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Full Length Research Paper

Screening of barley genotypes for drought tolerance by agro-physiological traits in field condition

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In a two-year experiment, 11 barley genotypes from ICARDA and one landrace from Iran were tested under optimum and drought stress conditions. Phenological and physiological traits such as relative water content (RWC), osmotic adjustment (OA), stay-green (SG), plant height (PLH), days to heading (DHE), days to maturity (DMA) and seed indexes such as 1000-grain weight (TGW), number of grain per spike (G/S) and grain yield (GY) were evaluated. Variations were observed in DHE, DMA, G/S, TGW, PLH, RWC, OA and length of stay-green period. DHE and DMA were the phenological traits that most influenced yield during water stress conditions. Negative correlation was observed under water stress between yield, DHE, and DMA under drought stress. The average reduction in yield caused by drought stress was 28.05%. Under drought stress condition, TGW, G/S, RWC and SG correlated positively with yield, while under both stress conditions, the correlation of yield and PLH was lower than other correlations. Yield was significantly correlated with osmotic adjustment (P<0.05). Among the genotypes, L6 possessed the greatest OA capacity, and L3, L8, L9 and L10 the smallest. The genotypes that show higher OA capacities therefore, are those that are most drought tolerant. Genotype L6 performed well under water stress condition as it attained a reasonable plant height, precocity, RWC, OA and SG, gave higher grain yields and seed index as compared with other genotypes.

Key words: Barley, drought stress, phenological and physiological traits, yield.

INTRODUCTION

Drought occurs around the world, every year, often with devastating effects on crop production (Ludlow and Muchow, 1990). Water deficit (commonly known as drought) is defined as the absence of adequate moisture necessary for normal plant growth and completion of the life cycle (Zhu, 2002). The lack of adequate moisture leading to water stress is a common occurrence in rain-fed areas, caused by infrequent rains and poor irrigation (Wang et al., 2005). Traits affected by the water relations of the plant, such as relative leaf water content (RWC) and osmotic adjustment clearly indicate water extraction patterns. Adapting the growth cycle to water availability therefore, becomes priority. Developing well-adapted crops could improve yields under such conditions, as observed in barley (Gonzalez et al., 2007). Leaf water potential (WP) and osmotic adjustment capacity are characteristics that can be selected to improve the drought tolerance of different crops (Teulat et al., 1997; Nayyar et al., 2005). The water status of a crop plant is defined in terms of its water content, water potential or the components of WP (Turner, 1986). Osmotic adjustment is increasingly gaining recognition as an efficient drought tolerance mechanism in cultivated plants (Teulat et al., 1997; Hamidou et al., 2007), and either directly or indirectly, it has a positive effect on productivity during drought (Ludlow and Muchow, 1990). Osmotic adjustment refers to the reduction in WP due to the net accumulation of solutes, as a response to water deficit (Nayyar and Walia, 2004). This allows the turgor potential (TP) to be maintained at higher levels and helps limit the effects of water stress on the opening of the stomata, photosynthesis and growth. Increase in crop biomass contributes to the improvement of cereal yield. At

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Abbreviations: DHE, Days to heading; **DMA,** days to maturity; **G/S,** number of grain per spike; **GY,** grain yield; **PLH,** plant height; **RWC,** relative water content; **OA,** osmotic adjustment; **SG,** stay-green; **TGW,** thousand grain weight.

anthesis, a proportion of this biomass corresponds to the ear. This proportion must be high to obtain better yields and a higher harvest index (Siddique et al., 1989). Apart from environmental conditions, the final grain yield of barley is determined by the product of three components: the number of ears per meter squaree, the number of grains per ear, and individual grain weight (currently expressed as 1000- grain weight). The duration of grain filling and the growth cycle also contribute greatly to crop yield (Garcia del Moral et al., 1991). Yield components are successively determined during plant development, exerting compensatory effects on one another. This complicates the selection of a trait as complex as yield. Each yield component could be affected by temporary water deficits, the extent of which would depend upon the stage of plant development when these conditions occur. The reproductive development of cereals is vulnerable to water shortage, while environmental conditions during anthesis mainly affect the number of grains and final yield due to the number of grains produced *per ear* (Christen et al., 1995). Late water stress shortens the grain-filling period as it leads to premature desiccation of the endosperm and limits the embryo size. Reduction in yield is therefore, mainly due to a reduction in the weight of the grains produced (Gibson and Paulsen, 1999).

Retention of green leaf area at maturity, termed stay-green, is considered an indicator of post anthesis drought resistance in plant breeding programs in USA and Australia (Borrell et al., 2000). The trait may indicate the presence of drought avoidance mechanisms, but probably does not contribute to yield per se if there is no water remaining in the soil profile by the end of the cycle to support leaf gas exchange. It may be detrimental if it indicates lack of ability to remobilize stem reserves (Blum, 1998). However, research in sorghum has indicated that stay-green is associated with higher leaf chlorophyll content at all stages of development, and both were associated with improved yield and transpiration efficiency under drought (Borrell et al., 2000). The contribution of stem reserves to grain yield was greater in a tall barley cultivar than in a short one. Subhani and Chowdhry (2000) reported that under drought stress conditions, grain yield was significant and positively correlated with flag leaf area, plant height and 1000-grain weight. Gupta et al., (2001) and Muzammil (2003) also observed substantial decline in plant height when irrigation was withheld at the booting stage; however, tolerant genotypes attained more plant height. To study the importance of identifying real water stress-tolerant barley genotypes, in this study, genotypes were planted during well rain-fed and warm conditions. Although several studies have examined the effects of phenology, physiology, and alterations in yield components on final yield under field conditions, few have simultaneously examined the outcome in controlled conditions of irrigation and water stress over a number of years. Thus, the aims of the present research were to screen drought tolerant barley genotypes, to determine the effect of water stress on yield and yield contributing traits and to examine its relationship with phenological and agronomic traits.

MATERIALS AND METHODS

Area/field studies and genotypes origins

All trials were performed over two years (2005-2007) at the dry land agriculture research station, Gachsaran (50°50′N, 30°17′W, altitude 710 m), in calcareous-type soil with a Silty Clay Loam texture, pH: 7.3, organic matter less than 1% and an available water-holding capacity of 150 mm/m of depth. Figure 1 shows the temperature and relative rainfall over the crop growth period. Each point represents the average temperature and rainfall for 30 days. The experimental material included 12 barley genotypes, one landrace (L11) from Iran, and 11 genotypes (L1 to L10 and L12) and from ICARDA (their row types shown in Table 1). These genotypes were used to compare the responses of different, widely used varieties and breeding lines selected for their tolerance to water stress. A compound fertilizer (10 N: 4 P: 8 K) was applied at the rate of 20 g/m^2. Both the control and treatment area were divided into three blocks (complete random block design), each containing six rows (4.37 m length of the rows) corresponding to each of the 12 barley genotypes. Plant density was 100 plants per m^2 and a border of 17.5 cm width. During the two years of this study, precipitation was generally below the long-term average. Water was withheld in the water stress treatment subplots when the flag leaves were fully expanded (stage 41 on the Zadoks scale) (Zadoks et al., 1974). During anthesis and grain-filling period, the soil water content was 11 and 7.4%, respectively, (SWC about 50 - 40% of soil water field capacity) and was the same for all stress treatment plots. The control subplots were maintained at field capacity by irrigation, until the beginning of maturity (stage 78 on the Zadoks scale).

Phenological and yield evaluations

The phenological characteristics of the different genotypes were determined considering the number of days elapsed between sowing and heading [that is, days to heading (DHE), taken when 50% of the shoots had the entire spike showing above the flag leaf]. When the crops were matured, spikes from the centre square meter of each subplot were counted, cut and threshed, to obtain the grain yield (g/m^2). To estimate days to maturity (DMA), the number of days between the sowing date and the time at which 50% of the spikes had matured, was counted. After the spikes were harvested, the stalks were cut at ground level. The thousand-grain weight (TGW) was obtained by counting out 1000 grains in each micro plot with a grain counter and weighing them. The number of grains per spike (G/S) was calculated from the equation: Yield=number of spike/m^2×G/S× (TGW) ×10^{-3}. Mean plant height in centimeter from the base to the top of the main stand was thus estimated.

Disease, RWC, osmotic adjustment and stay-green studies

During growing season, reaction to disease such as Scald [e.g. immune, resistance, medi-resistance, tolerant, medi-sensitive and sensitive (as O, R, MR, T, MS and S)], Powdery mildew (PM) and Barley dwarf virus (BDV) were recorded (Saari and Prescott, 1975). Many variables associated with the water stress of the leaf: osmotic potential (OP) and RWC were measured from the beginning of the stress treatment until the beginning of crop ripening. To do this, weekly samples of flag leaves (from one plant in each subplot subjected to each treatment) were collected at 07:15 A.M, placed in a sealable plastic bag, and transported to the laboratory (10 min away). These

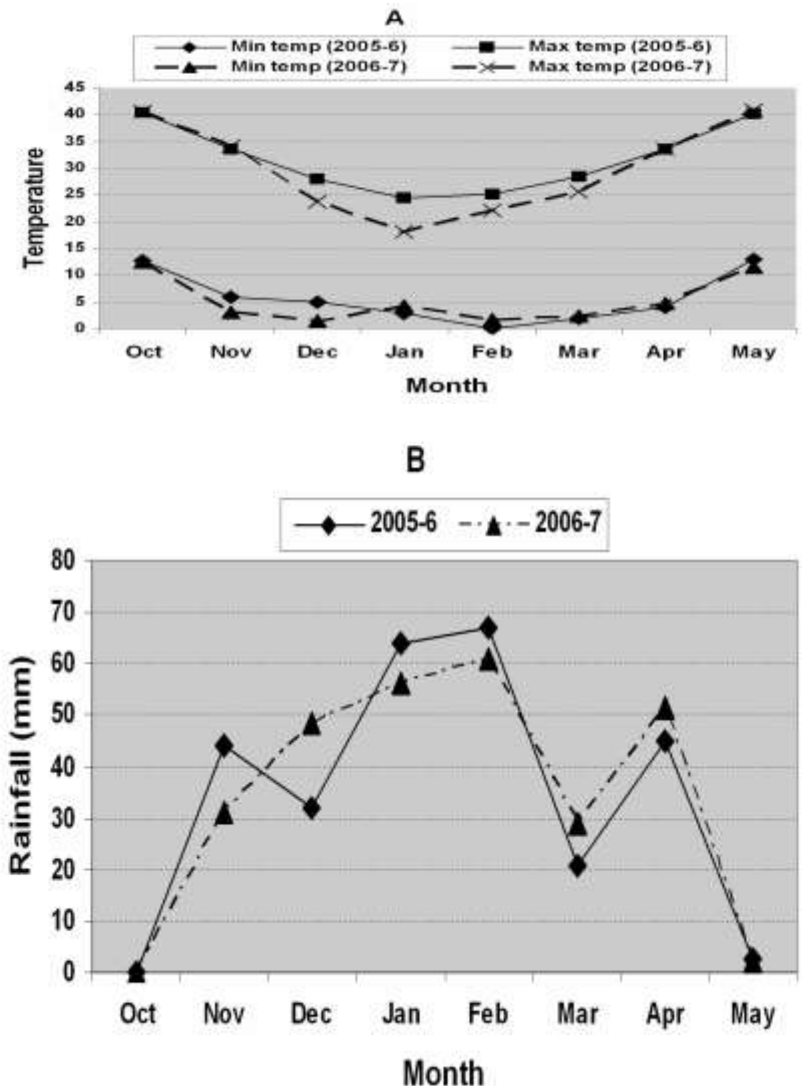

Figure 1. Temperature (A) and rainfall (B) status during the experiments, averaged over 30-day periods for the 2005-2006 to 2006-2007 growing seasons.

Table 1. Barley promising lines involved in this study, their pedigrees and row types.

Lines	Origin	Pedigrees	Row type
L1	ICARDA	Alanda/5/Aths/4/Pro/Toli//Cer *2/Toli/3/5106/6/Avt/. -8G -3 G	6
L2	ICARDA	Bda/Cr. 115/Pro/Bc/3/Api/Cm67/4/ Giza121/... -9G -2 G	2
L3	ICARDA	Emir/Nacta//As907/3/Avt_(9-9)ACSAD-1290-6AP-OTR-OAP-6AP-OAP-OAP	2
L4	ICARDA	Lth/3/Nopal//Prol/11012-2/4/Kabaa-03ICB94-0498-OAP-3AP-OAP-OAP	6
L5	ICARDA	Himalaya-12/Plaisant ICBH95-0630-OAP-OAP-16AP	6
L6	ICARDA	MK1272//Manker/Arig8/3/Alanda ICB93-0448-OAP-6AP-OAP	6
L7	ICARDA	Hyb 85-6//As46/Aths*2	6
L8	ICARDA	Alanda/Harma-01/7/Gustoe/6/M64-76/Bon....	6
L9	ICARDA	Zanbaca/3/H.spont.21-3/Arar84//Wi2291/Bgs ICB 94-0314-OAP	2
L10	ICARDA	Pld10342//Cr.115/por/3/Bahtima/4/DS	2
L11	Iran	Izeh. - (CONTROL)	6
L12	ICARDA	wi2291	2

Table 2. Combine analysis of variance for important traits for evaluation tolerance of advance barley lines to drought stress during two seasons (2005-2006 and 2006-2007).

					(Drought condition)					
S.O.V	**(df)**	**PLH**	**DHE**	**DMA**	**G/S**	**SG**	**RWC**	**OA**	**TGW**	**GY**
Year (Y)	1	7.09 ^{ns}	3.6 ^{ns}	2.01 ^{ns}	6.4 ^{ns}	2.1 ^{ns}	61.2 ^{ns}	0.008 ^{ns}	4.4 ^{ns}	0.01 ^{ns}
Rep × Y	4	1.27 ^{ns}	0.9 ^{ns}	0.9 ^{ns}	1.1 ^{ns}	1.2 ^{ns}	3.4 ^{ns}	0.015 ^{ns}	3.2 ^{ns}	1.3 ^{ns}
Line (L)	11	280.9**	44.3**	89.5**	1087.3**	55.2**	376.4**	0.87**	158.1**	20.9**
L × Y	11	88.7**	10.9*	14.1*	10.2**	18.3**	28.3**	0.35**	9.3 ^{ns}	0.35 ^{ns}
Treatment	1	90.2**	81.2**	94.8**	194.3**	38.23**	101.7**	0.96**	90.7**	224.9**
Error	44	21.07	3.47	3.67	3.53	3.21	125.4	0.04	4.8	0.60
CV%		5.28	1.91	1.47	5.03	1.37	15.2	12.3	4.77	21.6

*, ** and "ns" represented significant at 5%, 1% and non significant at 5% probability levels respectively.

leaves were then cut longitudinally into two symmetrical halves. One was immediately weighed to determine the RWC using the formula:

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

The turgid weight was obtained by leaving the leaf immersed in distilled water at 5°C, in the dark. Dry weight was recorded by placing the leaves for 72 h in oven and then weighing.

The other half was used to determine the OP. Thermocouple psychrometers were used, employing the method of Martin et al. (1995). For OP measurement, a sample consisting of three 1-cm long midleaf segments was sealed in a thermocouple psychrometer cup (2-mL volume) and freeze-killed at -20°C. Prior to measurement, samples were thawed for 30 min at room temperature. The OP of the tissues was then measured using a micro voltmeter (HR33T; Wescor, Logan, UT, USA). To estimate the osmotic adjustment of each genotype, the correlation between the OP and the RWC was determined from the corresponding linear regressions. The RWC values for an OP of -3MPa were recorded using the criteria of Morgan (1983). Higher RWC at a given OP indicates a relatively higher OA. The stay-green rating was visually scored at or soon after physiological maturity on a plot basis. Scoring was done on a 1 - 5 scale based on the proportion of leaf area of normal-sized leaves that had prematurely senesced and died. A rating of one indicated essentially no leaf death; three indicated approximately 50% mature leaf area dead, while five indicated 100% plant (leaves and stem) death (Xu et al., 2000). In addition, the days to 100% flag leaf death were recorded.

Statistical analysis

SAS software was used for the combined analyses of variance. Homogeneity of trial variance errors was verified using the Bartlett's test. Mean comparisons were carried out to estimate the differences between treatments, and genotypes using the LSD tests.

RESULTS AND DISCUSSION

The trials were conducted in two successive years. The climatic conditions in two years were almost the same and the results obtained from the trials after two years were not different. Therefore, only the results of a combined analysis of two years are shown. Statistical analysis was carried out to determine the differences among 12 barley genotypes, in response to drought stress condition. The mean squares from the analysis of variance (Table 2) revealed that the main effects (genotypes and stress treatments) were significant for all the characters studied. The significance of genotypes and stress treatments indicated that the varieties responded differently, and stress treatments significantly affected the plant traits. None of the genotypes expressed symptoms of the disease studied.

Rainfall distribution

Rainfall distribution patterns vary considerably among locations and over the years, and additional stresses may include heat and cold stress, soil microelement deficiency or toxicity, and a range of biotic stresses. Physiological assessment of drought tolerance characteristics in the field is therefore a complex task. Research, using a line source gradient to create different intensities of drought stress, demonstrated a linear relationship between grain yield and water application (Sayre et al., 1995). Breeding work for moisture-stressed environments has been largely empirical to date, but recent emphasis on breeding for marginal environments has increased the focus on dry and warm environments, and a multidisciplinary effort has been initiated to improve drought tolerance. The rainfall and temperature levels were almost the same in the two years of study (Figure 1). Therefore, there were no significant differences between results obtained from traits evaluated in the two years of our study.

Yield

Results from this study reveal that the differences in yield between barley genotypes grown in optimal and water stress conditions were the least in earlier genotypes that

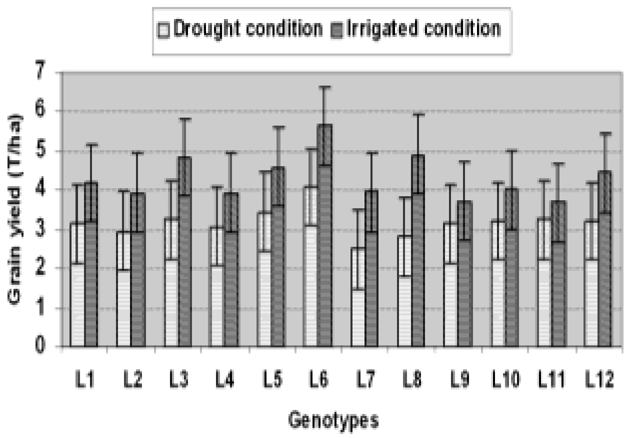

Figure 2. Grain yields of 12 barley genotypes under drought stress condition. The amounts of yield represented are the means of 2 years.

show a longer grain-filling period. The breeding lines yielded by these traits were more than common barley varieties. The results showed that the yield was greater under irrigated than under water stress conditions (Figure 2). Average yields (calculated as t ha^{-1}) in irrigated plots varied from 4.24 to 5.95 t ha^{-1} and in drought conditions, they varied from 2.68 to 4.37 t ha^{-1} (Figure 2). Grain yield as its component, which was affected by stress, showed a reduction in drought stress compared to the irrigated condition from 4.99 t ha^{-1} to 3.59 t ha^{-1} (that is, 28.05%). Under both water stress and irrigated conditions, L6 revealed the highest grain yield for two years, although there was no significant difference among the genotypes, with the exception of L7, that showed lowest yield compared with the other genotypes under stress condition (Table 3).

As shown in Table 3, L5, L6, L11and L12 not only revealed the best mean yields under the drought stress, but they also had very good yield potentials under irrigated conditions. This suggests that they possess wide-range adaptation to the environment. The yield performance of a genotype under stress reflects both its yield potential and its response to stress (Sadiq et al., 1994). These researchers also concluded that an empirical approach based on grain yield criteria will remain a reliable way of improving yield in water-stressed environments, at least until drought tolerance mechanisms for a particular crop, are better understood, both functionally and genetically. Genotypes of L5 and L8 showed the greatest differences in mean yield between stress and well-watered conditions (between 6-rowed) suggesting that these genotypes are more sensitive to drought. However, some genotypes systemically showed small differences in yield under both conditions, e.g. L4, L1, L6 and L11 (Table 6). L9 and L10 also showed less reduction in yield among 2-rowed genotypes. On an average during the stress environment, L6, which produced maximum grain yield (6.325%), compared to control (L11) (Table 6) is less affected, thus, being the more water stress tolerant genotype. Solomon et al. (2003) and Ozturk and Aydin (2004) found yield reductions of 79.7 and 65.5% when water stress was imposed either at the earlier stages or at grain formation. It might be concluded that a high potential yield and high TGW are the most significant traits of when selecting barley plants to improve yield under water stress conditions. Yield is a very important trait, but has high effect with environment conditions.

Thousand grain weight

The TGW was higher under irrigated than water stress conditions for all genotypes (Figure 3). In the present study, TGW was the yield component most affected by drought. These results are corroborated by other studies in cereals reporting the influence of individual grain weight on yield under stress conditions, showing that the responses of different genotypes to drought during grain filling lead to differences in individual grain weight (Giunta et al., 1993; Lopez-Castaneda and Richards, 1994; Voltas et al., 1999). In wheat, drought during grain filling reduces the individual grain weight (Mogensen et al., 1985). The 6-rowed genotypes had lower TGW than did the 2-roweds, but L6 was an exception since it had a TGW similar to some of the 2-rowed genotypes. Seed index is also regarded as one of the most important indicators of stress tolerance via grain weight. In the stress environment compared to the well-watered condition, seed index declined subsequently as 4% for all genotypes (Table 3). On an average of all the genotypes, the maximum seed index was noted in L6, as being highly tolerant to water stress conditions. Seed index results therefore, suggested that stress may be avoided at grain formation, and genotype L6 may be preferred in water deficit environments. In earlier studies, reduced yields were attributed mostly to lower grain weight and only minimally to lower grain number (Sofield et al., 1977; Tashiro and Wardlaw, 1990). We conclude that grain weight during the maturity stage is affected by water stress. The effect is as important as the critical water level (stress level) is high and long. It is independent of the grain number/m^2 and grain number/spike.

Number of grain per spike

The G/S for many genotypes was significantly greater in the irrigated than in the water stress conditions (Figure 4). Substantial losses in wheat grain yield have been reported due to water deficiency, depending on the developmental stages at which crop plants experiences stress. Water stress at various stages, especially before anthesis, can reduce the number of ear heads and number of grain per spike (Dancic et al., 2000; Mary et al., 2001). The number of grain per spike of the 6-rowed

Table 3. Means† of agronomic traits of evaluation advance barley lines to drought stress at 2005-2006 and 2006-2007.

Line no	Row type	Diseases			DHE (day)	PLH (cm)	DMA (day)	TGW (g)	G/S	Yield (t/ha)	SG (day)
		SCA	PM	BDV							
Stress condition											
1	6	0	0	0	94 F	95.7 A	128 CD	41 EF	46 B	3.37 AB	127 D
2	2	0	0	0	99 BC	90.2 B	129 CD	51.3 A	23 C	3.17 AB	129 CD
3	2	0	0	0	97 CDE	73.5 D	128 CD	51.9 A	22 C	3.53 AB	127 D
4	6	0	0	0	96 DEF	92.4 AB	128 CD	45.5 CD	47 B	3.31 AB	129 CD
5	6	0	0	0	98 CD	88.9 BC	134 B	39.9 F	46 B	3.69 AB	133 B
6	6	0	0	0	95 EF	88.7 BC	128 CD	50 AB	50 A	4.37 A	135 AB
7	6	0	0	0	103 A	84.3 C	137 A	36 G	47 B	2.68 B	136 A
8	6	0	0	0	101 AB	84.2 C	138 A	44.6 D	52 A	3.5 AB	135 AB
9	2	0	0	0	95 EF	88.9 BC	127 D	47.8 BC	21 C	3.97 A	127 D
10	2	0	0	0	96 DEF	90.9 AB	129 CD	51.7 A	23 C	4.05 A	130 C
11	6	0	0	0	97 CDE	91.4 AB	130 C	43 DE	45 B	4.0 A	130 C
12	2	0	0	0	95 E**F**	74.4 D	128 CD	47.5 BC	21 C	3.89 A	128 CD
LSD value (a=5%)					2.169	5.341	2.228	2.549	2.185	0.9036	2.086
(Irrigated condition)											
Line no	Row type	Diseases SCA	PM	BDV	DHE (day)	PLH (cm)	DMA (day)	TGW (g)	G/S	Yield (t/ha)	SG (day)
1	6	0	0	0	92 F	103 ABCD	103F G	44.35 D	50.83 C	4.56 CD	129 FG
2	2	0	0	0	95 DE	102A BCDE	130F G	53.76 A	24 D	4.28 D	131 DE
3	2	0	0	0	95 DE	97 DE	129 GH	53.8 A	24 D	5.26 ABC	130 EF
4	6	0	0	0	95 DE	103 ABCD	132 DE	47.8 BC	53 B	4.24 D	131 DE
5	6	0	0	0	96 D	106 A	133 CD	41.33 E	52 BC	5.95 A	136 B
6	6	0	0	0	95 DE	105 AB	133 CD	52.23 A	56 A	5.62 AB	139 A
7	6	0	0	0	104 A	102 ABCDE	138 B	37.10 F	50 C	4.29 D	134 C
8	6	0	0	0	102 B	101 ABCDE	140 A	46.90 BC	56 A	5.34 ABC	138 A
9	2	0	0	0	94 E	96 E	128 H	51.93 A	24 D	4.86 BCD	128 G
10	2	0	0	0	96 D	99 BCDE	131 EF	52.06 A	24 D	4.86 BCD	131 DE
11	6	0	0	0	98 C	104 ABC	134 C	45.7 DC	53 B	5.43 AB	132 D
12	2	0	0	0	96 D	98 CDE	133 CD	48.33 B	24 D	5.15 ABC	132 D
LSD value (a=5%)					1.866	6.472	1.529	2.31	2.015	0.8153	1.795

† Means followed by the same letter are not significantly different at the 5% probability level.

genotypes, both in the irrigated and water stress conditions was significantly greater than that of the 2-rowed genotypes. L6 produced the highest number of grain per spike, followed by L8 in irrigated and stress conditions (Table 3). For 2-rowed genotypes, L10 had the highest G/S, both in well watered and under stress conditions. While water stress imposed during the later stages could additionally induce a reduction in number of grains/spikes and grain weight. The degree of sensitivity to water deficit exists at all stages of plant development, although in barley there appear to be several critical stages of sensitivity.

The first stage appears at the germination (bad stand and low density), the second one coincides with the floral initiation, which reduces both primordia number per surface unit and tillers number, and the third level is seen at the anthesis (reduction of grain number per spike, due to pressure on reproduction efficiency). The fourth critical

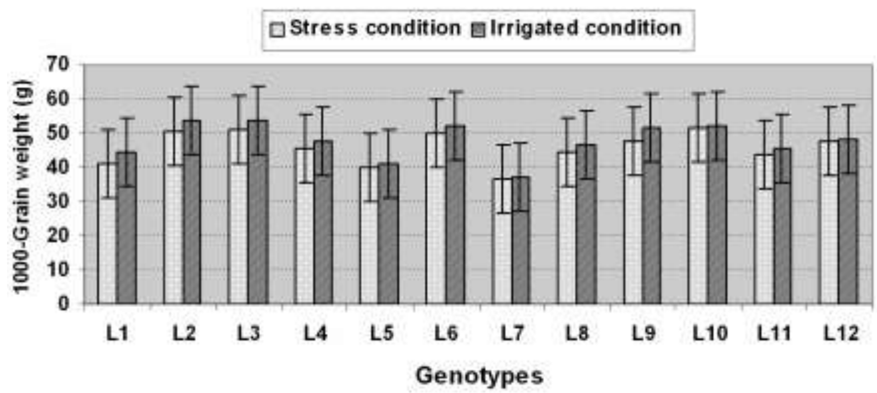

Figure 3. Number of grain per spike for 12 barley.

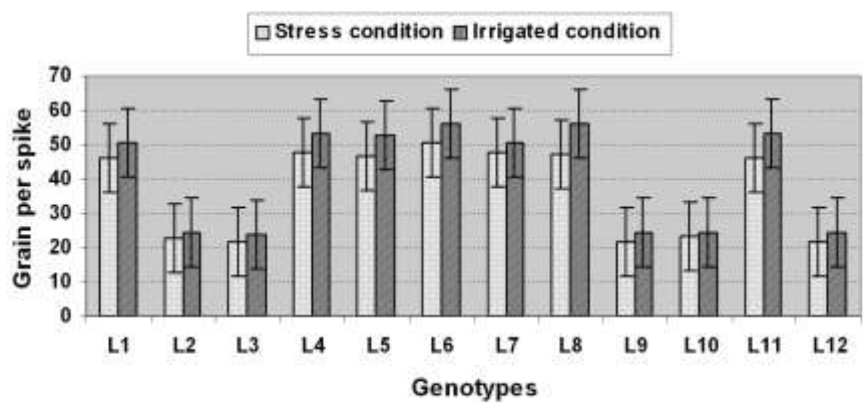

Figure 4. 1000-Grainweights of 12 barley genotypes grown in drought stress condition. The amounts of weights represented are the means of 2 years.

stage is located at the beginning of the milky stage of the grain and reduces grain weight. Varlet Granchet and Pluchard (1986), in their study on bread wheat, found that the number of grains per spike is determined quite early, at the shooting stage. Unfavorable conditions during the shooting stage mainly affect the grain number per spikelet. According to Ceccarelli (1987), water deficit during the early stage of plant development induces a reduction in spikelets primordia, while water deficit late in the plant development increases death of the flower and the entire spikelet. The number of grains per spike (fertility) depends on the water availability during the early vegetative phase and during the shooting stage. If water deficit occurs after the flowering stage, it induces a decrease of grain weight and thus its yield.

Days to heading and maturity

In our experiment, irrigation was stopped for each genotype at the same phenological stage (flag-leaf stage). Therefore, the earlier genotypes received less water in total than the later ones, thus reducing the drought escape effect and the advantages of earliness with respect to field conditions. Despite that, the present data show that in barley, precocity continued to be a positive trait for yield under stress conditions. Table 3 shows the number of days from sowing to heading (DHE) for each genotype. There were no variations among replications. The days required for heading were similar in the two years of study. The difference between the earliest (L1), and latest (L7) genotype for DHE was 10 days. However, results obtained by several authors show that the number of days to heading and yield were negatively correlated under stress conditions (Acevedo et al., 1991; Mitchell et al., 1996). According to Mitchell et al. (1996), variation in the number of days required to reach anthesis explains 48 - 72% of the difference in grain yield between barley genotypes. In pearl millet, this average was 57% under terminal water stress conditions (Bidinger et al., 1987). Sensitive genotypes responded with earlier heading, and therefore a shortened lifecycle to stress. Table 3 shows days to maturity (DMA) for the two years under irrigated and water stress conditions. The

Table 4. Linear regression coefficients for the osmotic potential (OP) and relative water content (RWC) in 12 barley genotypes grown under terminal water-stress condition.

Genotypes	OA (estimate)	RWC (%)	r†
L1	0.041 C	67.9 ABC	0.87**
L2	0.048 BC	65.4 ABC	0.86**
L3	0.042 C	49.1 D	0.87**
L4	0.055 B	70.6 A	0.70**
L5	0.047 BC	69.5 AB	0.82**
L6	0.063 A	72.04 A	0.95**
L7	0.047 BC	70.4 AB	0.54**
L8	0.043 C	56.02 CD	0.57**
L9	0.054 B	57.5 BCD	0.78**
L10	0.024 D	45.8 D	0.82**
L11	0.057 AB	64.4 ABC	0.83**
L12	0.048 BC	64.6 ABC	0.77**

OA, Osmotic adjustment; r, correlation coefficients.
Values followed by the same letter do not differ significantly ($P<0.05$).
†: ** Significant at $P < 0.01$.

L7 and L8 genotypes needed a longer time to reach heading and maturity than did the remaining genotypes. L1, L3, L4, L6, L9 and L12 took the least time to reach heading and maturity (Table 3). Early heading permits a long grain-filling period, during which photosynthetic components remain green, improving grain filling because the contribution to grain yield of post-anthesis assimilate is important in cereals.

Plant height

Plant height is directly linked to the productive potential of plant in terms of grain yield. In the present investigation, a significant reduction in plant height was noticed due to water stress. All varieties suffered strong depression (average of 14.6%) from the irrigated condition (Table 3). Gupta et al., (2001) and Muzammil (2003) observed substantial decline in plant height when irrigation was withheld at the booting stage; however, tolerant genotypes attained more plant height. Developmentally, potential stem storage as a sink is determined by stem length and stem weight density. Plant height (stem and spike length), as affected by the height genes significantly affects stem reserve storage. The Rht_1 and Rht_2 dwarfing genes of wheat reduced the reserve storage by 35% and 39%, respectively, because of a 21% reduction in stem length (Borrell et al., 1993). Stem size appears to play an important role in plant storage and the capacity of the grain to mobilize storage. Genotypes L1, L4, L11 and L10 showed the highest while L6, L5, L9 and L2 revealed medium plant height under water stress condition. There were no significant differences among the genotypes for PLH in irrigated conditions with the exception of L10, L12, L3 and L9 in that they had lower PLH (Table 3). Clarke et al., (1984) clearly demonstrated that simple relationships between stem reserve storage or remobilization and varietal drought resistance in terms of yield (such as by the “stress susceptibility index”) are not to be expected.

Relative water content and osmotic adjustment

Table 4 shows the mean RWC values for all 12 genotypes. Results show that, with respect to RWC, in the stress-treated L3, L8, L9 and L10 plants, significant differences were noted ($P < 0.05$), and were lower than other genotypes. The behaviour of the different genotypes with respect to RWC was similar in two years. Relative water content among genotypes differed from 45.8 to 72% for L10 and L6, respectively (Table 4). The RWC was demonstrated to be a relevant screening tool of drought tolerance in cereals, as well as a good indicator of plant water-status (Teulat et al., 2003) relative to their fully turgid condition. During the drought stress, relative growth rates were more reduced. The genotypes differed by the relative water content (Altinkut et al., 2001). Maintenance of relative water content and high osmotic adjustment contribute to the increased yield and yield stability under drought, in cereals (Clarke and McCiag, 1982). To estimate the osmotic adjustment of the different genotypes, the variation in OP was measured with respect to the RWC. During water stress, a significant linear correlation was found between these two variables for all genotypes (Table 4). The correlation coefficients (r) were high for all regression lines ($P<0.01$), indicating close relationship between OP and RWC, in all

Table 5. Correlation coefficients between grain yield (GY) and other traits under stress and non-stress conditions for 2005-2006 and 2006-2007.

Traits	Drought and irrigated trials	
	r (Stress)	r (Irrigated)
Plant height	0.003^{ns}	0.20^{ns}
Days to heading	-0.59*	0.01^{ns}
Days to maturity	-0.47^{ns}	0.20^{ns}
Grain per spike	0.29^{ns}	0.22^{ns}
1000-grain weight	0.47^{ns}	0.40^{ns}
Relative water content	0.52^{ns}	---
Osmotic adjustment	0.58*	---
Stay-green	0.4^{ns}	0.43^{ns}

*, P<0.05; **, P<0.01; ns, not significant at P<0.05.

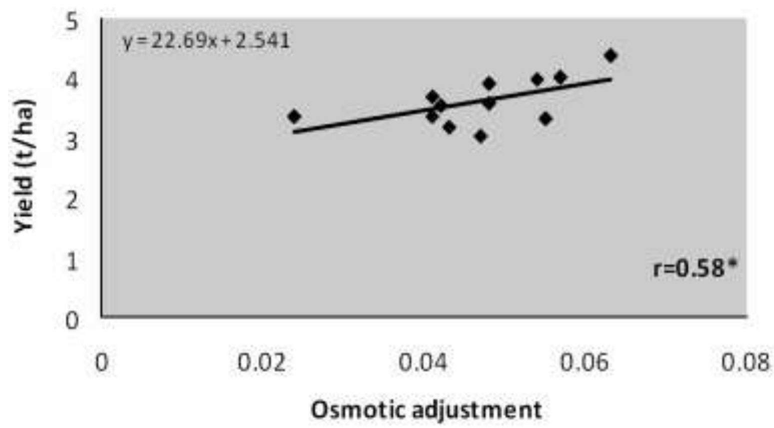

Figure 5. Correlation between yield and osmotic adjustment under water-stress conditions for the 12 barley genotypes in two years.

the genotypes studied. According to Morgan (1983), the RWC values recorded at an OP of -3 MPa can indicate an osmotic adjustment capacity, and the values recorded at this reference OP were notably different for the different genotypes (45.8–72.04; Table 4). The reference value itself was within the range of minimum OPs observed. Lines L6 and L4, followed by L7 and L5, indicate the highest RWC values at this OP. The overall mean RWC values at the reference OP of –3 MPa were 62.77%. This value can also be used as an indicator of osmotic adjustment capacity as it represents the variation in OP, with respect to RWC over the water stress period.

Table 5 shows the correlation coefficients for osmotic adjustment and yield, as well as of yield-related traits, for all the genotypes studied. Yield was significantly correlated with osmotic adjustment (P<0.05). Among the genotypes, L6 showed the greatest OA capacity followed by L11, while L10 had the smallest (Table 4). This indicates that the latter favours maintaining the turgor as water stress worsens, allowing cells to maintain their metabolic functions at low levels of hydration. The genotypes that reveal this trait are therefore those that are most droughts tolerant, as has been reported for wheat, chickpea, pea and cowpea (Sanchez et al., 1998; Hamidou et al., 2007; Gonzalez et al., 2007). In concordance with the results of the above-mentioned authors, the yield was highest in L6 and smallest in L10 (Table 3). The reduction in yield under stress compared with the watered controls was 22% for L6 and 38% for L5. These results are explained by the correlation between osmotic adjustment capacity and yield (P<0.05) (Figure 5). These results are to be expected, as water stress increases during the grain-filling period. Osmotic adjustment contributed to better grain filling, as shown by the positive correlation between yield and grain weight (r = 0.47); this of course led to better yields. Thus, L6 had the greatest osmotic adjustment capacities of all the genotypes studied as well as the highest grain weight and highest yield under water-stress conditions. The present results show wide variability in the osmotic

Table 6. grain yield status of barley lines in drought trail comparing to landrace variety (L11) at 2005-2006 and 2006-2007.

Lines	Yield (%) of genotypes comparing to yield of L11		Mean of 2 trials	Deference† to (L11)	Average reductions ‡
	Stress	Irrigated			
1	83.92	84.09	84.005	-15.995	1192
2	78.73	79.15	78.94	-21.06	1108
3	96.85	88.19	92.52	-7.48	1731
4	78.12	82.77	80.445	-19.555	930
5	99.53	92.31	95.92	-4.08	2255
6	109.25	103.48	106.325	6.325	1552
7	79.08	66.97	73.025	-26.975	1615
8	98.34	87.42	92.88	-7.12	1843
9	89.54	99.13	94.335	-5.665	898
10	89.54	101.65	95.595	-4.405	981
11	100.00	100.00	100	0	1429
12	94.87	97.10	95.985	-4.015	1266

† Deference of grain yields of 12 genotypes to landrace (L11) for 2 conditions.
‡ Average reduction of grain yield of 12 barley lines caused by drought stress (k/ha).

adjustment capacity of the 12 genotypes studied. Further, this is positively correlated with yield under terminal water-stress conditions. Therefore, genotypes with high capacity for osmotic adjustment should be selected for programmes designed to improve the drought tolerance of barley.

Stay-green

Stay-green (syn. 'non-senescence') is considered an important component in sustaining yield potential as well as to sustain yield under stress during grain filling (Sanchez et al., 2002). The average reduction in the stay-green period under stress conditions as compared with the irrigated condition was about 2.2 days (Table 3). Stay-green is an important factor in sustaining positive nitrogen balance in plants like wheat (Fokar et al., 1998), maize (Ma and Dwyer, 1998) and sorghum (Borrell and Hammer, 2000). However, at the same time, non-senescent genotypes retain more of their photosynthate in the leaves (Borrell and Hammer, 2000) and stems, while rapid leaf senescence may indicate reserve mobilization to the grain under stress (Yang et al., 2001). This appears to be linked with an accelerated export of nitrogen from the leaves (Pell and Dann, 1991). The total photosynthesis over the life of annual crops can be increased by extending the duration of active photosynthesis. Further, maintaining the supply of assimilated carbon to the grain during the grain-filling period of determinate crops ensures maximizing the mass per grain. Delaying leaf senescence (stay-green) is one of the methods to achieve this. Genetic variation exists in the timing and rate of leaf senescence, both between species and genotypes. The genotypes differed in stay-green; thus, L7, L6 and L8 took a longer time to reach leaf senescence under stress conditions (Table 3). Also in the well-watered condition, L6 and L8 had the longest stay-green period, more than the other genotypes (Table 3). Remobilization of nutrients from the leaves to the grain during senescence had a limited effect on grain status. The extended period of flag leaf photosynthetic competence is associated with the production of larger grains, presumably because of the increased carbohydrate content. By increasing the plant's capacity to photosynthesize and produce assimilates during the later phase of grain filling, and thereby delaying the onset of senescence the potential grain yield in plants can be enhanced.

Relationship between yield and other traits evaluated

A correlation was estimated between yield and traits evaluated, as well as yield and yield components (Table 5). As only a few genotypes were used in the trials and also because of the limited degree of freedom, some correlation coefficients were not significant, although they were quite high. Considering all the genotypes, in drought stress condition, the correlation between DHE and yield under stress conditions was strong ($r = -0.59^*$). This confirms the findings in barley by Gonzalez et al., (2007) and in other crops by Lopez-Castaneda and Richards (1994); the genotypes with the earliest ear emergence therefore, provide the highest yields when there is drought at the end of the growth cycle. The negative correlation between days to heading and yield may be due to the early lines, which have higher yield potential, or they may escape from terminal water stress. Therefore, genetic improvement of the drought tolerance in barley can identify and select the varieties that may not necessarily escape the stress, but can maintain normal metabolism, growth rate, and yield under serious stress situations. Registering the time of heading proved to be a useful indicator to characterize the genotypes. Negative correlation was observed between yield and days to maturity (r = -0.47). The association between precocity and yield under stress conditions was also observed in other cereals like wheat (Talbert et al., 2001) and triticale (Giunta et al., 1993). Under irrigated conditions, both correlations were weaker, thus indicating that precocity is advantageous under drought stress. Under drought stress conditions, TGW, G/S, RWC and SG were positively correlated with yield; however, these coefficients were not significant. In this study, the correlation coefficient between yield and number of grains per spike under stress (Table 5) was lower than that between yield and 1000-grain weight. These data show that the reduction in yield under terminal water stress conditions is mainly due to individual grain weight.

This is a logical deduction, as the effects of water stress were felt at quite an advanced stage of plant growth. Therefore, it mainly affected the number of secondary shoots. The inability of the secondary shoots to compensate for the effects of water stress could also possibly prevent the grain from filling adequately, and thus influence the reduction in yield as evident in bread wheat (Gonzalez et al., 2007). The compensatory effect between yield and yield components is therefore necessary to increase the yield and yield stability (Garcia del Moral et al., 1991; Mitchell et al., 1996). Considering the high correlation found between yields under irrigated and stress conditions (Table 5) it might be concluded that high potential yield, earliness, and high TGW are the most significant traits in the selection of barley plants to improve the yield under terminal water stress conditions. Plant height showed low positive correlation with yield, although it should have been higher (Table 5). In an earlier study, it was reported that under non-irrigated condition, yield showed a positive moderate correlation with culm diameter, and plant height (Okuyama et al., 2004). Subhani and Chowdhry (2000) reported that under drought stress conditions, grain yield was significant and positively correlated with flag leaf area, plant height and 1000-grain weight.

Conclusion

Yield parameters are the most important agronomical traits in selecting drought-tolerant genotypes. The depression in grain number and total grain yield was significantly smaller in tolerant genotypes. Grain yield, as its component, which was affected by stress, showed reduction in drought stress compared with none-stress condition. Generally, genotype L6 performed well in water stress condition as it attained a reasonable plant height, precocity, relative water content and stay-green, as well as gave higher grain yields and seed index as compared with other genotypes. Thus, we can conclude that a high osmotic adjustment capacity would also be beneficial as it helps maintain cells turgid when water stress increases during the grain-filling period. In addition to testing these traits by crossing suitable genotypes from these field trials, future work will aim to characterize these genotypes with molecular markers so that breeders can more easily identify the progeny that will carry the desired combinations of gene alleles for high-yield components.

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