

## Potential of Iranian Wild Barley (*Hordeum vulgare* ssp. *spontaneum*) in Breeding for Drought Tolerance

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The vast genetic resources of wild barley (*Hordeum vulgare* ssp. *spontaneum*, hereafter WB) may hold unique assets for improving barley (*H. vulgare* ssp. *vulgare*) cultivars for drought stress. To evaluate genetic potential and characterization of variation among a diverse collection of barley and WB genotypes, mostly originated from Iran, a field experiment was performed under three moisture environments (control, mild and intense drought stress) during two years (2012–2014). Considerable variation was observed among the wild and cultivated genotypes for drought tolerance and agronomic traits. Principal component analysis (PCA) grouped genotypes studied into three groups (WB, two-row barley and six-row barley groups). However, Iranian and foreign WB genotypes were not completely separated, showing a high variation within both gene pools. The high significance of genotype by environment interaction, confirms importance of using accurate target environments for drought stress breeding. A number of WB genotypes with the highest values of the number of tillers, number of seed per spike, seed weight, grain yield and yield stability index under stressed environments were identified as superior genotypes. Most of these genotypes originate from Iran, highlighting the importance of this germplasm in barley breeding.

**Keywords:** barley, tolerance index, wild relatives, grain yield

### Introduction

Barley (*Hordeum vulgare* ssp. *vulgare*) is an important crop for feed and food and also as a model plant for genetic studies in *Triticeae* tribe (Nevo and Chen 2010). Among the different abiotic stresses, drought is by far the most complex and devastating on a global scale (Tuberosa 2012). Wild barley (*H. vulgare* ssp. *spontaneum* C. Koch, hereafter WB), the progenitor of cultivated barley, has a predominantly Mediterranean and Irano-Turanian distribution (Harlan and Zohary 1966). Because wild and cultivated barley are cross-compatible, the wild accessions with valuable traits are interesting for barley breeders (Nevo and Chen 2010).

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In the past years, genetic diversity and drought stress response of barley and WB genotypes belonging to different geographic zones including Israel, Jordan and Tibet was investigated (Ivandic et al. 2000; Shakhatareh et al. 2001; Shakhatareh et al. 2010; Zhao et al. 2010). Their results have shown the existence of high variability among the WB accessions for the studied traits, especially for adaptive traits (plant height, earliness, peduncle length and peduncle extrusion) under drought conditions. Regarding Iranian germplasm, Nevo et al. (1986) reported high genetic variation in wild barley populations of Iran. Therefore, a much fuller exploitation of these genetic resources by breeding for economically important agronomic traits is warranted. Barati et al. (2015) also evaluated the root characteristics of a cultivated and WB germplasm, mostly originated from Iran, and identified genotypes with high drought tolerance at each developmental stage based on root-related traits. In a recent study, Barati et al. (2018) evaluated agro-morphological and yield-related traits associated with drought tolerance in 80 barley genotypes belonging to 15 wild species and identified wild barley genotypes with favorable characteristics and high drought tolerance. Despite such efforts, evaluation of genotypes native to Iran has been neglected to some extent. Iran is very rich in WB (Nevo et al. 1986), so it is expected that Iranian germplasm harbors important genes particularly to improve tolerance to drought stress. Therefore, the objectives of this research were to assess genetic diversity among and between Iranian and foreign WB and cultivated barley accessions using morphological traits, and to identify WB accessions with favorable characteristics to improve yield and stability of barley under different drought stress levels.

### Materials and Methods

A total of 64 barley genotypes belonging to three distinctive genotypic groups were used in this study (Table 1 and Table S1\*). The first group included 35 wild barley (*H. vulgare* ssp. *Spontaneum*, WB) accessions that were mainly native to Iran. The second and third groups consisted of sixteen two-rowed barley (TRB) and 13 six-rowed barley (SRB) genotypes, respectively. The experiment was performed on a silty clay loam soil on Isfahan University of Technology Research Farm (32°30' N, 51°20' E), Isfahan, Iran. The soil was non-saline and non-sodic. The mean annual temperature and precipitation are 14.5 °C and 140 mm, respectively. A combined analysis with three moisture environments and two years (2012–2013 and 2013–2014) was used in this study. The moisture environments were named control, mild drought stress (MDS) and intense drought stress (IDS). A completely randomized block design with 64 treatments (genotypes) and three replications was used in each environment. The seeds of all 64 genotypes were planted through mid-November in the farm; each plot contained two 1.5 m rows, with 20 cm between the rows and 2 cm between the plants in each row. All plots were irrigated from the sowing date until the onset of stem elongation (beginning of February at Isfahan), when the water treatments were applied to the end of growing season (May 20). After that, the irrigation was totally stopped for the IDS environment, while for the control and MDS

\*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

*Table 1.* Information on wild barley (numbers 1–35), two-row barley (numbers 36–51) and six-row barley (numbers 52–64) genotypes assessed for drought tolerance in field experiments during two years (2012–2013 and 2013–2014)

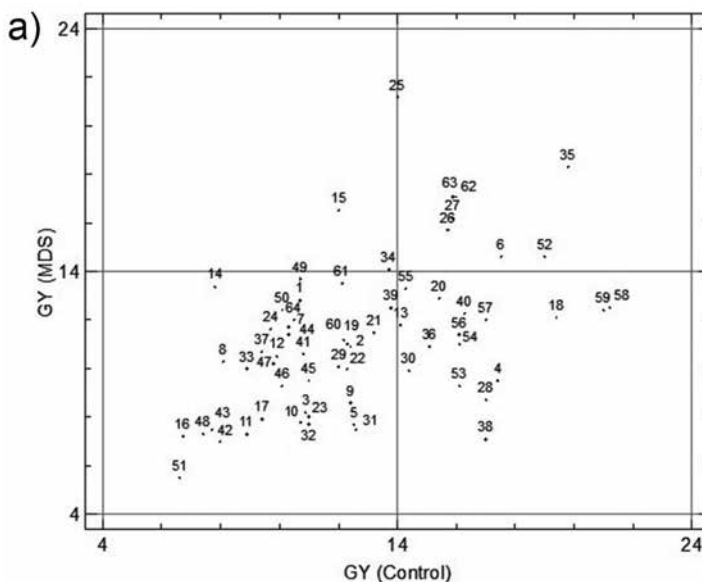
Number	Genotype name	Origin	Number	Genotype name	Origin
1	Hsp01	Cyprus	33	Hsp78	Iran – Kermanshah
2	Hsp02	USA	34	Hsp79	Iran
3	Hsp03	Israel	35	Hsp80	Iran – Ivan
4	Hsp04	Israel	36	Hvu204	Canada
5	Hsp05	Azerbaijan	37	Hvu207	Germany
6	Hsp06	Tajikistan	38	Hvu215	Iran
7	Hsp07	Israel	39	Hvu216	Iran
8	Hsp08	Iran	40	Hvu223	Iran
9	Hsp09	Turkmenistan	41	Hvu224	Iran
10	Hsp10	Turkmenistan	42	Hvu228	Iran
11	Hsp11	Turkmenistan	43	Hvu229	Iran
12	Hsp12	Iran	44	Hvu230	Iran
13	Hsp13	Iran	45	Hvu235	Iran
14	Hsp15	Libya	46	Hvu236	Iran
15	Hsp16	Azerbaijan	47	Hvu238	Iran
16	Hsp17	Cyprus	48	Hvu239	Iran
17	Hsp18	Iran	49	Hvu240	Iran
18	Hsp19	India	50	Hvu241	Iran
19	Hsp21	Turkmenistan	51	Hvu258	Iran
20	Hsp33	Iran	52	Hvu603	USA
21	Hsp45	Iran	53	Hvu605	USA
22	Hsp47	Iran	54	Hvu617	Iran
23	Hsp68	Iran – Azna	55	Hvu626	Iran
24	Hsp69	Iran – Kermanshah	56	Hvu627	Iran
25	Hsp70	Iran – Islamabad-e-gharb	57	Hvu651	Iran – cultivar Yousef
26	Hsp71	Iran – Khoramabad	58	Hvu653	Iran – cultivar Nosrat
27	Hsp72	Iran – Ilam	59	Hvu654	Iran – cultivar Reihan03
28	Hsp73	Iran – Kamyaran	60	Hvu659	Iran
29	Hsp74	Iran – Urmia	61	Hvu660	Iran
30	Hsp75	Iran – Mamulan	62	Hvu663	Iran
31	Hsp76	Iran – Saheb	63	Hvu664	Iran
32	Hsp77	Iran – Azna	64	Hvu665	Iran

environments, irrigation was applied when 50% and 80% of the total available water was depleted from the root zone (Allen et al. 1998), as used by Barati et al. (2018).

Days to heading (DH), days to ripening (DR), plant height (PH), spike length (SPL), number of fertile tillers (TN), and number of seed per spike (NS) were measured on ten plants in each plot at grain filling stage. After physiological ripening of each plot, ten plants were harvested and total above ground biomass yield (BY), the means grain yield per plant (GY) and hundred kernel weight (HKW) were measured. Finally harvest index (HI) was calculated for each plot with the formula  $HI = GY/BY$ . Yield stability index (YSI) (Bouslama and Schapaugh 1984) was calculated as the ratio of yield under stress/yield under control. Analysis of variance (ANOVA) was done after normality test using procedure GLM of SAS (SAS Institute 2008) to determine differences among years, moisture treatments and genotypes for each trait. Least significant differences (LSD) test ( $P < 0.05$ ) was used for mean comparisons. Correlation analysis and principal component analysis (PCA) were performed using Statgraphics centurion XVI (<http://www.statgraphics.com/>).

## Results

Based on the results of analysis of variance (Table S2), the difference between two years (Y) was significant for all of the traits except biomass yield and harvest index. The differences between three moisture treatments (E) were significant for all of the measured traits. The interaction between year and moisture treatments (Y\*E) was significant for all of the traits (except days to heading). Genotype (G) and its interactions with year and moisture environment (G\*Y, G\*E and G\*Y\*E) were significant for all of the traits. Based on the means of the genotypes (Table S3), the WB genotypes with desirable characteris-



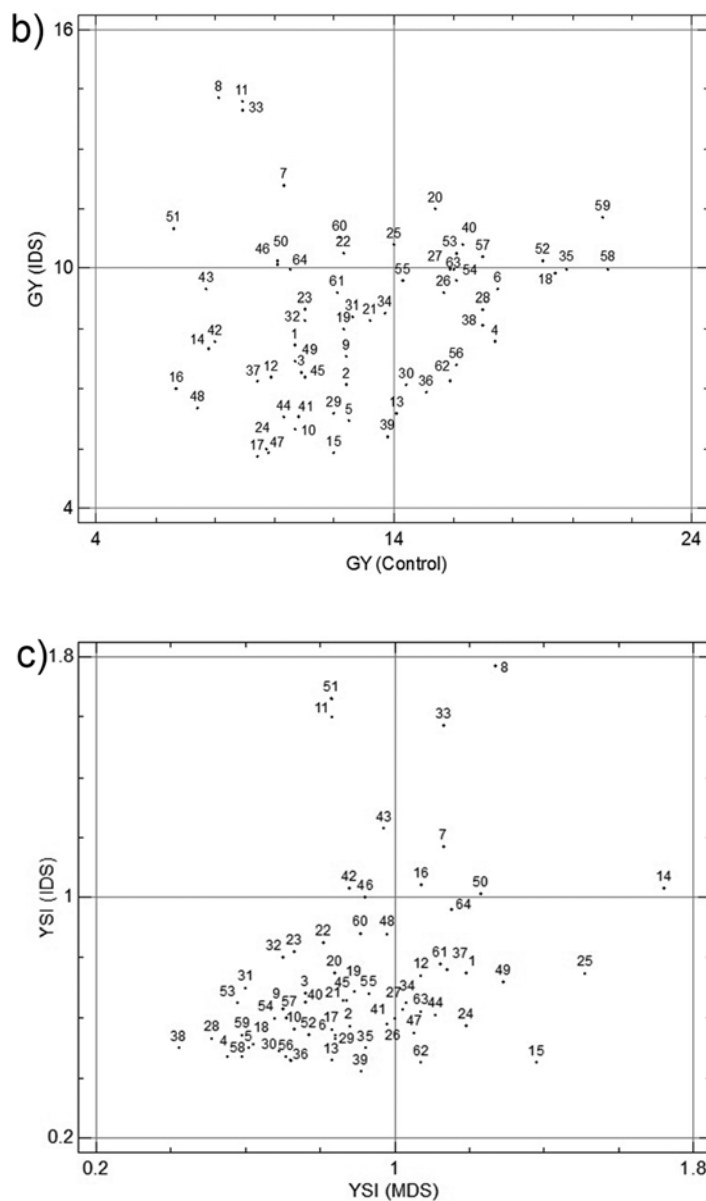


Figure 1. a) The biplot of the grain yield (GY) under control to GY under mild drought stress (MDS), b) The biplot of the GY under control to GY under intense drought stress (IDS), c) The biplot of the yield stability index (YSI) under mild drought stress (MDS) to YSI under intense drought stress (IDS). The means of two years has been used. Definition of origin of the genotypes can be seen in Table 1

tics such as earliness (Genotypes Hsp11 and Hsp47), higher number of fertile tillers (Genotypes Hsp79 and Hsp33), higher number of seed per peduncle (Genotype Hsp45, Hsp07 and Hsp47) and higher hundred kernel weight (Genotypes Hsp02 and Hsp03) were identified.

The biplot of grain yield under control vs. grain yield under MDS (Figure 1a) separated the genotypes into four groups. The first group contained the genotypes with low yield under both water stress and non-stress conditions. Most of the genotypes were placed in this group. The second group consisted of genotypes with high yield under control and low yield under water stress environments. The third group contained only one genotype (number 15) with low yield under control but high yield under water stress conditions. The last group included the genotypes with high yield under both control and water stress environments. Genotypes 6, 25, 26, 27, 35, 52, 62 and 63 were placed in this group. The biplot of grain yield under control vs. grain yield under IDS was also obtained (Figure 1b). Similarly, most of the genotypes were placed within the first and the second groups. Genotypes 7, 8, 11, 22, 33, 46, 50, 51, 60 and 64 were located within third group and the genotypes 20, 25, 40, 52, 53, 57 and 59 were placed in the fourth group.

Yield stability index (YSI) was calculated based on the means of yield under control and drought stress for each genotype (Figure 1c). Under mild drought stress, YSI ranged from 0.51 to 1.72 for WB genotypes, from 0.42 to 1.29 in TRB genotypes and from 0.58 to 1.15 in SRB genotypes. While under intense stress, YSI ranged from 0.45 to 1.77 in WB genotypes, from 0.42 to 1.66 in TRB genotypes and from 0.45 to 0.96 in SRB genotypes. The biplot of YSI under MDS to YSI under IDS environments was prepared (Figure 1c). The genotypes with high drought tolerance at mild (numbers 8, 14, 15, 25 and 49) and intense drought stress (numbers 8, 11, 33, 43 and 51) environments were identified. Some genotypes were identified with relatively high YSI in both MDS and IDS environments (numbers 7, 8, 14, 16, 33 and 50). Finally genotypes with YSI around 1 in MDS and IDS environments, which have almost the same yield in three moisture environments were identified (numbers 7, 16, 46, 48, 50 and 64).

The Pearson correlation coefficients were calculated between the traits for each moisture environment separately (Table S5). Under control environment, grain yield had positive correlation with days to ripening, number of seed per peduncle, biomass yield and harvest index and negative correlation with spike length. In the MDS environment, grain yield was positively correlated with days to ripening, number of seed per peduncle, hundred kernel weight, biomass yield and harvest index. Under intense drought stress environment, grain yield had positive correlation with number of seed per peduncle and biomass yield and negative correlation with spike length.

Based on the results of principal component analysis (PCA), the two first components explained 52, 53 and 48% of the total variation in the control, MDS and IDS environments, respectively. Under control conditions, on the scatter plot of the genotypes within PC1 (highly correlated with biomass yield and number of seed per peduncle) vs. PC2 (highly correlated with grain yield) (Figure S1,a), the genotypes were clustered into four main groups. Group 1 with high PC1 and moderate to high PC2 included most of SRB genotypes. Group 2 with moderate to low PC1 and low PC2 included most of TRB geno-

types. The two other groups consisted mostly of WB genotypes. Group 3 has low PC1 and moderate to high PC2. Group 4 has moderate PC1 and PC2. Similar to control conditions, in the biplot of PC1 vs. PC2 under MDS (Figure S1,b) and IDS (Figure S1,c) conditions also the TRB and SRB genotypes were tightly grouped together and separated from WB genotypes; however, more variations between the WB genotypes were observed which separated in 3 and 4 groups under MDS and IDS conditions, respectively.

## Discussion

Previous studies on evaluation of genetic diversity in barley germplasm showed a high genetic variation for agro-morphological traits, yield-related traits and tolerance to drought stress (Ivandic et al. 2000; Shakhathreh et al. 2001; Shakhathreh et al. 2010; Zhao et al. 2010; Jedmowski et al. 2015). Similarly, high genetic variation of these traits was observed among and between the barley and WB groups studied here. High genetic variation between barley cultivars and WB genotypes is a key factor aiming at the successful improvement of current barley cultivars (Talame et al. 2004; Ma et al. 2012). On the other hand, the superior genotypes which were identified for earliness (Genotypes Hsp11 and Hsp70), number of fertile tillers (Genotypes Hsp79 and Hsp33), number of seed per peduncle (Genotype Hsp80) and hundred kernels weight (Genotypes Hsp02 and Hsp03), may have novel genes useful for barley improvement.

Genotype by environment interaction was highly significant for grain yield, therefore different response to levels of irrigation was observed for most of the genotypes. Regarding to selection tolerant genotypes for drought stress environments, there are some possibilities: the first one is selection based on the performance of genotypes under stress environment. Considering this method, the high yielding genotypes under mild drought stress were Hsp16, Hsp70, Hsp71, Hsp72, Hsp80 and the ones under intense drought stress were Hsp07, Hsp08, Hsp11, Hsp33 and Hsp78. The second method is to select the genotypes based on performance under both control and stress environments (Fernandez 1993). Based on this method, the genotypes Hsp06, Hsp70, Hsp71, Hsp72, Hsp80, Hvu603, Hvu663 and Hvu664 under MDS (Figure 1a) and the genotypes Hsp33, Hsp70, Hvu223, Hvu603, Hvu605, Hvu651 and Hvu654 under IDS are preferred (Figure 1b). Thirdly, the genotypes that can keep their performance under stressed environments are preferred, even if they have low yield under control environment (Naim-Feil et al. 2017). The use of stability indices such as YSI facilitates the selection of tolerant genotypes (Bousslama and Schapaugh 1984). Using YSI values calculated for each genotype (Figure 1c), the genotypes Hsp07, Hsp08, Hsp15, Hsp17 and Hsp78, which had relatively high YSI in both stress levels could be selected. However, by all of these methods, most of the drought tolerant genotypes were from the WB group. Similar results have been reported in previous studies of root-related traits (Barati et al. 2015), which most of genotypes with vigorous root system (higher root dry weight, depth and area) were from the wild barley accessions. In the other study on wild barley species, the genotypes from *H. murinum* and *H. marinum* had more yield stability on drought stressed environment compared to the cultivated one (Barati et al. 2018).

The significant positive correlation between grain yield with other traits which have more simple genetic control (such as number of seed per peduncle in this study) may help breeder to improve yield through indirect selection for these traits (Abdolshahi et al. 2015). The positive significant correlation between grain yield with days to heading and days to ripening means a higher yield in long-lived genotypes. However, when the correlation coefficients were calculated based on the data from IDS environment, the correlations between grain yield with days to heading and days to ripening were not significant. This is maybe because one of the most susceptible stages of plant development to drought stages is flowering stages (Tuberosa 2012). Regarding days to ripening, drought escape via a short life cycle is one of the most important mechanisms of drought resistance; because earliness plants are less imposed to drought stress during grain filling (Van Oosterom et al. 1992; Shakhatareh et al. 2001). This is maybe one of the reasons that the earliest WB genotypes Hsp11 and Hsp70 have relatively high tolerance to drought (Figure 1).

The results of principal component analysis (PCA) completely separated cultivated and wild barley genotypes from each other, as well as the TRB and SRB genotypes. Concerning WB genotypes, the Iranian and exotic WB genotypes did not completely separate, confirming considerable variation within both Iranian and foreign genotypes. In the biplot of PC1 vs. PC2, the genotypes with same response to drought stress are placed in the same group, such as some of the genotypes that were being collected from west of Iran (Hsp70, Hsp71, Hsp72, Hsp73 and Hsp80).

In conclusion, considerable variation was observed among the wild and cultivated genotypes for drought tolerance and agronomic traits. A number of WB genotypes with the highest values of agronomic traits and drought tolerance under stressed environments were identified. Most of these genotypes have been originated from Iran. There is potential in these accessions to be used by breeders for improvement of the barley crop drought stress tolerance. In the other hand, study of physiological traits on the present germplasm and comparison with these results could help to understand more about the mechanisms of drought stress in barley.

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### Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at <https://akademai.com/loi/0806>

Electronic Supplementary *Table S1*. Information on wild barley (numbers 1–35), two-row barley (numbers 36–51) and six-row barley (numbers 52–64) genotypes assessed for drought tolerance in field experiments during two years (Y) (2012–2013 and 2013–2014)

Electronic Supplementary *Table S2*. Analysis of variance and mean squares for agro-morphological and yield-related traits in 64 genotypes (G) evaluated in three moisture environments (E) during two years (Y) (2012–2013 and 2013–2014)

Electronic Supplementary *Table S3*. Means of three environments and two years evaluated for each trait in 64 barley and wild barley genotypes

Electronic Supplementary *Table S4*. Means of wild barley (WB), two-row barley (TRB) and six-row barley (SRB) calculated for averages of two years for each moisture environment separately for evaluated traits

Electronic Supplementary *Table S5*. Correlation coefficients between the evaluated traits calculated based on means of two years and three irrigation levels

Electronic Supplementary *Figure S1*. Biplot of PC1 vs. PC2, obtained from principal component analysis on the data of a) control, b) mild drought stress and c) Intense drought stress conditions. The means of two years have been used. DH; Days to heading, DR; Days to ripening, PH; Plant height, TN; Number of fertile tiller, SPL; Spike length, NS; Number of seed per peduncle, HKW; Hundred kernel weight, GY; Grain yield, BY; Total above ground biomass yield, HI; Harvest index. Definition of origin of the genotypes can be seen in Table 1