

# Genetic Diversity and Potential High Temperature Tolerance in Barley (*Hordeum vulgare*)

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**Abstract** Heat stress is an important abiotic stress causing the major threat to the growth and development of most crop plants. A panel of 326 barley genotypes comprises of 320 wild barley accessions and six local cultivars were evaluated for days to heading (DTH), days to flowering (DTF), number of tillers per plant (NoT), plant height (PH), Chlorophyll content (CC), spike length (SL), thousand kernel weight (TKW) and single plant yield (SPY) under optimum sowing and heat-stressed conditions. All measured traits exhibited highly significant differences both among evaluated genotypes and between the optimum sowing and the heat-stressed conditions. Plants exposed to high temperatures flowered earlier. A drastic reduction in morphological and yield contributing traits, i.e., PH, NoT, SL, TKW, SPY and CC under heat stress conditions was observed. Cluster analysis revealed two distinct groups based on heat stress tolerance with substantial diversity among the heat tolerant genotypes.

**Keywords:** barley, *Hordeum*, heat-stress, temperature stress, cluster analysis

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## 1. Introduction

Barley (*Hordeum vulgare*), which is an ancient cereal grain originating in the Fertile Crescent of the Middle East [1], is one of the world's most important crops with uses ranging from food and feed production representing the fourth most abundant cereal in both area and harvested tonnage. Barley is considered an excellent crop for physiological and genetic studies because of i) it is an annual species with a relatively short life cycle, ii) its staple crop, particularly in Mediterranean regions, iii) its diploid nature with a relatively small number of large chromosomes, iv) it has a high degree of self-fertility and it has cross compatibility with species within the primary gene pool, v) it presents a high genetic and morphophysiological diversity [2,3]. Cultivated barley is among the world's earliest domesticated crop species and is widely adapted to diverse environmental conditions [4] (Purugganan and Fuller 2009).

*Hordeum vulgare* subsp. *spontaneum* is the progenitor of cultivated barley (*Hordeum vulgare* subsp. *vulgare* L.). Since wild and cultivated barley share a common genome and are cross compatible, useful alleles in the wild progenitor are easily available for crop improvement [5]. Since, due to selection processes the degree of genetic diversity in many breeding programs is determinate [6], a wide and representative collection of germplasm is essentially required to enrich genetic diversity. As a consequence of the interfertility between wild and cultivated barley, increasing the level of genetic diversity of barley using the wild ancestor as a progenitor in crosses

with the cultivated barley is of great importance [2,6,7,8,9,10,11]. Almost about 40% of wild barley alleles are represented in cultivated barley [8]. There are quite sophisticated evidences that the wild ancestor is a source of favorable alleles for a number of agronomic and physiological traits such as plant height, root morphology, grain yield and tolerance to abiotic stresses [2,7,8,10,11].

In the light of climate changes and global warming, which indicate a gradual increase in the atmosphere temperature and an increase in the frequency and extent of heat stress periods in the near future for the Mediterranean [12], high temperature is considered as a key stress factor with high potential impact on crop yield. Furthermore, high temperatures are also commonly accompanied by extended periods of drought, and the simultaneous occurrence of these two abiotic stresses under field conditions could have a negative impact on crop productivity worldwide and have terrible consequences on food security [13,14].

Heat stress is an important abiotic stress causing substantial crop losses worldwide. Therefore, understanding the genetic control of heat tolerance is of a great importance for the application of marker-assisted breeding in the development of cultivars with improved tolerance. Heat tolerance is a complex polygenic trait involving epistatic interactions among loci and powerful genotype × environment interactions. Numerous genetical and physiological studies have been performed to elucidate the genetic basis of heat tolerance in barley [14,15,16]. Heat shock proteins are low-molecular-weight proteins which are generally produced only in response to environmental stress, particularly high temperature and

belong to a larger group of molecules called chaperones [18]. Heat shock proteins have a role in stabilizing the structure of other proteins and have been reported to serve as molecular chaperones that participate in adenosine triphosphate-dependent protein unfolding or assembly/disassembly reactions and prevent protein denaturation during stress [19].

Flowering time is a determinant developmental stage of crop adaptation to the environment, particularly when the growing season is restricted by terminal high temperatures. As early maturity helps the crop to avoid the period of stress, the development of short-duration cultivars is the most efficient strategy for minimizing yield loss from terminal heat stress in low-value cropping systems [20]. However, yield is generally correlated with the length of the growing season under favorable growing conditions, and any decline in crop duration below the optimum would lead to substantial reduction in crop productivity [21].

The present study aimed to identify barley heat tolerant accessions to increase the level of genetic diversity of cultivated barley and as a prospective goal to employ association mapping analysis to investigate the association between molecular markers and heat stress related traits in a panel of 326 barley accessions.

## 2. Materials and Methods

### Plant material and experiments

A panel of 320 six-row spring barley accessions (*Hordeum vulgare* subsp. *spontaneum*) collected from 74

different countries (Suppl. Table 1) provided by National Small Grains Germplasm Research Facility, USDA, ARS, Idaho, USA and 6 barley (*Hordeum vulgare* subsp. *vulgare*) cultivars were included in the current study. Experiments were conducted in 2014/2015 at Assiut University Experimental Farm (lat 27° 03' N, long 31° 01' and alt 70 m asl). Accessions were sown in the field with plant to plant distance of 8-10 cm and row to row distance of 30 cm. The experiments were arranged in a split-plot design with three replications (10-12 plants per replicate), heat stress treatments assigned to main plots and accessions to sub-plots. Accessions were sown in two sowing dates: i) the optimum sowing date (OSD) on November 17, 2014, and ii) the heat stress sowing date (HSD) on February 7, 2015 where plants have experienced terminal high temperature during anthesis (Figure 1). All standard agronomic practices were applied during the whole season.

### Phenotypic Evaluation

Data was recorded on 8 phenotypic, agronomic and physiological traits including; days to heading (DTH; date when 50 % of plants have begun heading), days to flowering (DTF; date when 50 % of plants have begun flowering), number of tillers per plant (NoT), plant height (PH; height of main stem at maturity), spike length (SL; length from neck node to the tip of spike at maturity), thousand kernel weight (TKW) and single plant yield (SPY). Leaf chlorophyll content was measured using portable chlorophyll meter (SPAD502, Japan) at the flowering stage.

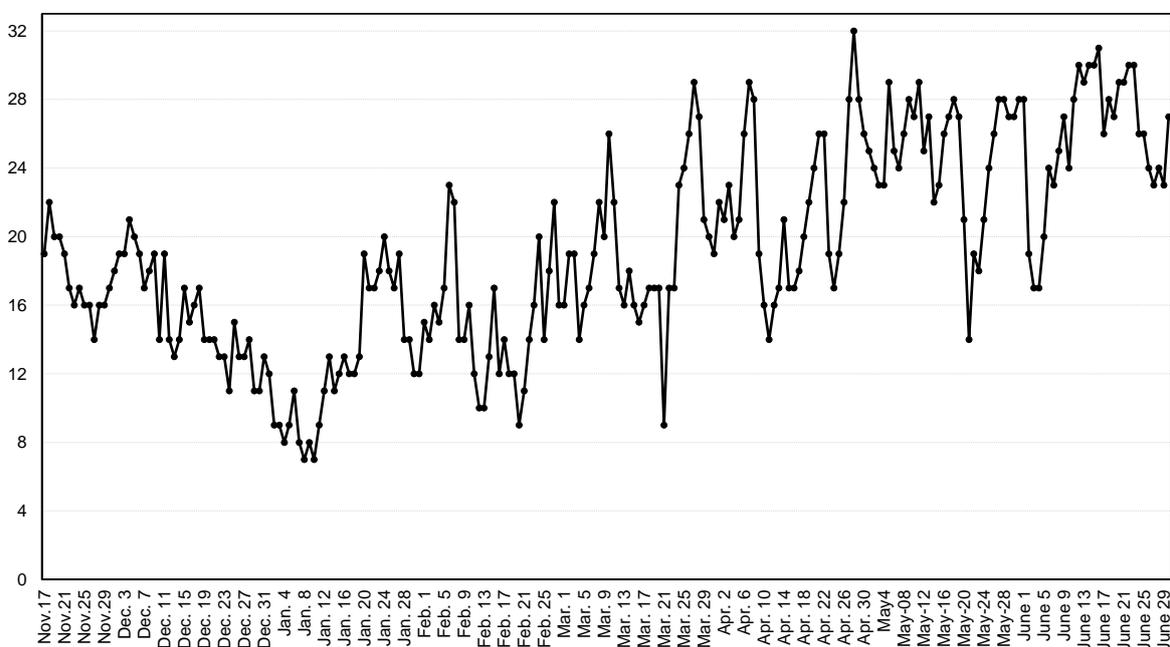


Figure 1. Average day and night temperatures in the field from November 17, 2014 to June 30, 2015

### Statistical Analysis

Analysis of variance (ANOVA), Fisher's least significant difference (LSD) and Pearson correlation coefficients (R) among measured traits were performed with Proc Mixed of SAS package version 9.2 [22]. For cluster analysis, heat tolerance indices (HTIs) were

calculated for all studied traits by dividing the trait value under heat stress by the trait value under control. The Euclidean distance matrix with un-weighted pair-group method based on arithmetic averages (UPGMA) in the software NTSYS-pc ver 2.1 [23] was implemented to develop a dendrogram. The output was analyzed using an agglomerative hierarchical clustering method with complete linkage strategy. Data was subjected to analysis

to produce a matrix of dissimilarity values and the phenotypic distance between each pair of accessions was estimated as Euclidean distance.

### 3. Results

#### Phenotypic Evaluation

All measured phenotypic traits exhibited highly significant differences both among evaluated accessions and between the optimum sowing date (OSD) on November 17, 2014 and the heat stress sowing date (HSD) on February 7, 2015 (Table 1). A continuous phenotypic variations in all measured traits were found (Figure 3: Suppl. Table 2 and Table 3), indicating a quantitative inheritance. The ANOVA revealed highly significant genotype  $\times$  environment interaction (Table 1). Under heat stress, sowing date only 214 accessions out of the 326 accessions were flowered and pursued until maturity.

**Table 1. Analysis of variance (ANOVA) of evaluated traits for the 326 barley genotypes under optimum sowing and heat-stressed conditions**

Trait	DTH	NoT	PH	SL	TKW	SPY
	F Value	F Value	F Value	F Value	F Value	F Value
Genotypes (G)	10645**	23465**	86756**	4533.8**	2345**	4781**
Heat-stress (H)	209.4**	31.56**	67.98**	30.75**	27.10**	17.25**
G $\times$ H	214.10**	33.56**	63.76**	27.98**	19.67**	12.86**

\*\*; highly significant differences ( $P \leq 0.01$ )

#### Days to heading (DTH)

The number of days to heading (DTH) was recorded as 50% of plants have begun heading. DTH exhibited highly significant differences between accessions, environments and the interaction as well. DTH was significantly decreased under heat stress sowing date with an average of 76.74 days (Suppl. Table 2). Under the optimum sowing, the accession S 7244 has the lowest number of days to heading (59 days), while the accession Forjara has the highest number of days to heading (119). Under the heat stress sowing date, the accession No. 7421 was the earliest to heading (61 days), meanwhile the accession QB 138.28 was the latest to heading (105 days) (Suppl. Table 3).

#### Days to flowering (DTF)

The number of days to flowering (DTF) was recorded as 50 % of plants have begun flowering. Days to flowering was highly significantly affected by either the accessions, sowing date and the interaction. Heat stress sowing date led to a significant delay in flowering time (Suppl. Table 2). Under the optimum sowing the accession Reids Triumph flowered earlier (65.67 days), while the accession Jawowas the latest in flowering (125.67). Under the heat stress sowing date the accession No. 4147 exhibited the lowest number of days to flowering of 70 days, meanwhile the accession Criolla Blanca exhibited the highest number of days to flowering of 114 days (Suppl. Table 3).

#### Number of tillers/ plant (NoT)

Number of tillers per plant was significantly influenced by accessions, sowing date and the interaction (Table). Number of tillers was significantly reduced under heat stress sowing date from an average of 25.22/ plant to 7.04/ plant (Suppl. Table 2). The lowest number of tillers (6 tillers/ plant) produced by the accession No. 1230-2 and the highest number of tillers (6 tillers/ plant) was produced by the accession HOR 492 under the optimum sowing. Meanwhile, under heat stress conditions the accession Albany produced the lowest number of tillers (2 tillers/ plant) and the accession No. 4064 produced the highest number of tillers (14 tillers/ plant) (Suppl. Table 3).

#### Plant height (PH)

Plant height (PH) was measured as the height of main stem at maturity. Plant height was significantly decreased under heat stress conditions as tested by ANOVA with an average of 53.07 cm (Table 1). Under the optimum cultivation conditions the accession Arbi exhibited the lowest plant height (58.00 cm), while the accession MOR 7/5 exhibited the highest plant height of 148.33 cm. Under heat stress conditions superiority in plant height was recorded to CI7498 (80 cm) meanwhile the lowest plant height (25 cm) was produced by NB-1003-3 (Suppl. Table 2 and Table 3).

#### Spike length (SL)

Spike length (SL) was measured as the length from neck node to the tip of spike at maturity. Heat stress conditions led to a significant decrease in spike length. The average spike length was reduced from 8.16 cm under optimum conditions to 6.89 cm under heat stress conditions (Suppl. Table 2). The shortest spike (4.00 cm) produced by the accession No. 2824 while the longest spike (14.00 cm) was produced by the accession N90B under the optimum sowing. Meanwhile, under heat stress conditions the accession No. 2867 produced the shortest spike (3.00 cm) while the accessions Carre 42 and B-112 produced the longest spikes (11.00 cm) (Suppl. Table 3).

#### Thousand kernel weight (TKW)

The average weight of thousand kernel was significantly reduced under heat stress conditions from 37.42 gm to 42.03 gm (Suppl. Table 2). Under optimum conditions, superiority was recorded to Cenad 345 with an average 1000KW of 62.00 gm, while the accession QB 138.28 produced the lowest 1000KW (17.90 gm). Under heat stress condition the superiority (57.70 gm) was achieved by H-2227, while the lowest value (15.80 gm) was recorded to Akyurek 32 (Suppl. Table 3)

#### Single plant yield (SPY)

Single plant yield was estimated as an average of the yield of three individual plants in each replicate. AS expected, single plant yield exhibited a highly significant reduction under heat stress condition. The average plant yield was reduced from 56.96 gm/ plant under optimum conditions to 18.34 gm/ plant under heat stress conditions (Suppl. Table 2). Accession H-2227 produced the lowest plant seed yield (14.67 gm/ plant) under optimum

conditions, while accession Kamaore 1 produced the highest seed yield (133.53 gm/ plant). Under heat stress conditions, accession CI7503 was superior with an

average seed yield of 38.14 gm/ plant while accession Ensenada produced the lowest seed yield (7.08 gm/ plant) (Suppl. Table 3).

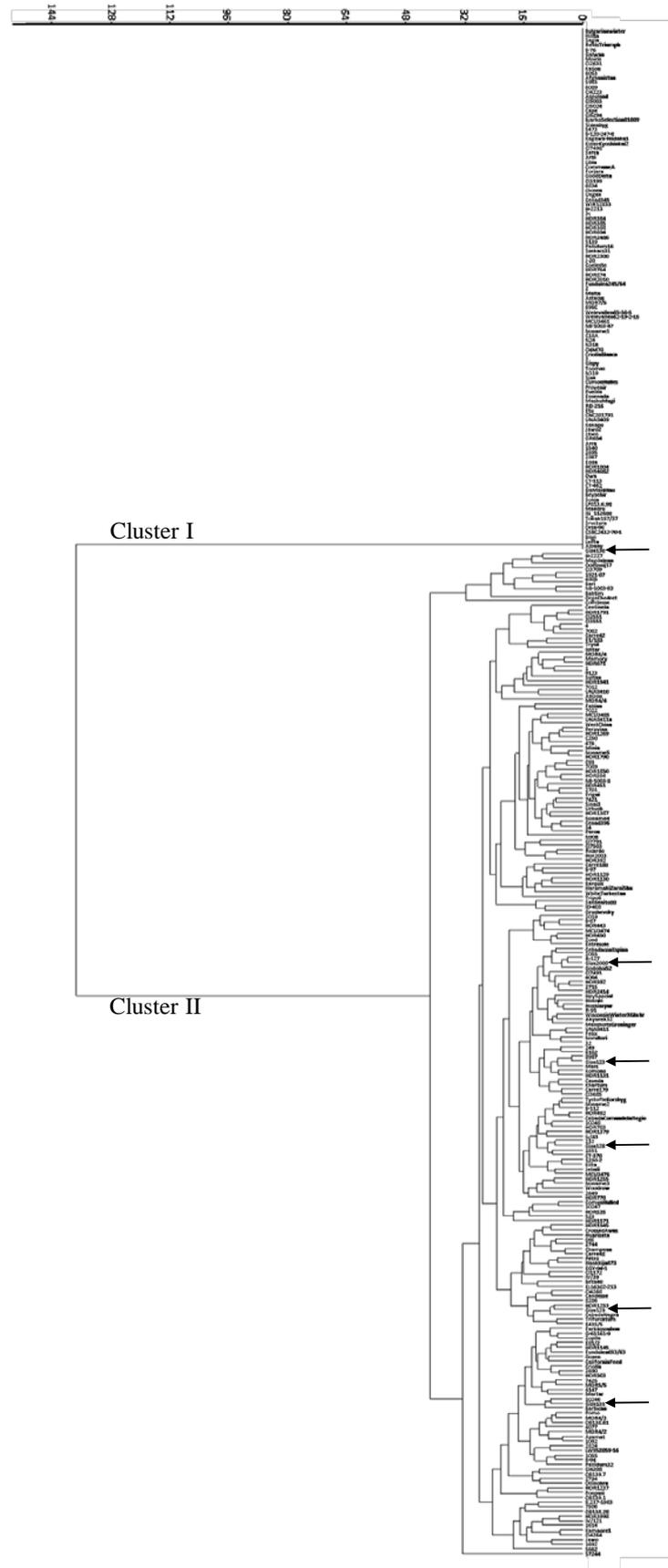


Figure 2. Dendrogram generated by UPGMA cluster analysis showing relationship among 326 barley genotypes based on Nei and Li similarity estimate [24]. Arrows indicated the six barley cultivars

## Leaf chlorophyll content (CC)

Leaf chlorophyll content was measured using portable chlorophyll meter (SPAD502, Japan) at the flowering stage. Heat stress conditions led to a highly significant decrease in leaf chlorophyll content (Table 1). Under optimum sowing conditions the accession No. 4064 exhibited the highest chlorophyll content (58.50), while

the accession Ricardo exhibited the lowest chlorophyll content (28.30). Under heat stress conditions the accession HOR 556 exhibited the highest value of chlorophyll content of 41.50, while the accessions Pallidum 32 and Beysehri exhibited the lowest chlorophyll content values of 11.30 (Suppl. Table 2 and Table 3).

**Table 2. Pearson correlation coefficient (R) calculated between studied traits under optimum sowing conditions (OSD) and heat-stressed conditions (HSD) in the 326 barley accessions**

Trait		DTH	DTF	NoT	CC	PH	SL	TKW
DTF	OSD	1.00**						
	HSD	1.00**						
NoT	OSD	0.592**	0.432**					
	HSD	0.872**	0.875**					
CC	OSD	0.269	0.216	0.133				
	HSD	0.925**	0.928**	0.811**				
PH	OSD	0.554**	0.478**	0.283	0.109			
	HSD	0.926**	0.930**	0.342**	0.917**			
SL	OSD	0.102	0.402**	0.128	0.293	0.0176		
	HSD	0.903**	0.907**	0.835**	0.882**	0.952**		
TKW	OSD	0.438**	0.482**	0.499**	0.870**	0.077	0.254	
	HSD	0.921**	0.926**	0.862**	0.893**	0.311**	0.299**	
SPY	OSD	0.789**	0.761**	0.718**	0.522**	0.157	0.483**	0.496**
	HSD	0.845**	0.850**	0.789**	0.845**	0.166	0.849**	0.907**

\* and \*\* are the significance levels at  $P < 0.05$  and  $P < 0.01$ , respectively  
OSD: optimum sowing conditions and HSD: Heat stressed conditions

## Cluster analysis and correlation

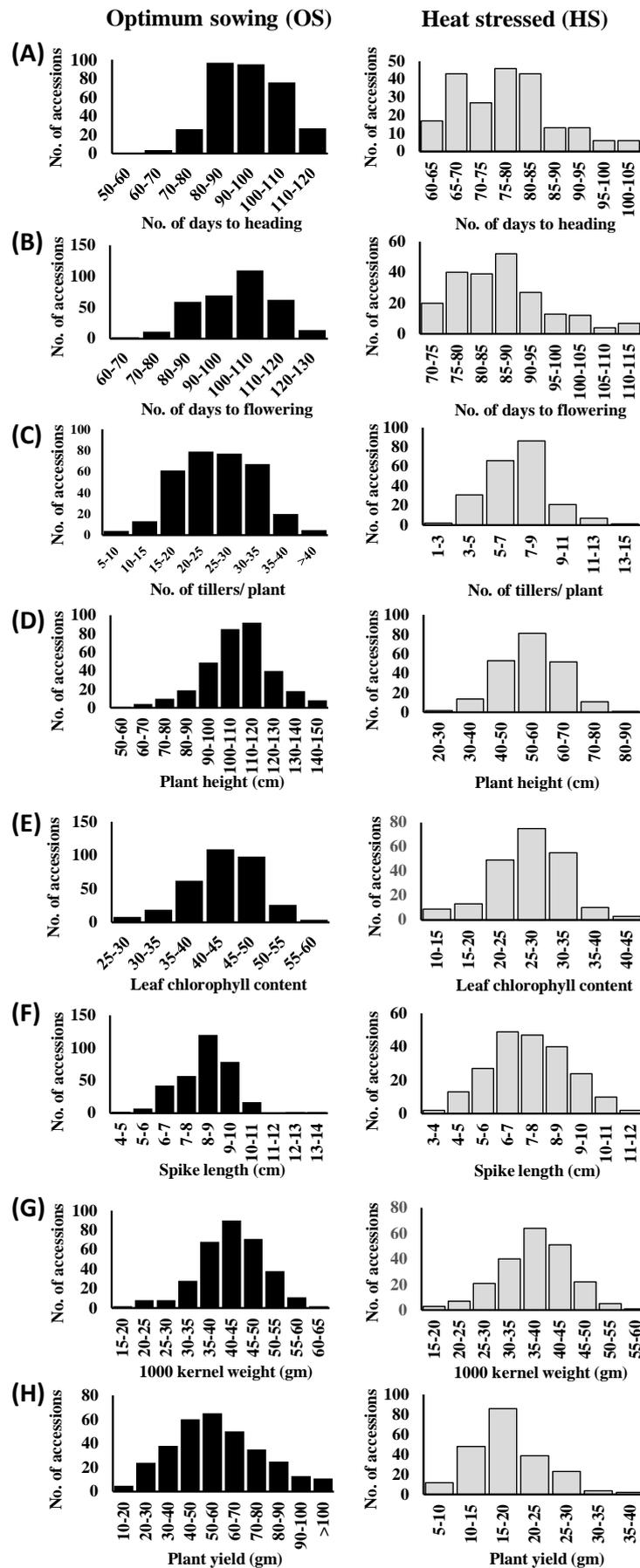
Cluster analysis using heat tolerance indices (HTIs) for all measured traits was performed based on the Euclidean distance matrix with un-weighted pair-group method based on arithmetic averages (UPGMA) in the software NTSYS-pc ver 2.1 (23). Cluster analysis based on heat tolerance indices of measured morphological and physiological characters revealed two distinct classes in regard to heat tolerance with substantial diversity among the heat tolerant genotypes (Figure 2). Cluster I having 224 accessions which are heat stress tolerant, while cluster II comprise 112 accessions susceptible to heat stress. Pearson correlation coefficients (R) between studied traits under either the heat stressed conditions (HSD) and the optimum conditions (OSD) was calculated. With a few exceptions, all studied traits exhibited highly significant positive correlations with each other (Table 2).

## 4. Discussion

Increased ambient temperature as a result of global warming and climate changes is emerging as a great threat to the growth and development of most crop plants. In the current study, a structured barley population consists of 326 genotypes which have wide differences in morphological characters and agronomic traits were evaluated for heat stress-associated quantitative traits, i.e.,

days to heading (DTH), days to flowering (DTF), number of tillers per plant (NoT), plant height (PH), Chlorophyll content (CC), spike length (SL), thousand kernel weight (TKW) and single plant yield (SPY). For heat stress treatment, plants were on February 7, 2015 where plants have been exposed to terminal high temperature during anthesis (Figure 1). The measured phenotypic traits responded differently to raising temperature during anthesis. Continuous phenotypic variations for all studied traits were observed, indicating a quantitative inheritance of heat stress tolerance. Variable levels of coefficient correlations were observed among studied phenotypic traits either under the optimum sowing or the heat-stressed conditions. The impact of temperature stress is a complex function of intensity, duration, and rate of temperature change. High temperature stress can alter multiple aspects of cellular physiology such as membrane fluidity, nucleic acid and protein structures, as well as metabolite and osmolyte concentrations [18,25,26]. Hot temperature stress can result in oxidative damage and, potentially, cell death as a result of production of elevated concentrations of ROS (reactive oxygen species) [27].

Our results revealed that plants exposed to high temperatures flowered earlier (Figure 3) which is consistent with the effect of heat stress on reproductive transition in flowering plants. Reproductive phase in flowering plants is often highly sensitive to hot temperature stresses, with even a single hot day sometimes being fatal to reproductive success [28].



**Figure 3.** Phenotypic evaluation of 326 barley genotypes under optimum sowing (OSD) and heat-stressed (HSD) conditions. Genotypes were evaluated for wilting score days to heading (A), days to flowering (B), number of tillers per plant (C), plant height (D), Chlorophyll content (E), spike length (F), thousand kernel weight (G) and single plant yield (H)

The drastic reduction in morphological and yield contributing traits, i.e., plant height, number of tillers/plant, spike length, thousand kernel weight and plant yield, under heat stress conditions could be due to the inhibition of photosynthesis as one of the most striking consequences of heat stress on photosynthetic tissues which is reflected by the reduction in chlorophyll content of the leaves under heat stress conditions (Figure 3). High temperatures damage the OEC (oxygen evolving complex) of PSII (photosystem II) [29], reduce Rubisco activity [30], and cause disorganization of the thylakoid membranes [31]. With respect to reproductive transition, a reduction of photosynthetic capacity ultimately results in a reduction of parental resources available for reproduction.

Cluster analysis revealed two distinct groups based on heat stress tolerance. The first class consists of 112 barley accessions which are mainly originated from cold regions. Meanwhile, the second group which consists of 224 barley genotypes revealed variable degrees of tolerance to heat stress as indicated by heat tolerance indices (Suppl. Table. 4) as shown by cluster analysis (Figure 2).

The current study has demonstrated that wild barley is a useful gene source for improving quantitative heat stress tolerance related traits through enriching the gene pool of cultivated barley. The panel of barley accessions investigated in this study represent a wide range of variability for heat tolerance associated traits. The results are promising in applying association mapping approach, which is a feasible alternative approach to identify QTLs associated with heat tolerance related traits, for studying the genetic basis of phenotypic variation for traits evaluated under heat stress conditions.

In conclusion, screening the primary gene pool of barley for heat tolerance identified a panel of accessions representing variable degrees of tolerance to heat stress which is a great threat for crop production in the future. Furthermore, the valuable phenotypic data shown here will be used for association mapping to identify molecular markers that are significantly associated with heat tolerance related traits. Besides, our data could serve as a vitally important tool in improving barley breeding for heat stress conditions by exploiting the identified heat tolerant genotypes in barley breeding programs.

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