DOI: 10.32615/ps.2020.041 PHOTOSYNTHETICA 58 (4): 1059-1067, 2020

Evaluation of morpho-physiological traits under drought stress conditions in barley (*Hordeum vulgare* L.)

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Abstract

Drought stress is one of the major constraints limiting the production and productivity of barley. We tried to identify some indicators related to plant productivity by analyzing some agro-morphological and physiological traits in recombinant inbred lines (RILs). Plants were exposed to three water treatments: well-watered at 70% available water content (AWC), mild drought stress at 35% AWC, and severe drought stress at 10% AWC. Our results revealed that drought significantly affected most of the studied morpho-physiological traits resulting in strong decreases in yield and the studied traits. We did not observe a significant difference in water-use efficiency between severe and mild drought conditions. Therefore, the mild stress treatment was considered as the most suitable water level in our study. Genotypes with the most tillers and spikes, the highest 1,000-grain mass, and biological yields achieved higher grain yields under all water stress conditions. Therefore, these traits could be considered as useful tools in breeding programs.

Additional key words: chlorophyll fluorescence; photosynthesis; stomatal conductance; water stress treatment; yield component.

Introduction

Barley (*Hordeum vulgare* L.) is a major cereal crop. It is well adapted to various abiotic stresses in the dry areas of West Asia and North Africa (Grando *et al.* 2005, Baum *et al.* 2007). Barley is cultivated on more than 40 million hectares in developing countries. In most developing countries, where it is subjected to extreme water deficits during dry seasons, it is often the only rain-fed crop that farmers can grow (Ceccarelli 1994). As a result, barley has been selected or bred for specific adaptations to abiotic stresses in geographically distinct areas of the world. This adaptation of genetically diverse germplasm to similar environmental conditions over a wide geographical range can be exploited for breeding and germplasm exchange. For example, barley germplasm bred by the International Center for Agricultural Research in the Dry Areas, Syria (ICARDA) for the marginal environments of West Asia and Northern Africa (WANA) showed good adaptation to dry southern Australian environments and *vice versa* (Rollins *et al.* 2013b).

Drought affects plant morphological and physiological traits, and causes metabolic modifications, which may lead to a decreased grain yield (Ludlow and Muchow 1990). Yield losses due to drought are estimated to vary from 17 to 70% (Ahmadizadeh *et al.* 2011). Stress adaptation in barley has been attributed to genetic variation in morphological traits (von Korff *et al.* 2008, Rollins *et al.* 2013a) and morpho-physiological traits (Rollins *et al.* 2013b).

Many morphological and physiological characteristics are affected by drought stress (Ahmadizadeh *et al.* 2011, Lonbani and Arzani 2011, Rollins *et al.* 2013b). Selection efficiency could be improved if a particular physiological and/or morphological trait related to improved seed yield under drought is identified and used in the selection process (Ludlow and Muchow 1990, Jatoi *et al.* 2011). Li *et al.* (2006), Kalaji and Guo (2008), and Kalaji *et al.* (2011a,b; 2012) reported that chlorophyll content (Chl) and some chlorophyll fluorescence (ChF) parameters could be considered as reliable indicators for drought tolerance in barley germplasm. Chl fluorescence has been used as a rapid technique to estimate the quantum efficiency of photosynthetic apparatus, and PSII performance (Roohi

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Received 20 January 2019, *accepted* 13 May 2020.

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Abbreviations: ARKE – Arta Keel population; AWC – available water content; BY – biological yield; ChF – chlorophyll fluorescence measurements; DM – dry mass; *E* – transpiration; FM – fresh mass; F_v/F_m – maximum photochemical efficiency; GM – 1,000-grain mass; g_s – stomatal conductance; GY – grain yield; HI – harvest index; MS – mild stress treatment; OA – osmotic adjustment; OP – osmotic potential; PH – plant height; P_N – net photosynthetic rate; QTL – quantitative trait locus; RILs – recombinant inbred lines; RT – relative turgidity; SL – spike length; SN – number of spikes per plant; SS – severe stress treatment; Ti – number of tillers; TM – turgid mass; WUE – water-use efficiency; WW – well-watered treatment.

Acknowledgments: This work was supported by the strategic program funding of the Biodiversity and Crop Improvement (BCI) at the International Center for Agricultural Research in the Dry Areas (ICARDA). Authors are grateful to the Arab Fund for Economic and Social Development (AFESD) and the CGIAR Research Program on Grain Legumes and Dryland cereals for their partial financial support to this research.

et al. 2013, Kalaji *et al.* 2014, 2017). Several studies reported that a water deficit leads to a significant inhibition of photosynthesis (Li *et al.* 2006, Roohi *et al.* 2013, Chéour *et al.* 2014, Dunic *et al.* 2015). Stomatal closure is one of the earliest plant responses to drought stress limiting the CO_2 diffusion to chloroplast, and reducing photosynthetic activity substantially (Pinheiro and Chaves 2011). Chéour *et al.* (2014) reported that barley plants subjected to water stress showed lower relative water and Chl contents and higher contents of proline. Rollins *et al.* (2013b) indicated that leaf proteins were differentially regulated in response to drought, heat, and combined stresses in the context of the morphological and physiological changes for two genotypes: Arta (Syrian landrace) and Keel (Australian cultivar). These genotypes, the parents of the RIL population used in this study, demonstrated significant reductions in biomass and yield under drought, reduction in photosynthetic performance and protein under heat and combinations of heat and drought. In another study for the same RIL population ARKE, which identified QTL-influencing agronomic performance in rain-fed Mediterranean environments at two locations in Syria for four years with two sowing dates (autumn and winter), obtained results showing the genotypic variability in annual yields, presumably caused by interannual differences in rainfall distribution (Rollins *et al.* 2013a).

Plants relative turgidity (RT), relative water content (RWC) as well as osmotic potential (OP) are commonly used indicators to assess plant water status. The former is a measure of plant water status resulting from a cellular water deficit, while osmotic adjustment (OA) is probably the most important mechanism for maintaining physiological activity (Ludlow and Muchow 1990). It is an appropriate estimate of plant water status as affected by leaf water potential and OA (Baum *et al.* 2007). Many studies have examined the relationship between OA and grain yield under water stress conditions and the results show a positive relationship between OA and grain yield in wheat (Moinuddin *et al.* 2005) and barley (González *et al.* 2008, Behbahanizadeh *et al.* 2014).

Some morphological and agronomic traits, which are correlated to yield under drought, may be used in breeding programs to improve yield under drought stress conditions (Lonbani and Arzani 2011). Drought stress treatments reduced the grain yield, the number of tillers, spikes, and grains per plant (Samarah 2005). A strong positive correlation has been observed between the Chl content, some Chl fluorescence parameters, and yield under water stress in barley under drought conditions (Li *et al.* 2006). As previously mentioned, several studies have indicated the effect of drought stress on barley in response to morpho-physiological parameters. However, since physiological parameters are more sensitive than morphological parameters in distinguishing drought stress treatments, it is necessary to know which of these parameters is more responsive to drought stress conditions. Therefore, the objective of this experiment was to identify indicators related to productivity (drought tolerance) through an analysis of morpho-physiological and yield traits in barley (*Hordeum vulgare* L.).

Materials and methods

Plant material and growth conditions: The present study was conducted during seasons 2008–2009 at ICARDA, Tel Hadya, Aleppo, Syria, in a greenhouse (16/8-h day/night at 27/18°C day/night) using a randomized complete block design (RCBD), with four replications. A total of 50 seeds from each of the RILs were planted in a plastic tray, filled by mixture of soil and peat moss in a volume ratio of 3:1. After germination, 36 seedlings from each of the RILs and 4-week-old parents were vernalized and transferred into a 2.5-kg pot capacity with three plants in each pot (15 cm in height and 16 cm in diameter), filled with 2 kg of sterilized soil and containing approximately 6% of water. The soil field capacity, wilting point, and AWC were measured at ICARDA soil laboratory according to a protocol described by Ryan *et al.* (2001). Out of 499 F_7 RILs derived from a cross between the Syrian susceptible line 'Arta' and the Australian tolerant line 'Keel', only the first 40 RILs were used for this study. Arta is a two-row pure high-yielding line selected from the Syrian white-seed landrace 'Arabi Abiad'. It is well adapted to Syrian conditions and combines a high number of tillers and high kernel mass, but is susceptible to lodging under high-yielding conditions. Keel is a two-row spring feed barley variety, which was developed by the South Australian Barley Improvement Program. It is a high-yielding line, which is adapted to severe drought stress and is resistant to lodging, early flowering, and plant height. Both parents are well adapted to low rainfall conditions (250–375 mm) with a high yield stability. The main objective of this cross was to develop lines combining the tillering ability of the Arta line with the plant height and severe drought adaptation of the Keel line (Grando *et al.* 2001, Rollins *et al.* 2013a). At the grain-filling stage, plants were subjected to three drought stress levels: 10, 35, and 70% of the soil AWC – representing severe stress (SS), mild stress (MS), and well-watered (WW) conditions, respectively.

Pots were weighed daily and maintained at the desired soil moisture content. The days under drought stress were counted after the AWC in the soil reached the desired percentage to allow measurements at precisely determined intervals.

Physiological traits: Relative Chl content, maximum photochemical efficiency of PSII (F_v/F_m), net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), relative turgidity (RT), and water-use efficiency (WUE) were measured/calculated at the grain-filling stage on the flag leaf for one selected plant of each genotype per treatment and replication.

The relative Chl content was determined using a chlorophyll meter (*SPAD-502*, *Minolta*, Japan). The average of three measurements in the middle of the flag leaf was collected randomly on the 7th day after the drought stress was applied.

Chl fluorescence parameters were measured using a portable fluorometer *HandyPEA* (*Hansatech Instruments Ltd.*, Norfolk, UK), following the manufacturer's instruction and Kalaji *et al.* (2014). The dark-adaptation period was about 25 min. Fluorescence parameters (F_0, F_m)

were recorded and F_v/F_m ($F_m - F_0/F_m$) parameter, which represents the maximum quantum yield of PSII, was automatically calculated.

Plant gas-exchange parameters (P_N, g_s, and E) were measured starting from the 7th day after water stress was applied. The fully expanded flag leaf was selected for one plant per pot per treatment using a *CIRAS-2* infrared gas analyzer system manufactured by *PP Systems Co.* (MA, USA). The measurements were set up with the following adjustments: the measured leaf surface area was 4.50 cm^2, ambient CO_2 concentration (C_{ref}) of 380 $\mu mol(CO_2)\ mol^{-1}$, the temperature of each leaf varied from 24.4 to 25.9°C, and the leaf chamber gas flow rate (v) was *ca.* 300 ml min^{-1}. Ambient pressure ranged from 967–973 mbar, and the PAR at the leaf surface reached a maximum of about 1,000 $\mu mol(photon)\ m^{-2}\ s^{-1}$. The value of water-use efficiency was calculated as P_N/E.

Plant relative turgidity (RT) was measured using leaf pieces collected from the flag leaf after imposing drought stress. Immediately after cutting the base of lamina, leaves were sealed within plastic bags and transferred quickly to the laboratory. Fresh mass (FM) was determined within two hours after excision. Turgid mass (TM) was obtained after soaking leaves in distilled water in test tubes for 24 h in the fridge at 4°C and in the dark. After that, leaves were quickly and carefully dried by tissue paper in preparation for determining turgid mass. Dry mass (DM) was obtained after oven drying the leaf sample for 48 h at 80°C. The RT was calculated according to a flowing formula devised by Barrs and Watherley (1968): RT = [(FM – DM)/(TM – DM)] × 100.

Leaf osmotic potential (OP) was obtained *in situ* from the leaf material collected at noon. This material was frozen inside an Eppendorf tube with liquid nitrogen. Later on, the material was thawed and placed in a syringe (1 mL) and the cellular juice was obtained by mechanical pressure. An aliquot of 10 µL was used to saturate a disc of filter paper and the osmolality [mmol kg^{-1}] was measured with a *Wescor 5520* osmometer (*Wescor Inc.*, Utah, USA). These values were converted to a pressure unit according to the following equation: OP [MPa] = –R × T × C, where R is the gas constant (0.008314), T is the temperature measured in the Kelvin scale (298 K in these measurements), and C is the osmolality [mmol kg^{-1}]. The OP was corrected (OP + 0.1 OP) for the dilution of symplastic sap by apoplastic water, assuming 10% apoplastic water. The OP at full turgor (OP_{100}) was calculated, according to Wilson *et al.* (1979), by the following equation: OP_{100} = (corrected OP × RT)/100. OA was expressed as the difference between OP_{100} of leaves of irrigated and stressed plants.

Agro-morphological traits*:* Plant height (PH), number of tillers (Ti), number of spikes per plant (SN), spike length (SL), 1,000-grain mass (GM), grain yield (GY), biological yield (BY), and harvest index (HI) were recorded during the experiment. At the maturity stage (grain-filling duration), PH, SL, SN, and Ti were recorded on three plants from each replication (total of 12 plants for each tested genotype). The average of three plants for each genotype was used for the analysis. At harvesting time, the average of three plants of each genotype was harvested to measure GM, GY, BY, and HI.

Statistical analysis: The experiment was laid out under RCBD (randomized complete block design) with four replications. Data were subjected to analysis of variance using a *GENSTAT v. 18* statistical software. The mean comparisons ± SD were carried out to estimate the differences between treatments and genotypes using least significance differences (LSD). A simple correlation analysis was performed to express the relationship among variables of interest.

Results

Under water stress conditions, the frequency distribution for grain yield among the 40 tested RILs and parental lines Arta and Keel showed a normal distribution (Fig. 1). Cultivar Keel yielded 34% more than that of cv. Arta (1.85 and 1.38 g per plant, respectively). Around 38% of the tested RILs produced a similar grain yield as the parental line Keel; and 56% of the progeny produced yields with values that were somewhere between those generated by both parental lines.

Morphological parameters: Variations analysis indicated a high significant difference ($P<0.001$) between genotypes, treatments, and genotype × treatment interaction for all studied traits (Table 1). This interaction revealed that genotypes performed inconsistently over the treatments. Drought stress treatments during the grain-filling stage significantly decreased values of morphological parameters and grain yield. Except the spike length and 1,000-grain mass, all the morphological parameters were significantly affected by both moderate drought and severe stress (Table 1). As compared to the control treatment, both drought stress levels, MS and SS, affected the plant height, resulting in an average decrease of 10 and 18.5%, respectively.

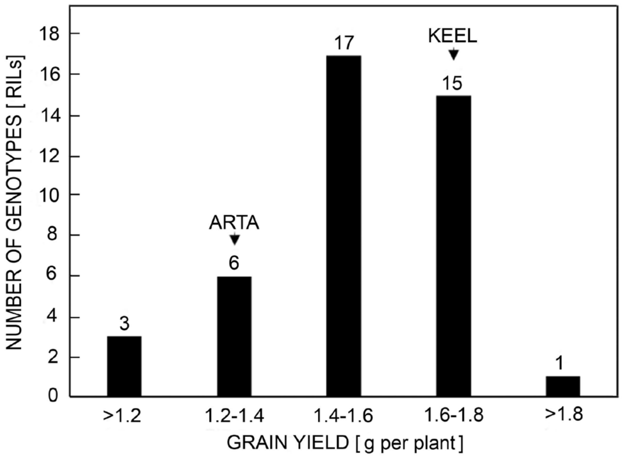

Fig. 1. Frequency distribution of grain yield. The mean performance of the parental lines Arta and Keel is indicated above the bars.

Table 1. Analysis of variance (mean square) and mean performance with their standard deviation for morphological traits of 40 RILs population (Arta × Keel) under drought stress conditions. PH – plant height [cm]; Ti – number of tillers; SN – number of spikes per plant; SL – spike length [cm]; GM – 1,000-grain mass [g]; GY – grain yield [g per plant]; BY – biological yield [g per plant]; HI – harvest index [%]. Treatment means followed by *different letters* indicate significant difference according to the least significant difference (LSD) test at probability level 0.05. *, **, *** – significance at 0.05, 0.01, 0.001 level, respectively. WW – well-watered treatment; MS – mild stress treatment; SS – severe stress treatment.

Traits	Treatments	Mean	Replications D.F. = 3	Genotypes [RILs] D.F. = 41	Treatments [T] D.F. = 2	T × RILs D.F. = 82	Error D.F. = 375	CV [%]
PH	WW	47.05 ± 4.89^{a}	9.93	178.56^{***}	$3{,}198.94^{***}$	30.11^{***}	5.19	5.4
	MS	42.32 ± 4.33^{b}						
	SS	38.34 ± 4.12^{c}						
Ti	WW	5 ± 0.66^{a}	1.17	1.44^{***}	180.18^{***}	0.77^{***}	0.22	11.5
	MS	4 ± 0.56^{b}						
	SS	3 ± 0.50^{c}						
SN	WW	5 ± 0.69^{a}	3.13	1.59^{***}	243.12^{***}	1.21^{***}	0.27	14.3
	MS	4 ± 0.55^{b}						
	SS	3 ± 0.67^{c}						
SL	WW	5.46 ± 0.73^{a}	0.49	3.068^{***}	2.65^{***}	0.55^{***}	0.08	5.2
	MS	5.39 ± 0.51^{a}						
	SS	5.24 ± 0.49^{a}						
GM	WW	1.54 ± 0.26^{a}	1.02	0.23^{***}	3.08^{***}	0.108^{***}	0.06	16.1
	MS	1.59 ± 0.16^{a}						
	SS	1.33 ± 0.13^{a}						
GY	WW	2.21 ± 0.28^{a}	0.80	0.42^{***}	63.97^{***}	0.22^{***}	0.03	11.7
	MS	1.67 ± 0.34^{b}						
	SS	0.98 ± 0.22^{c}						
BY	WW	5.37 ± 1.00^{a}	3.16	2.05^{***}	212.08^{***}	1.38^{***}	0.14	8.7
	MS	4.16 ± 0.30^{b}						
	SS	3.13 ± 0.30^{c}						
HI	WW	0.42 ± 0.06^{a}	0.046	0.045^{***}	0.537^{***}	0.0045^{***}	0.002	11.0
	MS	0.41 ± 0.08^{a}						
	SS	0.31 ± 0.06^{b}						

Both MS and SS drought treatments decreased the tiller number (Ti) and the spike number (SN) by 20 and 40%, respectively (Table 1). Spike length (SL) and 1,000-grain mass (GM) were not significantly different between treatments. MS treatment had a higher 1,000-grain mass than that in WW and SS treatments (Table 1). The effect of drought caused 1.3 and 4% reduction in the spike length under MS and SS treatments, respectively. The RILs with the highest value for the number of tillers, the number of spikes, spike length, and 1,000-grain mass were also characterized by the highest grain yield under three water stress treatments (Tables 1S–3S, *supplement*). Among yield component traits, the SN was significantly and positively correlated with PH ($r = 0.33^{*}$), Ti ($r = 0.34^{*}$), GM ($r = 0.32^{*}$), and harvest index ($r = 0.74^{***}$) (Table 2).

An important decrease in grain yield was recorded for the different genotypes studied under different water stress levels (Table 1). In comparison to the control treatment, average grain yield for RILs decreased by 24.4 and 55.7% under MS and SS drought conditions, respectively. No difference was recorded for both parental lines Arta and Keel in terms of the grain yield decrease (31%) under moderate drought stress (MS), while a clear difference was observed under severe drought stress (SS) resulting in respective decreases of 61 and 46% in grain yields for Arta and Keel, respectively (Fig. 2). The highest values for GY and HI were recorded in the tolerant parent Keel under three water treatments, whereas RILs had higher values of GY and HI than the susceptible parent Arta under these treatments (Tables 1S–3S).

A significant difference was observed for biological yield (BY) between water stress conditions, while there were no significant differences in HI between WW and MS treatments (Table 3). After 7 d of drought, a decrease of 22.5 and 41.7% was recorded for the BY under MS and SS treatments, respectively; whereas the harvest index was reduced by 2.3 and 26.2% under both MS and SS, respectively. The highest value for BY was observed for the genotype that showed the highest GY under three water stress treatments (Tables 1S–3S).

Physiological parameters: Results showed highly signifi-

Table 2. Simple coefficients of correlation among morpho-physiological traits under severe stress condition (10% AWC) in barley. Chl – chlorophyll content; F_v/F_m – maximum photosynthetic efficiency of PSII; P_N – photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; OP – osmotic potential; RT – relative turgidity; WUE – water-use efficiency; PH – plant height; Ti – number of tillers; SN – number of spikes per plant; SL – spike length; GM – 1,000-grain mass; GY – grain yield; BY – biological yield; HI – harvest index. *, **, *** – significance at 0.05, 0.01, 0.001 level, respectively.

Traits	Chl	F_v/F_m	P_N	E	g_s	OP	RT	WUE	PH	SL	SN	Ti	GM	GY	BY
F_v/F_m	–0.07														
P_N	0.18	–0.16													
E	0.14	–0.20	0.17												
g_s	0.20	–0.10	0.20	0.82***											
OP	–0.26	0.02	–0.04	–0.38*	–0.49**										
RT	–0.06	–0.01	0.22	–0.12	–0.32*	0.68***									
WUE	–0.03	–0.01	0.45**	–0.68***	–0.53***	0.20	0.15								
PH	0.09	–0.04	–0.26	0.01	0.08	–0.20	–0.23	0.07							
SL	0.04	–0.34*	–0.03	0.41**	0.37*	0.05	0.01	–0.35*	0.14						
SN	0.32*	–0.11	0.19	0.18	0.18	–0.46**	–0.24	0.09	0.33*	–0.13					
Ti	–0.24	0.16	0.04	–0.07	–0.05	0.11	0.15	0.22	0.12	–0.21	0.34*				
GM	0.28	0.05	0.27	0.00	–0.08	0.10	0.19	0.22	0.09	–0.28	0.32*	0.07			
GY	0.38*	0.01	0.15	0.34*	0.30	–0.46**	–0.28	0.01	0.42**	0.03	0.83***	0.25	0.27*		
BY	0.01	0.24	0.11	0.12	0.21	–0.19	–0.07	0.11	0.40*	0.03	0.50***	0.34*	0.38*	0.56***	
HI	0.45**	–0.14	0.14	0.33*	0.25	–0.45**	–0.29	–0.04	0.30	0.02	0.74***	0.13	0.13	0.90***	0.16

cant differences between the 40 studied RILs in response to the different treatments. The significant interaction of treatment × genotype was observed also for all studied parameters except RT, which indicates a difference between RILs in their response to drought stress (Table 3). No significant differences were observed between WW and MS treatments for the Chl content and between MS and SS for the WUE. An average decrease of 1.0 and 7.3% of the Chl were recorded for MS and SS treatments, respectively, as compared to the control treatment (Table 3). The maximum efficiency of PSII (F_v/F_m) was significantly affected in three water treatments, although these differences were not very large (Table 3). The reduction of F_v/F_m after 7 d of imposing drought treatments varied from 3.6% in SS treatment (10% AWC) to 2.4% for MS (35% AWC) treatment as compared to WW treatment (70% AWC). After 7 d of drought stress, the highest values of Chl content and F_v/F_m were observed in RILs which had lower grain yields under SS treatment and higher grain yields under MS and WW treatments (Tables 1S–3S). In our study, there were significant differences in the average of the photosynthetic indicators under three water stress treatments (Table 3). At the grain-filling stage, the decrease in P_N was 66.8 and 47.9% under SS and MS treatments, respectively. The decrease in g_s was 81.9 and 70% and 74.4 and 58.5% in E, respectively. After 7 d of drought stress, the highest values for P_N, g_s, and E were observed among the RILs showing a high grain yield under three water stress treatments (Tables 1S–3S). Obviously, stressed plants had a lower RT than the nonstressed plants. Water stress significantly influenced the RT, OP, and WUE traits. It reduced the RT by, on average, 31.4 and 11.4% and OP by, on average, 50.5 and 36.4% under SS and MS treatments, respectively, compared to WW treatments. The WUE increased by 29 and 23.8% under SS and MS treatments, respectively, as compared to WW treatments (Table 3). These values can also be used as an indicator of OA capacity, as it represents the variation in OP, with respect to RT over the water stress period. Our results indicated a decline in OP and RT when the severity of water stress increased (Table 4). The values of osmotic adjustment were 0.50 and 0.18 MPa under SS and MS treatments, respectively. After 7 d of drought stress, the highest value of OP was observed in RIL, which had the lower grain yield under SS treatment and the higher grain yield under MS and WW treatments, while the highest value of RT was observed in RILs that had the lower grain yields and higher grain yields in the WUE under three water treatments (Tables 1S–3S). At

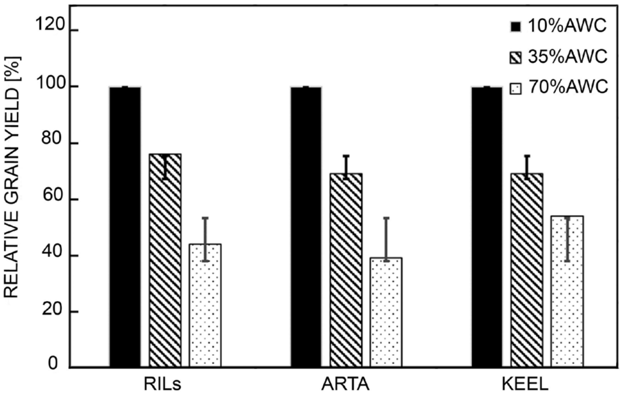

Fig. 2. Relative grain yields of two parents (Arta, Keel) and 40 RILs under three water stress treatments. Values are means ± SE. 10% AWC, 35% AWC, and 70% AWC – severe stress (SS), mild stress (MS), and well-watered (WW) conditions, respectively.

Table 3. Analysis of variance (mean square) and mean performance with their standard deviation for physiological parameters of 40 RILs population (Arta × Keel) under drought stress conditions. Chl – chlorophyll content [relative unit]; F_v/F_m – maximum photosynthetic efficiency of PSII [relative unit]; P_N – photosynthetic rate [μmol(CO_2) m^{-2} s^{-1})]; E – transpiration rate [μmol(H_2O) m^{-2} s^{-1})]; g_s – stomatal conductance [μmol(H_2O) m^{-2} s^{-1})]; OP – osmotic potential [MPa]; RT – relative turgidity [%]; WUE – water-use efficiency [mol(CO_2) mol^{-1}(H_2O)]. Treatment means followed by *different letters* indicate significant difference according to the least significant difference (LSD) test at probability level 0.05. *, **, *** – significance at 0.05, 0.01, 0.001 level, respectively. ns – not significant. WW – well-watered treatment; MS – mild stress treatment; SS – severe stress treatment.

Traits	Treatments	Mean	Replications D.F. = 3	Genotypes [RIL] D.F. = 41	Treatments [T] D.F. = 2	T × RILs D.F. = 82	Error D.F. = 375	CV [%]
Chl	WW	41.90 ± 3.34^{a}	6.09	114.59^{***}	471.35^{***}	25.58^{***}	1.86	3.3
	MS	41.52 ± 3.88^{a}						
	SS	38.84 ± 3.90^{b}						
F_v/F_m	WW	0.83 ± 0.01^{a}	0.00043	0.00096^{***}	0.0203^{***}	0.00077^{***}	0.0002	1.6
	MS	0.81 ± 0.02^{b}						
	SS	0.80 ± 0.02^{c}						
P_N	WW	5.61 ± 0.78^{a}	1.86	1.72^{***}	630.64^{***}	1.59^{***}	0.17	12.1
	MS	2.92 ± 0.65^{b}						
	SS	1.86 ± 0.44^{c}						
E	WW	0.94 ± 0.29^{a}	0.16	0.17^{***}	26.65^{***}	0.12^{***}	0.006	14.4
	MS	0.39 ± 0.13^{b}						
	SS	0.24 ± 0.07^{c}						
g_s	WW	82.74 ± 24.93^{a}	0.80	$1,200.05^{***}$	$210,475.62^{***}$	838.48^{***}	1.19	2.7
	MS	24.81 ± 8.58^{b}						
	SS	14.97 ± 4.91^{c}						
OP	WW	-1.78 ± 0.32^{a}	0.03	0.55412^{***}	148.13^{***}	0.4750^{***}	0.021	5.7
	MS	-2.29 ± 0.12^{b}						
	SS	-3.60 ± 0.52^{c}						
RT	WW	0.70 ± 0.04^{a}	0.14	0.012^{*}	1.98^{***}	0.0046^{ns}	0.008	14.9
	MS	0.62 ± 0.03^{b}						
	SS	0.48 ± 0.05^{c}						
WUE	WW	6.1 ± 1.58^{a}	26.57	27.64^{***}	2.66^{***}	22.28^{***}	2.36	20.1
	MS	8.0 ± 2.41^{b}						
	SS	8.6 ± 3.20^{b}						

Table 4. Calculation of osmotic adjustment (OA) under drought stress conditions in barley. WW – well-watered treatment; MS – mild stress treatment; SS – severe stress treatment.

Treatments	Relative turgidity [%]	Osmotic potential [MPa]	Osmotic potential at full turgor [MPa]	Osmotic adjustment [MPa]
WW	70	–1.68	–1.18	-
MS	62	–2.19	–1.36	0.18
SS	48	–3.50	–1.68	0.50

grain filling, the WUE of plants increased significantly with water supply, irrespective of genotype (Table 3).

Correlation analysis: The application of correlation analysis among yield component traits showed that the SN was significantly and positively correlated with PH ($r = 0.33^{*}$), Ti ($r = 0.34^{*}$), GM ($r = 0.32^{*}$), and HI ($r = 0.74^{***}$) (Table 2). A positive and significant correlation was recorded between GY and PH ($r = 42^{**}$), SN ($r = 0.83^{***}$), BY ($r = 0.56^{***}$), GM ($r = 0.27^{*}$), and HI ($r = 0.90^{***}$) (Table 2). BY was significantly and positively correlated with PH ($r = 0.40^{*}$), Ti ($r = 0.34^{*}$), SN ($r = 0.50^{***}$), and GM ($r = 0.38^{*}$) (Table 2). The Chl content exhibited positive and significant association with SN ($r = 0.32^{*}$), GY ($r = 0.38^{*}$), and HI ($r = 0.45^{**}$), while F_v/F_m showed a negative significant association with SL ($r = -0.34^{*}$) (Table 2).

A positive and significant correlation was recorded between E and g_s ($r = 0.82^{***}$), GY ($r = 0.34^{*}$), HI ($r = 0.33^{*}$), and SL ($r = 0.40^{**}$) (Table 2). A negative and significant correlation was found between g_s and OP ($r = -0.49^{**}$), RT ($r = -0.32^{*}$), and WUE ($r = -0.53^{***}$), while this trait correlated positively and significantly

($r = 0.37^{*}$) with SL (Table 2).

OP was associated negatively and significantly with E ($r = –0.38^{*}$), GY ($r = –0.46^{**}$), SN ($r = –0.46^{**}$), and HI ($r = –0.45^{**}$), while it showed positive and significant correlations with RT ($r = 0.68^{***}$) (Table 2). A negative and significant correlation was recorded between WUE and E ($r = –0.68^{***}$) and SL ($r = –0.35^{*}$), whereas a positive and significant correlation was found with P_N ($r = 0.45^{**}$) (Table 2).

Discussion

The effect of water stress during the grain-filling stage decreased the grain yield and yield components, and these results are consistent with previous studies (Samarah 2005, Bogale *et al.* 2011, Ahmadizadeh 2013, Rollins *et al.* 2013b, Saeidi and Abdoli 2015). Drought caused strong reductions in plant height, compared to control in Arta, but not in tolerant parent (Keel) (Tables 1S–3S). Ahmadizadeh (2011) reported that tall genotypes have this ability to extract more water from the soil. Many plants including cereals store carbohydrates in the vegetative organs such as stems and leaves before reproductive stage and then remobilize them into the grains during grain-filling stage under drought conditions. The coincidence of main effect QTL for plant height and yield indicated that average yield was mainly determined by plant height (von Korff *et al.* 2008), and the number of tillers and spikes decreased under MS and SS treatments. Similar to these findings, Samarah (2005) reported that drought stress reduced plants tillers by 25 and 4.2%, fertile spikes by 50 and 50%, and grain yield by 57 and 51% under severe and mild stress conditions, respectively, compared to plants under well-watered conditions. A significant and positive correlation was found between SN and Ti, GM, GY, BY, and HI (Table 2). Traits, which have a positive and significant correlation with grain yield, can be considered as enhancing performance under water stress. Rollins *et al*. (2013a), who also studied the same population, reported a significant and positive correlation between 1,000-grain mass and grain yield per plant ($r = 0.42^{***}$), which was higher than we identified in our study (0.27^{*}), but they found a lower correlation between 1,000-grain mass and biological yield (0.16^{*}) compared to our study (0.38^{*}). Grain yield (GY) per plant is the ultimate result of all physiological and agronomical responses of cultivars to drought stress conditions (Jatoi *et al.* 2011). Drought treatments caused significant reductions in GY and BY, compared to control (Table 1). Similar results were found in previous studies under both field and greenhouse experiments (Ceccarelli 1994, Li *et al.* 2006, Guo *et al.* 2009, Rollins *et al.* 2013a,b). Some RILs (9.2%) had a higher relative GY than the two parents Arta and Keel under MS treatment (Fig. 2). According to our results, mild stress treatments are more suitable water level in this study. Significant differences were found in HI under water stress conditions, while no significant differences in HI were found between WW and MS treatments (Table 1). This indicates that HI expresses the ability of plants to allocate photosynthetic assimilates to produce grain (Saeidi *et al.* 2015). Ahmadizadeh *et al.* (2011) found that wheat cultivars with the high biological yield and harvest index have high grain yields under stress conditions.

During the grain-filling stage, water deficits significantly decreased the Chl content by 7.3% (Table 3). This is in agreement with the results earlier obtained in barley – 20% (Li *et al.* 2006), 22.2% (Guo *et al.* 2009), and 7.8% in bread wheat (Moradi *et al.* 2015). Positive and significant correlations ($r = 0.32^{*}$, $r = 0.38^{*}$, and $r = 0.45^{**}$) were found between Chl and SN, GY, and HI respectively. It was reported by Li *et al.* (2006) that Chl content positively correlated ($r = 0.67^{*}$) with GY under drought stress conditions, which is 43% higher than that we observed in our study. The use of Chl fluorescence measurements as a nondestructive method proved to be reliable for monitoring photosynthesis events and judging the physiological status of the plant (Li *et al.* 2006, Kalaji and Guo 2008). The ratio of F_v/F_m indicates the potential photochemical yield of PSII and quantum efficiency, and it is an important index for evaluating photosynthesis efficiency (Baum *et al.* 2007). At the grain-filling stage, maximum efficiency of PSII (F_v/F_m) was significantly affected under three water treatments (Table 3). Many studies indicated that the latter parameter decreases under limited soil moisture (Baum *et al.* 2007, Guo *et al.* 2009, Moradi *et al.* 2015). The fluorescence ratios and yield values were altered most significantly after 7 d of stress imposition. Therefore, Kalaji *et al.* (2011a) did not recommend this parameter for early detection under such stress.

Photosynthesis is an important factor controlling growth and yield production in plants. Reduction on photosynthesis after flowering due to drought stress affected the dry matter production and therefore the grain yield was reduced (Table 3). Bogale *et al.* (2011) showed that water deficit treatments at grain-filling stage significantly affected gas-exchange parameters. Photosynthesis, transpiration, and stomatal conductance were reduced under water regime conditions (Table 3). These findings come in agreement with previous barley and wheat research (Samarah 2005, Roohi *et al.* 2013, Kalaji *et al*. 2014, 2017; Saeidi and Abdoli 2015). A positive and high significant correlation was recorded between E and g_s ($r = 0.82^{***}$; Table 2). Touchan *et al.* (2010) reported that decreasing leaf water content initially induces stomatal closure, imposing a decrease in the supply of CO_2 to mesophyll cells and consequently decreasing the rate of leaf photosynthesis. A high reduction in g_s of 81.9 and 70% under SS and MS conditions, respectively, is considered the best indicator for drought stress, compared to other traits used in this study (Table 3). Monitoring gas exchange in plants is a common approach, with g_s reported as one of the most sensitive indicators under drought stress (Medrano *et al.* 2002). This suggests an increased susceptibility of stomatal conductivity to water stress, resulting in an increase in the apparent mesophyll resistance, which can be differentiated among different genotypes of cereals (Roohi *et al.* 2013). A negative correlation was found between g_s and RT ($r = –0.32^{*}$; Table 2). The genotypes manifested minimum g_s, and although these cultivars exhibited higher RT, the E was much lower under stress conditions, enhancing survival in drought environments (Jatoi *et al.* 2011). According to Saeidi and Abdoli (2015), a greater decline in g_s was observed in tolerant cultivars compared to susceptible ones, and it can therefore be concluded that under water stress, the P_N of tolerant cultivars is primarily limited by stomatal rather than nonstomatal factors.

The water content at full turgor should be used as the basis for relative turgidity – not dry mass, nor fresh mass, or area (Barrs and Watherley 1968). RT is useful for determining physiological water status in plants, and it is high at the initial stages of leaf development (Ahmadizadeh 2013). In our study, the RT and OP were reduced under water stress treatment (Table 3). A positive and highly significant correlation was recorded between RT and OP ($r = 0.68^{***}$), while a negative and insignificant correlation was observed between RT and E ($r = -0.12$; Table 2) indicating that the RT was most likely reduced by an increase in E (Rollins *et al.* 2013b). Bogale *et al.* (2011) reported that water deficit caused a reduction of the RT of leaves, but genotypes demonstrated variation in the maintenance of RT under both water deficit and well-watered conditions. The values of OA were 0.50 and 0.18 MPa under SS and MS treatments, respectively (Table 4). A negative and significant correlation was found between OP and g_s, OP and E ($r = -0.49^{**}$, $r = -0.38^{*}$), respectively. This could be explained by a lowering of the cell OP, which in turn attracts water into the cell and thereby maintains its turgor (González *et al.* 2008). Accumulation of solutes in roots leads to a lowering of the OP of the root, which maintains the driving force for extracting soil water under water deficit conditions (Moinuddin *et al.* 2005). Thus, OA helps plants perform better in drought in terms of growth and productivity by maintaining turgor and water supply to the plant, which thereby maintains a comparatively higher photosynthetic rate and growth (Ludlow and Muchow 1990). WUE significantly increased as compared to control conditions at the grain-filling stage. A negative and significant correlation was recorded between WUE and E, WUE and g_s ($r = -0.68^{***}$; $r = -0.53^{*}$), respectively (Table 2). An increase in WUE could be due to higher reduction of E than P_N under water deficit conditions (Bogale *et al.* 2011).

According to the three parameters (OA, RT, and WUE), 12 tolerant genotypes (RILs) performed well under severe stress treatment (Table 1S). A higher WUE may be related to either higher growth and photosynthesis or to lower growth rates due to stomatal closure (Pita *et al.* 2005).

Conclusion: Drought stress during the grain-filling period reduced morpho-physiological traits and grain yield. Chlorophyll content affected the grain yield and was associated positively with yield increases under three water treatments. The results of stomatal conductance showed a higher reduction under drought stress conditions compared to other physiological parameters. Stomatal conductance is one of the most sensitive indicators under drought stress. The great decline in stomatal conductance was observed in tolerant genotypes compared to susceptible ones. Stomatal limitation of the net photosynthetic rate under stress conditions may vary in susceptible and tolerant genotypes. Well-watered plants produced more tillers and consequently more fertile spikes and grains compared with mild and severe stress treatments. The highest values of tillers, spikes, 1,000-grain mass, and biological yields were found in genotypes with the higher grain yields under three water stress treatments. Therefore, these traits could be considered as useful tools during crop breeding procedures. Under mild stress treatment (35% AWC), the genotypes (RILs) had higher grain yields than the two parents (Arta and Keel) and there were no significant differences in the means of RILs for WUE traits between severe and mild stress conditions. Therefore, our study suggests the mild stress treatment should be considered as a suitable water level. Correlation analysis showed that the correlation between morphological and yield traits was positive and significant, while the correlation was negative and significant between water-related parameters and stomatal conductance, transpiration and also between osmotic potential and number of seed, grain yield, harvest index. Therefore, the relationships could serve as the selection criteria to screen genotypes for drought tolerance and potentially higher yields under water stress treatments.

References

Ahmadizadeh M.: Physiological and agro-morphological response to drought stress. – Middle East J. Sci. Res. **13**: 998-1009, 2013.

Ahmadizadeh M., Shahbazi H., Valizadeh M., Zaefizadeh M.: Genetic diversity of durum wheat landraces using multivariate analysis under normal irrigation and drought stress conditions. – Afr. J. Agr. Res. **6**: 2294-2302, 2011.

Barrs H.D., Watherley P.E.: A re-examination of the relative turgidity technique for estimating water deficit in leaves. – Aust. J. Biol. Sci. **15**: 413-428, 1968.

Baum M., von Korff M., Guo P. *et al.*: Molecular approaches and breeding strategies for drought tolerance in barley. – In: Varshney R.K., Tuberosa R. (ed.): Genomic-Assisted Crop Improvement. Vol. 2. Genomics Applications in Crops. Pp. 51-79. Springer, Dordrecht 2007.

Behbahanizadeh S.A., Akbari G.A., Shahbazi M., Alahdadi I.: Relations between barley root traits and osmotic adjustment under terminal drought stress. – J. Agr. Sci. **6**: 112-119, 2014.

Bogale A., Tesfaye K., Geleto T.: Morphological and physiological attributes associated to drought tolerance of Ethiopian durum wheat genotypes under water deficit condition. – J. Biodivers. Environ. Sci. **1**: 22-36, 2011.

Ceccarelli S.: Specific adaptation and breeding for marginal conditions. – Euphytica **77**: 205-219, 1994.

Chéour F., Kaddachi I., Achouri D. *et al.*: Effects of water stress on relative water, chlorophylls and proline contents in barley (*Hordeum vulgare* L.) leaves. – J. Agr. Vet. Sci. **7**: 13-16, 2014.

Dunic J.A., Lepedus H., Simic D. *et al.*: Physiological response to different irradiation regimes during barley seedlings growth followed by drought stress under non-photoinhibitory light. – J. Agr. Sci. **7**: 69-83, 2015.

González A., Martín I., Ayerbe L.: Yield and osmotic adjustment capacity of barley under terminal water-stress conditions. – J. Agron. Crop Sci. **194**: 81-91, 2008.

Grando S., Baum M., Ceccarelli S. *et al.*: QTLs for straw quality characteristics identified in recombinant inbred lines of a *Hordeum vulgare* × *H. spontaneum* cross in a Mediterranean environment. – Theor. Appl. Genet. **110**: 688-695, 2005.

Grando S., von Bothmer R., Ceccarelli S.: Genetic diversity of barley: use of locally adapted germplasm to enhance yield and yield stability of barley in dry areas. – In: Cooper H.D., Spillane C., Hodgink T. (ed.): Broadening the Genetic Base of Crop Production. Pp. 351-372. CABI Publishing, Wallingford 2001.

Guo P., Baum M., Grando S. *et al*.: Differentially expressed genes between drought-tolerant and drought-sensitive barley genotypes in response to drought stress during the reproductive stage. – J. Exp. Bot. **60**: 3531-3544, 2009.

Jatoi W.A., Baloch M.J., Kumbhar M.B. *et al*.: Effect of water stress on physiological and yield parameters at anthesis stage in elite spring wheat cultivars. – Sarhad J. Agric. **27**: 59-65, 2011.

Kalaji H.M., Bosa K., Kościelniak J., Hossain Z.: Chlorophyll *a* fluorescence – A useful tool for the early detection of temperature stress in spring barley (*Hordeum vulgare* L.). – Omics **15**: 925-934, 2011a.

Kalaji H.M., Carpentier R., Allakhverdiev S.I., Bosa K.: Fluorescence parameters as an early indicator of light stress in barley. – J. Photoch. Photobio. B **112**: 1-6, 2012.

Kalaji H.M., Govindjee, Bosa K. *et al*.: Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. – Environ. Exp. Bot. **73**: 64-72, 2011b.

Kalaji H.M., Guo P.: Chlorophyll fluorescence: A useful tool in barley plant breeding programs. – In: Sanchez A., Gutierrez S.J. (ed.): Photochemistry Research Progress. Pp. 439-463. Nova Science Publishers, New York 2008.

Kalaji H.M., Schansker G., Brestic M. *et al*.: Frequently asked questions about chlorophyll fluorescence, the sequel. – Photosynth. Res. **132**: 13-66, 2017.

Kalaji H.M., Schansker G., Ladle R.J. *et al*.: Frequently asked questions about *in vivo* chlorophyll fluorescence: practical issues. – Photosynth. Res. **122**: 121-158, 2014.

Li R., Guo P., Baum M. *et al*.: Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. – Agr. Sci. China **5**: 751-757, 2006.

Lonbani M., Arzani A.: Morpho-physiological traits associated with terminal drought stress tolerance in triticale and wheat. – Agron. Res. **9**: 315-329, 2011.

Ludlow M.M., Muchow R.C.: A critical evaluation of traits for improving crop yields in water-limited environments. – Adv. Agron. **43**: 107-153, 1990.

Medrano H., Escalona J.M., Bota J. *et al*.: Regulation of photosynthesis of C_3 plants in response to progressive drought: Stomatal conductance as a reference parameter. – Ann. Bot.-London **89**: 895-905, 2002.

Moinuddin, Fischer R.A., Sayre K.D., Reynolds M.P.: Osmotic adjustment in wheat in relation to grain yield under water deficit environments. – Agron. J. **97**: 1062-1071, 2005.

Moradi Z., Farshadfar E., Shirvani H.: Screening of drought tolerant genotypes in bread wheat (*Triticum aestivum*) using morpho-physiological traits and integrated selection index. – Biol. Forum **7**: 163-172, 2015.

Pinheiro C., Chaves M.M.: Photosynthesis and drought: Can we make metabolic connections from available data? – J. Exp. Bot. **62**: 869-882, 2011.

Pita P., Cañas I., Soria F. *et al*.: Use of physiological traits in tree breeding for improved yield in drought-prone environments. The case of *Eucalyptus globulus*. – Invest. Agrar. Sist. Recur. For. **14**: 383-393, 2005.

Rollins J.A., Drosse B., Mulki M.A. *et al*.: Variation at the vernalisation genes *Vrn-H1* and *Vrn-H2* determines growth and yield stability in barley (*Hordeum vulgare*) grown under dryland conditions in Syria. – Theor. Appl. Genet. **126**: 2803-2824, 2013a.

Rollins J.A., Habte E., Templer S.E. *et al*.: Leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley (*Hordeum vulgare* L.). – J. Exp. Bot. **64**: 3201-3212, 2013b.

Roohi E., Tahmasebi-Sarvestani Z., Modarres-Sanavy S.A.M., Siosemardeh A.: A comparative study on the effect of soil water stress on photosynthesis function of triticale, bread wheat and barley. – J. Agr. Sci. Tech. **15**: 215-228, 2013.

Ryan J., Estefan G., Rashid A.: Soil and Plant Analysis Laboratory Manual. 2nd Edition. Pp. 172. ICARDA, Aleppo 2001.

Saeidi M., Abdoli M.: Effect of drought stress during grain filling on yield and its components, gas exchange variables, and some physiological traits of wheat cultivars. – J. Agr. Sci. Tech. **17**: 885-898, 2015.

Saeidi M., Ardalan S., Jalali-Honarmand S.: Evaluation of drought stress at vegetative growth stage on the grain yield formation and some physiological traits as well as fluorescence parameters of different bread wheat cultivars. – Acta Biol. Szeged. **59**: 35-44, 2015.

Samarah N.H.: Effects of drought stress on growth and yield of barley. – Agron. Sustain. Dev. **25**: 145-149, 2005.

Touchan H., Hussein M.H., Aldarir A.N. *et al*.: Evaluation of photosynthetic parameters in recombinant inbred lines of barley (*Hordeum vulgare* L.) in relation to grain yield under water-stress conditions. – Arab. J. Arid Environ. **3**: 34-46, 2010.

von Korff M., Grando S., Del Greco A. *et al*.: Quantitative trait loci (QTL) associated with agronomic performance of barley under drought. – Theor. Appl. Genet. **117**: 653-669, 2008.

Wilson J.R., Fischer M.J., Schulze E.-D. *et al*.: Comparison between pressure volume and dew point hygrometry techniques for determining the water relations characteristics of grass and legume leaves. – Oecologia **41**: 77-88, 1979.