A novel tolerance index to identify heat tolerance in cultivated and wild barley genotypes

Forouzan Bahrami, Ahmad Arzani* and Mehdi Rahimmalek

Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan 8415683111, Iran

*Corresponding author;

E-mail: a_arzani@iut.ac.ir

ORCID ID: 0000-0001-5297-6724

Tel: +98 311 3913453

Fax: +98 311 3912254

Number of table: 6 + 3 supplementary tables

Number of figure: 4

Abstract

Thermal stress at the reproductive stage poses a substantial constraint on cereal production worldwide. A two-year field study was conducted to assess tolerance to terminal heat stress in cultivated (*Hordeum vulgare* ssp. *vulgare* L.) and wild (*H. vulgare* ssp. *spontaneum* L.) barley genotypes using phenological and agronomic traits as well as selection indices based on grain yield. A new heat-tolerance index was tested while a simultaneous study was also carried out of both phenological and grain yield-related variables as well as previously defined indices. Results of analysis of variance showed the significant genotypic and high-temperature stress (environment) effects on all the traits studied. In contrast to the cultivated genotypes, the wild ones were found less affected by high-temperature stress. Moreover, both cultivated and wild genotypes were observed to use the life cycle shortening as a mechanism to evade heat stress. In addition, supplementary tolerance mechanisms were also found likely to contribute to heat-stress evasion in the wild germplasm. Grain yield showed a strong relationship with both stress tolerance index (STI) and heat tolerance index (HTI) among the wild genotypes. However, multivariate analysis highlighted the feasibility of HTI to screen high-temperature tolerant wild genotypes under harsh environments with the most high-temperature tolerant wild genotypes identified originating from warm climates.

Keywords Wild relatives · Global warming · High temperatures · Yield-based tolerance index

Introduction

Among the enormous global challenges facing plant breeders are included genetic erosion and climate change as the ones of utmost importance; there is, therefore, an urgent need to exploit genetic resources as an effective solution to tackle these challenges. Successful crop improvement for heat tolerance mainly relies on the availability of genetic variability, identification of the genetic and physiological mechanisms involved in heat tolerance, reliable screening approaches, efficient genetic manipulation of desired genetic materials, and ultimate development of heat-tolerant cultivars with suitable agronomic and quality attributes (Arzani and Ashraf 2016). As an immediate progenitor of barley (*Hordeum vulgare* ssp. *vulgare* L.) cultivars, wild barley, the *H. vulgare* ssp. *spontaneum* L. (hereafter referred to as *H. spontaneum*) species, is believed to be drought and heat-tolerant (Hubner et al. 2009; Bahrami et al. 2019; Arzani and Ashraf 2016). High temperatures and aridity have been suggested as the main selective pressures behind the evolution of wild barley (*H. spontaneum*) across the Fertile Crescent (Hubner et al. 2009).

The recent global warming phenomenon is giving rise to an aggravating climatic instability that adversely affects ecosystem quality, plant growth, and agricultural production (Schauberger et al. 2017; Hassan et al. 2020). Terminal heat stress is a term referring to the progressive rise of temperature during the day and in consecutive days during the reproduction stage in both spring and winter type cereals (Bahrami et al. 2019). Grain yield reduction due to high temperatures during the grain-filling stage has been reported in such cereals as wheat (Dwivedi et al. 2017) as well as barley and oat (Klink et al. 2014). Terminal heat-tolerant genotypes of *H. spontaneum* have already been found to employ protective strategies to reduce impairments in photosynthetic pigments, photosynthetic

performance, chlorophyll fluorescence parameters, and cell membrane integrity (Bahrami et al. 2019). Since photosystem II and Rubisco protein complexes are the initial targets of thermal stress during the reproductive stage, photosynthesis is largely limited by carbon fixation that limits the resources (i.e., the assimilates available to grains), thereby resulting in grain yield losses (Bahrami et al. 2019; Dwivedi et al. 2017).

Effective breeding of plants tolerant to abiotic stresses (drought, salinity, heat, and cold) depends upon the plant breeder's ability to find a compromise among the three conflicting yield response factors of grain yield obtained under normal conditions, grain yield obtained under stressful conditions, and grain yield losses to stress. Despite the numerous research efforts directed at the mechanisms involved in heat stress tolerance in crop plants, little is known about heat tolerance indices. Yield-based stress tolerance indices must ensure plant breeders they can safely select genotypes not only of high yield but also with reasonably consistent yields for cultivation under stressful conditions. This is while heat tolerance indices reported to date exclusively used drought tolerance indices for assessing heat tolerance in crop plants such as wheat (Aziz et al. 2018), durum wheat (Kamrani et al. 2017), and common bean (Chavez-Arias et al. 2018; Porch 2006).

In the face of the current climate change, genetic erosion, and inadequate diversity in modern barley cultivars, the available genetic diversity of the wild *spontaneum* subspecies need to be fully exploited to develop barley cultivars especially improved for heat tolerance. Such a breeding program might offer the following two key advantages: i) adaptation of wild subspecies to heat (Bahrami et al. 2019) and drought stresses (Barati et al. 2017), and ii) sharing the complete chromosome homology (similar genome, HH) that will ultimately result in fully fertile offspring after hybridization with cultivated barley. Based on these considerations, the current study was designed and conducted to: 1) investigate differences among the agronomic responses induced by genetic diversity and delayed sowing in Iranian *H. spontaneum* germplasm to high-temperature stress during the grain-filling period and 2) to employ yield-based selection indices, including a new index proposed in this article, to screen barley germplasm for heat tolerance under field conditions.

Materials and methods

Plant materials and growth conditions

For the purposes of this study, forty-nine barley genotypes comprising 45 *H. spontaneum* genotypes originated from western Iran (i.e., the five provinces of Lorestan, West Azarbaiyjan, Ilam, Kurdestan, and Kermanshah) and the four barley cultivars of 'Mona', 'Reyhan', 'Nosrat', and 'Fajr30' were used (Supplementary Table 1).

Two separate experiments differing only in their sowing dates (normal: October, and delayed: January) were carried out using a square lattice design (7 × 7) with two replications at the research farm of Isfahan University of Technology located at Lavark, Najaf-Abad, Iran (40 km south-west of Isfahan, 32º 30´ N, 51º 20´ E, 1630 m asl) in two consecutive years (2015–2016 and 2016–2017). The field site is characterized by a mean annual precipitation of140 mm and a silty clay loam soil (typic Haplargids) with a pH 7.3–7.8. The mean temperature and relative humidity at the experimental site are reported in Table 1. The sowing dates and the overall means of the

phenological stages during the two growing seasons are reported in Table 2. Identical crop management practices such as fertilizer application, weed control, and irrigation regimes (to prevent confounding drought effects) were exercised in both experiments.

Phenological and agronomic traits

Days to anthesis (DA) was defined as the number of days from the planting date until one-half of the plants had at least one extruded anther. Days to physiological maturity, hereafter referred to as 'days to maturity' (DM), was measured as the number of days from the planting date to the appearance of peduncle senescence in the whole plot. Moreover, the length of the reproductive growth period (RGP) was calculated by DA subtracted from DM. Spike length (SPL), plant height (PH), number of fertile tillers (NT), and number of grain per spike (NGS) were recorded at the physiological maturity stage on 10 plants per plot.

Finally, plants were harvested from the three middle rows at the two dates of May12th, 2016 and May 10th, 2017 for normal planting date and on June7th, 2016 and June 5th, 2017 for the delayed planting date. At the seed moisture content of about 14%, 1000-grain weight (GW) and grain yield were determined.

Heat tolerance indices

Five selection indices including stress tolerance index (STI) (Fernandez 1992), tolerance index (TOL) (Rosielle and Hamblin 1981), stress susceptibility index (SSI) (Fischer and Maurer 1978), yield stability index (YSI) (Bouslama and Schapaugh 1984), and heat susceptibility index (HSI) were calculated based on grain yield under normal and heat stress conditions according to the following equations:

1. Stress tolerance index (STI)

$$\mathrm{STI} = \frac{(\mathrm{Yp} \times \mathrm{Ys})}{\mathrm{Ymp}^2}$$

2. Tolerance index (TOL)

$$\mathrm{TOL} = \mathrm{Yp} - \mathrm{Ys}$$

3. Stress susceptibility index (SSI)

$$\mathrm{SSI} = \frac{[1 - (\frac{\mathrm{Ys}}{\mathrm{Yp}})]}{\mathrm{SI}}$$

$$\mathrm{SI} = 1 - (\frac{\mathrm{Yms}}{\mathrm{Ymp}})$$

3. Yield stability index

$$\mathrm{YSI} = \frac{\mathrm{Ys}}{\mathrm{Yp}}$$

5. Heat susceptibility index (HSI)

$$\mathrm{HSI} = 1 - [\frac{\mathrm{Ymp}}{\mathrm{Yp}} - \frac{\mathrm{Yms}}{\mathrm{Ys}}]$$

In which, Ys and Yp represent each genotype's yields under high-temperature stress and normal conditions, respectively, while Yms and Ymp denote each genotype's mean yields under normal and delayed sowing (high-temperature) conditions, respectively.

In addition, a new yield-based heat tolerance index (HTI) was developed and used to identify genotypes with superior heat tolerance.

6. Heat tolerance index (HTI)

$$\mathrm{HTI} = 1 - \left[\frac{(\mathrm{Yp} - \mathrm{Ys})}{\mathrm{Ys}}\right]$$

Statistical analysis

Combined analysis of variance (ANOVA) was carried out for the traits studied using the GLM procedure of SAS software (Version 9.3; SAS Institute 2011). Also, the efficiency of the lattice design was compared (tested) with that of the randomized complete block design (RCBD) based on the ratio of lattice mean square of residuals (error) × 100/RCBD mean square residual for each trait. Due to the slight differences between the two, the RCBD design was used for data analysis. Comparison of means was performed using the Fisher's least significant difference ($LSD_{5\%}$) test. Phenotypic correlations among the traits studied and the selection indices were estimated using the CORR procedure of SAS software (Version 9.3; SAS Institute 2011). Biplot graphs based on principal component analysis (PCA) were employed using Stat Graphics to discriminate between sensitive genotypes and tolerant ones according to yield attributing traits. The Sigma Plot software was then used to develop a three-dimensional graph for identifying superior wild genotypes based on two of the best selection indices identified and grain yield. To determine the associations of grain yield and the other traits studied with the best tolerance index selected, simple linear regression and path-coefficient analysis were also carried out using SPSS software. Finally, the Ward's method was employed to conduct cluster analysis based on phenological and agronomic traits as well as HTI using the SPSS statistics.

Results

Results of the analysis of variance showed that high temperature had significant effects on all the traits studied (Table 3). Moreover, highly significant (P<0.01) variations in phenological and agronomic traits were detected in both the wild and cultivated genotypes. The two growing years (Y) showed significant differences only for the NGS and GW traits. The genotype by environment (G×E) interaction was also found significant for all the traits except DA, SPL, and NGS. Furthermore, the environment by year (E×Y) interaction was significant for PH and yield components.

Mean comparisons of the traits studied indicated a high genetic variation among the wild genotypes (Supplementary Table 2). Results also showed that, compared to normal conditions, heat stress caused significant reductions in the means of all the traits studied, including grain yield, in both wild and cultivated genotypes (Table 4). Moreover, significant differences were observed between wild and cultivated genotypes with respect to all the traits studied except for DM and SPL under both treatments. More specifically, the cultivated genotypes had the

lowest means of DA under both treatments while RGP recorded higher values only in the cultivated genotypes. Under heat stress, however, RGP in the cultivated barley exhibited a reduction twice as much as that measured in the wild ones (Table 4). Regarding plant height, the wild genotypes grew taller than the cultivated ones under both treatments. In contrast to the cultivated genotypes, the wild ones were less affected by heat stress in their NT, NGS, and GW (Table 4). While the highest grain yields were recorded for the four cultivated genotypes under both treatments, these same genotypes exhibited greater grain yield reductions under heat stress than did the wild ones (Table 4).

Six yield-based indices, including the new Heat Tolerance Index (HTI), were employed to screen superior wild genotypes for heat tolerance. Comparison of means of grain yield and selection indices for both cultivated and wild genotypes are reported in Table 5 and Supplementary Table 3, respectively. Clearly, higher values of Yp, Ys, TOL, and STI were recorded for the barley cultivars, the wild genotypes proved superior with respect to the yield-stability based indices (YSI and HTI). The STI, YSI, and HTI correlated positively with Ys in the wild genotypes (0.91**, 0.77** and 0.85**, respectively). This is while, grain yield under high temperature-stress (Ys) had a high association with STI and TOL in the cultivated genotypes (0.98** and 0.85**, respectively).

PCA was carried out for all the traits studied under both normal and heat stress conditions. Results showed that the first and second components, respectively, explained 43.03% and 28.71% of the total yield variation under heat stress conditions (Fig. 1). The correlations of the traits based on the angles between the vectors in the PC1 vs. PC2 biplot, which were consistent with the results of correlation coefficients between the traits (data not shown), showed that TOL, NGS, STI, and Yp correlated positively and highly with each other but negatively with PH under heat stress (Fig. 1). Moreover, positive correlations were observed among YSI, SPL, HTI, NT, DM, and RGP. Based on the biplot derived, PC1 was termed ‘yield potential’ to reflect its high and positive correlations with NT, DM, HTI, SPL, RGP, YSI, and Ys while PC2 was named ‘stress susceptibility’ due to its strong and negative association with HSI, SSI, and TOL. In this respect, the heat-tolerant genotypes located in the high PC1 and PC2 regions of the biplot while the heat-sensitive ones located in the low PC1 and PC2 regions. Accordingly, the genotypes subjected to heat stress conditions were classified into the three groups located in the regions enclosed by circular lines in Fig. 1. The first group consisted of genotypes with high PC1 and PC2 values and included those located closest to HTI; they mostly originated from Illam and Kermanshah provinces. The second group consisted of the three ‘Reyhan’, ‘Nosrat’, and ‘Fajr30’ cultivated barley genotypes. Finally, the third heat-sensitive group (i.e., with the lowest PC1 and PC2 values) group consisted of seven genotypes some of which had been collected from Kamyaran and Sanandaj regions in Kurdestan Province. The remaining genotypes located in the center of the graph.

Due to the strong inter-correlations among Ys, STI, and HTI, a 3-dimensional graph was plotted to illustrate the relationships among the studied wild genotypes based on these variables (Fig. 2). The genotypes were placed into four zones (A, B, C, and D) representing the three variables of the two tolerance indices and grain yield under high temperature stress conditions. Mean comparisons of the four groups included 9, 7, 2, and 27 wild barley genotypes for grain yield; the selection indices are reported in Table 6. The results of mean comparisons of the four groups showed that about one-fourth of the wild genotypes with the highest yield stability and adaptability values

located in the ‘A’ zone, whereas those in the ’D’ zone exhibited the highest susceptibility to thermal stress and mostly originated from Lorestan, Kurdestan, and West Azarbaiyjan provinces.

The results of linear regression analysis showed a significantly positive relationship between Ys and HTI in both the wild and cultivated genotypes (Fig. 3). Compared to the wild genotypes, the cultivated ones had higher Ys values, but higher HTI values were recorded for the heat-tolerant wild genotypes. To discover the causal relationships of the phenological and agronomic traits with HTI under stress conditions, a path coefficient analysis was performed among the wild genotypes to find that DA, SPL, and GW had the highest positive direct effects (0.35, 0.45, and 0.33, respectively) on HTI. On the other hand, despite the fact that NGS had a negative direct effect (-0.31), its indirect effect via SPL was positive (0.26) on HTI.

Cluster analysis categorized the 45 wild barley genotypes into three groups based on the traits studied under stress conditions and HTI at a Euclidean distance of 10 (Fig. 4). The results of mean comparisons indicated significant differences among the groups in all the traits studied except for PH, NGS, and GW (data not shown). The third cluster comprised 13 wild barley genotypes mostly originating from a warmer climate (Ilam and Kermanshah provinces) and exhibited the highest Ys, HTI, DA, DM, RGP, SPL, and NH values (data not shown). This group of genotypes was identified as the most heat-tolerant one. On the other hand, 18 out of the 45 wild barley genotypes were included in group II characterized by the lowest HTI and Ys values and was, hence, considered as the most heat-sensitive group.

Based on multivariate analysis, the wild genotypes No. 1, 2, 7, 15, 16, 17, 19, 21, and 41 were classified with the heat-tolerant groups.

Discussion

Winter cereal crops are generally constrained by heat stress at their terminal growth stage due to the detrimental effects on their reproductive organs and the limited carbon availability during their grain-filling period. It has been postulated that, compared to their cultivated counterparts, wild cereal species that are distributed over a vast are from the Mediterranean regions to central Asia might contain different tolerance genes and that they might evolve different mechanisms for abiotic stress tolerance (Rampino et al. 2018). This hypothesis is confirmed by the results of the current study that showed the wild barley germplasm studied to exhibit a rich genetic variation for heat stress tolerance. Likewise, Hu et al. (2019) reported a high genetic variation for heading date in wild barley in comparison with cultivated one in three different sowing dates. It has also been hypothesized that *H. spontaneum* that has evolved efficient heat tolerance strategies to adapt to the hot climate in south-west Iran could be exploited as a rich genetic resource for improving cultivated barley with respect to its heat stress tolerance. Recently, this rich genetic resource of *H. spontaneum* germplasm originated from West-Iran has been demonstrated to underlie the physiological basis of its tolerance to both heat (Bahrami et al. 2019) and salinity (Ebrahim et al. 2020) stresses.

Reduction in grain yield might be attributed to the pollen infertility and seed abortion due to terminal heat stress during the grain-filling stage (Rezaei et al. 2010; Bita and Gerats 2013). Although in this experiment, the *H. spontaneum* germplasm did not exhibit a high yield productivity under either treatment, it showed less susceptibility

to thermal stress than did the cultivated genotypes as evidenced by its much lower yield loss than those observed in the cultivated ones. These findings are in agreement with those of Barati et al. (2017) who reported that, compared to the cultivated ones, the *H. spontaneum* genotypes had been less affected by drought stress. Weaker effects of high temperatures were also observed in the present study on RGP, NGS, and GW than those on the other traits in the wild genotypes, when compared with the cultivated ones. It might be, therefore, concluded that the higher reductions in grain yield due to heat stress observed in the cultivated genotypes could have been caused by the shortened reproduction period and the declining yield components. These results, in turn, imply that the heat escape by shortening the life cycle to avoid heat stress led to reduced yield in the cultivated barley, when compared with the wild one. Lohaniand and coworkers (2020) noted that high temperatures during anthesis caused the seed filling duration to shorten, which resulted in a smaller grain size and, thereby, a yield decline. Likewise, Zheng et al. (2016) reported the phenological acceleration in wheat under high temperature led to reduced photosynthesis due to smaller leaves and fewer tillers.

Since the genes involved in grain yield components are expressed at different developmental stages, they are influenced differently by variations in the environment. In the current study, the lower losses of grain yield in the wild genotypes could not only be attributed to the more stable reproduction growth period but also to the stable production of yield components. Jedmowski et al. (2015) found that heat stress had no significant effect on the total number of spikes per plant in *H. spontaneum* but that the number of seeds per spike and seed weight declined under high temperatures. In the wild genotypes, secondary tiller production and percentage of fertile florets exhibited stronger effect of terminal heat stress more than did seed size. These results are consistent with those reported by Jedmowski et al. (2015) who noted that disturbances in floral development due to heat stress affected grain productivity in wild barley.

Various strategies for improving plant yield under stressful environments have been proposed to increase the efficiency of plant breeding programs. Given the fact successful breeding programs can be measured against proper indices, an ideal tolerance index should have a high discriminative power to identify superior genotypes with long-term yield stability (Bahrami et al. 2014). The need to screen for stress-tolerant germplasms has, therefore, urged plant breeders to search for reliable indices. A novel tolerance index was proposed in this paper that incorporates the most important indicators of performance including yield stability and yield loss to stress. In addition, TOL, STI SSI, YSI, HIS, and HTI selection indices were used to identify the most heat-tolerant wild genotypes. The results showed a high variation in the wild barley germplasm and a discriminative screening judgment was made using each of the six indices.

Despite the much higher yield of the cultivated genotypes compared to the wild ones studied, the highest yield reduction due to thermal stress, or the highest TOL, was found to belong to four barley cultivars. In addition, the highest STI values were recorded for the three cultivated barley genotypes of ‘Reyhan’, ‘Nosrat’, and ‘Fajr30’. The results followed an expected pattern with an STI index that took into account the yields in both environmental treatments. These findings are in agreement with those of a recent study showing higher TOL and STI values in cultivated genotypes (*H. vulgare*) compared to wild ones (Barati et al. 2020) subjected to drought stress. Indeed, each of the tolerance indices used imposes important restrictions on the screening process so that the strategies used

may not be uniformly practicable. It is, therefore, essential to employ a combination of two supplementary indices that will not overemphasize only certain aspects of the genotypic attributes at the coast of a reasonable overall evaluation of genotype performance. In this regard, the strong correlations among Ys, STI, and HTI were used to identify the wild genotypes Nos. 1 and 2 with the highest STI and HTI values as the ones tolerant to thermal stress. On the other hand, the genotypes Nos. 22, 23, and 42 with the highest HSI and SSI values were identified as the most susceptible to thermal stress among all the wild genotypes.

PCA is a powerful statistical procedure to reduce the dimensions of the variables and to divulge constructive evidence-driven feedback from a highly-correlated dataset (Bahrami et al. 2014). The two-dimensional plot obtained from the two PC (PC1 and PC2) scores proved HTI as the most powerful selection index to identify tolerant genotypes capable of maximum sustainable yield. Under heat stress, the positive correlations among HTI, NT, DM, SPL, and RGP concentrated in the optimum region in the plot; i.e., they had high PC1 and PC2 scores suggesting that these traits were important in defining heat-tolerant wild genotypes. Using a combination of drought tolerance indices, Bahrami et al. (2014) and Barati et al. (2020) identified drought-tolerant genotypes in safflower and in wild barley, respectively. The results of the current study showed that the heat-tolerant barley genotypes exhibited greater numbers of tillers, taller spike heights, and sufficient grain-filling durations when exposed to high temperatures in the reproductive stage. These findings are consistent with those reported by Bergkamp et al. (2018) on wheat, indicating that terminal heat stress can shorten the grain-filling period via accelerated grain filling rate, thereby causing a limited amount of carbohydrates to be transferred from the source tissues to the grains and reducing grain yield.

Moreover, the results indicated that shortened life cycle to escape high temperatures is a common defense mechanism adopted by both cultivated and wild genotypes of barley, with the negative effect of drastically reduced yield but the positive one of dramatically enhanced yield stability. It was found that HTI could be exploited to identify the cultivated and wild barley genotypes with the highest thermal tolerance by making a reasonable compromise between yield and its stability. In addition to the development of a useful and novel tolerance index, the experiment revealed the challenges associated with screening genotypes tolerant to abiotic stresses. It was also found that the overall performance of wild populations could be viewed as an evolutionary compromise between two advantageous features of plant evolution – that is, increased productivity in an extremely stressful ecosystem and its long-term sustainability.

Conclusions

Although either yield loss or yield potential under stressful conditions is normally used to identify genotypes tolerant to abiotic stresses under field conditions, neither can exhaustively disclose the tolerant nature of a plant genotype. A novel abiotic tolerance index, HTI, was introduced as a scoring system to be used in combination with previously reported indices for identifying superior tolerant genotypes under field conditions. The results of multivariate analysis revealed that, among the six indices used in the present study, the novel HTI could be used alone to identify the most heat-tolerant wild barley genotypes under field conditions. Moreover, fertile florets and

tillers accompanied by longer reproductive periods to allow an improved grain filling process were found to play key roles in the thermal tolerance of *H. spontaneum* germplasm. The novel index introduced maybe tested in future research to determine its screening power in identifying superior tolerant genotypes under such other abiotic stresses as drought and salinity.

Acknowledgement

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

References

Arzani A, Ashraf M (2016) Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. Crit Rev Plant Sci 35:146–189

Aziz A, Mahmood T, Mahmood Z, Shazadi K, Mujeeb-Kazi A, Rasheed A (2018) Genotypic variation and genotype environment interaction for yield-related traits in synthetic hexaploid wheats under a range of optimal and heat-stressed environments. Crop Sci 58:295–303

Bahrami F, Arzani A, Karimi V (2014) Evaluation of yield-based drought tolerance indices for screening safflower (*Carthamus tinctorius* L.) genotypes. Agron J 106:1219–1224

Bahrami F, Arzani A, Rahimmalek M (2019) Photosynthetic and yield performance of wild barley (*Hordeum vulgare* ssp. *spontaneum*) under terminal heat stress. Photosynthetica 57:9–17

Barati M, Majidi MM, Mostafavi F, Mirlohi A, Safari M, Karami Z (2017) Evaluation of wild barley species as possible sources of drought tolerance for arid environments. Plant Genetic Resources 16:209–217

Barati M, Majidi MM, Safari M, Mostafavi F, Mirlohi A, Karami Z (2020) Comparative physiological attributes of cultivated and wild relatives of barley in response to different water environments. Agron J 112:36–43

Bergkamp B, Impa SM, Asebedo AR, Fritz AK, Krishna Jagadish SV (2018) Prominent winter wheat varieties response to post-flowering heat stress under controlled chambers and field based heat tents. Field Crops Res 222:143–152

Bita CE, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci 4:1–18

Bouslama M, Schapaugh WT (1984) Stress tolerance in soybeans I. Evaluation of three screening techniques for heat and drought tolerance. Crop Sci 24:933–937

Chavez-Arias CC, Ligarreto-Moreno GA, Restrepo-Diaz H (2018) Evaluation of heat stress period duration and the interaction of daytime temperature and cultivar on common bean. Environ Exp Bot 155:600–608

Dwivedi SK, Basu S, Kumar S, Kumar G, Prakash V, Kumar S, Mishra JS, Bhatt BP, Malviya N, Singh GP, Aror A (2017) Heat stress induced impairment of starch mobilization regulates pollen viability and grain yield in wheat: Study in Eastern Indo-Gangetic Plains. Field Crops Res 206:106–114

Ebrahim F, Arzani A, Rahimmalek M, Sun D, Peng J (2020) Salinity tolerance of wild barley *Hordeum vulgare* ssp. *spontaneum*. Plant Breed 139:304–316

Fernandez GCJ (1992) Effective selection criteria for assessing plant stress tolerance. In: Kuo CG (ed) Proceedings of the international symposium on adaptation of vegetables and other food crops in temperature and water stress. Asian Vegetable Research and Development Center, Taiwan, pp: 257–270

Fischer RA, Maurer R (1978) Drought resistance in spring wheat cultivars. Part 1: grain yield response. Aust J Agric Res 29:897–912

Hassan MU, Chattha MU, Khan I, Chattha MB, Barbanti L, Aamer M, Iqbal MM, Nawaz M, Mahmood A, Ali A, Aslam MT (2020) Heat stress in cultivated plants: nature, impact, mechanisms, and mitigation strategies - A review. Plant Biosyst. https://doi.org/10.1080/11263504.2020.1727987

Hu H, Ahmed I, Choudhury S, Fan Y, Shabala S, Zhang G, Harrison M, Meinke H, Zhou M (2019) Wild barley shows a wider diversity in genes regulating heading date compared with cultivated barley. Euphytica 215, 75. https://doi.org/10.1007/s10681-019-2398-1

.

Hubner S, Hqffken M, Oren E, Haseneyer G, Stein N, Graner A, Schmid K, Fridman E (2009) Strong correlation of wild barley (*Hordeum spontaneum*) population structure with temperature and precipitation variation. Mol Ecol 18:1523–1536

Jedmowski C, Ashoub A, Momtaz O, Brüggemann W (2015) Impact of drought, heat, and their combination on chlorophyll fluorescence and yield of wild barley (*Hordeum spontaneum*). J Bot 1–9, Article ID 120868; https://doi.org/10.1155/2015/120868

Kamrani M, Hoseini Y, Ebadollahi A (2017) Evaluation for heat stress tolerance in durum wheat genotypes using stress tolerance indices. Arch Agron Soil Sci 64:38–45

Klink K, Wiersma JJ, Crawford CJ, Stuthman DD (2014) Impacts of temperature and precipitation variability in the Northern Plains of the United States and Canada on the productivity of spring barley and oat. Int J Climatol 34:2805–2818

Lohani N, Singh MB, Bhalla PL (2020) High temperature susceptibility of sexual reproduction in crop plants. J Exp Bot 71:555–568

Porch TG (2006) Application of stress indices for heat tolerance screening of common bean. J Agron Crop Sci 192:390–394

Rampino P, Gulli M, Pascali MD, Caroli MD, Marmiroli N, Perrotta C (2018) Wild and cultivated *Triticum* species differ in thermo tolerant habit and HSP gene expression. Plant Biosyst 153:337–343

Rezaei M, Arzani A, Sayed-Tabatabaei BE (2010) Meiotic behavior of tetraploid wheats (*Triticum turgidum* L.) and their synthetic hexaploid wheat derivates influenced by meiotic restitution and heat stress. J Genet 89:401–407

Rosielle AA, Hamblin J (1981) Theoretical aspects of selection for yield in stress and non-stress environment. Crop Sci 21:943–946

Schauberger B, Archontoulis S, Arneth A, Balkovic J, Ciais P, Deryng D, Elliott J, Folberth C, Khabarov N, Müller C, Pugh TAM, Rolinski S, Schaphoff S, Schmid E, Wang X, Schlenker W, Frieler K (2017) Consistent negative response of US crops to high temperatures in observations and crop models. Nat Commun 8:1–9

Zheng B, Chenu K, Chapman SC (2016) Velocity of temperature and flowering time in wheat-assisting breeders to keep pace with climate change. Global Change Biol 22:921–933

Table 1 Daily mean air temperature and relative humidity at the experimental site during the three growth stages

Time	Year	Temperatures (^{o}C)				Relative humidity (%)	
		Minimum		Maximum			
		Normal planting	Delayed planting	Normal planting	Delayed planting	Normal planting	Delayed planting
Planting date	2015-16	12.4	5.0	21.0	14.8	63	65
	2016-17	9.8	0.6	22.2	14.6	40	30
Anthesis	2015-16	5.0	19.2	20.4	29.4	35	30
	2016-17	2.8	17.0	19.0	30.4	40	57
Maturity	2015-16	14.0	21.6	24.2	36.1	57	19
	2016-17	11.0	22.4	27.6	37.6	42	20

Table 2 Planting dates and overall means of phenological stages in barley genotypes grown under field conditions during two growing seasons

Year	Planting date		Days to anthesis		Days to maturity	
	Normal planting	Delayed planting	Normal planting	Delayed planting	Normal planting	Delayed planting
2015-16	23/10/2015	04/01/2016	163	123	199	151
2016-17	23/10/2016	03/01/2017	162	121	197	149

Table 3 Combined analysis of variance for phenological and agronomic traits in wild and cultivated barley genotypes grown under normal and high temperat stress field conditions in two consecutive years (2016 and 2017)

Source of variation	df	Days to anthesis	Days to maturity	Reproductive growth period	Plant height	Spike length	Number of fertile tillers	Number of grain per spike	1000-grain weight (g)	Grain yield (kg ha^{-1})
Environment (E)	1	94178**	130487**	2953.5**	15576.1**	153.7**	5408**	4560.1**	4130.7**	70489670**
Year (Y)	1	47.1^{ns}	79.1^{ns}	4.1^{ns}	59.7^{ns}	9.6^{ns}	12.5^{ns}	163.3**	262.5**	13396^{ns}
E × Y	1	0.0001^{ns}	0.04^{ns}	0.04^{ns}	158.1**	1.6*	52.8**	222.0**	95.2**	552^{ns}
Block (E × Y)	4	220.5**	194.4**	0.9^{ns}	252.1**	3.5**	84.1**	5.0^{ns}	3.9**	239502**
Genotype (G)	48	118.8**	248.8**	151.5**	753.9**	5.4**	79.9**	623.1**	161.2**	3395654**
Wild	44	68.9**	256.0**	80.5**	85.8**	63.1**	85.7**	63.1**	66.3**	2370631**
Cultivated	3	28.6**	205.3**	82.0**	10.7**	1521.0**	10.7**	1521**	2.5**	580168**
Wild vs. Cultivated	1	27.14**	26.83**	0.0009^{ns}	136.2**	0.12^{ns}	38.4**	297.8**	113.8**	641943**
G × E	48	0.0001^{ns}	5.5**	5.5**	15.3**	0.1^{ns}	4.2**	1.9^{ns}	17.3**	304590**
G × Y	48	0.6**	0.2^{ns}	0.9**	11.4^{ns}	0.2^{ns}	1.2*	1.1^{ns}	3.1**	10249^{ns}
G × E ×Y	48	0.0001^{ns}	0.04^{ns}	0.04^{ns}	14.5**	0.04^{ns}	0.8^{ns}	1.6^{ns}	3.3**	4312^{ns}
Residual	192	0.11	0.31	0.42	8.50	0.37	0.82	3.36	0.64	12369
Coefficient of variation		0.2	0.3	1.8	2.7	6.3	5.4	7.1	2.4	8.4

ns, not significant;* $P< 0.05$, ** $P< 0.01$

Table 4 Means of phenological and agronomic traits separately in wild and cultivated barley genotypes grown under normal and high temperature-stress field conditions in two consecutive years (2016 and 2017)

Traits	Normal		High temperature-stress		Reduce percentage	
	Wild	Cultivated	Wild	Cultivated	Wild	Cultivated
Days to anthesis	157.13[a†]	147.40[b]	126.13[a]	117.25[b]	19.72	20.45
Days to maturity	194.70[a]	199.50[a]	159.00[a]	156.75[a]	18.33	21.42
Reproductive growth period	37.57[b]	52.10[a]	32.86[b]	39.50[a]	12.53	24.18
Plant height	114.68[a]	86.18[b]	101.87[a]	75.81[b]	11.17	12.03
Spike length	10.17[a]	10.87[a]	8.84[a]	8.99[a]	13.00	17.30
Number of fertile tillers	19.25[b]	21.93[a]	12.85[b]	13.38[a]	33.24	39.00
Number of grains per spike	26.01[b]	56.50[a]	21.15[b]	40.65[b]	18.18	28.01
1000-grain weight (g)	34.09[b]	50.16[a]	28.99[b]	36.11[a]	14.95	28.11
Grain yield (kg ha^{-1})	1417.2[b]	4247.4[a]	819.0[b]	2069.1[a]	42.20	51.28

[†] Means in each row under either normal or stress conditions followed by the same letter are not statistically different at $P < 0.01$

Table 5 Means of grain yield under normal (Yp) and high temperature-stress (Ys) conditions and selection indices evaluated in four barley cultivars grown under normal and high temperature-stress field conditions in two consecutive years (2016 and 2017)

Genotype	Yp (kg ha^{-1})	Ys (kg ha^{-1})	TOL	STI	YSI	SSI	HIS	HTI
Mona	3156.6^{b†}	1643.7^{c}	1512.9^{b}	1.71^{b}	0.52^{a}	0.99^{b}	0.99^{b}	0.07^{a}
Reyhan	4699.1^{a}	2427.8^{a}	2271.3^{a}	3.76^{a}	0.51^{a}	1.00^{ab}	1.00^{ab}	0.06^{a}
Nosrat	4591.8^{a}	2377.9^{ab}	2213.9^{a}	3.60^{a}	0.51^{a}	1.00^{ab}	1.00^{ab}	0.06^{a}
Fajr30	4536.4 ^{a}	2167.8^{b}	2368.6^{a}	3.24^{a}	0.47^{b}	1.08^{a}	1.03^{a}	-0.09^{b}

TOL, tolerance index; STI, stress tolerance index and stress; YSI, yield stability index; SSI, stress susceptibility index; HIS, heat susceptibility index; HTI, heat tolerance index

† Means in each row followed by the same letter are not statistically different at $P < 0.01$

Table 6 Means of grain yield under normal (Yp) and high temperature-stress (Ys) conditions and selection indices evaluated in four groups of wild barley genotypes based on 3-dimensional diagram data

Group	Yp (kg ha^{-1})	Ys (kg ha^{-1})	TOL	STI	YSI	SSI	HIS	HTI
A	1837.7^{a†}	1188.2^{a}	649.51b^{c}	0.72^{a}	0.64^{a}	0.73^{c}	0.82^{b}	0.43^{a}
B	1832.7^{a}	909.33^{b}	923.34^{a}	0.56^{b}	0.49^{c}	1.05^{a}	1.06^{a}	0.02^{c}
C	1113.9^{b}	606.21^{c}	507.69^{c}	0.23^{c}	0.54^{b}	0.94^{b}	0.92^{b}	0.20^{b}
D	1391.5^{b}	673.74^{c}	717.72^{b}	0.31^{c}	0.48^{c}	1.07^{a}	1.10^{a}	-0.07^{d}

TOL, tolerance index; STI, stress tolerance index and stress; YSI, yield stability index; SSI, stress susceptibility index; HIS, heat susceptibility index; HTI, heat tolerance index

† Means in each row followed by the same letter are not statistically different at $P < 0.01$

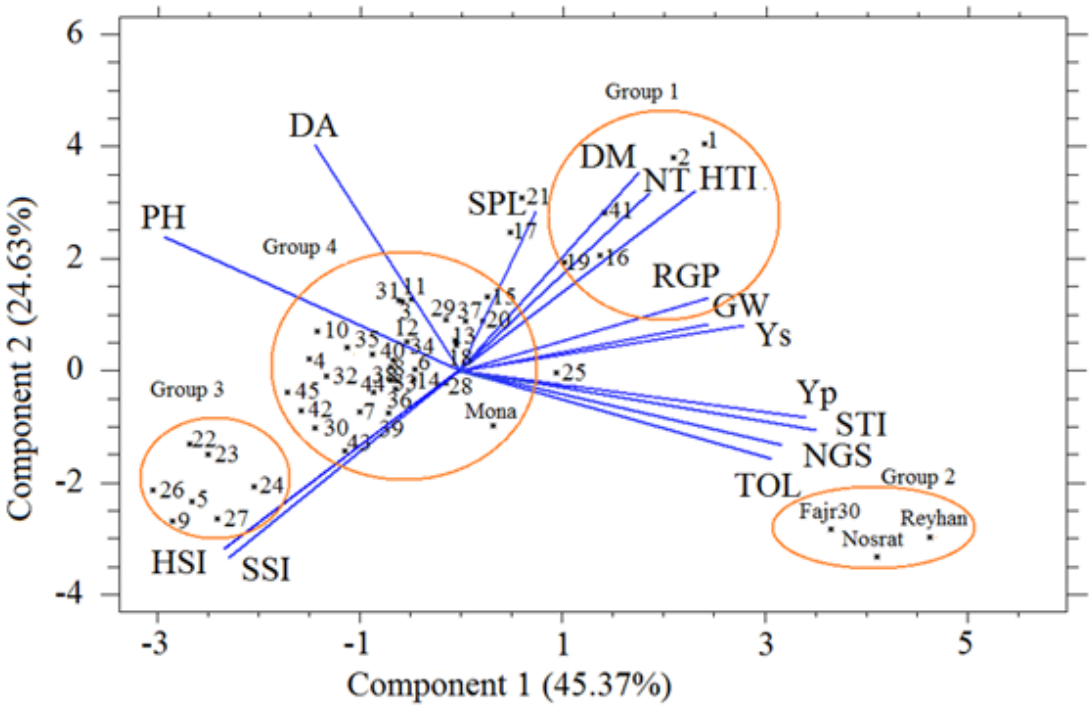

Fig. 1 Biplot of PC1 versus PC2 based on the mean values obtained for each trait during the two-year study period for the high temperature treatment. DA, days to anthesis; DM, days to maturity; RGP, reproductive growth period; PH, plant height; SPL, spike length; NT, number of fertile tillers; NGS, number of grain per spike; GW, 1000-grain weight; Yp, grain yield under normal conditions; Ys, grain yield under heat stress conditions; TOL, tolerance index; STI, stress tolerance index and stress; YSI, yield stability index; SSI, stress susceptibility index; HSI, heat susceptibility index; HTI, heat tolerance index. Numbers in the figure are wild genotypes with details of geographical origins provided in Supplementary Table 1

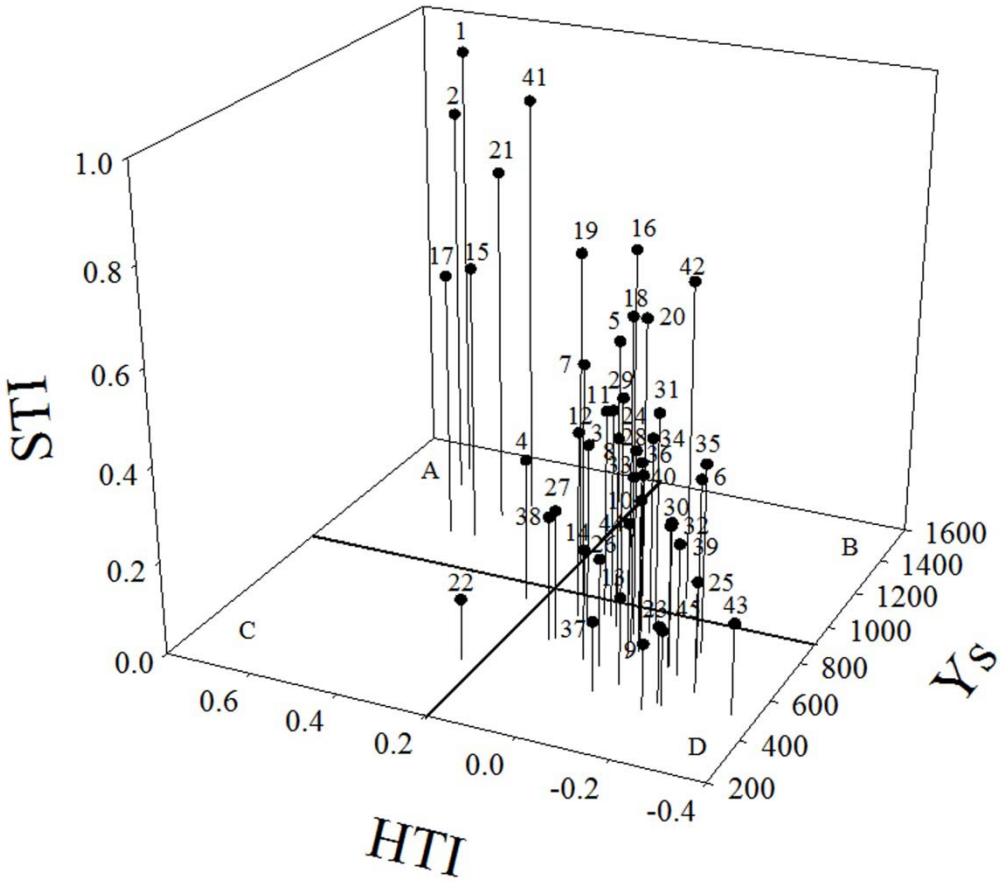

Fig. 2 Three-dimensional diagram for detecting heat-tolerant wild barley genotypes based on grain yield under high temperature-stress conditions (Ys), values obtained stress tolerance (STI) and heat tolerance (HTI) indices are also provided. Numbers represent wild genotypes with details of geographical origins provided in Supplementary Table 1

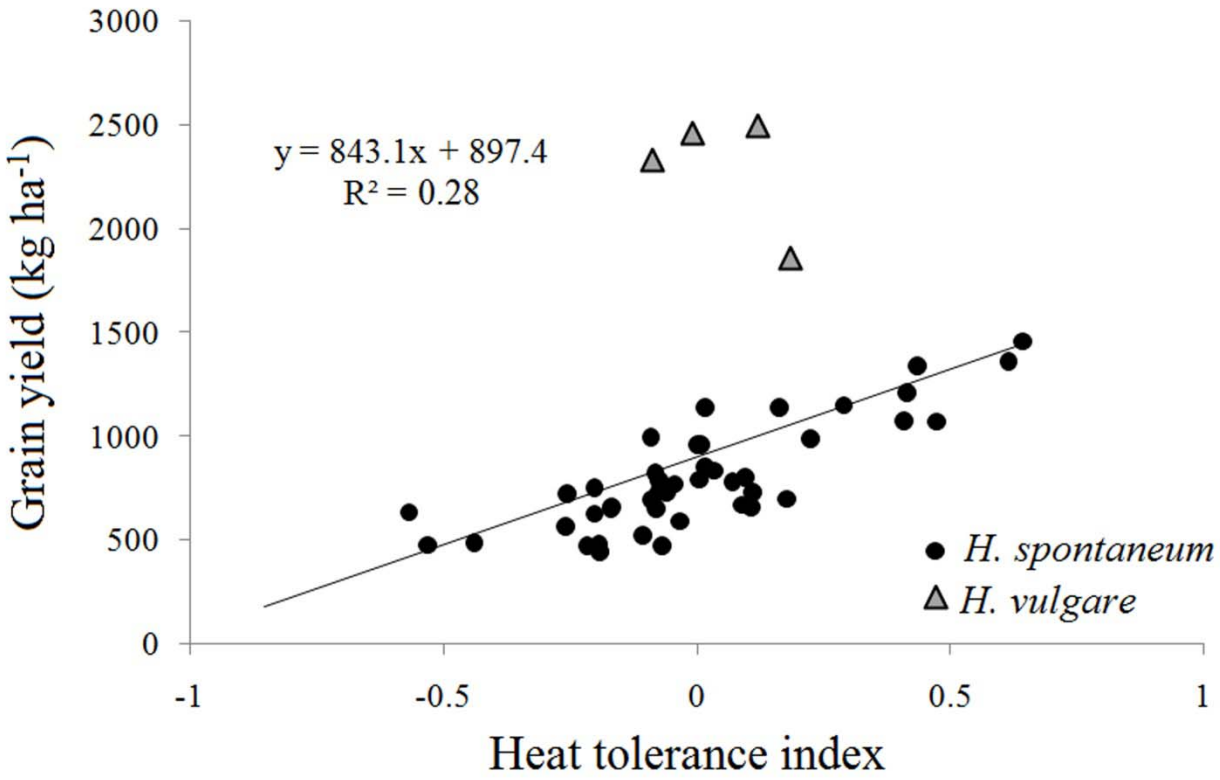

Fig. 3 Relationship between grain yield and heat tolerance index (HTI) of 49 barley (cultivated and wild) genotypes under high temperature-stress field conditions

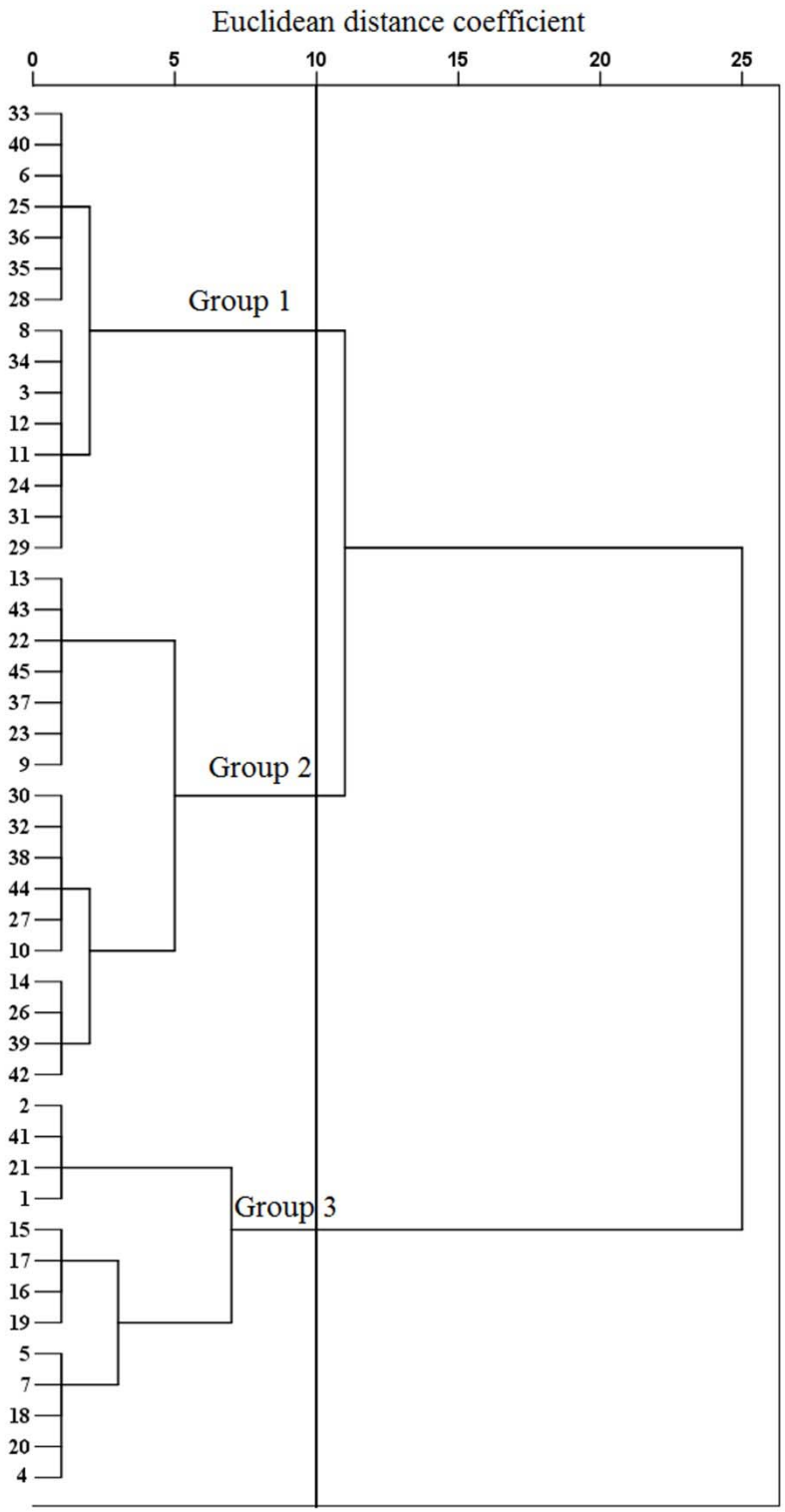

Fig. 4 Dendrogram prepared for the wild barley genotypes based on phenological and agronomic traits under heat stress conditions as well as heat tolerance index (HTI) using Ward's method. Numbers represent wild genotypes with details of geographical origins provided in Supplementary Table 1