Response of Barley Varieties to Drought Stress Imposed At Different Developmental Stages

Maisa’a F. Haddadin1,2, Adel H. Abdel-Ghani1,2, Nedal F. Al-Majali2

ABSTRACT

This study was carried out to examine the response of 14 barley varieties to drought imposed at different developmental stages (DDS). To examine the effect of drought stress (DS) on germination rate and seedlings growth, the seeds were exposed to induced osmotic potentials (OP; -0.75 and -1.2 Mpa), in addition to the control. Er/Apm variety did not show any significant decrease in germination rate in response to OP. The most drought tolerant varieties at seedling stage were Morocco9-75, Yarmouk, Acsad 176, and WI2291 with minimal biomass losses. Varieties were also tested against four DS treatments: continuous DS (Dcon), early DS (Dearly), late DS (Dlate) and a combination of Dearly and Dlate (Dearly+late) treatments. Grain yield in the control treatment (continuous irrigation) was almost two times greater than Dcon treatment, while the reductions in Dearly, Dlate and Dearly+late treatments were 12.9, 26.1, and 33.6% compared to the control treatment, respectively. WI2291 and Yarmouk showed the minimal grain yield losses in response to DS at DDS and their ranks in response to DS remained almost unchanged at DDS, and consequently they could be considered as a potential source of genes for drought tolerance.

Keywords: germination; seedling growth, grain yield; drought stress; barley.

Abbreviations: DDS, different developmental stages; DS, drought stress; DSI, a drought susceptibility index; GRI, germination rate index; OP, Osmotic potential; WANA, West Asia and North Africa

INTRODUCTION

Barley is a major crop of small scaled farmers in arid and semi-arid regions in West Asia and North Africa (WANA). In this region, both grain and straw are utilised as animal feed for small ruminants. In WANA, barley is mainly grown under rainfed conditions. It is the predominant crop below 300 mm rainfall in areas which are characterised by high inter- and intra-seasonal variation (Ceccarelli, 1987; Abdel-Ghani et al., 2004). Drought is often accompanied by relatively high temperature, which promotes evapotranspiration and hence accentuates its effects (Ceccarelli and Grando, 1996). Drought stress (DS) is a main abiotic stress that limits crop production (Forster, 2004; Alqudah et al., 2011). The grain yield achieved by farmers in WANA is low (about 1000 kg ha⁻¹) with large variability from year to year, ranging from 320 to 2300 kg ha⁻¹ (FAO, 1989-2000).

Drought tolerance is a complex trait involving several interacting phenol-morpho-physiological mechanisms for drought escape, drought (dehydration) avoidance and drought (dehydration) tolerance (Levitt et al., 1972; Turner, 1979). Grain yield and biological yield are the most important and most complex agronomic trait to be...
considered for selection under stress environment. In accordance, yield potential is defined as the yield of a genotype when grown in environments with non-limiting levels of water and nutrients (Evans and Fischer, 1999; van Ginkel et al., 1998). As such, it is distinguished from potential yield, which defined as the maximum yield that could be reached by a crop in the stress environment (Evans et al., 1999). Two selection philosophies were used to assess the level of drought tolerance. One is to select genotypes with high yield potential in optimum condition (water non-limiting environment), since genotypes with high yield potential under optimum condition had high potential yield under stressed environment (van Ginkel et al., 1998; Evans et al., 1999), the other is to select genotypes with minimal yield losses under DS environment compared to non-limiting water environment (Ceccarelli et al., 1992; Van Oosterom et al., 1993). Good performance of a genotype over a range of environments will be useful in developing a stable variety (Abbasi et al., 2003). Yield stability is a measure of variation between potential and actual yield of a genotype across changing environments (Brukner and Frohberg, 1987). A drought susceptibility index (DSI), which provides a measure of drought stress tolerance based on minimization of yield loss under stress, as compared to optimum condition, rather than on yield stress per se which has been used to characterize relative drought tolerance (Fisher and Maurer, 1978; Ceccarelli et al., 1987). Its values are used for differentiating the overall stress tolerance of genotypes (Brukner and Frohberg, 1987). The DSI index was highly negatively correlated with grain yield, indicating that larger yields were associated with higher levels of drought tolerance or with high stability under drought conditions (Ceccarelli et al., 1987; Shakhatreh et al., 2001). Genotypes identified as stress tolerance using DSI values should possess tolerant mechanism which are needed to improve high yielding varieties under DS (Bruckner and Frohberg, 1987).

Indirect selection for morphological traits usually associated with drought tolerance could be led to considerable improvement in yield (Ceccarelli et al., 2004). The traits more consistently associated with higher grain yield in environments with prevailing drought conditions are early growth vigor, earliness, tall plants, long peduncle and a short-grain filling duration (Acevedo and Ceccarelli, 1989). Other traits related to drought tolerance include germination percentage, germination rate, seedling root and shoot attributes (Kpoghomou et al., 1990; Grando and Ceccarelli, 1995; Al-Karaki et al., 2007; Sahnoune et al., 2004; Liu et al., 2007), physiological/biochemical traits such as proline content, stomatal conductance, relative water content, transpiration efficiency, water-use efficiency, retranslocation (Ceccarelli et al., 2004), water potential and gross photosynthetic rate (Samarah et al., 2009).

Drought tolerance is a highly stage-specific trait and changes during the life cycle (Szira et al., 2008). It has been found that quantitative trait loci (QTLs) that are linked to tolerance at one stage of plant development can differ from those linked to tolerance at other stages (Foolad, 1999). According to Shpiler and Blum (1991), the most sensitive growth period under DS, regarding to grain yield, is from double ridge to anthesis due to its negative impact on spikelet number and kernels spike^{-1}. In addition, DS from anthesis to maturity, especially if accompanied with high temperatures hastens leaf senescence, reduces the duration and rate of grain filling, and hence reduces mean kernel weight (Royo et al., 2000). Drought tolerance at germination is easy to measure, but the reports on the relationship between drought tolerance at germination and that of the seedling or the adult plant stages is conflicting (Foolad, 1999; Szira et al., 2008). However, a difference in the drought tolerance among barley genotypes may also occur at different growth stages (Shakhatreh et al., 2001; Abbasi et al., 2003; Szira et al., 2008; Samarah et al., 2009).
Varietal differences for yield under drought were reported in several studies in both pot (Ivandic et al., 2000; Abbasi et al., 2003; Li et al., 2006; Samarah et al., 2009) and field (Ceccarelli, 1987; Shakhatreh et al., 2001; Samarah et al., 2009) experiments, indicating the presence of potentially useful genetic variation for drought tolerance. Future climate changes are expected to increase risks of drought, which already represent the most common stress factor for stable barley production in WANA region (Rizza et al., 2004). Because water resources for irrigating crops are declining worldwide, the development of more drought-tolerant cultivars with high yield and water-use efficiency is a global concern (Ludlow and Muchow, 1990; Alqudah et al., 2011). In a typical Mediterranean environment, years with ample water availability during the main cereal-growing season alternate with years in which terminal drought occurs during grain filling as well as years with early drought during seedling development, vegetative growth and flowering (Ceccarelli and Grando, 1996; Ceccarelli et al. 2004). Breeding strategy for drought-prone environments has to consider the timing and intensity of the DS events that vary significantly from year to year. In the current study, fourteen barely varieties were screened in response to DS stress imposed at different developmental stages to achieve the following objectives: (i) to screen the most tolerant varities to set recommendations on their possible use in drought tolerance breeding programs and (ii) to identify varities could tolerate drought at different developmental stages.

MATERIALS AND METHODS

Plant material

Fourteen barley varieties were used in this study as follows: a) five Jordanian registered barley varieties: Rum, Acsad 176, Athroh, Yarmook and Muta’ obtained from the National Center for Agricultural Research and Extension (NCARE), Baqa, Jordan, b) one Jordanian barley landrace (Arabi Abiad) collected from a local farmer at Rabba, Karak district (31° 16' N, 35° 45' E and ca 920 meters above sea level) and c) eight ICARDA’s improved varieties (Tadmor, Arta, Morocco 9-75, WI2291, Zanbaka, Harmel, Furat2 and ER/Apm) kindly presented by Dr Stefania Grando, ICARDA. Rum, Acsad 176 and Athroh are six-rows barley, while the other varieties are from two-rows. Names, pedigree, and some characteristics of the tested barley varieties used in this study are presented in Table 1.

Germination and seeding test

This experiment includes 14 barley varieties and three DS levels (control, mild DS and severe DS). The experimental design was a complete randomized design (CRD) with five replicates arranged in split-plot. Drought stress levels were the main plots and barley varieties were the sub-plots. Seeds were first surface sterilized with Clorox® solution (6% sodium hypochlorite) for 15 minutes then washed two times with distilled water. Thereafter, ten sterilized seeds were placed on two layers of whatman filter paper in 25 cm petri dishes. Osmotic stress was induced using PEG-6000 (Michael and Kaufman 1973). PEG-6000 with half strength of Murashige and Skoog medium (MS) solution was added at concentrations of 15 % (w/v) (mild stress) and 18 % (w/v) (severe stress) resulting in an osmotic potential (OP) of -0.75 and -1.20 MPa, respectively (Molnar et al., 2004), while distilled water with half strength of MS medium solution was added to unstressed seeds (control). The petridishes were place in a growth chamber for 10 days under a photoperiod of 16/8 h (light/darkness) at 20/16 ºC with photosynthetically active radiation of 200 µmol photons m⁻² s⁻¹ (Wheeler and Sager, 2006). The relative humidity in the growth chamber was maintained at 70%. Counts of germinated...
seeds were made daily during the course of the experiment to determine the final germination percentage and to estimate the germination rate index (GRI). Seeds were considered germinated when the radical reached at least 2 mm in length (ISTA, 1999). Final germination percentage was calculated from the data collected at the 7th day of counting from the total number of seeds germinated divided by the total number of seeds used. GRI was calculated using Maguire’s equation (Maguire, 1962):

\[
GRI = \frac{\sum \text{number of germinated seeds at days } x}{\text{Days } x}
\]

Where \( n \) is the number of germinated seeds at the day number \( x \).

Data for root length, shoot length, coleoptile length, shoot fresh weight, root fresh weight and seminal root number were obtained from five seedlings in each replication from 10-days old seedlings.

**Pot experiment**

The experiment was conducted at Rabba agricultural research station (Elevation 920 m, longitude 35° 45’ and Latitude 31° 16’) belongs to NCARE, in an open greenhouse used as rainout shelter house in winter and for protection from bird attacks at grain filling period. Plastic pots, 26 cm in diameter and 40 cm deep were filled with 8 kg air-dried soil taken from a field at Ghweer agricultural research station (arid, 31° 14' N, 35° 45' E and ca 820 meters above sea level) which left fallow for many years. The soil used in the pot experiment contained 21.3% sand, 52.8% silt, 25.9% clay, alkaline pH of (7.8), 1.07% organic matter, 0.058% total nitrogen (N), 3.7 mg kg⁻¹ available (Olsen) phosphorus (P), 21.9% calcium carbonate, 386.2 mg kg⁻¹ a available potassium cation exchange capacity of 45 meq 100 g⁻¹, and electrical conductivity (1 : 1) of 0.4 dS m⁻¹.

On January 1st 2009 barley seeds were sown at the rate of 7 seeds per pot. Diammonium phosphate (DAP) was applied at a rate 0.4 g per pot (equivalent to 100 kg ha⁻¹) at planting date. The available soil moisture was determined as the difference between the soil water content at field capacity and the permanent wilting point (PWP at -15 bar). Field capacity was estimated as the difference obtained between soil saturated with water (soil weight after drainage was stopped at - 0.33 bar) and oven dry weight of saturated samples after soil being dried at 105 °C for 24 h. The permanent wilting point was estimated by subtracting oven dry weight from soil samples subjected to -15 bar. Soil moisture content was measured using the gravimetric method. Soil samples were weighed before and after oven drying at 105 °C for 24 h and the weights divided by the weight of the oven-dry soil.

All pots was irrigated to 90% of available water from seeding date until emergence and then seedlings were thinned to five healthy plants per pot when the second leaf expanded. The following stress treatments were performed to simulate the type of drought generally encountered in WANA region following BBCH-scale (Witzenberger et al., 1989; Lancashire et al., 1991):

- **Control** = continuous irrigation at 90% available water.
- **Dcon** = continuous DS or sustain deficit irrigation 30% available water.
- **Dearly** = early DS or early stage deficit irrigation at 30% available water from seedling (BBCH-scale=12) until tillering (BBCH-scale=25 )
- **Dlate** = late DS or late stages deficit irrigation at 30 % available water from the late boot stage (BBC-scale=45) until ripening (BBC-scale=89).
- **Dearly+late** = a combination of Dearly and Dlate.

Pots were irrigated once every other day and were kept free of weeds by hand weeding. The moisture statuses were determined gravimetrically by weighing and watering the pots. To correct for the fresh weight increment of the plants during the experiment, the difference due to plants’ weight in the pots was estimated based on the results previously
presented by Noggle and Fritz (1976). Irrigation continued every other day as required by the treatments until April 27th (BBC-scale=92), after that, irrigation withholds on all pots until May 15th. At this date, plants in each pot were hand harvested and consequently threshed to separate seeds from the spikes. The experimental design was Randomized Complete Block Design (RCBD) with three replicates arranged randomly in split plot, where the main plots were the DS treatments, and the sub plots were the varieties.

Data were collected for the following seven agro-morphological and phenological traits: grain yield (g plant⁻¹), biological yield or above ground biomass (g plant⁻¹), straw yield (g plant⁻¹), number of spikes plant⁻¹, hundred kernel weight (g), days to heading from sowing until 50% of spikes fully emerged from flag leave and days to maturity from sowing until 50% of peduncles turned yellow. Moreover, two additional traits were also derived from the previous data: number of kernels spike⁻¹ and grain filling period (days).

**Drought susceptibly index (DSI)**

A drought susceptibly index (DSI) for grain yield and other collected traits was calculated using the following formula (Fischer and Maurer, 1978):

\[
DSI = \frac{1 - \frac{Y_D}{Y_P}}{1 - \frac{X_D}{X_P}}
\]

Where, \(Y_D\) is the mean quantitative value of individual genotype under DS treatment, \(Y_P\) is the mean quantitative value of individual genotype under control treatment, \(X_D\) is the mean quantitative value of all varieties under DS treatment and \(X_P\) is the mean quantitative value of all varieties under control treatment.

**Statistical analysis**

Analysis of variance (ANOVA) was used to test variety (V) and DS treatment (T) effects and their interaction (V × T). Data was analyzed by two ways ANOVA using the statistical package MSTAT-C (Michigan State University, East Lansing, MI, and USA) statistical software. The differences between the means were compared using least significant differences at (LSD) \(P \leq 0.05\).

<table>
<thead>
<tr>
<th>Name</th>
<th>Row type</th>
<th>Pedigree/source</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rum</td>
<td>6</td>
<td>Harbin-Arivat*Attiki CYB 19-1A-0A-0A-0A</td>
<td>An old released Jordanian cultivar.</td>
</tr>
<tr>
<td>Acsad 176</td>
<td>6</td>
<td>(CN872-3Y-1B-2Y-1BXY-OB)*(Cr.366/16/2)</td>
<td>An old released Jordanian cultivar.</td>
</tr>
<tr>
<td>Athroh</td>
<td>6</td>
<td>Kathraia</td>
<td>A new released Jordanian cultivar.</td>
</tr>
<tr>
<td>Yarmouk</td>
<td>2</td>
<td>ESP/808 Harmal</td>
<td>A new released Jordanian cultivar.</td>
</tr>
<tr>
<td>Muta'</td>
<td>2</td>
<td>Roho-A. Abiad-6250</td>
<td>A new released Jordanian cultivar.</td>
</tr>
<tr>
<td>Tadmor</td>
<td>2</td>
<td>A pure line selection from the Syrian black-seeded landrace Arabi Aswad, adapted to the driest part of the country.</td>
<td>A black seeded, with prostrate growth habit, lodging susceptible, well adapted to dry conditions</td>
</tr>
<tr>
<td>Arta</td>
<td>2</td>
<td>A pure line selected from the Syrian landrace Arabi Abiad. It was released in Syria in 1993, with the name of &quot;Improved Arabi Abiad&quot;.</td>
<td>A high yielding white seeded. Well adapted to Syrian conditions, and combines high number of tillers and high kernel weight, and high yield stability, but becomes very short under dry conditions.</td>
</tr>
<tr>
<td>Morocco 9-75</td>
<td>2</td>
<td>-</td>
<td>A white-seeded line, with long head and large kernels</td>
</tr>
<tr>
<td>Name</td>
<td>Row type</td>
<td>Pedigree/source</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>WI2291</td>
<td>2</td>
<td>A breeding line produced at the Waite Institute in South Australia from the cross CI3576/Union*2.</td>
<td>A white-seeded cultivar. Well adapted to the dry areas of Syria. Susceptible to scald.</td>
</tr>
<tr>
<td>Zambaka</td>
<td>2</td>
<td>Derives from a single head collected from Arabi Aswad in a village in the Hassakeh province.</td>
<td>A black-seeded line. Long even in dry years and in dry environments.</td>
</tr>
<tr>
<td>Harmel</td>
<td>2</td>
<td>A white-seeded improved ICARDA breeding line deriving from the cross Union/CI03576/Coho.</td>
<td>Early maturing spring barley with short culms and large kernels, Well adapted to low rainfall conditions (250-375 mm) and characterized by high yield stability.</td>
</tr>
<tr>
<td>Furat2</td>
<td>2</td>
<td>Produced at the Waite Institute in South Australia (WI 2355) and released in Syria with the name of Furat 2.</td>
<td>A white-seeded variety. Adapted to moderate rainfall areas.</td>
</tr>
<tr>
<td>ER/Apm</td>
<td>2</td>
<td>A breeding line with a yield potential of about 6 t/ha in favourable conditions.</td>
<td>Short, lodging resistant and shows a particularly good adaptation to North Africa where it has been released in Morocco, Tunisia and Libya with the names of Aglou, Faiz and Iraween.</td>
</tr>
<tr>
<td>Local landrace</td>
<td>2</td>
<td>Old variety</td>
<td>Adapted to harsh environmental condition, tolerate powdery mildew</td>
</tr>
</tbody>
</table>

Figure 1. Germination of 14 barley genotype seeds as a function of time (i.e, germination rate) by increasing polyethylene glycol percentage (PEG%). 15 % PEG (mild stress) and 18 % PEG% (severe stress) resulting in an osmotic potential of -0.75 and -1.2 MPa, respectively.
RESULTS

Effect of PEG on germination and germination rate

The varieties and OP levels were significantly (P = 0.01 and 0.05 respectively) differed for final germination percentage with non-significant V × T interaction (data is not shown). The interactive effect of varieties and OP levels on germination rate was highly significant (P = 0.01, Fig. 1). Er/Apm variety did not show any significant decrease in germination rate over the OP treatments compared to their respective control. Rum, Acsad 176, Athroh, Yarmouk, Muta’, Harmel, Furat 2 and ER/Apm showed a slight reduction in germination rate at mild and severe OP; reductions ranged from 1.06 to 12.77% day\(^{-1}\) and from 8.6 to 29.79% day\(^{-1}\) at mild and severe OP, respectively. Germination rate in Morocco 9-75 and W12291 (reductions = 57.45 and 55.91% day\(^{-1}\), respectively) was dramatically reduced at severe OP, with slight effect of mild OP (reductions = 5.32 and 2.15% day\(^{-1}\), respectively). However, Tadmor, Arta, Zanbaka, and local barley landrace showed high significant (P = 0.01) reductions in germination rate at the two OP levels.

Effect of PEG on seedling growth and drought susceptibility index estimates for seedling traits

Mild OP reduced seedling related traits to a lesser extent than sever osmotic stress (data is not shown). Root-to-shoot length and root-to shoot fresh weight ratios increased by 17.5 and 60.0% under mild OP and 45 and 70% under sever OP compared to the control treatment respectively. The interactive effects of varieties and OP levels were highly significant (P = 0.01) on coleoptile length, shoot, and root length as well as on shoot and root fresh weight. DSI values calculated under mild and severe DS for 10 days seedlings are presented in Table 2. The mean DSI values based on seedling traits ranged from 0.33 to 1.67 and from 0.54 to 1.40 under mild and severe OP, respectively. The most tolerant varieties were Morocco9-75, Yarmouk, Acsad 176, and W12291 with DSI value of 0.33 and 0.57 in Morocco 9-75, 0.45 and 0.69 in Yarmouk, and 0.59 and 0.70 in W12291 under mild and sever OP, respectively. However, the least tolerant varieties were Harmel, Zanbaka and local landrace genotype with DSI values of 1.67, 1.21 and 1.21 under mild OP and 1.40, 1.28 and 1.20 under severe OP, respectively. The other varieties exhibited intermediate drought tolerance at seedling stage. Based on the mean DSI value under mild and severe OP for the five seedling characteristics, varieties could be ordered according to drought tolerance as follows: Morocco9-75 (0.45), Yarmouk (0.57), Acsad 176 (0.61), W12291 (0.70), Rum (0.80), ER/Apm (0.91), Athroh (1.02), Arta (1.07), Tadmor (1.08), Furat2 (1.10), Muta’ (1.13), local landrace (1.21), Zanbaka (1.25) and Harmel (1.54).

Table 2 Drought susceptibility index (DSI) based on seedling shoot and root attributes of the 14 barley varieties under mild and severe osmotic potentials

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mild osmotic potential (OP1(^{+}))</th>
<th>Drought susceptibility index</th>
<th>Severe osmotic potential (OP2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coleoptile length (mm)</td>
<td>Shoot length (mm)</td>
<td>Root length (mm)</td>
</tr>
<tr>
<td>Rum</td>
<td>1.40</td>
<td>0.99</td>
<td>1.63</td>
</tr>
<tr>
<td>Acsad 176</td>
<td>1.27</td>
<td>0.70</td>
<td>1.19</td>
</tr>
<tr>
<td>Athroh</td>
<td>1.69</td>
<td>1.40</td>
<td>0.88</td>
</tr>
<tr>
<td>Yarmouk</td>
<td>0.52</td>
<td>0.45</td>
<td>0.79</td>
</tr>
</tbody>
</table>
Effect of drought stress treatments on grain yield and yield contributing traits

The results showed a wide range of variation for all traits recorded in this study (data is not shown). For example, the average grain yield ranged from 1.79 g plant$^{-1}$ in Zanbaka to 3.05 g plant$^{-1}$ in Athroh. All varieties headed within 20 days starting 73 days after sowing, they reached maturity within 108 to 123 days and grain-filling period ranged from 27.6 to 31.4 days. In general, varieties earlier in heading were also early in maturity with somewhat longer grain-filling period. The earliest-headed variety was Athroh (73.7 days), while the latest headed varieties were Tadmor and Arta (92-93 days). Grain yield and its components (100-kernel weight, number of spike plant$^{-1}$ and number of kernels spike$^{-1}$) significantly ($P = 0.01$) decreased in response to DS treatments (data is not shown).

The ranking of grain yield and their components among DS treatments showed that control ranked first followed by Dearly, Dlate, Dearly+late and Dcon. The average yield at control was almost two times greater than at Dcon, while the reductions in Dearly, Dlate and Dearly+late treatments were 12.9, 26.1, and 33.6% less than control treatment, respectively. Dearly had a simulative effect on biological yield and straw yield. The average biological yield and straw yield plant$^{-1}$ increased by 4.1% and 20.9% under Dearly respectively, while average reductions of about 43.3 and 41.5%, 19.8 and 13.6% and 24.4 and 15.3% were recorded under Dcon, Dlate and Dearly+late, respectively, compared to control treatment.

In control and DS treatments, grain yield plant$^{-1}$ varied substantially among the 14 barley varieties tested in the pot experiment (Fig. 2), and all varieties managed to continue growing throughout the life cycle using the available water. DS treatments induced yield losses in all varieties except for Dearly treatment. The lowest effect of Dcon treatment on grain yield was observed in WI2291, Yarmouk, Zanbaka and the local barley landrace (reductions = 20.9, 35.6, 38.7 and 38.0%, respectively). However, Dcon caused severe grain yield losses in Rum, Acsad 176, Morocco 9-75 and Harmel (reductions = 60.7, 50.8, 50.6 and 53.8%, respectively). Dlate caused maximal grain yield losses in Tadmor and Arta (reductions = 40.2 and 44.7%, respectively), followed by Athroh (35.9%),

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mild osmotic potential (OP1)</th>
<th>Severe osmotic potential (OP2)</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coleoptile length (mm)</td>
<td>Shoot length (mm)</td>
<td>Root length (mm)</td>
</tr>
<tr>
<td>Mutat</td>
<td>1.66 0.36 1.55 1.35 0.91 1.17</td>
<td>1.37 0.79 1.55 0.83 0.91 1.09</td>
<td>1.13</td>
</tr>
<tr>
<td>Tadmor</td>
<td>0.01 1.52 1.01 1.21 1.60</td>
<td>1.07 0.49 1.30 1.01 1.02 1.60</td>
<td>1.08</td>
</tr>
<tr>
<td>Arta</td>
<td>0.16 0.30 1.48 1.34 1.65</td>
<td>1.07 0.37 0.88 1.48 0.91 1.06</td>
<td>1.07</td>
</tr>
<tr>
<td>Morocco 9-75</td>
<td>0.44 1.00 -0.27 0.16 0.31</td>
<td>0.33 0.54 1.12 -0.27 1.17 0.31</td>
<td>0.57</td>
</tr>
<tr>
<td>WI2291</td>
<td>0.16 0.75 0.65 0.16 1.72</td>
<td>0.59 0.16 1.11 0.65 0.98 1.21</td>
<td>0.81</td>
</tr>
<tr>
<td>Zanbaka</td>
<td>0.14 1.91 0.39 1.56 1.82</td>
<td>1.21 1.12 1.15 0.89 1.24 1.82</td>
<td>1.28</td>
</tr>
<tr>
<td>Harmel</td>
<td>2.16 1.16 1.35 1.81 1.85</td>
<td>1.67 1.51 1.25 1.35 1.05 1.85</td>
<td>1.40</td>
</tr>
<tr>
<td>Furat2</td>
<td>2.04 1.72 0.71 1.16 0.13</td>
<td>1.15 1.56 1.39 0.71 1.10 0.13</td>
<td>1.04</td>
</tr>
<tr>
<td>ER/Apm</td>
<td>1.94 0.53 0.24 0.75 1.10</td>
<td>0.91 1.55 0.95 0.24 0.72 1.10</td>
<td>0.91</td>
</tr>
<tr>
<td>Local landrace</td>
<td>0.22 0.57 1.54 2.15 1.55</td>
<td>1.21 0.76 0.97 1.54 1.19 1.55</td>
<td>1.20</td>
</tr>
</tbody>
</table>

$^{+}$OP1, 15 % PEG (w/v) (mild stress, -0.75 MPa); OP2, 18 % (w/v) (severe stress, -1.2 Mpa).
while WI2291 showed the minimal grain yield loss (7.98%) followed by Acsad 176 (12.1%) and Yarmouk (12.5%). The maximal losses in grain yield in response to Dearly+late treatment were recorded in Athroh, Arta, local landrace and Muta’ (reductions = 51.1, 46.2, 41.6 and 40.5% compared to their respective controls), while the minimal losses were recognized in Acsad 176 and Harmel (reductions= 15.1 and 14.3%, respectively). With Dearly, grain yield of Yarmouk, Tadmor, Morocco 9-75, Zanbaka and Furat2 were almost unchanged, while grain yield in WI2291 was slightly increased (8.37%), although non-significant (Fig. 2). The maximal grain yield losses in response to Dearly treatment was obtained in Athroh (39.1%), while the other seven varieties exhibited moderate yield losses (range=14.7 to 19.7%).

Similar results were obtained with yield contributing traits. The effect of DS treatment on number of spikes plant\(^{-1}\) and number of kernels spike\(^{-1}\), which both initiate during early growth stages, had greater influence on final grain yield than 100-kernel weight (data is not shown). 100-kernel weight under Dcon, Dearly, Dlate and Dearly+late was significantly (\(P = 0.01\)) reduced by 9.4, 1.5, 6.2 and 7.9%, respectively. The reductions were about 22.7, 4.5, 15.9, and 13.6% for the number of spikes plant\(^{-1}\) and 22.3, 3.80, 9.2 and 14.7% for the number of kernels spike\(^{-1}\) under Dcon, Dearly, Dlate and Dearly+late compared to their respective controls, respectively.

**Drought susceptibility index for grain yield and yield contributing traits**

The mean DSI values based on grain yield ranged from 0.46 to 1.34, from -0.65 to 3.03, from 0.31 to 1.71 and from 0.43 to 1.38 in response to Dcon, Dearly, Dlate and Dearly+late, respectively (Table 3). The most tolerant varieties under different DS treatments were WI2291 and Yarmouk varieties. DSI values under Dcon, Dearly, Dlate and Dearly+late were 0.46, -0.65, 0.31 and 0.75 in WI2291 and 0.79, -0.03, 0.48 and 0.96 in Yarmouk, respectively. However, the least tolerant varieties were Athroh followed by Arta and Rum with DSI values ranging from 1.04 to 1.34, from 1.23 to 3.03, from 1.07 to 1.71 and from 1.09 to 1.52 under Dcon, Dearly, Dlate and Dearly+late, respectively. Zanbaka and Furat2 varieties were more drought tolerant at early stages of plant development (DSI at Dearly= -0.07 and -0.06, respectively) than at flowering and grain filling stages (DSI at Dlate= 0.95 and 0.82, respectively). Acsad 176 was more drought tolerant during late boot and grain filling (DSI= 0.46 and 0.45 at Dlate and Dearly+late respectively) compared to other varieties. Depending on the mean DSI values based on grain yield, varieties could be ordered according to drought tolerance as follow: WI2291 (0.22), Yarmouk (0.55), Furat2 (0.61), Zanbaka (0.62), Morocco9-75 (0.79), Acsad176 (0.89), Harmel (0.90), Tadmor (0.98), local landrace (1.0), ER/Apm (1.11), Muta’ (1.12), Rum (1.23), Arta (1.34) and Athroh (1.75).

Minimal DSI values were also recorded for yield components in most cases in the least susceptible varieties (Yarmouk and WI2291), while high DSI values were recorded in the least tolerant ones (Table 3). DSI values for 100- kernel weight were 1.09, -0.29, 0.58 and 0.91 for Yarmouk variety and 0.53, -0.10, 1.16 and 1.28 for WI2291 under Dcon, Dearly, Dlate and Dearly+late, respectively, while DSI values for number of spikes plant\(^{-1}\) were 0.34, 0, 0.50 and 0.50 for Yarmouk and 0.34, 0, 0 and 0.5 for WI2291 under Dcon, Dearly, Dlate and Dearly+late, respectively. Minimal DSI values based on the number of kernels spike\(^{-1}\) were also recorded in the least susceptible varieties, DSI values were 0.72, 0.05, 0.07 and 0.59 for Yarmouk and 0.51, -1.31, 0.53 and 0.30 for WI2291 under Dcon, Dearly, Dlate and Dearly+late, respectively. Minimal DSI values for yield components were observed in the least tolerant varieties; for example DSI values for Rum ranged from 1.02 to 3.17 and from 1.29 to 2.01 and from -0.94 to 1.52 under different DS treatments for 100-kernel weight, number of spikes plant\(^{-1}\) and number of
kernels spike\(^{-1}\), respectively.

**Yield potential and potential yield**

Grain yield of varieties ranged from 2.17 to 4.68, from 1.23 to 2.41, from 2.19 to 3.1, from 1.63 to 3.0 and from 1.64 to 2.59 under control, Dcon, Dearly, Dlate and Dearly+late treatments, respectively (Fig. 2). Among the 14 varieties, Athroh followed by Rum, ER/Apm, local landrace and Acsad 176 had the highest yield potentials (yield = 4.68, 3.79, 3.05, 3.53 and 3.13 g plant\(^{-1}\), respectively) and at the same time they found to be the top yielding varieties under DS treatments. Athroh variety potential yield was 2.41, 2.85, 3.0 and 2.29 g plant\(^{-1}\) in Dcon, Dearly, Dlate and Dearly+late, respectively, although it had highest yield losses in response to DS treatments compared to its respective control (reductions= 48.50, 39.1, 35.9 and 51.07 % in Dcon, Dearly, Dlate and Dearly+late, respectively). Rum was also from the highest yielding varieties in Dearly, Dlate and Dearly+late (3.10, 2.73 and 2.41 g plant\(^{-1}\)); it showed low potential yield in Dcon (1.49 g plant\(^{-1}\)) but high yield losses in response to DS treatments (reductions were 60.69, 18.21, 27.97 and 36.41% in Dcon, Dearly, Dlate and Dearly+late, respectively). With the same trend, Acsad 176, ER/Apm and local landrace had moderate to high yield potentials in control and exhibited moderate to high potential yields under stress treatments. However, WI2291 and Zanbaka which showed low DSI values, showed low yield potential (2.63 and 2.17g plant\(^{-1}\)) under continuous irrigation but they exhibited the minimal losses in grain yield under DS treatments; the reductions were 20.91, -8.37, 7.98 and 25.1% in WI2291 and 38.71, -0.92, 24.88 and 24.42% in Zanbaka under Dcon, Dearly, Dlate and Dearly+late, respectively.

![Figure 2](image-url)
Table 3 Drought susceptibility index (DSI) for the yield and yield components of the fourteen genotypes under different drought stress treatments GY, grain yield; HKW, hundred kernel weight; SN, fertile spikes plant-1; KS, number of kernels spike-1

<table>
<thead>
<tr>
<th>Variety</th>
<th>Continuous drought stress (Dcon)</th>
<th>Early drought stress (Dearly)</th>
<th>Late drought stress (Dlate)</th>
<th>Combination of early and late drought stress (Dearly+late)</th>
<th>Mean based on GY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GY</td>
<td>HKW</td>
<td>SN</td>
<td>KS</td>
<td>GY</td>
</tr>
<tr>
<td>Rum</td>
<td>1.34</td>
<td>1.02</td>
<td>1.46</td>
<td>1.52</td>
<td>1.41</td>
</tr>
<tr>
<td>Acsad 176</td>
<td>1.13</td>
<td>1.59</td>
<td>0.94</td>
<td>1.02</td>
<td>1.52</td>
</tr>
<tr>
<td>Athroh</td>
<td>1.07</td>
<td>0.36</td>
<td>0.87</td>
<td>1.73</td>
<td>3.03</td>
</tr>
<tr>
<td>Yarmouk</td>
<td>0.79</td>
<td>1.09</td>
<td>0.34</td>
<td>0.72</td>
<td>-0.03</td>
</tr>
<tr>
<td>Muta'</td>
<td>0.92</td>
<td>0.69</td>
<td>1.36</td>
<td>0.38</td>
<td>1.23</td>
</tr>
<tr>
<td>Tadmor</td>
<td>0.98</td>
<td>1.03</td>
<td>0.34</td>
<td>1.39</td>
<td>-0.29</td>
</tr>
<tr>
<td>Arta</td>
<td>1.04</td>
<td>1.41</td>
<td>0.94</td>
<td>1.00</td>
<td>1.23</td>
</tr>
<tr>
<td>Morocco 9-75</td>
<td>1.12</td>
<td>0.81</td>
<td>1.36</td>
<td>1.23</td>
<td>0.22</td>
</tr>
<tr>
<td>WI2291</td>
<td>0.46</td>
<td>0.53</td>
<td>0.34</td>
<td>0.51</td>
<td>-0.65</td>
</tr>
<tr>
<td>Zambaka</td>
<td>0.86</td>
<td>1.84</td>
<td>1.02</td>
<td>0.71</td>
<td>-0.07</td>
</tr>
<tr>
<td>Harmel</td>
<td>1.19</td>
<td>0.82</td>
<td>1.46</td>
<td>0.73</td>
<td>1.17</td>
</tr>
<tr>
<td>Furat2</td>
<td>0.99</td>
<td>1.15</td>
<td>0.94</td>
<td>0.75</td>
<td>-0.06</td>
</tr>
<tr>
<td>ER/Apm</td>
<td>1.04</td>
<td>1.26</td>
<td>1.08</td>
<td>1.24</td>
<td>1.21</td>
</tr>
<tr>
<td>Local landrace</td>
<td>0.84</td>
<td>0.36</td>
<td>1.17</td>
<td>0.38</td>
<td>1.14</td>
</tr>
</tbody>
</table>

DISCUSSION

Osmotic stress treatments slightly reduced final germination percentage but delayed seed germination with considerable genetic variation among tested varieties. PEG-induced drought affects seed germination by decreasing the ease at which the seeds take up water, and consequently germination get either delayed or proceed at a reduced rate (e.g. Murillo-Amador et al., 2002; Dhanda et al., 2004; Al-Karaki et al., 2007; Lobato et al., 2008). High germination percentage and high germination rate under DS are essential requirements for vigorous stand establishment at early stages of seedlings development (Al-Karaki et al., 2007; Abdel-Ghani, 2008). From this perspective, ER/Apm genotype exhibited the highest germination rate, which could be advantageous over other varieties for drought adaptation at least at this stage of plant cycle.

Seedlings root and shoot attributes were significantly reduced by decreasing OP, except for the seminal root number which remained unchanged. These results were in agreement with Dhanda et al. (2004) and Sahnoune et al. (2004), who reported significant \( P = 0.01 \) reductions in seedling attributes by imposing osmolyte DS at early growth stage of plant development. Decreasing water potential during germination and seedling emergence will lead to inhibition of water imbibitions and reduction in embryonic axes growth, which are actually dehydrated (Poljakoff-Mayber et al., 1994). Root-to-
shoot length ratio and root-to-shoot fresh weight ratio were significantly increased under mild and severe OP, which could be due to the root plasticity potential under DS. Under DS, plants adapted by rapidly increasing their root system to exploit moisture from larger soil volumes (Pirdashti et al., 2003; Dhanda et al., 2004). Mean DSI based on seedling attributes showed that Morocco9-75, Yarmouk, Acsad176 and WI2291 were the most tolerant varieties, which might be recommended for areas with frequent early DS. In accordance, various authors showed high level of variability among barley genotypes in response to DS at germination and seedling stage (Dhanda et al., 2004; Sahnoune et al., 2004; Al-Karaki et al., 2007).

DS treatments significantly ($P = 0.01$) reduced yield and yield contributing traits and shortened days to heading and maturity with considerable genetic variability among the 14 barley varieties. These results confirmed the earlier findings of Shakhatreh et al. (2001), Samarah (2005) and Samarah et al. (2009) and consistent with other authors who worked on other crops species such as wheat (Al-Rjoub, 2006; Abdel-Ghani, 2008), Sorghum (Imma 2006) and Maize (Mathias, 2002). The grain yield significantly decreased when DS treatments were imposed as compared with the non-stress treatment (control). Yield reduction was more prominent under sustain DS (Dcon) followed in order by Dearly+$+$late, Dlate and Dearly. All yield components were significantly contributed in grain yield losses. The reduction in yield and yield components in barley in response to late and continuous DS was previously reported by other authors (Mogensen, 1992; Mary, 2001; Garcia, 2003; Samarah, 2005; Samarah et al., 2009).

Imposing late DS (Dlate and Dearly+$+$late) in our experiment shortened the grain filling period by 3 to 4 days which could be one reason of grain yield reductions. These results in agreement with Moustafa et al. (1996) who relieved that mid and late season DS shortened the grain filling period by 10 to 11 days. Similarly, Samarah (2005) and Samarah et al. (2009) showed that post anthesis DS shortened grain filling period and reduced the grain yield regardless of the stress severity. Number of spikes plant$^{-1}$ and number of kernel spike$^{-1}$ were more sensitive to drought than 100-kernel weight. These results in agreement with other authors (Giunta et al., 1993; Zhong-hu and Rajaram, 1994; Samarah et al., 2009) who found that number of kernels spike$^{-1}$ and spikes m$^{-2}$ were the most yield components sensitive to drought while kernel weight remains relatively stable due to high remobilization of stored preanthesis assimilates to grains.

Drought stress might fluctuate across years, and consequently might affect barley at different growth stages in WANA region (Ceccarelli, 1987; Abdel-Ghani et al., 2004). However, in WANA region, rainfall is usually scarce at the end of the growth cycle and consequently grain filling phase is frequently the most affected by DS. This will cause a drastic reduction in grain yield and might also lead to crop failure in seasons combined with severe terminal drought (Garcia del Moral et al., 2003; Samarah et al., 2009). Selection of genotypes adapting to terminal DS is therefore a priority in WANA. In the current study, a high level of variability was detected among tested barley genotypes when the stress was performed during grain filling (Dlate). Under Dlate, the least tolerant genotypes were Tadmor and Arta with average yield reductions of 40.2 and 44.7% (and also high DSI), respectively, followed by Athroh with 35.9% grain yield reduction. However, The most tolerant genotypes were WI2291 with minimum reduction percentage (7.98%) followed by Acsad 176
and Yarmouk with 12.1 and 12.5% grain yield reductions, respectively.

From high potential yield under stress environment, drought tolerance could be attributed to high yield potential under non-limiting DS condition with high potential yield under limiting-water environment (van Ginkel et al., 1998; Evans et al., 1999; Ismail et al., 1999) and/or low susceptibility to stress (less yield reduction) (Ismail et al., 1999; Shakhatreh et al., 2001). In this study, grain yield showed significant genotype × environment interaction indicating that the varieties responded to drought differentially. Among all tested varieties, Athroh followed by Rum, ER/Apm, barley local landrace and Acasad176 had the highest yield potential under water non-limiting treatment (Control) and high potential yield when subjected to DS at different developmental stages (Dcon, Dearly, Dlate and Dearly+late); however they showed moderate to high reductions in grain yield ranging from 38.02 to 60.69%, from 14.70 to 19.67%, from 12.13 to 29.18% and from 15.08 to 42.17% under Dcon, Dearly, Dlate and Dearly+late, respectively. So from the concept of high yield potential/potential yield, Athroh, Rum, ER/Apm, barley local landrace and Acasad 176 varieties could be declared as the most tolerant varieties because they exhibited high yield potential and high potential yield under non-limiting water and DS treatments, respectively. Results obtained by other authors (van Ginkel et al., 1998; Rizza et al., 2004) showed that selection for high grain yield under favorable conditions could lead to identify genotypes suitable in both stress and non-stress environments. On the other hand, from the concept of DSI (less grain yield losses), W12291, Yarmouk, Furat2 and Zanbaka were the most tolerance varieties because they exhibited low to moderate grain yield losses over all water-limiting treatments.

The grain yield were remained unchanged in three varieties (Yarmouk, Zanbaka and Furat2) and slightly increased in W12291 in response to Dearly. The yield increase in W12291 was mainly attributed to slight increases in the number of spikes plant⁻¹ and number of kernels spike⁻¹. This result may be due either to the stimulative effect of shoot primordial after rewatering (i.e. 2.5% increase in number of spikes plant⁻¹) or to elongated grain filling in response to Dearly. Longer grain filling duration might increase grain yield if later stages of grain filling are not affected by terminal DS (Garcia et al., 2003). Another explanation of the higher grain yield in Dearly compared to the control could be the changes in root morphology that becomes very relevant in the efficient absorption of water by early drought by increasing the root volume (Pirdashti et al., 2003; Dhanda et al., 2004), which might lead to better stand establishment that could be reflected in higher grain yields. However, in the current study, non-significant association was obtained between grain yield and seedling root traits.

Results from this study suggested that drought tolerance is a stage-specific trait and changes during the life cycle. So drought tolerance at the germination stage and early seedling stage does not guarantee tolerance in later stages. Moreover, the ranking pattern of varieties was almost changed with the stage of DS application. Therefore, it is important to test the DS tolerance at different growth stages, especially at reproductive stage. In accordance, results obtained by other authors (Mano et al., 1996; Zhu et al., 2005; De Leonardis et al., 2007; Szira et al., 2008) revealed that drought tolerance has been identified as a stage-specific phenomenon of tolerance at one stage of plant development being poorly correlated with
tolerance at other stages. W12291 and Yarmouk consistently showed relatively low biomass losses in seedling growth and grain yield when DS imposed at different growth stages, and consequently exhibited relatively low DSI values. Accordingly, they might represent a valuable genetic resource for enlarging the genetic variation of barley breeding programs for drought tolerance.

In conclusion, wide range variability was observed in response to DS. Among all tested varities, Athroh followed by Rum, ER/Apm, barley local landrace and Acsad 176 had high yield potential under continuous irrigation (control) and high potential yield when subjected to different water stresses (Dcon, Dearly, Dlate and Dearly+late); however they showed moderate to high reductions in grain yield. From the concept of DSI (less grain yield losses), W12291 and Yarmouk were the least susceptible since their ranks remained almost unchanged at different growth stages and accordingly they could be considered as a potential genes source for drought tolerance in barley.

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