2DH lines carrying the *Hsp*/*Hv* genotype or *Hv*/*Hsp* decreased PH non-significantly approximately at the four loci of epistatic interaction effects of PH. Only one pair ($HvGI_{[3H]}$*bPb-1366 $_{[1H]}$) of epistatic QTLs showed QTL ×

marker interaction. In this study, the marker $HvGI_{[3H]}$ was observed to be associated to a QTL (QHI.S42.3H.b) underlying HI.

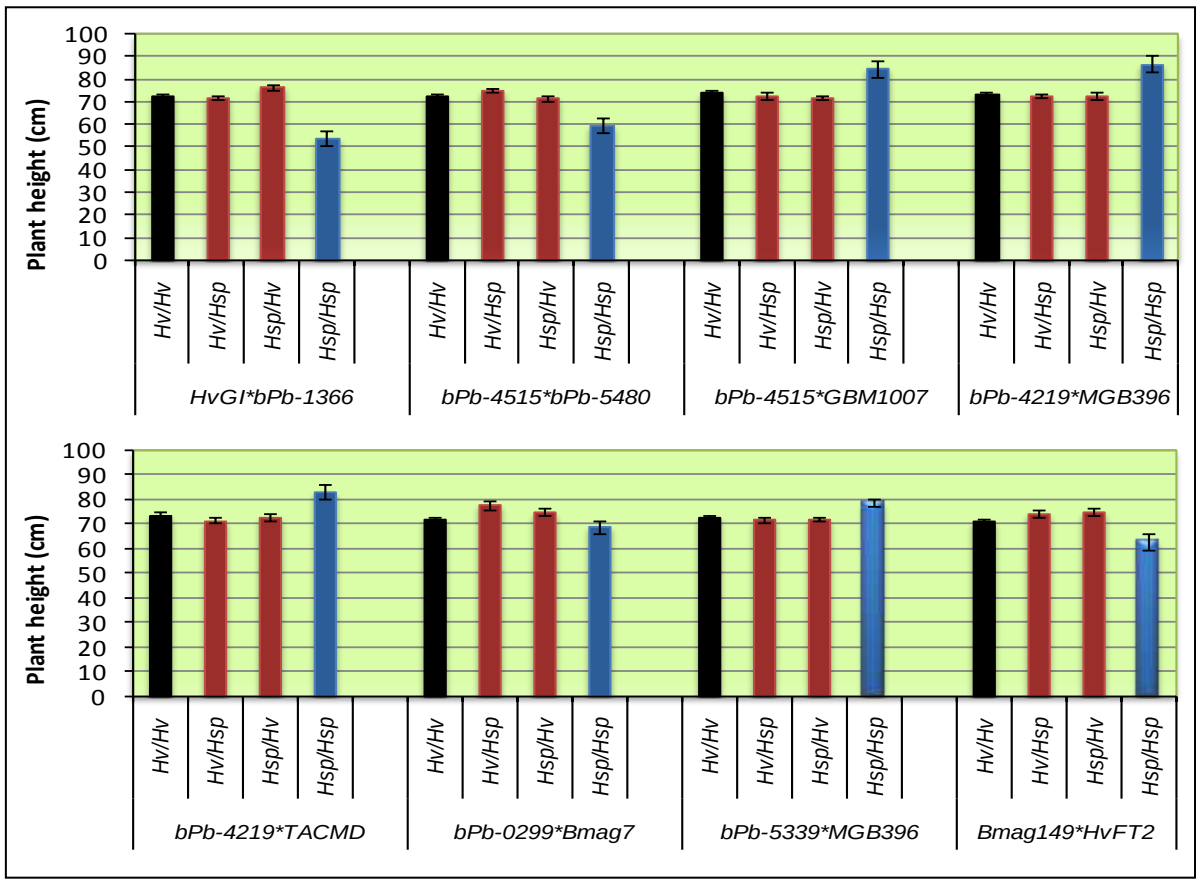


Figure 34 Digenic interaction effects for plant height. Lsmeans of four genotypes, *Hv/Hv* (elite allele at locus1 and 2), *Hv/Hsp* (elite allele at marker locus 1 and exotic allele at locus 2), *Hsp/Hv* (exotic allele at marker locus 1 and elite allele at locus 2), *Hsp/Hsp* (exotic allele at locus1 and 2).

Wilting score (WS)

Digenic epistatic interactions have been tested among BC_2DH genotypes that revealed two interaction effects for WS (Table 7 and Figure 44). The first interaction effect has been identified between marker locus bPb5339 (1H) and HvFT2 (3H). The combination of *Hv/Hv* or *Hv/Hsp* both resulted to a drought susceptible WS (almost 4.5) that has been converted to almost drought resistant WS (2.3) as the elite allele were replaced with exotic allele are both loci. It has been considered as an additive response of exotic allele in decreasing WS. In the second interaction, a similar effect has been detected where the combination of exotic alleles at marker locus bPb0353 and bmac316 resulted in a drought resistant WS (1.9) see Figure 35.

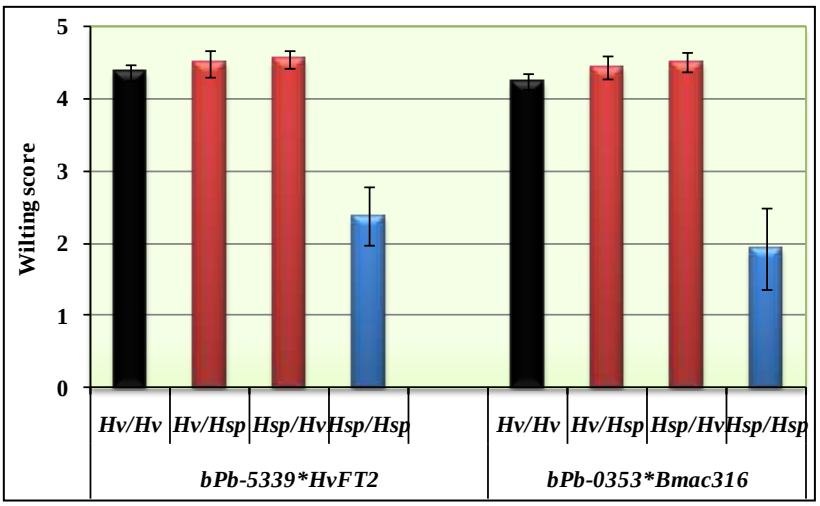


Figure 35 Digenic interaction effects for wilting score. Lsmeans of four genotypes, Hv/Hv (elite allele at locus1 and 2), Hv/Hsp (elite allele at marker locus 1 and exotic allele at locus 2), Hsp/Hv (exotic allele at marker locus 1 and elite allele at locus 2), Hsp/Hsp (exotic allele at locus1 and 2). Interaction 1 (marker locus bPb-5339 by marker locus HvFT2), interaction 2 (marker locus bPb-0353 by marker locus Bmac316).

Number of tillers/plant (TILS)

In the present study, the epistasis analysis revealed only one significant epistatic QTL pair (*bPb-6676*bPb-2225*) for TILS and distributed on chromosomes 5H and 2H respectively. It is worthwhile to note that the positive epistatic interaction (*bPb-6676*bPb-2225*) increased number of tillers/plant by 0.80 and explained 3.12% of genetic variance (R^2) (Table 7, Table 8 and Figure 34). (Figure 36*)*. The BC_2DH lines having the *Hsp*/*Hsp* genotype at locus (*bPb-6676*bPb-2225*) higher in no. of tillers/plant than lines with the allelic combination *Hv*/*Hv*. Comparing with other allelic combinations of this epistatic effect locus, we found that at the same locus, the BC_2DH lines having the *Hsp*/*Hv* genotype or *Hv*/*Hsp* decreased TILS (Figure 36).

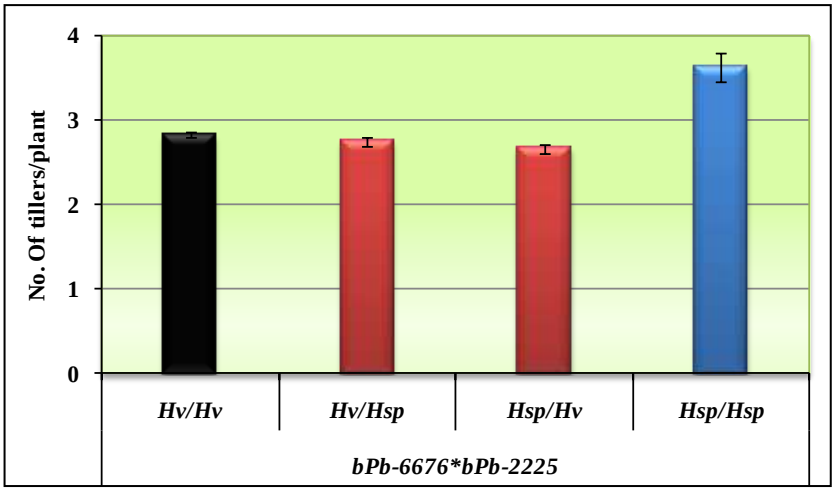


Figure 36 Digenic interaction effects for number of tillers/plant. Lsmeans of four genotypes, Hv/Hv (elite allele at locus1 and 2), Hv/Hsp (elite allele at marker locus 1 and exotic allele at locus 2), Hsp/Hv (exotic allele at marker locus 1 and elite allele at locus 2), Hsp/Hsp (exotic allele at locus1 and 2).

Shoot dry weight (SDW)

The epistasis analysis revealed three pairs of epistatic QTLs which were associated significantly with SDW, and mapped on chromosomes 1H, 3H and 6H (Table 7 and Figure 44). Those pairs of intervals had positive effects of epistatsis to increase shoot dry weight up to 1.25 g. They had high F value and the contribution in genetic variation (R^2) ranged between 2.43 and 4.90%.The BC_2DH lines carrying the *Hsp*/*Hsp* genotype at these loci were more weight on average 1.06 g than lines with the allelic combination *Hv*/*Hv* (Figure 37). Comparing with other allelic combinations of these loci, we found that, the BC_2DH lines carrying the *Hsp*/*Hv* genotype or *Hv*/*Hsp* decreased SDW non-significantly approximately at all loci of epistatic interaction effects of SDW. Among these digenic epistatic interactions, one pair (bPb-$0443_{[6H]}$*bPb-$3605_{[1H]}$) showed QTL × marker interaction. The DArT marker bPb-$3605_{[1H]}$ was associated significantly with the QTL QPH.S42.1H affecting on PH.

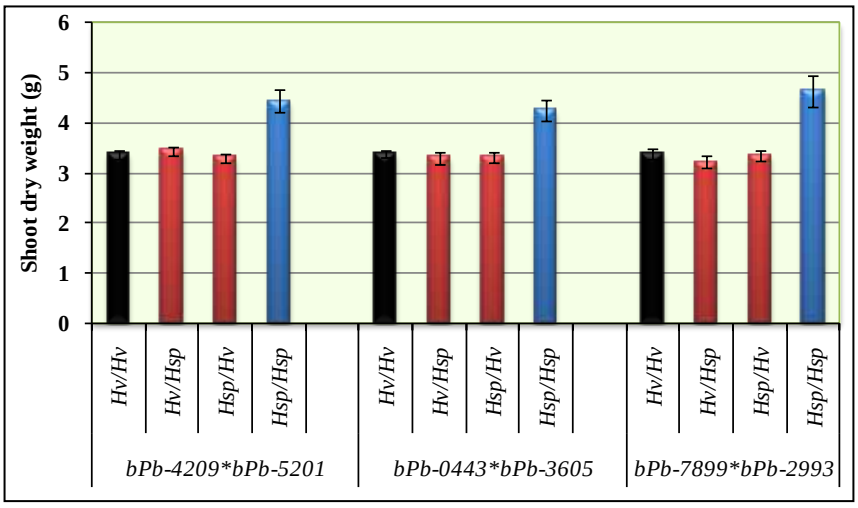


Figure 37 Digenic interaction effects for shoot dry weight. Lsmeans of four genotypes, Hv/Hv (elite allele at locus1 and 2), Hv/Hsp (elite allele at marker locus 1 and exotic allele at locus 2), Hsp/Hv (exotic allele at marker locus 1 and elite allele at locus 2), Hsp/Hsp (exotic allele at locus1 and 2)

No. of kernels/spike (KERS)

Three pairs of epistatic QTLs were associated significantly with KERS, and mapped on chromosomes 1H, 3H, 4H, 6H and 7H (Table 7 and Figure 44). Two pairs of intervals (*bPb-5480 *HvFT2 and bPb-7899 * bPb-3020*) had positive effects of epistatsis to increase number of kernels/spike up to 19.2. At both loci, the BC_2DH lines having the *Hsp*/*Hsp* genotype were higher KERS with value up to 5,7% than lines with the allelic combination *Hv*/*Hv*. While the other pair (*bPb-6477 * MGB410*) of eppistatic led to decrease number of kernels/spike (Figure 38).

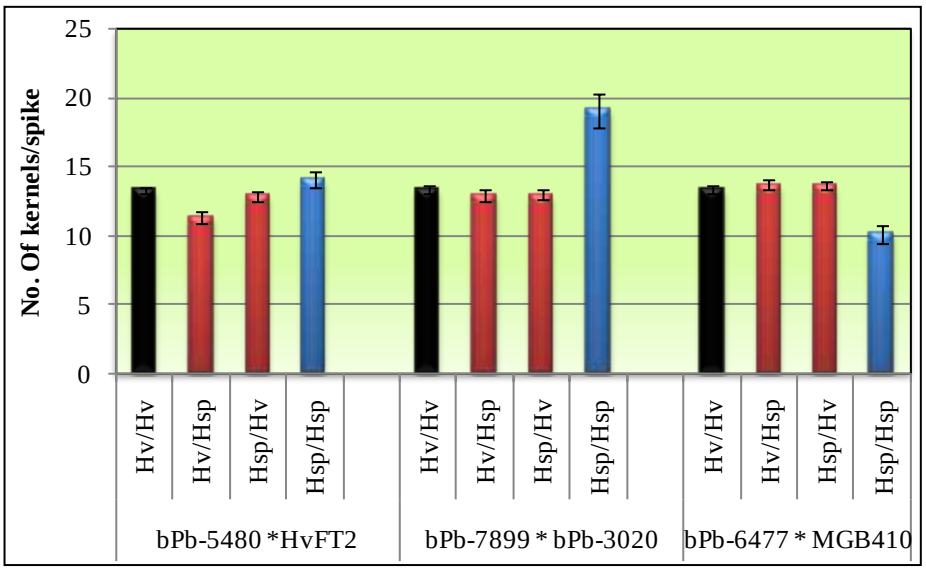


Figure-38Digenic interaction effects for No. of kernels/spike. Lsmeans of four genotypes, Hv/Hv (elite allele at locus1 and 2), Hv/Hsp (elite allele at marker locus 1 and exotic allele at locus 2), Hsp/Hv (exotic allele at marker locus 1 and elite allele at locus 2), Hsp/Hsp (exotic allele at locus1 and 2).

Harvest index (HI)

Only two significant epistatic QTL pairs were identified for HI, of which one pair QTL (*bPb-4577*VrnH1*) had positive epistatic effects with increasing HI by 9.01% and the other one (*HvGI*bPb-4389*) had negative epistatic effects with decreasing HI by 7.55%. Both pairs displayed QTL × marker interaction. At epistatic locus (*bPb-4577*VrnH1*), the BC_2DH lines having the *Hsp*/*Hsp* genotype were higher HI with value 9.02% than lines with the allelic combination *Hv*/*Hv*. Comparing with other allelic combinations of this epistatic effect locus, we found that, the BC_2DH lines having the *Hsp*/*Hv* genotype or *Hv*/*Hsp* decreased HI significantly. While at epistatic locus (*HvGI*bPb-4389*), the BC_2DH lines having the *Hsp*/*Hsp* genotype were showed the opposite result, where the lines were lower in HI by 7.56% than lines with the allelic combination *Hv*/*Hv*. Comparing with other allelic combinations of this epistatic effect locus, found that, the BC_2DH lines having the *Hsp*/*Hv* genotype decreased HI significantly by 2.13% while lines having Hv/*Hsp* increased HI significantly by 1.85% (Figure 39). The vernalisation gene $VrnH1_{[5H]}$ was associated significantly with RL. While the *Hordeum vulgare* gene $HvGI_{[3H]}$ was found to be associated with the QTL (QHI.S42.3H.b) influencing HI.

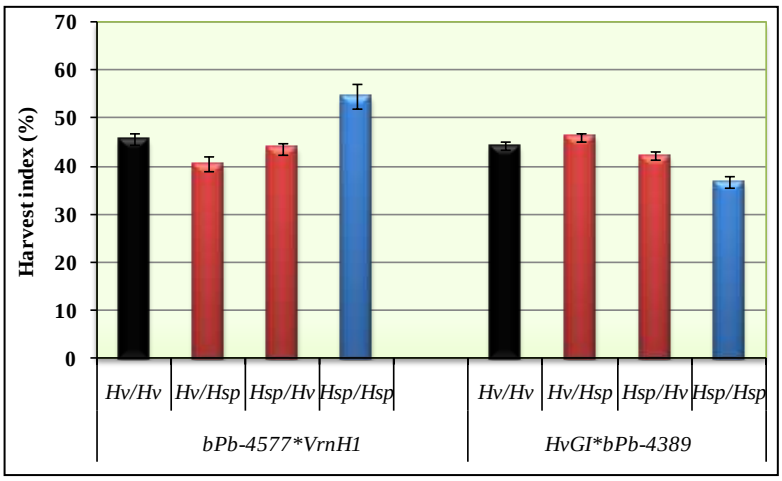


Figure 39 Digenic interaction effects for harvest index. Lsmeans of four genotypes, Hv/Hv (elite allele at locus1 and 2), Hv/Hsp (elite allele at marker locus 1 and exotic allele at locus 2), Hsp/Hv (exotic allele at marker locus 1 and elite allele at locus 2), Hsp/Hsp (exotic allele at locus1 and 2).

Other shoot traits

The other shoot traits (SPS, GY and TGW) did not show epistatic effects.

3.8.1.2 Epistatic effects for root traits

Eleven digenic epistatic interactions were detected for root dry weight and root shoot ratio. Among them, six digenic epistatic pair showed QTL × marker interaction. Almost all these loci showed favorable epistatic effects. In the following, the detected epistatic QTLs are described for each trait.

Root dry weight (RDW)

Seven significant QTLs epistatic pairs were detected for RDW and covered the whole genome of the S42 population (Table 7 and Figure 44). Among these loci, six pairs of epistatic QTLs had positive and favorable epistatic effects on RDW and showed an increase in root dry weight by values ranged between 1.06 and 2.40 g (Table 8) . The contribution of these loci in genetic variation ranged between 3.13 and 5.88% (Table 7). The most important epistatic pairs of QTLs detected for RDW and showed favorable effects was (*bPb-8779* Bmag357*) which distributed on chromosomes 2H (77.41 cM) and 5H (68 cM). The BC_2DH lines carrying the *Hsp*/*Hsp* genotype at these loci were more weight on average 1.34 g than lines with the allelic combination *Hv*/*Hv* (Figure 40). Epistatic QTL pair *(bPb-0353*HVM67*) had negative epistatic and unfavorable effects on RDW and located on chromosomes 3H and 4H (Table 7 and Figure 44). Four pairs out of seven showed QTL × marker interaction. The marker SSR $Bmac40_{[6H]}$ was participated the digenic interaction (bPb-1681*Bmac40) and

was found to be associated with the QTL (QKER.S42.6H) which influencing KERS. The marker HVM67$_{[4H]}$ in the digenic interaction (bPb-0353*HVM67) was associated significantly with the QTLs *QTGW.S42.4H.b* and *QOP.S42.4H* which affecting positively on TGW and OP respectively. Another marker (bPb-8779$_{[2H]}$) was involved in the digenic epistatic interaction pair (bPb-8779* Bmag357) was associated with *QKER.S42.2H.b*, this QTL was affecting KERS. The SSR marker MGB338 was involved in the interaction (bPb-7763 *MGB338) was found to be associated with the QTL *QPC.S42.5H* which influencing PC.

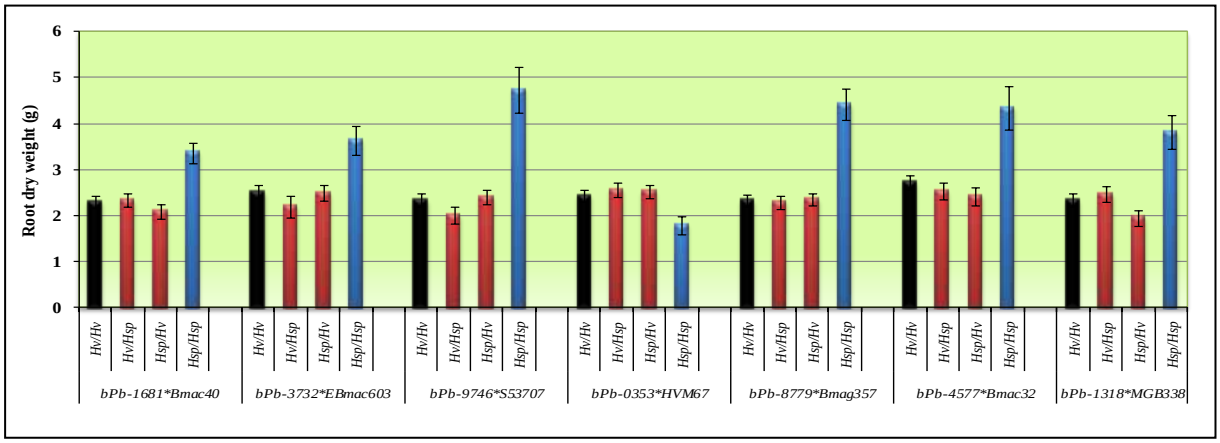


Figure 40 Digenic interaction effects for root dry weight. Lsmeans of four genotypes, *Hv/Hv* (elite allele at locus1 and 2), *Hv/Hsp* (elite allele at marker locus 1 and exotic allele at locus 2), *Hsp/Hv* (exotic allele at marker locus 1 and elite allele at locus 2), *Hsp/Hsp* (exotic allele at locus1 and 2).

Root shoot ratio (RSR)

Four pairs of epistatic QTLs were associated significantly with RSR, and mapped on chromosomes 1H, 5H, 6H and 7H (Table 7 and Figure 44). Two pairs of them showed QTL × marker interactions. All loci had positive epistatic and favorable effects on RSR and showed an increasing in RSR up to 5.92%. The strongest digenic epistatic pair accounted up to 5.71% of genetic variance (Tables 7). The BC_2DH lines carrying the *Hsp*/*Hsp* genotype at these loci were on average 3.97% higher than lines with the allelic combination *Hv*/*Hv*, comparing with other allelic combinations of these loci, we found that, the BC_2DH lines carrying the *Hsp*/*Hv* genotype or *Hv*/*Hsp* decreased RSR non-significantly in most of cases (Figure 41).The marker GMS61$_{[5H]}$ in the digenic interaction (bPb-4531* GMS61) was associated significantly with the QTLs *QHI.S42.5H.b* which affecting on HI. Another marker

(MGB338$_{[5H]}$) was involved in the digenic epistatic interaction pair (bPb-2862* MGB338) was associated with *QPC.S42.5H*, this QTL was affecting PC.

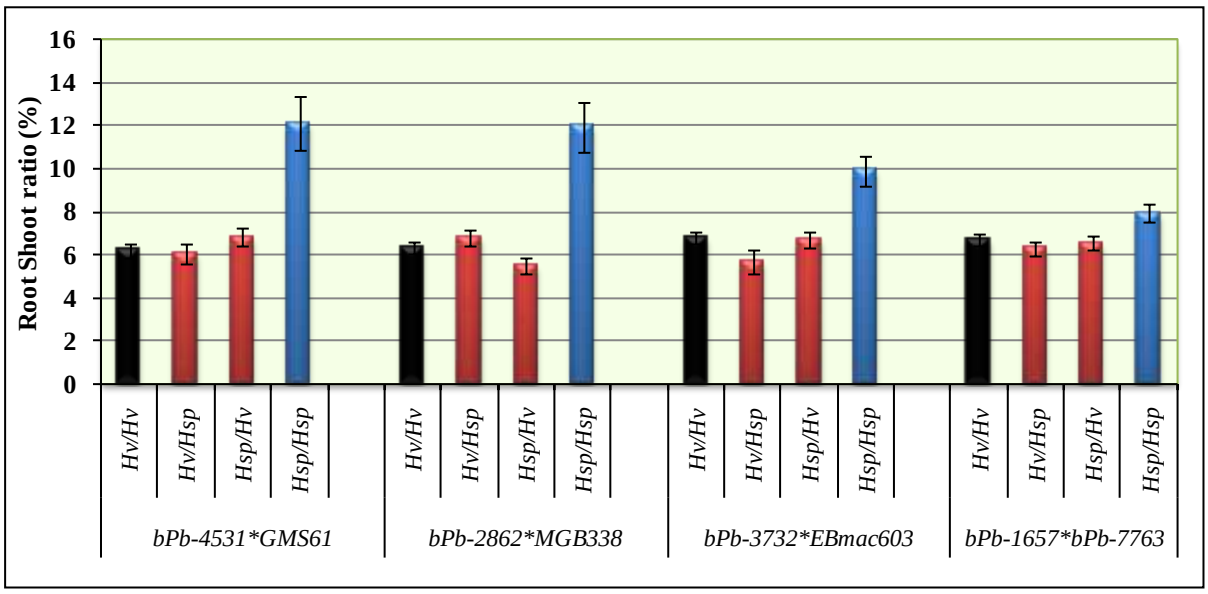


Figure 41 Digenic interaction effects for root shoot ratio. Lsmeans of four genotypes, Hv/Hv (elite allele at locus1 and 2), Hv/Hsp (elite allele at marker locus 1 and exotic allele at locus 2), Hsp/Hv (exotic allele at marker locus 1 and elite allele at locus 2), Hsp/Hsp (exotic allele at locus1 and 2).

Root Length (RL)

There is no epistatic effects detected for RL.

3.8.1.3 Epistatic effects for physiological traits

Only three digenic epistatic interactions were detected for relative water content and osmotic potential. In the following, the detected epistatic QTLs are described for each trait.

Relative water content (RWC)

In the present study, the epistasis analysis revealed only one significant epistatic QTL pair (*bPb-6676*bPb-2225*) was detected for RWC and distributed on chromosomes 5H and 2H respectively. This QTL pair was the same QTL pair which was identified for TILS. This pair of epistatic QTL had positive but small epistatic and favorable effects to increase RWC by 0,245 as well as explained very small percentage 0.07% of genetic variance, The BC_2DH lines having the *Hsp*/*Hsp* genotype were higher in RWC with percentage of 0.25% than lines with the allelic combination *Hv*/*Hv* (Figure 42). Comparing with other allelic combinations of

this epistatic effect locus, we found that, the BC_2DH lines having the *Hsp*/*Hv* genotype increased RWC by 0.41% and lines *Hv*/*Hsp* genotype decreased RWC by 0.93%.

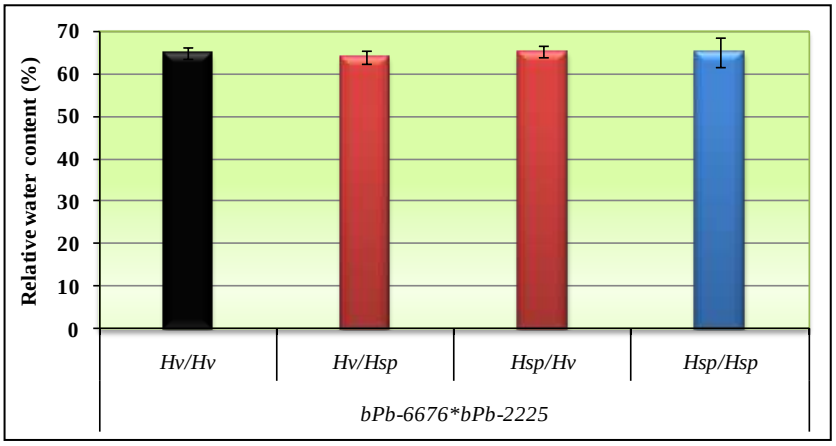


Figure 42 Digenic interaction effects for relative water content. Lsmeans of four genotypes, *Hv/Hv* (elite allele at locus1 and 2), *Hv/Hsp* (elite allele at marker locus 1 and exotic allele at locus 2), *Hsp/Hv* (exotic allele at marker locus 1 and elite allele at locus 2), *Hsp/Hsp* (exotic allele at locus1 and 2).

Osmotic potential (OP)

Two pairs of epistatic QTLs (*HvGI*bPb-3427* and *bPb-0202* bPb-8283*) were associated significantly with OP. Both pairs mapped on chromosomes 3H, 6H and 7H. One of them showed QTL × marker interaction Both loci had positive epistatic and favorable effects on OP, they showed an increasing in OP with same value 0.083 osmol/kg and were accounted up to 9.47% of genetic variance (Table 7). All loci had positive epistatic and favorable effects on OP, they showed an decreasing in OP by increasing osmolality. This means, the contribution of the two types of exotic alleles (recessive genes) decreased the osmotic-potential values and the BC_2DH lines carrying the *Hsp*/*Hsp* genotype were accumulate more particles and small molecules than lines with the allelic combination *Hv*/*Hv*. Comparing with other allelic combinations of these epistatic loci, the BC_2DH lines carrying the *Hsp*/*Hv* genotype or *Hv*/*Hsp* increased OP (Figure 43). The *Hordeum vulgare* gene $HvGI_{[3H]}$ was found to be associated with the QTL (*QHI.S42.3H.b*) which influencing HI. This marker was involved in the digenic epistatic interaction pair (*HvGI *bPb-3427*).

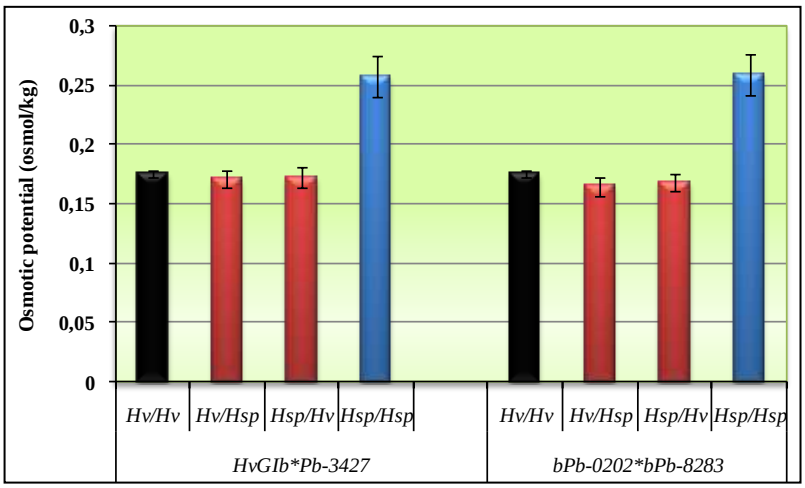


Figure 43 Digenic interaction effects for osmotic potential. Lsmeans of four genotypes, *Hv/Hv* (elite allele at locus1 and 2), *Hv/Hsp* (elite allele at marker locus 1 and exotic allele at locus 2), *Hsp/Hv* (exotic allele at marker locus 1 and elite allele at locus 2), *Hsp/Hsp* (exotic allele at locus1 and 2).

Proline content (PC)

There is no epistatic effects detected for PC

Table 7 Estimated of Lsmeans of 19 pairs of digenic interactions and epistatic effects (*additive* × *additive*) for shoot, root and physiological traits.

Trait	Effect			Marker 1			Marker 2			F value	Sign	P_{FDR}	R^2 %	Ls means of digenic interactions				*Hsp/Hsp-Hv/Hv*		*Hsp/Hv-Hv/Hv*		*Hv/Hsp-Hv/Hv*	
				Marker	Chrom	Pos	Marker	Chrom	Pos					*Hv/Hv*	*Hv/Hsp*	*Hsp/Hv*	*Hsp/Hsp*	*AA**	Pr > t	*AA**	Pr > t	*AA**	Pr > t
Shoot traits																							
PH	HvGI	×	bPb-1366	HvGI	3H	63	bPb-1366	1H	95,08	40,56	**	< 0,01	2,80	72,64	72,16	76,71	54,01	-18,63	< 0,01	4,065	< 0,01	-0,484	NS
	bPb-4515	×	bPb-5480	bPb-4515	1H	106,22	bPb-5480	4H	72,21	17	*	< 0,05	1,11	72,96	75,19	71,55	59,59	-13,36	< 0,01	-1,406	NS	2,231	< 0,05
	bPb-4515	×	GBM1007	bPb-4515	1H	106,22	GBM1007	1H	19	13,5	*	< 0,05	1,30	74,08	72,55	71,96	84,66	10,586	< 0,01	-2,119	< 0,05	-1,527	NS
	bPb-4219	×	MGB396	bPb-4219	7H	73,89	MGB396	4H	95	14,47	*	< 0,05	1,44	73,24	72,9	72,78	86,82	13,573	< 0,01	-0,466	NS	-0,346	NS
	bPb-4219	×	TACMD	bPb-4219	7H	73,89	TACMD	4H	125	14,33	*	< 0,05	1,32	73,77	71,74	72,92	83,26	9,487	< 0,01	-0,849	NS	-2,028	NS
	bPb-0299	×	Bmag7	bPb-0299	2H	157,13	Bmag7	7H	16	13,74	*	< 0,05	1,30	72,33	77,82	75,34	69,01	-3,322	NS	3,011	< 0,05	5,495	< 0,01
	bPb-5339	×	MGB396	bPb-5339	1H	76,78	MGB396	4H	95	16,88	*	< 0,05	1,47	73,02	71,95	72,26	78,73	5,716	< 0,01	-0,759	NS	-1,065	NS
	Bmag149	×	HvFT2	Bmag149	1H	63,2	HvFT2	3H	64	16,82	*	< 0,05	1,22	71,44	74,55	75,15	63,06	-8,383	< 0,05	3,706	< 0,05	3,11	< 0,05
WS	bPb-5339	×	HvFT2	bPb-5339	1H	76,78	HvFT2	3H	64	29,61	**	< 0,01	3,38	4,37	4,49	4,55	2,38	-1,994	< 0,01	0,176	< 0,05	0,118	NS
	bPb-0353	×	Bmac316	bPb-0353	3H	84,38	Bmac316	6H	6	23,47	*	< 0,05	2,5	4,24	4,44	4,51	1,94	-2,298	< 0,01	0,267	< 0,01	0,2	NS
TILS	bPb-6676	×	bPb-2225	bPb-6676	5H	81,39	bPb-2225	2H	67,35	31,28	**	< 0,01	3,12	2,83	2,75	2,67	3,63	0,803	< 0,01	-0,157	< 0,01	-0,073	NS
SDW	bPb-4209	×	bPb-5201	bPb-4209	3H	111,69	bPb-5201	1H	141,3	23,69	*	< 0,05	4,9	3,38	3,44	3,3	4,44	1,061	< 0,01	-0,075	NS	0,065	NS
	bPb-0443	×	bPb-3605	bPb-0443	6H	137,67	bPb-3605	1H	62,23	23,71	*	< 0,05	3,77	3,39	3,3	3,3	4,27	0,877	< 0,01	-0,085	NS	-0,085	NS
	bPb-7899	×	bPb-2993	bPb-7899	1H	86,3	bPb-2993	3H	51,59	20,8	*	< 0,05	2,43	3,39	3,21	3,36	4,64	1,251	< 0,01	-0,023	NS	-0,172	NS
KERS	bPb-5480	×	HvFT2	bPb-5480	4H	72,21	HvFT2	3H	64,00	18,5	*	< 0,05	2,86	13,3	11,3	12,9	14,1	0,8	NS	-0,4	NS	-2,0	< 0,01
	bPb-7899	×	bPb-3020	bPb-7899	1H	86,30	bPb-3020	7H	159,2	23,4	*	< 0,05	2,36	13,4	13,0	13,0	19,1	5,7	< 0,01	-0,4	NS	-0,4	NS
	bPb-6477	×	MGB410	bPb-6477	6H	107,69	MGB410	3H	65,00	29,6	**	< 0,01	2,47	13,4	13,7	13,6	10,1	-3,2	< 0,01	0,3	NS	0,4	NS

*,** indicate the significance level at 0.05 and 0.01 respectively to declare the putative epistatic QTL positions

Table 7 Continued.

Trait	Effect			Marker 1			Marker 2			F value	Sign	P_{FDR}	R^2 %	Ls means of digenic interactions				*Hsp/Hsp-Hv/Hv*		*Hsp/Hv-Hv/Hv*		*Hv/Hsp-Hv/Hv*	
				M.name	Chrom	Pos	M.name	Chrom	Pos					*Hv/Hv*	*Hv/Hsp*	*Hsp/Hv*	*Hsp/Hsp*	*AA**	Pr > t	*AA**	Pr > t	*AA**	Pr > t
HI	bPb-4577	×	VrnH1	bPb-4577	2H	108,72	VrnH1	5H	125,1	24,56	*	< 0,05	1,97	45,6	40,55	43,71	54,62	9,017	< 0,01	-1,895	< 0,01	-5,055	< 0,05
	HvGI	×	bPb-4389	HvGI	3H	63	bPb-4389	7H	125,4	24,08	*	< 0,05	2,05	44,33	46,18	42,2	36,77	-7,558	< 0,01	-2,131	< 0,05	1,845	< 0,01
Root traits																							
	bPb-1681	×	Bmac40	bPb-1681	3H	87,77	Bmac40	6H	120	25,89	**	< 0,01	5,20	2,29	2,33	2,1	3,36	1,069	< 0,01	-0,20	NS	0,039	NS
	bPb-3732	×	EBmac603	bPb-3732	7H	3,48	EBmac603	7H	40	16,78	*	< 0,05	3,13	2,51	2,19	2,49	3,63	1,116	< 0,01	-0,02	NS	-0,33	NS
	bPb-9746	×	S53707	bPb-9746	3H	54,8	S53707	1H	18	24,09	**	< 0,01	4,62	2,33	2,01	2,41	4,73	2,403	< 0,01	0,084	NS	-0,32	< 0,05
RDW	bPb-0353	×	HVM67	bPb-0353	3H	84,38	HVM67	4H	141	19,81	**	< 0,01	3,79	2,43	2,55	2,53	1,79	-0,64	< 0,01	0,097	NS	0,123	NS
	bPb-8779	×	Bmag357	bPb-8779	2H	77,41	Bmag357	5H	68	35,7	**	< 0,01	5,88	2,33	2,29	2,35	4,41	2,08	< 0,01	0,013	NS	-0,04	NS
	bPb-4577	×	Bmac32	bPb-4577	2H	108,7	Bmac32	1H	80	20,23	**	< 0,01	3,43	2,72	2,53	2,43	4,34	1,623	< 0,01	-0,29	< 0,05	-0,19	< 0,05
	bPb-1318	×	MGB338	bPb-1318	1H	13,14	MGB338	5H	95	18,92	**	< 0,01	3,36	2,34	2,47	1,95	3,83	1,491	< 0,01	-0,389	< 0,01	0,129	NS
	bPb-4531	×	GMS61	bPb-4531	1H	60,21	GMS61	5H	126	17,72	*	< 0,05	3,60	6,2	6,03	6,83	12,12	5,924	< 0,01	0,628	< 0,05	-0,16	NS
RSR	bPb-2862	×	MGB338	bPb-2862	1H	4,3	MGB338	5H	95	21,62	*	< 0,05	4,28	6,34	6,79	5,53	11,95	5,603	< 0,01	-0,81	< 0,05	0,443	NS
	bPb-3732	×	EBmac603	bPb-3732	7H	3,48	EBmac603	7H	40	26,73	**	< 0,01	5,71	6,81	5,69	6,67	9,92	3,11	< 0,01	-0,13	NS	-1,12	NS
	bPb-1657	×	bPb-7763	bPb-1657	6H	68,22	bPb-7763	5H	71	15,26	*	< 0,05	3,38	6,68	6,29	6,53	7,93	1,247	< 0,01	-0,15	NS	-0,39	NS
Physiological traits																							
RWC	bPb-6676	×	bPb-2225	bPb-6676	5H	81,39	bPb-2225	2H	67,4	11,5	**	< 0,01	0,07	64,87	63,94	65,28	65,12	0,245	NS	0,409	NS	-0,93	NS
OP	HvGI	×	bPb-3427	HvGI	3H	63	bPb-3427	6H	38	18,79	**	< 0,01	7,16	0,17	0,17	0,17	0,26	0,083	< 0,01	-0,003	NS	-0,004	NS
	bPb-0202	×	bPb-8283	bPb-0202	7H	106,6	bPb-8283	3H	69,6	25,03	**	< 0,01	9,47	0,18	0,17	0,17	0,26	0,083	< 0,01	-0,008	NS	-0,011	NS

,* indicate the significance level at 0.05 and 0.01 respectively to declare the putative epistatic QTL positions

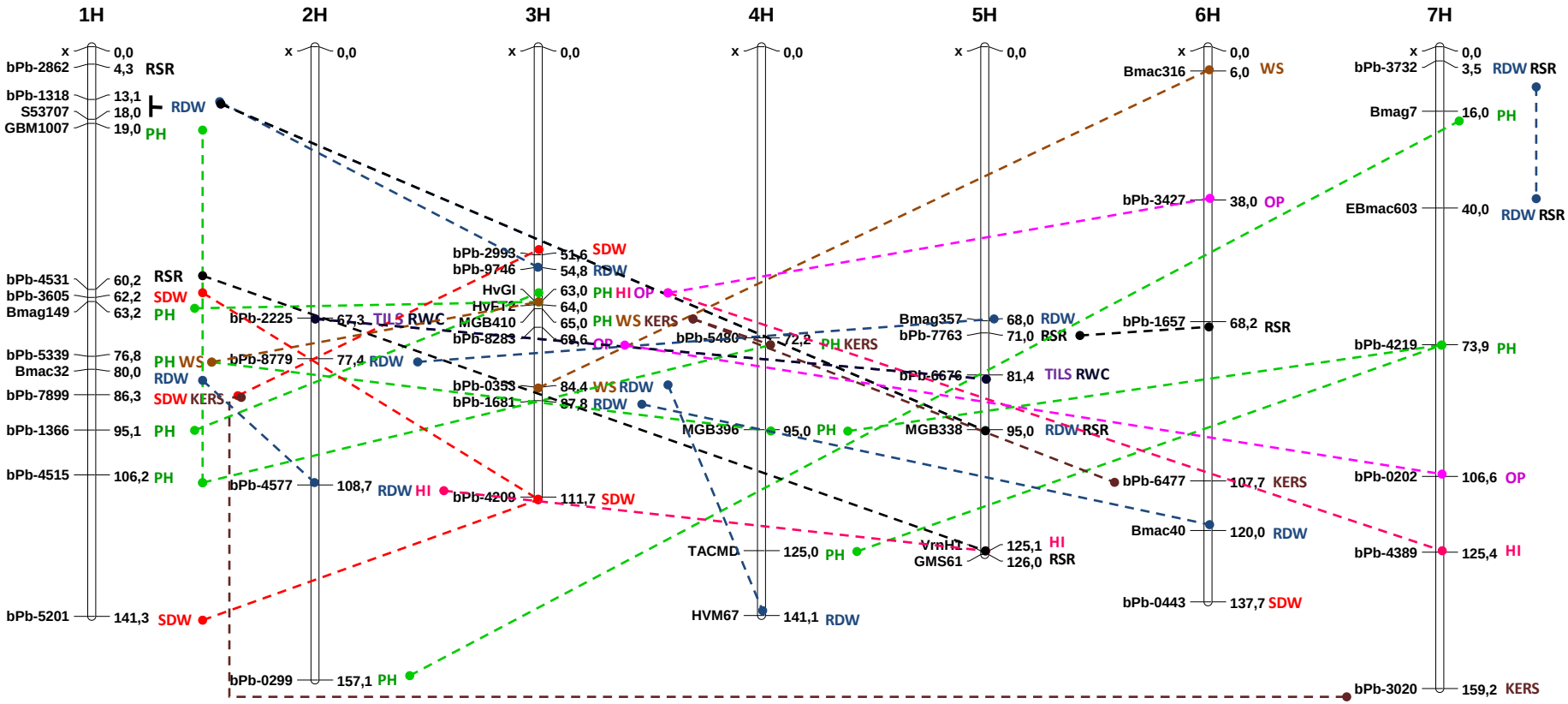


Figure 44 Positions of 33 pairs of epistatic effects controlling shoot, root and physiological in S42 population. Digenic epistatic interactions have been highlighted with dotted lines, arrow heads indicate associated markers on both sides.

4 Discussion

Drought is an increasingly constraint that limits barley world production. Drought tolerance, however, is a complex character resulting from many interacting components (traits). Therefore, improving the drought tolerance of barley is one of the most important objectives of barley breeders. In development of drought tolerance, the identification and characterization of QTLs controlling the adaptive traits for drought tolerance are necessary to understand the control and expression of these traits. Backcrossing is a way by which these genetically inherent barley characteristics can be transferred to an elite line. Marker aided simultaneous discovery and transfer of valuable QTLs from unadapted germplasm to an elite breeding line was demonstrated by Tanksley and Nelson 1996.

This study has been carried out in plastic tunnels during the summer seasons 2007, 2008 and 2009 at Bonn University, Germany. In this study the advanced backcross Quantitative Trait Loci (AB-QTL) analysis was applied using 371 SSR and DArT markers to identify favorable exotic alleles that improve drought tolerance in an advanced backcross population derived from a cross between the German spring barley cultivar ‘’Scarlett’’ (*H. vulgare* ssp. *vulgare*) and an exotic accession of *H. vulgare ssp. spontaneum* (ISR42-8). The goal of the present work was to detect favorable QTL alleles from the wild donor, which may lead to an improvement of drought tolerance related traits in this population. In the following discussion, the phenotypic variation and QTL-results have been presented.

4.1 Phenotype evaluated

Crop tolerance to drought is complex both genetically and physiologically. Many morpho-physiological traits putatively contribute to drought tolerance and each of these traits is typically controlled by multiple genes or quantitative trait loci (QTLs). They are influenced by environment to a great extent (Lang and Buu 2008). In current study, the population S42 which consists of 301 BC_2DH lines, was tested for tolerance to drought. This investigation was done under control and drought stress conditions in three successive summer seasons (2007, 2008 and 2009). A total of 15 quantitative traits were investigated for drought tolerance which are grouped in nine shoot traits (PH, WS, TILS, SPS, SDW, GY, KERS, TGW and HI), three root traits (RL, RDW and RSR) and three physiological traits (RWC, PC and OP).

In present study, components of variance revealed a wide range of variability for most of the traits. High significant differences between Scarlett and ISR 42-8 were detected for all investigated traits except OP and SPS. As a logical consequence, the parent Scarlett was exhibited high significant differences and superior in the yield and its attributes such as grain GY, KERS, TGW and HI, while the wild accession ISR 42-8 showed significant differences and superior in the vegetative and root traits such as PH, WS, RL, RDW, RSR and RWC. The comparison of phenotypic means, indicated that Scarlett was greatly influenced by drought stress. The analysis of variance (ANOVA) revealed highly significant differences among BC_2DH lines except for RSR, PC and OP. Variance arising due to differences between treatments as well as years were highly significant for all of the studied traits. The interaction between accessions and treatments was significant in most of the traits. All studied traits were greatly influenced by water stress, since the majority of the studied traits were reduced under drought conditions except WS, RL, PC and OP were increased. Under control and drought conditions high diversity of means was observed for most of the studied traits in particular PH, KERS, TGW, HI, RL and RWC. Different magnitudes of the agronomic performance of the BC_2DH lines have been observed when compared to their parents under both treatments.
The comparison of the means between BC_2DH lines and both parents revealed that the population means of agronomic and yield-related traits were excelled the recurrent parent Scarlett. That may due to the strong correlation among them, which has been observed in this study. On the other hand, the population means of root characteristics were inferior to Scarlett and showed the performance of the adaptive parent. We could expect that the presence of large chromosomal segments from elite cultivated barley would exhibit positive effects on agronomic traits. By other word we could expect some introgressions alleles coming from wild barley lead to reduce the agronomical traits and increase the adaptive traits such as RL, RDW and RWC. Several reports have been reported the negative effects of wild alleles on the agronomic traits (Pillen *et al*. 2003, 2004; Saal *et al*. 2010). In a study of El Soda *et al.* (2010) on barley, significant differences between lines and the recurrent parent were observed for leaf area, shoot dry weight, and tiller number means across all environments.

4.2 Correlations between studied traits

Information on association of yield and yield contributing traits could be useful in selection of drought tolerant/resistant genotypes. Further correlation studies among yield

contributing traits may help in indirect selection of yield components. Correlation is a pragmatic approach to develop selection criteria for accumulating optimum combination of yield contributing traits in a simple genotype (Munir *et al.* 2007). Correlation analysis was undertaken for fourteen drought tolerance- related traits including shoot, root and physiological traits in S42 population under control and drought conditions.

In the present work, strong positive correlations were found between GY and with all of TILS, SPS, SDW, KER and HI under both treatments. Very strong correlations between yield traits were observed. Pillen *et al.* (2003) mentioned that plant height (PH) displayed medium positive correlations with above ground biomass (MAS), while grain yield (GY) displayed medium positive correlations with KER and HI. von Korff *et al.* (2006) observed that grain yield was positively correlated with all of ears per m^2 and harvest index, while ears per m^2 showed a positive correlation with harvest index and yield. The very strong correlation observed between grain yield (GY) under stress and harvest index (HI) under stress indicates that the yield differences we observed under drought stress were mostly the result of a large difference in the accumulation of biomass. High significant and strong positive correlation has been observed between SDW and TILS under both treatment. El-soda *et al.* (2010) reported that the correlation between shoot dry weight and tiller number was statistically not significant. The positive correlations with the yield-related traits KERS and HI have been frequently observed in other studies. For the correlation between GY and root and physiological traits ranged between weak and relatively strong, where the correlation between GY and with all of RL, RDW, RSR and RWC was negative, and with all of PC and OP was positive under drought conditions. While under control, it was positive and highly significantly with RL, RDW, RWC and PC. Whereas with RSR and OP was negative and highly significant and non significant respectively. Weak positive correlation but highly significant between TILS and RL under control while it was very weak and non-significant under drought stress. This result agrees with obtained by El-Soda *et al.* (2010) they reported that tiller number was significantly correlated to total root lengths. A positive and high significant correlation between WS and PC was observed under both treatment, the same result has been obtained by Mohamed (2009). Babu *et al.* (2003) reported that leaf drying scores had negative correlations with yield and harvest index under stress. Strong, positive and highly significant correlations were detected among root traits RL, RDW and RSR under

both treatments. Root length density is very strongly correlated with root dry weight (RDW) (Yadav *et al.* 1997). Negative correlations were detected among the physiological traits RWC, PC and OP and ranged from weak and strong correlation under both treatments, and it has been observed that RWC was correlated negatively with PC under control and drought conditions. El-Soda *et al.* (2010) found a weak but positive correlation between tiller number and root lengths. The result of the correlation analysis indicated the possibility to select secondary traits related to yield and adaptation under drought conditions.

4.3 Clustering of QTLs detected in this study

Improving the drought tolerance of barley is one of the most important objectives of plant breeders focusing on this crop to minimize the yield losses resulting from moisture stress, which is a regular feature of most barley growing environments. In the past, plant breeders dealt with drought stress in crops through field observations and standard breeding practices. The evolution to molecular breeding has yielded a deeper understanding of the interacting quantitative trait loci (QTLs) of the drought tolerance related traits (complex traits) and has exposed underlying genetic variation useful in marker-assisted breeding (Holloway and Li 2010). Mapping quantitative trait loci (QTL) in bi-parental populations allows the detection of chromosome segments controlling traits of agronomic interest with the opportunity to dissect complex traits into component loci (Marza *et al.* 2006).

The present study was conducted in order to identify the drought tolerance exotic alleles /QTLs in BC_2DH lines of S42 by means of AB-QTL analysis. Tanksley and Nelson (1996a) developed a strategy, which allows a targeted transfer of favorable exotic alleles into elite breeding material. Through this approach, specific exotic alleles derived from the exotic donor are tagged with molecular markers and tested for association with agronomic traits. Favorable QTL-alleles are useful as a breeding resource after they have been fixed in nearly isogenic lines. However, these favorable QTLs often lose their effects after they are purified into elite lines (Pillen *et al.* 2003).

To our knowledge, this study represents the biggest double haploid population in combination with a high resolution genetic map of barley. The strength of a QTL analysis primarily depends upon the size of mapping population and the density of genetic map (Collard *et al.* 2005).The parents, Scarlett and ISR42-8 showed a significant variation for most investigated traits that segregates in BC_2DH population, thus indicating the suitability of

this population for the QTL analysis of selected traits. In this investigation, 79 putative QTLs for all studied traits were detected among 5,194 marker × trait combinations in the population S42 under study, and can be divided into 55 QTLs for shoot traits, 15 QTLs for root traits and 9 QTLs for physiological traits. The detected putative QTLs were clearly localized in clusters on all seven chromosomes. Overall 27 (34.1 %) QTLs showed favorable effects derived from the presence of exotic alleles of the homozygous *Hsp* genotype in population S42. That means more than one-third of the introgressed alleles from the exotic parent are in the genetic background of this population. The questions that arise strongly, is this percentage of the introgressed exotic alleles leads to genetic improvement for tolerance to drought in barley?. Which trait(s) has affected significantly by the presence of the exotic alleles?. Our result is matching with previous studies conducted on barley, whereas 34% of the QTLs identified had favorable effect in one population, 48% of the putative QTLs derived from the wild species *H. vulgare* ssp. *spontaneum* C. Koch were favorable in another population. In total, 26%of the putative QTLs were detected simultaneously in both the populations (Pillen *et al.* 2004). In another study of Pillen *et al.* (2003), they detected 29 (34%) favorable QTL effects are coming from the presence of the homozygous *Hsp* genotype alleles, and most of the favorable QTLs were located on chromosomes 1H, 2H and 4H (8, 6 and 7, respectively). Thus, in general, 30 to 50% of the QTLs identified from the wild species have been reported to be beneficial. To answer our questions, the QTL-results have been discussed separately for each trait and compared with the previous studies as follow:

4.3.1 QTLs detected for shoot traits

A total of 55 putative QTLs were detected for nine shoot traits (PH, WS, TILS, SPS, SDW, GY, KERS, TGW and HI) in S42 population. Among these loci, 17 (30.9 %) QTLs for shoot traits were identified with favorable effects. Most of putative QTLs were located on chromosomes 1H, 2H, 4H and 5H by one, seven, eight and one QTL for each respectively. However, most of favorable effects of the *Hsp* alleles were detected on chromosomes 2H and 4H. In recent years, large numbers of QTL have been reported in diverse cereals for a range of agronomic traits: for example, in barley, QTL have been reported for yield under drought environments (Comadran *et al.* 2008; Talame` *et al.* 2004), and in wheat, QTL for plant height, maturity, and grain yield (Kato *et al.* 2000; Kuchel *et al.* 2007; Marza *et al.* 2006; McCartney *et al.* 2005; Snape *et al.* 2007).

QTLs for Plant height (PH)

Plant height is an important morphological character directly linked with the productive potential of plant in terms of grain yield (Alam *et al.* 2007). A reduction in plant height can improve lodging resistance and indirectly increase yield. PH appears to be controlled by many genes, including dwarfing, semi dwarfing, and other plant height genes (Yu *et al.* 2010). Genes of plant height have been mapped to the long arm of chromosome 4H of barley Hackett *et al.* (1992). Our study revealed three QTLs (*QPH.S42.2H, QPH.S42.4H.a and QPH.S42.4H.b*) were exhibited a favorable performance of shortening PH by 11.03, 7.5 and 7.42 %. These QTLs were acted additively in the inheritance of PH. These QTLs explained up to 12.96 % of the genetic variance respectively. The contribution percentage of the QTLs in the genetic variance reflects variation in genotypes transmitted from one of the parents to the progeny that causes phenotypic variance in the trait.

Although the wild parent (*Hsp*) was taller than the elite parent (*Hv*) under both treatment. The *Hsp* allele revealed a decreased value of plant height (PH) and may contribute the drought escape allele, and become very useful to decrease plant height under drought stressed conditions. Comparing with previous study on S42 population, our results of plant height (PH) were confirmed that the *Hsp* allele was associated with a significantly reduced plant height and yield in BC_2DH lines. These results are in agreement with Wang *et al.* (2010). However, one QTL out of three was exhibited an increase in PH by maximal 16,96 cm at *QPH.S42.3H.b* at position 118.72 cM and explained up to 59.16 % of the genetic variance (Table 6). No significant interaction effects were recorded for plant height. In another work on the same population 'S42', Saal *et al.* (2010) have identified four QTLs associated with PH and were uncovered on chromosomes 2H, 4H and 6H. In this study, we have identified that, the SSR marker GBM1043 3H (100.7 cM) was associated with PH as marker main effect and affected positively on plant height, this result in agreement with von Korff *et al.* (2010) they have detected the same marker 'GBM1043' but at different position 3H (130 cM) and interacted with another marker 'BMAG125' 2H (122 cM). The allelic combination of *Hsp*/*Hsp* at this locus increased plant height significantly as compared to the combination Hv/Hv. Also, the SSR marker GMS3 2H (81 cM) was associated highly significantly with PH and affected negatively on this trait, the same trend of this marker has been observed by von korff *et al.* (2006) and Pillen *et al.* (2003 and 2004). Pillen *et al.* (2003) identified 17 putative QTLs for PH were located on four chromosomes and eight putative QTLs were located on

five chromosomes (Pillen, 2004). All these loci exhibited significant marker main effects. For five and six QTLs respectively, a favorable effect of the *Hsp* allele on PH was observed. At these loci, the presence of the *Hsp* allele led to a reduction in plant height of up to 10.4% (GMS3$_{[2H]}$) and 19.8% (GMS6$_{[6H]}$) respectively.

In another population of barley, QTLs with major effects have been identified by Baum *et al.* (2003) on chromosomes 2H, 3H and 7H. Under rainfed Mediterranean environments in a recombinant inbred line (RIL) population derived from the barley cultivars ER/Apm and Tadmor, von Korff *et al.* (2008) identified six QTLs for plant height on chromosomes 3H, 4H and 6H. The Tadmor allele increased height at five out of six loci by maximal 4.3 cm at *Qph-tera_3H.a* in range 101-118 cM, which explained 19.4% of the genetic variance. In the same previous population, major QTLs for plant height were located on 2H, 3H, 4H and 6H (Teulat *et al.* 2001). Chloupek *et al.* (2006) identified four QTLs for PH and were located on chromosomes 3H, 4H, 5H and 7H. Gyenis *et al.* (2007) reported five QTLs for PH on chromosomes 1H, 2H, 5H and 7H. Forster *et al.* (2004) detected QTL for plant height on 7H between 89 and 120 cM. Li *et al.* (2006) detected thirteen QTLs affected significantly plant height. In rice, Gomez *et al.* (2006) detected five QTLs for plant height (PH) under drought conditions and distributed on chromosomes 1, 4 and 5. While Li *et al.* (2010) detected four QTLs associated with plant height and acted additively.

Wilting score (WS)

Change in leaf shape or form has often been enumerated as a means of reducing transpiration rate by plants experiencing water deficit. Leaf wilting and leaf rolling are the first visible syndromes of plant exposure to drought in the vegetative phase (Boyer, 1982). Plant wilting occurs due to the inability of leaves to sustain the transpiration demand of the plant (Blum, 1988). Leaf rolling is the most important criteria found useful in assessing levels of drought tolerance in large scale screening (Chang *et al.* 1974), and potentially useful drought avoidance mechanism in arid areas (Clarke, 1986).

The QTL analysis revealed four QTLs for WS. At two loci, the introgression of exotic alleles from the drought tolerant parent, ISR42-8 was responsible for reduced WS. It agrees with hypothesis of introgression exotic allele from a resistant wild-accession. However, the inferior performance of exotic alleles at *QWS.S42.2H* and *QWS.S42.3H* suggest that susceptible parent, Scarlett also harbors useful alleles for WS. Hence, it is tempting to

speculate that the associated loci may underlie essential components of plant performance and their replacement with the detrimental exotic alleles might be a reason of superior elite alleles. von Korff *et al.* (2008) identified one QTL for wilting and was located at the marker pHva1(1H) where the allele from ER/Apm increased the susceptibility to wilting. In a structured population of barley, Mohamed N (2009) has detected five markers associated significantly with WS and located on the chromosomes 3H, 4H, 5H, 6H and 7H. the markers which located on 3H and 5H affected negatively on this trait. In rice, Cairns *et al.* (2009) detected QTLs associated with leaf drying in 13 regions on chromosomes 1, 2, 4, 5, 7, 8, 11 and 12 and five regions with QTLs for leaf rolling on chromosomes 1, 2, 5, 7 and 12.

Number of tillers/plant (TILS)

Tillering is an important agronomic trait, as the tiller number per plant determines the spikes number which is a key component of barley grain yield (Sinha and Aggarwal, 1981). High tiller numbers are often the goal for genetic improvement and breeding in cereals, which seek to maximize the crop yield. In the present study, the QTL analysis revealed five QTLs for number of tillers/plant as marker main effects and located on chromosomes 2H, 4H and 6H. Four QTLs exhibited favorable performance of exotic alleles to increase number of tillers/plant. The four favorable QTLs placed on 2H (81-90 cM) and 4H (127.5 – 140.2 cM) are likely to be dominating the tiller and spikes number in this population. Another important point, the QTL, *QTILS.S42.2H.a*, which explained 39.86% of the genetic variance and the exotic alleles increased TILS by 22.12%. The strong contribution of the exotic alleles in the genetic variability demonstrates the strength of the impact of these alleles in the gene expression of TILS. Results of the present study indicate that introgressions from wild barley may increase number of tillers/plant in S42 population. Similar result has been obtained by El-Soda *et al.* (2010). A QTL for the number of fertile tillers on 4H at HVM67 was detected previously by Teulat *et al.* (2001). Baum *et al.* (2003) detected a QTL for tiller number on 4H (27 cM) upwards of HVM67 in a *H. vulgare* ssp. *vulgare* × *H. vulgare* ssp. *spontaneum* cross.

Several QTL for tillering have been described in rice. Li *et al.* (2010) detected nine QTLs associated with TILS in two groups of hybrids of rice and displayed different gene effects between additive up to complete dominance effect. QTL for tiller number were detected on chromosome 03 (Cairns *et al.* 2009; Liu *et al.* 2009; Quarry *et al.* 1997). Syntheny between the rice chromosome 03 and barley chromosome 4H are described in Thiel

et al. (2009). A homolog of the wheat tiller inhibition gene *tin3* was mapped on chromosome 01 in rice (Kuraparthy *et al.* 2008). *Tin3* is located on chromosome 3A in *T. monococcum* and the mutant is responsible for monoculm growth habit (Kuraparthy *et al.* 2007). The *HIGH-TILLERING DWARF1* (*HTD1*) and *DWARF10* (*D10*) genes were mapped on rice chromosomes 04 and 01, respectively, and are orthologs of the *Arabidopsis MAX3* and *MAX4* genes. *D10* controls lateral bud outgrowth and is upregulated in high tillering mutants (Arite *et al.* 2007) while *HTD1* negatively regulates tiller bud outgrowth (Zou *et al.* 2006). Another gene, *FINE CULM 1*, a homolog of teosinte branched 1 (*tb1*), controlling lateral bud outgrowth, was mapped on chromosome 03 (Takeda *et al.* 2003). *Tb1* is responsible for tillering suppression during maize domestication (Doebley *et al.*1997).

Number of spikes/plant (SPS)

A trait spikes per plant is one of yield related attributes in cereals generally. In present study, the QTL analysis revealed seven QTLs for SPS and located on chromosomes 2H, 3H, 4H and 6H Among these, four QTLs showed favorable performance of the exotic genotype in the enhancement plant spikes. The present study revealed significant and positive correlation between tillers and spikes number per plant. The *QTLs, QSPS.S42.2H.a* and *QSPS.S42.2Hb* explained 40.95 and 34.80% of the genetic variance respectively. The high contribution in the genetic variance indicating that, these QTLs are likely to be dominating number of spikes per plant. The introgressions from wild barley may increase number of spikes/plant in S42 population. In another work on the same population 'S42', Saal *et al.* (2010) have identified three QTLs as marker main effects were associated with SPS and localized on chromosomes 1H (HVABAIP), 6H (GMS6) and 7H (BMAG7). The presence of the exotic allele at locus HVABAIP increased SPS by 6.8%.

In wheat, among five QTLS were detected for SPS by Ibrahim *et al.* (2010), one QTL (QSpk.D84-3B.a) increased SPS by 10.8% and 16.3% under well-watered and drought stress treatment, respectively. Zhao *et al.* (2010) have detected two QTLs which were associated significantly with number of panicales per plant in rice population and mentioned that the alleles of ''Nagdong '' parent had increased effects on the number of panicles per plant.

Shoot dry weight (SDW)

Above ground dry matter production is an important criterion to judge drought tolerance in crop breeding (Morgan *et al.* 1993). Shoot dry weight is one of important agronomic traits when the plants were grown under soil water deficit conditions. Locations close to the five chromosomal regions on 2H, 5H and 6H, probably influencing shoot dry weight. The presence of the exotic allele at locus *QSDW.S42.5H.a* increased SDW by 12.04%. Despite the strong positive correlation between SDW and each of TILS and SPS under both treatment in S42 population, a QTL for shoot dry weight, no. of tillers/plant and no. of spikes/plant and plant height was detected at GMS3. This linked QTL decreased SDW and PH while it increased TILS and SPS. Pillen *et al.* (2003 and 2004) detected only one QTL for MAS in each study separately. Markers HvA22S $_{[7H]}$ and EBmac0679 $_{[4H]}$ were exhibited a significant main effect. The negative effect of the *Hsp* allele resulted in a 5.1% of the above ground biomass. The explained phenotypic variance for HvA22S $_{[7H]}$ amounted to 0.6%, while EBmac0679$_{[4H]}$ exhibited a favorable effect of the *Hsp* allele resulted in a 3.8% increase in the above-ground biomass and explained 0.4% of the phenotypic variance. In wheat, Ibrahim *et al.* (2010) identified five QTLs for biomass with marker main effects and associated significantly with this trait, they found that the exotic allele *QBm.D84-3D.a* located on chromosome 3D increased BM under both well-watered and drought-stress treatment by 5.8% and 9.7%, respectively.

Grain yield/plant (GY)

Yield is assumed to be influenced by multiple component traits, where each with their own genetic architecture (Cooper *et al.* 2009). For over a decade, with development of molecular approaches, QTL analysis was used to detect yield and fecundity-related traits. Many QTLs affecting yield were mapped on seven chromosomes throughout the whole genome of barley. Yield QTLs derived from related wild species have also been mapped in wheat, barley and other crops (Swamy and Sarla 2008).

In this investigation, six QTLs were identified for GY and located on chromosomes 2H, 3H and 6H. All QTLs alleles showed unfavorable effect with an explained genetic variance up to 14.34%. The relative performances of the exotic genotype led to reducing GY with range between -17.90% and -8.96%. The reduction of GY in the population S42 may due to the presence of large or small specific segments of the wild genotype. This assumption has

been emphasized by several QTL studies (Pillen *et al*. 2003, 2004). Comparing with previous study on S42 population, our results of grain yield (GY) were confirmed that the *Hsp* allele was associated with a significantly reduced yield in S42ILs. These results are in agreement with Wang *et al.* (2010) as well as with the fact that 'Scarlett' is a spring cultivar with high yield performance. In another work on the same population 'S42', Saal *et al.* (2010) have identified eight new QTLs for yield and detected on all chromosomes except 3H and 5H. All new QTLs revealed M × E effects. In another study, three yield-enhancing QTLs were mapped on chromosomes 2H and 3H (von Korff *et al.* 2006). Yield QTLs were identified on all but one chromosome (6H) in the wild species of barley *Hordeum vulgare* ssp. *spontaneum*. They were frequently present on chromosomes 4H, 3H and 2H, and mostly exerted a negative effect on yield. But, three other QTLs located on chromosome 2 enhanced yield (Pillen *et al.* 2003, 2004). Pillen *et al.* (2003 and 2004) identified 31 putative QTLs for GY, 24 loci exhibited a significant marker main effect by 11 and 13 loci respectively. While, nine loci showed a significant M x E interactions. Most QTL alleles from *Hsp* resulted in yield reductions with a maximum of 21.0% $EBmac0824_{[5H]}$ and up to a maximum of 22.6% $EBmac0378_{[2H]}$ respectively. In present investigation, we have identified that, the SSR marker Bmag603 3H (66 cM) was associated with GY and affected negatively on yield, this result in agreement with von Korff *et al.* (2010) they have detected 9 out of 12 interactions, the allelic combination of exotic and elite reduced yield and found that the allelic interaction between *Hv/Hsp* at the markers $S53707_{[1H]}$ and $Bmag603_{[3H]}$ was associated with a yield reduced by 8 dt/ha. Similar results have been obtained by Li *et al.* (2006), they have detected six QTLs for GY and in most cases, the donor parent of barley segment decreased total grain yield. In a population of a cross Steptoe × Morex (SM) of barley, Hayes *et al.* (1993) identified 14 QTLs for yield were mapped on seven chromosomes, of them, only five on 2H, 3H, 5H, and 6H were confirmed in the same cross by Zhu *et al.* (1999a), Romagosa *et al.* (1999a and 1996) and Han *et al.* (1999), respectively.

In rice, Kato *et al.* (2009) detected two QTLs for grain yield on chromosomes 1 and 2 with negative additive effects (-0.66 and -0.81) and explained 16.3 and 12.2% of genetic variation under limited and full irrigation respectively. Li *et al.* (2010) have investigated six QTLs controlling grain yield in rice with two showing an additive effect. In wheat, Ibrahim *et al.* (2010) detected four QTLs for GY. Where the exotic allele introgressed chromosome 5D

(*QYld.D84-5D.a*) decreased YLD by 18.3% under well-watered and increased YLD by 4.0% under drought-stress treatments.

Number of kernels/spike (KERS)

Kernels or grains number/spike is one of the main components of yield in cereals (Araus *et al.* 2008). Like in the case of GY, six QTLs were detected for KERS and distributed on chromosomes 2H, 3H, 4H and 6H. Only one QTL, at locus *QKERS.S4264H,* the exotic genotype showed a favorable increase of KERS by 6.44% and explained 3.5% of the genetic variance.

Pillen *et al.* (2003 and 2004) detected only one QTL for KERS in each study separately. At markers loci $GMS21_{[1H]}$ and $Bmag0113_{[5H]}$ significant main effect has been observed. The negative effect of the *Hsp* allele resulted in a 6.5% and 16.0% reduction of kernels per spike respectively. Weak contribution percentage of these QTLs in the genetic variance has been detected. The SSR marker $GBM1049_{[6H]}$ (55 cM) was associated significantly with the reduction of KERS due to the presence of *Hsp* alleles. The same trend of this marker has been observed by von korff *et al.* (2006) but with trait 1000-grain weight.

Thousand grain weight (TGW)

Thousand-grain weight (TGW), known as a representative quantitative trait, is important to yield component and determined by synthesis and accumulation of starch in grain endosperm (You *et al.* 2006 and Mei *et al.* 2005). It is clear that wild barley yield less and has lower grain weight than cultivated barley. Locations close to the six chromosomal regions on whole genome of barley except chromosomes 2H and 5H, probably influencing weight of thousand grain. The exotic alleles only at marker locus *HvFT3* on chromosome 1H revealed positive effects on TGW, while it revealed negative effects for the five remaining QTLs in relation to TGW. By other words, those five QTLs carry Scarlett alleles and increase the weight of grains. Nine QTLs were detected for TGW and located on chromosomes 2H to 7H (von Korff *et al.* 2006). The SSR marker *BMS64* was associated to the QTL *QTGW.S42.7H,* this QTL led to reduce TGW, the same result with same marker have been identified by von Korff *et al.* 2006. Several QTLs have been detected by Pillen *et al.* 2003 and 2004.

Harvest index (HI)

Several studies carried out on wheat and barley genotypes showed that harvest index (HI) is mainly and directly associated with increasing in grain yield potential of the plant from about 30 up to 55% (Singh *et al.* 1998c, Slafer *et al.* 1994; Cattivelli *et al.* 1994). Locations close to the nine chromosomal regions on whole genome of barley except chromosome 7H, probably influencing harvest index. Among these, the QTLs, *QHI.S42.2H* and *QHI.S42.4H* showed favorable performance of the exotic genotype and accounted up to 7.78% of the genetic variance. Results of the present study indicate that introgressions from wild barley may increase harvest index in S42 population. Similar result has been obtained by von korff *et al.* (2006), they have detected twelve QTLs with a marker main effect for HI and distributed on all seven chromosomes, the exotic allele decreased HI at seven QTLs. The SSR marker EBmac701 on 3H (130 cM) was detected as marker main effect for HI in this study and was associated positively and significantly with harvest index (HI), the same trend of this marker has been identified for HI by von korff *et al.* (2006) and revealed an increasing in HI. Wang *et al.* (2010) have reported that, the closely linked genes HvGI and HvFT2 on chromosome 3H both were associated with significant effects on HEA, EAR, HEI, HI, LAH and YLD, the same gene or marker *HvGI* at the new position 63 cM on 3H was highly significantly associated with HI in the present study and revealed unfavorable effect and affected negatively on this trait. The *Hsp* alleles resulted in a reduced performance for HI in both study. Pillen *et al.* (2003) identified five putative QTLs for HI and located on chromosomes 4H, 5H and 7H. In another study of Pillen *et al.* (2004), seven putative QTLs were located for HI on chromosomes 2H, 3H and 5H and a maximum favorable *Hsp* effect of 5.0% was reached at the three linked loci-*HVM36*$_{[2H]}$, *GMS3*$_{[2H]}$ and *HvBKASI*$_{[2H]}$. In the two study the presence of the *Hsp* allele resulted in a HI decrease of up to 15.6% (*EBmac0824* $_{[5H]}$) and 7.3% (*HvLOXC*$_{[5H]}$) respectively. In wheat, Ibrahim *et al.* (2010) identified three QTls with a significant M*T interaction for HI. A QTL out of them (*QHi.D84-2A.a*), increased HI by 1.9% under drought-stress treatment.

4.3.2 Detection of QTLs for root traits in the population S42

Plants have different mechanisms to minimize the effects of drought. Adaptive mechanisms involve different root and shoot characteristics that allow plants to maintain high internal water status when available water is less than the evaporative demand (O'Toole and Chang 1979; Nguyen *et al.* 1997; Zhang *et al.* 1999). A root system that enables the crop to

extract more soil water has the potential to increase yield under drought (Mambani and Lal 1983). Individual root characteristics, such as thickness, depth of rooting and the ability to penetrate through compacted soils, have been associated with drought avoidance (O'Toole and Chang 1979). Chloupek *et al.* (2006) reported that large genetic variation therefore exists for root traits in the barley gene pool. In the current investigation, Large variation in root characters was observed as indicated by the large standard deviations. The important detected QTLs for root traits are discussed as follow:

Root Length (RL)

A deep root system able to extract water at depth and respond to evaporative demand, provided there is water in the profile. Root length (RL) is the most consensual of the traits contributing to drought avoidance (Courtois *et al.* 2009). Quantitative trait loci (QTL) mapping has been used to analyze the genetic basis of several root traits which might be involved in drought resistance (Li *et al.* 2005, Yue *et al.* 2005). In present study, the QTL analysis revealed three QTLs for root length and distributed on chromosomes 2H, 3H and 5H. The QTLs at, *QRL.S42.2H* and *QRL.S42.3H* showed unfavorable performance of the exotic genotype and revealed shortening of RL with values 15.09 and 7.69% respectively. In contrast, the presence of exotic alleles at marker locus *VrnH1* $_{[5H]}$ led to increase root length by 9.17 % under drought conditions. This result indicate that the introgression from wild barley may increase root length in S42 population. The present study revealed a weak but positive correlation between tillers number and root length. This result is matching with those obtained by El-Soda *et al*. (2010), since they reported that there is a direct relation between root system size and tillering, because nodal roots, which may dominate root system size, emerge directly from stem bases. Chen *et al.* (2010) detected four QTLs for RL on chromosome 2H (55 and 120.3 cM), 5H (187.4 cM), and 6H (83.8 cM). They reported that the WQ23-38 alleles at the four QTLs increased RL trait value. In rice, Cairns *et al.* (2009) identified two significant and three putative QTLs for root density at the upland site on chromosomes 3, 4, 6 and 7. Deep root per tiller QTL were detected in rice (Yadav *et al.* 1997). Recently, Obara *et al*. (2010) mapped *qRL6.1*, a major QTL for root length, on chromosome 6 in rice seedlings grown under hydroponic conditions. A novel major QTL *Dro1* (*Deeper rooting* 1) on chromosome 9 that controls deep rooting was reported by several

reports that this QTL is responsible for deep rooting under upland field conditions (Yonemaru *et al.* 2010, Uga *et al.* 2009, 2010, 2011)

Root dry weight (RDW)

The ability of genotypes with large root systems to better maintain water uptake may explain their relatively high transpiration efficiency under drought stress. Recent years some reports have demonstrated that root dry weight is an important trait related to water use efficiency long term drought (Songsri *et al.* 2009) and they suggested that root dry weight should be useful selection criteria for high water use efficiency long term drought. Li *et al.* (2005) identified three additive QTLs for RDW in rice. Li *et al.* (2009) detected one QTL (*qRDW8*) contributed by IRAT109, explaining 13.88% of the trait variation. In present study, we identified seven QTLs were associated significantly with RDW and located on chromosomes 1H, 2H, 3H, 4H, 5H and 7H. Among these loci, four QTLs *QRDW.S42.1H.a, QRDW.S42.1H.b, QRDW.S42.5H* and *QRDW.S42.7H* with positive additive effects, explained 6.50, 7.85, 4.21 and 6.91% of the genetic variance, and showed favorable performance of the exotic genotype and revealed an increasing of RDW with values ranged between 16.13 and 36.76%. All of these QTLs would be useful for drought resistance breeding in barley.

Root shoot ratio (RSR)

The root-shoot ratio is usually given as the ratio of the weight of the roots to the weight of the top of a plant (Harris, 1993). The varieties with high root : shoot ratios were more drought resistant (Yamauchi and Aragones, 1997). Li *et al.* (2009) detected two QTLs (*qRS8b* and *qRS9*) for RSR. Li *et al.* (2005) identified three additives for RDW/SDW. Our study revealed five QTLs were associated significantly with RSR and distributed on chromosomes 1H, 3H, 5H and 7H. At four QTLs, the exotic genotype showed favorable performance of the exotic genotype and revealed an increasing of RSR with values ranged between 16.26 and 30.87 %. The strongest effect was identified at the QTL, *QRSR.S42.1H.b* and explained 8.26% of the genetic variance.

Generally, four additive QTLs at marker loci GBM1042 (1H), bPb-2240 (1H), bPb-0071 (5) and VrnH3 (7H) were found to governed RDW and RSR, and led to increase both traits under drought conditions. In contrast, at marker locus bPb-9110 (3) was found to be governed RL, RDW and RSR and led to reduce these traits under drought conditions. In conclusion, root

length (RL), root dry weight (RDW) and root shoot ratio (RSR) were significantly and positively correlated each other under drought conditions. Therefore, a deeper root system with high RL, high RDW and high RSR should be the breeding objective when selecting for drought-resistant plants. Marker assisted selection (MAS) for these root traits would be extremely useful because they cannot be measured directly.

4.3.3 Detection of QTLs for physiological traits in the population S42

Breeding for drought tolerance based on traits associated with drought resistance, but easier to select for than grain yield, has been and still is very popular. Some physiological responses have been observed in plants induced by drought stress (Ludlow and Muchow, 1990). However, relatively few studies have examined QTL for physiological traits and their co-location with effects on crop yield and quality. In the following, the detected QTLs are discussed for each trait as follow:.

Relative water content (RWC)

Relative water content (RWC) is a measure of plant water status resulting from a cellular water deficit, and is an appropriate estimate of plant water status as affected by leaf water potential and osmotic adjustment (OA). Relative water content (RWC) has been proposed as a selection criterion for drought tolerance in many crops (Matin *et al.* (1989) in barley; Schonfeld *et al.* (1988) in wheat. In present work, four chromosomal regions related to variation in water status were detected on chromosomes 2H, 3H and 4H. Three QTLs exhibited significant marker main effects, while one QTL exhibited significant marker by trait interaction. The relative performances of the exotic genotype led to reducing RWC with values ranged between -20.17% and -8.27%. Previously ten genomic regions for RWC were identified in barley chromosomes 1, 2, 4, 5, 6, and 7H (Teulat *et al.* 2003). Diab *et al.* (2004) have detected six QTLs for RWC under irrigation treatment and three were detected under conditions of water stress. Further two QTLs for RWC were detected by Diab (2006) on 5H and 7H under irrigated and stress conditions respectively. Chen *et al.* (2010) detected three QTLs for RWC on chromosome 1H, 2H and 6H. The allele on chromosome 2H from xeric parent contributed the positive effect on relative water content of drought-stressed leaves. The QTL effect for RWC on chromosome 1H was collocated with an effect for relative water content in drought-stressed plants (Teulat *et al.* 2001) and a QTL effect for plant drought tolerance (Cattivelli *et al.* 2002). The QTL effect for RWC on chromosome 6H was

coincident with a QTL effect for RWC in field grown barley (Teulat *et al.* 2003). In rice, eleven QTLs on nine genomic regions for RWC measured in two different environments (Courtois *et al.* 2000) and eight QTLs for RWC scored in three different environments (Price *et al.* 2002b) were identified. Carns *et al.* (2009) detected QTLs in nine different regions for RWC by four QTLs were detected at the hydromorphic site on chromosomes 3, 7 and 8, while at the upland site, five QTLs were identified on chromosome 1, 2, 4, 7 and 11.

Proline content (PC)

Proline accumulates in many plant species under a broad range of stress conditions such as water shortage, salinity, extreme temperatures, and high light intensity (Aspinall *et al.* 1981; Mansour *et al.* 2000), and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants, and normally accumulates in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment (Leigh *et al.* 1981; Binzel *et al.* 1987; Ketchum *et al.* 1991). The level of proline accumulation in plants varies from species to species and can be 100 times greater than in control situation (Verbruggen and Hermans 2008). Although, we have utilized exotic parent, ISR42-8 as a source of drought tolerance but it has shown a reduced level of PC as compared to Scarlett. Proline accumulation has been considered as the marker for drought tolerance in different species (Kishor *et al.* 1995, Roosens *et al.* 2002, Yamada *et al.* 2005, Simon-Sarkadi *et al.* 2005). However, higher proline accumulation in drought susceptible parent Scarlett suggest that proline accumulation might be a consequence of drought and hence, plant that suffers more in drought can accumulate more proline for survival. Stewart (1978) reported proline accumulation in wilted barley leaves. His studies indicate that wilting caused a 40 fold stimulation of proline biosynthesis in nonstarved leaves than in starved leaves. He has found the role of carbohydrates in the process of proline accumulation and suggested that carbohydrate metabolism supplies precursors for the proline bio-synthesis. Thus, a low level of proline accumulation in ISR42-8 might be due to its inferior carbohydrate metabolism as compared to Scarlett. Hence, in the wilting leaves of Scarlett the conversion of glutamate to proline might be higher than in ISR42-8. However, the superior performance of exotic allele at marker locus MGB338 on chromosome 5H suggests a transgression effect. Interesting, this exotic QTL allele responded favorably under drought conditions only that indicates the possibility of underlying a novel drought inducible gene. The previous data and current

inferences suggested that the leaf wilting can influence proline accumulation. In general, this relationship can be viewed like a cause and consequence. Hitherto, the genetics behind these processes seems quite independent and diverse. This study has highlighted the role of a two-way evaluation of elite and exotic allele for the detection of favorable leads for drought tolerance. Subsequently, a combinatory approach for the selection of favorable elite and exotics allele can be employed to develop a better shield against the adverse effects of drought. In a structured population of barley, Mohamed (2009) identified two markers (bpb-3217 and bpb-8833) for PC and located on chromosomes 1H (40.53 cM) and 7H (147.17 cM and had positive main effect with predicted values 7.67 and 5.77, respectively

Osmotic potential (OP)

Under water-limiting environments, leaf water potential and osmotic potential (OP) are usually used for measuring the capability of osmotic adjustment (OA) in plants (Teulat *et al.* 1997). Osmotic adjustment refers to the lowering of osmotic potential due to a net accumulation of solutes in response to water deficit, and is distinct from the change in osmotic potential due to increased solute concentration associated with reductions in cell water content under drought Chimenti *et al.* (2006). A high OSM means a low osmotic potential that results in postponing plant wilting Chen *et al.* (2010). We found that there was no significant difference between elite cultivar Scarlett and wild accession ISR 42-8 in osmotic potential. The same result has been obtained by Chen *et al.* (2010), they reported that osmolarity and RWC traits showed no significant difference between two parents, xeric *H. spontaneum* WQ23-38 and mesic *H. spontaneum* MA10-30. QTL analysis of the present trait, revealed one QTL (*QOP.S42.4H*) for OP and located on chromosome 4H at positions 141.1 cM. The QTL exhibited favorable effect with an explained genetic variance of 6.75% and was responsible of reducing OP with value of 10.15%. The presence of exotic alleles led to reduce OP. Diab (2006) mapped two QTL for osmotic potential at full turgor were placed, one on chromosome 4H and one on 3H. In another study of Diab *et al.* (2004) they have identified seven QTLs for OP in the irrigated group and three were detected under conditions of water stress, one of them on 4H (126.8 cM) is near to the QTL which has been detected in the present study and showed negative additive affect on this trait.

4.4 QTL ×Treatment interactions

The primary objective of plant breeders is to produce genotypes with high and consistent performance across environments. The genetic dissection of complex traits still presents a challenge. The oligo/polygenic character of complex traits, combined with interactions between loci, makes the task a priori difficult and intricate. In addition, environmental factors will trigger and modify gene actions, and thereby further complicate the analysis. In present study, the majority (72 QTLs) of the detected QTLs were acted as marker main effect, which is considered to be stable across control and drought treatments. On other hand, seven QTLs were exhibited marker × treatment interaction effects, where the effect is considered to depend on a particular treatment. Six QTLs revealed unfavorable effects on SDW, RWC and PC. Only one QTL showed favorable interaction.

For more details, the first marker × treatment interaction was observed for SDW and mapped on 2H, since the exotic alleles were responsible for the reduction of the dry mass of shoots by 6.44% and explained 4.73% of the genetic variance (Table 6 and Figure 45). Pillen *et al.* 2003 detected a QTL associated with negative effect of *Hsp* alleles which resulted reduction in above ground mass

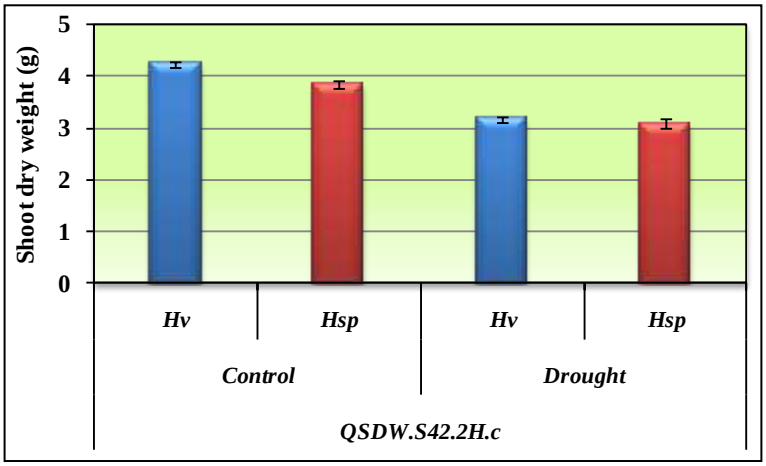


Figure 45 Ls-means of the QTL (*QSDW.S42.2H.c*) which showed marker × treatment interaction effects for shoot dry weight (SDW) under both treatments

At marker locus HvNAM2 on the long arm of 2H, a QTL associated with decreasing RWC and acted as marker × treatment interaction has been found for RWC. The performance of BC_2DH line carrying the elite and exotic alleles are quite the same under control, while little bit different under drought conditions. Relatively, water content percentage was higher in BC_2DH lines carrying the elite alleles than lines having exotic alleles. Teulat *et al*. 2003 has detected two QTL × environment interaction and mapped on the long arms of chromosomes 7H and 1H (Table 6 and Figure 46).

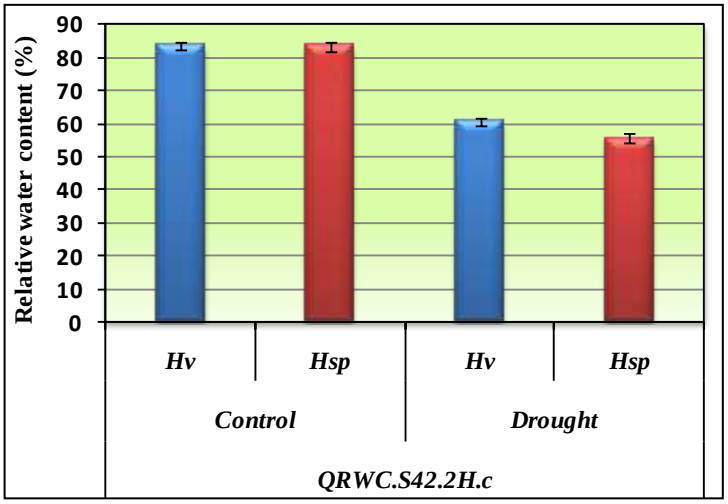


Figure 46 Ls-means of the QTL (*QRWC.S42.2H.c*) which showed marker × treatment interaction effects for relative water content (RWC) under both treatments

Four marker by treatment interactions have been detected for proline content. It has been observed that proline was accumulated many folds in S42 population under drought conditions. Only at marker locus MGB338$_{[5H]}$ the exotic alleles had huge effect on the increasing proline content in BC_2DH lines carrying *Hsp* alleles. This QTL''*QPC.S42.5H*'' may be useful as a target for crop drought tolerance improvement via marker-assisted selection (Figure 47).

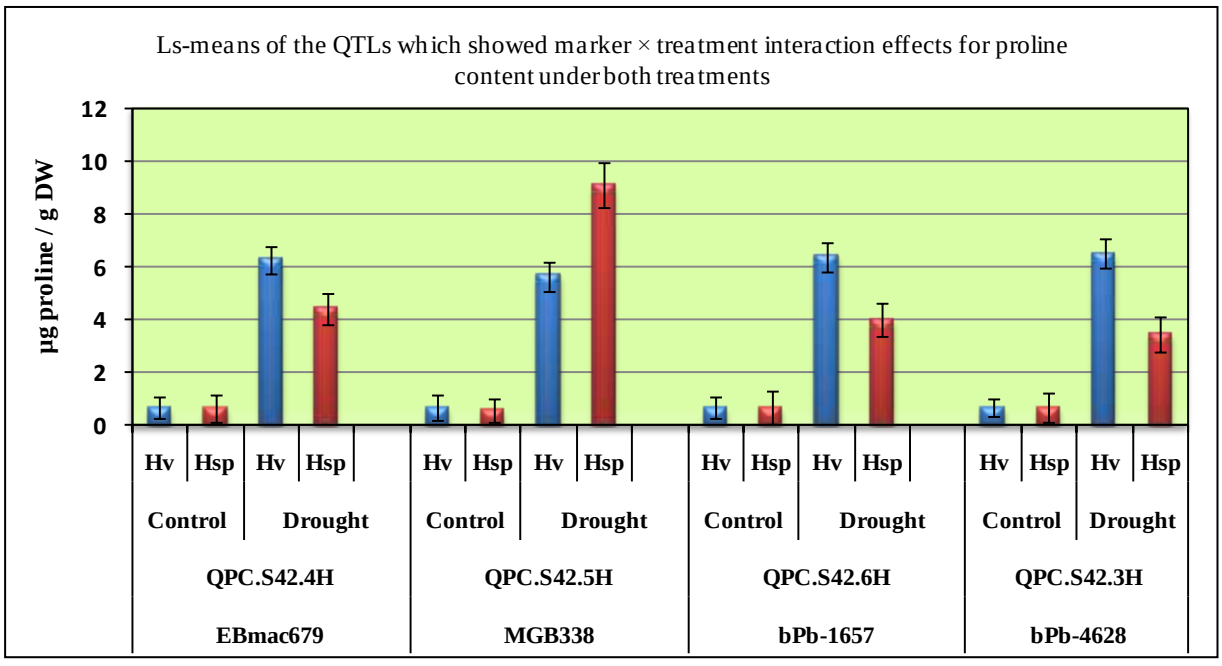


Figure 47 Ls-means of the QTLs which showed marker × treatment interaction effects for relative water content (RWC) under both treatments

4.5 QTL overlap among different traits

The colocation of QTLs for different traits implies the likely presence of pleiotropic or closed linkage between the QTLs control the traits (Lebreton *et al.* 1995; Agrama and Moussa 1996; Tuberosa *et al.* 2002b). In the present study, It was found that some QTLs controlling different shoot, root and physiological traits were located in the same chromosome regions or tightly linked together (Table 8). Two QTL regions on chromosome 1H at locus position GBM1042 (39 cM) and on 7H at locus position VrnH3 (42,50 cM) governing RDW and RSR, another two QTL regions were detected on chromosomes 1H (bPb-2240) and 5H (bPb-0071) were governing RDW and RSR with another trait PH and SDW respectively (Table 8). Five QTL regions were located along chromosome 2H and exhibited pleiotropic effects, while on chromosome 4H, four tightly linked QTLs in interval *Mlo* - *VrnH2* were exhibited pleiotropic effects and governing approximately the shoot traits. Interestingly, the QTL locus bPb-9110 on 3H (118,72 cM) is the most important QTL, hence, it was associated and governed with eight traits (GY, HI, PH, RDW, RL, RSR and WS). For yield and its attributes, several QTL regions were exhibited pleiotropic effects and governing two or more from yield components, for example on chromosome 3H all QTLs regions exhibited pleiotropic effects and were controlling yield and its traits, the QTL loci; bPb-7989 (50,43 cM), Bmag603 (66 cM) and bPb-9110 (118,72 cM) were governing grain yield/plant (GY), Harvest index (HI) and number of spikes/plant (SPS) and other traits (Table 8). QTL locus HvGI (63 cM) was controlling HI and KERS, QTL locus Mlo (127,50 cM) was controlling SPS and TILS, while QTL locus GBM1015 (140 cM) was controlling KER and SPS. It is worth mentioning in this study that yield and its attributes are highly positively correlated under both treatment. Pleitropic or tightly linked QTLs may be the genetic basis of phenotypic correlation. These QTLs will be helpful in MAS. In this investigation, the SSR marker $GMS3_{[2H]}$ showed a cluster of putative QTL effects for four traits. The similar results have been obtained by Pillen *et al.* (2004), they have detected three makers including the same marker $GMS3_{[2H]}$ revealed clusters of putative QTL effects for seven traits and one marker $GMS27_{[5H]}$ showed a cluster of putative QTL effects for six traits. Diab *et al.* (2004) have found several genomic regions, where QTL for different traits overlapped, for example, QTL for OA, OP and $DWSC_{100}$ were all mapped to approximately the same chromosomal location around caaaccO. Saal *et al.* (2010) observed different QTL regions showing co-localization, for example at locus HVABAIP on chromosome 1H for traits TGW and YLD, on 3H for HEI and YLD, on 4H for

HEI and TGW, and finally on 7H for HEI, TGW and YLD. In a study of Xing *et al.* (2002) on rice, three loci with pleiotropic effects were observed.

Table 8 Colocation of QTLs for drought tolerance related traits

Chrom	Marker	Type	Pos.	Traits							
1H	GBM1042	SSR	39,00	RDW	RSR						
	bPb-2240	DArT	123,09	RDW	RSR	PH					
2H	PpdH1	SSR	41,10	HI	KER	RL	SDW				
	bPb-4261	DArT	44,79	GY	RDW	WS					
	GMS3	SSR	81,00	PH	SDW	SPS	TILS				
	HvNAM2	SSR	90,00	RWC	SPS	TILS					
	bPb-8143	DArT	98,21	GY	SDW						
3H	bPb-7989	DArT	50,43	GY	HI						
	HvGI	SSR	63,00	HI	KER						
	Bmag603	SSR	66,00	GY	SPS						
	GBM1043	SSR	100,70	KER	PH						
	bPb-9110	DArT	118,72	GY	HI	PH	RDW	RL	RSR	RWC	WS
4H	Mlo	SSR	127,50	SPS	TILS						
	EBmac635	SSR	131	PC	PH	RDW					
	GBM1015	SSR	140,00	KER	SPS						
	VrnH2	SSR	140,20	TILS	WS						
5H	bPb-0071	DArT	126,77	RDW	RSR	SDW					
6H	Bmag613	SSR	75,00	KER	PC	SPS					
7H	VrnH3	SSR	42,50	RDW	RSR						

4.6 Detection of Epistasis

QTL mapping is one experimental approach to explore the role of epistasis in the genetic basis of complex traits (Carlborg and Haley 2004). Determining the contribution of epistasis is important for understanding the genetic basis of complex traits. Hence, genetic models for QTL mapping assuming no epistasis can lead to a biased estimation of QTL parameters. A large number of epistatic effects have recently been detected in rice (*Oriza sativa* L.) using polymorphic markers in the whole genome (Hua *et al.* 2002; Mei *et al.* 2003, 2005). Thus, in the present study, we have employed a QTL analysis using REML forward selection approach for simultaneous estimation of main effects of all individual markers and epistatic effects of all pairs of markers, which allows detecting interactions with a higher power. The present study used a BC_2DH population derived from a cross between cultivated and wild barley. Several studies have suggested that epistatic interactions play a larger role in crosses involving exotic germplasm than in elite by elite crosses (von Korff *et al.* 2010). The reason behind that may be due to the selection and conservation of different allele combinations in wild and elite barley as an adaptation to natural and agricultural environments, respectively.

Altogether 33 pairs of digenic epistatic QTLs as *additive* × *additive* effects were detected for nine studied traits related to drought tolerance in S42 population. Among them, eleven pairs displayed QTL by marker interaction and twenty two displayed marker by marker interaction. It will be interesting to study the relationships between additive QTLs and epistatic QTLs identified. Only 33% of main-effect QTLs for shoot, root and physiological traits were involved in epistatic effects. This indicates that several loci involved in epistatic interactions may not have significant effects for these traits and may affect the trait expression by epistatic interactions with other loci. Similarly, Ma *et al.* (2007) observed that 37% of the main-effect QTLs were involved in the epistatic interactions in maize grain yield and its components. Zhang *et al.* 2008) found 25% of main-effect QTLs for wheat plant height were involved in epistatic effects.

Epistatic effects for plant height (PH)

Epistasis is an important genetic characteristic of quantitative traits such as plant height (PH). The majority of epistatic interactions detected for plant height involved markers, which were not significant in the single marker analysis. Only one pair ($HvGI_{[3H]}$*bPb-1366 $_{[1H]}$) of epistatic QTLs showed QTL × marker interaction. The marker $HvGI_{[3H]}$ was observed to be associated to a QTL (*QHI.S42.3H.b*) underlying HI. In the current study, the wild barley parent is significantly taller than Scarlett under both treatments. The QTL analysis revealed that the exotic allele increased plant height at the half of loci. Since The BC2DH lines carrying the Hsp/Hsp genotype at these loci were on average 10.92 cm shorter than lines with the allelic combination Hv/Hv. For example, the most favorable pair of epistatic QTLs for shortening plant height was (HvGI*bPb-1366) and located on chromosomes 3H (63 cM) and 1H (95.08 cM). The phenotypic value of plant height is expressed better in case of the double introgression of the exotic genotype. Similar result has been obtained by (von Korff *et al*. 2006). In previous study on the same population of von Korff *et al.* (2010) they have detected four epistatic interactions between exotic alleles *Hsp/Hsp* introgressed from wild barley (*H. vulgare* ssp. *spontaneum* C. Koch) which increased plant height significantly as compared to the combination *Hv/Hv*. In Wheat, Zhang *et al.* (2008) identified five pairs of epistatic effects for the plant height (PH), and located on chromosomes 3A, 4B, 5A, 6A, 7B, and 7D. All the five pairs of epistatic effects reduced the plant height. In rice, Li *et al.* (2003) identified ten epistatic QTL pairs for PH. six of the 11 epistatic QTL pairs were exhibited significant *AAijE*

effects and these *AAijE* effects differed greatly in both direction and magnitude across the environments. Mei *et al.* (2005) detected seven epistatic QTLs affecting plant height in two different populations of rice. Zhao *et al.* (2009) detected 11 QTLs and 23 digenic interactions for plant height and its components, and mentioned that both additive and epistasis effects are involved in the inheritance of plant height in rice. In Maize, Qiu *et al.* (2007) detected five QTL pairs for PH, contributing from 4.62 % to 11.81 % of the variance.

Epistatic effects for wilting score (WS)

Leaf rolling is an interesting adaptation to conserve internal water by reducing transpiration losses. Inability of this process may result in leaf wilting and death of leaves because of failure to cope with the transpiration demands of plants (Blum 1988). The genetics of leaf wilting seems complex but it offers a straightforward determination of drought tolerance in plants and therefore can be used in large scale screening as a criterion of drought tolerance (Clarke 1986). The wild barley (*Hordeum vulgare* ssp. *spontaneum* C. Koch) is adapted to drought environments. The expectation of that exotic genotype has genes or QTL alleles for drought tolerance (Suprunova *et al.* 2007; Nevo and Chen 2010) has become true. In this investigation, the adaptive parent (ISR42-8) showed significantly lower WS than Scarlett under drought condition. Only two significant epistatic QTL pairs were found for WS and located on chromosomes 1H, 3H and 6H. The epistatic interaction for WS presented additive role of exotic alleles in the development of tolerance against drought. Here, the elite allele seems dominant and therefore, the homozygous exotic alleles were responsible in reducing WS. The results indicate that the alleles of the exotic parent (ISR 42-8) had favorable effects to reduce leaf wilting in S42 population.

Epistatic effects for number of tillers/plant (TILS)

The number of productive tillers per plant plays an important role in the formation of grain yield in cereals. Tiller number per plant is a quantitative trait with a relatively low heritability of 29.8-49.6% (Xiong 1992). The genetics of final tiller number at the maturity stage have been well documented by traditional statistical analysis. In the present study, the adaptive parent (ISR42-8) had significantly a larger number of tillers than Scarlett under drought condition. reported the role of the additive by additive interaction in the inheritance of tiller number. The QTL analysis revealed one significant epistatic QTL pair (*bPb-*

*6676*bPb-2225*) for TILS. Here, the exotic allele seems dominant and therefore, the homozygous exotic alleles were responsible in increasing TILS. The results indicate that the alleles of the exotic parent (ISR 42-8) had favorable effects to increase tillers number in S42 population. Murai and Kinoshita (1986) considered the additive gene effects to be more important than the non-additive effects, whereas Perera *et al.* (1986) suggested that both the number of tillers at maturity and the number of panicles per plant were controlled by genes with additive, dominant, and epistatic effects. Xing *et al.* (2002) detected eight digenic interactions for the number of tillers per plant, involving 16 loci distributed on seven chromosomes in rice.

Epistatic effects for shoot dry weight (SDW)

In present study, both parents produced approximately the same quantity of shoot dry weight under drought conditions, while the exotic parent had higher SDW than Scarlett under control. The epistasis analysis revealed three pairs of epistatic QTLs which were associated significantly with SDW and had positive effects in increasing shoot dry weight. The BC_2DH lines carrying the *Hsp*/*Hsp* genotype at these loci had higher weight than lines with the allelic combination *Hv*/*Hv*. Highly positively significant correlation between SDW and TILS was observed in this study. The result suggests that the increasing in SDW in BC_2DH lines having the alleles *Hsp/Hsp* might due to the increasing tiller number. In rice, Liang *et al.* (2010) have identified seven pairs of epistatic QTLs affected dry matter accumulation (DMA) in the total of the plants of wheat. Under P-deficiency condition in rice Li *et al.* (2009) detected 3 pairs of epistatic QTLs for shoot dry weight (SDW), which explained 4.15%, 3.10%, and 6.89% of the trait variation, respectively. In Maize, Qiu *et al.* (2007) detected two pairs of epistatic loci in SDW and TDW involved two intervals both having a significant putative QTL, and eight epistatic QTL involved one interval having a significant putative QTL.

Epistatic effects for number of kernels/spike (KERS)

The epistasis analysis revealed three pairs of digenic epistatic QTLs were associated significantly with KERS. Two pairs of them were affected positively on KERS. At both loci, the BC_2DH lines having the *Hsp/Hsp* genotype were higher KERS with value up to 5,70% than lines with the allelic combination *Hv/Hv*. This result suggests that *additive* by *additive* effects contributed significantly to the inheritance of kernels per spike in this population. This

result is in agreement with results of Sharma *et al*. (2002) they explained that epistatic effects were present in the inheritance of spikelets per spike.

Epistatic effects for harvest index (HI)

Two significant digenic interactions effects (epistatic QTLs) were identified for HI in this study. Contrast effects have been observed for both pairs. Since, at epistatic locus (*bPb-4577*VrnH1*), the BC_2DH lines having the *Hsp*/*Hsp* genotype were higher HI than lines with the allelic combination *Hv*/*Hv*. The opposite case the epistatic locus (*HvGI*bPb-4389*). The result showed additive gene effects determined the inheritance of harvest index.

Epistatic effects for root traits

Eleven digenic epistatic interactions were detected for root dry weight and root shoot ratio. Six pairs out of them showed QTL by marker interaction Highly positive correlation between RDW and RSR has been observed in this study. Ten digenic epistatic interactions effects were acted positively in increasing both traits. Since, the BC_2DH lines carrying the *Hsp/Hsp* genotype at these loci had higher RDW and RSR than lines with the allelic combination *Hv/Hv*. The result considers the additive gene effects to be more important in the inheritance of root traits. Li *et al*. (2005) detected three and four pairs of epistatic QTLs for RDW and RSR respectively.

Epistatic effects for Relative water content (RWC)

Leaf relative water content (RWC) has been proposed as a more important indicator of water status than other water potential parameters under drought stress conditions (Carter and Patterson 1985; Sinclair and Ludlow 1985). Maintenance of higher relative water content has been suggested as screening criterion for drought resistance (Matin *et al.* 1989; Ritchie *et al.* 1990). In the present study, the epistasis analysis revealed only one significant epistatic QTL pair (*bPb-6676*bPb-2225*) was detected for RWC and distributed on chromosomes 5H and 2H respectively and was the same QTL pair which was identified for TILS. The BC_2DH lines having the *Hsp*/*Hsp* genotype were higher in RWC with percentage of 0.25% than lines with the allelic combination *Hv*/*Hv*. kumar and Sharma (2007) studied the genetic of excised-leaf water loss and relative water content in bread wheat under rainfed and irrigated conditions, they confirmed the importance of existance of both of digenic interactions

(*additive* × *additive*) and (*dominance* × *dominance*) for RWC under irrigated conditions, while *additive* × *dominance* is important for RWC under rainfed conditions. Schonfeld *et al.* (1988) reported additive, dominance as well as additive x additive genetics effects for RWC in wheat. Ahmad *et al.* (2009) reported additive, dominance and interactions for morpho-physiological traits in cotton.

Epistatic effects for Osmotic potential (OP)

Osmotic potential (OP) is a component of osmotic adjustment (OA), and the later is defined as a decrease of osmotic potential within the cells, due to solute accumulation during a period of declining leaf water potential (Ludlow and Muchow 1990). Osmotic adjustment to water stress has been identified as an important physiological mechanism contributing to improved adaptation in a number of crop species grown under water-limited conditions (Ackerson *et al.* 1980; Morgan 1980; Ludlow and Muchow 1990, 1992). At low soil moisture, OA maintains cell turgor, permits survival and maintenance of vital processes and contributes to increase yield and yield stability (Santamaria *et al.* 1990) and can sustain root growth (Reynolds *et al.* 2008) under drought. It has been claimed that growth and yield under water-limited conditions can be improved by selecting for lines with higher levels of osmotic adjustment in wheat (Morgan 1980), sorghum (Ludlow and Muchow 1990, 1992), and barley (Blum, 1989). Two pairs of epistatic QTLs (*HvGI*bPb-3427* and *bPb-0202* bPb-8283*) were associated significantly with OP, and mapped on chromosomes 3H, 6H and 7H. All loci had positive epistatic and favorable effects on OP; they showed an decreasing in OP by increasing osmolality. This means, the contribution of the two types of exotic alleles (recessive genes) decreased the osmotic-potential values and the BC_2DH lines carrying the *Hsp*/*Hsp* genotype were accumulate more particles and small molecules than lines with the allelic combination *Hv*/*Hv*. Teulat *et al.* (1998) detected two chromosomal regions for osmotic potential (OP) and could be considered as regions controlling OA, these regions were present on chromosome 1 (7H) and chromosome 6 (6H).

The epistasis analysis demonstrated that epistatic interactions play an important role in shaping shoot, root and physiological performance in BC_2DH population of barley. Our results suggest that some of the additive QTLs may be detected with effects confounded by epistatic effects, if the epistatic effects were ignored in QTL mapping. Thus, breeders have to

take into account such complexity and examine the effects of individual loci in the targeted genetic background to obtain the expected phenotypes of the interested genes.

4.7 Conclusion

The identification of genomic regions associated with drought tolerance would enable breeders to develop improved cultivars with increased drought tolerance using marker-assisted selection. In addition, the expression of QTLs can be measured under various drought stress treatments. However, QTL × environment interaction can hamper the utilization of closely linked markers for genetic improvement. Detection of single QTLs in classical QTL mapping methods is compromised by linked and interacting QTLs. This problem can be mitigated to some extent by fitting multiple QTL models involving epistatic interaction and QTL × environment interaction (e.g. Baierl *et al*. 2006; Manichaikul *et al*. 2009). The advanced backcross quantitative trait locus (AB-QTL) analysis has proven its usefulness to identify and localize favorable alleles from exotic germplasm and to transfer those alleles into elite varieties. Several reports on the application of the AB-QTL strategy are available for tomato (Fulton *et al*. 2002) and rice (Brondani *et al*. 2002), maize (Ho *et al*. 2002), wheat (Huang *et al*. 2003) and barley (Pillen *et al*. 2003). However, the potential use of the wild germplasm for the improvement of agronomic traits is different between crop species. For example, favorable exotic alleles were responsible for increasing the tomato yield by 50% (Gur and Mazir 2004). The rice yield has increased by 18% due to the introgression of the favorable exotic alleles (Xiao *et al*. 1998). While the effects of wild-type QTL alleles on yield were less pronounced in maize, wheat and barley but still reached levels of 11%, 15% and 7%, respectively (Pillen *et al*. 2004). In current study, we have utilized the exotic parent ISR42-8 (*Hordeum vulgare* ssp. s*pontaneum* C. Koch) as a source of drought tolerance as well as to identify favorable QTL alleles from the wild barley donor which improve the respective shoot, root and physiological traits under drought conditions. The QTL analysis revealed six QTLs were identified for GY and located on chromosomes 2H, 3H and 6H. At All loci the exotic alleles resulted in yield reductions with a maximum -18.77%. This result has been confirmed by Wang *et al*. (2010) and Saal *et al*. (2010) as well as with the fact that 'Scarlett' is a spring cultivar with high yield performance.

A variety of factors may affect the outcome of a QTL analysis. For example, the selection of the cross, population structure and size, number of measured replications and

environments and type, number and density of markers (Pillen *et al*. 2003). In addition, the selection of the statistical method exerts a major impact on the results of a QTL experiment. In this work, we used a multiple QTL model iteratively extended and reduced by forward selection and backward elimination, respectively, using the PROC MIXED procedure in SAS software (SAS version 9.2, SAS, 2008). The forward selection strategy (REML forward selection approach) is very effective to detect QTLs influencing the interested traits (Bauer *et al*. 2009). However, as expected, the forward selection analysis seems to be more powerful for QTL mapping. Since the markers with the most significant effect in previous estimation rounds are included as fixed cofactors in the statistical model of the next estimation cycle, similar to composite interval mapping, the forward selection approach accounts for multiple marker loci in the analysis (Bauer *et al*. 2009). In each round of the forward selection process, the selection of the most significant and informative marker was added as a fixed factor (QTL) into the model according to the F value with the probability of false discovery rate (FDR $\leq$ 0.05) and then all remaining markers were scanned with the respective model containing the previously found QTLs. The process of the following iterations of this model was continued until no more additional QTL could be detected. Therefore, the detection of QTL for studied traits by using REML forward selection approach and false discovery rate is very restricted.

In order to conduct the AB-QTL analysis, a doubled haploid population consists of 301 lines has been used. The S42 population is considered to be one of the biggest mapping population used in QTL analysis. The strength of a QTL analysis primarily depends upon the size of mapping population and the density of genetic map (Collard *et al*. 2005). Thus, a DH population is an ideal population for AB-QTL analysis because the same genotypes could be tested in different environments and in subsequent years. The genetic background plays a very important role in QTL detection. The population S42 has been genotyped successfully with 106 SSRs, 255 DArT and 10 gene-specific DNA markers in order to perform QTL analysis and this resulted a high resolution genetic map.

A more direct way to exploit novel allelic diversity is to cross elite material with genetic resources of the same genome. Wild barley H. vulgare ssp. spontaneum accession (ISR 42-8) has been originated from abiotically stressed environments. The current study has demonstrated that wild barley *H. vulgare* ssp. *spontaneum* does harbor favorable alleles, which have the potential to improve quantitative shoot, root and physiological traits and can

enrich the genetic basis of cultivated barley. In this study, the wild parent contributed the beneficial alleles for 27 (34.1%) out of 79 QTLs that affected shoot, root and physiological including with exception of grain yield and relative water content. The favorable QTL alleles were located mainly on chromosomes 1H, 2H, 4H, 5H and 7H. Novel exotic alleles with a favorable effect on some drought-adaptive traits such as root characteristics and proline content. For instance, the presence of exotic alleles at marker locus *VrnH1* [5H] led to increase root length by 9.17 % under drought conditions. This result indicate that the introgression from wild barley may increase root length in S42 population. For proline accumulation, the QTL ‘*’QPC.S42.5H*’’, the exotic alleles at marker locus MGB338 [5H] revealed favorable effects on increasing proline content (PC) under drought conditions. This QTL may be useful as a target for crop drought tolerance improvement via marker-assisted selection. Babu *et al.* (2003) proposed yield improvements in water-limited environments could be achieved by identifying secondary traits contributing to drought resistance and selecting for those traits within a breeding program. Although, the QTL analysis revealed 55 QTLs for shoot traits (PH, WS, TILS, SPS, SDW, KERS, TGW, GY and HI). Out of them 17 (30.9 %) QTLs were found to be associated with favorable exotic alleles effects. These secondary traits are strongly correlated with grain yield; therefore these QTLs may be useful as a target for crop drought tolerance improvement via marker-assisted selection. Favorable exotic alleles were identified for yield component traits including number of tillers per plant, number of spikes per plant, shoot dry weight, number of kernels per spike and harvest index. However, most of these QTLs were mapped in particular on the short arm of chromosome 2H and the long arm of chromosome 4H. These secondary traits are strongly correlated with grain yield; therefore these QTLs may be useful as a target for crop drought tolerance improvement via marker-assisted selection. The identification of markers linked to the favorable QTL alleles as well as the advanced backcross population structure employed in this study will allow us to rapidly isolate these QTLs in NILs.

5 Summary

Cultivated barley (*Hordeum vulgare* ssp. *vulgare* L.) is an established model species for genetic and physiological studies (Koorneef *et al*. 1997). It is a convenient experimental organism because: (1) is an annual with a short life cycle; (2) it is diploid with only seven pairs of chromosomes; (3) it is true breeding allowing multiple testing; (4) it exhibits wide diversity in physiology, morphology and genetics; (5) a wide range of genetic stocks is available; and (6) it has well-defined genetic maps. Barley is also an important cereal crop species ranking fourth after rice, wheat and maize. The improvement of abiotic stress tolerance in the barley crop (Robinson *et al*. 2000) depends on understanding the range of genetic variation possessed by cultivated barley and its wild ancestor (*H. vulgare* ssp. *spontaneum* C. Koch.). The main objective of the present study was to identify favorable exotic QTL alleles for the improvement of drought tolerance via shoot, root and physiological traits in the BC2DH population and that can enrich the genetic basis of cultivated barley.

A double haploid mapping population containing 301 BC_2DH lines was used for QTL analysis. This population was designated as S42 and has been derived by hybridization of the German spring barley cultivar Scarlett (*H. vulgare* ssp. vulgare L.) with the exotic accession ISR42-8 (*H. vulgare* ssp. *spontaneum* C. Koch) originating from Israel. The development of the BC_2DH population was according to von Korff *et al.* (2004). The population S42 was genotyped with simple sequence repeats (SSRs), diversity array technology (DArT) and gene-specific marker systems. A linkage map of 371 genetic markers has been established that contains 106 SSRs, 255 DArT and 10 gene-specific DNA markers. The SSRs markers and gene-specific markers were according to von Korff *et al.* (2004) and Wang *et al*. (2010), respectively. The chromosomal positions of the DArT markers (Diversity Array Technology, www.diversityarrays.com) are according to Wenzl *et al.* (2006). By using DArT, SSR and specific genes positions, the linkage map has been drawn by using MapChart ver.2.2 (Voorrips 2002).

The genotyped markers were distributed over all seven chromosomes and covered 1154.31 cM of the barley genome in this population with an average of 164,90 cM. The average distance between markers was 3.20 cM. However, the chromosome 7H had largest number of markers (67 markers), while the chromosome 4H had the smallest number (40 markers) of markers, the distribution of DArT markers ranged from 20 to 47 with an average of 36.43, while the distribution of SSR markers ranged from 11 to 20 with an average of

16.57. Only two gaps (> 20 cM) were observed on chromosomes 2H and 3H. 21 gaps (> 10 cM) were observed in this population and distributed on all chromosomes with an average 3 gaps per chromosome except chromosome 7H had no gaps exceeded 10 cM.

The population S42, which consists of 301 BC_2DH lines and their parents (Scarlett and ISR 42-8), were tested for tolerance to drought; this investigation was done under control and drought stress conditions in three successive summer seasons (2007, 2008 and 2009). The experiments were arranged in a split-plot design where BC_2DH lines and parents have been assigned randomly. 15 quantitative traits were investigated for drought tolerance and grouped in nine shoot traits (PH, WS, TILS, SPS, SDW, GY, KERS, TGW and HI), three root traits (RL, RDW and RSR) and three physiological traits (RWC, PC and OP). The marker by trait associations were carried out using multiple QTL model iteratively extended and reduced by forward selection and backward elimination, respectively, using the PROC MIXED procedure in SAS software (SAS version 9.2, SAS, 2008). The REML forward selection approach is very effective to detect QTLs influencing the interested traits (Bauer *et al*. 2009).

In present study, components of variance revealed a wide range of variability for most of the traits. High significant differences between Scarlett and ISR 42-8 were detected for all investigated traits except osmotic potential (OP) and no. of spikes/plant (SPS). As a logical consequence, the parent Scarlett was exhibited high significant differences and superior in the yield and its attributes such as GY, KERS, TGW and HI, while the wild accession ISR 42-8 showed significant differences and superior in the vegetative and root traits such as PH, WS, RL, RDW, RSR and RWC. The elite parent Scarlett was higher in PC than the exotic parents. For the variation with population S42, high significant differences were detected among BC_2DH lines and between control and drought treatments in the majority of studied traits. The interaction between BC_2DH lines and treatments was highly significant in most cases.

The parents, Scarlett and ISR42-8 showed a significant variation for most investigated traits that segregate in BC_2DH population, thus indicating the suitability of this population for the QTL analysis of selected traits. In total, 79 putative QTLs for 15 studied traits were detected among 5,565 marker by trait combinations in the population S42 under study. They can be divided into 55 QTLs for shoot traits, 15 QTLs for root traits and 9 QTLs for physiological traits. Out of 79 putative QTLs, 72 QTLs were significant as marker main effects, 4 QTLs were significant as marker by treatment interaction effects and 3 QTLs had both effects. The number of QTLs for each trait ranged between one and nine QTLs. 16

common QTLs have been found to be governing different traits and covered the whole genome of S42 population except chromosome 6H. The highest number of the common QTLs was found on 2H (five QTLs) followed by chromosome 4H (4 QTLs). Overall 27 (34.1 %) QTLs showed favorable effects derived from the presence of exotic alleles. Most of putative QTLs were located on chromosomes 1H, 2H, 4H and 5H by one, seven, eight and one QTL for each respectively. However, most of favorable effects of the *Hsp* alleles were detected on chromosomes 2H and 4H. Out of 55 QTLs only 17 (30.9 %) QTLs for shoot traits were identified with favorable effects of the exotic alleles. Nine (60 %) QTLs out of fifteen were detected for root traits with favorable effects of the exotic alleles. Two (22.2 %) QTLs were detected for physiological traits favorable effect of the exotic alleles.

Numerous interesting QTLs were detected in this study that displaying beneficial effects of the exotic alleles. For instance, two QTLs (QWS.S42.1H and QWS.S42.4H) had favorable effects due to the presence of the exotic alleles (Hsp) that were responsible for decreasing plant wilting score by 17%. The SSR markers GMS3 $_{[2H]}$, HvNAM2 $_{[2H]}$ and M1o $_{[4H]}$ were associated with QTLs influencing number of tillers/plant and number of spikes/plant. These QTLs are likely to be dominating both traits and the introgressions from wild barley may increase number of tillers/plant and number of spikes/plant in S42 population. Also for root length, the vernalization gene *VrnH1* $_{[5H]}$ was associated significantly with the .QTL (*QRL.S42.5H*). The presence of exotic alleles at this marker locus led to increase root length by 9.17 % under drought conditions. This result indicates that the introgression from wild barley may increase root length in S42 population. For proline accumulation, the superior performance of exotic allele at marker locus MGB338 on chromosome 5H suggests a transgression effect of the exotic alleles and led to increase proline content BC_2DH lines carrying *Hsp* alleles by 53% under drought conditions.

Altogether 33 pairs of digenic epistatic QTLs as *additive* × *additive* effects were detected for nine studied traits related to drought tolerance in S42 population. Among them, eleven pairs displayed QTL by marker interaction and twenty two displayed marker by marker interaction. It will be interesting to study the relationships between additive QTLs and epistatic QTLs identified. Only 33% of main-effect QTLs for shoot, root and physiological traits were involved in epistatic effects. This indicates that several loci involved in epistatic interactions may not have significant effects for these traits and may affect the trait expression by epistatic interactions with other loci. Similarly, Ma *et al.* (2007) observed that 37% of the

main-effect QTLs were involved in the epistatic interactions in maize grain yield and its components. Zhang *et al.* 2008) found 25% of main-effect QTLs for wheat plant height were involved in epistatic effects. Our results suggest that some of the additive QTLs may be detected with effects confounded by epistatic effects, if the epistatic effects were ignored in QTL mapping. Thus, breeders have to take into account such complexity and examine the effects of individual loci in the targeted genetic background to obtain the expected phenotypes of the interested genes.

Interesting, this exotic QTL allele responded favorably under drought conditions only that indicates the possibility of underlying a novel drought inducible gene. The majority of the digenic epistatic interaction pairs which were detected in current study had favorable effects on the phenotypic values of the studied traits which showed epistatic interactions. This study has highlighted the role of the exotic alleles for the detection of favorable leads for drought tolerance. Subsequently, a combinatory approach for the selection of favorable exotics alleles can be employed to develop a better shield against the adverse effects of drought.

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7 Abbreviations

Abbreviation	Explanation
A×*A*	*Additive* × *Additive* interaction effect (Epistasis)
AB	advanced backcross
AFLP	Amplificated fragment length polymorphism
ANOVA	Analysis of variance
BC_2DH	Backcross (second generation)-doubled haploid
CIM	Composite interval mapping
cM	centiMorgan
cm	centimetre
DArT	Diversity array technology
DH	Doubled haploid
DNA	Deoxyribonucleic acid
EST	Expressed sequence tag
et al.	*et al*eri
F_2	Second generation after a cross
FDR	False discovery rate
GY	Grain yield per plant (g)
HI	Harvest index (%)
Hsdr4	Gene of *H. spontaneum dehydration responsive*
Hsp	*Hordeum spontaneum*
Hv	*Hordeum vulgare*
Hz	hertz
ISR	ISR 42-8 (a wild accession of barley)
KER	Number of kernels per plant
M	Marker main effect
M*T	Marker- treatment interaction
MAS	Marker-assisted selection
mm	millimetre
NIL	Near isogenic line
OA	Osmotic adjustment
OP	Osmotic potential (osmol/kg)
PC	Proline content (µmol proline/g DW)
PCR	polymerase chain reaction
PH	Plant height (cm)
Pos.	position
Ppd	Gene associated with photoperiod (flowering)
QE	QTL × environment interaction

QTL	Quantitative trait locus
R^2	Coefficient of determination
RAPD	Random amplified polymorphic DNA
RDW	Root dry weight (g)
REML	Restricted maximum likelihood method
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
RL	Root length (cm)
RNA	Ribonucleic acid
RP[*Hsp*	Relative performance of the exotic genotype (ISR 42-8)
rpm	random per minute
RSR	Root/shoot ratio (%)
RWC	Relative water content (%)
S42	‘Scarlett × ISR 42-8‘ population of barley
SAS	Statistical Analysis System software
SCA	Scarlett (a german elite cultivar of barley)
SDW	Soot dry weight (g)
sdw	Gene associated to semi-dwarf
SIM	Simple interval mapping
SNP	Single/simple nucleotide polymorphism
SPS	Number of spikes per plant
SS	Sum of squares
ssp.	subspecies
SSR	Simple sequence repeat
T42	‘Thuringia x ISR42-8’ population of barley
TILS	Number of tillers per plant
Tris	2-Amino-2 (hydroxymethyl)-1,3-propandiol
Vrn	Gene associated with vernalisation requirement
Vrn	Gene associated with vernalisation requirement
WS	Wilting score
µl	microliter

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