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# Association mapping for drought stress related traits in a structured population with wild and cultivated barley

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**Abstract (in Deutsch)**

Assoziationskartierung ist eine Alternative zur Kartierung in einer bi-parentalen Population. Schlüssel zu einem erfolgreichen Mapping ist es, übergeordnete Verbindungen durch Kontrolle der Populationsstruktur und der Verwandtschaftsbeziehungen zu berücksichtigen.

In dieser Studie wurde eine strukturierte Gerstenpopulation aus Wild- und Kulturgersten genutzt (98 Wildgerstenakzessionen *H. vulgare ssp. spontaneum* aus einer Core-Collection und 21 deutsche Sommergerstensorten). Die Experimente wurden während der Sommervegetationszeiten der Jahre 2007 und 2008 in Kunststofffolientunneln am Standort der Universität Bonn-Poppelsdorf (Nordrhein-Westfalen, Deutschland) durchgeführt. Die Gefäße wurden in einer Spaltanlage ohne Wiederholungen arrangiert; die Tropfbewässerungsvarianten waren „Voll Bewässert“ und „Trockenstress“ mit etwa der Hälfte der Wassermenge in einer Periode von 21 Tagen angefangen 40 Tage nach der Saat. Danach (vor der Blüte) wurden die Pflanzen analysiert und phänotypische Daten von insgesamt 18 Trieb-, Wurzel- und physiologischen Merkmalen erhoben. Genotypisiert wurden die 119 Akzessionen mit 1081 DarT-Markern. Die Assoziationsanalyse wurde unter Einbeziehung der Q- und K-Matrix zur Berücksichtigung von Populationsstruktur und Vewandtschaftsbeziehungen mittels eines Mixed-Linear-Model (MLM) durchgeführt.

Die Triebmerkmale Welkegrad (WS), Triebfrischgewicht (SFW), die Wurzelmerkmale Wurzellänge (RL), Wurzelfrischgewicht (Gesamtwurzelfrischgewicht RFW, Teilwurzelfrischgewicht 0-10cm Wurzellänge FWa, FWb 10-20cm Länge, FWc größer 20cm Länge) und Wurzeltrockengewicht (RDW, DWc) sowie die physiologischen Mermale Relativer Wassergehalt (RWC) und Prolingehalt (PC) wiesen höchst signifikante Differenzen zwischen beiden Bewässerungsvarianten in beiden Jahren auf.

In der Assoziationsanalyse waren 79 Marker signifikant mit allen untersuchten Merkmalen korreliert. Sie fanden sich über das gesamte Genom der strukturierten Gerstenpopulation verteilt. Verschiedene QTLs für verschiedene Trieb-, Wurzel- und physiologische Merkmale wurden identifiziert. Sie zeigten Haupt- und / oder Interaktionseffekte, die die Merkmale unter „Voll Bewässert“ und „Trockenstress“ sowohl verbesserten als auch reduzierten.

Es wurden 30 Co-Lokationen von QTLs gefunden, von denen 18 Regionen mit zwei Merkmalen, 6 Regionen mit 3 Merkmalen und 6 Regionen mit mehr als 3 Merkmalen assoziiert waren.

Die wichtigsten Co-Lokationen waren bpb-3574 auf Chromosom 2H (49.03 cM) assoziiert mit RL und RWC, bpb-2910 auf Chromosom 3H (51.59 cM) assoziiert mit RWC und Gesamttriebtrockengewicht (SDW) und bpb-1408 auf Chromosom 4H (60.04 cM) assoziiert mit WS, RL, FWc, RWC and PC.

**Abstract (in English)**

Association mapping is an alternative to mapping in a biparental population. A key to successful association mapping is to avoid superior associations by controlling the population structure and the kinship relations.

A structured population of 119 wild and cultivated barley genotypes (98 accessions of wild barley *H. vulgare ssp. spontaneum* from a core collection and 21 german spring barley cultivars) was used in this study. The experiments were carried out in plastic green house tunnels at Bonn University (Nord-Rhine-Westfalia, Germany) during the summer seasons 2007 and 2008. Pots were arranged in a split-plot design with non-replications; drip irrigation treatments were “well-watered” and “drought stress” with about half of the water amount in a period of 21 days starting 40 days after sowing. After that (before anthesis) plants were analysed and phenotypic data from in total 18 shoot, root and physiological traits were measured. The 119 accessions were genotyped by using 1081 DArT markers and the association analysis was performed with a mixed linear model (MLM) including Q and K matrix considering the population structure and the kinship relations.

The shoot traits wilting score (WS), shoot fresh weight (SFW), the root traits root length (RL), root fresh weight (total fresh weight RFW, FWa 0-10cm length, FWb 10-20cm length, FWc greater 20cm length) and root dry weight (RDW, DWc) and the the physiological traits relative water content (RWC) and proline content (PC) exhibited highly significant differences between the two irrigation treatments “well-watered” and “drought stress” in both seasons.

In the association analysis 79 markers were significantly correlated with the studied traits covering the whole genome of the structured Barley population. Different QTLs have been identified for different shoot, root and physiological traits. They are located all over the whole barley genome. These QTLs had main and / or interaction effects on improving or reducing the traits under well-watered and drought stress conditions.

Thirty co-locations of QTLs were found correlating with the studied traits covering the whole genome of the tested Barley population. Among these co-locations 18 regions were found to be associated with two traits, six co-locations with three traits and six co-locations were affected by more than three traits.

The most important co-locations which have been obtained in the current study were bpb-3574 on chromosome 2H (49.03 cM) associated with RL and RWC, bpb-2910 on chromosome 3H (51.59 cM) associated with RWC and total shoot dry weight (SDW) and bpb-1408 on chromosome 4H (60.04 cM) associated with WS, RL, FWc, RWC and PC.

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## 1. Introduction, objectives and Literature review

Association mapping of a trait is an approach to identify chromosomal regions that contain genes affecting the trait. The discovery of dense polymorphic markers covering the entire genome provides us an opportunity to localize these regions by determination the markers closest to the genes of interest.

### 1.1 Introduction

For the past decade, there has been success in using conventional map-based strategies in identification and cloning of quantitative trait loci (QTL) in model plant species including tomato and arabidopsis. These quantitative traits are generally the products of many loci with varying degrees of effect upon the observed phenotypes. Recently a new approach to genetic mapping has emerged called association mapping. This new technique takes into account the thousands of genes to evaluate for QTL effect and is a more efficient approach that does not require generation of segregating populations/large numbers of progeny. As it can utilize all of the historic recombination events in a diverse population of individuals it can generate higher resolution genetic maps and, is needed to complement current map based cloning methods.

Association analysis in plants provides both basic and advanced understanding of association mapping and an awareness of population genomics tools to facilitate mapping and identification of the underlying causes of quantitative trait variation in plants. It acts as a useful review of the marker technology, the statistical methodology, and the progress to date. It also offers guides to the use of single nucleotide polymorphisms (SNPs) in association studies. As a complement to traditional linkage studies, association mapping or linkage disequilibrium (LD) mapping offers a powerful alternative approach for fine-scale mapping of flowering time in maize (Thornsberry et al. 2001), yield traits in barley (Kraakman et al. 2004), Iron deficiency in soybean (Wang et al. 2008), and disease resistance in rice (Garris et al. 2003), potato (Gebhardt et al. 2004; Simko et al. 2004) and corn (Szalma et al. 2005). Drought is the major cause of crop yield reduction in the world today. Breeding crops with improved drought tolerance is one approach to alleviate this problem. However, progress towards this goal has been slow because of the complexity of the trait and its quantitative inheritance. Barley is an excellent crop for studies on both the inheritance and physiology of this trait, because it is a diploid (2n = 14) and a predominantly self-pollinated crop.

## 1.2 Literature review

Most of drought traits in plants are quantitative in nature, and controlling by polygenes. These traits have interaction between environments, which is makes, the inheritance of these traits more complicated and difficult to understand. The procedure which identify the association between the marker close proximity to genetic factors affecting quantitative traits (QTL) and traits and analyzing their magnitude of genetic effect and interaction with treatments as well as drought conditions, are called association mapping, the following reviews contain the drought stress mechanism, genetic markers, linkage disequilibrium, and association analysis.

### 1.2.1 Barley

Barley is characterized by being relatively high drought tolerance, where it can grow with lesser soil moisture. 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. Traits that have been investigated include physiological/biochemical and developmental/morphological traits. Yield performance under drought is particularly a complex phenomenon, and plants exhibit a diverse range of genetically complex mechanisms for drought resistance (Baum et al. 2007).

Barley is a diploid (2n = 14) and a predominantly self-pollinated crop. Consequently, its variation is structured in true breeding lines. Hundreds of modern varieties and thousands of landraces are known.

### 1.2.2 Differences between wild barley and cultivated barley

The wild form *H. vulgare* ssp *spontaneum* grow in open habitats with comparatively low competition from other species (Von Bothmer 1992). Wild barley is distributed over the eastern Mediterranean area and in Southwest Asia across a wide range of climates and soils. It is particularly common in the Near East Fertile Crescent (Zohary 1969). In general, wild barley is not tolerant to extreme low temperatures and is rarely found above 1500 m altitude. However, it is more drought resistant than the wild wheat and penetrates relatively deeply into the warm steppes and deserts (Zohary & Hopf 1988).

Wild barley and cultivated 2-rowed barley have quite similar morphology. The most notable differences are wild barley’s brittle rachis and its hulled grain. Six-rowed barley

has evolved during domestication, the trait being controlled by a single gene on chromosome 2 (Komatsuda et al. 1999, Tanno et al. 2002). Wild barley subspecies *spontaneum* is the only wild *Hordeum* species that can produce fully fertile hybrids (with normal chromosome pairing and segregation in meiosis) when crossed with cultivated barley. Hybrids can also be formed in nature when these two occur at the same location (Asfaw & Von Bothmer 1990).

Studies with wild and cultivated barley have shown that there is more variation within the wild than in the cultivated barley (Saghai Maroof et al. 1995), although in some cases the opposite has been reported for some isozymes and mitochondrial DNA (Nevo, 1992). The larger genetic variation within wild barley gives the opportunity to use this variation for breeding purposes.

### 1.2.3 Wild barley

Wild barley 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. *Hordeum vulgare* ssp*. spontaneum* (wild barley) is the ancestor of cultivated barley. It belongs to the poaceae-family of grasses and within it to the triticeae-tribe. *Triticeae* is a temperate plant group mainly concentrated around central and South-eastern Asia, although the species belonging to it are distributed around the world. *Triticeae* includes many economically important cultivated cereals and forages but also about 350 wild species. The wild species are of great interest as potential gene donors for commercial breeding (Vanhala 2004).

Wild ancestry: The wild ancestor of the cultivated barley is well known. The crop shows close affinities to a group of wild and weedy barley forms which are traditionally grouped in *Hordeum spontaneous* C. Koch, but which are in fact, the wild race or subspecies of the cultivated crop. The correct name for this wild is therefore *H. vulgare* L. ssp. *spontaneum* (C. Koch). These are annual, brittle, two-rowed, diploid (2n = 14), predominantly self-pollinated barley forms and the only wild Hordeum stock that is cross compatible and fully interceptive with the cultivated barley, *vulgare* x *spontaneum* hybrids show normal chromosome pairing in meiosis (von Bothmer 1992). Also

morphologically, the similarity between wild spontaneous and cultivated two-rowed distichal varieties is rather striking. They differ mainly in their modes of seed dispersal. Spontaneous ears are brittle and maturity disarticulates into individual arrow-like triplets. These are highly specialized devices, which ensure the survival of the plant under wild conditions. Under cultivation this specialization broke down and non-brittle mutants were automatically selected for in the man-made system of sowing, reaping and threshing (Harlan and Zohary 1966, Zohary 1969).

The development of new barleys tolerant of abiotic and biotic stress is an essential part of the continued improvement of the crop. The domestication of barley, as in many crops, resulted in a marked truncation of the genetic variation present in wild populations. This process is significant to agronomists and scientists because a lack of allelic variation will prevent the development of adapted cultivars and hinder the investigation of the genetic mechanisms underlying performance. Wild barley would be a useful source of new genetic variation for abiotic stress tolerance if surveys identify appropriate genetic variation and the development of marker-assisted selection allows efficient manipulation in cultivar development, there are many wild barley collections from all areas of its natural distribution, but the largest are derived from the Mediterranean region (Ellis et al. 2000).

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

### 1.2.4 Barley breeding

Barley is grown for fodder, human consumption, and the brewing of beer and whisky. The main breeding objectives are high yield, and resistance to biotic and abiotic stresses. Furthermore, malting cultivars need to have high malting quality, which includes plump kernels, rapid and uniform germination, and optimal values for protein content and enzymatic activity (Kraakman 2005).

Barley lines are almost completely homozygous. $F_1$-hybrids are produced by emasculation of the female parent and adding the pollen of the male parent one to three days later to the bagged female spike. The $F_1$ can be developed into inbred lines by self-fertilization, but also by the production of doubled haploids (DH). The most frequently applied techniques to obtain DHs are the bulbosum method (Kasha and Kao 1970), and the anther culture (Friedt and Foroughti-Wehr 1981). DHs are a fast road to homozygosity, but selection is only possible after the DHs have been created. Selfing is time consuming, as at least 7 or 8 cycles of selfing are necessary to reach homozygosity, but in the later stages of this process many inadequate lines can be discarded already.
Resistance to biotic and abiotic stresses has been the main area of study on the phenotypic variation in wild barley, as these are important targets for improvement in barley breeding (Ellis et al. 2000).

### 1.2.5 Development of detecting QTLs for abiotic stress tolerance in barley

In barley and many other crops, greater variation to abiotic stresses exists in primitive landraces and related wild species gene pools. Wild barley (*Hordeum spontaneum* C. Koch) is the progenitor of cultivated barley (*Hordeum vulgare* L.) and is easily hybridized with *H. vulgare*. The processes of domestication and selection have resulted in a drastic narrowing of the genetic variation of crop species (Tanksley and McCouch, 1997), including barley (Powell, 1997). In the recent years breeders have become increasingly interested in exploiting genetic markers: ‘molecular breeding’, ‘accelerated breeding, ‘marker assisted selection’ are terms used to describe new breeding methodologies based on genotypic selection. Once genetic markers can be used to evaluate the variation available to breeders (Forster et al. 2000).

Many of genetic markers have been applied to diversity studies in wild barley. Interesting results came from some of the early work using biochemical markers (Nevo et al. 1992) which showed that wild barley possessed considerably more variation than the cultivated species, and that many alleles are associated with adaptation to specific environments. It has been shown that wild barleys from Israel possess seven isoforms of enzyme β-amylase of which only tow have been found in European cultivars (Chalmers et al. 1992). The association of β-amylase with adaptation to dry environments in wild barley may be due to linkage with variation at the *sh* locus. It may be that, as in cultivars, developmental genes in wild barley have major effects on adaptation and tolerance to abiotic stresses and control of development may provide an avoidance mechanism.

There is specific genetic variation associated with specific environments. The wild barleys used in the AFLP study have been used for responses to a number of abiotic stresses including, salinity, drought, N-starvation, cold, ozone, and day length (Forster et al.1997). Of the AFLPs associated with salt tolerance only three were mappable. Interest has centred on SSRs as a genetic marker system (Tautz and Renz 1984). SSRs are PCR-based markers, which have the advantages of being single locus markers, co-dominant, multi-allelic, and widely dispersed over the genome.

The variation of SSRs in cultivars, landraces and wild barley shows that landrace and wild barley have unique alleles not found in the cultivated gene pool (Powell 1997). The results show the wild barley offers a rich source of genes of enormous potential for crop improvement. Genetic loci known to be involved in the control of specific traits in cultivated barley can now be targeted and investigated in the wild gene pool to seek out novel and rare alleles.

### 1.2.6 Drought stress

Drought stress is the main limited factor of crop productivity; drought like many other environmental stresses has adverse effects on crop yield. Low water availability is one of the major causes for crop yield reductions affecting the majority of the farmed regions around the world. As water resources for agronomic uses become more limiting, the development of drought-tolerant lines becomes increasingly more important (Bruce et al. 2002).

Improving the tolerance of crops to drought compared with other abiotic stresses, requires a broader interdisciplinary approach, involving an understanding of the factors (e.g. availability of water during the crop cycle) determining yield in a particular target population of environments (Collins et al. 2008).

Plant water deficits may occur as a consequence of a seasonal decline in soil water availability, developing in the long term, or may result from drought spells. An increased evaporative demand of the atmosphere occurring mostly on a daily basis, affects total carbon gain by the crops, even irrigated ones. The timing, intensity and duration of stress episodes are pivotal to determine the effects produced by drought. Plant strategies to control water status and resist drought are numerous (Schulze 1986). Consequently, efforts are directed towards a better understanding of the genetic basis of the adaptive response of plants to drought and how best to exploit this knowledge for breeding purposes.

The essence of good drought management is to use this range of responses to best advantage. Five distinct categories of drought affecting crop production in the dry lands, depending on the time of occurrence of drought and general climatic conditions of the region (Hafid et al. 1998).

**a) Early season drought**

The early season droughts occur in association with the delay in commencement of sowing rains. Characterization of early season droughts in any agroclimatic region requires precise information on (1) optimum sowing periods for the different crops and their varieties grown in the region under rainfed conditions, (2) amount of rainfall needed to complete the sowing in a given region and (3) the initial amount of rainfall required for safe germination and establishment of the crop stand to minimize the adverse effect of dry spells immediately after sowing.

**b) Mid-season drought**

Mid-season droughts occur in association with the breaks in the southwest monsoon. If the drought conditions occur during the vegetative phase of crop growth, it might result in stunted growth, low leaf area development, and even reduced plant population. Mid-

season droughts for crops grown under rainfed conditions can be characterized by (1) the relationship between leaf area index and water use of the crop, depending on the water availability to the crop, and (2) the relationship between the actual leaf area index and effective leaf area index of the crop under moisture stress conditions.

**c) Late season or terminal drought**

If the crop encounters moisture stress during the reproductive stage because of early cessation of the rainy season, there may be an increase in temperature, hastening the process of crop development to forced maturity. Therefore, late-season droughts have to be characterized on the basis of the relationship between water availability to the crop during the reproductive stage of crop growth and grain yield.

**d) Apparent drought**

Rainfall in the region may be adequate for one crop but not for others. Therefore, apparent drought conditions are encountered because of mismatching of the cropping patterns to the rainfall/moisture availability patterns in some of the regions.

**e) Permanent drought**

Drought is a recurring feature in arid regions, as it is in virtually all climate regimes. Even the drought-resistant crops grown in these regions are likely to be subjected to moisture stress, even during years with above-normal rainfall. Alternate land use systems have to be introduced in these regions for sustainable agriculture.

### 1.2.7 Drought stress mechanisms

Water deficits result from low rain fall, poor soil water storage and when the rate of transpiration exceeds water uptake by plants. Plants have developed various strategies to acquire stress tolerance. These strategies include changes in metabolic processes, structural changes of membranes, expression of specific genes and production of secondary metabolites.

In genetic sense, the mechanisms of drought resistance can be grouped into three categories, drought escape, drought avoidance and drought tolerance. However, crop plants use more than one mechanism at a time to resist drought (Mitra 2001).

**1.2.7.1 Drought escape:**

Drought escape is the ability of a plant to complete its lifecycle before serious soil and plant water deficits develop. The plants can escape from drought by early flowering and maturity before the stress occur (Turner 1979). Xu et al. (2005) studied QTls for drought escape and tolerance in set of introgression lines of rice, they found twelve main-effects QTL (M-QTLs) for heading date (HD) were identified and mapped to ten rice chromosomes except chromosomes 2 and 11. In addition, five pairs of epistatic QTL (eQTLs) affecting HD were identified including two pairs detected under the irrigated condition, one pair under stress and two pairs by the HD differences across water levels.

**1.2.7.2 Drought tolerance at high water potential (Drought Avoidance)**

The ability of plant to endure periods without significant rainfall, whilst maintaining a higher water status. The plant can withstand the drought stress by either reduce the ***water loss*** in these mechanisms (I) increase in stomatal and cuticular resistance (Cohen 1970, Cowan 1982, Schulze 1986, Dawson &Ehleringer 1993, Meinzer 1993) (II) reduce of radiation absorbed throw rolled leaves (Ehleringer and Cooper 1992) (III) reduce leaf area. and/or increase ***water uptake*** by the roots using two procedures (I) increase root density and length (II) increase liquid phase conductance. (Cruz et al. 1992, Jackson et al. 2000)

**1.2.7.3 Drought tolerance at low water potential**

The ability to endure periods without significant rainfall and to endure low tissue water potentials. In this mechanism the plant can resist the drought stress by reserve ***high turgor*** with Osmotic adjustment, increase elasticity, or small cell size and/or desiccation tolerance by protoplasmic tolerance (Morgan 1984).

**1.2.8 Traits for drought tolerance**

Many traits have been considered for drought tolerance screening that could eliminate the need for field testing in unpredictable environments. Some of these traits include osmotic adjustment, relative water content, water loss rate, and water use efficiency (Rampino et al. 2006, Grony 1999) all of which influence the plant's ability to maintain high turgor and normal growth with less water available.

### 1.2.8.1 Physiological traits

**a) Relative water content (RWC)**

Relative water content is a measure of the amount of water held in the leaves relative to full turgor. Maintaining high water content allows normal growth to occur as water becomes scarce. Relative one way a plant can stay closer to full turgor is to lose water at a slower rate by closing stomata or accumulating insulating wax layers. (Suprunova et al. 2004), furthermore, the aim of breeding drought tolerance is to develop cultivars that have high yield potential under drought conditions instead of merely being able to survive them. Unfortunately, these traits are not always associated with economic yield. Plants that are able to maintain high levels of RWC under drought stress should be less affected by the stress and be able to maintain more normal growth and yield.

Kocheva and Georgiev (2003) were studied the RWC in leaves of barley and found that tow cultivars of barley decreased the RWC with increasing water stress. Teulat et al. (2001) mapped six QTL for RWC on chromosomes 2H (1 QTL), 6H (1 QTL), and 7H (4 QTLs) of barley in a growth chamber study. Three of these were detected under well-watered conditions and three were detected under stress. Only one stress QTL, on the long arm of chromosome 7H is located close to a non-stress QTL. A field study in mediterranean environments in Europe and North Africa tested the consistency of QTL across environments (Teulat et al. 2003). The QTL on chromosomes 2H and 6H in the 2001 study were still detected, but only 6H was consistent across environments in 2003. QTLs for RWC were also mapped in upland rice by Price et al. (2002). They identified QTLs on chromosomes 1, 3, 4, 5, 6, 8, 9, 10, and 11. The QTL on chromosomes 4 and 5 are in homologous locations to those showing QTL by environment interaction on 2H and 1H, respectively, identified by Teulat et al. (2003).

**b) Osmotic Potential (OP)**

Osmotic adjustment (OA) acts to maintain cell turgor pressure by accumulation of solutes to generate a more negative cellular water potential and maintain a favourable gradient of water potential (Ludlow and Muchow 1990). Although OA may influence WUE through maintaining turgor-driven processes influencing growth, stomatal conductance, and root development under drought, there is conjecture of a positive

contribution of OA (Serraj and Sinclair 2002). Teulat et al. (2003) have identified an OA locus on chromosome 6H coincident with plant water status traits in Tadmor x ER/Apm. An increasing number of reports provide evidence on the association between high rate of osmotic adjustment (OA) and sustained yield or biomass under water-limited conditions across different cultivars of crop plants. Since OA helps to maintain higher leaf relative water content (RWC) at low leaf water potential (LWP), it is evident that OA helps to sustain growth while the plant is meeting transpiration demand by reducing its LWP. Osmotic adjustment sustained turgor maintenance and hence the yield-forming processes during moderate and severe water stress (Ali et al. 1999 and Blum 2005).
Significant associations between molecular markers and putative QTL that were analyzed for the 167 barley RILs were detected for all traits that were measured by (Teulat et al. 2001, 2002) several chromosomal regions related to variation in OA and water status were detected. Two QTL were identified for OA, one on chromosome 3H and one on 5H. Seven QTL were identified for OP in the irrigated group and three were detected under conditions of water stress. QTLs for $OP_{100}$ were also detected for both treatments. A total of seven QTL were detected on all chromosomes except 7H. One of them was identified under drought stress and six under irrigated conditions.

**c) Proline content**

Proline accumulation plays a highly protective role in plants that are exposed to abiotic stresses, conferring osmotic adjustment together with an increase in the levels of other osmolytes. Other suggested functions of proline are as antioxidants, as reactive oxygen species (ROS) and interaction with the hydrophobic residue of proteins (Valliyodan and Nguyen 2006).

The involvement of proline in the response to water deficits has been demonstrated in transgenic tobacco that over expressed proline biosynthesis enzymes (Kavi kishor et al. 1995 and Rosens et al. 2002). The suppression of proline synthesis in transgenic plants that contain the pyrroline-5-carboxylate reductase (P5CS) gene in the antisense direction resulted in increased sensitivity to water deficit (De Ronde et al. 2002). Recently, it was reported that transgenic petunia plants that over expressed the At P5CS gene from Arabidopsis and the OsP5CS gene from rice can withstand drought conditions for longer durations than wild type plants (Yamada et al. 2005).

The sense transformants, which demonstrated the earliest proline accumulation, experienced the least water loss when compared to the antisense transformants, which possessed the slowest proline accumulation (Simon-Sarkadi et al. 2005). Singh et al. (1972) reported that drought stress triggered the accumulation of proline in barley seedlings grown and stressed under controlled conditions. There was a strong correlation between the amounts of proline accumulated in 60 hr in stressed seedlings of 10 varieties.

#### 1.2.8.2 Shoot traits

Biomass production reflects the amount of water used and the efficiency of its water use efficiency at the canopy level is the proportion of water used to produce biomass as a ratio of water loss to the atmosphere through evapotranspiration (Richards et al. 2002). Germination of seeds in PEG solutions during a period of 5 day caused a growth reduction of shoots of barley seedling, its shoot dry matter (Leinhos et al. 1996). Root and shoot weights of all wheat cultivars were reduced when osmotic potential was decreased, but the extent of reduction in root growth was less than that for shoot (Baalbaki et al. 1999). Also there were decreases in total leaf blade length and shoot dry weight of both two wild barley lines P10-30 and P23-38, when exposed to water stress (Guoxiong et al. 2002).

In a study for Teulat et al. (1997) a large variations were obtained in the RILs, and a water effect was found for studied shoot traits between the water-stressed and the irrigated treatments, the decrease of tillers number NT, number of leaves NL in main stem, total shoot fresh matter TSFM and total shoot dry mutter TSDM was observed in the stress treatment compared with the irrigated, also two QTls were detected for TSFM on chromosome 1H and 6H, and two QTLs on chromosomes 1H and 6H for TSDM.
Shoot fresh weight especially leaves fresh weight correlated positively with grain yield in barley (*Hordeum vulgare* L.), and two QTLs were detected for leaves fresh weight on chromosome 7H and 6H (Mickelson et al. 2003). Pillen et al. (2003, 2004) detected two QTLs associated with dry biomass on 7H, and 4H. Ivandic et al. (2003) detected three QTLs for total dry matter one on chromosome 3H under well-watered and two on 4H under well-watered and drought stress condition (each one).

### 1.2.8.3 Root traits

Roots are a vital organ for absorbing soil moisture and nutrients and influence drought resistance, where the root traits are commonly considered drought tolerance traits because the ability of a plant to reach and extract the water from the soil should impact its ability to continue normal growth during periods of low moisture. Increased root biomass and root/shoot ratio has been reported under drought stress. The identification of quantitative trait loci (QTLs) with molecular markers may allow the estimation of parameters of genetic architecture and improve root traits by molecular marker-assisted selection (MAS). (Blum et al. 1983 and Qu et al. 2008).

Drought stress has effected in wild barley lines in Israel, where as no significant difference between wild and cultivar barley in root number and root thickness, however, within the wild barley accessions group, P23-38 and P20-05 had thinner root than P10-30, which imply that desert barley has thinner roots, hence more ability rooting deeper than mesic barley. Soil drying decreased slightly P10-30 root dry weight but increased P23-38 root dry weight. P23-38 root growth was enhanced by drought; therefore P23-38 is of drought resistance in comparison with P10-30 (Guoxiong et al. 2002).

Ping et al. (2003) found a significant negative correlation between root number (RN) and basal root thickness (BRT), Very significant or significant correlations were found between BRT, MRL, RFW, RDW, RFW/SFW and RDW/SDW, and reported that this result indicted that a good root system was with some characteristics of thicker BRT, longer MRL, heavier RFW and RDW, higher RFW/SFW and RDW/SDW.

In a study for Xiong et al. (2006) exposed arabidopsis seedlings to water stress (20% of soil water-holding capacity) for three weeks, and found that seedlings under the drought stress treatment had a significantly smaller root mass (fewer lateral roots) than those growing under well-watered conditions. Therefore, drought stress also inhibits lateral root development of soil-grown plants.

The root traits associated with drought tolerance is important for further understanding drought tolerance mechanisms of the whole plant. Six tall fescue cultivar were examined for root physiological with drought resistance, drying reduced root length and

dry mass in the 0- to 20 cm layer for all six cultivars. Root length and dry mass in 40- to 60 cm layer was enhanced for Houndog v, Flacon II, and Kentucky-31 cultivars, and was not affected for Phoenix and Bonsai; and was reduced for Rebel Jr. by soil drying. Drought stress increased root mortality in the 0- to 20, 20- to 40, and 40- to 60 cm layers, but the increase was most dramatic in the surface soil layer. Root depth of tall fescue cultivars during drought was positively correlated with root desiccation, as evidenced by severe leakage of organic solutes from roots in drying soil. Carbohydrate supply to roots was not a contributor to root depth during drought stress. This was supported by the increased or unaffected total non-structural carbohydrates in both shoots and roots, and the increased C allocation to roots under soil drying conditions (Huang and Gao 2000).

A total of 38 QTLs were observed in recombinant inbred line population of rice for the seven root traits under drought stress conditions, including 6 detected in two years and 32 detected in only 1 year. The effects of QTLs detected in 2004 were not necessarily larger than those resolved in 2003 for the QTLs detected simultaneously in both years. Alleles from IRAT109 at 23 of the 38 QTLs contributed to the increase of the trait measurements, whereas at the other 15 QTLs, alleles from cultivar Zhenshan 97 were in the direction of increasing the trait measurements. Of the 22 QTLs each explaining .10% of phenotypic variation, alleles from IRAT109 at 17 loci had positive effects on these root traits (Yue et al. 2006).

### 1.2.9 Genotype x environment interaction

Selection for many traits is not only being complicated by their quantitative nature, but also by the interaction between genotype and environment (GE). As a result of this interaction, the ranking order of varieties may change as the growing conditions (environments) change. Yield is a complex, polygenic trait that is strongly influenced by environmental factors. The changes of yield in relation to environmental changes are studied in the context of the concepts yield adaptability. Adaptability can be described as the reaction of the genotype to environmental factors, often defined in terms of linear or quadratic functions (Lin et al.1986). A well known measure for adaptability is the slope of the regression of yield for an individual cultivar on the mean yield (over all cultivars) across environments (Finlay and Wilkinson 1963; Eberhart and Russell 1966).

Several researchers have conducted multi-environment trials for various traits in different plant species, e.g. drought resistance in cotton (Saranga et al. 2001),growth and yield in rice (Hittalmani et al. 2003), and yield in barley (Teulat et al. 2001; Romagosa et al. 1996; Voltas et al. 2001; Malosetti et al. 2004). They all succeeded in identifying loci that interacted with the environment, so loci underlying GE. Some loci for GE co-localized with loci for the trait mean expression, while others appeared at positions where no QTLs for the mean expression were found.

### 1.2.10 DArT Markers

DArT is one of the recently developed molecular techniques and it has only been used in rice (Jaccound et al. 2001), barley (Wenzl et al. 2004), eucalyptus (Lezar et al. 2004), Arabidopsis (Wittenberg et al. 2005), cassava (Xia et al. 2005), wheat (Akbari et al. 2006; Semagn et al. 2006), and pigeon-pea (Yang et al. 2006).

Diversity array technology is one of a range of new microarray based molecular markers in the early stages of use. Unlike oligonucleotide arrays, the printed diversity arrays do not require prior genome sequence knowledge, instead using a subset of genetic information from a pool of genomes representing genetic diversity in a species or genus, for example, a range of cultivars, breeding germplasm and wild relatives (Jaccoud et al. 2001). Individuals can be genotyped by hybridisation to the array, with the genetic variation between tested genotypes evident in the presence or absence of hybridisation to array elements. The key attraction of microarray technology platform is the promise of high throughput capability and this is clearly evident with DArT. 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).

#### 1.2.10.1 DArT Markers applications

The pattern of hybridisation to the array 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 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. If alleles from a genotype are under-represented on an array, then DArT will indicate potentially greater differences from the population average. DArT is especially suited to QTL mapping (Wittenburg et al. 2005), and can be used to construct medium-density linkage maps relatively quickly.
Wenzl et al. (2004) gives 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 is dealt with 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 & 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). One shortcoming of DArT is the number of positions on a DArT array that are consistently non-polymorphic, i.e. non-marker clones. This has been recognised since the inception of this technology (Jaccoud et al. 2001), and recent studies detail how polymorphic markers can be identified in an initial discovery array process, then re-arrayed for genotypic applications as polymorphism-enriched arrays (Wenzl et al. 2004, Xia et al. 2005).

#### 1.2.10.2 The advantages and limitations 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).

*This technique, however, has also its own limitations*:

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).

### 1.2.11 Linkage Map

A linkage map is a genetic map of a species or experimental population that shows the position of its known genes and/or genetic markers relative to each other in terms of recombination frequency, rather than as specific physical distance along each chromosome. The breeding process can be enhanced by using the linkage between markers and traits, which enables indirect selection on markers avoiding the phenotypic assessment of traits. An important step towards the establishment of such linkages is the development of genetic maps. One of the first well developed classical genetic maps for barley included isozymes and morphological markers (Sogaard and von-Wettstein-Knowles 1987). Later on, molecular markers were added, beginning with RFLP and PCR markers (Shin et al. 1990), and these maps became more dense (Graner et al. 1991, Heun et al. 1991, and Kleinhofs et al. 1993) enabling the mapping

of many important agronomic qualitative and quantitative traits. New molecular markers were developed, improving the barley genetic map with AFLP markers (Waugh et al. 1997, Qi et al. 1998a, and Yin et al. 1999), and with microsatellite markers (Ramsay et al. 2000, Pillen et al. 2000, and Holton et al. 2002).

### 1.2.12 Method of Association mapping

There are many types of different methodologies that have been developed and initially are widely used for association mapping studies in human (comprehensively reviewed by Schulze and McMahon 2002), yet perfectly applicable without change or case-to-case modifications for wide range of organisms, including plants. Lately, some considerably successful achievements have been made to develop powerful, more precise and unbiased population-based association mapping methodology for plants.

1) The classical methodology and design of association mapping is “case and control” (also referred to as “casecontrol”) approach that identifies the causative gene tags in the comparison of allele frequencies in a sample of unrelated affected (referred to as “cases”) individuals and a sample of uninfected or healthy individuals (referred to as “controls”) (Schulze and McMahon 2002, Ohashi et al. 2001). This design requires an equal numbers of unrelated and unstructured “case-control” samples for accurate mapping. Case and control approach is seriously affected by the existence of population structure and stratification that caught the attention of scientist (Schulze and McMahon 2002).

2) Falk and Rubinstein (1987) developed a haplotype relative risk (HRR) approach that minimizes, but not eliminates population stratification issues in association mapping (Spielman and Ewens 1996). In that, first a “pseudocontrol” group (containing combination of two alleles that are not transmitted to affected offspring) is created; then the marker allele frequencies in case and “pseudocontrol” groups are correlated (Schulze and McMahon 2002)

3) To efficiently eliminate the confounding effects coming from population structure and stratification, Spielman et al. (1993) developed transmission disequilibrium test (TDT) method that compares transmission versus non-transmission of marker alleles to affected offspring by using chi-square test (Schulze and McMahon 2002), assuming a linkage between marker and trait. The TDT design requires genotyping of markers from three individuals: one heterozygous parent, one homozygous parent, and one affected

offspring. Although HRR performs better with unstructured sample than TDT because of its power to completely eliminate spurious association with good experimental design.
4) recently, Yu et al. (2006) developed new methodology, a mixed linear model (MLM) that combines both population structure information (Q-matrix) and level of pairwise relatedness coefficients—"kinship" (K-matrix) in the analysis, where the mixed linear model (MLM) approach found to be effective in removing the confounding effects of the population in association.

Although the overall approach of population-based association mapping in plants varies based on the methodology chosen (as above), assuming structured population samples, the performance of association mapping includes the following steps as described by Abdurakhmonov and Abdukarimov 2008.
(1) Selection of a group of individuals from a natural population or germplasm collection with wide coverage of genetic diversity. (2) Recording or measuring the phenotypic characteristics (yield, quality, tolerance, or resistance) of selected population groups. (3) Genotyping a mapping population individuals with available molecular markers. (4) Assessment of the population structure (the level of genetic differentiation among groups within sampled population individuals) and kinship (coefficient of relatedness between pairs of each individual within a sample). And (5) based on information gained through population structure, correlation of phenotypic and genotypic/haplotypic data with the application of an appropriate statistical approach that reveals, consequently a specific gene(s) controlling a QTL of interest can be cloned using the marker tags and annotated for an exact biological function.

### 1.2.13 Applications of Association Mapping

Association mapping is an alternative to mapping in a biparental population. A key to successful association mapping is to avoid spurious associations by controlling for population structure and/ or relatedness relationship between individuals. Compared to linkage mapping in traditional bioparental populations, association mapping offers three main advantages: increased mapping resolution, reduced research time, and greater allele numbers (Reich et al. 2001).

Association mapping, also known as linkage disequilibrium mapping, is a relatively new and promising genetic method for complex trait dissection. Association mapping has the promise of higher mapping resolution through exploitation of historical recombination events at the population level that may enable gene level mapping on non-model organisms where linkage based approaches would not be feasible (Varshney and Tuberosa. 2007).

The objective of genetic mapping is to identify simply inherited markers in close proximity to genetic factors affecting quantitative traits (Quantitative trait loci, or QTL). This localization relies on processes that create a statistical association between marker and QTL alleles and processes that selectively reduce that association as a function of the marker distance from the QTL. When using crosses between inbred parents to map QTL, we create in the $F_1$ hybrid complete association between all marker and QTL alleles that derive from the same parent. Recombination in the meioses that lead to doubled haploid, $F_2$ or recombinant inbred lines reduces the association between a given QTL and markers distant from it. Unfortunately, arriving at these generations of progeny requires relatively few meioses such that even markers that are far from the QTL (e.g., 10 cM) remain strongly associated with it. Such long-distance associations hamper precise localization of the QTL. One approach for fine mapping is to expand the genetic map, for example through the use of advanced intercross lines, such as $F_6$ or higher generational lines derived by continual generations of outcrossing the $F_2$ (Darvasi and Soller 1995, Jannink and Walsh 2002). In such lines, sufficient meioses have occurred to reduce disequilibrium between moderately linked markers. When these advance generation lines are created by selfing, the reduction is disequilibrium is not nearly as great as that under random mating. The central problem with any of the above approaches for fine mapping is the limited number of meioses that have occurred and (in the case of advanced intercross lines) the cost of propagating lines to allow for a sufficient number of meioses.

An alternative approach is “association mapping”, taking advantage of events that created association in the relatively distant past. Assuming many generations and therefore meioses have elapsed since these events, recombination will have removed

association between a QTL and any marker not tightly linked to it. Association mapping thus allows for much finer mapping than standard bi-parental cross approaches.
The most difficulties and problems with association mapping is that population structure can lead to spurious association between a candidate marker and a phenotype. One common solution has been to abandon case-control studies in favour of family-based test of association, but this comes at a considerable cost in the need collect DNA from close relative of affected individuals (Pritchared et al. 2000).

Analysis of genetic distance and population structure provided evidence of significant population structure in the *G. arboretum* accessions and identified the highest likelihood at k=6 . A total of 30 marker-trait association were identified with 19 SSR markers located on 11 chromosomes, the association analysis identified marker-trait associations (P=0.05) for all traits evaluated. Lint%, lint colour, elongation, micronaire and perimeter were associated with four markers each, length with three markers, and strength and maturity with tow and five markers respectively, Furthermore the LD ($R^2$ values) between markers ranged from 10% to 20%. Of the 30 marker-trait associations, four identified 15% or more of the total variation for lint% (BNL0256 and BNL1122), lint colour (BNL0542) and length (BNL1122) (Kantartzi and Stewart. 2008).

Yu et al. (2006) observed six gene expression phenotypes as phenotypic traits in mapping expression quantitative trait loci (eQTL). For the sample containing complex familial relationships and population structure, and they studied three quantitative traits measured on 277 diverse maize inbred lines, representing the diversity present in public breeding programs around the world. The population differentiation (Fst) among the major subgroups in our sample ranged from 0.047 (SSR) to 0.073 (SNP)., Although 80% of the pair-wise kinship estimates were close to 0, the remaining estimates were distributed from 0.05 to 1.0, as expected from complex familial relationship and population structure. Furthermore they found 37.6% of SNPs were associated with flowering time at $P < 0.05$ by the simple model, compared with 14.1% by the Q model, 6.1% by the K model and only 6.0% by the Q+K model. For flowering time and ear height, the Q+k model had the highest power. For ear diameter, the k model yielded a slightly higher power than the Q+k model did. The most benefit of the Q+K model is able to systematically account for multiple levels of relatedness among individuals.

Wang et al. (2008) investigated association mapping of iron deficiency chlorosis (IDC) loci in tow independent populations of soybean (*Glycine max* L. Merr.) three AM carried out with the TASSEL 2.0, a single factor analysis of variance (SFA) and mixed linear model (MLM) analysis to discover marker/trait associations. The MLM analysis, which includes population structure, kinship or both factors, reduced the number of markers significantly associated with IDC by 50% compared with SFA. With the MLM approach, three markers were found to be associated with IDC in the first population. Two of these markers, *Satt* 114 and *Satt* 239, were also found to be associated with IDC in the second populations, those lines with the tolerance allele at both these tow marker loci had significantly lower IDC scores than lines with one or no tolerant alleles.

Breseghello and Sorrells (2006) studied Association mapping (AM) in wheat for identification of genetic markers associated with kernel morphology and milling quality. They used in their study a population of 149 cultivars of soft winter wheat (*Triticum aestivum* L.), were genotyping with 93 SSR markers, Association between markers and traits was tested using a linear mixed-effect model, where the marker being tested was considered as a fixed effects factor and subpopulation was considered as a random-effects factor. Significant markers were detected in the three chromosomes tested; kernel width was associated with the locus Xwmc111-2D in both Ohio (OH) and New York (NY) and with Xgwm30-2D in NY only. A tow-marker model including both loci was significantly (P = 0.0002) more informative for KW in NY than either marker separately. The locus Xgwm539-2D was associated with kernel length in NY, although in this location it did not achieve the corrected threshold. Six loci in the LD block near the centromere of 5A were associated with kernel area, length, and weight, but not with kernel width.

### 1.2.14 Linkage disequilibrium

Linkage disequilibrium is the non-random association of alleles in a sample population and forms the basis for the construction of genetic maps and the localization of genetic loci for a variety of traits. The principles leading to LD apply to both biparental mapping populations ($F_2$, RILs, etc) and natural populations. Therefore LD mapping is the method of choice for genetic analysis in organisms like humans and animals, where

experimental populations are either not available or difficult to establish (Reich et al. 2001). Because of its inherent advantages, LD mapping approaches are increasingly being applied for plant species, in particular maize. Due to the out-breeding character of this species, LD extends only over a few kb and thus leads to a high genetic resolution, up to the level of individual candidate genes that can be associated with a given trait (Rafalski and Morgante 2004, Gupta et al. 2005).

The use of association genetic analyses in inbreeding species such as barley has been limited so far. However, recent studies have shown that LD extends over much longer genetic distances in barley than in maize. A European germplasm collection of 146 two-rowed spring barley cultivars was used to carry out LD mapping of yield traits using 236 AFLP markers (Kraakman et al. 2004). Associated markers were identified that are located in similar regions where QTLs for yield had been found in barley. (Romagosa et al. 1999 and Li et al. 2006).

A systematic survey of 953 gene bank accessions representing a broad spectrum of the genetic diversity in barley genetic resources revealed that LD extends up to 50 cM but is highly dependent on population structure (Kraakman et al. 2004 and Malysheva-Otto et al. 2006). On the one hand, the high level of LD in barley is due to the inbreeding mating type of this species; on the other hand, the selection of germplasm plays an important role Analysis of a germplasm collection of European cultivars, land races and wild barley accession from the Fertile Crescent region provided hints that the level of LD increases from cultivars to landraces to wild barley (Caldwell et al. 2006). Similarly, Morell et al. (2005) reported low levels of LD in wild barley by examining LD within and between 18 genes from 25 accessions.

Local differences in LD have been observed at the barley grain hardness locus comprising four closely linked genes (hinb, hina, GSP and PG2). Here, a high level of LD was observed in the intergenic region between hinb-1 and hina probably due to transposable elements present in this region, which influence the local recombination rate (Rae et al. 2007).

In a recent whole genome LD-mapping approach, Steffenson et al. (2007) used 318 wild barley accessions to perform association mapping studies using DArT markers to

identify rust resistance genes. In addition LD analysis has been performed based on haplotypes derived from 131 accessions by covering 83 SNPs within 132 kb around the gene HveIF4E, which confers resistance to barley yellow mosaic virus.

The genotyping database for 953 cultivated barley accessions profiled with 48 SSR markers was established. The PCoA revealed structuring of the barley population with regard to (i) geographical regions and (ii) agronomic traits. Geographic origin contributed most to the observed molecular diversity. Genome-wide linkage disequilibrium (LD) was estimated as squared correlation of allele frequencies ($r^2$). The values of LD for barley were comparable to other plant species (conifers, poplar and maize). The pattern of intrachromosomal LD with distances between the genomic loci ranging from 1 to 150 cM revealed that in barley LD extended up to distances as long as 50 cM with $r^2 > 0.05$, or up to 10 cM with $r^2 > 0.2$. Few loci mapping to different chromosomes showed significant LD with $r^2 > 0.05$. The number of loci in significant LD as well as the pattern of LD was clearly dependent on the population structure.

The LD in the homogenous group of 207 European 2-rowed spring barleys compared to the highly structured worldwide barley population was increased in the number of loci pairs with $r^2 > 0.05$ and had higher values of $r^2$, although the percentage of intrachromosomal loci pairs in significant LD based on $P < 0.001$ was 100% in the whole set of varieties, but only 45% in the subgroup of European 2-rowed spring barleys (Malysheva-Otto et al. 2006).

### 1.2.15 Effects of population admixture and selection on association

Population stratification exists when the total population has been formed by admixture between subpopulations and when admixture proportions (defined as the proportions of the genome that have ancestry from each subpopulation) vary between individuals (Hoggart et al. 2003).

Studies to determine association between a marker allele and the phenotype can take two forms. In one form, groups are distinguished on the basis of their divergent phenotypes (diseased vs. healthy; low vs. high trait value) and allele frequencies are compared across groups. Such studies are often referred to as case-control studies in

the human genetics literature since they contrast disease-affected individuals (cases) with unaffected (control) individuals. The second type of study uses groups distinguished on the basis of their marker genotypes, and phenotypic means are compared across groups. An example of this is Beer et al. (1997) analysed 13 quantitative traits on 64 North American oat varieties and landraces grouped according to RFLP genotype at 48 loci. Significant associations between RFLP fragments and group means occurred for 11.2% of fragments when testing at a 1% type I error rate, indicating many more associations than expected by chance alone. Some caution is in order, because the observed marker-trait association does not necessarily imply that markers showing a significant effect on the phenotype are linked to QTL. Rather, the marker-trait disequilibrium may exist in the absence of linkage, and instead may have arisen simply as a consequence of population structure.

The relationship between the putative quantitative trait locus (QTL) and phenotype is the one of interest, but it can be confounded by other variables. First, note that QTLs and individual admixture can be directly influenced by random variation due to meiosis. In addition, both the phenotype and measured admixture are potentially subject to measurement error. Furthermore, measured admixture is directly affected by individual admixture, which in turn is affected by individual ancestry. Naturally, the ancestry of the parents, represented by $P_1$ and $P_2$ affects individual ancestry. Individual ancestry can directly affect the putative QTL, which in turn can affect the phenotype, so individual ancestry has an indirect affect on the phenotype via the putative QTL (Redden et al. 2006).

### 1.3 Objectives of this study

The main goal of this research was to apply AM approaches to identify DArT markers associated drought tolerance traits in structured barley population, and determine a marker-based kinship matrix based on REML for drought related Traits.

Another goal is to identify and develop barley with improved adaptation to low rainfall environments, and to develop molecular markers for key traits associated with drought stress tolerance.

## 2. Material and Methods

### 2.1 Plant material

Plant material was taken from 98 accessions of wild barley (H. *vulgare* ssp. *spontaneum*) from the ICBB core collection (gene banks in Gatersleben and Braunschweig) and from 21 spring barley cultivars representative for the breeding pool of spring barley in North Rhine Westphalia (NRW), Germany, (Reetz and Leon 2004). These cultivars were provided by the Institute of Crop Science and Resource Conservation (INRES), chair of plant breeding.

### 2.2 The experiment

The experiments were carried out in plastic green house tunnels during the summer seasons 2007 and 2008 at the Poppelsdorf Experimental Station, Institute of Crop Science and Plant Resource conservation, Faculty of Agriculture, Rheinische Friedrich-Wilhelms-University Bonn. The experiments were arranged in a split-plot design with non-replications, drought treatments assigned to main plots and accessions to sub-plots.

The seeds of all accessions have been germinated in Petri dishes onto welted tissue paper in refrigerator at 4 $^{0}$C for 7 days, after that 12 seeds in two rows were sown in plastic pots of 22 x 22 cm with 25 cm depth, with 4 holes pierced at the bottom for drainage. Plastic pots contained sandy soil. The plants were fertilized three times with 250 ml of NPK liquid fertilizer containing 7 % N, 3% P2O5, and 6% K2O each pot in both seasons. They grew from on the 10$^{th}$ and 1$^{st}$ of April in 2007 and 2008, respectively.

In the well-watered treatment the plants got 330 ml water each pot daily per drip irrigation, while in the drought stress treatment the plants got 165 ml for each pot daily for 21 days. The application of drought treatment was applied after 40 days from sowing and continued for 21 days. After that the phenotypic measurements of the traits took place.

### 2.3 Phenotypic data measurements

#### 2.3.1 Shoot traits

Table (1) shows the studied traits determined in both seasons under drought stress and well-watered conditions.

**Table (1) list of 18 studied traits in both seasons**

| Traits | Abbreviations | Breeding goal |
|---|---|---|
| **Shoots** | | |
| **Wilting Score** | WS | - |
| **No. of Tillers** | TILS | + |
| **Shoot fresh weight (g)** | SFW | + |
| **Shoot Dry weight (g)** | SDW | + |
| **Roots** | | |
| **Root length (cm)** | RL | + |
| **Root Volume ( $cm^3$ )** | RV | + |
| **Total root fresh weight (g)** | RFW | + |
| **Root fresh weight a (g)** | FW a | + |
| **Root fresh weight b (g)** | FW b | + |
| **Root fresh weight c (g)** | FW c | + |
| **Total root Dry weight (g)** | RDW | + |
| **Root dry weight a (g)** | DW a | + |
| **Root dry weight b (g)** | DW b | + |
| **Root dry weight c (g)** | DW c | + |
| **Root / Shoot ratio (%)** | RSR | + |
| **Physiological traits** | | |
| **Relative water content (%)** | RWC | + |
| **Osmotic potential** (**Osmol**/kg FW) | OP | + |
| **Proline Content** (**µmoles**/g DW) | PC | + |

The value of the trait should be increased (+) or reduced (–) under water stress conditions with respect to the breeding goal.

**Wilting Score (WS)** Scored from 0 up to 9. 0 with no symptoms of stress effect and 9 with all plants apparently dried. (Dedatta et al. 1988).

**Number of Tillers / plant (TILS)** Average of tillers per plant was calculated from the tillers of four of the twelve plants.

**Shoot fresh weight / plant (SFW)** After 61 days from sowing, the middle four plants in each pot were cut out and weighted immediately, then the average per one plant has been calculated.

**Shoot dry weight / plant (SDW)** The shoot fresh mass of the middle four plants from each pot dried in oven at 80 $^0$C for 48 hours, and then the average per one plant was calculated.

### 2.3.2 Root traits

Roots were washed free of soil and then the root traits were measured.

**Root Length (RL)** Measured manually by ruler in cm from the base of roots to the end of roots.

**Root volume (RV)** Measured by imbedding the twelve roots in a cylinder (2000 ml) filled with water up to 600 ml and then the root volume was calculated by the difference between the initially volume in the cylinder (600 ml) and the observed volume after imbedding the roots in the cylinder according to the water-replacing method (Price and Tomos 1997).

**Root fresh weight** All roots of each pot were washed out, cleaned from soil and dried with absorbing paper. After that they were weighted as a whole (RFW) and then divided into 3 parts as follows

**Part a**: the first 10 cm root length from the base of roots, **Part b** 10 – 20 cm, and **Part c** > 20 cm. These three parts were also weighted as fresh weight (FWa, FWb and FWc).

**Root dry weight**

After determining the fresh weight the root parts were dried in the oven at 80 $^0$C for 72 hours, and the dry weight for each part (DWa, DWb and DWc) was determined and summarized to the total dry weight (RDW).

**Root / Shoot ratio (RSR)** Ratio between RDW and SDW.

### 2.3.3 Physiological Traits

**Relative water content (RWC)** The relative water content was calculated from the two upper fully developed leaves of the main stem from two plants.
RWC % = (FW- DW)/ (TW-DW) x 100 according to Barrs and Weatherly (1962), where FW is leave fresh weight, TW is the turgid weight obtained after floating the leaves in distilled water for 4 hours and DW is the dry weight of the leaves measured after drying the samples in the oven at 80 $^0$C for 24 hours.

**Osmotic potential (OP)** For determination the OP, the second upper fully developed leaf of the main tiller and the first biggest other tiller were cut and wrapped in plastic foil, and 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 small mixer and incubated in refrigerator at 4 $^0$C for 1 hour and centrifuged at 13000 U/min (Heraeus Biofuge Pico) for 10 min and finally stored at -20 $^0$C until the measurement. 15 µl from each sample were taken and measured with an Osmomat 300 (Gonotec, Berlin) with sterile water as a standard.

**Proline Content (PC)** The first upper fully developed leaf of the main tiller and the first biggest other tiller were cut and wrapped in plastic foil, frozen in liquid nitrogen, freeze-dried (Lyophilizer Leybold Heraeus Lyovec G12) and ground in a Wiley mill machine (Retsch MM 2000) into a fine powder. 30 mg were taken, 2 ml sulfosalicylic acid solution added, mixed 3 times for 15`` with a Vortex machine. 250 µl from this solution were taken and completed to 1 ml with Sulfosalicylic acid. Then 1 ml ninhydrin reagent and 1 ml Glacial acetic acid were added to the sample and then well mixed. After that the tubes were put into a water bath for one hour at 100 $^0$C and then left for cooling for 5 min. 2 ml Toluene were added to the sample, mixed for 15`` with Vortex machine and left for 5 min for sedimentation. Finally the upper 2 ml from the sample, which contain the Toluene including the proline, were taken for the proline measurement. The Proline concentration (µg proline/ml) was measured by using a Spectrophotometer at 520 nm using a standard curve. The Proline content was calculated as follows: Proline (µmoles proline / g dry weight) = ((µg proline/ml x8 x10) / (0.03 x 115.1) (Bates et al. 1973).

## 2.4 DNA extraction

DNA has been extracted from 10 mg freeze dried leaves by using “Kit” procedure according to DNeasy Plant Handbook 07/2006 as follows:

The leaf tissue was collected from young plants. It was freeze-dried and stored in a refrigerator at -80 $^0$C until extraction. 10 mg were cooled in liquid nitrogen, grind by tungsten carbide in microtubes for 1 min at 20 Hz in a TissueLyser and cooled again in liquid nitrogen. Then a clear cover was placed over each rack of collection microtubes and the racks were knocked upside down against the bench 5 times to ensure that all tungsten carbide beads could move freely within the microtubes. The racks were grind again in the TissueLyser for 1 min at 20 Hz. Then the caps of the microtubes were carefully removed and 400 µl working lysis solution (Combine Buffer 90 ml AP1, 250 µl RNase A, 250 µl Reagent DX) were added into each collection microtube. The microtubes were sealed with new caps. A clear cover was replaced over the racks with the microtubes. Then the racks were knocked vigorously up and down for 15 sec. To collect any solution from the caps they were shaken. The microtubes were centrifuged by SIGMA laboratory centrifuges until centrifuge reached 3000 rpm. 130 µl Buffer AP2 were added to each, the racks were shaken vigorously up and down for 15 sec. The microtubes were centrifuged until centrifuge reached 3000 rpm and then incubated for 10 min at –20 $^0$C, then centrifuged again for 5 min at 6000 rpm. 400 µl of each supernatant were transferred to new racks of collection microtubes, ensuring that the new tubes are in the correct orientation. 1.5 volumes (typically 600 µl) of Buffer AP3/E were added to each sample. The racks were shaken vigorously up and down for 15 sec and again centrifuged till the centrifuge reached 3000 rpm.

The DNeasy 96 plates were placed on top of S-Blocks. 1 ml of each sample was transferred carefully to each well of the DNeasy 96 plates. Then the DNeasy 96 plates were centrifuged for 4 min at 6000 rpm, after that sealed with an AirPore Tape Sheet (provided). 800 µl Buffer AW were added to each sample, centrifuged for 15 min at 6000 rpm to dry the DNeasy membranes. The DNeasy 96 plates were placed in correct orientation on a new rack of Elution Microtubes RS. 100 µl Buffer AE were added to each sample, sealed with new AirPore Tape Sheets and incubated for 1 min at room temperature (20 $^0$C) and centrifuged for 2 min at 6000 rpm. The previous step was repeated with another 100 µl Buffer AE. Finally the Elution Microtubes RS were sealed with new caps for storage in -20 $^0$ C.

### 2.5 DArT Marker analysis

The produced DNA was sent to the Australian lab of Diversity Arrays Technology P/L - Triticarte P/L, 1 Wilf Crane Crescent, Yarralumla ACT 600, AUSTRALIA for doing the marker analysis with their hybridization based markers.

Their technology involves reducing the complexity of the DNA sample by cutting the DNA 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. See the Diversity Arrays website at www.diversityarrays.com for more information.

DArT markers are biallelic dominant markers. Each marker was scored for each sample as 0, 1, and x, whereas 0 stands for absent, 1 for Present, and x stands for missing data.

### 2.6 Statistical analysis

The statistical analysis was conducted in three parts as follows

#### 2.6.1 Phenotypic data

The phenotypic data were analysed each season separately by a one way ANOVA using Proc GLM procedure (SAS version 9.1, SAS Institute 2003). The Pearson correlation coefficients (r) between the traits under well-watered and drought stress conditions were calculated by the SAS Procedure too.

The combined analysis for the two years was carried out using a mixed linear model

$$\mathbf{Y_{ijkm} = \mu + Y_i + T_j + Y_i{*}T_j + A_k + Y_i{*}A_k + T_j{*}A_k + \varepsilon_m}$$

Where $\mu$ is the general mean, $Y_i$ is the fixed effect of *ith* year, $T_j$ is the fixed effect of *jth* drought treatment, $Y_i{*}T_j$ is the fixed interaction effect of *ith* year with *jth* drought treatment, $A_k$ is the random effect of *kth* accession, $Y_i{*}A_k$ is the random interaction effect of the *ith* year with the *kth* accession, $T_j{*}A_k$ is the random interaction effect of the *jth* drought treatment with *kth* accession, and $\varepsilon_m$ is the residual effect.

### 2.6.2 Structure analysis and relatedness relationships

Population structure (Q-matrix) analysis was carried out using Software package "*STRUCTURE"* version 2.2. (Pritchard et al. 2000). The recognized subgroups from the 119 accessions are shown in table (2). Based on the suggestions of Pritchard and Wen (2007) for each run the burn-in time was 50.000 and the number of replications (MCMC) was 100.000.

The relative kinship coefficients (K-matrix) among all pairs of accessions were calculated using 1081 DArT marker data by "TASSEL" Software version 2.0.1.

Table (2): List of 119 Accessions of the structured barley population.

| No | Accession | Country of origin | Type | Subpop. | | No | Accession | Country of origin | Type | Subgroups. |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ICB180051 | AFGHANISTAN | Wild | 4 | | 61 | ICB180092 | PALASTIN | Wild | 8 |
| 2 | CCS004 | GERMANY | Cultivated | 10 | | 62 | ICB180102 | PALASTIN | Wild | 8 |
| 3 | CCS010 | GERMANY | Cultivated | 10 | | 63 | ICB180109 | PALASTIN | Wild | 8 |
| 4 | CCS012 | GERMANY | Cultivated | 10 | | 64 | ICB180117 | PALASTIN | Wild | 4 |
| 5 | CCS018 | GERMANY | Cultivated | 10 | | 65 | ICB180131 | PALASTIN | Wild | 10 |
| 6 | CCS023 | GERMANY | Cultivated | 10 | | 66 | ICB180148 | PALASTIN | Wild | 5 |
| 7 | CCS041 | GERMANY | Cultivated | 10 | | 67 | ICB180172 | PALASTIN | Wild | 8 |
| 8 | CCS049 | GERMANY | Cultivated | 10 | | 68 | ICB180199 | PALASTIN | Wild | 8 |
| 9 | CCS052 | GERMANY | Cultivated | 10 | | 69 | ICB180231 | PALASTIN | Wild | 8 |
| 10 | CCS060 | GERMANY | Cultivated | 10 | | 70 | ICB180260 | PALASTIN | Wild | 8 |
| 11 | CCS067 | GERMANY | Cultivated | 10 | | 71 | ICB180329 | PALASTIN | Wild | 8 |
| 12 | CCS081 | GERMANY | Cultivated | 10 | | 72 | ICB180372 | PALASTIN | Wild | 8 |
| 13 | CCS083 | GERMANY | Cultivated | 10 | | 73 | ICB180389 | PALASTIN | Wild | 9 |
| 14 | CCS084 | GERMANY | Cultivated | 10 | | 74 | ICB180410 | PALASTIN | Wild | 8 |
| 15 | CCS086 | GERMANY | Cultivated | 10 | | 75 | ICB180430 | PALASTIN | Wild | 8 |
| 16 | CCS089 | GERMANY | Cultivated | 10 | | 76 | ICB180508 | PALASTIN | Wild | 8 |
| 17 | CCS095 | GERMANY | Cultivated | 10 | | 77 | ICB180554 | PALASTIN | Wild | 8 |
| 18 | CCS096 | GERMANY | Cultivated | 10 | | 78 | ICB180573 | PALASTIN | Wild | 9 |
| 19 | CCS109 | GERMANY | Cultivated | 10 | | 79 | ICB180593 | PALASTIN | Wild | 6 |
| 20 | CCS121 | GERMANY | Cultivated | 10 | | 80 | ICB180631 | PALASTIN | Wild | 9 |
| 21 | CCS140 | GERMANY | Cultivated | 10 | | 81 | ICB180743 | PALASTIN | Wild | 8 |
| 22 | CCS141 | GERMANY | Cultivated | 10 | | 82 | ICB180973 | PALASTIN | Wild | 6 |
| 23 | ICB180046 | IRAK | Wild | 12 | | 83 | ICB180982 | PALASTIN | Wild | 7 |
| 24 | ICB180049 | IRAK | Wild | 3 | | 84 | ICB180994 | PALASTIN | Wild | 8 |
| 25 | ICB180069 | IRAK | Wild | 9 | | 85 | ICB181150 | PALASTIN | Wild | 8 |
| 26 | ICB180052 | IRAN | Wild | 10 | | 86 | ICB180006 | SYRIA | Wild | 6 |
| 27 | ICB180072 | IRAN | Wild | 1 | | 87 | ICB180802 | SYRIA | Wild | 7 |
| 28 | ICB181154 | IRAN | Wild | 1 | | 88 | ICB180812 | SYRIA | Wild | 7 |
| 29 | ICB181156 | IRAN | Wild | 1 | | 89 | ICB180862 | SYRIA | Wild | 7 |
| 30 | ICB181158 | IRAN | Wild | 1 | | 90 | ICB180867 | SYRIA | Wild | 7 |
| 31 | ICB181160 | IRAN | Wild | 4 | | 91 | ICB180872 | SYRIA | Wild | 6 |
| 32 | ICB181162 | IRAN | Wild | 1 | | 92 | ICB180877 | SYRIA | Wild | 6 |
| 33 | ICB181164 | IRAN | Wild | 1 | | 93 | ICB180882 | SYRIA | Wild | 8 |
| 34 | ICB181166 | IRAN | Wild | 1 | | 94 | ICB180887 | SYRIA | Wild | 7 |
| 35 | ICB181168 | IRAN | Wild | 3 | | 95 | ICB180902 | SYRIA | Wild | 7 |
| 36 | ICB181172 | IRAN | Wild | 3 | | 96 | ICB180923 | SYRIA | Wild | 6 |
| 37 | ICB181176 | IRAN | Wild | 3 | | 97 | ICB181238 | SYRIA | Wild | 4 |
| 38 | ICB181180 | IRAN | Wild | 1 | | 98 | ICB181323 | SYRIA | Wild | 6 |
| 39 | ICB181182 | IRAN | Wild | 1 | | 99 | ICB181475 | SYRIA | Wild | 11 |
| 40 | ICB181184 | IRAN | Wild | 5 | | 100 | ICB181481 | SYRIA | Wild | 11 |
| 41 | ICB181186 | IRAN | Wild | 1 | | 101 | IG119424 | SYRIA | Wild | 9 |
| 42 | ICB180007 | JORDAN | Wild | 11 | | 102 | IG119443 | SYRIA | Wild | 1 |
| 43 | ICB180013 | JORDAN | Wild | 1 | | 103 | IG119451 | SYRIA | Wild | 1 |
| 44 | ICB180014 | JORDAN | Wild | 11 | | 104 | IG121857 | SYRIA | Wild | 11 |
| 45 | ICB180018 | JORDAN | Wild | 8 | | 105 | ICB181500 | TADSCHIKISTAN | Wild | 4 |
| 46 | ICB181216 | JORDAN | Wild | 11 | | 106 | ICB180063 | TURKY | Wild | 9 |
| 47 | ICB181268 | JORDAN | Wild | 8 | | 107 | ICB180070 | TURKY | Wild | 5 |
| 48 | ICB181381 | JORDAN | Wild | 8 | | 108 | ICB181228 | TURKY | Wild | 12 |
| 49 | ICB181387 | JORDAN | Wild | 6 | | 109 | ICB181230 | TURKY | Wild | 12 |
| 50 | ICB181412 | JORDAN | Wild | 9 | | 110 | ICB180211 | TURKYKMENISTAN | Wild | 4 |
| 51 | ICB181418 | JORDAN | Wild | 11 | | 111 | ICB180213 | TURKYKMENISTAN | Wild | 4 |
| 52 | ICB181424 | JORDAN | Wild | 11 | | 112 | ICB180215 | TURKYKMENISTAN | Wild | 8 |
| 53 | ICB181430 | JORDAN | Wild | 8 | | 113 | ICB180217 | TURKYKMENISTAN | Wild | 4 |
| 54 | ICB181442 | JORDAN | Wild | 11 | | 114 | ICB181492 | TURKYKMENISTAN | Wild | 4 |
| 55 | ICB181448 | JORDAN | Wild | 2 | | 115 | ICB180035 | UNKNOWN | Wild | 10 |
| 56 | ICB181454 | JORDAN | Wild | 2 | | 116 | ICB191338 | UNKNOWN | Wild | 4 |
| 57 | ICB181466 | JORDAN | Wild | 9 | | 117 | ICB181498 | USBEKISTAN | Wild | 4 |
| 58 | ICB180084 | PAKISTAN | Wild | 4 | | 118 | IG124000 | USBEKISTAN | Wild | 4 |
| 59 | ICB181243 | PAKISTAN | Wild | 4 | | 119 | IG124017 | USBEKISTAN | Wild | 4 |
| 60 | ICB180079 | PALASTIN | Wild | 8 | | | | | | |

### 2.6.3 Marker-Trait association analysis

A major problem with association mapping is the presence of a population structure, which can lead to false positives and failure to detect genuine associations (i.e. false negatives), particularly in highly selfing species (Iwata et al. 2007). Therefore we include the relatedness relationships and population structure of the tested accessions using the Q and K matrixes in our analysis.

The association analysis was performed with a mixed linear model (MLM) using "ASReml" Software version 2 according to Stich et al. (2008). The statistical model for the association analysis identifying DArT markers which are associated with the tested drought tolerance traits considering population structure and the relatedness relationships is as follows:

$$\mathbf{Y_{ijklmnf} = \mu + Y_i + T_j + Y_i{*}T_j + Q_k + M_L + Y_i{*}M_L + T_j{*}M_L + Y_i{*}T_j{*}M_L + A_m(M_L)K_n + Y_i{*}A_m(M_L)K_n + T_j{*}A_m(M_L)K_n + \varepsilon_f(ijkmn)}$$

where $\mu$ is the general mean, $Y_i$ is the fixed effect of the *ith* Year, $T_j$ is the fixed effect of the *jth* Drought treatment, $Y_i{*}T_j$ is the fixed interaction effect of *ith* year with *jth* drought treatment, $Q_k$ is the fixed effect of *kth* subgroup of the population structure (Q matrix), $M_L$ is the fixed effect of *Lth* marker, $Y_i{*}M_L$ is the fixed interaction of *ith* year with *Lth* marker, $T_j{*}M_L$ is the fixed interaction effect of *jth* drought treatment with *Lth* marker, $Y_i{*}T_j{*}M_L$ is the fixed interaction effect of *ith* year with *jth* drought treatment and *Lth* marker, $A_m(M_L)K_n$ is the random effect of *mth* accession nested in the *Lth* marker associated with *nth* kinship coefficient , $Y_i{*}A_m(M_L)K_n$ is the random interaction effect of *ith* year with *mth* accession nested in the *Lth* marker associated with nth kinship coefficient, $T_j{*}A_m(M_L)K_n$ is the random interaction effect of *jth* drought treatment with *mth* accession nested in the *Lth* marker associated with nth kinship coefficient, $\varepsilon_{f(ijkmn)}$ is the error.

## 3. Results:

The structured barley population was evaluated under well-watered and drought stress conditions under greenhouse for two successive seasons (2007 and 2008). In parallel, the population was genotyped with 1081 DArT Markers to identify DArT markers associated drought tolerance traits in structured barley population. Structure analysis was conducted using Structure software 2.2, and Kinship coefficients matrix calculated by TASSEL 2.0.1, and then the association analysis was achieved including population structure (Q-matrix) and relatedness relationship coefficients (K-matrix) to avoid the superiors association to detect the marker genotype which associated with studied traits by ASReml Software version 2. The following part presents the phenotypic variation, phenotypic correlation among traits, and the markers which associated with each trait.

### 3.1 Phenotypic measurements

In this study 119 accession were evaluated for quantitative traits (Wilting score, Shoot fresh weight, Shoot dry weight, Root traits, Root/Shoot ratio, Relative water content, Osmotic potential, and Proline content) the phenotypic differences between well-watered and stress conditions in 2007 and 2008 seasons are shown in table 3. The followed traits, WS, SFW, RWC, RL, FWa, FWb, FWc, RFW, DWc, RDW, and PC were exhibited highly significantly differences in both seasons, while DWb and OP had non-significant differences in both seasons. On the other hand the traits RV and DWa were highly significant in the first season and insignificant in the second season and traits TILS, SDW, and RSR were exhibited non-significant differences in the first season, while they were highly significant in the second season.

Table 4 presents a summary statistics of the studied traits under well-watered and drought stress treatments across to years. High significant differences were found between well-watered and drought stress treatment for all studied traits except TILS, RDWb, RDW, RSR, and OP, where RWC and RV decreased under drought stress condition with 25.6%, and 15.4 % respectively and the Proline content increased about 13 fold more than well-watered, and the Root length increased with 17.5 % under drought stress treatment (see also table 5).

The variation among accessions, treatments and the interaction between them are presented in table 5. The accessions showed high significant differences for WS, TILS,

SFW. SDW, RSR, RL, FWc and DWc traits under the two treatments and through the two years of the study, while for the other traits they showed non significant differences. With regarding to the interaction between treatments and the accessions was highly significantly only for the two traits DWc and FWc.

Table 3 Analysis of Variance of studied traits as an average under both treatments in 2007 and 2008 seasons.

| Trait | SOV | DF | 2007 | | 2008 | | |
|---|---|---|---|---|---|---|---|
| | | | MS | Sign | DF | MS | Sign |
| WS | Treat. | 1 | 812.90 | *** | 1 | 698.45 | *** |
| | Error | 234 | 2.51 | | 234 | 1.30 | |
| TILS | Treat. | 1 | 0.04 | Ns | 1 | 12.13 | * |
| | Error | 234 | 4.52 | | 234 | 2.11 | |
| SFW | Treat. | 1 | 397.14 | *** | 1 | 1215.89 | *** |
| | Error | 234 | 4.45 | | 234 | 2.99 | |
| SDW | Treat. | 1 | 0.63 | Ns | 1 | 26.82 | *** |
| | Error | 234 | 0.63 | | 234 | 0.30 | |
| RWC | Treat. | 1 | 20991.91 | *** | 1 | 45566.98 | ** |
| | Error | 234 | 121.25 | | 234 | 89.66 | |
| RV | Treat. | 1 | 41724.76 | *** | 1 | 246.10 | Ns |
| | Error | 234 | 1463.25 | | 234 | 33.92 | |
| RL | Treat. | 1 | 378.68 | *** | 1 | 1602.64 | *** |
| | Error | 230 | 27.33 | | 234 | 17.98 | |
| FWa | Treat. | 1 | 32100.68 | *** | 1 | 2278.94 | *** |
| | Error | 234 | 363.46 | | 234 | 59.64 | |
| FWb | Treat. | 1 | 3262.29 | *** | 1 | 284.24 | *** |
| | Error | 234 | 180.32 | | 234 | 20.96 | |
| FWc | Treat. | 1 | 1771.62 | *** | 1 | 1359.45 | *** |
| | Error | 234 | 19.93 | | 234 | 18.61 | |
| RFW | Treat. | 1 | 37710.68 | *** | 1 | 768.78 | * |
| | Error | 234 | 1065.54 | | 234 | 188.18 | |
| DWa | Treat. | 1 | 499.53 | *** | 1 | 0.16 | Ns |
| | Error | 234 | 25.37 | | 234 | 2.46 | |
| DWb | Treat. | 1 | 1.03 | Ns | 1 | 0.88 | Ns |
| | Error | 234 | 3.99 | | 234 | 0.42 | |
| DWc | Treat. | 1 | 41.74 | *** | 1 | 40.91 | *** |
| | Error | 234 | 0.27 | | 234 | 0.37 | |
| RDW | Treat. | 1 | 285.84 | ** | 1 | 48.15 | ** |
| | Error | 234 | 42.16 | | 234 | 5.44 | |
| RSR | Treat. | 1 | 3581.40 | Ns | 1 | 10993.01 | *** |
| | Error | 234 | 1117.58 | | 234 | 257.51 | |
| OP | Treat. | 1 | 0.002 | Ns | 1 | 0.001 | Ns |
| | Error | 231 | 0.003 | | 232 | 0.001 | |
| PC | Treat. | 1 | 7.16 | *** | 1 | 23202.68 | *** |
| | Error | 233 | 0.44 | | 234 | 117.99 | |

Where, *, ** and *** are significant at 0.05, 0.01 and 0.001 levels of probability, respectively. Ms is the mean square of the studied trait.

Table 4 Summary statistics of 18 evaluated traits under well-watered (W) and Drought stress treatments (D) across two years for structured barley populations.

| Trait | Treatment | Mean | Min. | Max. | SD | SE | Sign. |
|---|---|---|---|---|---|---|---|
| WS | W | 1.059 | 0.000 | 4.00 | 0.965 | 0.062 | ** |
| | D | 4.635 | 0.000 | 9.000 | 1.760 | 0.114 | |
| TILS. | W | 4.766 | 1.500 | 12.000 | 1.989 | 0.129 | Ns |
| | D | 4.552 | 0.000 | 12.750 | 2.182 | 0.142 | |
| SFW | W | 9.799 | 3.497 | 18.222 | 2.496 | 0.162 | ** |
| | D | 6.232 | 2.852 | 12.000 | 1.465 | 0.095 | |
| SDW. | W | 2.388 | 1.027 | 4.750 | 0.754 | 0.049 | ** |
| | D | 1.999 | 0.797 | 4.672 | 0.712 | 0.0463 | |
| RWC | W | 90.876 | 57.370 | 98.810 | 4.928 | 0.320 | ** |
| | D | 67.549 | 28.170 | 94.331 | 15.065 | 0.980 | |
| RV | W | 92.906 | 30.000 | 235.000 | 43.321 | 2.819 | ** |
| | D | 78.588 | 20.000 | 645.000 | 60.156 | 3.915 | |
| RL | W | 26.601 | 11.000 | 43.000 | 4.833 | 0.314 | ** |
| | D | 31.275 | 15.000 | 44.500 | 4.733 | 0.310 | |
| RFWa | W | 41.886 | 7.810 | 132.490 | 26.407 | 1.719 | ** |
| | D | 27.116 | 4.380 | 88.530 | 14.626 | 0.952 | |
| RFWb | W | 21.062 | 1.390 | 77.570 | 14.690 | 0.956 | ** |
| | D | 16.247 | 0.800 | 42.680 | 8.796 | 0.572 | |
| RFWc | W | 2.711 | 0.000 | 18.050 | 2.594 | 0.168 | ** |
| | D | 7.851 | 0.000 | 31.280 | 5.646 | 0.367 | |
| RFW | W | 65.660 | 12.760 | 209.350 | 40.467 | 2.634 | ** |
| | D | 51.214 | 8.750 | 134.090 | 25.305 | 1.647 | |
| RDWa | W | 6.983 | 0.640 | 45.320 | 5.988 | 0.389 | ** |
| | D | 5.503 | 0.810 | 17.390 | 3.242 | 0.211 | |
| RDWb | W | 2.645 | 0.160 | 12.020 | 1.948 | 0.126 | Ns |
| | D | 2.640 | 0.120 | 8.270 | 1.477 | 0.096 | |
| RDWc | W | 0.264 | 0.00 | 1.240 | 0.222 | 0.014 | ** |
| | D | 1.101 | 0.000 | 3.850 | 0.770 | 0.050 | |
| RDW | W | 9.893 | 1.870 | 47.680 | 7.467 | 0.486 | Ns |
| | D | 9.244 | 1.480 | 23.850 | 4.712 | 0.306 | |
| RSR | W | 40.758 | 4.854 | 262.266 | 35.043 | 2.281 | Ns |
| | D | 43.688 | 6.562 | 131.216 | 23.522 | 1.531 | |
| PC | W | 0.833 | 0.001 | 15.133 | 1.602 | 0.104 | ** |
| | D | 10.967 | 0.008 | 83.577 | 14.956 | 0.975 | |
| OP | W | 0.157 | 0.060 | 0.3900 | 0.044 | 0.002 | Ns |
| | D | 0.158 | 0.041 | 0.356 | 0.0512 | 0.003 | |

Where, SD: standard deviation; SE: standard error; * and ** are significant effects at 0.05, and 0.01 levels of probability, respectively.

Table 5 Mean Squares of the studied traits under well-watered treatment and drought stress conditions across two successive seasons 2007 and 2008.

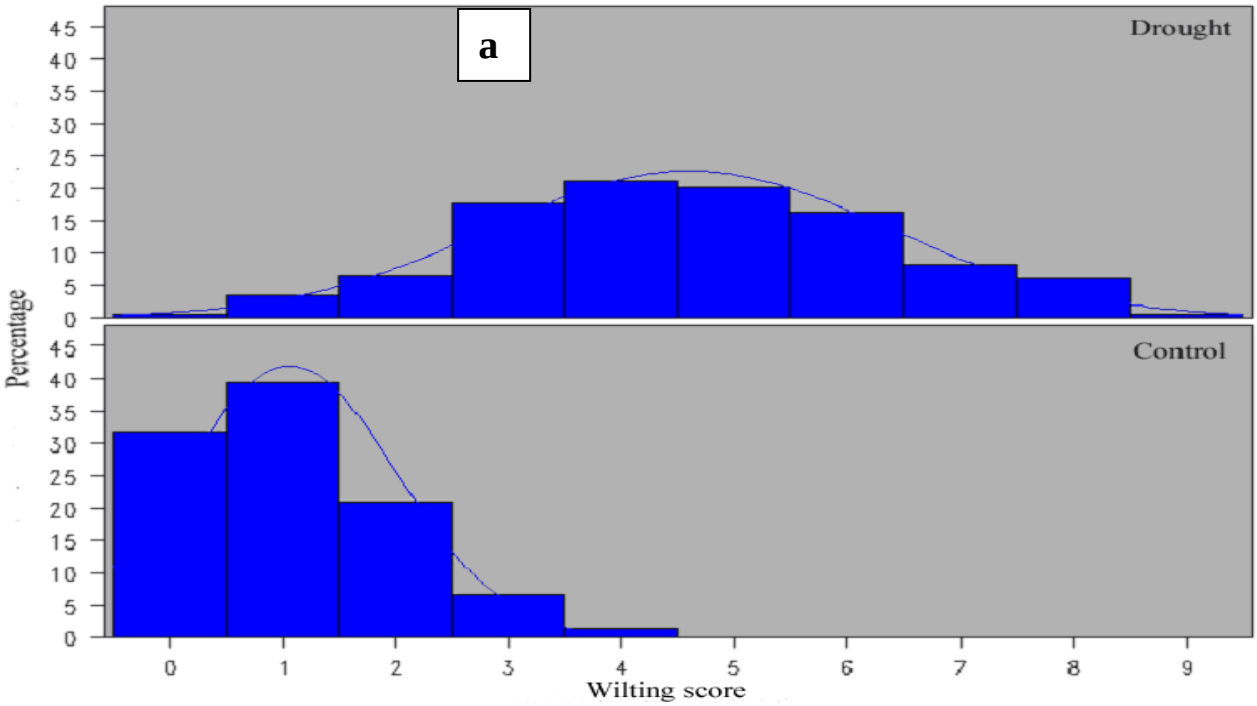
| Trait | S.V. | D.F | Ms. | Sign | Trait | S.V. | D.F. | Ms. | Sign. |
|---|---|---|---|---|---|---|---|---|---|
| WS | Acc. | 118 | 3.38 | *** | RV | Acc. | 118 | 3559.67 | NS |
| | Treatment | 1 | 1499.26 | *** | | Treatment | 1 | 24661.52 | ** |
| | Acc.x Treatment | 117 | 1.50 | NS | | Acc.x Treatment | 117 | 1969.80 | NS |
| TILS | Acc. | 118 | 7.96 | *** | FWa | Acc. | 118 | 389.65 | NS |
| | Treatment | 1 | 5.49 | NS | | Treatment | 1 | 26203.69 | *** |
| | Acc.x Treatment | 117 | 0.92 | NS | | Acc.x Treatment | 117 | 111.41 | NS |
| SFW | Acc. | 118 | 6.48 | *** | FWb | Acc. | 118 | 181.77 | NS |
| | Treatment | 1 | 1478.66 | *** | | Treatment | 1 | 2760.37 | *** |
| | Acc.x Treatment | 117 | 2.58 | NS | | Acc.x Treatment | 117 | 65.48 | NS |
| SDW | Acc. | 118 | 1.01 | *** | FWc | Acc. | 118 | 40.17 | *** |
| | Treatment | 1 | 17.55 | *** | | Treatment | 1 | 3127.69 | *** |
| | Acc.x Treatment | 117 | 0.21 | NS | | Acc.x Treatment | 117 | 15.37 | * |
| RWC | Acc. | 118 | 127.10 | NS | RFW | Acc. | 118 | 1323.19 | NS |
| | Treatment | 1 | 63963.4 | *** | | Treatment | 1 | 25118.77 | *** |
| | Acc.x Treatment | 117 | 110.81 | NS | | Acc.x Treatment | 117 | 259.67 | NS |
| RSR | Acc. | 118 | 1426.8 | ** | DWa | Acc. | 118 | 20.95 | NS |
| | Treatment | 1 | 875.50 | NS | | Treatment | 1 | 267.47 | * |
| | Acc.x Treatment | 117 | 314.86 | NS | | Acc.x Treatment | 117 | 10.30 | NS |
| RL | Acc. | 118 | 31.97 | *** | DWb | Acc. | 118 | 3.74 | NS |
| | Treatment | 1 | 2560.83 | *** | | Treatment | 1 | 0.01 | NS |
| | Acc.x Treatment | 117 | 20.40 | NS | | Acc.x Treatment | 117 | 1.46 | NS |
| PC | Acc. | 118 | 58.07 | NS | DWc | Acc. | 118 | 0.61 | *** |
| | Treatment | 1 | 12074.51 | *** | | Treatment | 1 | 82.70 | *** |
| | Acc.x Treatment | 117 | 61.47 | NS | | Acc.x Treatment | 117 | 0.32 | *** |
| OP | Acc. | 118 | 0.0020 | NS | RDW | Acc. | 118 | 42.96 | NS |
| | Treatment | 1 | 0.0006 | NS | | Treatment | 1 | 54.13 | NS |
| | Acc.x Treatment | 117 | 0.0021 | NS | | Acc.x Treatment | 117 | 13.20 | NS |

Where, *, ** and *** are significant effects at 0.05, 0.01 and 0.001 levels, respectively.
Ms is the mean squares of the studied trait.

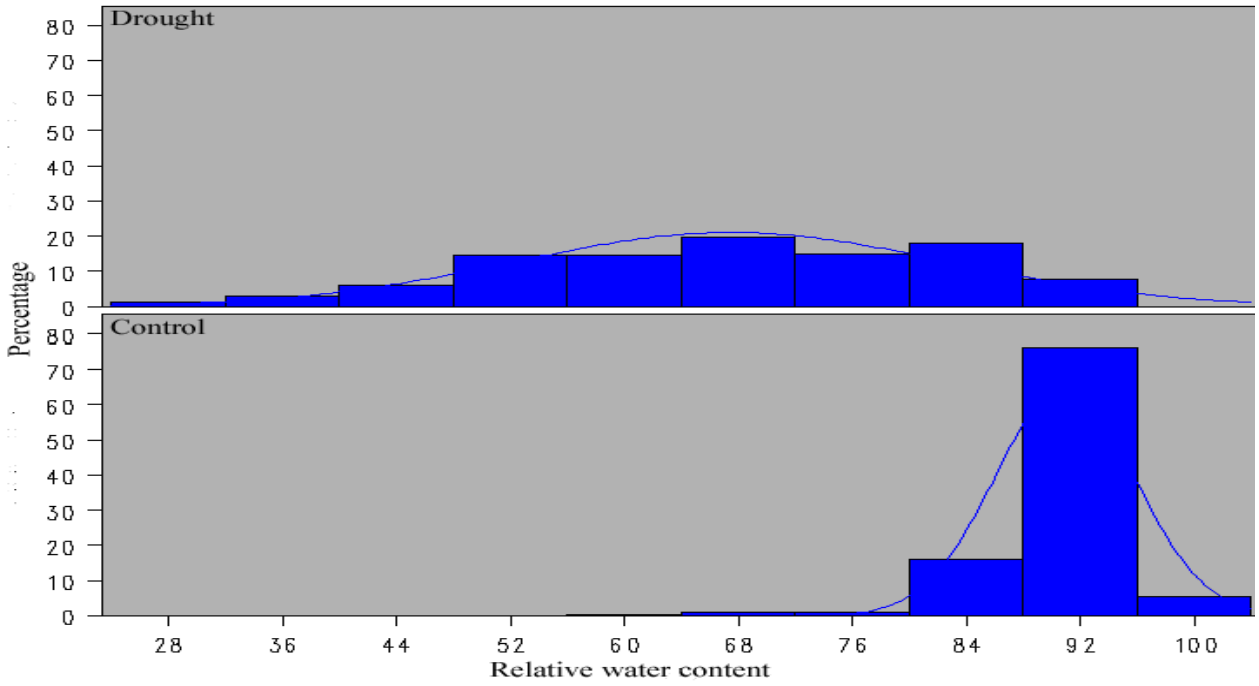
**Fig.1** (a, b, c, d, e and f) shows the normal distribution of WS, RWC, SFW, SDW, RL and RV as quantitative traits under well-watered treatment and drought stress conditions across 2007 and 2008 years, where the histogram **a** refers to the distribution analysis of WS which ranged from 0 up to 4 and from 1 to 8 under normal and drought

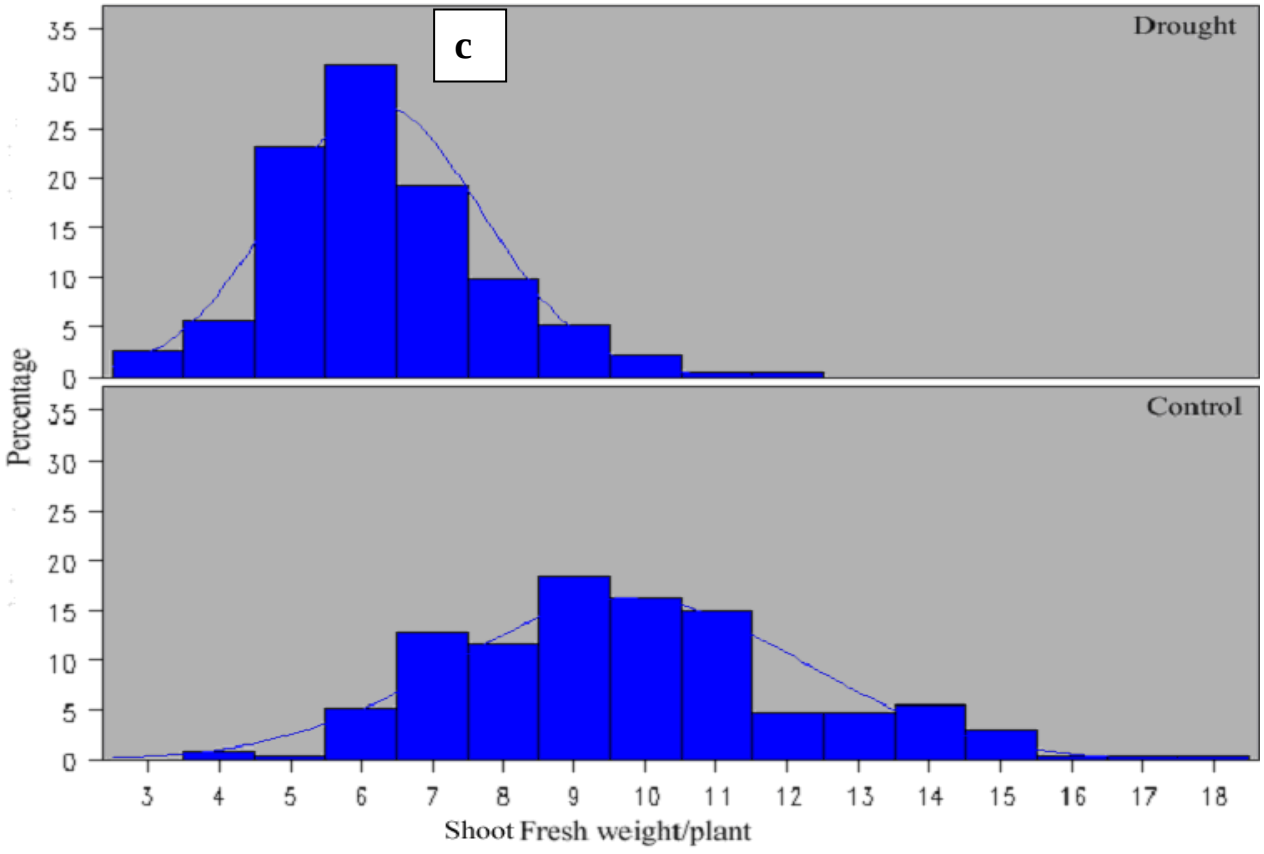
stress treatments respectively. In histogram **b**, RWC ranged from 28 up to 92% and from 76 up to 98.70% under drought and well-watered conditions, respectively.

SFW shown in histogram **c,** ranged from 3 to 18 g and from 3 until 10 gm under well-watered treatment and drought stress condition. With regard to SDW which presented in histogram **d**, ranged from 0.8 to 4.4 g and 1.2 to 4.8 g under drought and normal conditions, respectively. In histogram **e**, RL ranged from 24 up to 44 cm, and 16 to 44 under drought stress and well-watered and for RV (histogram **f)** ranged from 30 up to 150, and 30 to 210 $cm^3$ under drought stress and well-watered treatments respectively.

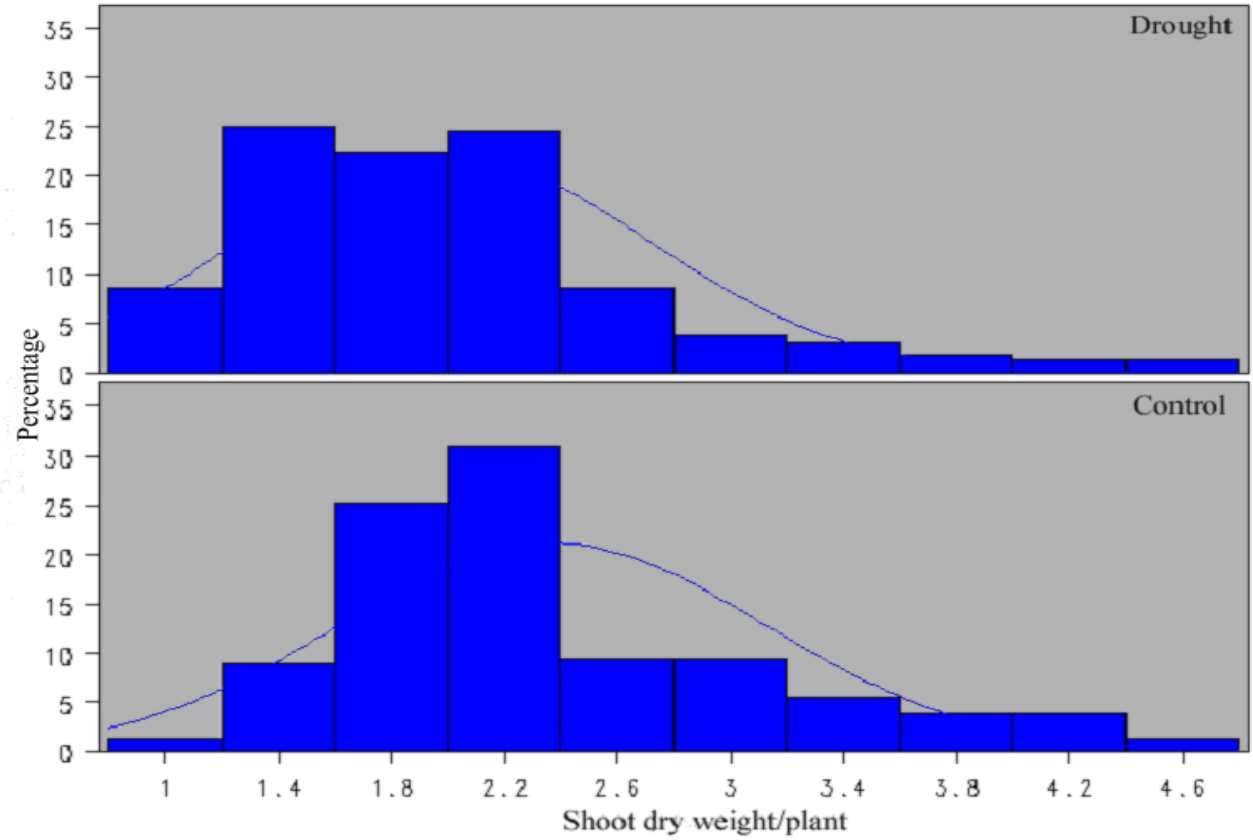


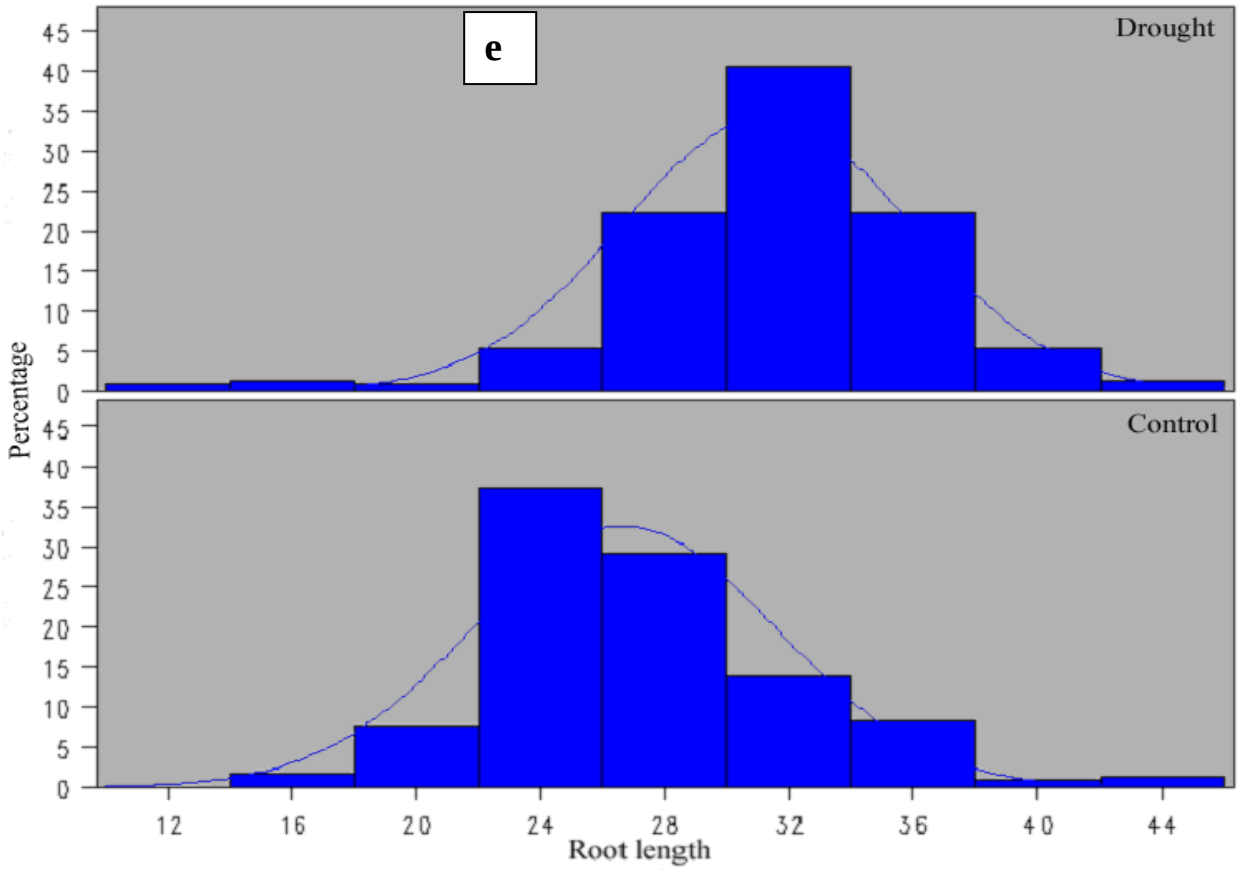

**b**


Drought
Control
Percentage
Relative water content


c
Drought
Control
Percentage
Shoot Fresh weight/plant

**d**


Drought
Control
Percentage
0
5
10
15
20
25
30
35
1
1.4
1.8
2.2
2.6
3
3.4
3.8
4.2
4.6
Shoot dry weight/plant


e
Drought
Control
Percentage
0
5
10
15
20
25
30
35
40
45
12
16
20
24
28
32
36
40
44
Root length

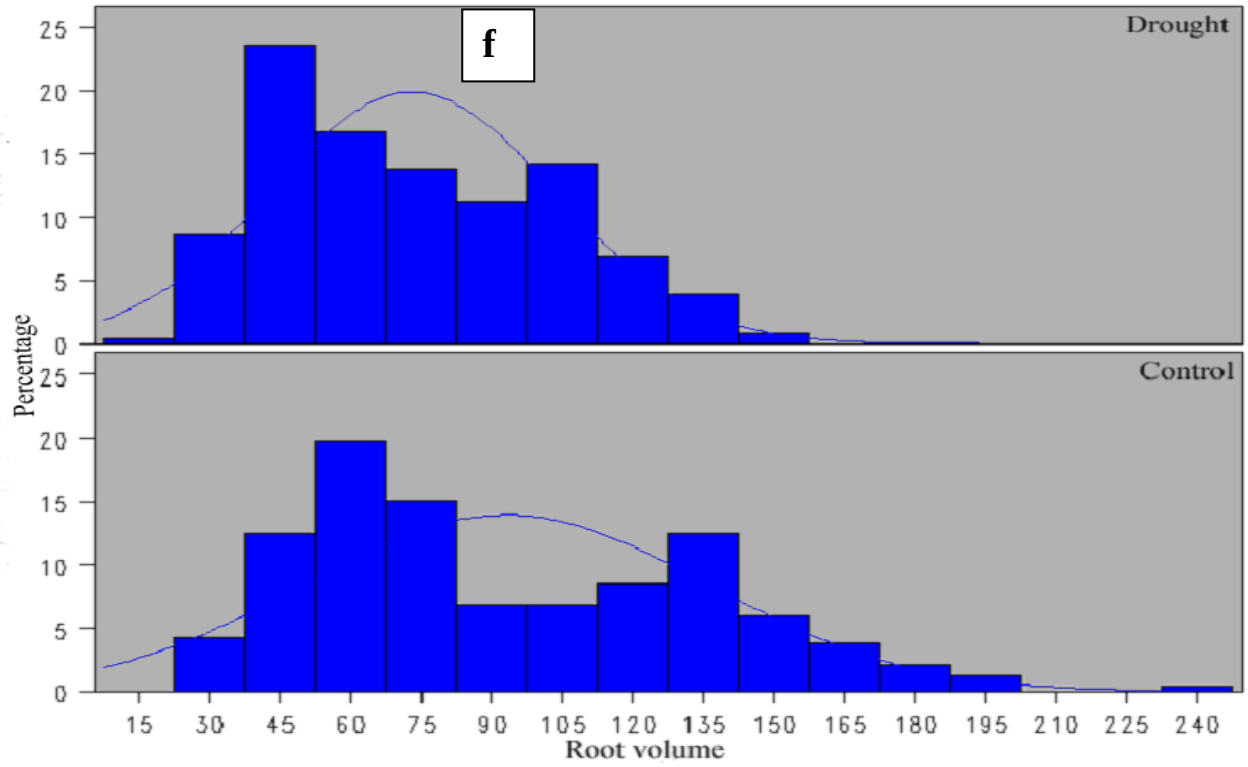



**Fig.1** Normal distribution for a: Wilting Score, b: Relative water content, c: fresh weight / plant, d: Dry weight / plant, e: Root length and f: Root volume under well-watered and drought stress treatments across 2007 and 2008 years.

**Fig.2**. illustrates the differences between fresh and dry weight of the roots under both treatments (see also table 5), there were significant differences between both treatments in the fresh weight in all the parts of the roots, while, the dry weight of roots in part a and c differed significantly, but total dry weight of roots and root dry weight part b had non significant differences.

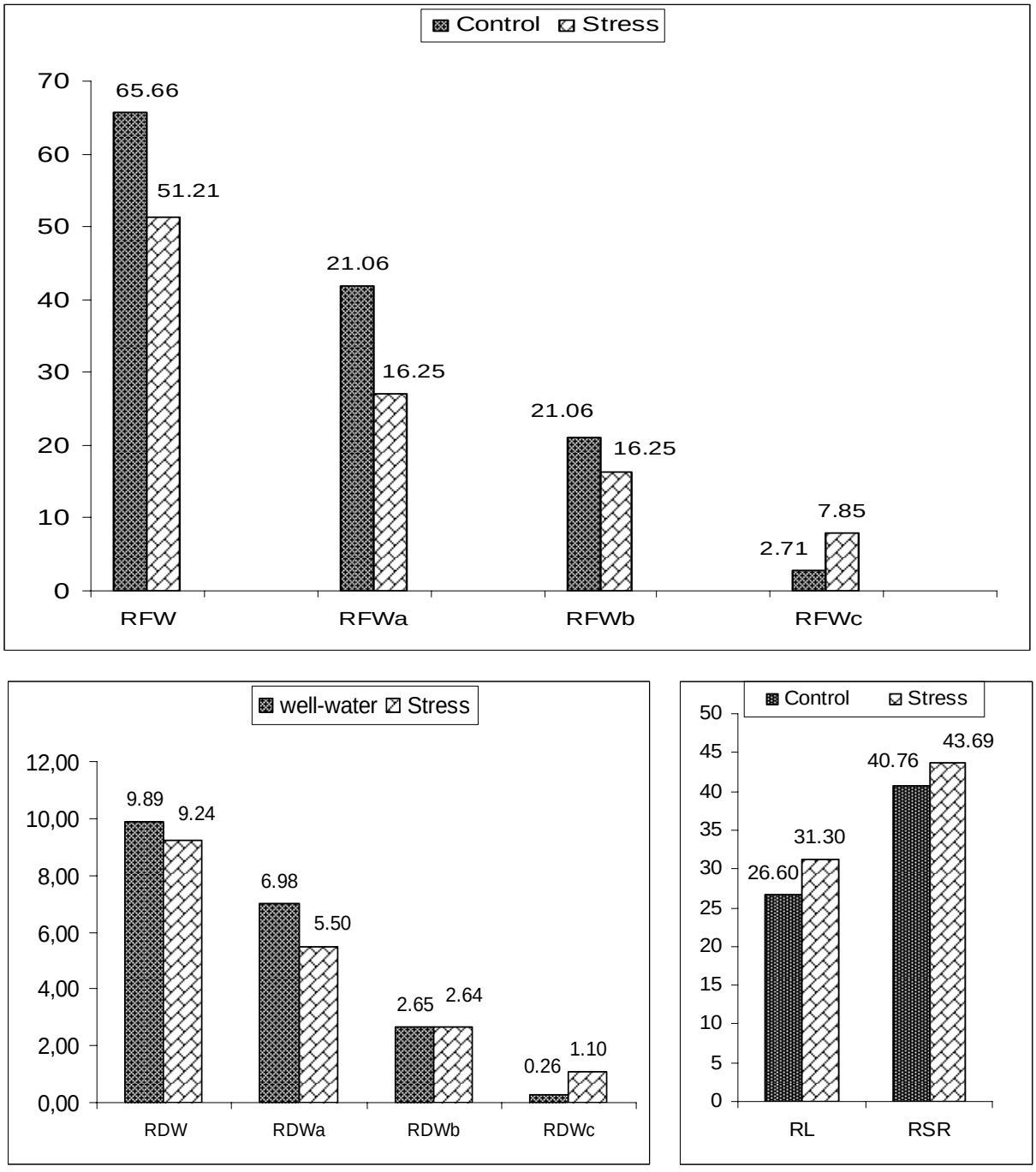



**Fig.2** The root fresh and dry weights, root length and root/shoot ratio under well-watered and drought stress conditions across 2007 and 2008 seasons.

One of the methods adaptation to sever drought stress increase the root length to extract any water in depth of soil, and decrease the water loss to the dry soil by the roots in the upper soil, via either cover its fine lateral roots with a layer relatively impermeable to water, or it must separate and a abscise them by a layer in order to cut down the loss of water to the dry soil (Xiong et al 2006, Peter and Neumann 2008). So

these results in fig. 2 the decreasing root weight in part a & b and increasing the root length and root weight in part c under drought stress conditions may be due to this adaptation.

### 3.2 Correlation between phenotypic traits

Table 6 present the Pearson correlation coefficients (r) between all possible pair-wise combination traits. Many traits correlated significantly each other in both seasons, and in overall two seasons.

**The phenotypic correlation across two years**

Table 6 shows the Pearson correlation coefficients (r) between 12 pairs of studied traits under drought-stress (D) and well-watered (W) treatment across 2007 and 2008 yeas, The number of Tillers / plant (TILS) correlated positively with all traits under well-watered and drought stress conditions except SFW, SDW and PC was negatively. Osmotic potential (OP) was correlated positively with TILS, SDW, RFW, RDW and RSR under drought stress treatment and with RV, RFW, and RDW under well-watered treatment, whereas it was correlated negatively with RL and PC under drought stress and with WS in well-watered treatment. PC was correlated negatively with each of TILS, RFW, RDW and RSR and positively with WS under both treatments, while it was negatively with RWC under drought stress conditions. RWC was correlated negatively with WS and RL and positively with RFW, TILS and RDW under both of treatments.

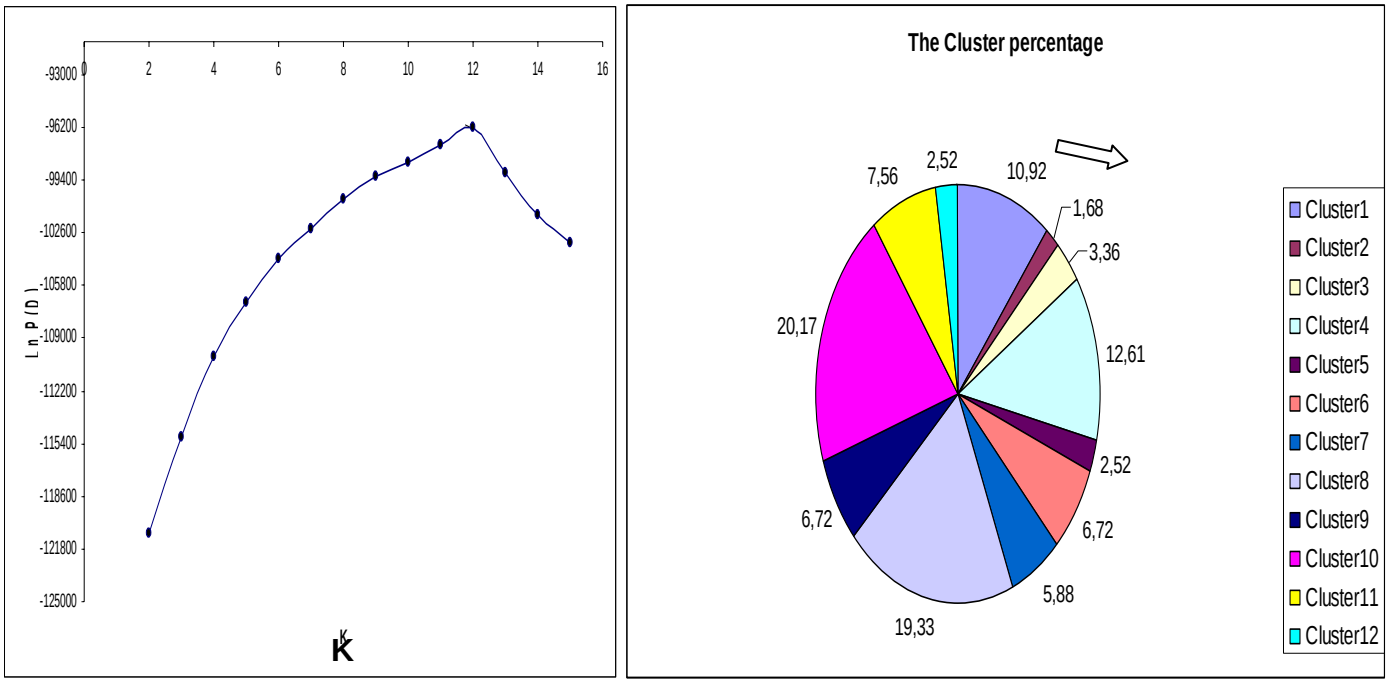
Table 6 Pearson correlation coefficient (r) calculated between 12 pairs of traits under well-watered (W) and under drought-stress (D) treatment in structured barley population over two seasons.

| Trait | | WS | TILS | SFW | SDW | RWC | RV | RL | RFW | RDW | RSR | PC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TILS | W | -0.257** | | | | | | | | | | |
| | D | -0.282** | | | | | | | | | | |
| SFW | W | -0.020 | -0.203** | | | | | | | | | |
| | D | -0.040 | -0.066 | | | | | | | | | |
| SDW | W | -0.068 | -0.208** | 0.746** | | | | | | | | |
| | D | 0.111 | 0.081 | 0.742** | | | | | | | | |
| RWC | W | -0.287** | 0.320** | -0.133* | -0.107 | | | | | | | |
| | D | -0.456** | 0.497** | -0.054 | -0.072 | | | | | | | |
| RV | W | -0.305** | 0.604** | -0.410** | -0.346** | 0.420** | | | | | | |
| | D | -0.058 | 0.237** | -0.205** | -0.091 | 0.067 | | | | | | |
| RL | W | 0.093 | 0.146* | -0.047 | -0.176** | -0.027 | 0.376** | | | | | |
| | D | -0.172** | 0.207** | -0.252** | -0.391** | -0.394** | 0.119 | | | | | |
| RFW | W | -0.328** | 0.586** | -0.393** | -0.210** | 0.398** | 0.904** | 0.302** | | | | |
| | D | -0.294** | 0.685** | -0.157* | 0.133 | 0.445** | 0.349** | 0.232** | | | | |
| RDW | W | -0.281** | 0.544** | -0.387** | -0.210* | 0.346** | 0.825** | 0.229** | 0.902** | | | |
| | D | -0.221** | 0.614** | -0.102 | 0.186** | 0.391** | 0.332** | 0.143* | 0.939** | | | |
| RSR | W | -0.241** | 0.481** | -0.567** | -0.441** | 0.345** | 0.791** | 0.191** | 0.812** | 0.935** | | |
| | D | -0.297** | 0.476** | -0.519** | -0.348** | 0.345** | 0.332** | 0.326** | 0.763** | 0.797** | | |
| PC | W | 0.164* | -0.167* | 0.146* | -0.032 | -0.129 | -0.264** | -0.082 | -0.298** | -0.269** | -0.236** | |
| | D | 0.177** | -0.439** | -0.007 | -0.288** | -0.479** | -0.055 | 0.065 | -0.445** | -0.417** | -0.236** | |
| OP | W | -0.145* | -0.042 | -0.099 | 0.078 | 0.032 | 0.159* | -0.021 | 0.171** | 0.156* | 0.128 | -0.123 |
| | D | -0.071 | 0.144* | 0.004 | 0.197** | 0.087 | 0.018 | -0.145* | 0.248** | 0.284** | 0.140* | -0.195* |

Where,*, ** and *** are the significances differences at 0.05, 0.01 and 0.001 levels. W: well-watered and D: Drought stress.

### 3.3 Population structure and Kinship coefficients

The Population structure analysis was conducted using genotypic data of 1081 DArT markers by using Structure Software 2.2 (Pritchard et al 2000), and the accessions subdivided into 12 subpopulations, base on the suggestion of Pritchard and Wen (2007), we used the burn-in time 50 000 and the number of replications (MCMC) was 100 000, the individuals placed into k clusters, we set k (the number of subpopulations) from 2 to 15 and performed 14 runs for k values, the population structure matrix (Q) was defined by running structure at K = 12 , where the highest likelihood has been obtained **(Fig.3a).**




(a) (b)

**Fig.3** (a) presents the number of clusters, which have the highest maximum likelihood, and (b) presents the percentage of the accessions in each cluster.

Table 3 presents the accessions, the country of origin, and the cluster number for each accession, the cluster 1 included 13 accessions (10.92%),10 out of them are Iranian accessions, 1 Jordan, and 2 accessions from Syria., Cluster 2 included 2 accessions (1.68%) from Jordan, Cluster 3 contained 4 accessions (3.36%) one from Iraq and 3 Iranian., 14 accession (12.61%) sited in cluster 4 one of them is unknown its origin, and all accession distribute in middle Asia, cluster 5 included 3 accessions (2.52%) from Palestine, Turkey, and Iran. 8 accessions (6.72%) in cluster 6 one from Jordan, one

from Palestine, and 6 from Syria, cluster 7 included 7 accessions (5.88%), one from Palestine and 6 from Syria. 23 (19.33%) and 8 (6.72%) accession originated in Middle East and cited in Cluster 8, and 9 respectively. On the other hand about 20.17 % of the population cited in cluster 10 (one Palestinian, one Iranian, one unknown, and 21 German Accessions). Nine accessions (7.56%) placed in cluster 11 from Jordan and Syria. Three accessions (2.52%) one from Iraq and tow turkey accessions placed in cluster 12. (See fig.3b).

**Figure 4** represents the distribution of pairwise kinship coefficients, more than 45 % of the values close to zero and more than 50% ranged from 0,04 up to 0,48.

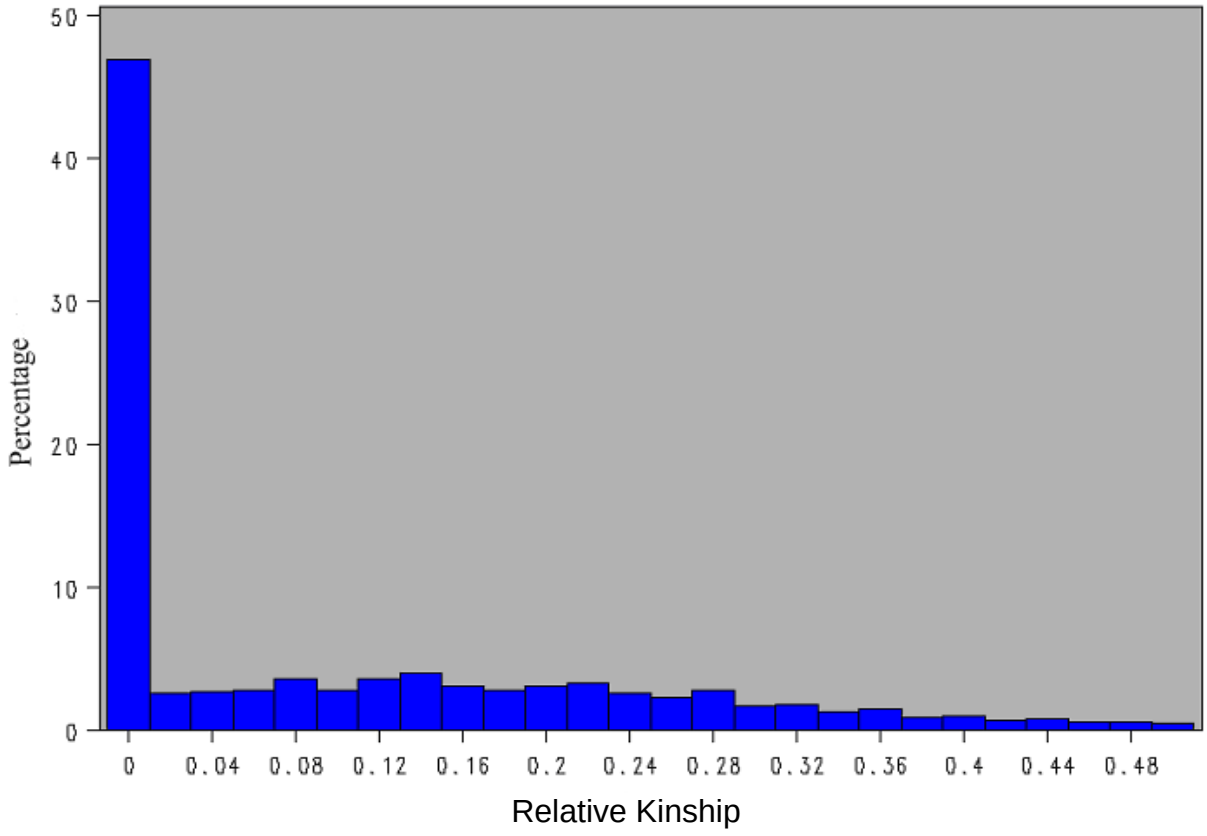



Fig. (4) The distribution of pairwise Loiselle et al (1995) kinship coefficients over the two years for barley population

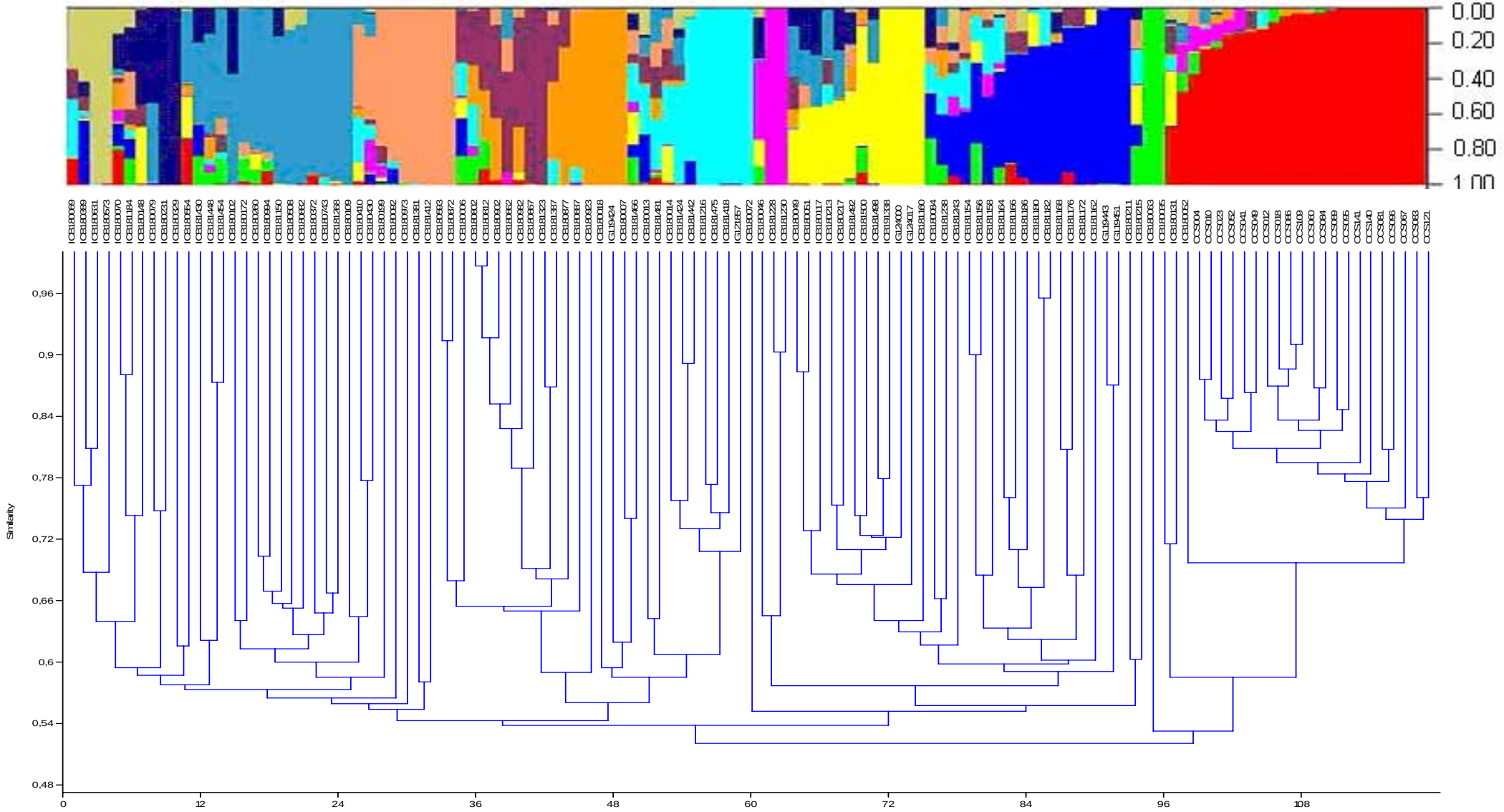



**Fig.5** The hierarchical clustering (UPGMA) of the accessions based on their genetic distances and the subdivision into 12 Groups according to the structure analysis and geographical distribution.

**Figure 5** shows that all accessions were distributed within the 12 groups according to the relatively genetic distances using structure and cluster analysis, in the colored part above the diagram each individual is represented by a single vertical line broken into k colored segments, with lengths proportional to each of the k inferred clusters or subgroups. Whereas the part below of the diagram represents the cluster analysis based on the DICE dissimilarity index and the unweighted neighbour-jointing method was performed on the 1081 DArT markers for 119 Accessions, twelve main clusters were identified which correspond well with genetic distances and origin of the genotypes.

### 3.4 Marker-trait associations

A mixed liner model (MLM) implemented in ASReml Software Version 2 according to (Stich et al. 2008) was used to conduct the association analysis and to identify the DArT markers associated with the drought related traits in the structured barley population based on population structure (Q-matrix) and relatedness relationship (K-matrix). The association of DArT markers with the studied traits is described in table (7 and 8).

### 3.5 The main effects and the predicted values of the significant markers

Table 7 shows the main effects and predicted values of the markers at 0.01 significance onto different chromosomes of barley genome for 18 traits.

#### 1) Wilting Score (WS)

Five markers were associated significantly with WS and located on the chromosomes 3H, 4H, 5H, 6H and 7 H. the markers which located on 3H and 5H affected negatively on this trait, while the others affected positively. The lowest and the highest predicted values (2.45 and 3.41) were observed for marker allele M1 on 3H (41.78 cM) and 6H (74.3 cM) respectively.

#### 2) Number of tillers/plant (TILS)

12 markers were associated significantly with TILS and distributed on the whole genome of Barley except chromosome 4H did not contain any marker associated with this trait, 4 markers out of them ((bPb-6848 on chr. 2H), (bPb-9945, and bPb-3278 on 3H), and bPb-6311 on 6H) had negatively effects on TILS, and the presence of the allele M1 led to decline that trait. In contrast, 8 markers had significant positive effects

Table 7 The main effects and the predicted values of the significant markers at 0, 01

| Trait | No | Marker | Marker Name | Chr | Pos. | FDR | Prob | Effect | | Predicted Value | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $M_0$ | $M_1$ | $M_0$ | $M_1$ |
| WS | 1 | M0290 | bPb-0312 | 3H | 41.78 | 0.0080 | ** | -1.61 | -2.86 | 2.97 | 2.45 |
| | 2 | M0153 | bPb-6949 | 4H | 72.21 | 0.0100 | ** | 0.00 | 1.71 | 2.75 | 3.86 |
| | 3 | M0372 | bPb-7569 | 5H | 125.98 | 0.0100 | ** | -3.00 | -2.63 | 2.78 | 2.79 |
| | 4 | M0158 | bPb-3068 | 6H | 74.30 | 0.0075 | ** | 0.75 | 2.16 | 2.77 | 3.41 |
| | 5 | M0628 | bPb-9104 | 7H | 127.40 | 0.0067 | ** | 1.28 | 1.45 | 2.63 | 2.98 |
| TILS | 1 | M0533 | bPb-9414 | 1H | 16.100 | 0.0015 | *** | 3.01 | 3.11 | 4.62 | 5.07 |
| | 2 | M0278 | bPb-6848 | 2H | 14.400 | 0.0012 | *** | -1.54 | -1.22 | 4.44 | 5.05 |
| | 3 | M0465 | bPb-8779 | 2H | 77.410 | 0.0016 | *** | 2.28 | 3.15 | 4.71 | 4.91 |
| | 4 | M0045 | bPb-9945 | 3H | 10.200 | 0.0090 | ** | -1.08 | -0.49 | 4.43 | 4.76 |
| | 5 | M0105 | bPb-3278 | 3H | 100.760 | 0.0012 | *** | -3.27 | -2.66 | 4.30 | 5.37 |
| | 6 | M0212 | bPb-1961 | 3H | 118.720 | 0.0018 | *** | 1.63 | 1.35 | 5.02 | 4.59 |
| | 7 | M0286 | bPb-8021 | 3H | 147.950 | 0.0060 | ** | 2.56 | 2.01 | 4.86 | 4.69 |
| | 8 | M0216 | bPb-7247 | 3H | 178.600 | 0.0087 | ** | 2.86 | 0.66 | 4.89 | 3.78 |
| | 9 | M0372 | bPb-7569 | 5H | 125.98 | 0.0017 | ** | 1.38 | 1.10 | 5.04 | 4.47 |
| | 10 | M0790 | bPb-6311 | 6H | 19.420 | 0.0053 | ** | -3.30 | -3.20 | 4.62 | 4.63 |
| | 11 | M0297 | bPb-0730 | 6H | 68.220 | 0.0015 | *** | 1.89 | 1.97 | 4.65 | 4.82 |
| | 12 | M0344 | bPb-1140 | 7H | 10.210 | 0.0015 | ** | 1.55 | 1.29 | 5.00 | 4.67 |
| SFW | 1 | M0684 | bPb-1628 | 2H | 70.03 | 0.0120 | ** | 1.23 | 1.50 | 7.94 | 8.31 |
| | 2 | M0281 | bPb-4040 | 2H | 82.13 | 0.0075 | ** | -0.17 | -0.27 | 8.09 | 7.79 |
| | 3 | M0603 | bPb-1105 | 7H | 68.80 | 0.0060 | ** | 0.12 | -0.33 | 8.17 | 7.70 |
| SDW | 1 | M0788 | bPb-0395 | 1H | 141.29 | 0.0096 | ** | 0.14 | 0.48 | 2.15 | 2.37 |
| | 2 | M0299 | bPb-9757 | 2H | 14.40 | 0.0120 | ** | -0.30 | -0.46 | 2.18 | 2.13 |
| | 3 | M0572 | bPb-2203 | 3H | 35.93 | 0.0210 | ** | -0.35 | -0.49 | 2.26 | 2.10 |
| | 4 | M0715 | bPb-2910 | 3H | 51.59 | 0.0090 | ** | 0.42 | -0.02 | 2.39 | 2.14 |
| | 5 | M0665 | bPb-0522 | 6H | 142.51 | 0.0140 | ** | -0.17 | 0.11 | 2.15 | 2.24 |
| | 6 | M0223 | bPb-9865 | 7H | 159.19 | 0.0105 | ** | -0.15 | 0.21 | 2.12 | 2.30 |
| RWC | 1 | M0033 | bPb-3574 | 2H | 49.03 | 0.0100 | ** | -14.89 | -36.88 | 79.40 | 72.40 |
| | 2 | M0247 | bPb-5755 | 2H | 133.29 | 0.0050 | ** | -0.77 | -9.83 | 80.34 | 77.13 |
| | 3 | M0715 | bPb-2910 | 3H | 51.59 | 0.0050 | ** | -12.25 | -2.16 | 76.65 | 79.47 |
| | 4 | M0007 | bPb-1408 | 4H | 60.04 | 0.0090 | ** | 3.88 | 19.77 | 77.24 | 80.47 |
| | 5 | M0397 | bPb-0182 | 7H | 123.08 | 0.0075 | ** | 29.74 | 13.61 | 79.85 | 77.35 |
| RV | 1 | M0644 | bPb-5683 | 1H | 62.98 | 0.0080 | ** | -8.06 | -6.39 | 79.59 | 84.30 |
| | 2 | M0336 | bPb-0326 | 2H | 139.91 | 0.0135 | ** | 2.22 | 6.65 | 79.21 | 94.51 |
| | 3 | M0103 | bPb-1217 | 5H | 184.45 | 0.0003 | *** | -7.29 | -3.56 | 80.69 | 86.34 |
| RL | 1 | M0392 | bPb-3389 | 1H | 76.78 | 0.0060 | ** | -4.82 | -3.94 | 28.61 | 29.61 |
| | 2 | M0271 | bPb-4898 | 1H | 94.90 | 0.0003 | *** | 0.76 | -3.32 | 28.99 | 28.40 |
| | 3 | M0786 | bPb-9504 | 4H | 67.92 | 0.0060 | ** | 0.58 | 2.65 | 28.23 | 29.99 |
| FWa | 1 | M0336 | bPb-0326 | 2H | 139.91 | 0.0080 | ** | 1.39 | 3.22 | 32.57 | 38.32 |
| | 2 | M0103 | bPb-1217 | 5H | 184.45 | 0.0027 | ** | -4.47 | -4.49 | 32.88 | 32.60 |
| | 3 | M0333 | bPb-8135 | 6H | 9.10 | 0.0040 | *** | 9.56 | 10.95 | 34.76 | 35.39 |
| | 4 | M0518 | bPb-5923 | 7H | 140.94 | 0.0020 | *** | -4.52 | -9.55 | 35.19 | 32.47 |

.Where, ** and *** are the significances differences at 0, 01 and 0,001 levels.

Whereas, $M_0$ and $M_1$ refer to the effect of the first allele (absence) and the second allele (presence) of the marker, respectively, Where, FDR refers to the Falsh Descovery Rate (Benjamini and Yekutieli 2005).

Table 7 (Continued)

| Trait | No | Marker | Marker Name | Chr. | Pos. | FDR | Prob. | Effect | | Predicted Value | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $M_0$ | $M_1$ | $M_0$ | $M_1$ |
| FWb | 1 | M0136 | bPb-1566 | 2H | 149.44 | 0.0050 | ** | -7.04 | -6.21 | 18.53 | 18.92 |
| | 2 | M0420 | bPb-5312 | 3H | 148.83 | 0.0070 | ** | -0.57 | 1.35 | 16.76 | 20.35 |
| | 3 | M0507 | bPb-1807 | 5H | 21.50 | 0.0050 | ** | -5.07 | -13.12 | 18.40 | 16.94 |
| | 4 | M0798 | bPb-0050 | 5H | 30.98 | 0.0075 | ** | 4.62 | 0.16 | 17.32 | 19.97 |
| | 5 | M0145 | bPb-0572 | 6H | 17.86 | 0.0033 | ** | -3.69 | -6.87 | 18.42 | 17.87 |
| FWc | 1 | M0392 | bPb-3389 | 1H | 76.78 | 0.0090 | ** | -4.05 | -2.70 | 5.03 | 5.65 |
| | 2 | M0292 | bPb-3805 | 3H | 72.18 | 0.0080 | *** | -2.47 | -0.37 | 3.84 | 6.13 |
| | 3 | M0627 | bPb-7277 | 5H | 139.48 | 0.0004 | *** | -9.61 | -11.63 | 5.36 | 4.54 |
| | 4 | M0554 | bPb-3722 | 6H | 68.53 | 0.0080 | ** | 3.96 | -1.26 | 5.70 | 3.46 |
| RFW | 1 | M0052 | bPb-4531 | 1H | 60.21 | 0.0023 | ** | -9.76 | -12.93 | 59.03 | 55.44 |
| | 2 | M0298 | bPb-7229 | 2H | 38.97 | 0.0070 | ** | -12.57 | -8.47 | 55.02 | 67.95 |
| | 3 | M0420 | bPb-5312 | 3H | 148.83 | 0.0093 | ** | -1.79 | 2.09 | 53.77 | 62.54 |
| | 4 | M0798 | bPb-0050 | 5H | 30.98 | 0.0018 | ** | 4.34 | 8.24 | 55.88 | 61.08 |
| | 5 | M0132 | bPb-2960 | 5H | 134.93 | 0.0080 | ** | 1.89 | 4.47 | 48.88 | 60.52 |
| | 6 | M0333 | bPb-8135 | 6H | 9.10 | 0.0007 | *** | 15.95 | 19.86 | 58.47 | 60.64 |
| | 7 | M0518 | bPb-5923 | 7H | 140.94 | 0.0004 | *** | -8.94 | -15.59 | 59.03 | 55.81 |
| DWa | 1 | M0336 | bPb-0326 | 2H | 139.91 | 0.0120 | ** | 0.36 | 0.99 | 5.76 | 7.30 |
| | 2 | M0333 | bPb-8135 | 6H | 9.10 | 0.0080 | ** | 3.14 | 3.17 | 6.35 | 6.36 |
| | 3 | M0125 | bPb-8049 | 7H | 53.43 | 0.0080 | ** | 1.52 | -16.44 | 6.38 | 4.03 |
| | 4 | M0518 | bPb-5923 | 7H | 140.94 | 0.0060 | ** | -1.90 | -4.68 | 6.27 | 5.91 |
| DWb | 1 | M0420 | bPb-5312 | 3H | 148.83 | 0.0100 | ** | -0.64 | 0.52 | 2.38 | 2.85 |
| | 2 | M0507 | bPb-1807 | 5H | 21.50 | 0.0075 | ** | -0.19 | -0.85 | 2.61 | 2.34 |
| | 3 | M0398 | bPb-0786 | 5H | 57.00 | 0.0080 | ** | -1.24 | -1.30 | 2.57 | 2.65 |
| | 4 | M0145 | bPb-0572 | 6H | 17.86 | 0.0200 | ** | -0.92 | -0.71 | 2.53 | 2.60 |
| | 5 | M0125 | bPb-8049 | 7H | 53.43 | 0.0125 | ** | 0.00 | -0.99 | 2.74 | 1.72 |
| DWc | 1 | M0052 | bPb-4531 | 1H | 60.21 | 0.0105 | ** | -0.29 | -0.32 | 0.67 | 0.69 |
| | 2 | M0124 | bPb-8530 | 2H | 149.09 | 0.0070 | ** | -0.37 | -0.39 | 0.68 | 0.62 |
| | 3 | M0292 | bPb-3805 | 3H | 72.18 | 0.0070 | ** | -0.39 | -0.12 | 0.50 | 0.77 |
| | 4 | M0798 | bPb-0050 | 5H | 30.98 | 0.0070 | ** | 0.16 | 0.40 | 0.59 | 0.86 |
| | 5 | M0669 | bPb-6676 | 5H | 81.39 | 0.0080 | ** | -0.52 | -0.31 | 0.47 | 0.69 |
| | 6 | M0627 | bPb-7277 | 5H | 139.48 | 0.0070 | ** | -1.34 | -1.65 | 0.69 | 0.49 |
| | 7 | M0554 | bPb-3722 | 6H | 68.53 | 0.0070 | ** | -0.68 | 0.01 | 0.74 | 0.76 |
| RDW | 1 | M0038 | bPb-0429 | 1H | 52.77 | 0.0088 | ** | 2.62 | 3.44 | 9.68 | 10.11 |
| | 2 | M0052 | bPb-4531 | 1H | 60.21 | 0.0070 | ** | -2.84 | -3.02 | 9.74 | 8.83 |
| | 3 | M0336 | bPb-0326 | 2H | 139.91 | 0.0075 | ** | 0.59 | 0.88 | 8.99 | 10.87 |
| | 4 | M0507 | bPb-1807 | 5H | 21.50 | 0.0067 | ** | -1.55 | -3.21 | 9.48 | 8.62 |
| | 5 | M0333 | bPb-8135 | 6H | 9.10 | 0.0100 | ** | 4.20 | 4.66 | 9.62 | 9.92 |
| RSR | 1 | M0298 | bPb-7229 | 2H | 38.97 | 0.0075 | ** | -4.33 | 9.99 | 39.35 | 51.25 |
| | 2 | M0507 | bPb-1807 | 5H | 21.50 | 0.0060 | ** | -8.76 | -19.51 | 42.14 | 36.24 |
| | 3 | M0125 | bPb-8049 | 7H | 53.43 | 0.0060 | ** | 29.23 | -7.60 | 43.35 | 23.77 |
| OP | 1 | M0669 | bPb-6676 | 5H | 81.39 | 0.0080 | ** | 0.01 | 0.02 | 0.14 | 0.16 |
| | 2 | M0117 | bPb-1009 | 6H | 13.83 | 0.0080 | ** | -0.06 | -0.08 | 0.16 | 0.15 |
| PC | 1 | M0651 | bPb-3217 | 1H | 40.53 | 0.0090 | ** | -0.62 | 0.36 | 5.50 | 7.67 |
| | 2 | M0440 | bPb-8833 | 7H | 147.17 | 0.0140 | ** | 1.47 | 1.50 | 4.31 | 5.77 |

on TILS, The allele M1 of markers bPb-3278 and bPb-7247 on 3H had the lowest and highest predicted values (3.78 and 5.37), respectively. The marker bpb-6848 on 2H (14.40 cM) was found to be associated with SDW too (Fig. 6)

**3) Shoot fresh weight (SFW)**

Shoot fresh weight/plant was associated only with three markers, which were located onto chromosomes 2H (two markers) and one onto 7H. The marker (bPb-1628 on 2H) affected positively that trait, whereas markers (bPb-4040 on 2H and bPb-1105 on 7H) affected negatively. In regarding to the lowest and highest predicted values of that trait were (7.70 and 8.31) for markers bPb-1628 on 2H and bPb-1105 on 7H, respectively in relation to M1.

**4) Shoot dry weight (SDW)**

A total of six marker loci main effect were significantly associated with SDW, where three markers (bPb-0395 on 1H, bPb-0522 on 6H and bPb-9865 on 7H) had a positive effect and were increased the SDW with marker allele M1, while the others markers were affected negatively. Furthermore the highest predicted value (2.37) was obtained in the marker allele M1 (bPb-0395, which located in region 141.29 cM on 1H) and the lowest predicted value (2.10 cM) obtained in marker bPb-2203 on 3H.

**5) Relative Water Content (RWC)**

Five Marker loci were associated significantly with RWC and distributed in chromosomes 2H (tow markers), 3H, 4H and 7H, three markers on chromosome 2H and 3H were affected negatively, and two markers on chromosomes 4H and 7H had positive effect on RWC. The lowest and highest predicted values (72.40 and 80.47) of RWC were obtained for marker bPb-3574, on 2H, and bPb-1408 on 4H, respectively.

**6) Root volume (RV)**

Three markers bPb-5683, bPb-0326 and bPb-1217 on chromosome 1H, 2H and 5H respectively were associated significantly with root volume, whereas the first and the third one had negative effect while the second marker was affected positively with RV. The lowest and highest predicted values for RV (84.30 and 94.51) have been found on chromosomes 1H and 2H respectively. The marker bpb-0326 on 2H (139.91 cM) was

collocated with RV, FWa, DWa and RDW (Fig.6 and table 9); furthermore this marker was responsible of improving these traits under drought stress conditions.

**7) Root length (RL)**

Three marker loci associated with RL (bPb-3389 , bPb-4898) on 1H and bPb-9504 on 4H, the marker allele M1 was declined the trait on 1H and increased this traits on 4H, where the lowest and highest predicted values for RL (28.40 and 29.99) were obtained to marker allele M1 on 1H and 4H, respectively.

**8) Root fresh weight part a (FWa)**

Four markers were highly significantly associated with FWa and located on chromosomes 2H, 5H, 6H and 7H, the marker loci on 5H and 7H were correlated negatively, and the marker loci on 2H and 6H were correlated positively with this trait. The lowest and the highest predicted values for FWa (32.47 and 38.32) were observed for marker allele M1 of bPb-5923 in location 139.91 cM on 2H and bPb-0326 in 140.94 cM on 7H, respectively.

The markers bpb-8135 and bpb-5923 on chromosomes 6H (9.10 cM) and 7H (140.94 cM) respectively were collocated with RFW (Fig.6).

**9) Root fresh weight part b (FWb)**

Five marker loci were correlated significantly with FWb and detected on chromosomes 2H, 3H, 5H (two markers) and 6H, the marker bPb-1566 on 2H, bPb-1807 on 5H and bPb-0572 on 6H were negatively affected on this trait and the marker bPb-5312 on 3H and bPb-0050 on 5H had positive effect, the lowest and highest predicted values (16.94 and 20.35) were observed for marker allele M1 on 5H and 3H respectively. The marker bpb-5312 on 3H (148.83 cM) was collocated with FWb and RFW (Fig.6).

**10) Root fresh weight part c (FWc)**

A total of four markers were highly significantly associated with FWc, and these markers bPb-3389, bPb-3805, bPb-7277 and bPb-3722 were located on 1H, 3H, 5H and 6H respectively. These entire markers affected negatively on FWc, whereas the lowest and highest predicted values (3.46 and 6.13) were detected to marker allele M1 of bPb-3722 on 6H and bPb-3805 on 3H, respectively.

**11) Total root fresh weight (RFW)**

Among 7 marker loci associated significantly with RFW which located on all chromosomes except 4H, three markers located on 1H, 2H and 7H were affected negatively on this trait, and the others were affected positively. The chromosomes 1H and 2H were the responsible for giving the lowest and the highest predicted values (55.44 and 67.95) respectively.

**12) Root Dry Weight part a (DWa)**

DWa was associated significantly with four markers and these markers were distributed onto three chromosomes 2H, 6H and 7H, only two markers on 7H had negatively main effects on DWa, while the others had positive effects. Regarding to the predicted effects of the markers, the lowest and the highest values were 4.03 and 7.30 for chromosomes 7H and 2H, respectively. The markers bpb-0326 and bpb-8135 on chromosomes 2H (139.91 cM) and 6H (9.10 cM) respectively were found to be collocated with DWa and RDW (Fig.6).

**13) Root Dry Weight part b (DWb)**

In this trait, there were five markers correlated significantly with DWb, and distributed onto chromosomes 3H, 5H (two markers), 6H and 7H. Only the marker bpb-5312 on chromosome 3H affected positively on DWb, while the other markers were affected negatively on this trait. The lowest and highest predicted values (1.72 and 2.85) were detected for chromosome 7H and 3H respectively. The marker bpb-1807 on 5H (21.50 cM) was correlated with DWb and RDW (Fig.6).

**14) Root Dry Weight part c (DWc)**

Seven marker loci were correlated significantly with DWc and distributed on chromosomes 1H, 2H, 3H, 5H (three markers) and 6H. The majority of the markers had negative main effects, while only two markers bPb-0050 on 5H and bPb-3722 on 6H had positive main effect. The lowest and highest predicted values (0.49 and 0.86) were observed for marker allele M1 of bpb-7277 and bPb-0050 on 5H, respectively. The marker bpb-4531 on 1H (60.21 cM) was correlated with DWc and RDW (Fig.6).

**15) Total Root Dry Weight (RDW)**

Five marker loci were correlated significantly with RDW and distributed on chromosomes 1H (two markers), 2H, 5H and 6H, two markers out of them on 1H (60.21 cM) and 5H (21.50 cM) had negative main effect and the others had positive effect on this trait, the lowest and highest predicted values (8.83 and 10.87) were observed for marker allele M1 on 1H (60.21 cM) and 2H (139.91 cM), respectively.

**16) Root Shoot ratio (RSR)**

In this trait, there were three markers correlated significantly with RSR, and located on chromosomes 2H, 5H and 7H. The marker loci on 5H and 7H affected negatively and the marker locus on chromosome 2H affected positively with this trait, whereas the lowest and highest predicted values (23.77 and 51.25) were detected for markers on chromosomes 7H and 2H respectively. The marker bpb-1807 on 5H (21.50 cM) was associated with RSR, FWb and DWb (fig.6).

**17) Osmotic potential (OP)**

Only two markers were associated significantly with osmotic potential, where the marker bPb-6676 on 5H (81.39 cM) affected positively with highest predicted value (0.16), while the marker bPb-1009 on 6H (13.83 cM) affected negatively with lowest predicted value (0.15).

**18) Proline content (PC)**

Two markers bpb-3217 and bpb-8833, which were located on chromosomes 1H (40.53 cM) and 7H (147.17 cM), were correlated significantly with PC and had positive main effect with predicted values 7.67 and 5.77, respectively.

### 3.6 The interaction effects between markers and drought treatments

Table 8 shows the effects and predicted values of the significant markers at 0.01 levels under well-watered and drought stress treatments.

#### 1) Wilting score (WS)

Three markers were found to be associated with WS and located on chromosomes 4H, 5H and 7H and these markers had negative effects under well-watered treatment and positive effects under drought stress treatment, where the marker locus on 5H had the highest predicted values (4.77), while markers on 4H and 7H had the lowest predicted values under drought water stress treatment at presence of marker allele M1 comparing with the absence of the marker allele (M0). The marker bpb-1408 at 60.04 cM on 4H was found to be associated positively with RWC, RL, FWc and PC. Marker bpb-0786 at 57.00 cM on 5H was correlated negatively with WS and DWc and positively with RFW and DWb (table 9 and fig.6).

#### 2) Number of tillers/plant (TILS)

Among four markers which were correlated with this trait, only one marker (on 4H) had positive effect and highest predicted value (4.94), while the others had negative effects and lowest predicted values that was at the presence of marker allele M1 under drought stress treatment, comparing with the absence of the marker allele (M0). All markers had positive effects under well-watered treatment for this trait. The marker bpb-4990 on 4H (64.16 cM) was collocated with TILS and SDW (Fig.6 and table 9).

#### 3) Shoot Fresh Weight (SFW)

Total of five markers which were correlated with shoot fresh weight under drought stress treatment and distributed on 1H, 2H (two markers), 5H and 6H, were affected negatively and gave lower predicted values at the presence of marker allele M1 under drought stress treatment than those under well-watered treatment.

Table 8 the effect and predicted values of the significant markers under well-watered and drought stress conditions.

| Trait | No. | Marker | Marker name | Chr | Pos. | FDR | Prob | Effect | | | | Predicted Value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $M_0D$ | $M_1D$ | $M_0W$ | $M_1W$ | $M_0D$ | $M_1D$ | $M_0W$ | $M_1W$ |
| WS | 1 | M0007 | bPb-1408 | 4H | 60.04 | 0.0030 | ** | 2.05 | 1.65 | -2.75 | -1.28 | 4.88 | 4.48 | 0.71 | 1.14 |
| | 2 | M0398 | bPb-0786 | 5H | 57.00 | 0.0030 | ** | 1.68 | 1.94 | -0.5 | -1.42 | 4.51 | 4.77 | 1.27 | 0.74 |
| | 3 | M0065 | bPb-9912 | 7H | 94.41 | 0.0020 | ** | 2.03 | 1.2 | -2.49 | -1.4 | 4.86 | 4.03 | 1.06 | 1.1 |
| TILS | 1 | M0516 | bPb-4990 | 4H | 64.16 | 0.0053 | ** | -0.10 | 0.29 | 5.04 | 4.82 | 4.55 | 4.94 | 4.82 | 4.56 |
| | 2 | M0694 | bPb-3427 | 6H | 38.04 | 0.0090 | ** | 0.02 | -0.11 | 0.41 | 1.23 | 4.68 | 4.54 | 4.58 | 5.00 |
| | 3 | M0043 | bPb-7179 | 6H | 58.56 | 0.0040 | ** | -0.06 | -0.07 | 2.59 | 2.16 | 4.59 | 4.58 | 4.89 | 4.60 |
| | 4 | M0175 | bPb-8524 | 7H | 58.02 | 0.0040 | ** | -0.04 | -0.20 | 1.99 | 2.41 | 4.61 | 4.46 | 4.57 | 4.85 |
| SFW | 1 | M0287 | bPb-2055 | 1H | 12.96 | 0.0150 | ** | -1.76 | -1.80 | 1.71 | 2.83 | 6.24 | 6.20 | 9.72 | 10.09 |
| | 2 | M0299 | bPb-9757 | 2H | 14.4 | 0.0250 | ** | -1.65 | -2.07 | -2.56 | -1.50 | 6.35 | 5.93 | 9.55 | 9.98 |
| | 3 | M0739 | bPb-9199 | 2H | 145.95 | 0.0125 | ** | -1.84 | -1.72 | 1.31 | 1.87 | 6.16 | 6.28 | 8.99 | 10.11 |
| | 4 | M0811 | bPb-2314 | 5H | 163.75 | 0.0113 | ** | -1.78 | -1.72 | -0.45 | 0.89 | 6.22 | 6.28 | 9.29 | 10.29 |
| | 5 | M0297 | bPb-0730 | 6H | 68.22 | 0.0090 | ** | -1.76 | -1.71 | -1.80 | -1.05 | 6.24 | 6.29 | 9.33 | 10.14 |
| SDW | 1 | M0299 | bPb-9757 | 2H | 14.40 | 0.0120 | ** | -0.15 | -0.29 | -0.71 | 0.42 | 2.03 | 1.89 | 2.33 | 2.38 |
| | 2 | M0516 | bPb-4990 | 4H | 64.16 | 0.0004 | *** | -0.20 | 0.17 | 2.67 | 2.86 | 1.98 | 2.01 | 2.38 | 2.41 |
| | 3 | M0665 | bPb-0522 | 6H | 142.51 | 0.0200 | ** | -0.20 | 0.13 | -0.63 | 0.48 | 1.98 | 2.05 | 2.32 | 2.43 |
| | 4 | M0477 | bPb-2478 | 7H | 35.22 | 0.0120 | ** | -0.18 | -0.21 | -1.09 | 0.81 | 2.00 | 1.97 | 2.36 | 2.41 |
| RWC | 1 | M0381 | bPb-4830 | 3H | 138.85 | 0.0006 | *** | -5.57 | 9.66 | 5.63 | -7.12 | 63.64 | 69.55 | 91.72 | 89.29 |
| | 2 | M0007 | bPb-1408 | 4H | 60.04 | 0.0003 | *** | -6.65 | 8.93 | -4.57 | -19.20 | 62.56 | 70.28 | 91.92 | 90.67 |
| | 3 | M0559 | bPb-6029 | 7H | 16.21 | 0.0002 | *** | -9.83 | -14.35 | 2.20 | 13.68 | 69.38 | 64.86 | 89.68 | 92.42 |
| | 4 | M0341 | bPb-7915 | 7H | 87.55 | 0.0002 | *** | -10.33 | -15.26 | 0.00 | 12.46 | 68.88 | 63.95 | 90.16 | 92.72 |
| | 5 | M0397 | bPb-0182 | 7H | 123.08 | 0.0001 | *** | -10.17 | -16.27 | -25.97 | 1.29 | 69.04 | 62.94 | 90.67 | 91.75 |
| | 6 | M0022 | bPb-5898 | 7H | 149.4 | 0.0010 | ** | -7.19 | -12.98 | -7.55 | 1.14 | 72.02 | 66.23 | 90.18 | 91.30 |
| RV | 1 | M0028 | bpb-9767 | 1H | 63.32 | 0.0050 | ** | 8.31 | -8.96 | -8.91 | 0.50 | 91.45 | 74.18 | 79.36 | 94.64 |
| | 2 | M0268 | bpb-5519 | 2H | 15.76 | 0.0060 | ** | -2.11 | 5.83 | 6.11 | -6.79 | 73.03 | 77.31 | 94.96 | 93.66 |
| RL | 1 | M0052 | bPb-4531 | 1H | 60.21 | 0.0070 | ** | 2.48 | 1.27 | 4.04 | 8.03 | 31.44 | 30.23 | 26.23 | 28.03 |
| | 2 | M0033 | bPb-3574 | 2H | 49.03 | 0.0004 | *** | 2.46 | -1.20 | -14.69 | -7.50 | 31.42 | 27.76 | 26.49 | 29.51 |
| | 3 | M0216 | bPb-7247 | 3H | 178.60 | 0.0080 | ** | 2.40 | 1.10 | 5.56 | 9.00 | 31.36 | 30.06 | 26.56 | 28.60 |
| | 4 | M0007 | bPb-1408 | 4H | 60.04 | 0.0080 | ** | 2.64 | 0.63 | 2.08 | 6.29 | 31.60 | 29.59 | 26.51 | 27.73 |

Table 8 (Continued)

| Trait | No | Marker | Marker name | Chr | Pos. | FDR | Prob | Effect | | | | Predicted Value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $M_0D$ | $M_1D$ | $M_0W$ | $M_1W$ | $M_0D$ | $M_1D$ | $M_0W$ | $M_1W$ |
| FWa | 1 | M0517 | bPb-5289 | 3H | 35.93 | 0.0002 | *** | -6.42 | -10.93 | 0.00 | -20.69 | 27.86 | 23.35 | 45.25 | 29.70 |
| | 2 | M0103 | bPb-1217 | 5H | 184.45 | 0.0060 | ** | -3.62 | 4.68 | -16.03 | -12.93 | 25.66 | 29.60 | 40.11 | 41.61 |
| FWb | 1 | M0054 | bPb-8112 | 1H | 141.85 | 0.0001 | *** | -3.07 | 1.50 | 14.89 | -2.69 | 15.54 | 20.11 | 22.08 | 18.58 |
| $FW_C$ | 1 | M0007 | bPb-1408 | 4H | 60.04 | 0.0001 | *** | 0.56 | 3.31 | 3.67 | -0.42 | 5.84 | 8.59 | 3.50 | 2.34 |
| RFW | 1 | M0054 | bPb-8112 | 1H | 141.29 | 0.0003 | *** | -9.07 | 1.24 | 15.02 | -10.49 | 49.10 | 59.41 | 66.51 | 61.94 |
| | 2 | M0398 | bPb-0786 | 5H | 57.00 | 0.0015 | ** | -10.51 | 4.19 | 8.15 | -10.81 | 47.66 | 53.98 | 66.90 | 62.13 |
| | 3 | M0132 | bPb-2960 | 5H | 134.93 | 0.0090 | ** | -12.96 | 6.09 | 4.60 | 27.22 | 45.21 | 52.08 | 52.55 | 68.97 |
| DWa | 1 | M0517 | bPb-5289 | 3H | 35.93 | 0.0002 | *** | -0.47 | -1.30 | 0.00 | -4.81 | 5.73 | 4.90 | 7.84 | 4.28 |
| | 2 | M0125 | bPb-8049 | 7H | 53.43 | 0.0010 | ** | -0.50 | -2.53 | 6.44 | -17.90 | 5.70 | 3.67 | 7.06 | 4.40 |
| DWb | 1 | M0507 | bPb-1807 | 5H | 21.50 | 0.0010 | ** | 0.04 | -0.21 | -1.92 | -1.98 | 2.68 | 2.43 | 2.54 | 2.25 |
| | 2 | M0117 | bPb-1009 | 6H | 13.83 | 0.0002 | *** | 0.03 | -0.21 | -1.09 | 1.01 | 2.67 | 2.43 | 2.50 | 3.48 |
| DWc | 1 | M0038 | bPb-0429 | 1H | 52.77 | 0.0030 | ** | 0.39 | 0.36 | 0.11 | 0.36 | 1.07 | 1.04 | 0.12 | 0.37 |
| | 2 | M0124 | bPb-8530 | 2H | 149.09 | 0.0004 | *** | 0.37 | 0.35 | 0.41 | 0.32 | 1.05 | 1.03 | 0.31 | 0.22 |
| | **3** | M0398 | bPb-0786 | 5H | 57.00 | 0.0013 | ** | 0.42 | 0.36 | 0.25 | 0.56 | 1.10 | 1.04 | 0.15 | 0.40 |
| | 4 | M0554 | bPb-3722 | 6H | 68.53 | 0.0002 | *** | 0.53 | -0.07 | -0.36 | 0.31 | 1.21 | 0.61 | 0.26 | 0.31 |
| RDW | 1 | M0136 | bPb-1566 | 2H | 149.44 | 0.0090 | ** | -0.35 | 0.28 | -15.21 | -17.90 | 9.17 | 9.80 | 9.85 | 9.32 |
| | 2 | M0517 | bPb-5289 | 3H | 35.93 | 0.0002 | *** | -0.02 | -1.17 | 0.00 | -5.48 | 9.50 | 8.35 | 10.87 | 6.71 |
| RSR | 1 | M0258 | bPb-7199 | 3H | 13.67 | 0.0060 | ** | 0.84 | 2.26 | -62.50 | -61.02 | 42.95 | 44.37 | 38.61 | 42.62 |
| | 2 | M0061 | bPb-2828 | 7H | 36.53 | 0.0003 | *** | 1.28 | 1.98 | -20.92 | -21.11 | 43.39 | 44.09 | 39.86 | 40.05 |
| | 3 | M0175 | bPb-8524 | 7H | 58.02 | 0.0090 | ** | 1.89 | -0.46 | -50.81 | -57.52 | 44.00 | 42.57 | 42.82 | 37.54 |
| OP | 1 | M0701 | bPb-8884 | 1H | 53.19 | 0.0075 | ** | 0.01 | -0.01 | -0.02 | 0.02 | 0.17 | 0.15 | 0.14 | 0.16 |
| | 2 | M0446 | bPb-5334 | 1H | 67.88 | 0.0050 | ** | 0.01 | 0.01 | -0.09 | 0.07 | 0.17 | 0.17 | 0.14 | 0.16 |
| | 3 | M0136 | bPb-1566 | 2H | 149.44 | 0.0060 | ** | 0.01 | 0.01 | -0.13 | 0.12 | 0.17 | 0.17 | 0.15 | 0.16 |
| PC | 1 | M0651 | bPb-3217 | 1H | 40.53 | 0.0120 | ** | 4.38 | 8.63 | 0.37 | -0.49 | 10.26 | 14.51 | 0.73 | 0.82 |
| | 2 | M0836 | bPb-0870 | 3H | 1.48 | 0.0160 | ** | 5.74 | 4.43 | -0.20 | -0.51 | 11.62 | 10.31 | 1.42 | 0.67 |
| | 3 | M0539 | bPb-6228 | 3H | 147.95 | 0.0107 | ** | 4.45 | 5.20 | 1.23 | -0.21 | 10.33 | 11.08 | 1.03 | 0.11 |
| | 4 | M0007 | bPb-1408 | 4H | 60.04 | 0.0080 | ** | 2.75 | 6.91 | 0.29 | -0.14 | 8.63 | 12.79 | 0.88 | 0.66 |

Where, ** and *** are the significances differences at 0, 01 and 0,001 levels.
Whereas, M0 and M1 refer to the effect of the first allele (absence) and the second allele (presence) of the marker, respectively.
Where, FDR refers to the Falsh Discovery Rate (Benjamini and Yekutieli 2005).

**4) Shoot dry weight (SDW)**

For the trait SDW four marker loci were detected on chromosomes 2H, 4H, 6H and 7H, two markers which was located on 4H (64.16 cM) and 6H (142.51 cM) had positive effect and highest predicted values (2.01 and 2.05) for marker allele M1 under water stress, while the other two markers on 2H and 7H had negative effects and lowest predicted values (1.89 and 1.97) at the presence of marker (M1) under water stress comparing with absence of marker (M0). While all these markers had positive effects and high predicted values under well-watered treatment.

**5) Relative water content (RWC)**

A total of six marker loci associated significantly with RWC as Interaction effects and located on chromosomes 3H, 4H and 7H (four markers), the first two markers on chromosome 3H and 4H had positive and negative effect with high (69.55 and 70.28) and low (89.29 and 90.67) predicted values, while the others markers had negative and positive effect with low and high predicted values at the presence of marker allele M1 under drought and well-watered treatment, respectively comparing with absence of the marker allele (M0).

**6) Root volume (RV)**

Only two markers on chromosomes 1H (63.32 cM) and 2H (15.76 cM) associated significantly with root volume as interaction effect, the first marker locus affected negatively and declined the trait under water stress, where the predicted values for marker alleles M1 and M0 under water stress condition were 74.18 and 91.45 respectively. While the second marker locus affected positively and improved the trait under drought stress, where the predicted values for both marker alleles M1 and M0 were 77.31 and 73.03 under drought stress.

**7) Root length (RL)**

Among four marker loci were associated with root length and located on chromosomes 1H, 2H, 3H and 4H. Only the marker bpb-3574 on 2H (49.03 cM) affected negatively and had low predicted value (27.76 and 29.51) for marker allele M1 under drought stress and well-watered conditions, respectively, while the predicted values of the other markers for marker allele M1 were higher under drought stress conditions than under well-watered conditions.

**8) Root fresh weight part a (FWa)**

Two marker loci associated significantly with FWa and located on chromosome 3H (35.93 cM) and 5H (184.45 cM), the marker bpb-1217 on 5H affected positively on this trait and had the higher predicted value (29.60), while the marker bpb-5289 on 3H affected negatively and had the lowest predicted value (23.35) at the presence of marker allele M1 under drought stress conditions. Furthermore, they had negative effect on this trait under well-watered treatment.

**9) Root fresh weight part b (FWb)**

The marker bpb-8112 was identified on chromosome 1H (141.85 cM), associated significantly and affected positively on this trait, whereas it exhibited the highest predicted values (20.11) under drought stress for marker allele M1, on the other hand this marker affected negatively and declined this traits under well-watered conditions at the presence of marker allele M1, and had low predicted value (18.58).

**10) Root fresh weight part c (FWc)**

Single marker locus bpb-1408 was associated with FWc on chromosome 4H (60.04 cM), this marker had positive and negative effects and the predicted values for marker allele M1 were (8.59 and 2.34) in drought and well-watered treatments, respectively.

**11) Total root fresh weight (RFW)**

A total of three markers on chromosomes 1H and 5H (two markers) were affected positively and gave high predicted values for marker allele M1 under drought stress conditions, and affected negatively except the marker bpb-2960 on 5H (134.93 cM) affected positively on this trait with high predicted value (68.97) under well-watered treatment at the presence of marker (M1).

**12) Root dry weight part a (DWa)**

Among two marker loci were detected on chromosomes 3H (35.93 cM) and 7H (53.43 cM), were affected negatively and had low predicted values (4.90 and 3.67) and (4.28 and 4.40) on drought and well-watered treatments at the presence of marker allele M1, respectively.

**13) Root dry weight part b (DWb)**

Two marker loci which associated significantly with this trait and located on chromosomes 5H (21.50 cM) and 6H (13.83 cM), were affected negatively and had low predicted values under drought stress for marker allele M1, The marker on 5H had negative effect with predicted value 2.25 and the other marker on 6H had positive effect with predicted value 3.48 under well-watered treatment.

**14) Root dry weight part c (DWc)**

Four markers on chromosome 1H, 2H, 5H and 6H were associated significantly with DWc, and the first three marker of them had positive effect and high predicted values, but the last one affected negatively with low predicted value (0.61) under drought stress at marker allele M1, and all of them had higher predicted values under drought stress than under well-watered at the presence of marker allele M1.

**15) Total root dry weight (RDW)**

Two marker loci were detected on chromosome 2H (149.44 cM) and 3H (35.93 cM) and correlated with RDW, and had positive and negative effect with high and low predicted value (9.80 and 8.35) respectively, while both of them had a negative effect and low predicted values (9.32 and 6.71) at marker allele M1 under drought stress and under well-watered treatment, respectively.

**16) Root shoot ratio (RSR)**

Among three marker were associated with RSR on chromosomes 3H and 7H (two markers), the first two markers on 3H and 7H affected positively and had high predicted values (44.37 and 44.09) at the presence of marker allele M1 under drought stress treatment, but the third one on 7H affected negatively and had the lowest predicted values (42.57 and 37.54) at marker allele M1 under drought and well-watered treatment respectively, however, the presence of marker allele M1 gave high predicted values for all markers under drought stress conditions.

**17) Osmotic potential (OP)**

Three markers loci on chromosome 1H (two markers) and 2H were associated significantly as interaction effect with trait OP, the second and third one had positive effect with high predicted values, while the first marker on chromosome 1H (53.19 cM)

had negative effect with low predicted values (0.15 and 0.16) in the presence of marker allele M1 under both of drought stress and well-watered conditions, respectively.

**18) Proline content (PC)**

A total of four marker loci were associated significantly with proline content as interaction effect and located on chromosomes 1H, 3H (two markers) and 4H, affected positively and negatively with high and low predicted values at marker allele M1 under drought stress and well-watered conditions respectively. Generally, the predicted values of PC were higher under drought stress than under well-watered conditions.

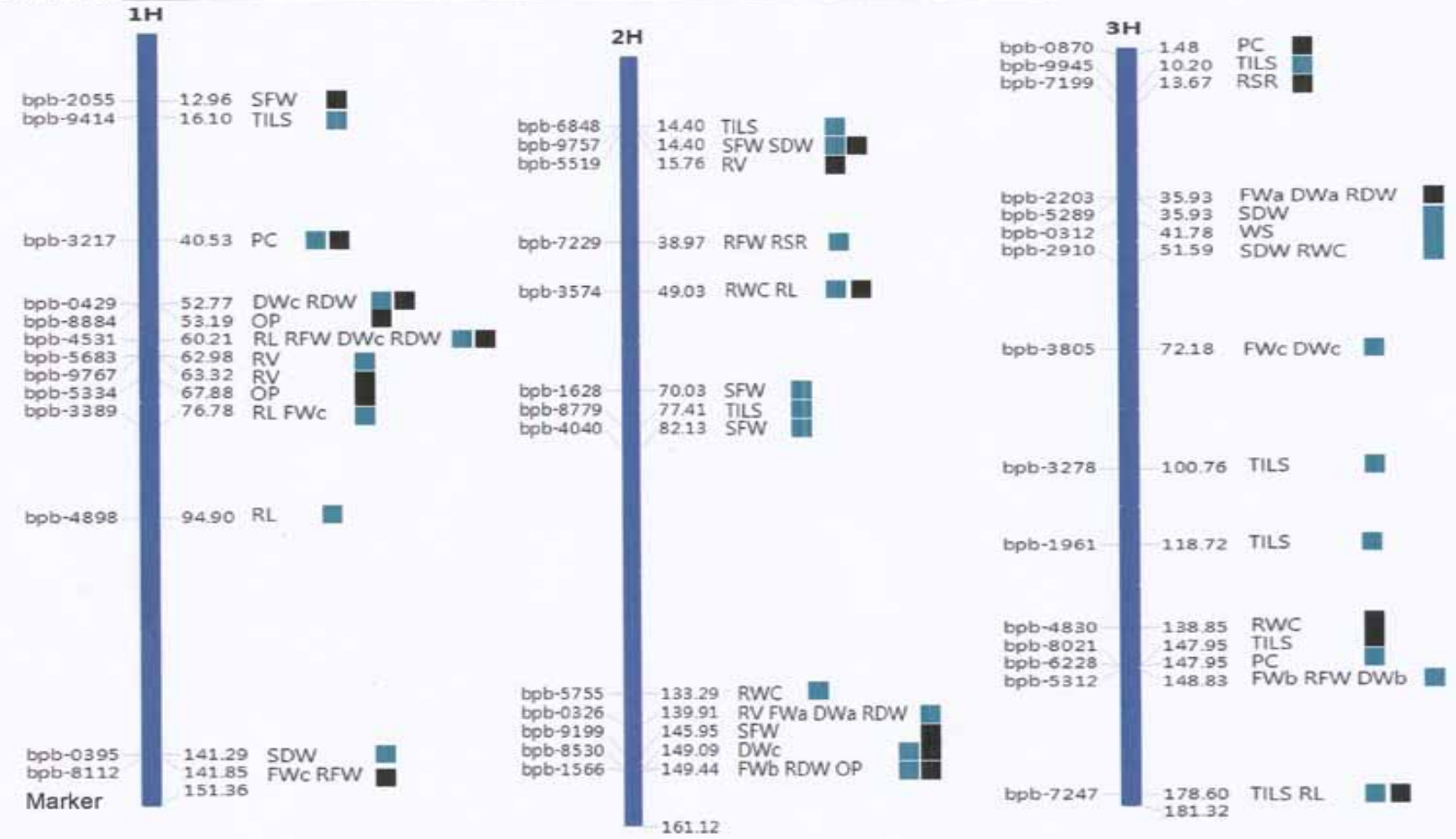
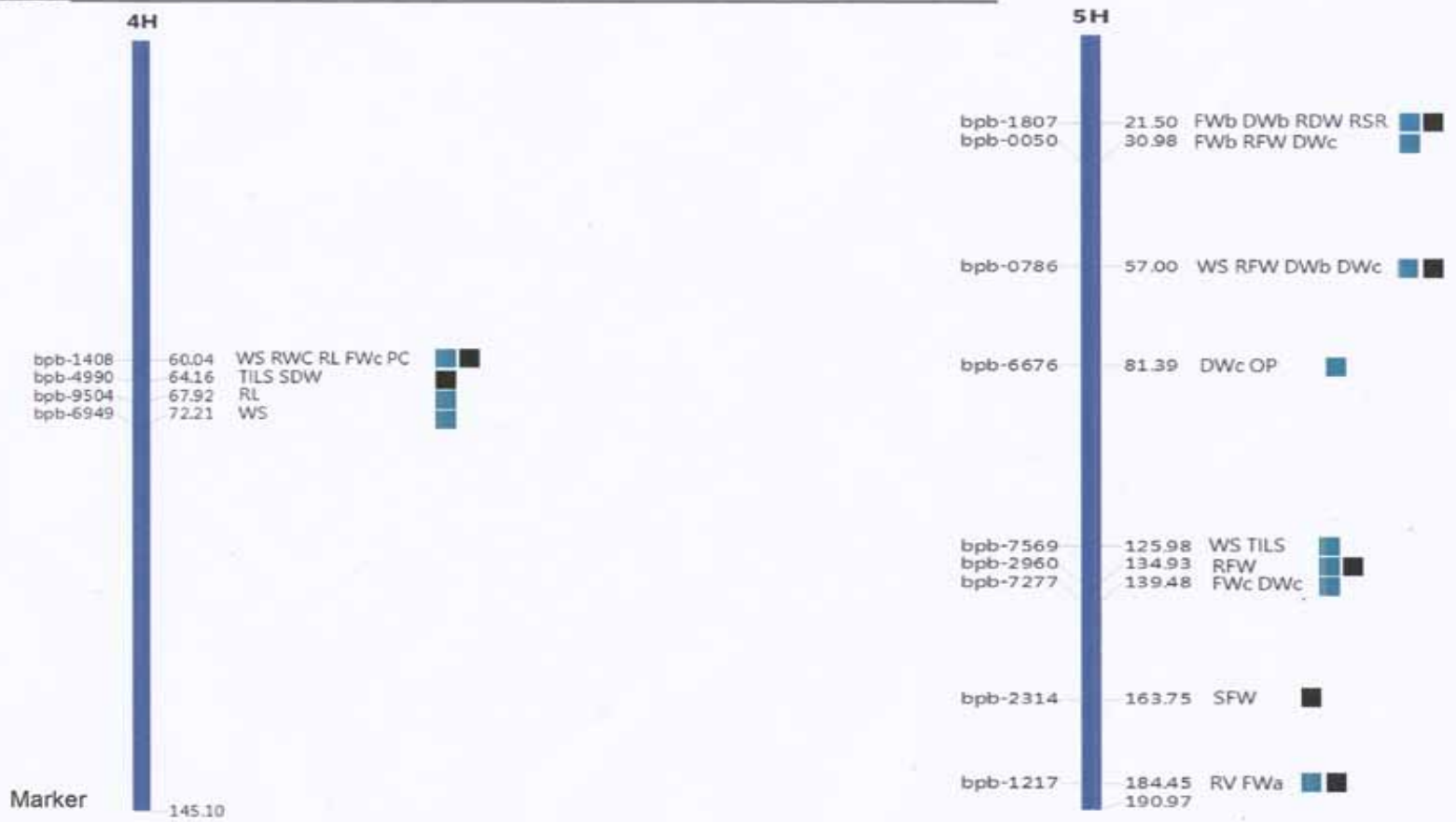



**Fig. (6)** DArT map for structured Barley population, presents the detected QTLs associated with drought tolerance traits. (see table 9). On the right hand, the traits and their positions (cM): wilting score (WS); no. of tillers/plant (TILS); shoot frsh weight/plant (SFW); Shoot dry weight/plant (SDW); Relative water content (RWC); Root volume (RV); Root length (RL); Root fresh weight parts a, b & c (FWa, b & c); Total root fresh weight (RFW); Root dry weight parts a, b, c (DWa, b & c) ; Total root dry weight (RDW); Root shoot ratio (RSR); Osmotic potential (OP) and Proline content (PC).

Main effect Interaction effect

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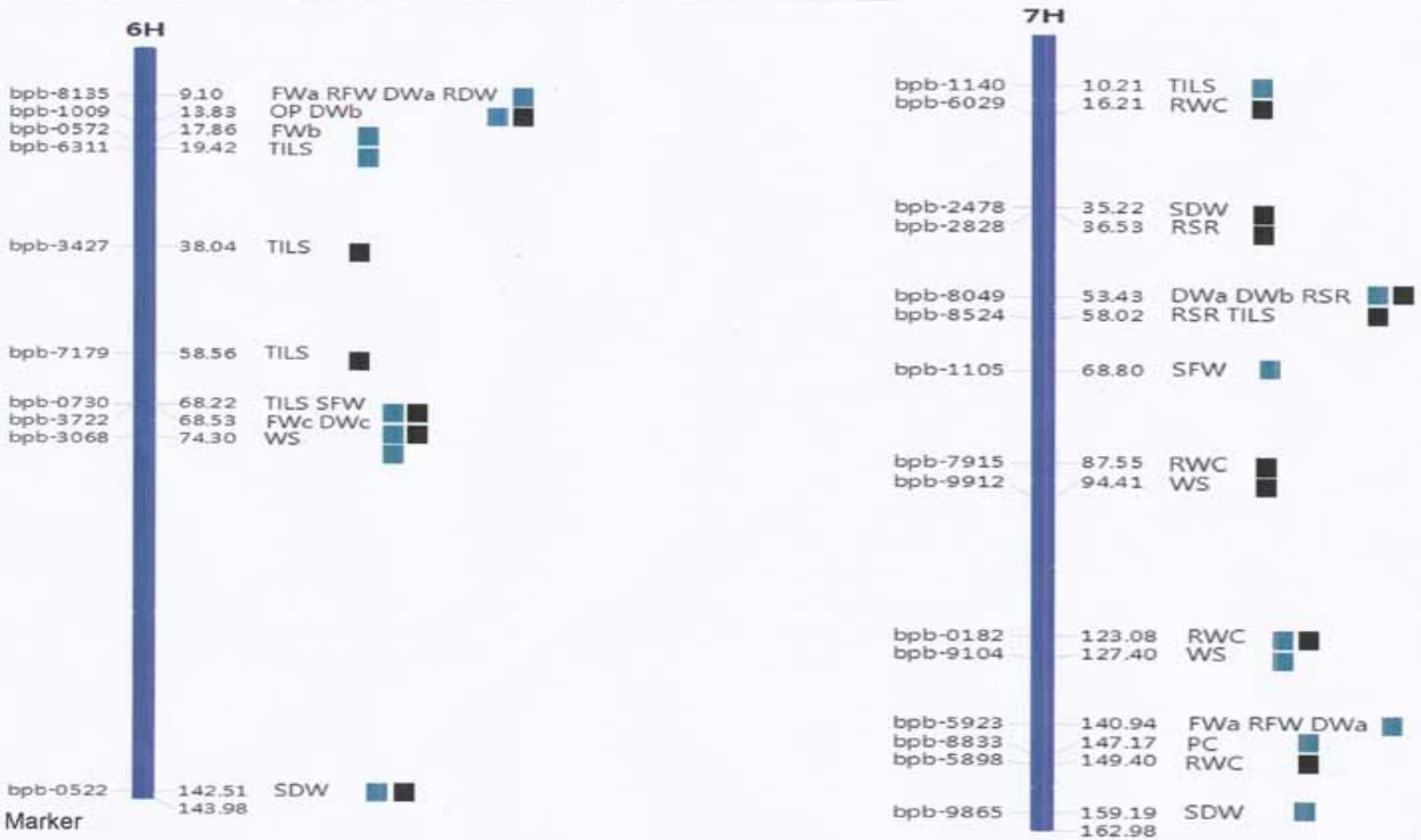
■ Main effect ■ Interaction effect

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■ Main effect ■ Interaction effect

# 4. Discussion

Application of association-mapping approaches in plants is complicated by the population structure present in most germplasm sets (Flint-Garciaet et al. 2003) to overcome this problem, linear models with fixed effects for subpopulations (Breseghello and Sorrells 2006) or a logistic regression-ratio test (Prichard et al. 2000, Thornsberry et al. 2001) can be employed. Owing to the large germplasm sets required for dissecting complex traits, the probability increases that partially related individuals are included. This applies in particular when genotypes selected from plant-breeding populations are used for association mapping (Thornsberry et al. 2001, Kraakman et al. 2004). Association mapping identifies quantitative trait loci (QTLs) by examining the marker-trait associations that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse germplasm (Zhu et al. 2008).

Association analysis was applied using QK mixed-model approach, which proposed by Yu et al. (2006) that promises to correct for linkage disequilibrium (LD) caused by population structure and relatedness relationship. The suitability of this approach has to be evaluated in breeding germplasm of autogamous species, because their population structure is presumably high and levels of relatedness relationship are diverse (Garris et al. 2005).

## 4.1 The identified QTLs by association analysis

Different QTLs have been identified for the shoot, root and some physiological traits, and located on the whole barley genome, these QTLs had main and/or interaction effects on improving or reducing the traits of interest under well-watered and drought stress conditions, (Table 7, 8 and 9) and figure 6. The number of markers associated with the traits and the QTLs for each trait will discuss as follow

### 1) Wilting score (WS)

Eight QTLs were detected for WS and distributed on chromosomes 3H, 4H, 5H, 6H and 7H. The chromosomes 3H and 6H contained one QTLs for each, while chromosomes 4H, 5H and 7H involved two QTLs for each. Among these QTLs, two QTLs located at regions 60.04 and 94.41 cM on 4H and 7H, respectively, led to improving drought tolerance by reducing wilting score, while the others QTLs led to reduce drought

tolerance by increasing wilting score. Von Korff et al (2008) mapped one QTL for wilting score at position (195.7- 206.5 cM) on 1H. Gomez et al. (2006) studied QTLs linked to drought stress related traits in rice and detected five QTLs associated to leaf rolling located on chromosomes 5 (at 57.5 and 85.2 cM), 9 (at 65.6 cM) and 11 (at 46.3 and 103.9 cM) and four QTLs for leaf drying, distributed on chromosomes 1 (at 76.7 cM), 3 (at 14.1 and 91.4 cM) and 11 (at 29.5 cM). Price et al (2002) mapped quantitative trait loci (QTLs) for the visual scores of leaf rolling and leaf drying in upland Rice. Courtois et al. (2000) identified 11 QTLs for leaf rolling and 10 QTls for leaf drying in upland rice, among the eleven possible QTLs for leaf rolling, three QTLs (on chromosomes 1, 5 and 9) were common across the three trials and four additional QTLs (on chromosomes 3, 4 and 9) were common across two trials, and only one QTL on chromosome 4 for leaf drying was detected. Champoux et al. (1995) conducted an early QTL study in rice and found Twelve of the 14 QTL associated with leaf rolling. Yue et al. (2006) mapped six QTLs in rice for leaf drying score (LDS) on chromosomes 1, 2, 3 (two QTLs), 8 and 9.

**2) Number of Tillers per plant (TILS)**

16 QTls were identified to be associated with TILS, and these QTL regions covered the whole genome of Barley population. The chromosomes 3H and 6H contained 5 and 4 QTLs along chromosome respectively, while chromosomes 1H, 2H, 4H, 5H and 7H comprised 1, 2, 1, 1 and 2 QTLs respectively. Two QTLs 38.04 and 58.02 cM on chromosomes 6H and 7H respectively were the responsible of improving this trait under well-watered conditions, while they reduced this trait under stress. In contrast, the QTL 64.16 cM on 4H was improved this trait under drought and reduced it under well-watered conditions, whereas the QTL 58.56 on 6H was reduced this trait under well-watered treatment and had no effects under drought. In a QTL study in barley, Gyenis et al. (2007) identified three QTLs for tiller number; one QTL was detected on 1H (45.7 cM) and two on 2H and 6H. Two QTLs were detected on 6H (103 cM) and 7H (85 cM) and associated with tiller number under well-watered and drought stress, respectively (Ivandic et al 2003). With regard to candidate genes, Vinod et al. (2006) identified a candidate gene (EXP15) on chromosome 1 controlling number of tillers under well-watered conditions.

**3) Shoot fresh weight (SFW)**

Eight QTLs for SFW were detected on chromosomes 1H, 2H, 5H, 6H and 7H, four QTLs on 2H and one for each other chromosomes. The QTL at 14.40 cM on 2H and QTL at 12.96 cM on 1H led to decline the trait under drought stress and improved it under well-watered, while all the others improved SFW under both treatments. Similar results were obtained by Chloupek and Forster (2006); they detected three QTLs for plant weight on chromosome 3H (192 cM), 5H (16.5 cM and 7H (51 cM). Shoot fresh weight especially leaves fresh weight correlated positively with grain yield in barley (*Hordeum vulgare* L.), and two QTLs were detected for leaves fresh weight on chromosome 7H (27.8 cM) and 6H (72-96 cM) (Mickelson et al. 2003). In a study for drought stress in barley two QTls were detected for total shoot fresh weight on chromosome 1H (92.4 cM) and 6H (0.00 cM) by Teulat (1997).

**4) Shoot dry weight (SDW)**

A total of eight QTLs for SDW were detected on chromosomes 1H, 2H, 4H, 5H (one each), 3H and 7H (tow each). The QTLs on chromosomes 2H (14.40 cM), and at 35.22 cM on 7H increased SDW under well-watered and decreased under drought stress conditions, the QTLs on chromosomes 3H (at 35.93 and 51.59 cM) led to decline this traits under both of treatments, while the QTLs on 4H, 6H and at 159.19 cM on 7H were found to be favourable locations in this trait under both of treatment. Similar results were found by many Authors, Teulat (1997) detected two QTLs on chromosomes 1H (92.4 cM) and 6H (0.00 cM). Pillen et al. (2003 and 2004) detected two QTL associated with dry biomass on 7H (120 cM), and 4H (83 cM9). Ivandic et al. (2003) detected three QTLs for total dry matter one on chromosome 3H (172 cM) under well-watered and two on 4H (21 and 118 cM) under well-watered and drought stress conditions (each one). Bálint et al. (2008) detected five QTLs associated with shoot dry weight; three out of them located at (61.05, 69.40 and 76.75 cM) on 1H, 5H and 7H, respectively for shoot dry weight under Osmotic stress and two QTLs at 88.26 and 82.60 cM on chromosomes 2H and 7H associated with shoot dry weight under control and under osmotic stress (SDWC, SDWT) together. Also these results agreement with those obtained by Li et al. (2001) and Yin et al. (1999). With regard to candidate genes, Vinod et al. (2006) identified a candidate gene (EXP13) on chromosome 1 controlling shoot dry weight in

rice under well-watered conditions. Gomez et al. (2006) found one QTL for Straw yield ($g/m^2$) located at 75.6 cM on chromosome 12 in rice.

Ibrahim (2007) detected eighteen QTLs in two wheat populations (D84 and T84 ) for dry weight of biomass on chromosomes 2A, 4A, 2B, 6B, 7B, 3D and 6D, and were found to increase dry weight of biomass under drought and well-watered treatments.

**5) Relative water content (RWC)**

In this study nine QTLs were associated with RWC and located on chromosomes 2H, 3H (tow each), one on 4H and four QTLs on 7H. Where the QTLs on 2H decreased the RWC under both treatments, QTLs on 3H and 4H improved this trait only under drought stress, while all QTLs on chromosomes 7H led to decline this trait under drought stress and increased RWC under well-watered conditions. Teulat et al. (2001) mapped six QTL for RWC to chromosomes 2H (1 QTL), 6H (1 QTL), and 7H (4 QTLs) of barley in a growth chamber study. Three of those markers were detected under well watered conditions and three were detected under stress. Three QTLs for RWC were detected by Teulat et al. (1998) in genomic regions A, B and J on chromosomes 1H and 6H. Teulat et al. (1997) identified two QTLs associated with RWC on 1H (29.4 cM) and 6H (190.8 cM) under water stress treatment and single QTL on 1H under irrigated treatment. Courtois et al. (2000) identified one QTL on chromosome 1 for relative water content in rice. similar results were obtained by Forster et al. (2004), where they mapped six QTLs associated with RWC from growth chamber experimentation , three QTLs on chromosomes 1H (29.41 cM), 6H (176.9 cM) and 7H (58.0 cM) for drought stress and three QTLs on 7H (45.6 and 97.4 cM)) and one QTL on 2H (32.02 cM) for irrigated conditions. In the same study they mapped also five QTLs measured in the field, two QTLs on 1H and 7H associated with RWC as GxE interaction effect, and three QTLs on 2H, 4H and 6H as main effect. Diab (2006) mapped two QTLs into the consensus AD-2005 map on chromosomes 5H (75.2 cM) and 7H (97.4 cM) for relative water content (RWC) under irrigated and drought stress conditions, respectively.

QTLs for RWC were also mapped in upland rice by Price et al. (2002), they identified QTLs on chromosomes 1, 3, 4, 5, 6, 8, 9, 10, and 11.

**6) Root volume (RV)**

Five QTLs for root volume were mapped on chromosomes 1H, 2H (tow each) and 5H (one QTL). The QTLs on 1H and 5H had improved this trait under well-watered and declined the size of roots under drought stress conditions, while QTLs on 2H had the contrast effects. In a study on root system size of barley, chloupek et al. (2006) mapped four QTLs for root size on chromosomes 1H (116 cM), 3H (176 cM), 4H (162 cM) and 7H (187 cM). In a study on rice to identify the genes which responsible of drought tolerance. Vinod et al. (2006) detected a candidate gene (CIS) controlled root volume located on chromosome 1 under low moisture stress in rice. Yue et al. (2006) identified six QTLs for root volume (RV) of rice on chromosomes 1, 3, 4, 6, 7 and 8 under control, and five QTLs on chromosomes 1, 3, 4, 7 and 8 under drought stress. Ibrahim (2007) identified five QTLs for root volume in population T84 and detected on chromosomes 1A (116 cM), 2D (73.1 cM), 5D (44 and 57.8 cM) and 7D (91.5 cM) under both treatments, whereas he detected four QTLs on chromosomes 2A (52.5 cM), 3A (83.3 cM), 4A (9.9 cM) and 5D (82 cM) in population D84 under both treatments too.

**7) Root Length (RL)**

Seven QTLs were identified to be correlated with root length and distributed on chromosomes 1H (three QTLs), 2H (one QTL), 3H (one QTL) and 4H (two QTLs). All of them were considered as favourable regions in improving root length under drought stress, QTL on 2H were showed the contrast effect. In a study for barley seedling root length, Jefferies et al. (1999) detected three QTls at the low (B0) and high (B100) boron concentration on the long arm of chromosome 5H, long arm of chromosome 4H and the short arm of chromosome 3H were strongly associated with relative root length (RRL).
Bálint et al. (2008) found two QTLs associated with root length; one QTL at 82.60 cM on chromosome 7H associated with root length (RLc) under normal conditions and QTL at position 61.05 cM on 1H associated with root length under control and osmotic stress (RLc& RLT). Thanh et al. (2006) mapped four QTLs for maximum root length (MRL). One QTL on chromosome 2 (30 cM) flanked by AFLP marker AVM43.1 and SSR marker RM250; two QTLs on chromosome 3 (5 cM) and chromosome 9 (0.0 cM) flanked by AFLP markers AVM56.2-AVM8.6 and AVM62.5-AVM77.7, respectively; the QTL on chromosome 12 (0.0 cM) flanked by SSR marker RM270 and AFLP marker AVM28.17. The positions of these QTLs are very close to the flanked markers (2.1 cM

from RM250, 5 cM from AVM56.2, 0 cM from AVM62.5 and RM270). Similar results were obtained by Champoux et al. (1995) used different mapping population, and detected 3 QTL in common for maximum root length (chromosomes 2, 11, 9 or 5), 2 for root thickness (chromosomes 2, 3), and 1 for root volume (chromosome 12). Redona and Mackill (1996) identified a root length QTL that corresponded to a QTL in the Ray et al. (1996) study for root length at 14 days. In a study of Vinod et al. (2006) to identify the candidate genes for drought tolerance in rice, they had detected (LTP &KCDL) and Exp15 as a candidate genes on chromosomes 1 and 6 for maximum root length under well-watered and low moisture stress, respectively. Ping et al (2003) reported that QTLs for maximum root length (MRL), two pairs of epistatic QTLs were detected on chromosome 2 (0.0 cM), 5 (0.0 cM), 3 (0.0 cM), 8 (12 cM), respectively. Epistatic QTLs mrl3 and mrl8 had a positive effect of 8.147 and high general contribution of 21.51%. Ibrahim (2007) mapped four QTLs for root length of wheat to chromosomes 1B (91.5 cM), 2A (126 cM), 6B (47.7 cM) and 7A (29.6 cM) in population T84 under drought and well-watered conditions.

In a study of drought resistance on rice Yue et al. (2006) detected five QTLs on chromosomes 2, 4, 5, 9 and 11 for maximum root depth under control (MRDC), and four QTLs on chromosomes 4 ( two positions of QTL), 11 and 9.

**8) Root fresh Weight traits (Fwa, FWb, FWc and RFW)**

Out of twenty QTLs were found to be correlated with RFW traits covered all seven chromosomes of structured barley population, three QTLs were distributed on 1H, where two QTLs were associated with RFW and FWc (each one), and one QTL (bpb-8112) was associated with RFW and FWb. Chromosome 2H contained three QTLs correlated with RFW, FWa and FWb. For chromosome 3H, contained three QTLs were correlated with FWa, FWc (each one), FWb and RFW were associated with one QTL (bpb-5312). Only one QTL was associated with FWc and located on 4H. On chromosome 5H, six QTLs were detected and correlated with RFW traits, one QTL for FWb, two QTLs for RFW, one QTL for FWa, one QTL for FWc and one QTL (bpb-0050) in region 30,98 cM was identified for RFW and FWb. Three QTLs were mapped on 6H, one QTL for FWb, one QTL for FWc and one QTL for FWa and RFW. One QTL was detected on 7H for FWa and RFW. Among these QTLs and as an average main effect, there were seven QTLs led to decrease root fresh weight traits, whereas the other QTLs

led to improve that traits (see table 8, 9). With regarding to the effects of the QTLs under treatments, four QTLs led to increase root fresh weight traits only under drought stress treatment, two QTLs out of them for FWb abd RFW on chromosome 1H, while the other two QTLs were located on 4H and 5H for FWc and RFW respectively. Under both treatments, there were two QTLs on 5H led to improve FWa and RFW traits, whereas one QTL on 3H was declined FWa under both treatments. Ping et al. (2003) detected five additive QTLs and one pair of epistatic QTLs for RFW on chromosomes 1 (10 cM), 2 (2 cM), 3 (0.0 cM), 7 (22 cM) and 10 (4 cM). Of the five additive QTLs, three QTLs (rfw1b, rfw2 and rfw10) had positive effects, 0.583, 0.568 and 0.645, respectively and two QTLs (rfw1c, rfw3) had negative effects, -0.447 and -0.331 respectively. Two additive QTLs rfw1b and rfw2 had GxE effects with a GxE contribution of 19.90% and 9.62%, respectively.

**9) Root dry weight traits (Dwa, Dwb, DWc and RDW)**

Eighteen QTLs were detected for root dry weight traits and mapped on all chromosomes except 4H. Chromosome 1H contained two QTLs for RDW and DWc together; the first QTL at 52.77 cM decreased DWc and increased RDW, while the second one at 60.21 had contrast effect. Three QTLs were identified on 2H, one QTL located at 139.9 cM and improved DWa and RDW, Tow QTLs at 149.1 and 149.4 decreased DWc and RDW respectively. Chromosomes 3H contained three QTLs, two at 72.18 cM and 148.83 cM improved DWc and DWb, respectively and one QTL at 35.93 cM decline both of DWa and RDW. On chromosome 5H had been found three QTLs mapped at (30.98, 81.39 and 139.5 cM) the first tow improved DWc and the third one decreased this trait, one QTL at 21.5 cM increased DWb and decreased RDW, while the QTL at 57 cM increased DWb and decreased DWc. Three QTLs were mapped on 6H at positions (9.10, 13.83 and 68.53 cM) and improved the traits DWa, RDW, DWb and DWc under water stress conditions. On 7H had been found two QTLs located at 53.43 and 140.9 cM; decreased DWa, DWb and increased Dwa under drought stress treatment, respectively.

In general, there were 13 QTLs had positive effects in improving root dry weight traits. For traits improvement under both treatments, there were five QTLs led to decrease those traits under both treatments and located on chromosomes 2H, 3H, 5H and 7H, four QTLs on chromosomes 1H, 5H and 6H were decreased the RDW traits under

drought stress treatment, while they were increased these traits under well-watered treatment, whereas there was one QTL on 2H led to improve root dry weight traits under water stress treatment and exhibited the contrast under well-watered treatment. In a study for phenotype-genotype association for yield and salt tolerance in barley, Ellis et al. (2002) detected two QTLs associated with root dry weight (RWt); the SSR Bmag 337 at 40 cM on chromosome 5H showed main effects for RWt and Bmag 13 at 12 cM on chromosome 3H. Li et al. (1999) developed NILs for 4 different rice chromosome regions associated with total root weight and deep root weight. Ping et al (2003) detected QTLs for RDW. The 2 QTLs on chromosome 1 had opposite effects. Additive QTLs on chromosome 3, chromosome 7 and chromosome 9 had negative effects but QTLs on chromosome 5 and chromosome 11 had positive effects. Epistatic QTLs rdw11a and rdw12 had a high effect of 0.179 and accounted for 25.64% of phenotypic variation. Three QTLs rdw1a, rdw5b and rdw9 were detected to have G x E interactions with the effect varied from 0.038 to 0.062. Ibrahim (2007) mapped two QTLs for dry weight of roots of wheat to chromosomes 2A (63.1 cM) and 5D (16 cM) in population D84, and five QTLs were detected on chromosomes 3A (115.8 cM), 4D (54 cM), 5B (61.2 cM), 6B (59.3 cM) and 7B (35.2 and 40 cM) in population T84, these QTLs were found to increase dry weight of roots under both treatments.

**10) Root/shoot ratio (RSR)**

Six QTLs were identified for RSR on chromosomes 2H, 3H, 5H (each one) and 7H (three QTLs). The QTLs on 2H and 5H were increased and decreased RSR as main effects respectively, while QTL at position 53.43 cM on 7H led to decrease RSR as general mean. Two QTLs on 3H (13.67 cM) and 7H (36.53 cM) were increased this trait under both treatments, while the QTL at region 58.02 cM on 7H was decreased RSR under both treatments.

Ping et al. (2003) detected QTLs for RDW/SDW in rice, four QTLs as main effects and three pairs of epistatic QTLs were detected. Two QTLs rrsd3 and rrsf3 were common QTLs for RFW/SFW and RDW/SDW with high LOD scores of 14.56 and 17.32 respectively. The QTL rrsd11a had a high LOD score of 8.85 and GxE general contribution of 6.60%. Thanh et al. (2006) detected four QTLs associated with root weight to shoot ratio under water stress in upland rice and were located: two on chromosome 9 (0.0 cM), one on chromosome 2 (10 cM), and one on chromosome 8 (10

cM). Ibrahim (2007) mapped three QTLs for root/shoot ratio of wheat to chromosomes 2A (63.1 cM), 3A (115.8 cM) and 5A (34.3 cM) in population D84, and four QTLs were detected on chromosomes 2A (54 cM), 5D (3.5 and 82 cM) and 7D (135.9 cM) in population T84, these QTLs were associated with a positive effect increasing root/shoot ratio under both treatments.

**11) Osmotic potential (OP)**

Five QTLs were detected for OP, two QTLs on 1H and one QTL for each 2H, 5H and 6H. One QTL at 67.88 cM on 5H led to increase OP under both of treatments, while the other one at 53.19 cM decreased OP under water stress conditions, QTL at 149.4 cM on 2H improved this trait only under well-watered treatment, QTL at 81.39 cM on 5H had positive main effect, and the QTL at 13.83 cM on chromosome 6H had negative main effect. Forster et al. (2004) mapped Six QTLs associated with osmotic potential under drought stress conditions on chromosomes 1H, 2H, 4H, 7H (one each ) and 5H (two QTLs)., also in the same study they mapped four QTLs associated with Osmotic adjustment (OA) on chromosomes 2H, 4H, 5H and 6H. Teulat et al. (1998) mapped other osmoregulation genes in barley on 6H (136.8 cM), on1H (97.4 cM) and on 2H (0.00 cM). Lilley et al. 1996 identified a single locus on chromosome 8 near RG1 and RZ66 was found to be associated with osmotic adjustment in rice at 70% water potential. Diab (2006) incorporated three into the consensus AD-2005 map, two for Osmotic potential at full turgor placed on chromosomes 3H (38.8 cM) and 4H (13.0 cM) and one for osmotic potential under irrigated condition on chromosome 2H (32.0 cM).

**12) Proline content (PC)**

Five QTLs were detected for PC and mapped on 1H, 3H (2 QTLs), 4H and 7H. The QTLs at (40.53 on 1H, 1.48 and 147.95 on 3H, 60.04 cM on 4H) increased proline content under water stress treatment and decreased it under well-watered treatment, while the QTL located at 147.2 cM on chromosome 7H had positive effect and increased this trait as main effect. In view of fact that the accumulation of Proline is tightly controlled by genes and cDNA encoding osmolyte biosynthesis and only achieved when the rate of synthesis prevails over that degradation, probably because too much Proline is toxic to cell plant (Yokota et al. 2006). In addition to Shivkumar et al. (1998) and Silverira et al. (2003) showed that proline accumulation was indeed a

heritable trait and they concluded that selection for high proline had been effective and played an important role in rehydration of protoplasm and osmotic adjustment are hypothesize to enhance drought tolerance in plants. In a study to determine chromosomal location of osomoregulation genes of wheat, Galiba et al. (1992) observed positive correlations between the accumulation rate of proline during cold hardening in chromosomes 5A and 5D chromosomes, and they mentioned that these chromosomes contain the genes which have roles in drought (osmoregulation of proline as amino acid) and in freezing tolerance. Genes controlling osmoregulation are primarily located on chromosomes 5A and 5D although the contribution of other chromosomes, e.g., 1A and 2D, cannot be ignored. Ma et al. 2008 identifed the gene TaP5CR on chromosome 3D increasing proline content at higher levels in radicles, flowers and leaves than other organs under salt, PEG, ABA, heat and water stress conditions .

Table (9): The performance of the detected QTLs for the studied traits on the defferent chromosomes over two years.

| Chr. | Marker | M.Name | Pos. | Effe. | WS | TILS | SFW | SDW | RWC | RV | RL | FWa | FWb | FWc | RFW | DWa | DWb | DWc | RDW | RSR | OP | PC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1H | M0287 | bPb-2055 | 12.96 | M.T | | | - | | | | | | | | | | | | | | | |
| | M0533 | bPb-9414 | 16.10 | M | | + | | | | | | | | | | | | | | | | |
| | M0651 | bPb-3217 | 40.53 | M+MT | | | | | | | | | | | | | | | | | | + |
| | M0038 | bPb-0429 | 52.77 | M+MT | | | | | | | | | | | | | | - | + | | | |
| | M0701 | bPb-8884 | 53.19 | MT | | | | | | | | | | | | | | | | | - | |
| | M0052 | bPb-4531 | 60.21 | M+MT | | | | | | | + | | | | - | | | + | - | | | |
| | M0644 | bPb-5683 | 62.98 | M | | | | | | - | | | | | | | | | | | | |
| | M0028 | bPb-9767 | 63.32 | MT | | | | | | - | | | | | | | | | | | | |
| | M0446 | bPb-5334 | 67.88 | MT | | | | | | | | | | | | | | | | | + | |
| | M0392 | bPb-3389 | 76.78 | M | | | | | | | + | | | + | | | | | | | | |
| | M0271 | bPb-4898 | 94.90 | M | | | | | | | + | | | | | | | | | | | |
| | M0788 | bPb-0395 | 141.29 | M | | | | + | | | | | | | | | | | | | | |
| | M0054 | bPb-8112 | 141.85 | MT | | | | | | | | | + | | + | | | | | | | |
| 2H | M0278 | bPb-6848 | 14.40 | M | | + | | | | | | | | | | | | | | | | |
| | M0299 | bPb-9757 | 14.40 | M+MT | | | - | - | | | | | | | | | | | | | | |
| | M0268 | bPb-5519 | 15.76 | MT | | | | | | + | | | | | | | | | | | | |
| | M0298 | bPb-7229 | 38.97 | M | | | | | | | | | | | + | | | | | + | | |
| | M0033 | bPb-3574 | 49.03 | M+MT | | | | | - | | - | | | | | | | | | | | |
| | M0684 | bPb-1628 | 70.03 | M | | | + | | | | | | | | | | | | | | | |
| | M0465 | bPb-8779 | 77.41 | M | | + | | | | | | | | | | | | | | | | |
| | M0281 | bPb-4040 | 82.13 | M | | | + | | | | | | | | | | | | | | | |
| | M0247 | bPb-5755 | 133.29 | M | | | | | - | | | | | | | | | | | | | |
| | M0336 | bPb-0326 | 139.91 | M | | | | | | + | | + | | | | + | | | + | | | |
| | M0739 | bPb-9199 | 145.95 | MT | | | + | | | | | | | | | | | | | | | |
| | M0124 | bPb-8530 | 149.09 | M+MT | | | | | | | | | | | | | | - | | | | |
| | M0136 | bPb-1566 | 149.44 | M+MT | | | | | | | | | + | | | | | | - | | + | |

Table (9) continued

| Chr. | Marker | M.Name | Pos. | Effe. | WS | TILS | SFW | SDW | RWC | RV | RL | FWa | FWb | FWc | RFW | DWa | DWb | DWc | RDW | RSR | OP | PC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3H | M0836 | bPb-0870 | 1.48 | MT | | | | | | | | | | | | | | | | | | + |
| | M0045 | bPb-9945 | 10.20 | M | | + | | | | | | | | | | | | | | | | |
| | M0258 | bPb-7199 | 13.67 | MT | | | | | | | | | | | | | | | | + | | |
| | M0517 | bPb-2203 | 35.93 | MT | | | | | | | | - | | | | - | | | - | | | |
| | M0572 | bPb-5289 | 35.93 | M | | | | - | | | | | | | | | | | | | | |
| | M0290 | bPb-0312 | 41.78 | M | + | | | | | | | | | | | | | | | | | |
| | M0715 | bPb-2910 | 51.59 | M | | | | - | + | | | | | | | | | | | | | |
| | M0292 | bPb-3805 | 72.18 | M | | | | | | | | | | + | | | | + | | | | |
| | M0105 | bPb-3278 | 100.76 | M | | + | | | | | | | | | | | | | | | | |
| | M0212 | bPb-1961 | 118.72 | M | | - | | | | | | | | | | | | | | | | |
| | M0381 | bPb-4830 | 138.85 | MT | | | | | + | | | | | | | | | | | | | |
| | M0539 | bPb-6228 | 147.95 | MT | | | | | | | | | | | | | | | | | | + |
| | M0286 | bPb-8021 | 147.95 | M | | - | | | | | | | | | | | | | | | | |
| | M0420 | bPb-5312 | 148.83 | M | | | | | | | | | + | | + | | + | | | | | |
| | M0216 | bPb-7247 | 178.60 | M+MT | | - | | | | | + | | | | | | | | | | | |
| 4H | M0007 | bPb-1408 | 60.04 | M+MT | + | | | | + | | + | | | + | | | | | | | | + |
| | M0516 | bPb-4990 | 64.16 | MT | | + | | + | | | | | | | | | | | | | | |
| | M0786 | bPb-9504 | 67.92 | M | | | | | | | + | | | | | | | | | | | |
| | M0153 | bPb-6949 | 72.21 | M | - | | | | | | | | | | | | | | | | | |
| 5H | M0507 | bPb-1807 | 21.5 | M+MT | | | | | | | | | - | | | | + | | - | - | | |
| | M0798 | bPb-0050 | 30.98 | M | | | | | | | | | + | | + | | | + | | | | |
| | M0398 | bPb-0786 | 57.00 | M+MT | - | | | | | | | | | | + | | + | - | | | | |
| | M0669 | bPb-6676 | 81.39 | M | | | | | | | | | | | | | | + | | | + | |
| | M0372 | bPb-7569 | 125.98 | M | - | - | | | | | | | | | | | | | | | | |
| | M0132 | bPb-2960 | 134.93 | M+MT | | | | | | | | | | | + | | | | | | | |
| | M0627 | bPb-7277 | 139.48 | M | | | | | | | | | | - | | | | - | | | | |
| | M0811 | bPb-2314 | 163.75 | MT | | | + | | | | | | | | | | | | | | | |
| | M0103 | bPb-1217 | 184.45 | M+MT | | | | | | - | | + | | | | | | | | | | |

Table (9) continued

| Chr. | Marker | M.Name | Pos. | Effe. | WS | TILS | SFW | SDW | RWC | RV | RL | FWa | FWb | FWc | RFW | DWa | DWb | DWc | RDW | RSR | OP | PC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6H | M0333 | bPb-8135 | 9.10 | M | | | | | | | | + | | | + | + | | | + | | | |
| | M0117 | bPb-1009 | 13.83 | M+MT | | | | | | | | | | | | | + | | | | - | |
| | M0145 | bPb-0572 | 17.86 | M | | | | | | | | | - | | | | | | | | | |
| | M0790 | bPb-6311 | 19.42 | M | | + | | | | | | | | | | | | | | | | |
| | M0694 | bPb-3427 | 38.04 | MT | | - | | | | | | | | | | | | | | | | |
| | M0043 | bPb-7179 | 58.56 | MT | | - | | | | | | | | | | | | | | | | |
| | M0297 | bPb-0730 | 68.22 | M+MT | | + | + | | | | | | | | | | | | | | | |
| | M0554 | bPb-3722 | 68.53 | M+MT | | | | | | | | | | - | | | | + | | | | |
| | M0158 | bPb-3068 | 74.30 | M | - | | | | | | | | | | | | | | | | | |
| | M0665 | bPb-0522 | 142.51 | M+MT | | | | + | | | | | | | | | | | | | | |
| 7H | M0344 | bPb-1140 | 10.21 | M | | - | | | | | | | | | | | | | | | | |
| | M0559 | bPb-6029 | 16.21 | MT | | | | | - | | | | | | | | | | | | | |
| | M0477 | bPb-2478 | 35.22 | MT | | | | - | | | | | | | | | | | | | | |
| | M0061 | bPb-2828 | 36.53 | MT | | | | | | | | | | | | | | | | + | | |
| | M0125 | bPb-8049 | 53.43 | M+MT | | | | | | | | | | | | - | - | | | - | | |
| | M0175 | bPb-8524 | 58.02 | MT | | - | | | | | | | | | | | | | | - | | |
| | M0603 | bPb-1105 | 68.80 | M | | | + | | | | | | | | | | | | | | | |
| | M0341 | bPb-7915 | 87.55 | MT | | | | | - | | | | | | | | | | | | | |
| | M0065 | bPb-9912 | 94.41 | MT | + | | | | | | | | | | | | | | | | | |
| | M0397 | bPb-0182 | 123.08 | M+MT | | | | | - | | | | | | | | | | | | | |
| | M0628 | bPb-9104 | 127.40 | M | - | | | | | | | | | | | | | | | | | |
| | M0518 | bPb-5923 | 140.94 | M | | | | | | | | - | | | - | + | | | | | | |
| | M0440 | bPb-8833 | 147.17 | M | | | | | | | | | | | | | | | | | | + |
| | M0022 | bPb-5898 | 149.40 | MT | | | | | - | | | | | | | | | | | | | |
| | M0223 | bPb-9865 | 159.19 | M | | | | + | | | | | | | | | | | | | | |

**Chr**: chromosome number; **M Name:** real marker name; **Pos.:** the marker position; **Effe.** : The significant effect of marker; **M:** Main effect of the marker; **MT**: the interaction effect between marker and treatments; **(+)** and **(-)** indicate that the favourable and unfavourable performance of the marker under drought stress or under both of treatments.

### 4.2 Co-location of specific QTLs

The co-location of specific genes with QTLs could be a better way to understand the molecular basis of drought tolerance or of traits related to drought response. The co-location of the QTLs detected for the different traits allow us to identify the important genomic regions for traits related to drought tolerance and several other regions specific for one trait (Teulat et al. 1998), several chromosomal locations with effects on more than one trait are found by Hackett et al. (2001). In this study, table (9) and figure (6) refer to several co-locations of QTLs have been found for studied traits on different chromosomes as follow:

Thirty co-locations of QTLs were correlated with all studied traits and covered the whole genome of barley population of interest. Among these co-locations 18 regions were found to be associated with two traits, these regions were distributed on chromosomes 1H, 2H, 3H (3 QTLs for each), 4H (one QTL), 5H (4 QTLs), 6H (3 QTLs) and 7H (one QTL). A total of six co-locations have been identified and were found to be co-located with three traits, these co-locations were detected on 2H and 5H (one each), 3H and 7H (two QTLs each one). Six co-locations were detected on chromosomes 1H, 2H, 4H and 5H one for each, and two QTLs co-located on 5H. Each location of them was affected more than three traits. The most important co-locations which have been obtained in the current study were bpb-3574, bpb-2910 and bpb-1408 on chromosomes 2H (49.03 cM), 3H (51.59) and 4H (60.04), respectively, these positions were correlated with the most important traits related to drought tolerance in Barley, where the co-position of QTL bpb-3574 was found to be associated with root length (RL) and relative water content (RWC), while the second co-location bpb-2910 was correlated with RWC and SDW and the third co-location bpb-1408 was associated with five traits WS, FWc, RL, RWC and PC. These results supported by the significant Pearson correlation coefficients (r) between the pairwise of these traits under drought stress conditions over the two years table (6), where WS trait correlated negatively with RWC, RL and RFW, and positively with PC, while RDW associated positively with RWC and RL. RWC was correlated negatively with PC and RL and positively with RDW. Ping et al (2003) studied QTL mapping of the root traits and their correlation analysis with drought resistance using DH rice lines and detected 18 additive QTLs and 18 pairs of epistatic QTLs associated with root traits, and found that some QTLs controlling different root traits were located in

the same chromosome regions or tightly linked together. There were two QTL regions on chromosomes 1 (C813-C955) and 7 (RM18-RM47) governing RFW and RDW, and 1 QTL region on chromosome 3 (G51-RM231) controlling RFW/SFW and RDW/SDW. QTL regions on chromosome 2 (RM208-RM48), chromosome 11 (RM287-RM209) governing BRT and RN, RN and MRL, respectively, were also found. Diab et al. (2004) identified the locus BM816463b on chromosome 3H coding for blue copper-binding protein co-segregated with QTLs for RWC, $WSC_{100}$, OP and $DWSC_{100}$. in his study, he found QTL for OA and $DWSC_{100}$ were positively associated and mapped to the same region (caaaccO) on chromosome 3H, and mentioned that these traits physiologically are components of drought tolerance, therefore, the co-localization of these QTL is most likely due to pleiotropic effects of the same gene(s).

Also Michael et al. (2006) identified QTLs for leaf drying, days to 50% flowering and number of productive tillers under drought stress co-located at certain of these regions. Further, QTLs for several root traits overlapped with QTLs for grain yield under stress in these RL lines, indicating the pleiotropic effects of root trait QTLs on rice performance under stress.

According to these results, this population posses several mechanisms to react or tolerance to drought stress such as drought avoidance and drought tolerance via controlling root, shoot and physiological traits. In the current population, nineteen accessions were exhibited a desirable performance under drought conditions in the most important traits related to drought tolerance (i.e. PC, WS, RL, RWC and RSR), these traits were associated with the QTLs overall Barley genome of the studied population, and the majority of them were co-located on different chromosomes (see appendix 1).

## 5. Summary

Barley is characterized by being relatively high drought tolerance, where it can grow with lesser soil moisture. Barley genotypes, in particular landraces and wild species, represent an important source of variation for adaptive traits that may contribute to increase yield under drought conditions. Association mapping of a trait is to identify chromosomal regions that contain genes affecting the trait. The discovery of dense polymorphic markers covering the entire genome provides us an opportunity to localize these regions by trying to find the markers closest to the genes of interest. Drought stress is the main limited factor of crop productivity. Most of drought traits in plants are quantitative in nature, and controlling by poly genes.

The objectives of this study were:

1) To apply association mapping approaches to identify DArT markers associated to drought tolerance traits in a structured of wild and cultivated barley population,
2) To determine a marker-based kinship matrix based on a REML for Drought Traits.
3) To identify and develop barley with improved adaptation to low rainfall environments, and to develop molecular markers for key traits associated with drought stress tolerance.

In the current study, 98 accessions of wild barley (*H. vulgare* ssp*. spontaneum*) from the ICBB core collection (gene banks in Gatersleben and Braunschweig) and 21 spring barley cultivars representative for the breeding pool of spring barley in the North Rhine Westphalia (NRW), Germany, (Reetz and Leon 2004). These cultivars were provided by the Institute of Crop Science and Resource Conservation (INRES), chair of plant breeding.

The experiments were carried out in plastic green house tunnels during the summer seasons of 2007 and 2008 at the Poppelsdorf Experimental Station, Dept. of Crop Science and Plant Breeding, Faculty of Agriculture, Rheinische Friedrich-Wilhelms-University Bonn. The experiments were arranged in a split-plot design with non-replications, drought treatments assigned to main plots and accessions to sub-plots. In the drought stress treatment, water (165 ml) was applied daily for each pot, whereas the

well-watered treatment was 330 ml water, The drought treatment was applied after 40 days from sowing and continued for 21 days. Phenotypic data were collected on 18 measured traits (table 1)., these traits were; wilting score (WS), no. of tillers/plant (TILS), shoot fresh weight (SFW), shoot dry weight, root length (RL), root volume (RV), root fresh weight (RFW) traits, root dry weight (RDW) traits, relative water content (RWC), osmotic potential (OP) and proline content (PC). In parallel, DNA has been extracted from 10 mg freeze drying of each accession by using “Kit” procedure according DNeasy Plant Handbook 07/2006. The produced DNA of the accessions was sent to Australia and genotyped by using 1081 DArT markers (YarralumlaACT, Australia). The phenotypic data were analysed each season separately as one way ANOVA using Proc GLM procedure and the Pearson correlation coefficients (r) between traits under well-watered and drought stress condition were calculated by SAS version 9.1 (SAS institute 2003). The combined analysis was carried out using the mixed liner model considering year and drought treatments and the interaction between them as fixed effect, while the accessions and the interactions between accessions with each of year and drought treatments as random effects. The population structure analysis was carried out for the all accessions using Software package Structure version 2.2. (Pritchard et al. 2000) to subdivide the 119 accessions into subgroups showed in table (2). The relative kinship coefficients (K matrix) among all pairs of accessions were calculated using 1081 DArT markers data by TASSEL Software version 2.0.1 to calculate the pair-wise kinship coefficients for all accessions. For the results of ANOVA, the followed traits, WS, SFW, RWC, RL, FWa, FWb, FWc, RFW, DWc, RDW, and PC were exhibited highly significantly differences in both seasons, Pearson correlation coefficients (r) between 12 pairs of studied traits have been detected under drought-stress (D) and well-watered (W) treatment across two years (table 6 ). With regarding to the population structure, the accessions were subdivided into 12 subpopulations, which correspond well with genetic distances and origin of the genotypes (table 3 and figure 4). Seventy nine markers were correlated significantly with the all studied traits and covered the whole genome of Barley population of interest. (Table 7, 8 and 9). Different QTLs have been identified for the shoot, root and some physiological traits, and located on the whole Barley genome, these QTLs had main and/or interaction effects on improving or reducing the traits of interest under well-watered and drought stress conditions (Table 7, 8, 9). A total of 8, 16, 8, 8, 9, 5, 7, 20, 18, 6, 5 and 5 QTLs were

detected for WS, TILS, SFW, SDW, RWC, RV, RL, RFW traits, RDW traits, RSR, OP and PC, respectively. The co-location of specific genes with QTLs could be a better way to understand the molecular basis of drought tolerance or of traits related to drought response; thirty co-locations of QTLs were correlated with all studied traits and covered the whole genome of Barley population of interest (table 9, fig.6). Among these co-locations 18 regions were found to be associated with two traits, these regions were distributed on chromosomes 1H, 2H, 3H (3 QTLs for each), 4H (one QTL), 5H (4 QTLs), 6H (3 QTLs) and 7H (one QTL). A total of six co-locations have been identified and were found to be co-located with three traits, these co-locations were detected on 2H, and 5H (one each), 3H and 7H (two QTLs each one). Six co-locations were detected on chromosomes 1H, 2H, 4H and 6H one for each, and two QTLs co-located on 5H. Each location of them was affected more than three traits. The most important co-locations which have been obtained in the current study were bpb-3574, bpb-2910 and bpb-1408 on chromosomes 2H (49.03 cM), 3H (51.59) and 4H (60.04) respectively, these positions were correlated with the most important traits related to drought tolerance in barley, where the co-position of QTL bpb-3574 was found to be associated with root length (RL) and relative water content (RWC), while the second co-location bpb-2910 was correlated with RWC and SDW and the third co-location bpb-1408 was associated with five traits DS, FWc, RL, RWC and PC.

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**7. List of tables**



**8. List of figures**

## 9. Abbreviations

| Abbreviations | Explanation |
|---|---|
| AFLP | Amplified fragment length polymorphism |
| AM | Association Mapping |
| ANOVA | Analysis of Variance |
| cM | Centi Morgan |
| DArT | Diversity array technology |
| DH | Double haploid |
| DNA | Deoxyribonucleic acid |
| DW | Dry weight |
| FW | Fresh weight |
| GLM | General linear model |
| K matrix | Kinship matrix |
| LD | Linkage disequilibrium |
| LWP | Leaf water potential |
| MLM | Mixed linear model |
| OA | Osmotic adjustment |
| OP | Osmotic potential |
| PC | Proline content |
| PCR | Polymerase chain reaction |
| PEG | Polyethylene glycol |
| Q matrix | Population structure matrix |
| QTL | Quantitative trait locus |
| RDW | Root dry weight |
| REML | Restricted maximum likelihood |
| RFLP | Restriction fragment length polymorphism |
| RFW | Root fresh weight |
| RIL | Recombinant inbred line |
| RL | Root length |
| RSR | Root shoot ratio |
| RV | Root volume |
| SA | Structure analysis |
| SDW | Shoot dry weight |
| SFA | Single factor analysis |
| SFW | Shoot fresh weight |
| SNP | Single nucleotide polymorphism |
| SSR | Simple sequence repeat |
| TDT | Transmission disequilibrium test |
| TILS | No. of tillers/plant |
| TW | Turgid weight |
| WS | Wilting score |

**10. Appendix 1** list of the Accessions which had desirable performance in most important traits related to drought tolerance under water stress conditions.

| Marker | | Acc. | PC | | | WS | | | RL | | | RWC | | | RSR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | W | D | D% | W | D | D% | W | D | D% | W | D | D% | W | D | D% |
| bpb-4531 ( 60.21 cM on 1H) | 0 | ICB180051 | 0.70 | 27.29 | 38.17 | 1.00 | 3.00 | 3.00 | 29.50 | 34.00 | 0.15 | 92.34 | 72.75 | 0.21 | 55.89 | 59.71 | 6.84 |
| | 0 | ICB180084 | 0.44 | 11.11 | 24.44 | 1.50 | 2.50 | 1.70 | 34.50 | 34.50 | 0.00 | 91.40 | 66.42 | 0.27 | 47.01 | 63.58 | 35.25 |
| | 0 | ICB180573 | 0.32 | 12.96 | 39.04 | 0.50 | 4.00 | 8.00 | 29.00 | 29.00 | 0.00 | 93.01 | 80.95 | 0.13 | 46.93 | 38.52 | -17.92 |
| | 0 | ICB180593 | 0.32 | 17.53 | 53.63 | 1.00 | 3.00 | 3.00 | 28.50 | 31.50 | 0.11 | 89.14 | 71.70 | 0.20 | 144.81 | 35.40 | -75.56 |
| | 0 | ICB180743 | 0.38 | 7.26 | 18.27 | 0.50 | 4.00 | 8.00 | 26.00 | 31.50 | 0.21 | 93.27 | 81.58 | 0.13 | 72.27 | 55.23 | -23.58 |
| | 0 | ICB180812 | 0.30 | 41.82 | 137.04 | 0.50 | 2.50 | 5.00 | 28.50 | 35.00 | 0.23 | 88.19 | 72.63 | 0.18 | 35.32 | 69.64 | 97.17 |
| | 0 | ICB180872 | 0.33 | 7.99 | 23.15 | 0.00 | 3.00 | 3.00 | 23.00 | 35.00 | 0.52 | 91.94 | 78.50 | 0.15 | 33.57 | 39.99 | 19.13 |
| | 0 | ICB180923 | 3.30 | 12.96 | 2.93 | 1.00 | 3.50 | 3.50 | 25.50 | 35.50 | 0.39 | 92.79 | 63.53 | 0.32 | 47.14 | 55.60 | 17.96 |
| | 1 | CCS010 | 0.55 | 15.31 | 26.97 | 0.50 | 5.00 | 10.00 | 23.50 | 30.50 | 0.30 | 92.21 | 71.79 | 0.22 | 26.26 | 19.93 | -24.10 |
| | 1 | CCS095 | 0.47 | 15.81 | 32.40 | 1.00 | 3.00 | 3.00 | 26.00 | 26.00 | 0.00 | 91.54 | 74.56 | 0.19 | 10.85 | 25.30 | 133.05 |
| | 1 | G119424 | 0.33 | 11.13 | 32.41 | 1.00 | 2.50 | 2.50 | 27.50 | 32.50 | 0.18 | 90.31 | 75.10 | 0.17 | 103.17 | 73.68 | -28.59 |
| | 1 | G119443 | 0.78 | 17.42 | 21.48 | 0.50 | 3.00 | 6.00 | 28.50 | 34.50 | 0.21 | 89.75 | 66.79 | 0.26 | 61.00 | 91.12 | 49.37 |
| | 1 | G124000 | 0.22 | 2.44 | 9.89 | 0.50 | 3.00 | 6.00 | 27.00 | 38.00 | 0.41 | 93.46 | 72.60 | 0.22 | 87.48 | 68.49 | -21.70 |
| | 1 | G124017 | 0.75 | 8.59 | 10.39 | 0.00 | 2.50 | 2.50 | 31.00 | 32.50 | 0.05 | 87.88 | 73.53 | 0.16 | 85.02 | 65.24 | -23.26 |
| | 1 | ICB181387 | 0.47 | 13.11 | 27.02 | 1.50 | 4.50 | 3.00 | 26.00 | 34.00 | 0.31 | 92.66 | 71.03 | 0.23 | 44.23 | 46.35 | 4.80 |
| | 1 | ICB181492 | 1.01 | 27.65 | 26.36 | 0.50 | 3.50 | 7.00 | 31.00 | 34.50 | 0.11 | 92.34 | 74.48 | 0.19 | 41.15 | 54.73 | 33.00 |
| | 1 | ICB181500 | 0.45 | 11.88 | 25.71 | 1.00 | 3.50 | 3.50 | 35.00 | 39.25 | 0.12 | 89.24 | 79.91 | 0.10 | 89.06 | 68.56 | -23.01 |

APPENDIX

Appendix 1 (Contd.)

| Marker | | Acc. | PC | | | WS | | | RL | | | RWC | | | RSR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | W | D | D% | W | D | D% | W | D | D% | W | D | D% | W | D | D% |
| bpb-0326 (139.91 cM on 2H) | 0 | CCS010 | 0.55 | 15.31 | 26.97 | 0.50 | 5.00 | 10.00 | 23.50 | 30.50 | 0.30 | 92.21 | 71.79 | 0.22 | 26.26 | 19.93 | -24.10 |
| | 0 | CCS095 | 0.47 | 15.81 | 32.40 | 1.00 | 3.00 | 3.00 | 26.00 | 26.00 | 0.00 | 91.54 | 74.56 | 0.19 | 10.85 | 25.30 | 133.05 |
| | 0 | G119424 | 0.33 | 11.13 | 32.41 | 1.00 | 2.50 | 2.50 | 27.50 | 32.50 | 0.18 | 90.31 | 75.10 | 0.17 | 103.17 | 73.68 | -28.59 |
| | 0 | G124000 | 0.22 | 2.44 | 9.89 | 0.50 | 3.00 | 6.00 | 27.00 | 38.00 | 0.41 | 93.46 | 72.60 | 0.22 | 87.48 | 68.49 | -21.70 |
| | 0 | G124017 | 0.75 | 8.59 | 10.39 | 0.00 | 2.50 | 2.50 | 31.00 | 32.50 | 0.05 | 87.88 | 73.53 | 0.16 | 85.02 | 65.24 | -23.26 |
| | 0 | ICB180051 | 0.70 | 27.29 | 38.17 | 1.00 | 3.00 | 3.00 | 29.50 | 34.00 | 0.15 | 92.34 | 72.75 | 0.21 | 55.89 | 59.71 | 6.84 |
| | 0 | ICB180573 | 0.32 | 12.96 | 39.04 | 0.50 | 4.00 | 8.00 | 29.00 | 29.00 | 0.00 | 93.01 | 80.95 | 0.13 | 46.93 | 38.52 | -17.92 |
| | 0 | ICB180743 | 0.38 | 7.26 | 18.27 | 0.50 | 4.00 | 8.00 | 26.00 | 31.50 | 0.21 | 93.27 | 81.58 | 0.13 | 72.27 | 55.23 | -23.58 |
| | 0 | ICB180923 | 3.30 | 12.96 | 2.93 | 1.00 | 3.50 | 3.50 | 25.50 | 35.50 | 0.39 | 92.79 | 63.53 | 0.32 | 47.14 | 55.60 | 17.96 |
| | 0 | ICB181387 | 0.47 | 13.11 | 27.02 | 1.50 | 4.50 | 3.00 | 26.00 | 34.00 | 0.31 | 92.66 | 71.03 | 0.23 | 44.23 | 46.35 | 4.80 |
| | 1 | G119443 | 0.78 | 17.42 | 21.48 | 0.50 | 3.00 | 6.00 | 28.50 | 34.50 | 0.21 | 89.75 | 66.79 | 0.26 | 61.00 | 91.12 | 49.37 |
| | 1 | ICB180084 | 0.44 | 11.11 | 24.44 | 1.50 | 2.50 | 1.70 | 34.50 | 34.50 | 0.00 | 91.40 | 66.42 | 0.27 | 47.01 | 63.58 | 35.25 |
| | 1 | ICB180172 | 0.59 | 2.38 | 3.05 | 0.50 | 2.50 | 5.00 | 26.50 | 32.50 | 0.23 | 93.85 | 78.90 | 0.16 | 46.72 | 71.18 | 52.35 |
| | 1 | ICB180593 | 0.32 | 17.53 | 53.63 | 1.00 | 3.00 | 3.00 | 28.50 | 31.50 | 0.11 | 89.14 | 71.70 | 0.20 | 144.81 | 35.40 | -75.56 |
| | 1 | ICB180812 | 0.30 | 41.82 | 137.04 | 0.50 | 2.50 | 5.00 | 28.50 | 35.00 | 0.23 | 88.19 | 72.63 | 0.18 | 35.32 | 69.64 | 97.17 |
| | 1 | ICB181492 | 1.01 | 27.65 | 26.36 | 0.50 | 3.50 | 7.00 | 31.00 | 34.50 | 0.11 | 92.34 | 74.48 | 0.19 | 41.15 | 54.73 | 33.00 |
| | 1 | ICB181500 | 0.45 | 11.88 | 25.71 | 1.00 | 3.50 | 3.50 | 35.00 | 39.25 | 0.12 | 89.24 | 79.91 | 0.10 | 89.06 | 68.56 | -23.01 |

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Appendix 1 (Contd.)

| Marker | | Acc. | PC | | | WS | | | RL | | | RWC | | | RSR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | W | D | D% | W | D | D% | W | D | D% | W | D | D% | W | D | D% |
| bpb-1408 (60.04 cM on 4H) | 0 | G119424 | 0.33 | 11.13 | 32.41 | 1.00 | 2.50 | 2.50 | 27.50 | 32.50 | 0.18 | 90.31 | 75.10 | 0.17 | 103.17 | 73.68 | -28.59 |
| | 0 | G119443 | 0.78 | 17.42 | 21.48 | 0.50 | 3.00 | 6.00 | 28.50 | 34.50 | 0.21 | 89.75 | 66.79 | 0.26 | 61.00 | 91.12 | 49.37 |
| | 0 | G124000 | 0.22 | 2.44 | 9.89 | 0.50 | 3.00 | 6.00 | 27.00 | 38.00 | 0.41 | 93.46 | 72.60 | 0.22 | 87.48 | 68.49 | -21.70 |
| | 0 | G124017 | 0.75 | 8.59 | 10.39 | 0.00 | 2.50 | 2.50 | 31.00 | 32.50 | 0.05 | 87.88 | 73.53 | 0.16 | 85.02 | 65.24 | -23.26 |
| | 0 | ICB180148 | 0.94 | 5.81 | 5.19 | 1.00 | 2.50 | 2.50 | 25.00 | 34.00 | 0.36 | 93.06 | 72.02 | 0.23 | 51.27 | 73.44 | 43.24 |
| | 0 | ICB180923 | 3.30 | 12.96 | 2.93 | 1.00 | 3.50 | 3.50 | 25.50 | 35.50 | 0.39 | 92.79 | 63.53 | 0.32 | 47.14 | 55.60 | 17.96 |
| | 0 | ICB181387 | 0.47 | 13.11 | 27.02 | 1.50 | 4.50 | 3.00 | 26.00 | 34.00 | 0.31 | 92.66 | 71.03 | 0.23 | 44.23 | 46.35 | 4.80 |
| | 0 | ICB181492 | 1.01 | 27.65 | 26.36 | 0.50 | 3.50 | 7.00 | 31.00 | 34.50 | 0.11 | 92.34 | 74.48 | 0.19 | 41.15 | 54.73 | 33.00 |
| | 0 | ICB181500 | 0.45 | 11.88 | 25.71 | 1.00 | 3.50 | 3.50 | 35.00 | 39.25 | 0.12 | 89.24 | 79.91 | 0.10 | 89.06 | 68.56 | -23.01 |
| | 1 | CCS010 | 0.55 | 15.31 | 26.97 | 0.50 | 5.00 | 10.00 | 23.50 | 30.50 | 0.30 | 92.21 | 71.79 | 0.22 | 26.26 | 19.93 | -24.10 |
| | 1 | CCS095 | 0.47 | 15.81 | 32.40 | 1.00 | 3.00 | 3.00 | 26.00 | 26.00 | 0.00 | 91.54 | 74.56 | 0.19 | 10.85 | 25.30 | 133.05 |
| | 1 | ICB180051 | 0.70 | 27.29 | 38.17 | 1.00 | 3.00 | 3.00 | 29.50 | 34.00 | 0.15 | 92.34 | 72.75 | 0.21 | 55.89 | 59.71 | 6.84 |
| | 1 | ICB180084 | 0.44 | 11.11 | 24.44 | 1.50 | 2.50 | 1.70 | 34.50 | 34.50 | 0.00 | 91.40 | 66.42 | 0.27 | 47.01 | 63.58 | 35.25 |
| | 1 | ICB180172 | 0.59 | 2.38 | 3.05 | 0.50 | 2.50 | 5.00 | 26.50 | 32.50 | 0.23 | 93.85 | 78.90 | 0.16 | 46.72 | 71.18 | 52.35 |
| | 1 | ICB180573 | 0.32 | 12.96 | 39.04 | 0.50 | 4.00 | 8.00 | 29.00 | 29.00 | 0.00 | 93.01 | 80.95 | 0.13 | 46.93 | 38.52 | -17.92 |
| | 1 | ICB180593 | 0.32 | 17.53 | 53.63 | 1.00 | 3.00 | 3.00 | 28.50 | 31.50 | 0.11 | 89.14 | 71.70 | 0.20 | 144.81 | 35.40 | -75.56 |
| | 1 | ICB180743 | 0.38 | 7.26 | 18.27 | 0.50 | 4.00 | 8.00 | 26.00 | 31.50 | 0.21 | 93.27 | 81.58 | 0.13 | 72.27 | 55.23 | -23.58 |
| | 1 | ICB180812 | 0.30 | 41.82 | 137.04 | 0.50 | 2.50 | 5.00 | 28.50 | 35.00 | 0.23 | 88.19 | 72.63 | 0.18 | 35.32 | 69.64 | 97.17 |
| | 1 | ICB180872 | 0.33 | 7.99 | 23.15 | 0.00 | 3.00 | 3.00 | 23.00 | 35.00 | 0.52 | 91.94 | 78.50 | 0.15 | 33.57 | 39.99 | 19.13 |

APPENDIX

Appendix 1 (Contd.)

| Marker | | Acc. | PC | | | WS | | | RL | | | RWC | | | RSR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | W | D | D% | W | D | D% | W | D | D% | W | D | D% | W | D | D% |
| bpb-0786 (57.00 cM on 5H) | 0 | CCS010 | 0.55 | 15.31 | 26.97 | 0.50 | 5.00 | 10.00 | 23.50 | 30.50 | 0.30 | 92.21 | 71.79 | 0.22 | 26.26 | 19.93 | -24.10 |
| | 0 | CCS095 | 0.47 | 15.81 | 32.40 | 1.00 | 3.00 | 3.00 | 26.00 | 26.00 | 0.00 | 91.54 | 74.56 | 0.19 | 10.85 | 25.30 | 133.05 |
| | 0 | ICB180051 | 0.70 | 27.29 | 38.17 | 1.00 | 3.00 | 3.00 | 29.50 | 34.00 | 0.15 | 92.34 | 72.75 | 0.21 | 55.89 | 59.71 | 6.84 |
| | 0 | ICB180084 | 0.44 | 11.11 | 24.44 | 1.50 | 2.50 | 1.70 | 34.50 | 34.50 | 0.00 | 91.40 | 66.42 | 0.27 | 47.01 | 63.58 | 35.25 |
| | 0 | ICB180573 | 0.32 | 12.96 | 39.04 | 0.50 | 4.00 | 8.00 | 29.00 | 29.00 | 0.00 | 93.01 | 80.95 | 0.13 | 46.93 | 38.52 | -17.92 |
| | 0 | ICB180593 | 0.32 | 17.53 | 53.63 | 1.00 | 3.00 | 3.00 | 28.50 | 31.50 | 0.11 | 89.14 | 71.70 | 0.20 | 144.81 | 35.40 | -75.56 |
| | 0 | ICB180812 | 0.30 | 41.82 | 137.04 | 0.50 | 2.50 | 5.00 | 28.50 | 35.00 | 0.23 | 88.19 | 72.63 | 0.18 | 35.32 | 69.64 | 97.17 |
| | 0 | ICB180872 | 0.33 | 7.99 | 23.15 | 0.00 | 3.00 | 3.00 | 23.00 | 35.00 | 0.52 | 91.94 | 78.50 | 0.15 | 33.57 | 39.99 | 19.13 |
| | 0 | ICB180923 | 3.30 | 12.96 | 2.93 | 1.00 | 3.50 | 3.50 | 25.50 | 35.50 | 0.39 | 92.79 | 63.53 | 0.32 | 47.14 | 55.60 | 17.96 |
| | 1 | G119424 | 0.33 | 11.13 | 32.41 | 1.00 | 2.50 | 2.50 | 27.50 | 32.50 | 0.18 | 90.31 | 75.10 | 0.17 | 103.17 | 73.68 | -28.59 |
| | 1 | G119443 | 0.78 | 17.42 | 21.48 | 0.50 | 3.00 | 6.00 | 28.50 | 34.50 | 0.21 | 89.75 | 66.79 | 0.26 | 61.00 | 91.12 | 49.37 |
| | 1 | G124000 | 0.22 | 2.44 | 9.89 | 0.50 | 3.00 | 6.00 | 27.00 | 38.00 | 0.41 | 93.46 | 72.60 | 0.22 | 87.48 | 68.49 | -21.70 |
| | 1 | G124017 | 0.75 | 8.59 | 10.39 | 0.00 | 2.50 | 2.50 | 31.00 | 32.50 | 0.05 | 87.88 | 73.53 | 0.16 | 85.02 | 65.24 | -23.26 |
| | 1 | ICB180172 | 0.59 | 2.38 | 3.05 | 0.50 | 2.50 | 5.00 | 26.50 | 32.50 | 0.23 | 93.85 | 78.90 | 0.16 | 46.72 | 71.18 | 52.35 |
| | 1 | ICB181387 | 0.47 | 13.11 | 27.02 | 1.50 | 4.50 | 3.00 | 26.00 | 34.00 | 0.31 | 92.66 | 71.03 | 0.23 | 44.23 | 46.35 | 4.80 |
| | 1 | ICB181492 | 1.01 | 27.65 | 26.36 | 0.50 | 3.50 | 7.00 | 31.00 | 34.50 | 0.11 | 92.34 | 74.48 | 0.19 | 41.15 | 54.73 | 33.00 |
| | 1 | ICB181500 | 0.45 | 11.88 | 25.71 | 1.00 | 3.50 | 3.50 | 35.00 | 39.25 | 0.12 | 89.24 | 79.91 | 0.10 | 89.06 | 68.56 | -23.01 |

APPENDIX

Appendix 1 (Contd.)

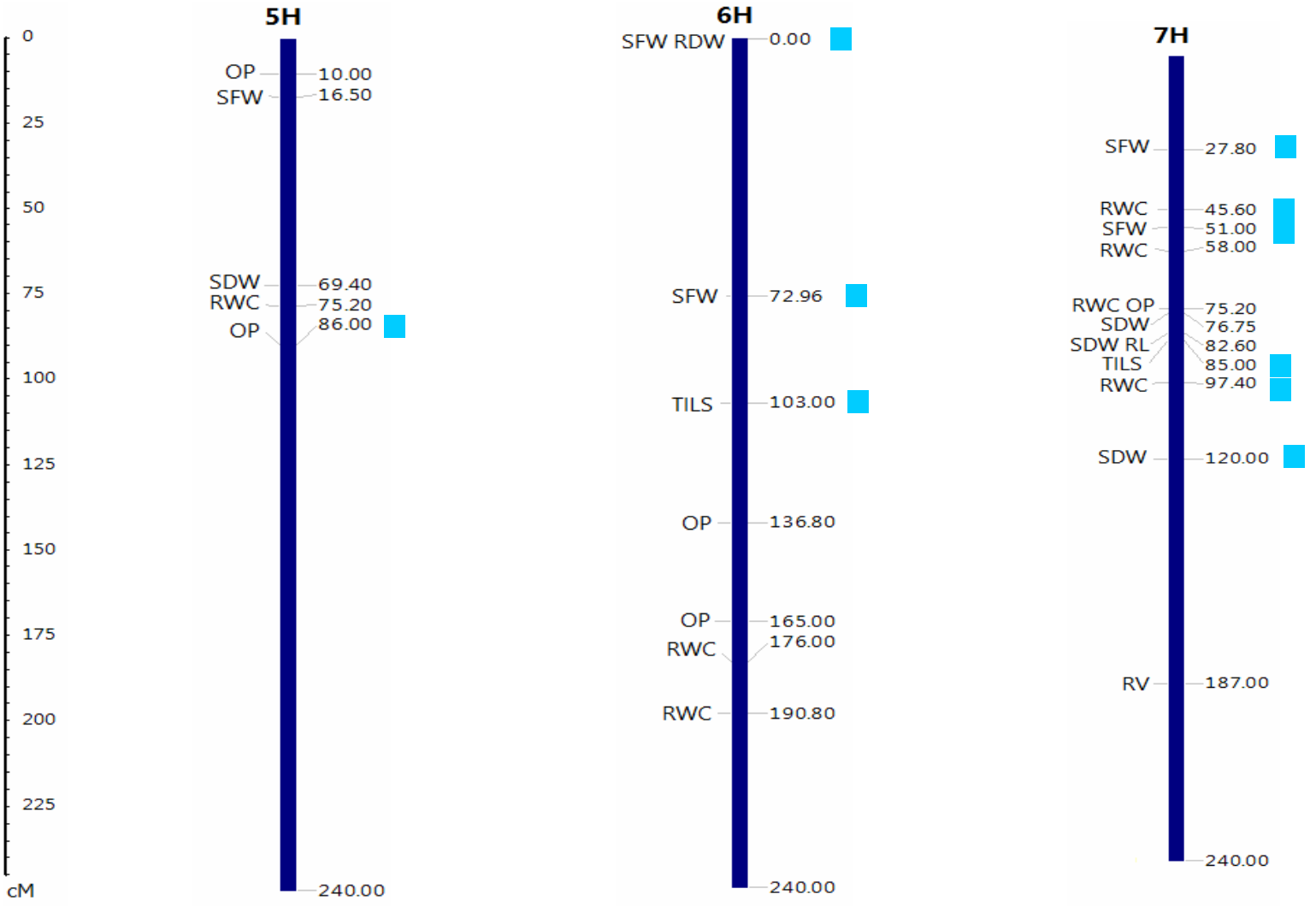
| Marker | | Acc. | PC | | | WS | | | RL | | | RWC | | | RSR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | W | D | D% | W | D | D% | W | D | D% | W | D | D% | W | D | D% |
| bpb-8049 (53.43 cM on 7H) | 0 | ICB180051 | 0.70 | 27.29 | 38.17 | 1.00 | 3.00 | 3.00 | 29.50 | 34.00 | 0.15 | 92.34 | 72.75 | 0.21 | 55.89 | 59.71 | 6.84 |
| | 0 | ICB180084 | 0.44 | 11.11 | 24.44 | 1.50 | 2.50 | 1.70 | 34.50 | 34.50 | 0.00 | 91.40 | 66.42 | 0.27 | 47.01 | 63.58 | 35.25 |
| | 0 | ICB180148 | 0.94 | 5.81 | 5.19 | 1.00 | 2.50 | 2.50 | 25.00 | 34.00 | 0.36 | 93.06 | 72.02 | 0.23 | 51.27 | 73.44 | 43.24 |
| | 0 | ICB180172 | 0.59 | 2.38 | 3.05 | 0.50 | 2.50 | 5.00 | 26.50 | 32.50 | 0.23 | 93.85 | 78.90 | 0.16 | 46.72 | 71.18 | 52.35 |
| | 0 | ICB180743 | 0.38 | 7.26 | 18.27 | 0.50 | 4.00 | 8.00 | 26.00 | 31.50 | 0.21 | 93.27 | 81.58 | 0.13 | 72.27 | 55.23 | -23.58 |
| | 0 | ICB180812 | 0.30 | 41.82 | 137.04 | 0.50 | 2.50 | 5.00 | 28.50 | 35.00 | 0.23 | 88.19 | 72.63 | 0.18 | 35.32 | 69.64 | 97.17 |
| | 0 | ICB180923 | 3.30 | 12.96 | 2.93 | 1.00 | 3.50 | 3.50 | 25.50 | 35.50 | 0.39 | 92.79 | 63.53 | 0.32 | 47.14 | 55.60 | 17.96 |
| | 1 | CCS010 | 0.55 | 15.31 | 26.97 | 0.50 | 5.00 | 10.00 | 23.50 | 30.50 | 0.30 | 92.21 | 71.79 | 0.22 | 26.26 | 19.93 | -24.10 |
| | 1 | CCS095 | 0.47 | 15.81 | 32.40 | 1.00 | 3.00 | 3.00 | 26.00 | 26.00 | 0.00 | 91.54 | 74.56 | 0.19 | 10.85 | 25.30 | 133.05 |
| | 1 | G119424 | 0.33 | 11.13 | 32.41 | 1.00 | 2.50 | 2.50 | 27.50 | 32.50 | 0.18 | 90.31 | 75.10 | 0.17 | 103.17 | 73.68 | -28.59 |
| | 1 | G119443 | 0.78 | 17.42 | 21.48 | 0.50 | 3.00 | 6.00 | 28.50 | 34.50 | 0.21 | 89.75 | 66.79 | 0.26 | 61.00 | 91.12 | 49.37 |
| | 1 | G124000 | 0.22 | 2.44 | 9.89 | 0.50 | 3.00 | 6.00 | 27.00 | 38.00 | 0.41 | 93.46 | 72.60 | 0.22 | 87.48 | 68.49 | -21.70 |
| | 1 | G124017 | 0.75 | 8.59 | 10.39 | 0.00 | 2.50 | 2.50 | 31.00 | 32.50 | 0.05 | 87.88 | 73.53 | 0.16 | 85.02 | 65.24 | -23.26 |
| | 1 | ICB180573 | 0.32 | 12.96 | 39.04 | 0.50 | 4.00 | 8.00 | 29.00 | 29.00 | 0.00 | 93.01 | 80.95 | 0.13 | 46.93 | 38.52 | -17.92 |
| | 1 | ICB180872 | 0.33 | 7.99 | 23.15 | 0.00 | 3.00 | 3.00 | 23.00 | 35.00 | 0.52 | 91.94 | 78.50 | 0.15 | 33.57 | 39.99 | 19.13 |
| | 1 | ICB181387 | 0.47 | 13.11 | 27.02 | 1.50 | 4.50 | 3.00 | 26.00 | 34.00 | 0.31 | 92.66 | 71.03 | 0.23 | 44.23 | 46.35 | 4.80 |
| | 1 | ICB181492 | 1.01 | 27.65 | 26.36 | 0.50 | 3.50 | 7.00 | 31.00 | 34.50 | 0.11 | 92.34 | 74.48 | 0.19 | 41.15 | 54.73 | 33.00 |
| | 1 | ICB181500 | 0.45 | 11.88 | 25.71 | 1.00 | 3.50 | 3.50 | 35.00 | 39.25 | 0.12 | 89.24 | 79.91 | 0.10 | 89.06 | 68.56 | -23.01 |

Abbreviations: Accessions (Acc), Proline content (PC), Wilting Score (WS), root length (RL), relative water content (RWC), root dry weight part c (DWc), the performance of Accessions under water stress (D %), well-watered treatment (W) and drought stress treatment (D)

APPENDIX


**Appendix 2** The barley map of the previous studies, whereas SFW: shoot fresh weight, SDW: shoot dry weight, OP: osmotic potential, TILS: number of tillers, RWC: relative water content, RL: root length, RV: root volume, RDW: root dry weight and WS: wilting score. ■ The agreement QTL with the current study.

APPENDIX




**Appendix 2** The barley map of the previous studies, whereas SFW: shoot fresh weight, SDW: shoot dry weight, OP: osmotic potential, TILS: number of tillers, RWC: relative water content, RL: root length, RV: root volume, RDW: root dry weight and WS: wilting score. ■ The agreement QTL with the current study.

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