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QTL ANALYSIS FOR DROUGHT TOLERANCE IN BARLEY AT NEWLY RECLAIMED SOILS IN EGYPT

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ABSTRACT

Water deficit is being the most devastating abiotic stress factor in newly reclaimed soils in Egypt. Identification of useful alleles in both cultivated varieties and wild relatives of these traits might be helpful in developing drought tolerant genotypes. 301 doubled haploid (DH) lines, their parents (Scarlett and ISR 42-8) and four check cultivars were investigated under two water regimes in the 2013/2014 season in newly reclaimed area at Wadi Al-Asyouti farm of the Faculty of Agriculture, Assiut University, Assiut, Egypt. The QTL analysis revealed 35 putative QTLs as maker main effect and marker × treatment interaction for six studied traits. 17 QTLs were identified with favorable effects of the exotic alleles. Numerous interesting QTLs were detected in this study that displaying beneficial effects of the exotic alleles (Hsp). For instance, the alleles of the QTL QHD.S42-2H exhibited a favorable performance of reducing days to 50% heading by -3.03%. A favorable QTL QPH.S42-7Ha effect was responsible for almost 6.29% decrease in plant height due to Hsp alleles. Interestingly, the QTL QGYP.S42-1Ha revealed that marker × treatment interaction effect and the relative performance of the exotic genotype led to increase grain yield per plant with 14.31%. Altogether 21 pairs of epistatic QTLs as additive × additive effects were detected in current study. The majority of the digenic epistatic interaction pairs had favorable effects on the phenotypic values of the studied traits. For example, the most favorable pair of epistatic QTL for reducing heading date was bPb-9111* bPb-8701 and located on chromosomes 3H (141.94 cM) and 4H (93.64 cM) and accounted for 19.36% of genetic variation. The DH lines carrying the Hsp/Hsp combination at two loci had heavier grain weight by maximum 0.87 g more than lines with the allelic combination Hv/Hv. This study has highlighted the role of the exotic alleles for the detection of favorable alleles lead that for drought tolerance in barley.

Key words: ***Cultivated and wild barley, Sandy soils, QTL mapping, Drought stress.***

INTRODUCTION

Egypt is located in the hyper-arid regions of North Africa and West Asia, of which only a small portion (3% of total area) is agriculturally productive with annual rainfall in most parts of less than 50mm (Hegazi *et al* 2005). A recent report has been published by Dakkak (2016) pointed out that Egypt has been suffering from severe water scarcity in recent years. Uneven water distribution, misuse of water resources and inefficient irrigation techniques are some of the major factors playing havoc with water security in the country. Being more or less an arid country, Egypt is heavily dependent on rain in other countries to support its rapidly growing population and development. In addition, Egypt is facing an annual water deficit of around 7 billion cubic metres and United Nations is already warning that Egypt could run out of water by the year 2025. Therefore, water deficit is being one of the major causes for crop loss in this area and worldwide including that of barley (Jana and Wilen 2005). However, the

agricultural regions that affected by drought can experience yield loss up to 50% or more (Wang *et al*. 2003 and Jenks and Hasegawa 2005). Genetically, drought stress tolerance is a quantitatively inherited trait, controlled by several genetic loci (QTL) and tolerance to drought involves a complex of mechanisms working in combination to avoid or tolerate water deficits (Diab 2004).

Barley (*Hordeum vulgare* ssp. *vulgare* L.) is one of the important crops worldwide and provides an excellent system for genome mapping and genetic studies, due to (1) its diploid nature, (2) low chromosome number (2n=14), (3) relatively large chromosomes (6-8 μm), (4) high degree of self-fertility, and (5) ease of hybridization (Hussain 2006 and Sreenivasulu *et al* 2008). It is widely grown in the arid and semiarid regions of the Mediterranean for forage purposes and as a grain crop (Al-Karaki 2001), and characterized by being relatively high drought tolerance, where it can grow with lesser soil moisture (Mishra and Shivakumar 2000). Barley genotypes, in particular landraces and wild species, represent an important source of variation for adaptive traits that may contribute to increase yield and yield stability under drought conditions, and that could be introgressed into improved varieties. Producing more drought-tolerant barley varieties and the other crops would be the most economical approach to improve agricultural productivity and to reduce agricultural use of fresh water resources in arid areas (Jenks and Hasegawa 2005).

Molecular markers can be used to explore germplasm through segregation and association mapping to identify useful alleles in both cultivated varieties and wild relatives (Cattivelli *et al* 2008). QTL analysis is a very popular and powerful tool to assign specific positions to genes contributing to traits related to drought tolerance by performing the statistical association analysis between markers and traits of interest. QTL maps have been made for traits thought to be involved in drought tolerance in many species including rice, barley, and wheat (Teulat *et al* 2003; Quarrie *et al* 2005 and Sayed *et al* 2012). Cattivelli *et al* (2008) reviewed progress of breeding for drought tolerance and suggested that markers tightly linked to traits conferring drought tolerance could improve breeding efficiency. The identification of these QTLs with linked markers allows the breeders to use marker-assisted selection as a complementary tool instead of traditional selection.

Genotype effects can be attributed to major genes, quantitative trait loci (QTL) and gene by gene interactions, which are also termed epistatic interactions. QTL analysis can explore the role of epistasis in the genetic basis of complex traits (Carlborg and Haley 2004). Many studies have suggested that epistatic interactions play a larger role in crosses involving exotic germplasm than in elite by elite crosses (von Korff *et al* 2010 and Sayed *et al* 2012). Thus, in the current study, we have employed a QTL

analysis using REML forward selection approach for simultaneous estimation of main effects of all individual markers and epistatic effects of all pairs of markers, which allows detecting interactions with a higher power. The present study used 301 BC_2DH lines derived from a cross between cultivated and wild barley. The objectives of this work were to identify the beneficial exotic alleles which are important for the expression of the drought tolerance related traits

MATERIALS AND METHODS

Plant material

A doubled haploid mapping population designated as S42 was used for QTL analysis in this study. It consisted of 301 BC_2DH lines derived from crossing between a German elite cultivar of *H. vulgare* ssp. *vulgare* 'Scarlett' with an exotic accession of *H. vulgare* ssp. *spontaneum* 'ISR42-8'. The cultivar Scarlett was used as the recurrent parent whereas ISR42-8 was utilized as the donor. More details about development of this population and proportion of donor genome are given in von Korff *et al* (2004) and Schmalenbach *et al* (2008). For comparison with barley local cultivars, four commercial cultivars of barley namely; *i. e*. Giza 123, Giza 127, Giza 129 and Giza 2000 were used in this study as check cultivars.

Experimental site

The experiment was carried out in the 2013/2014 growing season under two water regimes using sprinkler irrigation system in the newly reclaimed area (Wadi Al-Asyouti farm) at the Experimental Station of the Faculty of Agriculture, El-Ghorieb Farm, Assiut University (25 km South East of Assiut city) Egypt. This area is a sandy soil (EC 1.66 dsm^{-1}; pH 8.34; Total CaCO3 20.26%; Organic matter 0.097%; Ca^{++} 16.46 meq kg^{-1}; Mg^{++} 8.72 meq kg^{-1}; Na^{+} 1.86 meq kg^{-1}; K^{+} 0.22 meq kg^{-1}; HCO^{3-} 7.65 meq kg^{-1}; CL- 6.25 meq kg^{-1}; SO4= 3.54 meq kg^{-1}; Total nitrogen 0.018%). Each value of the physical and chemical properties represents the mean of three replications of the representative soil samples in the experimental site before sowing (0-30 cm depth) in 2013 - 2014 season.

Experimental design and water treatments

The experiment was arranged in strip plot design based on randomized complete block arrangement (RCBD) in two replications. Well-watered (WW) and drought stress (DS) treatments were arranged in main plots while DH lines, the two parents and check cultivars were arranged in subplots. Each plot consisted of one row 6 m long and 0.20 m apart. Total irrigation requirement (IR) was estimated as 4368 m^3 / hectare /season following Ainer *et al* (1999). We divided this amount by 50 irrigations each one 87.36 m^3/ hectare. Sprinkler irrigation was applied every three days along the growing season as two hours for well-watered treatment (100% IR) and 1.5 hours for drought stress treatment (75% IR). Sprinkler diameter was 3/4" and its discharge was 0.3 m^3/h distributed in 6 m service radius. In

case of having rains we omitted the amount of water gained by rains from the total irrigation requirements. The recommended doses of (NPK) fertilizers were added and normal cultural practices of growing winter cereals conducted in the usual manner followed by the farmers of this district.

Phenotypic data

Heading date (HD) for each genotype was recorded as the number of days from the sowing date until 50% of tillers had emergence of spikes from the flag leaf sheath (Zadoks *et al* 1974). At anthesis time, chlorophyll content (CC) of the flag leaf was measured using a self-calibrating SPAD chlorophyll meter (Model 502, Spectrum Technologies, Plainfield, IL) from 10 randomly flag leaves, then the average was scored. This measurement directly estimated the chlorophyll content of the flag leaf (Xu *et al* 2000). At maturity time, 10 individual plants were randomly chosen in the middle for each row to measure plant height (PH; cm). At harvest, 8-12 guarded individual plants were randomly harvested to measure the following traits: the average spikes number per plant (NSP), 100-grain weight (GW; g) and the average grain yield per plant (GYP; g).

Genotyping of population S42

The S42 population was genotyped with a total number of 371 genetic markers containing 106 SSRs according to von Korff *et al* (2004), 255 DArT following Sayed *et al* (2012) and ten gene-specific DNA markers referred to Wang *et al* (2010) in order to perform QTL analysis. The linkage map of this population was drawn using MapChart ver.2.2 (Voorrips 2002).

Analysis of variance

To detect the differences and variation in DH lines and between ISR 42-8 and Scarlett for the studied traits, ANOVA was performed with the Statistical Analysis System SAS (SAS Institute, ver. 9.2 2008), using PROC GLM procedure. The phenotypic correlations among trait performances were computed using the correlation procedure (PROC CORR). The LS-means of the investigated traits across the DH lines over replication were used for the calculation of the Pearson correlation coefficients (r).

Broad-sense heritability estimation

Broad-sense heritability (h^2_B) was computed as $h^2_B = \delta^2_G / (\delta^2_G + \delta^2_{G\times T}/t + \delta^2_E/tr)$, where δ^2_G, $\delta^2_{G\times T}$ and δ^2_E were the estimates of genetic, genotype × treatment interaction and error variances, respectively, derived from the expected mean squares of the combined analysis of variance. Also t and r were the number of treatments and replications, respectively.

Relative performance of the exotic parent $RP_{[Hsp]}$

The relative performance of the exotic parent $RP_{[Hsp]}$ was computed by the following formula:

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

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

QTL analysis

The QTL analysis was conducted using a multiple QTL model iteratively extended and reduced by forward selection and backward elimination, respectively using the PROC MIXED procedure in SAS software. More details about this model (see Sayed *et al* 2012). Starting point was the following mixed hierarchical model:

$$X_{ijkl}=\mu+Mi+L_j(M_i)+T_k+L_j*T_k+M_i*T_k+\varepsilon l_{(ijk)},$$

where the total of phenotypic value was sum of general mean μ, fixed effect Mi of the i-th marker genotype, random effect $L_j(M_i)$ of the j-th DH line nested in the i-th marker genotype, fixed effect T_k of the k-th treatment, fixed interaction effect L_j*T_k of the j-th DH line and the k-th treatment, fixed interaction effect M_i*M_k of the i-th marker genotype and the k-th treatment and residue $\varepsilon_{l(ijk)}$ of Xijkl. P values from F-tests were adjusted genome-wide across all single marker tests using the false discovery rate (FDR). The significant marker main effects as well as marker × treatment interaction with PFDR ≤ 0.05 were accepted as putative QTLs for the next iteration, however, the final model was:

$$X_{ijkl}=\mu+\sum QTL+M_i+L_j(M_i)+T_k+L_j*T_k+M_i*T_k+\varepsilon_{l(ijk)},$$

where ∑QTL represents the detected QTL from the forward/backward selection process. The contribution of a QTL to trait genotypic variance was estimated by the R2 coefficient (percentage of the explained genotypic variance) according to von Korff *et al* (2004).

Digenic epistatic effects

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

$$X_{ijklm}=\mu+\sum QTL+M1_i+M2_j+M1_i*M2_j+L_k(M1_i*M2_j)+Tl+Lj*Tk+ +\varepsilon l_{(ijkm)},$$

where M1i and M2j are the fixed effects of the i-th marker and j-th marker (M2). M1i*M2j is the fixed interaction effect of the *i-th* M1 genotype with *j-th* M2 genotype, $L_k(M1_i*M2_j)$ is the random effect of the *k-th* BC_2DH line nested in the *i-th* M_1 and *j-th* M_2 marker genotype interaction.

RESULTS AND DISCUSSION

Phenotypic variation of the parents and DH lines

Data in Table (1) shows the separate and combined analysis of variance of the investigated traits under well-watered and drought stress conditions and across both treatments, respectively. There were highly significant differences among the 307 genotypes (301 DH lines, their parents and four check cultivars) for all investigated traits under both treatments, except for days to 50% heading under drought stress, where the genotypes were not significant.

Table 1. Separate and combined analysis of variance and broad sense heritability (h^2_B) for the studied traits.

SOV	df	HD	PH	CC	GW	NSP	GYP
Under well-watered conditions							
Replications	**1**	**206.41****	**1.36**	**47.16**	**12.01****	**0.24**	**31.38****
Genotypes	**306**	**56.38****	**210.30****	**54.04****	**0.61****	**4.30****	**1.33****
Error	**306**	**24.38**	**47.92**	**15.87**	**0.01**	**0.14**	**0.03**
Under drought stress conditions							
Replications	**1**	**22.29**	**2.47**	**3.73**	**19.69****	**0.03**	**35.61****
Genotypes	**306**	**38.45**	**142.85****	**60.60****	**0.47****	**3.14****	**0.91****
Error	**306**	**37.72**	**94.66**	**12.41**	**0.01**	**0.21**	**0.00**
Combined analysis of variance over two treatments							
Treatments (T)	**1**	**162.71***	**6619.80****	**1603.64****	**46.72****	**202.53****	**187.90****
T(Rep)	**2**	**114.35***	**69.72**	**25.45**	**15.90**	**0.14**	**33.50**
Genotypes (G)	**306**	**55.41****	**180.67****	**64.64****	**0.53****	**3.94**	**1.20****
G×T	**306**	**39.44****	**172.44****	**50.00****	**0.55****	**3.50**	**1.03****
Error	**612**	**31.05**	**71.29**	**14.14**	**0.00**	**0.18**	**0.02**
Broad sense heritability (h^2) %		**11.16**	**56.22**	**72.70**	**90.06**	**95.62**	**87.72**

*** and **; significant at P values at 0.05 and 0.01, respectively.**

Furthermore, the combined analysis of variance revealed significant differences between both treatments and among genotypes for all studied traits. The interaction between genotypes and treatments was highly significant for all studied traits, except number of spikes per plant. This result reflected the existence of sufficient variation among genotypes. Presented data in Table (2) exhibits the mean, minimum, maximum and standard deviation values of S42 population, their parents (Scarlett and ISR 42-8) and check cultivars under well-watered and drought stress conditions. Results revealed that Scarlett was earlier heading, shorter height, less in chlorophyll content, heavier 100- grain weight, higher in spikes number per plant and higher in grain yield per plant than the wild accession ISR 42-8 under both treatments. Regarding to their progeny, it can be observed that DH lines showed extreme phenotypes of minimum and maximum values that fall outside the range of their parental lines. This finding reflected the existence of the transgressive segregation and indicated that S42 population showed more genetic variation and variation in gene expression than their parents. The transgressive segregation also indicated that both parents transmitted favorable alleles for each trait. All investigated traits of the both parents were reduced due to drought stress except of chlorophyll content in Scarlett, which was higher under drought conditions. Yield reduction percentages due to drought accounted 24.34, 76.71 and 21.05% for Scarlett, ISR 42-8 and DH lines, respectively (Table 2).

Table 2. Summary statistics of Scarlett, ISR 42-8, DH lines and check cultivars for the studied traits under well-watered and drought stress conditions.

Plant material	Statistics	Well-watered conditions						Drought stress conditions						Yield reduction %
		HD	PH	CC	GW	NSP	GYP	HD	PH	CC	GW	NSP	GYP	
Scarlett	Mean	89.50	69.00	46.25	4.49	5.92	4.52	88.50	59.34	47.45	3.64	5.33	3.42	24.34
	Min	89.00	65.00	39.10	4.34	5.67	4.31	88.00	58.88	41.80	3.47	4.83	3.18	
	Max	90.00	73.00	53.40	4.64	6.18	4.72	89.00	59.80	53.10	3.81	5.83	3.66	
	SD	0.71	5.66	10.11	0.21	0.36	0.29	0.71	0.65	7.99	0.24	0.71	0.34	
ISR 42-8	Mean	95.50	94.50	51.89	3.55	5.08	1.46	92.50	51.98	50.51	2.72	5.08	0.34	76.71
	Min	95.00	10.87	6.07	0.52	0.20	0.20	92.00	48.76	50.32	2.59	4.83	0.10	
	Max	96.00	105.8	66.90	6.10	9.50	4.70	93.00	55.20	50.69	2.85	5.33	0.58	
	SD	0.71	14.85	3.38	0.17	0.35	0.39	0.71	4.55	0.26	0.18	0.35	0.34	
S42 pop.	Mean	87.78	68.52	47.75	4.19	5.94	3.61	88.29	64.29	45.49	3.81	5.15	2.85	21.05
	Min	69.00	42.00	26.80	1.60	1.00	0.30	69.00	38.60	22.50	2.30	1.20	0.20	
	Max	100.00	115.00	67.10	6.30	10.30	7.20	100.0	105.80	66.90	6.10	9.50	4.70	
	SD	5.26	11.26	5.91	0.57	1.46	0.83	5.29	10.87	6.07	0.52	1.30	0.70	
Checks	Mean	72.63	77.25	51.06	4.34	8.34	5.14	90.75	52.33	45.81	3.61	5.31	3.23	37.16
	Min	69.00	63.00	42.90	3.60	6.52	3.90	88.00	46.00	40.20	2.97	4.00	2.46	
	Max	77.00	93.00	59.70	5.23	11.85	6.45	95.00	58.88	51.10	4.40	6.00	3.89	
	SD	3.16	10.01	5.49	0.59	1.98	0.89	2.60	4.19	3.72	0.45	0.70	0.51	
Overall mean		87.49	68.71	47.81	4.19	5.96	3.63	88.22	64.07	45.51	3.80	5.15	2.84	21.76
LSD 5%		9.7	13.6	7.8	0.07	0.74	0.35	12.0	19.1	6.9	0.08	0.90	0.05	
Overall SD		4.93	6.92	3.98	0.04	0.37	0.18	6.14	9.72	3.52	0.04	0.46	0.03	
Overall C.V.%		5.64	10.07	8.33	0.89	6.36	4.96	6.96	15.18	7.73	1.11	8.94	0.95	
Overall R^2		70.06	81.46	77.34	99.70	96.67	97.79	50.52	60.14	83.00	98.30	93.60	96.20	

On average, check local cultivars were earlier than Scarlett, wild accession and DH lines by 16.8, 22.8 and 15.1 days under WW conditions, respectively. In contrast under DS conditions, the check cultivars were late by average value one day. As an average, check cultivars (5.14 g/plant) yielded more than Scarlett under WW conditions, while under DS Scarlett yielded more than check cultivars. By maximum values comparison, it can be observed that the maximum values of the DH lines for grain yield/plant were higher (7.20 and 4.70 g) than the maximum grain yield values of check cultivars (6.45 and 3.89 g) under WW and DS, respectively. Since under well-watered conditions, 14 and 5 DH lines were yielded on average more than the cultivated parent Scarlett and the average of the check cultivars, respectively. Meanwhile, under drought stress conditions, 40 and 65 DH

lines were yielded on average more than the cultivated parent Scarlett and the average of the check cultivars, respectively. This result indicates the possibility of selection among S42 population for high yielding genotypes under well-watered and drought stress conditions. Coefficient of variation ranged from 0.89% (100-grain weight) to 10.07% (plant height) and from 0.95% (grain yield/plant) to 15.18% (plant height) under WW and DS conditions, respectively. Plant height was the most trait showed more variation under both treatments. Days to 50% heading explained the lowest R^2 value (70.06 and 50.52%), while 100-grain weight accounted the maximum value of the phenotypic variance (99.70 and 98.30%) under WW and DS conditions, respectively. Zhu *et al* (1999) found significant variation among DH lines within each environment and there were both positive and negative transgressive segregants. Xue *et al* (2008) studied chlorophyll content in barley and found significant transgressive distribution among DH lines.

Correlation among traits

Information on association of yield and its attributes could be useful in selection of drought tolerant genotypes and may help in indirect selection of yield components under water deficit conditions. Mutual correlation coefficients among studied traits under well-watered and drought stress conditions are presented in Table (3). There was negative and significant correlation between days to 50% heading and each of plant height (r=-0.343**), chlorophyll content (r=-0.327**), number of spikes per plant (r=-0.211**) and grain yield per plant (r=-0.162**) under well-watered conditions. While it was negative and significant with plant height (r=-0.277**), grain weight (r=-0.120*) and number of spikes per plant (r=-0.124*) under drought stress. von Korff *et al* (2006) found negative correlation between yield and plant height, and positive correlation with number of spikes and days until heading.

Table 3. Correlation coefficients (r) among studied traits under well-watered conditions (above) and drought stress conditions (below).

	HD	PH	CC	GW	NSP	GYP
HD		**-0.343****	**-0.327****	**-0.069**	**-0.211****	**-0.162****
PH	**-0.277****		**0.232****	**0.116***	**0.134***	**0.157****
CC	**-0.006**	**-0.124***		**0.023**	**0.231****	**0.209****
GW	**-0.120***	**-0.019**	**0.067**		**0.140***	**0.242****
NSP	**-0.124***	**0.083**	**0.070**	**0.139***		**0.635****
GYP	**-0.067**	**-0.018**	**0.105**	**0.407****	**0.497****	

*** and **; significant at P values at 0.05 and 0.01, respectively.**

QTL detection in S42 population

Altogether, 35 putative QTLs and marker × treatment interaction were detected for six studied evaluated under well-watered and drought stress conditions (Table 4 and Figure 1). Among these loci, 17 (48.5 %) QTLs for studied traits were identified with favorable effects of the exotic alleles. Most of putative QTLs were covered the whole genome (except chromosome 4H). Several studies on the same population revealed that detected QTL showed favorable effects derived from the presence of exotic alleles of the homozygous *Hsp* genotype in population S42 ranged between 26 to 34.1% (Pillen *et al* 2003 and 2004 and Sayed 2011)

Days to 50% heading (HD)

Time of flowering is a major trait of a crop adaptation to the environment, particularly when the growing season is restricted by terminal drought and high temperatures. Developing short-duration varieties has been an effective strategy for minimizing yield loss from terminal drought, as early maturity helps the crop to avoid the period of environmental stress (Kumar and Abbo 2001). Five putative QTLs for HD were mapped on chromosomes 2H, 3H, 5H and 6H (Table 4 and Figure 1). Four loci exhibited significant marker main effects, while one QTL showed main and QTL × treatment interaction effects. According to the relative performance of the exotic allele ($Rp_{[aa]}$), the alleles of four QTLs (QHD.S42-2H, QHD.S42-3Ha, QHD.S42-3Hb and QHD.S42-5H) were exhibited a favorable performance of reducing HD by -3.03, -2.76, -2.70 and -1.76%, respectively. These QTLs showed negative additive effects. The strongest QTL was QHD.S42-3Hb and explained 18.15% of the genetic variance. Furthermore, the QTL QHD.S42-6H was exhibited increase in HD due to presence of the exotic allele that increased HD by 1.12%. von Korff *et al* (2006) identified ten QTLs for days until heading and covered the whole genome except chromosome 5H, at five locations the exotic allele (Hsp) was associated with a reduced heading time of 7.9%. The marker locus EBmac415 on 2H where the exotic allele decreased time to heading and coincided with the major flowering QTL on chromosome arm 2HS detected by Pillen *et al* (2003), Li *et al* (2004) and von Korff *et al* (2006).

Plant height (PH)

Plant height is an important morphological trait, where shortening height of a plant can improve lodging resistance and may indirectly increase grain yield (Alam *et al* 2007). Three QTLs were detected for PH and distributed on chromosome 7H (Table 4 and Figure 1). These QTLs QPH.S42-7Ha, QPH.S42-7Hb and QPH.S42-7Hc exhibited significant marker main, marker × treatment and both effects, respectively. One favorable QTL (QPH.S42-7Ha) effect was influenced by the presence of exotic alleles, where this locus was responsible for almost 6.29% decrease in PH.

Table 4. Summary of the detected QTL for studied traits over well-watered and drought stress treatments.

[1]QTL	[2]Trait	[3]Marker	[4]Chr	[5]Pos	[6]Flanking	[7]F	[8]Pr.
QHD.S42-2H	HD	EBmac415	2H	144.00	138.23 - 150.10	9.9	0.002
QHD.S42-3Ha		bPb_9110	3H	118.72	115.50 - 118.72	24.7	0.000
QHD.S42-3Hb		bPb_1411	3H	160.19	153.54 - 165.54	20.1	0.000
QHD.S42-5H		bPb_6676	5H	81.39	81.39 – 87.00	7.3	0.007
QHD.S42-6H		bPb_1256	6H	74.34	68 - 75	4.0	0.048
QPH.S42-7Ha	PH	bPb_9753	7H	82.61	82 - 87.54	5.1	0.027
QPH.S42-7Hb		bPb_9563	7H	149.40	147.17 - 149.39	12.4	0.000
QPH.S42-7Hc		HVCHI26A	7H	159.20	159.05 - 165	20.9	0.000
QCC.S42-1Ha	CC	bPb_1213	1H	95.02	94.90 - 95.08	7.8	0.006
QCC.S42-1Hb		GBMS12	1H	134.00	133.11 - 140.85	12.3	0.001
QCC.S42-2H		bPb_3050	2H	30.24	26.23 - 30.23	6.3	0.000
QCC.S42-3H		bPb_9746	3H	54.80	50.43 - 55.63	11.8	0.001
QCC.S42-6H		HvCO2	6H	90.00	84.63 - 91.98	10.7	0.001
QCC.S42-7H		bPb_0108	7H	0.47	0.46 - 3.47	4.0	0.047
QNSP.S42-2H	NSP	bPb_8292	2H	27.07	27.06	10.3	0.000
QNSP.S42-4H		Mlo	4H	127.50	123.24 - 132	6.8	0.010
QNSP.S42-6H		bPb_1657	6H	68.22	68.1 - 75	7.9	0.000
QNSP.S42-5H		bPb_6363	5H	36.10	33 - 36.09	7.4	0.007
QNSP.S42-7Ha		bPb_4597	7H	68.80	68.67 - 73.88	8.7	0.000
QNSP.S42-7Hc		BMS64	7H	100.30	94.17 - 107	7.0	0.009
QNSP.S42-7Hb		HvCO1	7H	82.00	82 - 84.95	11.8	0.000
QNSP.S42-7Hd		EBmac755	7H	137.00	136.56 - 141.80	13.5	0.000
QGW.S42-1Ha	GW	MGB402	1H	0.00	0 - 4.82	8.2	0.004
QGW.S42-1Hb		GBM1061	1H	125.00	123.08 – 125	6.5	1E-02
QGW.S42-2H		bPb_8292	2H	27.07	25.73 - 30.23	13.2	0.000
QGW.S42-3Ha		bPb_7827	3H	148.34	146.78 - 153.54	11.6	0.000
QGW.S42-3Hb		bPb_7724	3H	179.49	175.24 - 181.32	12.9	0.000
QGYP.S42-1Ha	GYP	bPb_0589	1H	141.24	139.59 - 147.25	6.4	0.000
QGYP.S42-1Hb		bPb_1487	1H	147.25	140.85- 147.25	9.8	0.002
QGYP.S42-2H		bPb_7991	2H	101.27	97 - 102.37	5.6	0.019
QGYP.S42-6H		GMS6	6H	68.00	63 – 75	6.5	0.011
QGYP.S42-7H		bPb_3732	7H	3.48	0.46 - 7.52	6.8	0.010

Table 4. Cont.

[1]QTL	[2]Trait	[3]Marker	[4]Chr	[9]FDR	[10]R^2	[11]Effect	[12]*Hv*	[13]*Hsp*	[14]R [Hsp]	[15]Add.
QHD.S42-2H	HD	EBmac415	2H	0.016	3.45	M	88.14	85.47	-3.03	-1.34
QHD.S42-3Ha		bPb_9110	3H	0.000	13.33	M, M*T	88.46	86.02	-2.76	-1.22
QHD.S42-3Hb		bPb_1411	3H	0.001	18.15	M	88.54	86.15	-2.70	-1.19
QHD.S42-5H		bPb_6676	5H	0.046	8.33	M	88.18	86.62	-1.76	-0.78
QHD.S42-6H		bPb_1256	6H	0.161	1.80	M	87.61	88.58	1.12	0.49
QPH.S42-7Ha	PH	bPb_9753	7H	0.230	6.81	M	66.95	62.74	-6.29	-2.11
QPH.S42-7Hb		bPb_9563	7H	0.000	4.46	M*T	65.86	71.28	8.24	2.71
QPH.S42-7Hc		HVCHI26A	7H	0.001	35.91	M, M*T	65.77	71.84	9.23	3.04
QCC.S42-1Ha	CC	bPb_1213	1H	0.089	2.97	M	46.98	45.36	-3.45	-0.81
QCC.S42-1Hb		GBMS12	1H	0.036	3.71	M	47.17	45.36	-3.84	-0.91
QCC.S42-2H		bPb_3050	2H	0.000	2.79	M*T	47.28	44.88	-5.07	-1.20
QCC.S42-3H		bPb_9746	3H	0.036	3.38	M	46.40	49.08	5.79	1.34
QCC.S42-6H		HvCO2	6H	0.037	2.35	M	46.18	48.04	4.01	0.93
QCC.S42-7H		bPb_0108	7H	0.286	4.36	M	46.77	45.13	-3.50	-0.82
QNSP.S42-2H	NSP	bPb_8292	2H	0.000	1.20	M*T	5.49	5.79	5.53	0.15
QNSP.S42-4H		Mlo	4H	0.254	3.62	M	5.64	5.28	-6.46	-0.18
QNSP.S42-6H		bPb_1657	6H	0.000	2.12	M*T	5.42	5.65	4.24	0.12
QNSP.S42-5H		bPb_6363	5H	0.253	3.61	M	5.60	5.17	-7.67	-0.21
QNSP.S42-7Ha		bPb_4597	7H	0.000	13.10	M*T	5.14	5.34	3.89	0.10
QNSP.S42-7Hc		BMS64	7H	0.254	0.51	M	5.48	5.99	9.27	0.25
QNSP.S42-7Hb		HvCO1	7H	0.000	6.72	M*T	5.56	5.53	-0.61	-0.02
QNSP.S42-7Hd		EBmac755	7H	0.104	1.59	M	5.46	6.08	11.36	0.31
QGW.S42-1Ha	GW	MGB402	1H	0.499	2.93	M	3.98	4.25	6.62	0.13
QGW.S42-1Hb		GBM1061	1H	0.000	2.05	M*T	3.99	4.01	0.30	0.01
QGW.S42-2H		bPb_8292	2H	0.000	3.55	M*T	4.01	3.97	-0.95	-0.02
QGW.S42-3Ha		bPb_7827	3H	0.000	3.71	M*T	3.90	4.06	4.32	0.08
QGW.S42-3Hb		bPb_7724	3H	0.000	6.68	M*T	3.96	4.06	2.51	0.05
QGYP.S42-1Ha	GYP	bPb_0589	1H	0.000	2.69	M*T	2.95	3.37	14.31	0.21
QGYP.S42-1Hb		bPb_1487	1H	0.271	4.05	M, M*T	3.29	3.07	-6.69	-0.11
QGYP.S42-2H		bPb_7991	2H	0.360	2.94	M	3.29	3.08	-6.23	-0.10
QGYP.S42-6H		GMS6	6H	0.321	2.88	M	3.27	3.07	-5.98	-0.10
QGYP.S42-7H		bPb_3732	7H	0.293	3.71	M	3.27	3.04	-6.95	-0.11

1) Description of quantitative trait locus, 2) Studied trait, 3) Linked DNA marker revealing strongest F-value, 4) Chromosome, 5) DNA markers positions in cM, 6) Flanking positions in cM, 7) F-value of the given marker locus, 8) Probability at P < 0.05, 9) False discovery rate, 10) Genetic variance, 11) Main effects (M) and marker × treatment (M×T) interaction, 12) Trait value of homozygous cultivated genotype *[Hv]*, 13) Trait value of homozygous exotic genotypes *[Hsp]*. 14) Relative performance of the homozygous exotic allele, $RP_{[Hsp]}$ and 15). The additive effect is half the difference between the phenotypic means of the homozygous elite and exotic marker genotypes.

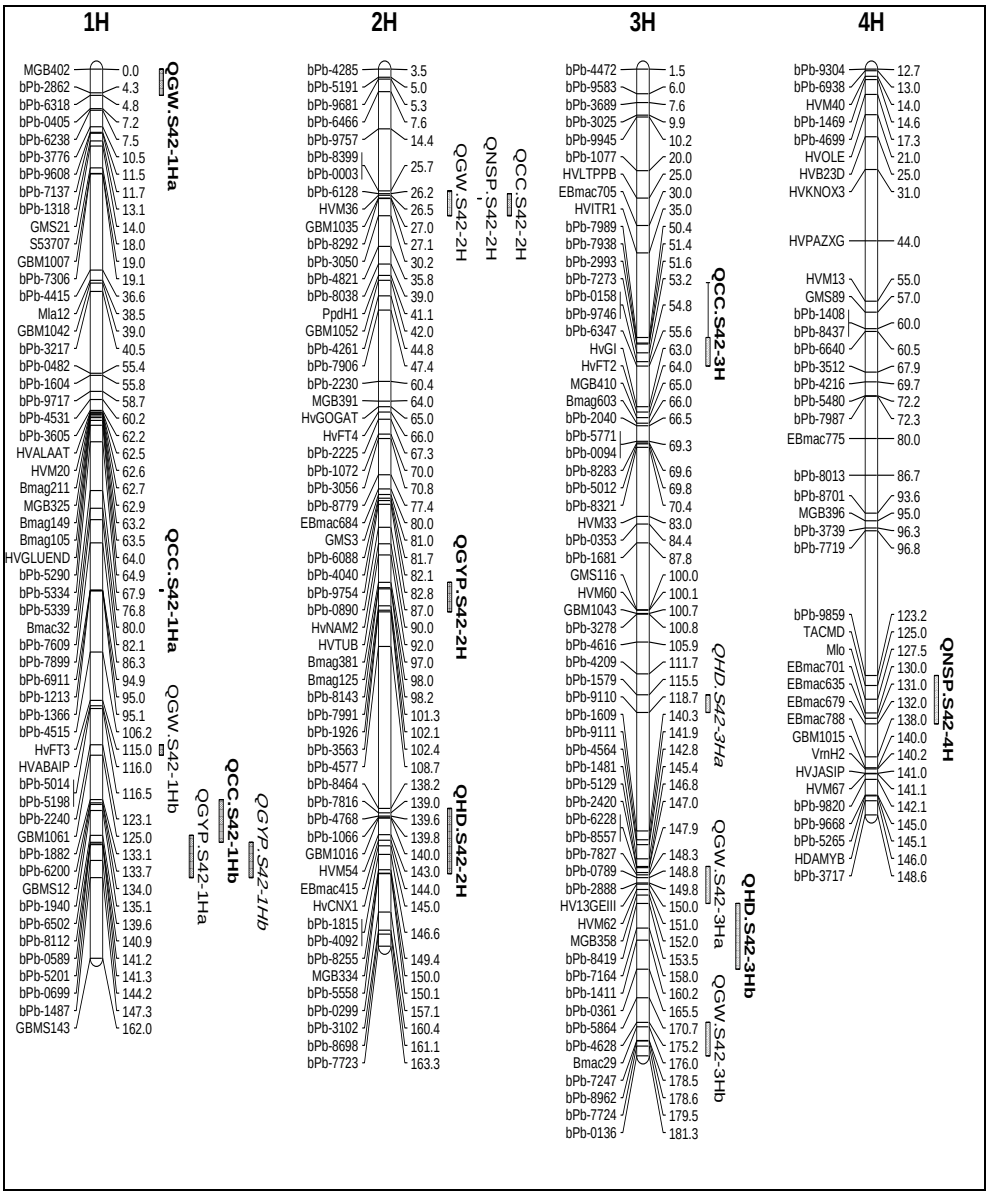

Fig. 1. Localization of 32 putative additive QTL detected days to 50% heading (HD), plant height (PH), chlorophyll content (CC), number of spikes per plant (NSP), grain weight (GW) and grain yield per plant (GYP) over well-watered and drought conditions. Bold QTL were specified as marker main effect, non-bold QTL specified as marker × treatment interaction effect and Italic-non bold QTL were assigned for QTL that showed both effects.

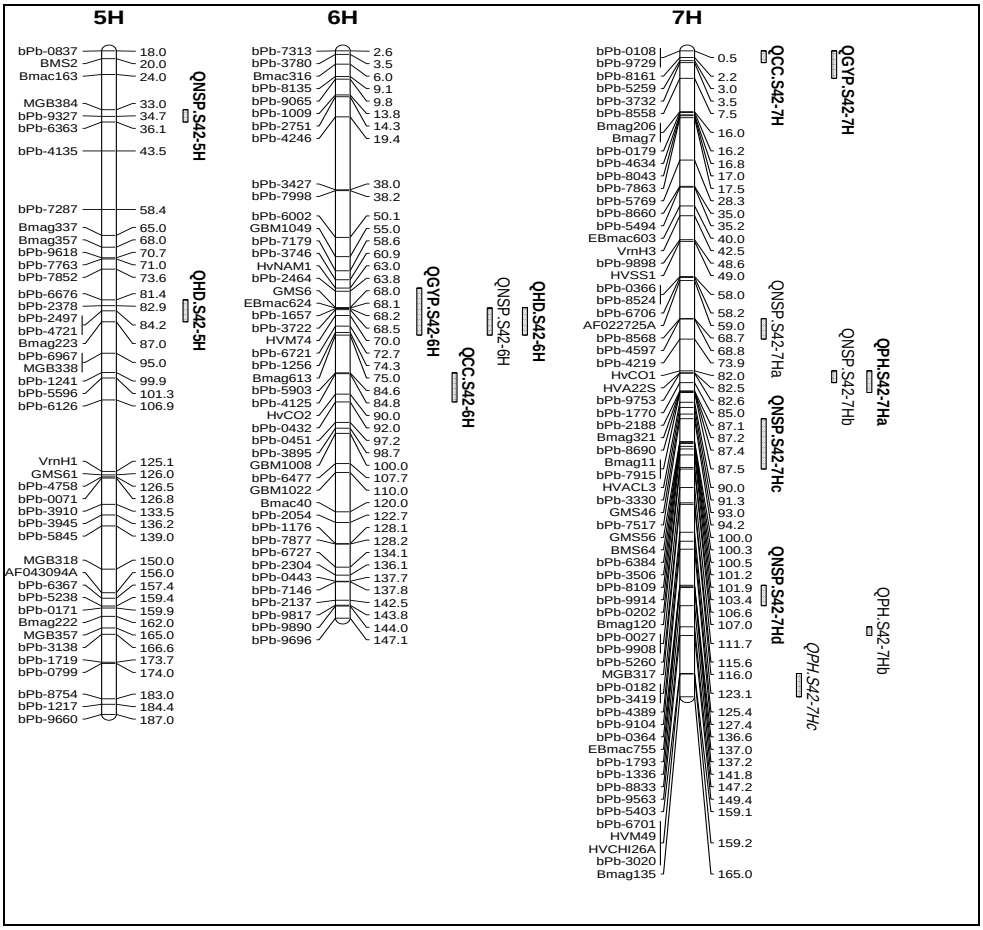

Fig. 1. Cont.

The exotic alleles explain 6.81% of the genetic variance. Negative additive effect was detected for this marker. In contrast, the exotic alleles at QTLs, QPH.S42-7Hb and QPH.S42-7Hc were associated to an enhancement of PH by 8.24 and 9.23%. The exotic allele at these two loci posed 4.46 and 35.91% of the genetic variation. This finding is agreement with that obtained by Forster *et al* (2004a) who detected QTL for plant height on 7H between 89 and 120 cM. Several studies detected QTL associated to PH on chromosome 7H, where Baum *et al* (2003) identified QTL with major effects on 2H, 3H and 7H. Also, Chloupek *et al* (2006) and Gyenis *et al* (2007) reported QTLs for PH on chromosomes 7H. The detected QTL for PH in this study are different than those obtained by Sayed (2011) and Wang *et al* (2010) in the same population. ISR 42-8 was taller than Scarlett under both treatments (Table 2), however the *Hsp* alleles led to shortening plant height in the DH lines. This result is in harmony with those obtained by Saal *et al* (2010) and von Korff *et al* (2010).

Chlorophyll content (CC)

Chlorophyll content is one of the important physiological traits of flag leaf in cereals which has been considered to be the important determinants of grain yield (Hirota *et al* 1990 and Chen *et al* 1995). Therefore, flag leaf is one of the greatest components in determining grain yield potential in cereal crops. Altogether six QTLs were associated significantly with CC, five showed marker main effects and located on chromosomes 1H, 3H, 6H and 7H while one locus showed marker × treatment interaction effect and mapped on 2H (Table 4 and Figure 1). Relative performances of the exotic genotype ranged between -5.07% and 5.79%. Two QTLs (QCC.S42-3H and QCC.S42-6H) exhibited favorable performance of exotic alleles and revealed an increasing of CC. The result of the additive effects of those QTLs indicates that the exotic alleles appeared to be desirable for CC as compared to elite alleles. The other QTLs showed unfavorable performance of the exotic alleles. Similar result was obtained by Xue *et al* (2008) who detected four putative QTLs for chlorophyll content on chromosomes 2H, 3H and 6H. Since, individual QTL explained the variation from 6.3% to 20.2% of the total phenotypic variation. Also, Guo *et al* (2008) detected 5 QTLs on chromosomes 2H and 4H associated with chlorophyll content in flag leaves at post-flowering stage under well-watered and drought conditions using an RIL population with 194 lines. Similar results were obtained by Eshghi *et al* (2013) who mapped five QTLs for chlorophyll content, with the *H. spontaneum* alleles contributing to increased chlorophyll content at two of the five loci. The QTL with largest effect was located at 43-45 cM of chromosome 1H (linked to Bmag0105) and exotic alleles from wild barley increased this character by 33.8%. The other four other QTLs for chlorophyll content were detected on chromosomes 2H, 5H and 6H.

Number of spikes/plant (NSP)

Spikes number is one of the main components of grain yield in cereals. Eight QTLs were detected for NPS and located on chromosomes 2H, 4H, 5H, 6H and 7H (Table 4 and Figure 1). Four QTLs showed significant marker main effects and the others showed marker × treatment interaction effects. The relative performances of the exotic genotype ranged between -7.67% and 11.36%. Among these, five QTLs showed favorable performance of the exotic genotype alleles and revealed an increasing of NSP. The strongest QTL (QNSP.S42-7Ha) explained 13.10% of the genetic variance. This result indicates that the introgressions from wild barley may increase number of spikes/plant in S42 population. Saal *et al* (2010) have identified three QTLs as marker main effects associated with NSP and localized on chromosomes 1H (HVABAIP), 6H (GMS6) and 7H (BMAG7). In wheat, Ibrahim *et al* (2010) detected five QTL associated to NSP, one of

them increased NSP by 10.8% and 16.3% under well-watered and drought stress, respectively.

100 - Grain weight (GW)

Grain weight or seed index, is known as a representative quantitative trait and one of important components of yield and determined by synthesis and accumulation of starch in grain endosperm (You *et al* 2006 and Mei *et al* 2005). Results revealed that ISR 42-8 yielded less and had lower 100-grain weight than Scarlett under well-watered and drought conditions. Locations close to the five chromosomal regions (QTL) on 1H, 2H and 3H were probably influencing 100-grain weight (Table 4 and Figure 1). Four QTLs exhibited significant marker × treatment interaction effects, while one QTL showed significant marker main effect. Four QTLs revealed a favorable increase in GW, and the exotic alleles explained maximum 6.68% of the genetic variance with favorably increased GW by 6.62%. von Korff *et al* (2006) detected nine QTL for thousand grain weight and located on chromosomes 2H to 7H. Several QTLs have been detected by Pillen *et al* 2003 and 2004.

Grain yield/plant (GYP)

Grain yield is assumed to be influenced by multiple component traits, where each with their own genetic makeup (Cooper *et al* 2009). Last decades, with development of molecular approaches, QTL analysis was used to detect yield and fecundity-related traits. Many QTL affecting yield were mapped on seven chromosomes throughout the whole genome of barley. Yield QTL derived from related wild species have also been mapped in barley and other crops (Swamy and Sarla 2008). In current study, five QTLs were identified for GYP and located on chromosomes 1H, 2H, 6H and 7H (Table 4 and Figure 1). Three QTLs revealed significant marker main effects and showed unfavorable effect with an explained genetic variance up to 4.05%. The QTL (QGYP.S42-1Ha) revealed marker × treatment interaction effect and the relative performance of the exotic genotype led to increase GYP with 14.31%. The QTL (QGYP.S42-1Hb) showed both effects and reduced GYP due to the presence of the exotic alleles. In contrast, the elite alleles at these QTLs were associated to an enhancement of GYP as compared to exotic alleles. It means elite alleles appeared to be desirable for GYP as compared to exotic alleles. This results are in harmony with those obtained by (Pillen *et al* 2003 and 2004; von Korff *et al* 2006 and 2010; Wang *et al* 2010 and Sayed 2011). The findings of this work and previous studies on barley, concluded that due to the presence of large or small specific segments of wild parent ISR 42-8 may contributed to GYP reduction in S42 population. However, the favorable detected QTL referred to possibility presence of *Hsp* regions may contribute to yield enhancement, especially under drought conditions.

Pleiotropic effect

The colocation of QTL for different traits implies the likely presence of pleiotropic or closed linkage between the QTL control the traits (Tuberosa *et al* 2002b). Current study revealed only one QTL region bPb-8292 (27.07 cM) on 2H was controlling number of spikes per plant and 100-grain weight. This locus may be useful for marker-assistant selection (MAS) in barley improvement. Diab *et al* (2004) have found several genomic regions, where QTL for different traits overlapped. Saal *et al* (2010) observed different QTL regions showing co-localization, for example at locus HVABAIP on chromosome 1H for traits thousand grain weight and grain yield.

Detection of Epistasis

For the epistatic effect, altogether 21 pairs of epistatic QTLs as additive × additive effects were detected for five studied traits related to drought tolerance in S42 population, while number of spikes per plant did not exhibit epistatic effects. Among them, eight pairs displayed QTL by marker interaction and thirteen displayed marker by marker interaction (Table 5). About 31.2% of main-effect QTL detected for studied traits were involved in epistatic effects. This indicates that several loci involved in epistatic interactions may not have significant effects for these traits and may affect the trait expression by epistatic interactions with other loci. Similarly, Ma *et al* (2007) observed that 37% of the main-effect QTLs were involved in the epistatic interactions in maize grain yield and its components. Zhang *et al* (2008) found 25% of main-effect QTLs for wheat plant height were involved in epistatic effects.

Days to 50% heading (HD)

Results revealed seven pairs of epistatic QTLs were associated significantly with days to 50% heading and mapped on 3H, 4H, 5H, 6H and 7H. Among these loci, six pairs of epistatic effects reduced the days to 50% heading up to -4.02 day. The most favorable pair of epistatic QTLs for reducing heading date was (bPb-9111* bPb-8701) and located on chromosomes 3H (141.94 cM) and 4H (93.64 cM) and had the highest F value and accounted for 19.36% of genetic variation (Table 5).

Table 5. Estimation of LS-means of 21 pairs of digenic interactions and epistatic effects (additive × additive) for studied traits.

Trait	Marker 1			Marker 2			F	Pr.	FDR	R^2
	M. name	Chr.	Pos.	M. name	Chr.	Pos.				
HD	bPb_9945	3H	10.20	bPb_8833	7H	147.17	8.1**	0.000	0.005	9.93
	bPb_9110	3H	118.72	bPb_8701	4H	93.64	8.0**	0.001	0.008	14.64
	bPb_9111	3H	141.94	bPb_8701	4H	93.64	12.4**	0.000	0.001	19.36
	HVB23D	4H	25.00	bPb_2137	6H	142.51	3.2*	0.025	0.074	7.70
	bPb_7763	5H	70.99	bPb_1009	6H	13.83	3.2*	0.042	0.101	10.39
	bPb_8135	6H	9.10	Bmag135	7H	165.00	3.8*	0.024	0.071	5.49
	bPb_0202	7H	106.63	bPb_9104	7H	127.40	5.7**	0.001	0.010	9.66
PH	bPb_1609	3H	140.29	bPb_6363	5H	36.10	3.5*	0.021	0.116	4.14
	bPb_9753	7H	82.61	HVCHI26A	7H	159.20	14.6*	0.013	0.080	40.43
CC	bPb_7609	1H	82.15	Bmag7	7H	16.00	5.5**	0.002	0.084	9.42
	GBMS12	1H	134.00	bPb_6466	2H	7.59	9.9**	0.000	0.037	15.57
	HVM36	2H	26.50	HvCO2	6H	90.00	5.8**	0.004	0.096	8.65
	bPb_7164	3H	157.98	bPb_9817	6H	143.80	3.1*	0.040	0.161	5.74
	bPb_9618	5H	70.75	HvCO2	6H	90.00	3.3*	0.026	0.138	5.02
GW	MGB402	1H	0.00	VrnH3	7H	42.50	4.2*	0.015	0.409	4.84
	bPb_3776	1H	10.47	Bmag223	5H	87.00	8.1**	0.000	0.409	40.63
	bPb_7989	3H	50.43	bPb_0366	7H	58.02	3.6*	0.023	0.409	13.08
GYP	bPb_1487	1H	147.25	GMS6	6H	68.00	6.1**	0.003	0.228	12.93
	HVM36	2H	26.50	bPb_3050	2H	30.24	6.3**	0.006	0.228	10.04
	GMS3	2H	81.00	Bmag206	7H	16.00	3.8*	0.015	0.228	9.88
	bPb_7938	3H	51.44	bPb_9111	3H	141.94	3.1*	0.049	0.228	9.87

Table 5. Cont.

Trait	Marker 1			Marker 2			(1) Ls means of digenic interactions				(2)*Hsp/Hv-Hv/Hv*	*Hv/Hsp-Hv/Hv*	*Hsp/Hsp-Hv/Hv*
	M. name	Chr.	Pos.	M. name	Chr.	Pos.	*Hv/Hv*	*Hsp/Hv*	*Hv/Hsp*	*Hsp/Hsp*			
HD	bPb_9945	3H	10.20	bPb_8833	7H	147.17	88.32	84.73	84.92	88.21	-3.59	-3.40	-0.11
	bPb_9110	3H	118.72	bPb_8701	4H	93.64	88.59	85.15	87.30	86.44	-3.44	-1.29	-2.15
	bPb_9111	3H	141.94	bPb_8701	4H	93.64	88.73	84.71	87.54	85.71	-4.02	-1.19	-3.02
	HVB23D	4H	25.00	bPb_2137	6H	142.51	88.02	89.50	86.44	88.35	1.48	-1.57	0.34
	bPb_7763	5H	70.99	bPb_1009	6H	13.83	88.19	86.79	86.28	85.50	-1.40	-1.92	-2.69
	bPb_8135	6H	9.10	Bmag135	7H	165.00	88.18	87.03	85.09	85.37	-1.15	-3.09	-2.81
	bPb_0202	7H	106.63	bPb_9104	7H	127.40	88.20	84.70	85.32	86.52	-3.50	-2.88	-1.68
PH	bPb_1609	3H	140.29	bPb_6363	5H	36.10	65.83	69.40	66.89	63.06	3.57	1.06	-2.77
	bPb_9753	7H	82.61	HVCHI26A	7H	159.20	66.10	62.29	88.54	87.18	-3.81	22.44	21.08
CC	bPb_7609	1H	82.15	Bmag7	7H	16.00	47.41	45.26	45.75	37.12	-2.16	-1.66	-10.20
	GBMS12	1H	134.00	bPb_6466	2H	7.59	47.20	47.01	49.26	41.74	-0.19	2.06	-5.46
	HVM36	2H	26.50	HvCO2	6H	90.00	46.42	44.92	48.17	45.37	-1.50	1.76	-1.05
	bPb_7164	3H	157.98	bPb_9817	6H	143.80	47.04	46.92	46.12	40.89	-0.12	-0.92	-6.14
	bPb_9618	5H	70.75	HvCO2	6H	90.00	46.10	45.99	48.25	46.71	-0.10	2.15	0.61
GW	MGB402	1H	0.00	VrnH3	7H	42.50	3.98	4.29	3.88	3.53	0.31	-0.10	-0.46
	bPb_3776	1H	10.47	Bmag223	5H	87.00	4.07	5.63	3.69	4.22	1.56	-0.37	0.15
	bPb_7989	3H	50.43	bPb_0366	7H	58.02	4.06	3.73	4.29	4.92	-0.33	0.23	0.87
GYP	bPb_1487	1H	147.25	GMS6	6H	68.00	3.35	3.06	3.04	3.20	-0.29	-0.31	-0.15
	HVM36	2H	26.50	bPb_3050	2H	30.24	3.28	2.28	3.56	3.09	-1.00	0.28	-0.19
	GMS3	2H	81.00	Bmag206	7H	16.00	3.23	3.29	3.35	2.46	0.06	0.12	-0.77
	bPb_7938	3H	51.44	bPb_9111	3H	141.94	3.21	3.10	3.43	2.71	-0.11	0.22	-0.50

***, ** indicate the significance level at 0.05 and 0.01, respectively to declare the putative epistatic QTL positions. (1) Least means of the allelic combinations of the cultivated genotype (Hv) and wild genotype (Hsp). (2) The differences between the genotype Hv/Hv and the other allelic combinations.**

At this locus, the BC_2DH lines carrying the *Hsp/Hsp* genotype at these loci were on average 3.02 day earlier than lines with the allelic combination *Hv/Hv*. Several studies on barley reported epistatic QTL for days to heading (Xu and Jia 2007 andSannemann 2013)

Plant height (PH)

Epistasis is an important genetic characteristic of quantitative traits such as plant height (PH). Epistatic interaction analysis revealed two interaction effects for PH (Table 5). The first interaction effect was identified between marker locus bPb-1609 (3H) and bPb-6363 (5H) and accounted 4.14% of the genetic variance. The combination of *Hsp/Hsp* led to reduce plant height by 2.77, while BC_2DH lines carrying the *Hsp/Hv* or *Hv/Hsp* genotype were on average 3.57 and 1.06 cm shorter than lines with the allelic combination *Hv/Hv*. In the second pair, showed QTL × QTL

interaction and was between marker locus bPb-9753 (7H) and HVCHI26A (7H) and explained 40.43% of the genetic variance. The combinations Hsp/Hsp and Hv/Hsp genotypes led to increase plant height by values 21.08 and 22.44 cm compared to the combination of *Hv/Hv*. Similar results were obtained by von Korff *et al* (2010) have detected four epistatic interactions between exotic alleles *Hsp/Hsp* introgressed from wild barley (*H. vulgare* ssp. *spontaneum* C. Koch) which increased plant height significantly as compared to the combination *Hv/Hv*. Sannemann (2013) found two significant epistatic interactions for plant height.

Chlorophyll content (CC)

Results revealed five pairs of epistatic QTLs were associated significantly with chlorophyll content and covered the whole genome except 4H. All loci explained maximum 15.57% of the genetic variance. Among these loci, all combination of the exotic alleles Hsp reduced chlorophyll content except in in the pair of interaction (bPb-9618 (5H) and HvCO2 (6H)), where the Hsp alleles led to increase CC in the DH lines carrying it. Zhang *et al* (2009) detected nine pairs of QTLs with epistatic effects and/or epistasis × environment effects for chlorophyll content in wheat.

100-grain weight (GW)

The epistasis analysis revealed four pairs of epistatic QTLs which were associated significantly with SI, and mapped on chromosomes 1H, 3H, 5H and 7H (Table 5). The BC_2DH lines carrying the Hsp/Hsp genotype at two loci were heavier weight by maximum 0.87 g than lines with the allelic combination *Hv/Hv*. Sannemann (2013) found two significant epistatic effects for thousand kernel weight.

Grain yield/plant (GYP)

Four pairs of epistatic QTLs were associated significantly with GYP, and mapped on chromosomes 1H, 2H, 3H, 6H and 7H (Table 5). All pairs had negative effects of epistatsis on GYP. At these loci, the BC_2DH lines having the *Hsp/Hsp* genotype were lower GYP with value up to 0.77 g than lines with the allelic combination *Hv/Hv*. von Korff *et al* (2010) detected 12 interaction effects the allelic combination exotic by exotic caused a strong decrease in grain yield.

In conclusion, 301 BC_2DH lines, their parents (Scarlett and ISR 42-8) and four local check barley cultivars were tested for tolerance to drought in new reclaimed soil in Assiut, Egypt. Significant differences among all genotypes under well-watered and drought stress conditions for all investigated traits. The QTL analysis revealed 35 putative QTL and marker × treatment interaction for six studied, 17 (48.5 %) QTL for studied traits were identified with favorable effects of the exotic alleles (ISR 42-8). Numerous interesting QTLs were detected in this study that displaying beneficial effects of the exotic alleles. For example, the alleles of four QTL (QHD.S42-2H, QHD.S42-3Ha, QHD.S42-3Hb and QHD.S42-5H) exhibited

a favorable performance of reducing days to 50% heading by -3.03, -2.76, -2.70 and -1.76%, respectively. One favorable QTL (QPH.S42-7Ha) effect was influenced by the presence of exotic alleles, where this locus was responsible for almost 6.29% decrease in plant height. Interestingly, the QTL (QGYP.S42-1Ha) revealed marker × treatment interaction effect and the relative performance of the exotic genotype led to increase grain yield per plant with 14.31%. Altogether 21 pairs of epistatic QTLs as *additive* × *additive* effects were detected for six studied traits related to drought tolerance in S42 population. Among them, eight pairs displayed QTL by marker interaction and thirteen displayed marker by marker interaction. The favorable QTL could be used for marker-assisted selection for these three traits. The exotic QTL allele responded favorably under drought stress conditions and the majority of the digenic epistatic interaction pairs had favorable effects on the phenotypic values of the studied traits. For example, the most favorable pair of epistatic QTLs for reducing heading date was (bPb-9111* bPb-8701) and located on chromosomes 3H (141.94 cM) and 4H (93.64 cM) and accounted for 19.36% of genetic variation. At this locus, the BC_2DH lines carrying the *Hsp/Hsp* genotype at these loci were on average 3.02 day earlier than lines with the allelic combination *Hv/Hv*. For 100-grain weight, the BC_2DH lines carrying the *Hsp/Hsp* genotype at two loci were heavier grain weight by maximum 0.87 g than lines with the allelic combination Hv/Hv. This study has highlighted the role of the exotic alleles for the detection of favorable leads for drought tolerance.

REFERENCES

Ainer, N.G., W.I Miseha, F.A. Abbas and H.M. Eid (1999). A new concept of rationalization of irrigation water use in Egypt. in Proceedings of the Third Conference of On-Farm irrigation Agroclimatologoy, Vol. 2, Giza, Egypt, January 25-27: 721-744.

Alam, M.Z., S.A. Haider and N.K. Paul (2007). Yield and yield components of barley (*Hordeum vulgare* L). cultivars in relation to nitrogen fertilizer. J App Sci Res 3(10).:1022-1026

Al-Karaki, G.N. (2001). Germination, sodium, and potassium concentrations of barley seeds as influenced by salinity. J Plant Nutr 24(3).:511-522

Baum, M., S. Grando, G. Backes, A. Jahoor, A. Sabbagh and S. Ceccarelli (2003). QTLs for agronomic traits in the Mediterranean environment identified in recombinant inbred lines of the cross 'Arta' x *H spontaneum* 41-1. Theor Appl Genet 107:1215-1225

Carlborg, O. and C.S. Haley (2004). Epistasis: too often neglected in complex trait studies? Nat Rev Genet 5:618-625

Cattivelli, L., F. Rizza, F.W. Badeck, E. Mazzucotelli, A.M. Mastrangelo, E. Francia, C. Mare, A. Tondelli and A.M. Stanca (2008). Drought tolerance improvement in crop plants: An integrated view from breading to genomics. Field Crops Res 105:1-14

Chen, W., Z. Xu and L. Zhang (1995). Physiological bases of super high yield breeding in rice. Liaoning Science and Technology Publishing Company, Shenyang, p.1-2.

Chloupek, O., B.P. Forster and W.T.B. Thomas (2006). The effect of semi dwarf genes on root system size in field-grown barley. Theor Appl Genet 112:779-786

Cooper, M, F.A. van Eeuwijk, G.L. Hammer, D.W. Podlich and C. Messina (2009). Modelling QTL for complex traits: detection and context for plant breeding. Plant Biology 12:231-240

Dakkak, A. (2016). Water scarcity in Egypt | EcoMENA http://www.ecomena.org/tag/water-scarcity-in-egypt/, (Retrieved 6th September 2016).

Diab, A.A., B. Teulat-Merah, T. Dominique, Z. Neslihan, D.B. Ozturk and E.S. Mark (2004). Identification of drought- inducible genes and differentially expressed sequence tags in barley. Theor Appl Genet 109:1417-1425

Eshghi, R., S. Salayeva, F. Ebrahimpour, M. Rahimi, M. Baraty and J. Ojaghi (2013). Advanced-backcross QTL analysis in hulless barley: I. Detection of exotic alleles for yield and yield components introgressed from *Hordeum vulgare* ssp. *spontaneum*. International Journal of Agriculture and Crop Sciences, 5(2):95-100.

Forster, B.P., R.P. Ellis, J. Moir, V. Talame, M.C. Sanguineti, R. Tuberosa, D. This, B. Teulat-Merah, I. Ahmed, S.A. Mariy, H. Bahri, M. El Ouahabi, N. Zoumarou-Wallis, M. El-Fellah and M. Ben Salem (2004a). Genotype and phenotype associations with drought tolerance in barley tested in North Africa. Ann Appl Biol 144:157-168

Guo, P., M. Baum, R. Varshney, A. Graner, S. Grando and S, Ceccarelli (2008). QTLs for chlorophyll and chlorophyll fluorescence parameters in barley under post-flowering drought. Euphytica 163(2).:203-214.

Gyenis, L., S.J. Yun, K.P. Smith, B.J. Steffenson, E. Bossolini, M.C. Sanguineti and G.J. Muehlbauer (2007). Genetic architecture of quantitative trait loci associated with morphological and agronomic trait differences in a wild by cultivated barley cross. Genome 50(8).:714-723

Hegazi, A.M., M.Y. Afifi, M.A. El Shorbagy, A.A. Elwan and S. El-Demerdashe (2005). Egyptian national action program to combat desertification. Arab Republic of Egypt. Ministry of Agriculture and Land Reclamation Desert Research Center (DRC). http://www.unccd.int/ActionProgrammes/egypt-eng2005.pdf

Hirota, O., M. Oka and T. Takeda (1990). Sink activity estimation by sink size and dry matter increase during the ripening stage of barley (*Hordeum vulgare*). and rice (*Oryza sativa*). Annals of Botany 65(4).:349-354.

Hussain, S.S. (2006). Barley genetics and genomics: a review. Proc Pakistan Acad Sci 43:63-84

Ibrahim, S.E., A. Schubert, K. Pillen and J. Léon (2010). Quantitaive trait loci analysis for drought tolerance in an advanced backcross population of spring wheat. Sudan J Agric Res 15:1-18

Jana, S. and R.W. Wilen (2005). Breeding for abiotic stress tolerance in barley. M Ashraf, and PJC Harris, eds Abiotic Stresses Plant Resistance Through Breeding and Molecular Approaches, Haworth Press, New York

Jenks, M.A. and P.M. Hasegawa (2005). Plant Abiotic Stress. John Wiley and Sons. 1st ed New York.

Kumar, J. and S. Abbo (2001). Genetics of flowering time in chickpea and its bearing on productivity in the semi-arid environments. Adv Agron 72:107-138

Li, J.Z., X.Q. Huang, F. Heinrichs, M.W. Ganal, M.S. Röder (2004). Analysis of QTLs for yield, yield components, and malting quality in a BC_3-DH population of spring barley. Theor Appl Genet. 110(2): 356-363.

Ma, X.Q., J.H. Tang, W.T. Teng, J.B. Yan, Y.J. Meng and J.S. Li (2007). Epistatic interaction is an important genetic basis of grain yield and its components in maize. Mol Breeding 20:41-51

Mei, H.W., L.J. Luo, C.S. Ying, Y.P. Wang, X.Q. Yu, L.B. Guo, A.H. Paterson and Z.K. Li (2005). Gene actions of QTL affecting several agronomic traits resolved in a recombinant inbred rice population and two backcross population. Theor Appl Genet 110:649−659

Mishra, B.N. and B.G. Shivakumar (2000). Barley In: Techniques and Management of Field Crop Production. Agrobios. Rothere, PS (Ed.), India

Pillen, K., A. Zacharias and J. Léon (2003). Advanced backcross QTL analysis in barley (*Hordeum vulgare* L). Theor Appl Genet 107:340-352

Pillen, K., A. Zacharias and J. Léon (2004). Comparative AB-QTL analysis in barley using a single exotic donor of *Hordeum vulgare* ssp *spontaneum.* Theor Appl Genet 108:1591-1601

Quarrie, S.A., A. Steed, C. Calestani, A. Semikhodskii, C. Lebreton, C. Chinoy, N. Steele, D. Pljevljakusic, E. Waterman, J. Weyen, J. Schondelmaier, D.Z. Habash, P. Farmer, L. Saker, D.T. Clarkson, A. Abugalieva, M. Yessimbekova, Y. Turuspekov, S. Abugalieva, R. Tuberosa, M.C. Sanguineti, P.A. Hollington, R. Aragues, A. Royo and D. Dodig (2005). A high-density genetic map of hexaploid wheat (*Triticum aestivum* L). from the cross Chinese Spring x SQ1 and its use to compare QTL for grain yield across a range of environments. Theor Appl Genet 110:865-880

Saal, B., M. von Korff, J. Lèon and K Pillen (2010). Advanced-backcross QTL analysis in spring barley: IV Localization of QTL 3 nitrogen interaction effects for yield-related traits. Euphytica 177 (2).:223-239

Sannemann, W. (2013). Marker-trait-sensor association in a multi-parent advanced generation intercross (MAGIC). population in barley (*Hordeum vulgare* ssp. *vulgare*). PhD diss., Universitäts-und Landesbibliothek Bonn. Germany.

SAS Institute (2008). The SAS System for Windows, release 9.2. Cary NC: SAS Institute.

Sayed, M.A., H. Schumann, K. Pillen, A.A. Naz and J. Léon (2012). AB-QTL analysis reveals new alleles associated to proline accumulation and leaf wilting under drought stress conditions in barley (*Hordeum vulgare* L). BMC Genetics 13:61.

Sayed, M.A.A., (2011). QTL analysis for drought tolerance related to root and shoot traits in barley *(Hordeum vulgare L.* PhD diss., Bonn, Univ., Diss., 2011, Germany).

Schmalenbach, I., N. Körber and K Pillen (2008). Selecting a set of wild barley introgression lines and verification of QTL effects for resistance to powdery mildew and leaf rust. Theor Appl Genet 117: 1093–1106

Sreenivasulu, N., A. Graner and U. Wobus (2008). Barley Genomics: An Overview, Inter J Plant Genomics, http://doi.org/10.1155/2008/486258

Swamy, M.B.P. and N. Sarla (2008). Yield-enhancing quantitative trait loci (QTLs). from wild species. Biotech Advances 26:106-120

Teulat, B., N. Zoumarou-Wallia, B. Rotter, M. Ben Salem, H. Bahri and D. This (2003). QTL for relative water content in field-grown barley and their stability across Mediterranean environments. Theor Appl Genet 108:181-188

Tuberosa, R., M.C. Sanguineti, P. Landi, M.M. Giuliani, S. Salvi and and S. Conti (2002). Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. Plant Mol Biol 48:697-712

von Korff, M., S. Grando, A.D. Greco, D. This, M. Baum and S. Ceccarelli (2008) Quantitative trait loci associated with adaptation to Mediterranean dryland conditions in barley. Theor Appl Genet 117:653-669

von Korff, M., H. Wang, J. Léon and K. Pillen (2004). Development of candidate introgression lines using an exotic barley accession (*H. vulgare* ssp. *spontaneum*). as donor. Theor Appl Genet 109:1736–45

von Korff, M., H. Wang, J. Léon and K. Pillen (2006). AB-QTL analysis in spring barley:II Detection of favourable exotic alleles for agronomic traits introgressed from wild barley (*H vulgare* ssp *spontaneum*). Theor Appl Genet 112:1221-1231

von Korff, M., J. Léon and K. Pillen (2010). Detection of epistatic interactions between exotic alleles introgressed from wild barley (*H. vulgare* ssp. *spontaneum*). Theor Appl Genet, 121(8): 1455-1464

Voorrips, R.E. (2002). MapChart: software for the graphical representation of linkage maps and QTLs. J. Hered. 93: 77-78

Wang, G., I. Schmalenbach, M. von Korff , J. Lèon, B. Kilian, J. Rode and K. Pillen (2010). Association of barley photoperiod and vernalization genes with QTLs for flowering time and agronomic traits in a BC_2DH population and a set of wild barley introgression lines. Theor Appl Genet 120:1559-1574

Wang, W, B. Vinocur and A. Altman (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1-14

Xu, S. and Z. Jia (2007). Genome wide analysis of epistatic effects for quantitative traits in barley. Genetics, 175(4): 1955-1963

Xu, W., D.T. Rosenow and H.T. Nguyen (2000). Stay green trait in grain sorghum: Relationship between visual rating and leaf chlorophyll concentration. Plant Breed. 119: 365-367.

Xue, D.W., M.C. Chen, M.X. Zhou, S. Chen, Y. Mao and G.P. Zhang (2008). QTL analysis of flag leaf in barley (*Hordeum vulgare* L.). for morphological traits and chlorophyll content. Journal of Zhejiang University Science B, 9(12): 938-943

You, A.Q., X.G. Lu, H.J. Jin, X. Ren, K. Liu, G.C. Yang, H.Y. Yang, L.L. Zhu and G.C. He (2006). Identification of quantitative trait loci across recombinant inbred lines and testcross populations for traits of agronomic importance in rice. Genetics 172:1287-1300

Zadok, J.C., T.T. Chang and F.C. Konzak (1974). A decimal code for growth stages of cereals. Weed Res 14: 415–421

Zhang, K., J. Tian, L. Zhao and S. Wang (2008). Mapping QTLs with epistatic effects and QTL × environment interactions for plant height using a doubled haploid population in cultivated wheat. J Genet Genomics 35:119-127

Zhang, K., T. Fang, Y. Liang and J. Tian (2009). Genetic dissection of chlorophyll content at different growth stages in common wheat. Journal of genetics 88(2): 183-189.

Zhu, H., G. Briceno, R. Dovel, P.M. Hayes, B.H. Liu, C.T. Liu and S.E. Ulrich (1999). Molecular breeding for grain yield in barley: An evaluation of QTL effects in a spring barley cross. Theor. Appl. Genet. 98:772-779

تحليل مواقع الصفات الكمية (QTL) لتحمل الجفاف في الشعير في الأراضي المستصلحة حديثاً في مصر

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يعد نقص الماء أحد أبرز عوامل الأجهاد غير الحيوي خطورة في الأراضي المستصلحة حديثاً في مصر. والذي يؤثر علي المحصول بالنقص في الصفات المرتبطة بالمحصول. لذا فإن تحديد الأليلات المفيدة من الأصناف المنزرعة و الأقارب البرية ربما يكون مفيداً في تحسين تحمل التراكيب الوراثية للجفاف. لهذا, فانه تم استخدام عشيرة من الشعير (ناتجة من تضاعف النباتات الأحادية لغرض رسم الخرائط الوراثية) مكونة من ٣٠١ سلالة. أختبرت هذه السلالات مع أبآئها Scarlett و ISR 42-8 مع أربعة أصناف شعير محلية للتحمل للجفاف في موسم ٢٠١٣/٢٠١٤ في أرض رملية مستصلحة حديثاً بمزرعة الوادي الأسيوطي التابعة لكلية الزراعة- جامعة اسيوط – محافظة أسيوط – مصر. أشار تحليل التباين إلي وجود اختلافات عالية المعنوية بين التراكيب الوراثية, ومعاملات الجفاف وايضاً التفاعل بينهما لمعظم الصفات تحت الدراسة. أعطي تحليل مواقع الصفات الكمية (QTL) عدد ٣٥ موقعاً كتأثير رئيسي للواسم الوراثي و كتفاعل بين الواسم الوراثي مع معاملات الجفاف للستة صفات المستخدمة في التحليل تحت الظروف المروية والجفافية. من بين هذه المواقع, كانت ١٧ موقعاً (٤٨,٥%) تحتوي علي تأثير مرغوب من وجود أليلات الأب البري علي الصفات المدروسة. تم تحديد عدد من المواقع الوراثية الهامة في هذه الدراسة والتي يمكن الاستفادة من أليلات الأب البري في تحسين التحمل للجفاف. مثلاً, أظهر الموقع QHD.S42-2H انخفاضاً في عدد الأيام حتي ٥٠% طرد نورات بواقع -٣,٠٣%, هذا الموقع قد يساهم في هروب النبات من الجفاف في نهاية الموسم. موقع أخر هو QPH.S42-7Ha كان مسئولاً عن نقص ارتفاع النبات بمقدار ٦,٢٩% نتيجة لوجود أليلات الأب البري. موقع أخر هاماً, هو QGYP.S42-1Ha أظهر تأثير تفاعلي مع معاملات الجفاف وأدي وجود أليلات الأب البري إلي زيادة محصول النبات الفردي بمقدار ١٤,٣١%. كما أشار التحليل الي ٢١ زوج من التأثيرات التفاعلية بنظام مضيف × مضيف في هذه الدراسة. معظم أزواج التفاعلات الأليلية كانت ذات تأثير مرغوب غلي الصفات تحت الدراسة. علي سبيل المثال, أكثر أزواج التفاعلات تأثيراً في خفض عدد الأيام حتي ٥٠% طرد السنابل هو bPb-9111(3H) × bPb-8701(4H) حيث أظهر ١٩,٣٦% من التباين الوراثي. السلالات التي ذات التركيب الوراثي Hsp/Hsp في هذه التفاعل كانت أبكر بواقع ٣,٠٢ يوم عن السلالات التي تحمل التركيب الوراثي Hv/Hv. بالنسبة لوزن ١٠٠ حبة, كانت السلالات الحاملة للتركيب الوراثي Hsp/Hsp في زوجين من التفاعلات أثقل وزناً بواقع ٠,٨٧ جرام عن السلالات الحاملة للتركيب الوراثي Hv/Hv. القت هذه الدراسة الضوء علي دور أليلات الأب البري في تحسين الشعير للتحمل للجفاف.

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