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# Association Mapping for Shoot Traits Related to Drought Tolerance in Barley

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***Abstract* – The shoot traits related drought tolerance is an important for understanding drought tolerance mechanisms of the whole plant. The current research was curried out to identify the QTLs associated with shoot traits related to drought tolerance in a structured barley population grown under well-watered and drought stress conditions during the summer seasons of 2007-08., Marker data collected from all accessions were used to establish population structure and kinship relationships. Mixed liner model (MIL) analysis, which include population structure and kinship used to discover marker-trait associations. 56 QTLs have been identified for the shoot 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, a total of 8, 16, 8, 8, 9, 5 and 5 QTLs were detected for wilting score (WS), no. of tillers/plant (TILS), shoot fresh weight/plant (SFW), Shoot dry weight/plant (SDW), Relative content (RWC), Proline content (PC) and Osmotic potential (OP) respectively. Six co-locations of QTLs were correlated with all studied traits and covered the whole genome of Barley population of interest.**

***Keywords* – Association Mapping, Drought Stress, Barley, QTL.**

## I. INTRODUCTION

Barley (Hordeum vulgare L.) is an economically important crop and is considered a model species for genetic and physiological studies [1]. It is diploid with seven chromosome pairs (2n=2x=14) and is predominantly inbreeding species with out crossing rates ranging from 0 to 9.6% [2].

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 [3]. 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, and shoot traits [4, 5] all of which influence the plant's ability to maintain high turgor and normal growth with less water available.

Most of drought tolerance traits in plants are quantitative in nature, and controlling by polygenes. 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). [6,7,8]. Linkage analysis in plants typically localizes QTLs to 10 to 20 cM intervals because of the limited number of recombination events that occur during the construction of mapping populations and the cost for propagating and evaluating a large number of lines [9,10]. 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 [11, 12, and 13].

Association mapping of a trait is an approach to identify chromosomal regions that contain genes affecting the trait. 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 [14], yield traits in barley [15], Iron deficiency in soybean (16), and disease resistance in rice [17], potato [18,19] and corn [20]. Application of association mapping to plant breeding seems to be a promising means of overcoming the limitations of conventional linkage mapping [21].

In this study, we investigate the association of 1081 diversity array technology (DArT) markers with Shoot and some physiological traits, which was related to Drought tolerance in barley population.

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

## II. MATERIAL AND METHODS

Ninety eight wild barley accessions (Hordeum vulgare ssp. spontaneum), mostly members of the International Barley Core Collection were provided by Dr. Helmut Knuepffer, IPK, Gatersleben (zitieren Buch mit Kapitel über IBCC:Diversity on Barley. Auswahl etc.) The accessions were from 10 different countries, mostly in the Fertile Crescent, but also Central Asia, and the Caucasus region (Table 1). The origin of two accessions (ICB180035, ICB191338) is not known.

Table 1: 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 | PALESTINE | Wild | 8 |
| 2 | CCS004 | GERMANY | Cultivated | 10 | | 62 | ICB180102 | PALESTINE | Wild | 8 |
| 3 | CCS010 | GERMANY | Cultivated | 10 | | 63 | ICB180109 | PALESTINE | Wild | 8 |
| 4 | CCS012 | GERMANY | Cultivated | 10 | | 64 | ICB180117 | PALESTINE | Wild | 4 |
| 5 | CCS018 | GERMANY | Cultivated | 10 | | 65 | ICB180131 | PALESTINE | Wild | 10 |
| 6 | CCS023 | GERMANY | Cultivated | 10 | | 66 | ICB180148 | PALESTINE | Wild | 5 |
| 7 | CCS041 | GERMANY | Cultivated | 10 | | 67 | ICB180172 | PALESTINE | Wild | 8 |
| 8 | CCS049 | GERMANY | Cultivated | 10 | | 68 | ICB180199 | PALESTINE | Wild | 8 |
| 9 | CCS052 | GERMANY | Cultivated | 10 | | 69 | ICB180231 | PALESTINE | Wild | 8 |
| 10 | CCS060 | GERMANY | Cultivated | 10 | | 70 | ICB180260 | PALESTINE | Wild | 8 |
| 11 | CCS067 | GERMANY | Cultivated | 10 | | 71 | ICB180329 | PALESTINE | Wild | 8 |
| 12 | CCS081 | GERMANY | Cultivated | 10 | | 72 | ICB180372 | PALESTINE | Wild | 8 |
| 13 | CCS083 | GERMANY | Cultivated | 10 | | 73 | ICB180389 | PALESTINE | Wild | 9 |
| 14 | CCS084 | GERMANY | Cultivated | 10 | | 74 | ICB180410 | PALESTINE | Wild | 8 |
| 15 | CCS086 | GERMANY | Cultivated | 10 | | 75 | ICB180430 | PALESTINE | Wild | 8 |
| 16 | CCS089 | GERMANY | Cultivated | 10 | | 76 | ICB180508 | PALESTINE | Wild | 8 |
| 17 | CCS095 | GERMANY | Cultivated | 10 | | 77 | ICB180554 | PALESTINE | Wild | 8 |
| 18 | CCS096 | GERMANY | Cultivated | 10 | | 78 | ICB180573 | PALESTINE | Wild | 9 |
| 19 | CCS109 | GERMANY | Cultivated | 10 | | 79 | ICB180593 | PALESTINE | Wild | 6 |
| 20 | CCS121 | GERMANY | Cultivated | 10 | | 80 | ICB180631 | PALESTINE | Wild | 9 |
| 21 | CCS140 | GERMANY | Cultivated | 10 | | 81 | ICB180743 | PALESTINE | Wild | 8 |
| 22 | CCS141 | GERMANY | Cultivated | 10 | | 82 | ICB180973 | PALESTINE | Wild | 6 |
| 23 | ICB180046 | IRAK | Wild | 12 | | 83 | ICB180982 | PALESTINE | Wild | 7 |
| 24 | ICB180049 | IRAK | Wild | 3 | | 84 | ICB180994 | PALESTINE | Wild | 8 |
| 25 | ICB180069 | IRAK | Wild | 9 | | 85 | ICB181150 | PALESTINE | 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 | TAJIKISTAN | Wild | 4 |
| 46 | ICB181216 | JORDAN | Wild | 11 | | 106 | ICB180063 | TURKEY | Wild | 9 |
| 47 | ICB181268 | JORDAN | Wild | 8 | | 107 | ICB180070 | TURKEY | Wild | 5 |
| 48 | ICB181381 | JORDAN | Wild | 8 | | 108 | ICB181228 | TURKEY | Wild | 12 |
| 49 | ICB181387 | JORDAN | Wild | 6 | | 109 | ICB181230 | TURKEY | Wild | 12 |
| 50 | ICB181412 | JORDAN | Wild | 9 | | 110 | ICB180211 | TURKMENISTAN | Wild | 4 |
| 51 | ICB181418 | JORDAN | Wild | 11 | | 111 | ICB180213 | TURKMENISTAN | Wild | 4 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 52 | ICB181424 | JORDAN | Wild | 11 | | 112 | ICB180215 | TURKMENISTAN | Wild | 8 |
| 53 | ICB181430 | JORDAN | Wild | 8 | | 113 | ICB180217 | TURKMENISTAN | Wild | 4 |
| 54 | ICB181442 | JORDAN | Wild | 11 | | 114 | ICB181492 | TURKMENISTAN | 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 | PALESTINE | Wild | 8 | | | | | | |

Additionally 21 spring barley cultivars representative for the breeding pool of spring barley in North Rhine Westphalia (NRW), Germany, [22, 23] were included into the group. Single plant selections were made from the raw accessions selfed twice, and then tested for their reaction to drought stress.

### *Genotyping:*

DNA has been extracted from 10 mg freeze dried leaves by using "Kit" procedure according to DNeasy Plant Handbook 07/2006. DArT markers were generated by Triticarte Pty. Ltd. (Canberra, Australia; http://www.triticarte.com.au), a whole-genome profiling service laboratory, as described by [24]. The locus designations used by Triticarte Pty. Ltd. were adopted in this article. We received 1081 DArT markers for the population.

### *Inference of population structure and kinship*

According to [25] the population structure as well as the relative kinship (Q matrix and K matrix) were included into the association analysis. A model-based approach, implemented in the software package STRUCTURE 2.2. for dominant markers, was used to identify significant clusters (for Q matrix). We used 'admixture' co-ancestry model under independent and correlated allele frequencies using the burn-in time of 50,000 and the number of replications at 100,000 with the k up to 14 Pair wise kinship estimates (K matrix) were calculated using the software package TASSEL 2.01. The K-matrices, and the Q-matrix describing the assignment of each accession to specific clusters, were used in mixed liner model association mapping [25].

### *Experiments*

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 a refrigerator for 7 days at 4 °C. After that 12 seeds were sown in two rows in plastic pots of 22 x 22 cm with 25 cm depth, with 4 holes pierced at the bottom for drainage. 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 10th and 1st of April in 2007 and 2008, respectively.

In the well-watered treatment the plants got 330 ml water per pot each day per drip irrigation, while in the drought stress treatment the plants got 165 ml per pot each day. The drought stress treatment started 40 days after sowing and was continued for 21 days.

After that shoot traits were measured as follows:

*Wilting Score (WS)* Scored from 0 up to 9. 0 with no symptoms of stress effect and 9 with all plants apparently dried. [26].

*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 0C for 48 hours, and then the average per one plant was calculated.

### *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 [27], 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 0C 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 0C for 1 hour and centrifuged at 13000 U/min (Heraeus Biofuge Pico) for 10 min and finally stored at -20 0C 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, freezedried (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 0C 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) [28]

*Marker-trait association*

Marker-trait association analysis was performed with a mixed linear model (MLM) using "ASReml" Software version 2 [29] according to [30]. In order to estimate the marker by drought treatment interaction which is essential in interpreting the reaction of gene regions to drought stress, we adapted ASReml to this problem. The statistical model for the association analysis identifying DArT markers which are associated with the tested drought tolerance traits considering population structure and the relative kinship is as follows:

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

where µ is the general mean, Yi is the fixed effect of the *ith* Year, Tj 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, $A_m(M_L)$ is the random effect of *mth* accession nested in the *Lth* marker associated with *nth* kinship coefficient , $Y_i*A_m(M_L)$ is the random interaction effect of *ith* year with *mth* accession nested in the *Lth* marker associated with *nth* kinship coefficient, $T_j*A_m(M_L)$ 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.

FDR, wann Marker significant

## III. RESULTS AND DISCUSSION

*Phenotypic Measurements*

In this study 119 accession were evaluated for quantitative traits (Wilting score, Number of tillers, Shoot fresh weight, Shoot dry weight, 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 2) The followed traits, WS, SFW, RWC and PC were exhibited highly significantly differences in both seasons, while OP had non-significant differences in both seasons and TILS was exhibited non-significant differences in the first season, while it was highly significant in the second season.

Table 2: 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 | |
| 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 3: Summary statistics of 7 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 | |
| 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 3 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 and OP, where RWC decreased under drought stress condition with 25.6%, and the Proline content increased about 13 fold more than well-watered.

*The phenotypic correlation across two years*

Table 4 shows the Pearson correlation coefficients (r) between 6 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 and SDW under drought stress treatment. PC was correlated negatively with each of TILS, and positively with WS under both treatments, while it was negatively with RWC under drought stress conditions. RWC was correlated negatively with WS and positively with TILS under both of treatments.

Table 4: 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 | 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 | | |
| PC | W | 0.164* | -0.167* | 0.146* | -0.032 | -0.129 | |
| | D | 0.177** | -0.439** | -0.007 | -0.288** | -0.479** | |
| OP | W | -0.145* | -0.042 | -0.099 | 0.078 | 0.032 | -0.123 |
| | D | -0.071 | 0.144* | 0.004 | 0.197** | 0.087 | -0.195* |

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

*Population structure and Kinship coefficients*

The Population structure analysis was conducted using genotypic data of 1081 DArT markers by using Structure Software 2.2 [31], and the accessions subdivided into 12 subpopulations, base on the suggestion of [32], 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.1).

Figure 2 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.

*Marker-trait associations*

A mixed liner model (MLM) implemented in ASReml Software Version 2 according to [30] 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).

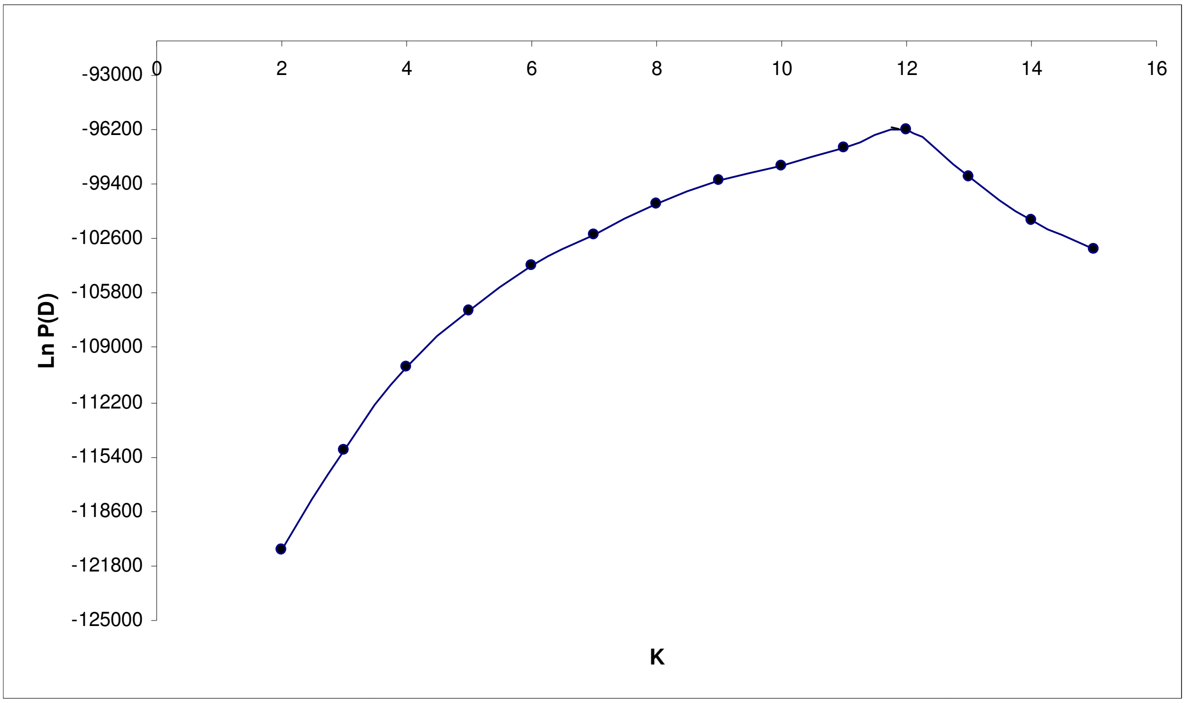

Fig.1. Presents the number of clusters, which have the highest maximum likelihood

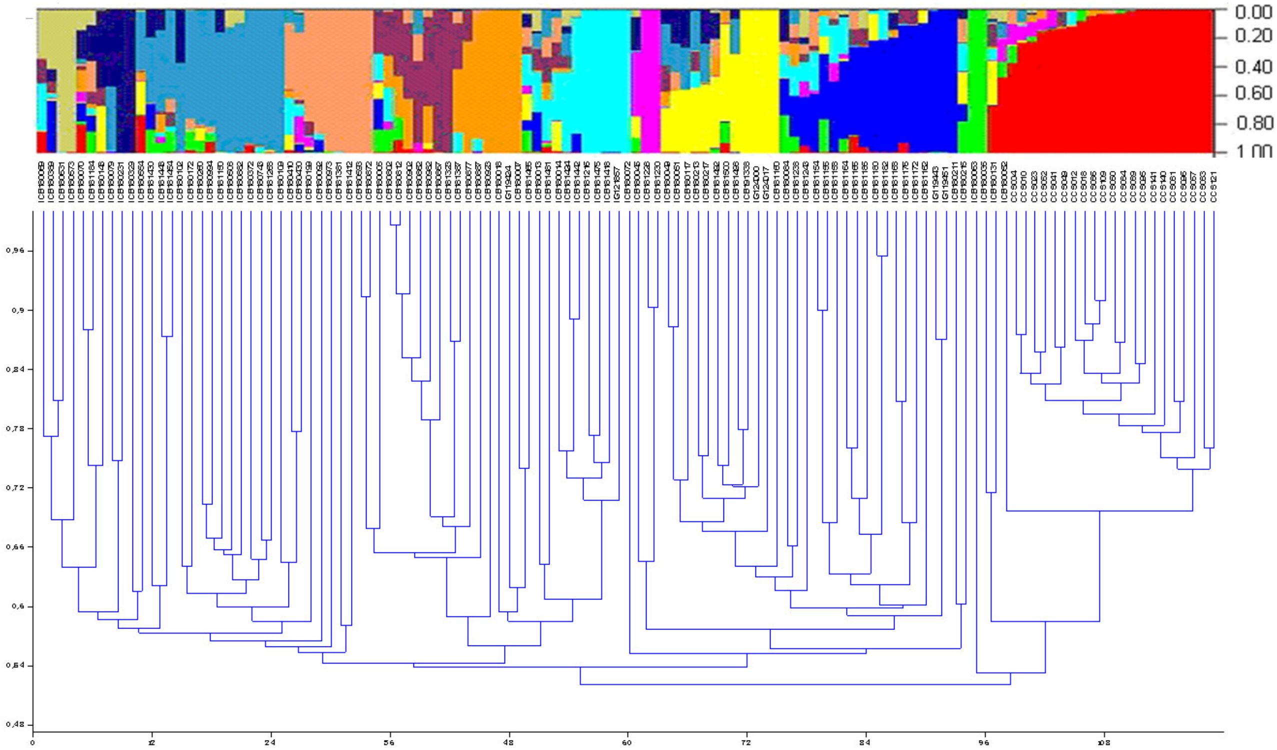

Fig.2. The hierarchical clustering (UPGMA) of the accessions based on their genetic distances and the subdivision into 12 Groups according to the structure analysis and geographical distribution

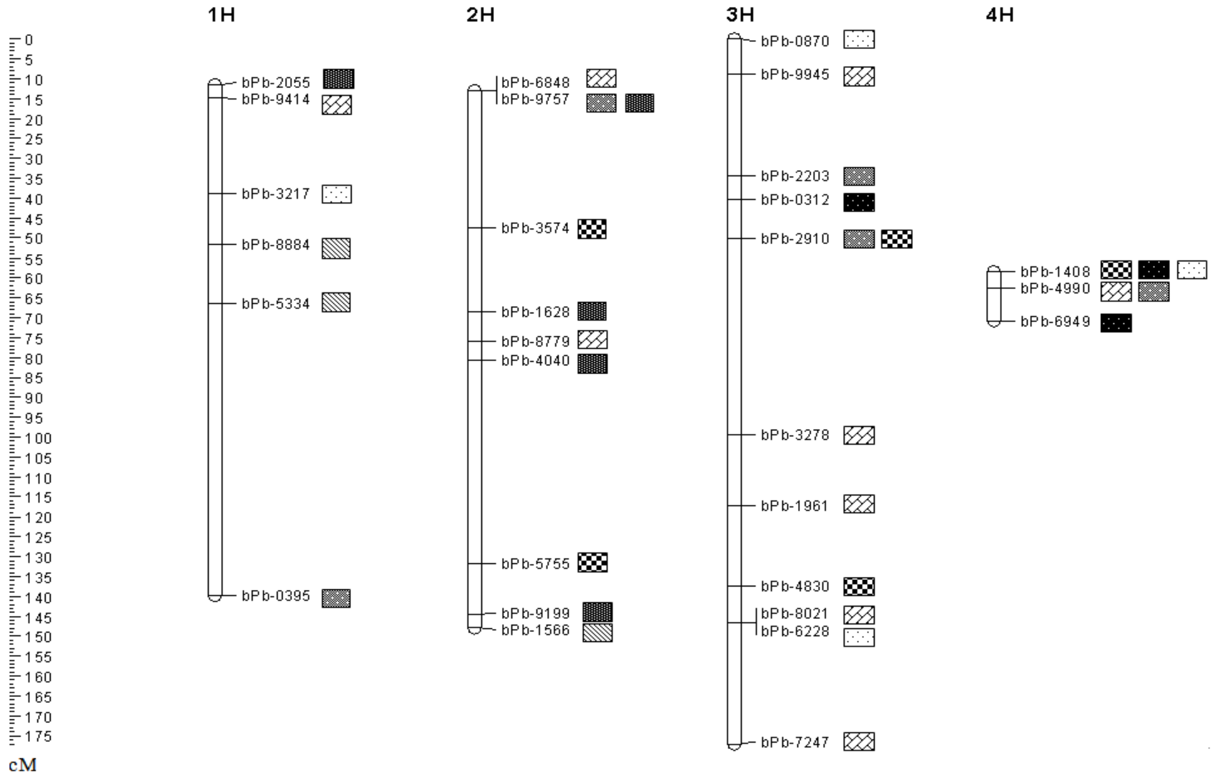

Fig.3. DArT map for structured barley population presents the detected QTLs associated with shoot drought tolerance traits. On the right hand, the traits and their positions (cM): ■ wilting score (WS); ▨ no.of tillers/plant (TILS); ▦ shoot frsh weight/plant (SFW); ▩ Shoot dry weight/plant (SDW); ▣ Relative content (RWC); ☐ Proline content (PC) and ▧ Osmotic potential (OP)

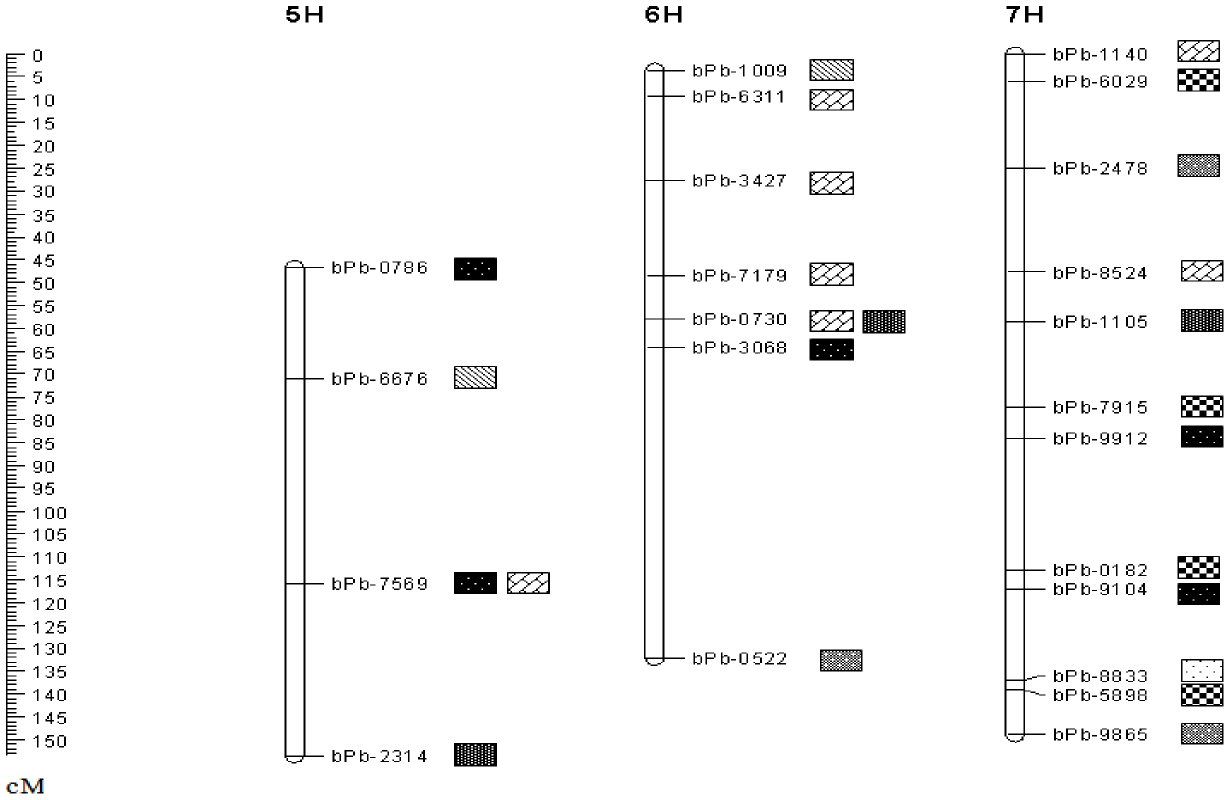

Fig.4. DArT map for structured barley population presents the detected QTLs associated with shoot drought tolerance traits. On the right hand, the traits and their positions (cM): ■ wilting score (WS); ▨ no.of tillers/plant (TILS); ▦ shoot frsh weight/plant (SFW); ▩ Shoot dry weight/plant (SDW); ▣ Relative content (RWC); ☐ Proline content (PC) and ▧ Osmotic potential (OP)

### *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 QTL for each, while chromosomes 4H, 5H and 7H involved two QTLs for each (Fig. 3 and fig. 4). 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 (Table 5 and 6). [33] mapped one QTL for wilting score at position (195.7- 206.5 cM) on 1H. [34] 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). [35] mapped quantitative trait loci (QTLs) for the visual scores of leaf rolling and leaf drying in upland Rice. [36] 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. [37] conducted an early QTL study in rice and found Twelve of the 14 QTL associated with leaf rolling. [38] Mapped six QTLs in rice for leaf drying score (LDS) on chromosomes 1, 2, 3 (two QTLs), 8 and 9.

Table 5: The real performance as main effect of Marker allele in studied traits

| Trait | DArT Marker | Chr. | Pos. (cM ) | Prob. | FDR | Difference Allele 1 minus Allele 0 | Rel. Performance of Allele 1 | Freq. Of Allele 1 in Population |
|---|---|---|---|---|---|---|---|---|
| WS | bPb-0312 | 3H | 41.78 | ** | 0.008 | -0.52 | -0.38 | 0.39 |
| | bPb-6949 | 4H | 72.21 | ** | 0.01 | 1.11 | 1.03 | 0.54 |
| | bPb-7569 | 5H | 125.98 | ** | 0.01 | 0.01 | -0.04 | 0.35 |
| | bPb-3068 | 6H | 74.3 | ** | 0.0075 | 0.64 | 0.58 | 0.49 |
| | bPb-9104 | 7H | 127.4 | ** | 0.0067 | 0.35 | 0.15 | 0.49 |
| TILS | bPb-9414 | 1H | 16.1 | *** | 0.0015 | 0.45 | 0.424 | 0.42 |
| | bPb-6848 | 2H | 14.4 | *** | 0.0012 | 0.61 | 0.404 | 0.45 |
| | bPb-8779 | 2H | 77.41 | *** | 0.0016 | 0.2 | 0.264 | 0.50 |
| | bPb-9945 | 3H | 10.2 | ** | 0.009 | 0.33 | 0.114 | 0.52 |
| | bPb-3278 | 3H | 100.76 | *** | 0.0012 | 1.07 | 0.724 | 0.34 |
| | bPb-1961 | 3H | 118.72 | *** | 0.0018 | -0.43 | -0.056 | 0.58 |
| | bPb-8021 | 3H | 147.95 | ** | 0.006 | -0.17 | -0.044 | 0.40 |
| | bPb-7247 | 3H | 178.6 | ** | 0.0087 | -1.11 | -0.866 | 0.45 |
| | bPb-7569 | 5H | 125.98 | ** | 0.0017 | -0.57 | -0.176 | 0.35 |
| | bPb-6311 | 6H | 19.42 | ** | 0.0053 | 0.01 | 0.016 | 0.49 |
| | bPb-0730 | 6H | 68.22 | *** | 0.0015 | 0.17 | 0.174 | 0.35 |
| | bPb-1140 | 7H | 10.21 | ** | 0.0015 | -0.33 | 0.024 | 0.44 |
| SFW | bPb-1628 | 2H | 70.03 | ** | 0.012 | 0.37 | 0.32 | 0.52 |
| | bPb-4040 | 2H | 82.13 | ** | 0.0075 | -0.3 | -0.2 | 0.48 |
| | bPb-1105 | 7H | 68.8 | ** | 0.006 | -0.47 | -0.29 | 0.38 |
| SDW | bPb-0395 | 1H | 141.29 | ** | 0.0096 | 0.22 | 0.19 | 0.45 |
| | bPb-9757 | 2H | 14.4 | ** | 0.012 | -0.05 | -0.05 | 0.45 |
| | bPb-2203 | 3H | 35.93 | ** | 0.021 | -0.16 | -0.08 | 0.40 |
| | bPb-2910 | 3H | 51.59 | ** | 0.009 | -0.25 | -0.04 | 0.39 |
| | bPb-0522 | 6H | 142.51 | ** | 0.014 | 0.09 | 0.06 | 0.40 |
| | bPb-9865 | 7H | 159.19 | ** | 0.0105 | 0.18 | 0.12 | 0.40 |
| RWC | bPb-3574 | 2H | 49.03 | ** | 0.01 | -7 | -6.81 | 0.50 |
| | bPb-5755 | 2H | 133.29 | ** | 0.005 | -3.21 | -2.08 | 0.41 |
| | bPb-2910 | 3H | 51.59 | ** | 0.005 | 2.82 | 0.26 | 0.39 |
| | bPb-1408 | 4H | 60.04 | ** | 0.009 | 3.23 | 1.26 | 0.55 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | bPb-0182 | 7H | 123.08 | ** | 0.0075 | -2.5 | -1.86 | 0.40 |
| OP | bPb-6676 | 5H | 81.39 | ** | 0.008 | 0.02 | 0.003 | 0.28 |
| | bPb-1009 | 6H | 13.83 | ** | 0.008 | -0.01 | -0.007 | 0.52 |
| PC | bPb-3217 | 1H | 40.53 | ** | 0.009 | 2.17 | 1.79 | 0.41 |
| | bPb-8833 | 7H | 147.17 | ** | 0.014 | 1.46 | 0.11 | 0.52 |

Table 6: The real performance as interaction effects of marker allele in studied traits

| Trait | DArT Marker | Chr. | Pos. (cM) | Prob. | FDR | Control | | Drought | | Freq. Of Allel 1 in Population |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Difference Allele 1 minus Allele 0 | Real perform. of Allele1 | Difference Allele 1 minus Allele 0 | Real perform. of Allele1. | |
| WS | bPb-1408 | 4H | 60.04 | ** | 0.0030 | 0.43 | 0.08 | -0.40 | -0.15 | 0.55 |
| | bPb-0786 | 5H | 57.00 | ** | 0.0030 | -0.53 | -0.32 | 0.26 | 0.14 | 0.42 |
| | bPb-9912 | 7H | 94.41 | ** | 0.0020 | 0.04 | 0.04 | -0.83 | -0.60 | 0.48 |
| TILS | bPb-4990 | 4H | 64.16 | ** | 0.0053 | -0.26 | -0.2 | 0.39 | 0.39 | 0.52 |
| | bPb-3427 | 6H | 38.04 | ** | 0.0090 | 0.42 | 0.24 | -0.14 | -0.01 | 0.29 |
| | bPb-7179 | 6H | 58.56 | ** | 0.0040 | -0.29 | -0.16 | 0.01 | 0.03 | 0.57 |
| | bPb-8524 | 7H | 58.02 | ** | 0.0040 | 0.28 | 0.09 | -0.15 | -0.09 | 0.47 |
| SFW | bPb-2055 | 1H | 12.96 | ** | 0.0150 | 0.37 | 0.30 | -0.04 | -0.03 | 0.42 |
| | bPb-9757 | 2H | 14.4 | ** | 0.0250 | 0.43 | 0.19 | -0.42 | -0.30 | 0.45 |
| | bPb-9199 | 2H | 145.95 | ** | 0.0125 | 1.12 | 0.32 | 0.12 | 0.05 | 0.45 |
| | bPb-2314 | 5H | 163.75 | ** | 0.0113 | 1.00 | 0.50 | 0.06 | 0.05 | 0.45 |
| | bPb-0730 | 6H | 68.22 | ** | 0.0090 | 0.81 | 0.35 | 0.05 | 0.06 | 0.35 |
| SDW | bPb-9757 | 2H | 14.40 | ** | 0.0120 | 0.05 | 0.01 | -0.14 | -0.10 | 0.45 |
| | bPb-4990 | 4H | 64.16 | *** | 0.0004 | 0.03 | 0.02 | 0.03 | 0.02 | 0.52 |
| | bPb-0522 | 6H | 142.51 | ** | 0.0200 | 0.11 | 0.04 | 0.07 | 0.06 | 0.40 |
| | bPb-2478 | 7H | 35.22 | ** | 0.0120 | 0.05 | 0.02 | -0.03 | -0.02 | 0.46 |
| RWC | bPb-4830 | 3H | 138.85 | *** | 0.0006 | -2.43 | -1.58 | 5.91 | 2.00 | 0.54 |
| | bPb-1408 | 4H | 60.04 | *** | 0.0003 | -1.25 | -0.2 | 7.72 | 2.73 | 0.55 |
| | bPb-6029 | 7H | 16.21 | *** | 0.0002 | 2.74 | 1.55 | -4.52 | -2.69 | 0.39 |
| | bPb-7915 | 7H | 87.55 | *** | 0.0002 | 2.56 | 1.85 | -4.93 | -3.6 | 0.33 |
| | bPb-0182 | 7H | 123.08 | *** | 0.0001 | 1.08 | 0.88 | -6.1 | -4.61 | 0.40 |
| | bPb-5898 | 7H | 149.4 | ** | 0.0010 | 1.12 | 0.43 | -5.79 | -1.32 | 0.33 |
| OP | bPb-8884 | 1H | 53.19 | ** | 0.0075 | 0.02 | 0.003 | -0.02 | -0.008 | 0.45 |
| | bPb-5334 | 1H | 67.88 | ** | 0.0050 | 0.02 | 0.003 | 0.00 | 0.012 | 0.34 |
| | bPb-1566 | 2H | 149.44 | ** | 0.0060 | 0.01 | 0.003 | 0.00 | 0.012 | 0.50 |
| PC | bPb-3217 | 1H | 40.53 | ** | 0.0120 | 0.09 | -0.013 | 4.25 | 3.55 | 0.41 |
| | bPb-0870 | 3H | 1.48 | ** | 0.0160 | -0.75 | -0.163 | -1.31 | -0.65 | 0.40 |
| | bPb-6228 | 3H | 147.95 | ** | 0.0107 | -0.92 | -0.723 | 0.75 | 0.12 | 0.32 |
| | bPb-1408 | 4H | 60.04 | ** | 0.0080 | -0.22 | -0.173 | 4.16 | 1.83 | 0.55 |

### *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 (Fig. 3 and 4). 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 (Table 5 and 6). In a QTL study in barley, [39] 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 [1]. With regard to candidate genes, [40] 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 (Fig. 3 and 4). 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 (Table 5 and 6). Similar results were obtained by [41]; 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) [42]. 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 (43).

### *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) (Fig. 3 and 4). 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 (Table 5 and 6). Similar results were found by many Authors, [43] detected two QTLs on chromosomes 1H (92.4 cM) and 6H (0.00 cM). [44, 45] detected two QTL associated with dry biomass on 7H (120 cM), and 4H (83 cM9). [1] 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). [46] 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 [47, 48]. With regard to candidate genes, [40] identified a candidate gene (EXP13) on chromosome 1 controlling shoot dry weight in rice under well-watered conditions. [34] found one QTL for Straw yield (g/m$^2$) located at 75.6 cM on chromosome 12 in rice.

[49] 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 (Fig. 3 and 4). 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 (Table 5 and 6)). [50] 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 [51] in genomic regions A, B and J on chromosomes 1H and 6H. [52] 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. [53] Identified one QTL on chromosome 1 for relative water content in rice. similar results were obtained by [54], 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. [55] 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 [35], they identified QTLs on chromosomes 1, 3, 4, 5, 6, 8, 9, 10, and 11.

### *6) Osmotic potential (OP)*

Five QTLs were detected for OP, two QTLs on 1H and one QTL for each 2H, 5H and 6H (Fig. 3 and 4). 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 (Table 5 and 6). [54] 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. [51] mapped other osmoregulation genes in barley on 6H (136.8 cM), on1H (97.4 cM) and on 2H (0.00 cM). [56] 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. [55] 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).

### *7) Proline content (PC)*

In the (Fig3 and Fig4) Five QTLs were detected for PC and mapped on 1H, 3H (2 QTLs), 4H and 7H (Fig.3 and 4). 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 (Table 5 and 6). 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 [57]. In addition to [58, 59] 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, [60] 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. [61] 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 .

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