

Photosynthetic and yield performance of wild barley (*Hordeum vulgare* ssp. *spontaneum*) under terminal heat stress

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Abstract

Terminal heat stress is one of the major constraints of cereal production. A two-year field investigation was performed to assess the response of *Hordeum vulgare* ssp. *spontaneum* genotypes to terminal heat stress using gas-exchange parameters, photosystem efficiency, proline accumulation, cell membrane leakage, and grain yield traits. Results of analysis of variance revealed the significant effects of heat stress (E), genotype (G), and $G \times E$ on the studied traits. The results of linear regression analysis showed that yield loss was inversely correlated with the maximum quantum yield of PSII photochemistry (F_v/F_m) and chlorophyll content. Path-coefficient analysis revealed that high Chl contents were either directly related to the grain yield or indirectly through the higher net photosynthetic rate and higher F_v/F_m values under high temperatures at the reproductive growth stage. Overall, the adapted wild genotypes exhibited physiological mechanisms capable of sustainably maintaining their yield capacity and plasticity flow, which could be exploited by crossing with cultivated barley to introgress heat tolerance.

Additional key words: abiotic stress; climate changes; genetic diversity, high-temperature stress; photosynthetic capacity; physiology.

Introduction

Thermal stress is a major abiotic stress likely to be worsening under global warming conditions (Bitá and Gerats 2013, Schaubberger *et al.* 2017). Based on climate model predictions, the global mean temperature will rise by 1–4°C above the present temperature by the end of the twenty-first century (Driedonks *et al.* 2016). Effects of heat stress on crops depend on heat duration and intensity, rate of temperature rise, and plant developmental stage (Wahid *et al.* 2007). Furthermore, plant growth and development can be affected by high temperatures due to vulnerability of plant architecture as well as its physiological and reproductive processes (Driedonks *et al.* 2016). The predicted average temperature rise of 3–4°C is expected to reduce crop production in the Middle East by 25–35% (Ortiz *et al.* 2008). Crop yield could, indeed, be affected by heat stress by inducing pollen sterility and seed abortion during the reproductive growth stage (Barnabás *et al.* 2008, Rezaei *et al.* 2010). Reduced crop productivity due to high temperatures during the reproductive stage has been reported in many cereals, such as barley (Klink *et al.* 2014), wheat (Dias *et al.* 2011, Dwivedi *et al.* 2017), and rice (Shi *et al.* 2016).

Despite the economic importance of cultivated barley (*Hordeum vulgare* ssp. *vulgare*) as a major cereal crop

worldwide, little is known about its thermal-tolerance mechanisms. Wild barley, the *H. vulgare* ssp. *spontaneum* L. species, is a widely distributed one (Thormann *et al.* 2016) that is cross-compatible with its immediate descendant, the domesticated barley subspecies. The progenitor of cultivated barley, *H. vulgare* ssp. *spontaneum*, primarily originated from Irano-Turanian and Mediterranean regions before it flowed outward as curtailed stable inhabitants in desert ecosystems (Harlan and Zohary 1966). Phenotypic plasticity in *H. vulgare* ssp. *spontaneum* is regarded as a feature that substantially motivated the spread and persistence of the plants in various environmental conditions across almost all western Iran; hence, it is proposed as a heat and drought-tolerant species (Arzani and Ashraf 2016). Genetic diversity analysis of 51 *H. spontaneum* populations using molecular markers revealed the effective roles of temperature and precipitation as selective pressures in the Fertile Crescent on the adaptation of wild barley (Hübner *et al.* 2009).

In general, sustainability in leaf gas exchanges and photosystem efficiency are directly related to heat tolerance in crop plants (Wahid *et al.* 2007, Bitá and Gerats 2013). Brestic and Živčák (2013) reported that temperature increase to 37°C could lead to reduced stomatal

Received 16 January 2018, accepted 1 June 2018.

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Abbreviations: Chl – chlorophyll; C_i – intercellular CO₂ concentration; F_0 – minimum fluorescence intensity; F_m – maximum fluorescence intensity; F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; MSI – membrane stability index; P_N – net photosynthetic rate.

Acknowledgement: This work was supported by funds from the Iran National Science Foundation (INSF) under Grant No. 96002328.

conductance (g_s). It is well-established that leaf photosynthesis, as the most heat-sensitive metabolic process, can be interrupted under high temperatures (Wang *et al.* 2015). The PSII and the carbon fixation by Rubisco are the prime targets of thermal stress on the plant photosynthetic apparatus (Allakhverdiev *et al.* 2008, Ashraf and Harris 2013, Oukarroum *et al.* 2016). Furthermore, chlorophyll (Chl) fluorescence has been found a reliable physiological parameter not only for measuring PSII efficiency, but also determining damage to the photosynthetic apparatus due to heat stress (Allakhverdiev *et al.* 1997, Allakhverdiev 2011, Jedmowski *et al.* 2015). Maximum quantum yield of PSII photochemistry (F_v/F_m) has been widely applied as a selection criterion for heat-tolerant genotypes in crops (Sharma *et al.* 2012, 2015). The reduced Chl content has been correlated with damage to the thylakoid membrane with the subsequent reduction in photosynthesis due to heat stress (Wang *et al.* 2015). Gupta *et al.* (2015) reported that high temperature at a grain-filling stage would lead to reductions in photosynthetic capacity, Chl content, and grain yield in wheat genotypes. Proline accumulation, which is involved in cellular osmoregulation and protection of cellular structures against increased temperatures by cell water balance conservation and stabilization of biological membranes, is an important adaptive response in crops exposed to high temperatures (Wahid *et al.* 2007, Bitá and Gerats 2013). Accumulation of proline has been reported to increase in response to heat stress in wheat genotypes (Dhyani *et al.* 2013, Truong *et al.* 2017).

Effective exploitation of heat-tolerant genetic resources in breeding crop plants is an urgent priority in the context of genetic erosion and climate change scenarios. At the end of the day, the fulfillment of this goal depends on a number of major factors such as crossability as well as both intra- and inter-genomic chromosome pairing in hybrids of domesticated and wild species. Thus, the use of *spontaneum* wild subspecies (*H. vulgare* ssp. *spontaneum*) seems meaningful as successful achievements in these aspects could likely lead to beneficial outcomes for improving heat-tolerant barley cultivars. The complete genome homology between cultivated barley *H. vulgare* ssp. *vulgare* ($2n = 2x = 14$, HH) and *H. vulgare* ssp. *spontaneum* ($2n = 2x = 14$, HH) as well as adaptation of the wild subspecies to harsh climatic conditions, including

high temperatures, could be quoted as the reasons for the successful hybridization and subsequent gene introgression of the thermal-tolerance attribute. Given the seriousness of global warming that requires enhanced heat tolerance through improved photosynthesis as the main goal in many C_3 crops and considering the limited data available on physiological thermal-tolerance responses in wild barley under field conditions, the current study was performed to (1) investigate the physiological responses of *H. vulgare* ssp. *spontaneum* genotypes to post-anthesis high-temperature stress and (2) determine an adaptation strategy for wild genotypes under field conditions.

Materials and methods

A total of 49 barley genotypes including 45 *H. spontaneum* genotypes collected from western Iran (namely Ilam, Kermanshah, Lorestan, Kordestan, and West Azarbaijan provinces) and four barley cultivars comprising ‘Mona’, ‘Reyhan’, ‘Nosrat’, and ‘Fajr30’ (Table 1S, *supplement*) were grown in two consecutive years (2015–2016 and 2016–2017) at the research farm of Isfahan University of Technology located at Lavark, Najaf-Abad, Iran (40 km south west of Isfahan, 32°32’N, 51°23’E, 1,630 m a.s.l. with a mean annual precipitation of 149 mm). The soil type of the experimental field was silty clay loam, typic Haplargids of the arid tropic, with pH 7.3–7.8.

Under both trial conditions (normal and heat stress), crop management was carried out uniformly with fertilizer application, weed control, and irrigation to avoid drought conditions. All the physiological studies were performed at the grain-filling stage on flag leaves for both conditions.

Table presents details of the sowing dates and phenological stage of the genotypes in the two growing seasons. A square lattice design (7×7) with two replications was used for each of the two sowing dates including October (normal) and January (terminal heat stress). The seeds were planted at 4-m long plots comprising five rows spaced 30 cm apart.

Leaf gas-exchange measurements were accomplished on intact flag leaves at the grain-filling stage. Net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were recorded using an

Temperature variations of experimental site at different growth stages of barley genotypes grown under normal and heat stress conditions.

Time	Year	Temperature [°C]						Relative humidity [%]	
		Minimum		Maximum		Mean		Normal sown	Late sown
		Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown		
Sowing	2015–16	12.4	5.0	21.0	14.8	16.7	9.9	63	65
	2016–17	9.8	-0.6	22.2	14.6	16.0	7.0	40	30
Anthesis	2015–16	5.0	19.2	20.4	29.4	12.7	24.3	35	30
	2016–17	2.8	11.0	19.0	23.4	10.9	17.2	40	57
Maturity	2015–16	14.0	21.6	24.2	35.1	19.1	28.3	57	19
	2016–17	11.0	22.4	27.6	37.6	19.3	30.0	42	9

Sowing dates and phenological stages of 49 barley genotypes grown in the field conditions.

Year	Sowing date		Time to anthesis [d]		Time to maturity [d]	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
2015–2016	23.10. 2015	04.01. 2016	163	123	199	151
2016–2017	23.10. 2016	03.01. 2017	162	121	197	149

open-system portable infrared gas analyzer (*LCA-4 ADC*, Analytical Development Company, Hoddesdon, UK). Measurements were made under constant conditions at temperatures in the range of 25–30°C, a relative humidity of 50–60%, a light intensity in the range of 1,400–1,700 $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$, and a CO_2 concentration of 400 $\mu\text{mol}\text{mol}^{-1}$ from 10:00 to 12:00 h for two consecutive days.

Chl fluorescence parameters were measured in the dark using a Chl fluorometer (*OS-30p*, Opti-Science, London). Minimum (F_0) and maximum (F_m) fluorescence intensity were measured in dark-adapted leaves using leaf clips (*FL-DC*, Opti-Science). In addition, maximum quantum yield of PSII photochemistry (F_v/F_m) was calculated $(F_m - F_0)/F_m$ when all PSII reaction centers were fully oxidized. Chl fluorescence measurements were made on the fully expanded flag leaves of barley plants at the grain-filling stage. All the measurements were conducted on field-grown plants under natural sunlight with PPFD $>1,200\ \mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$ at the temperature in the range of 25–30°C.

Chl and carotenoids (Car) were extracted in acetone 80% according to the standard method of Lichtenthaler (1983) using fresh leaves at the grain-filling stage. The absorbance of the solutions was measured at 663, 646, and 470 nm using a UV/visible spectrophotometer (*U-1800*, Hitachi, Japan). Concentrations (C) were then calculated and expressed as $\text{mg g}^{-1}(\text{FM})$ using the following equations:

$$\text{Chl } a: C_a = 12.21 \times A_{663} - 2.81 \times A_{646} \times V/1,000 \times W$$

$$\text{Chl } b: C_b = 20.13 \times A_{646} - 5.03 \times A_{663} \times V/1,000 \times W$$

$$\text{Car: } C_{x+c} = [(1,000 \times A_{470}) - (1.9 C_a) - (63.14 C_b)] / 214 \times V/1,000 \times W$$

where A is absorbance, V is the volume of acetone (ml), and W is the fresh mass (g).

Proline content was measured according to the ninhydrin method of Bates *et al.* (1973) using fresh leaves at the grain-filling stage. Leaf samples (0.2 g) were homogenized in 10 ml of 3% aqueous sulfosalicylic acid centrifuged for 10 min at 10,000 rpm. This supernatant was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid before being boiled at 96°C for 1 h. After stopping the reaction in an ice bath, the reaction mixture was extracted in 4 ml of toluene before the absorbance of the pink-red upper phase was measured at 520 nm using UV-visible spectrophotometer (*U-1,800*, Hitachi, Japan) against toluene blank. The proline content was finally calculated using the standard curve and expressed in $\text{mg g}^{-1}(\text{FM})$.

Cell membrane stability: Leaf electrolyte leakage was

estimated at the grain-filling stage by taking 0.2 g of fresh leaves in tubes containing 10 ml of double distilled water in a water bath at 40°C for 30 min, before the electrical conductivity of the solution was recorded on a conductivity bridge (C_1). The samples were subsequently boiled at 100°C at a boiling water bath for 10 min and their conductivity was measured on a conductivity bridge (C_2). The membrane stability index was then calculated as $\text{MSI} = [1 - (C_1/C_2)] \times 100$ (Sairam 1994).

Grain yield: Grains were harvested from the three middle rows in the normal (May 2016) and heat stress (June 2017) treatments, and the grain yield was determined and expressed as kg ha^{-1} .

Statistical analysis: The data were subjected to a combined analysis of variance (*ANOVA*) using the GLM procedure of SAS software (*Version 9.3*, SAS Institute 2011). The efficiency of the lattice design was compared (tested) against that of randomized complete block design (RCBD) by calculating the ratio of the lattice mean square of error $\times 100/\text{RCBD mean square error}$ for each trait. The results showed only slight gains in efficiency by using a lattice design rather than the RCBD for all the traits; hence, the RCBD design was used to analyze the data. Mean comparisons were carried out using the Fisher's least-significant difference ($\text{LSD}_{5\%}$) test. Linear regression and path-coefficient analysis were also carried out to determine the relationship between grain yield loss and physiological variables using SPSS software.

Cluster analysis was accomplished based on physiological traits, grain yield, and grain yield loss via the Ward's method as a measure of similarity using SPSS statistics. The optimal number of clusters was estimated by p -value obtained from Hotelling's T -square (T^2) test. Finally, group means obtained from the cluster analysis were compared using the Fisher's least-significant difference ($\text{LSD}_{5\%}$) test.

Results

Combined *ANOVA* results showed that all the traits studied were significantly influenced by the environment (heat stress), genotype, and genotype \times environment interaction (Table 1). However, no significant differences were found between the two study years in the traits studied except for g_s and MSI. There were highly significant variations in the traits studied among the wild genotypes, whereas no significant differences were observed in the Chl content and F_m among the four cultivars (Table 1).

Terminal heat stress caused significant reductions in P_N and g_s but a significant increase in C_i of 49 barley genotypes studied. In contrast to normal conditions, in

Table 1. Combined analysis of variance for gas exchange and chlorophyll fluorescence parameters, chlorophyll and carotenoid content, proline content, membrane stability index, and grain yield in barley genotypes grown normal and heat-stress conditions in 2016 and 2017. Chl – chlorophyll; Car – carotenoids; C_i – intercellular CO_2 concentration; F_0 – minimum fluorescence intensity; F_m – maximum fluorescence intensity; F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; MSI – membrane stability index; P_N – net photosynthetic rate. ns – insignificant; * – significant at $P < 0.05$; ** – significant at $P < 0.01$.

Source of variation	df	P_N [$\mu\text{mol}(\text{CO}_2)$ $\text{m}^{-2}\text{s}^{-1}$]	g_s [$\text{mmol}(\text{H}_2\text{O})$ $\text{m}^{-2}\text{s}^{-1}$]	C_i [$\mu\text{mol mol}^{-1}$]	F_0	F_m	F_v/F_m	Chl [$\text{mg g}^{-1}(\text{FM})$]	Car [$\text{mg g}^{-1}(\text{FM})$]	Proline [$\text{mg g}^{-1}(\text{FM})$]	MSI [%]	Grain yield [kg ha^{-1}]
Environment (E)	1	2,333.3**	0.43**	1,046,698*	13,731**	29,143.8*	0.04**	38.01*	3.8**	138.7**	348,701**	70,489,670**
Year (Y)	1	1.8 ^{ns}	0.006*	1,640.8 ^{ns}	15.52 ^{ns}	2,716.8 ^{ns}	0.0001 ^{ns}	0.18 ^{ns}	0.01 ^{ns}	1.5 ^{ns}	1,009.7**	13,396 ^{ns}
E × Y	1	2.3*	0.0007*	458.6 ^{ns}	10.4 ^{ns}	20,749.7**	0.002**	0.11 ^{ns}	0.04**	0.06 ^{ns}	527.7**	552 ^{ns}
Block (E × Y)	4	41.5**	0.001**	1,739.5 ^{ns}	439.1**	6,421.8**	0.0002 ^{ns}	0.89**	0.1**	1.01**	18.7**	239,502**
Genotype (G)	48	13.9**	0.001**	17,489.3**	624.5**	13,495.7**	0.001**	0.25**	0.04**	1.51**	183**	3,1395.654**
Wild (W)	44	13.5**	0.001**	18,865.8**	636.1**	13,365**	0.001**	0.25**	0.04**	1.59**	192**	2,370,631**
Cultivated (C)	3	16.1**	0.001*	2,386.7**	308.5**	1,868.1 ^{ns}	0.001**	0.03 ^{ns}	0.07**	0.7**	21.3**	580,168**
W vs. C	1	52.8**	0.0002 ^{ns}	35,210**	377**	1,175 ^{ns}	0.0009*	0.9**	0.1**	0.93**	0.08 ^{ns}	641,943**
G × E	48	4**	0.002**	18,598.4**	648.2**	13,103.6**	0.001**	0.11**	0.04**	0.6**	174.4**	304,590**
G × Y	48	0.4 ^{ns}	0.0001 ^{ns}	1,594 ^{ns}	12.70 ^{ns}	1,523.1**	0.0003 ^{ns}	0.08 ^{ns}	0.00003 ^{ns}	0.05 ^{ns}	5.90**	10,249 ^{ns}
G × E × Y	48	0.1 ^{ns}	0.0001 ^{ns}	1,595 ^{ns}	12.93 ^{ns}	1,848.5**	0.0002 ^{ns}	0.05 ^{ns}	0.00003 ^{ns}	0.04 ^{ns}	5.93**	4,312 ^{ns}
Residual	192	0.46	0.0002	1,583	15.7	846.9	0.0002	0.06	0.0009	0.07	3.5	12,369
Coefficient of variation		8.9	19.3	18.9	3.9	6.8	1.9	11.9	5.9	18.7	3.2	8.4

Table 2. Means of gas-exchange and chlorophyll fluorescence parameters, chlorophyll and carotenoid content, proline content, membrane stability index and grain yield in wild and cultivated barley genotypes grown under normal and heat stress field conditions. Chl – chlorophyll; C_i – intercellular CO_2 concentration; F_0 – minimum fluorescence intensity; F_m – maximum fluorescence intensity; F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; MSI – membrane stability index; P_N – net photosynthetic rate. Means in each row under normal or stress conditions followed by the same letter are not statistically different at $P < 0.01$.

Trait	Normal		Heat stress	
	Wild	Cultivated	Wild	Cultivated
P_N [$\mu\text{mol}(CO_2) m^{-2} s^{-1}$]	9.82 ^b	12.10 ^a	5.24 ^a	3.94 ^b
g_s [$\text{mmol}(H_2O) m^{-2} s^{-1}$]	0.11 ^b	0.13 ^a	0.05 ^a	0.02 ^b
C_i [$\mu\text{mol mol}^{-1}$]	162.67 ^a	114.50 ^b	259.58 ^b	290.31 ^a
F_0	92.52 ^b	102.25 ^a	104.93 ^b	107.31 ^a
F_m	427.68 ^b	472.88 ^a	410.80 ^b	451.43 ^a
F_v/F_m	0.780 ^a	0.779 ^a	0.763 ^a	0.716 ^b
Chl content [$\text{mg g}^{-1}(\text{FM})$]	2.45 ^a	2.43 ^a	1.83 ^a	1.40 ^b
Carotenoid content [$\text{mg g}^{-1}(\text{FM})$]	0.62 ^a	0.56 ^b	0.43 ^a	0.41 ^a
Proline content [$\text{mg g}^{-1}(\text{FM})$]	0.86 ^a	0.87 ^a	2.11 ^a	1.81 ^b
MSI [%]	88.00 ^a	86.99 ^a	29.96 ^a	23.09 ^b
Grain yield [kg ha^{-1}]	1,507.11 ^b	4246.00 ^a	893.23 ^b	2,139.14 ^a

which cultivated barley was superior with respect to P_N and g_s , the mean values of P_N and g_s were higher in the wild genotypes than in the cultivated ones under heat stress treatment (Table 2). In addition, the photosynthetic rate ranged from 3.24 to 8.44 $\mu\text{mol}(CO_2) m^{-2} s^{-1}$ in the wild genotypes but from 2.21 to 5.68 $\mu\text{mol}(CO_2) m^{-2} s^{-1}$ in the four barley cultivars exposed to heat stress. The mean enhancement found in C_i due to heat stress was 175.81 $\mu\text{mol mol}^{-1}$ (60%) in the cultivated barley, but it was only 96.91 $\mu\text{mol mol}^{-1}$ (37%) in the wild genotypes tested. The results of linear regression analysis between P_N and the grain yield loss in 45 wild genotypes indicated that the two traits were strongly negatively correlated ($R = -0.63^{**}$) under heat stress conditions.

Chl fluorescence parameters were significantly influenced by heat stress (Table 1). The values of F_m and F_v/F_m declined, while F_0 increased as a result of heat stress. Although the genotypes studied (wild + cultivated) exhibited significant variations in their Chl fluorescence parameters, the cultivated genotypes did not vary significantly

in their F_m (Table 1). Wild barley genotypes showed higher variations (0.723–0.798) in their F_v/F_m under heat stress conditions than that of the barley cultivars (0.700–0.722). Accordingly, the cultivated genotypes experienced greater reductions in F_v/F_m under heat stress than that of the wild ones (Table 2). In the current study, wild genotypes number 1, 2, 9, and 22 exhibited the highest F_v/F_m values (0.798, 0.787, 0.784, and 0.780, respectively) under heat stress. Moreover, linear regression analysis results showed a strongly negative correlation ($r = -0.84^{**}$) between F_v/F_m and the grain yield loss (Fig. 1).

Total Chl and Car content underwent a significant decrease under heat stress as compared with normal conditions (Table 2). Total Chl content ranged from 1.54 to 2.32 $\text{mg g}^{-1}(\text{FM})$ in the wild genotypes and 1.32–1.48 $\text{mg g}^{-1}(\text{FM})$ in the cultivated ones. Heat stress affected greatly total Chl in the cultivated genotypes when compared to the wild ones (Table 2). A high positive correlation was also found between total Chl and P_N under heat stress ($r = 0.77^{**}$), while the total Chl content and grain yield loss exhibited a negative relationship ($r = -0.83^{**}$) under heat stress (Fig. 2).

Terminal heat stress led to significant ($P < 0.01$) increases in the leaf proline content (Table 1) ranged from 1.29 to 3.64 $\text{mg g}^{-1}(\text{FM})$ in wild barley and 1.64–2.35 $\text{mg g}^{-1}(\text{FM})$ in cultivated ones. Compared to the cultivated genotypes, the wild ones displayed enhancements in their proline accumulation by 60% due to heat stress (Table 2). The highest proline accumulation was observed in the wild genotypes including 1, 4, 8, and 21.

Mean of MSI values in the barley genotypes decreased significantly under heat stress (Table 1). MSI values ranged from 21.1 to 56.7% among the wild barley genotypes and from 22.3 to 25.4% among the cultivated ones under heat stress. The mean comparisons showed that MSI values in the wild genotypes under heat stress were higher than those

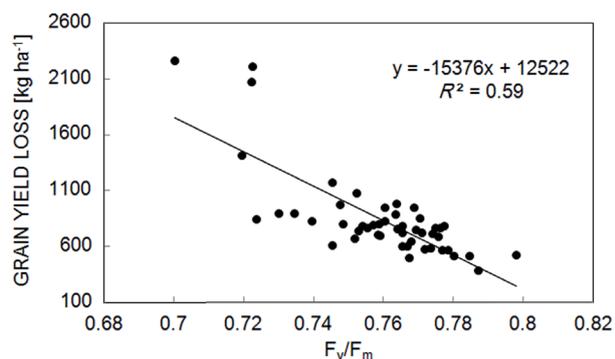


Fig. 1. Relationship between grain yield loss and maximum quantum yield of PSII (F_v/F_m) of 49 barley genotypes under heat stress conditions.

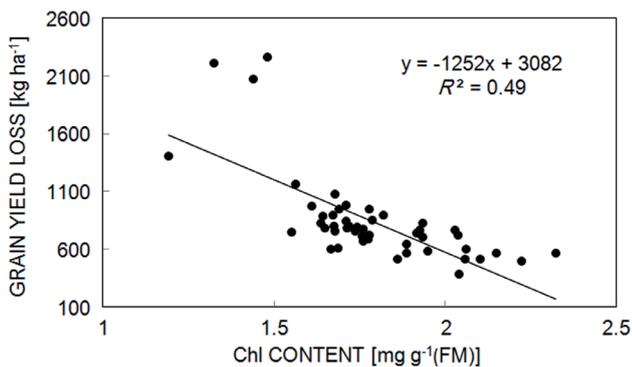


Fig. 2. Relationship between grain yield loss and chlorophyll (Chl) content of 49 barley genotypes under heat stress conditions.

in the four barley cultivars studied (Table 2). Moreover, the wild genotype 1, 2, and 41 with MSI reductions of 32, 36, and 37%, respectively, under heat stress exhibited the least membrane injury among the genotypes studied. A positive correlation was found between MSI and P_N under heat stress ($r = 0.73^{**}$). Path coefficient analysis revealed both direct and indirect effects of physiological variables on the grain yield (Table 3). The results also showed that the total Chl content exhibited the maximum negative direct effect (-0.46) on the grain yield loss in the wild barley genotypes followed by P_N (-0.35) and F_v/F_m (-0.22) under high temperature.

Cluster analysis of the data obtained on the plants exposed to heat stress classified the genotypes into two groups, at a low Euclidean distance, which exactly corresponded to the two wild and cultivated barley subspecies (Fig. 3). The 45 wild genotypes were further divided into three groups at a high Euclidean distance, which comprised the genotypes 11, 12, and 22. The cultivated barley genotypes exhibited much higher grain yield losses than that of the wild ones. Eleven wild barley genotypes categorized in the first group exhibited the highest values of P_N , g_s , and proline accumulation, MSI, grain yield, and grain yield loss among the wild genotypes. On the other hand, four cultivated genotypes exhibited the highest grain yield losses which might result from their lowest values of P_N , g_s , F_v/F_m , and total Chl content.

Table 3. Direct and indirect effects of photosynthetic rate, stomatal conductance, F_v/F_m , chlorophyll and carotenoid contents, proline content and membrane stability index on grain yield loss of wild barley genotypes under heat stress during grain-filling stage. Chl – chlorophyll; Car – carotenoids; F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; MSI – membrane stability index; P_N – net photosynthetic rate.

Trait	Direct effects	Indirect effects							Total
		P_N [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$]	g_s [$\text{mmol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$]	F_v/F_m	Chl [$\text{mg g}^{-1}(\text{FM})$]	Car [$\text{mg g}^{-1}(\text{FM})$]	Proline [$\text{mg g}^{-1}(\text{FM})$]	MSI [%]	
P_N	-0.35		-0.06	-0.16	-0.21	-0.11	-0.13	-0.19	-0.63
g_s	-0.12	-0.02		-0.01	-0.006	-0.008	-0.02	-0.002	-0.18
F_v/F_m	-0.22	-0.09	-0.02		-0.10	-0.03	0.01	-0.04	-0.59
Chl	-0.46	-0.27	-0.02	-0.23		-0.22	-0.06	-0.13	-0.66
Car	0.20	0.06	0.01	0.03	0.10		0.004	0.05	-0.18
Proline	0.16	0.05	0.03	-0.008	0.02	0.003		0.04	0.03
MSI	-0.002	-0.001	-0.00003	-0.0004	-0.0005	-0.0004	-0.0005		-0.28

Discussion

High temperatures cause adverse physiological modifications and responses in plants including their photosynthesis, respiration, and interacting processes, especially in the reproductive organs. Winter small-grain cereals are predominantly affected by terminal heat stress, implying that temperature generally rises during the grain development period. Wild relatives of cultivated crops naturally furnish an indispensable gene pool for tolerance to abiotic stress (Arzani and Ashraf 2016). In their study of population structure, Hübner *et al.* (2009) found that temperature and aridity gradients served as major selective pressures in the adaptation of wild barley (*H. vulgare ssp. spontaneum*). This is confirmed by the on-site observations made by the second author of the present article during his germplasm explorations in five provinces in western Iran (see also Arzani and Ashraf 2016).

Photosynthesis as the most sensitive physiological process is significantly affected by heat stress (Wang *et al.* 2015). The reductions in P_N and g_s due to high temperature observed in the current study are generally consistent with those reported elsewhere on bread wheat (Gupta *et al.* 2015, Dwivedi *et al.* 2017). Under high temperatures, the ability to sustain leaf gas exchange is directly associated with heat tolerance in all plant species (Bitá and Gerats 2013). The wild genotypes maintained their g_s values under heat stress better than did the cultivated ones, indicating that the $\text{H}_2\text{O}/\text{CO}_2$ exchange in the leaves of *H. vulgare ssp. spontaneum* is relatively less disturbed by high temperatures. It is argued that the increase in C_i under stress conditions may be more influenced by photosynthesis than by g_s (Feng *et al.* 2014). Since the initial targets of thermal damage are PSII and Rubisco, a large portion of the negative effect of high temperature on net photosynthesis is associated with decreased carbon fixation followed by net carbon assimilation in plants. Consequently, photosynthetic constraints due to high temperatures during the reproductive stage cause strict limitation on the availability of assimilates to the grains, thereby leading to reduced grain yield (Dwivedi *et al.* 2017). Nevertheless, the inverse relationship between photosynthetic rate and grain yield loss in our study can

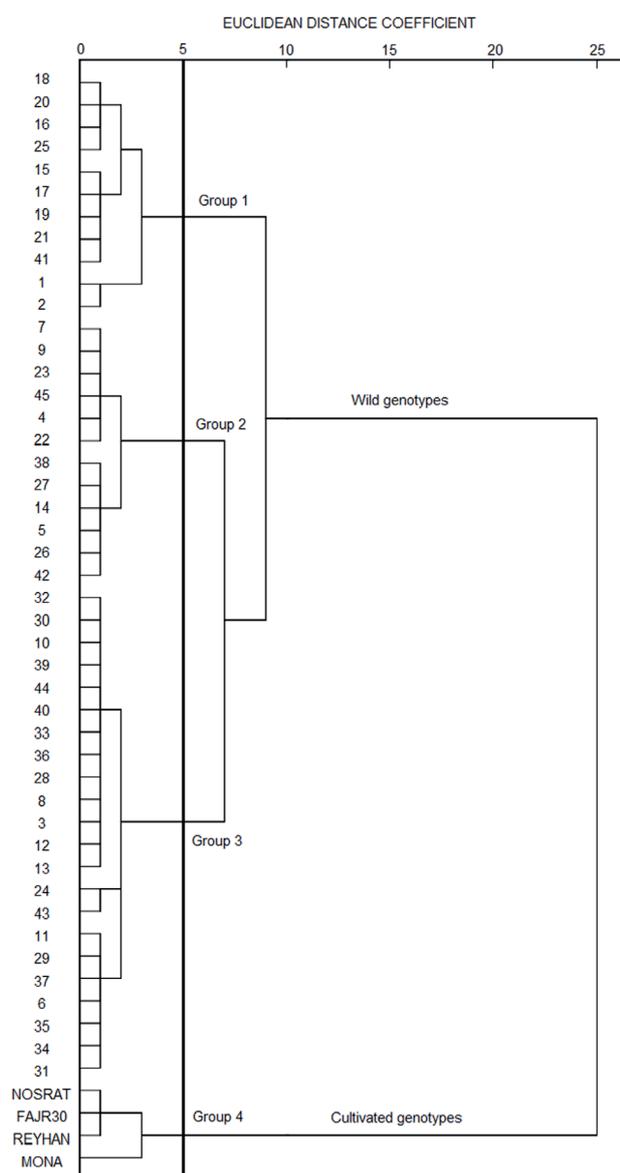


Fig. 3. Dendrogram generated for barley genotypes (Table 1S) based on grain yield loss, physiological traits and grain yield in barley genotypes under heat stress using *Ward's* method.

be explained by the negative effect of reduced P_N on grain yield under elevated temperatures.

Overall, the reduced photosynthetic electron transport under high temperatures can be attributed to the thermal stability of PSII (Brestic and Živčák 2013). According to our results, F_m decreases under heat stress, while F_0 increases. A similar trend was also observed in a previous study of ten varieties of barley (Oukarroum *et al.* 2016). Kalaji *et al.* (2011) suggested that the significant reduction in F_m under heat stress is most likely caused by the inactivation of the oxygen-evolving complex in the PSII complex. The observed reduction in F_v/F_m due to terminal heat stress is consistent with that reported by Sharma *et al.* (2015) for wheat cultivars exposed to heat stress during their grain-filling stage. The F_v/F_m value is generally

considered as a reliable criterion for evaluating the extent of damage caused by a given stress at the PSII complex, and the rate of photoprotective responses induced by the photosynthetic activity of the plants (Roháček *et al.* 2008, Brestic and Živčák 2013). The strong inverse relationship observed in the current study between F_v/F_m and grain yield loss further supports for the idea that thermal stress may hamper the efficient absorption of light, the energy flow of the light captured, and the reaction center of photosynthetic systems in using light. Evaluating 30 winter wheat genotypes, Brestic *et al.* (2012) reported that the PSII thermostability of genotypes was associated with their origin and acclimation capacity. In addition, it has been shown that the ability to maintain an efficient photoprotection under high temperatures is crucial for heat tolerance (Sharkey and Zhang 2010, Brestic *et al.* 2016). Congruently, it is unfavorable that the cultivated barley genotypes are poorly protected and, thus, vulnerable to the stress when compared with the wild barley genotypes.

Leaf Chl, a vital pigment involved in light-harvesting and energy dissipating functions, was found to be negatively influenced by heat stress. It is interesting to note that although both the cultivated and wild barley genotypes exhibited close average values of the total Chl content under normal conditions, heat stress led to significantly lower Chl degradation in the wild barley than that in the cultivated ones. The strong inverse relationship observed between the total Chl and grain yield loss under heat stress provides further evidence that leaf Chl content can be exploited as an indicator of either plant photosynthetic capacity or its yield potential under thermal-stress conditions (Gupta *et al.* 2013, Wang *et al.* 2015). The reduced Chl content under heat stress could be explained by the reduced expression of Chl synthase (*CS*) gene as reported by Saha *et al.* (2016) for the C_4 plant species *Setaria viridis*. Similar to our observations in barley, the reduced Car under heat stress (at temperatures above 35°C) was reported for bread wheat (Sarkar *et al.* 2016) and durum wheat (Dias *et al.* 2011). The high correlation observed between Chl content and P_N under high temperatures may be caused by both the downregulation of Chl synthesis in the chloroplast and the accumulation of reactive oxygen species, which lead to Chl degradation in plants and the subsequently reduced P_N (Dwivedi *et al.* 2017).

The greater proline accumulation in the leaves of *H. vulgare* ssp. *spontaneum* wild genotypes observed in this study is in agreement with the finding of Gosavi *et al.* (2014) who reported higher proline contents accumulated in wild sorghum genotypes; such germplasm could be exploited in breeding programs for introgressing heat tolerance into cultivated sorghum.

Under heat stress, permeability enhancement and changes in cell differentiation and elongation as well as expansion of cellular membranes or thylakoids cause injuries to such cellular processes as photosynthesis and respiration *via* alterations in chloroplast proteins and the performance of ion channels (Kalaji *et al.* 2011, Bitá and Gerats 2013). Thus, high temperatures increase the fluidity of membrane lipids and the subsequent loss of cellular membrane stability indicates the vital role of lipid

membranes in response to heat stress in crops (Horváth *et al.* 2012). Our results were consistent with those of Dwivedi *et al.* (2017) who observed significant declines in the MSI values of both late and very late sown wheat cultivars due to high temperatures. In addition, linear regression results revealed not only the superiority of wild genotypes over domesticated ones with respect to steady maintenance of cell membrane integrity but also the perceived contribution of wild genotypes under heat stress. Nevertheless, changes in the environment bring about changes in the lipid composition of plasma membranes with tremendous effects on the functionality, sustained fluidity, and integrity of membranes, especially under stress conditions (Yeilaghi *et al.* 2012). The impact of heat stress on the composition of membranes, therefore, appears to be more damaging than other abiotic stresses because the fluidity of membranes composed of straight-chain fatty acids can be easily disrupted by heat.

Heat tolerance is a complex trait related to the physiological and molecular bases of plant cells (Allakhverdiev *et al.* 2008). It seems difficult to dissect the photosynthetic regulatory mechanisms used by the wild barley to resist high temperatures (Brestic and Živčák 2013). However, the results of the current study suggest that the most important operations under heat stress is the control of the expression of genes involved in maintaining the functional properties of PSII, Chl contents, and cell membrane structure. In addition, it seems that the PSII thermostability and the stable cellular membrane structure observed in wild genotypes under heat stress can be related to their heat-tolerance genes including heat-shock proteins (Hsps) which are both genetically and epigenetically regulated. Regarding domestication, the barley cultivars investigated exhibited higher grain yield means than that of the wild ones under both environmental conditions. Consequently, thermally tolerant genotypes were characterized by the lowest grain yield loss as a heat tolerance index followed by the highest values for P_N , F_v/F_m , MSI, Chl content, and proline content under terminal heat stress. The path-coefficient analysis carried out in this study revealed the relative importance of physiological traits for grain yield loss in the wild genotypes under terminal heat stresses at the grain-filling stage. Brestic *et al.* (2016) stated that high temperatures had a greater impact on CO₂ assimilation capacity and photoprotection responses in wheat Chl *b*-deficient mutant lines. Our results showed that, under high temperatures, the higher Chl content in the wild genotypes affected grain yield preservation at the grain-filling period either directly or indirectly through higher P_N and F_v/F_m . It is revealed that the performance of wild genotypes at the grain-filling stage is significantly associated with their maintenance of the functional photosynthetic apparatus under heat stress. Wang *et al.* (2015) used cluster analysis based on photosynthetic rates and grain yield to identify heat-tolerant wheat cultivars. Based on the grain yield and photosynthetic performance observed in the current study, 11 out of the 45 wild barley genotypes in the first group could be selected as superior wild genotypes for adaptation to climate changes brought about by heat. Furthermore,

it is worth noting that the superior genotypes happened to originate from the southernmost province (*i.e.*, Ilam) which is characterized by the hottest temperatures among the five provinces selected as the seed-collection sites.

Conclusion: The increasing temperature as a consequence of global climate change and the limited variability for heat tolerance in crop cultivars require reliable criteria to be established for screening thermal-tolerant wild germplasm. Given its wide distribution due to its high genome plasticity and, in particular, its heat-stress-adapted germplasm from hot climatic regions, *H. vulgare* ssp. *spontaneum* may supply the ideal material for investigating climatic adaptations. Under field conditions, thermal-tolerant genotypes of *H. spontaneum* were found to employ various strategies to alleviate damages to their chlorophyll, cell membrane integrity, PSII, and photosynthetic rate. Considering the drastic effect of the global warming on the photosynthetic apparatus, the study of thermal-tolerant genotypes of wild barley can contribute tremendously to our understanding and improvement of carbon sequestration in cultivated barley by employing genetics and breeding tools.

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