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## Exploring Qualitative and Quantitative Genetic Variations of Barley (*Hordeum vulgare* L.) Genotypes Grown under Heat Stress Conditions

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**ABSTRACT:** Heat stress is a major abiotic constraint limiting barley (*Hordeum vulgare* L.) productivity in regions experiencing rising temperatures. This study evaluated the genetic variability and morphophysiological as well as yield responses of 50 barley genotypes under control and heat-stressed conditions to identify superior lines for thermotolerance breeding. A completely randomized design (CRD) with replications was used, and data was collected for ten morpho-physiological and yield traits. Analysis of variance (ANOVA) indicated highly significant ( $p \leq 0.001$ ) effects of genotype, treatment, and their interaction on most of the measured traits. Wide phenotypic variation was observed for grain yield ( $1.62\text{--}9.60\text{ g plant}^{-1}$ ) and thousand-grain weight ( $19.18\text{--}40.02\text{ g}$ ). High genotypic and phenotypic coefficients of variation, heritability, and genetic advance for grain yield  $\text{plant}^{-1}$ , total biomass accumulation  $\text{plant}^{-1}$ , and leaf area  $\text{plant}^{-1}$  indicated strong additive genetic control. Correlation and network analyses showed strong positive associations among plant height, total biomass accumulation  $\text{plant}^{-1}$ , chlorophyll content, leaf area  $\text{plant}^{-1}$ , thousand-grain weight, and grain yield  $\text{plant}^{-1}$ . Heatmap clustering and the multi-trait genotype-ideotype distance index (MGIDI) identified BD7194, BD7188, BD8579, BD9681, and IBON14 as best heat-tolerant genotypes. Principal component analysis (PCA) revealed variation primarily driven by grain yield  $\text{plant}^{-1}$ , leaf area  $\text{plant}^{-1}$ , and number grains spike $^{-1}$ . Overall, these findings demonstrate substantial genetic variation for heat tolerance and identify promising genotypes that can be used as valuable resources for developing climate-resilient barley cultivars suited to heating environments.

**KEYWORDS:** Barley; heat stress; genetic variability; multi-trait selection; yield stability; multivariate analysis

## 1 Introduction

The interaction between global climate change and the growth of the human population has resulted in a substantial impact on agricultural production [1]. Heat stress is a significant contributor to the adverse effects of climate change, as the average world temperature is projected to rise by 1.8–4°C in the 21st century [2] causing a noteworthy yield loss [3]. Elevated temperatures have a detrimental impact on agricultural productivity, rendering heat a significant abiotic stress [4]. The detrimental impact of heat on the reproductive system in various crops is a significant factor in the reduction of agricultural yields worldwide [5]. There is a considerable negative correlation between high seasonal temperatures and crop production, mostly due to heat stress [6].

Bangladesh is highly vulnerable to climate change, with rising temperatures and more frequent extreme heat events threatening crop growth. Changes in rainfall and rising soil salinity may further worsen heat-related damage. Barley (*Hordeum vulgare* L.) is a minor crop in Bangladesh, contributing approximately 0.10% to total cereal production [7]. Despite its limited cultivation, barley serves multiple agronomic purposes as forage and a cover crop and provides a rich source of dietary fiber, vitamins, minerals, and antioxidants. Increasing barley production alongside pulses and oilseeds could improve dietary balance and food security [8,9]. Bahrami et al. [10] defined terminal high-temperature stress as a continuous escalation in temperature, both within a single day and over consecutive days, during the reproductive stage of spring and winter cereals.

Yields in several cereal species, including wheat [11], barley [12], and oats [13] are negatively impacted by heat stress occurring during the grain-filling period. To adapt crops to future climate conditions, it is essential to understand their responses to elevated temperatures and identify strategies to improve heat tolerance [14]. In heat-prone regions of Bangladesh, identifying tolerant genetic material and uncovering its internal mechanisms is highly vital. The most viable strategy involves breeding heat-tolerant cultivars by merging physiological, molecular, and biochemical insights.

In the case of barley plants, increased temperature levels act as the main factor responsible for decreased fertility of florets [15]. In addition to that, heat stress during anthesis and grain development phases may result in early leaf senescence, a reduced grain-filling period, and finally, lowered grain weight [16]. Evaluating heat tolerance and identifying superior genotypes can be achieved through multi-trait selection tools such as the multi-trait genotype-ideotype distance index (MGIDI). By assessing both the strengths and weaknesses of breeding material, this index has proven valuable across a variety of crops, including wheat [17], rice [18], barley [19], and maize [20].

The present study targets to identify heat-tolerant barley genotypes with desirable morpho-physiological traits and high yield. In Bangladesh, no barley variety currently shows high heat tolerance, making it essential to screen diverse genotypes to select potential donor parents. Findings from multivariate analysis will support targeted breeding and the development of heat resilient barley varieties through conventional or biotechnological approaches.

## 2 Materials and Methods

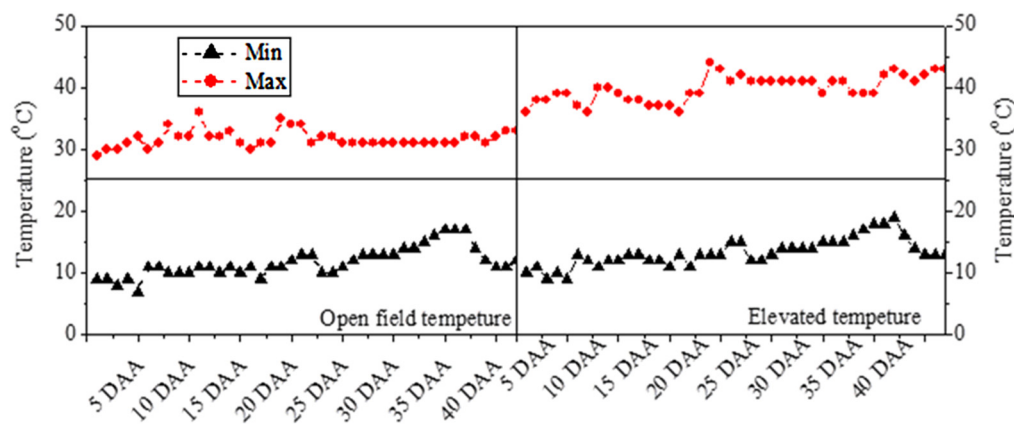
### 2.1 Experimental Site Description

The study was carried out during the winter (Rabi) seasons in the year 2018–2019 at the net house facility of the Plant Physiology Division of Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. Located within the Dhaka division region and positioned at a latitude of 23.60° N and longitude of 90.25° E, the study site is situated at an elevation of 8.4 m above mean sea level.

## 2.2 Plant Materials and Experimental Design

To evaluate heat tolerance, this study screened 50 barley (*Hordeum vulgare* L.) genotypes (Table S1). The germplasm consisted of 25 lines from the Plant Genetic Resource Centre (PGRC) and 4 sourced from the Plant Breeding Division at Bangladesh Agricultural Research Institute (BARI). The remaining 21 genotypes comprised commercial varieties and advanced lines provided by the International Centre for Agricultural Research in Dry Areas (ICARDA).

To assess the effect of temperature on the genotypes of barley, a factorial experiment was carried out under the completely randomized design (CRD) with three replications. The fertility of the soil and biological activities were increased in November 2018 by mixing the soil with organic substances at a ratio of 1:1. Plastic pots (diameter at the top of 17 cm, bottom diameter of 15 cm, and depth of 11 cm) were filled with 3 kg of this substrate. Prior to planting, seeds underwent surface sterilization in 2 H<sub>2</sub>O<sub>2</sub> for 30 min, followed by three rinses in distilled water. Initially, 10 seeds were sown in each pot, which were later thinned to three uniform seedlings per pot post-emergence. Temperature treatments were imposed during anthesis, defined as the period when yellow anthers were visible. Elevated temperature was applied using a polythene chamber (4 m × 3 m × 1.5 m height) constructed with a GI pipe frame and transparent polythene sheet, leaving a 30 cm opening at the base. Ambient temperature served as the control. When under heat stress, use a polythene chamber with a relative humidity of 45–50%, light intensity of 450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and VPD of 1.0–1.5 kPa, depending on the temperature. Temperature treatments were applied from 5 days after anthesis (DAA) until maturity, and air temperature inside the chambers was recorded daily from 14:00–15:00 h using a maximum-minimum thermometer (Fig. 1).



**Figure 1:** Barley genotypes received daily mean air temperatures (open field and elevated) ranging from 5 to 40 DAA, with a horizontal straight line indicating the critical temperature (25°C) for grain growth.

Just after anthesis, first few days we observed the polythene chamber temperature, when the thermometer reading comes in stable condition, we started to maintain reading, i.e., from 5 DAA. This data represents optimal temperature in open field condition and heat stress in elevated temperature under polythene chamber (for example, at anthesis stage, temperature exited 30°C which is higher than the optimal temperature for grain yield of barley).

Fertilizers were applied at a rate of 90 kg N ha<sup>-1</sup>, 15 kg P ha<sup>-1</sup>, 40 kg K ha<sup>-1</sup>, and 12 kg S ha<sup>-1</sup> [21]. The required dose for each pot was calculated based on pot area and applied through fertigation with water. Irrigation was maintained near field capacity to avoid drought stress. Field capacity of soil was determined at 0.33 atm pressure using a membrane extractor (Soil Moisture, Santa Barbara, CA, USA) as

described by Richards [22]. Measured values were also calibrated with a TDR 300 soil moisture probe (Spectrum Technologies, Aurora, IL, USA), and readings were made and used in subsequent soil water content measurements. No pest or disease incidence was observed during the experiment. At full maturity, plant growth and yield-related characteristics were recorded.

### 2.3 Data Collection

At harvesting stage, height and spike length of the plants were measured using the meter ruler in cm. The morphological measurements were total numbers of tillers plant<sup>-1</sup> and spikes plant<sup>-1</sup>. For each individual plant, their metrics such as total biomass accumulation in grams and grain yield in grams were calculated using a digital electric balance. The determination of total leaf area was carried out through automated leaf area meter, Model LI-3100C; LI-COR, Lincoln, NE, USA and expressed in (cm<sup>2</sup>). For the thousand-grain weight in g, it was measured electronically after the standardizing moisture level of the seeds to 14% using a digital grain moisture analyzer (Model TD-5, Ogawa Seiki Co., Ltd., Tokyo, Japan). Measurement of non-destructive chlorophyll content was done by measuring the Soil Plant Analysis Development (SPAD) value of leaves. The measurement was made using SPAD-502, Konica-Minolta, Japan in fully expanded, intact leaves (second from the apex of the leaf).

### 2.4 Heat Indexing Parameters

Six heat tolerance and susceptibility indices were calculated based on grain yield under control and stress conditions for each genotype using the following relationships:

- i. Tolerance index (TOL) =  $Y_p - Y_s$  [23]
- ii. Mean productivity (MP) =  $(Y_p + Y_s)/2$  [23]
- iii. Geometric mean productivity (GMP) =  $\sqrt{(Y_p \times Y_s)}$  [24]
- iv. Stress susceptibility index (SSI) =  $1 - (Y_s \div Y_p) \div 1 - (Y_s \div Y_p)$  [25]
- v. Stress Tolerance Index STI =  $(Y_p * Y_s)/\tilde{Y}_p$  [24]
- vi. Yield stability index (YSI) =  $(Y_s \div Y_p)$  [26]
- vii. Yield index (YI) =  $Y_s/Y_s$  [27]

Where,  $Y_s$  and  $Y_p$  are the mean yield of genotypes under stress and non-stress conditions, respectively.  $Y_s$  and  $Y_p$  are the mean yield of all genotypes under stress and non-stress conditions, respectively.  $\tilde{Y}_p$  and  $\tilde{Y}_s$ : Mean grain yield of all genotypes under control and heat stress conditions, respectively.

### 2.5 Statistical Analysis

The statistical analyses were conducted on the R Studio platform [28]. The ANOVA of the quantitative traits was carried out, and then separation of means was calculated by Fisher's least significant difference (LSD) post hoc test [29] at ( $p < 0.05$ ) through 'agricolae' R software [30]. The phenotypic and yield parameters were computed with the aid of quantitative genetics equations. The genotypic variance and phenotypic variance can be expressed as follows: Genotypic variance ( $\sigma_g^2$ ) =  $(GMS - EMS)/r$ ; where GMS represents the genotypic mean square, EMS denotes the error mean square, and  $r$  signifies the number of replications ( $r = 3$ ). And the phenotypic variance is  $(\sigma_p^2) = \sigma_g^2 + \sigma_e^2$ . To evaluate the extent of variation, the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were determined using the following expressions:  $GCV = \frac{\sigma_g^2}{\bar{X}} \times 100$ , where  $\sigma_g^2$  is genotypic variance and  $\bar{X}$  is mean of the parameter;  $PCV = \frac{\sigma_p^2}{\bar{X}} \times 100$ ; where  $\sigma_p^2$  is phenotypic variance and  $\bar{X}$  is population mean. Additionally, broad-sense heritability ( $h^2_b$ ) was computed as a percentage using the ratio of genotypic to phenotypic

variance:  $\sigma_g^2/\sigma_p^2 \times 100$ . Expected genetic advance (GA) was subsequently modeled using the standard formulation:  $GA = h_b^2 \cdot K \cdot \sigma_p$ . Within this equation,  $K$  represents the standardized selection differential (assigned a value of 2.06 at a 5% selection intensity threshold), while  $\sigma_p$  designates the phenotypic standard deviation. Furthermore, the genetic advance as a percentage of the mean (GAM) was calculated using the following relationship:  $GAM = \frac{GA}{\bar{X}} \times 100$ . The computation of the breeding values (Table 1) was carried out through the multi-trait genotype-ideotype distance index (MGIDI) algorithm, implemented in the ‘metan’ package in the R software suite [28].

**Table 1:** The formulas for determining the breeding values and genetic parameter using MGIDI to choose superior barley genotypes in heat.

| Selection Indices                                                                                                                                     | Equation No. | Reference |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|-----------|
| Multi trait genotype-ideotype index, $MGIDI_i = \left[ \sum_{j=1}^f (y_{ij} - \gamma_j)^2 \right]^{0.5}$                                              | (1)          | [31]      |
| Ideotype design and rescaling of traits, $rX_{ij} = \frac{\eta_{nj} - \phi_{nj}}{\eta_{oj} - \phi_{oj}} \times (\theta_{ij} - \eta_{oj}) + \eta_{nj}$ | (2)          |           |
| Factor analysis, $X = \mu + Lf + \varepsilon$                                                                                                         | (3)          |           |
| Factor loadings, $F = Z(A^T R^{-1})^T$                                                                                                                | (4)          |           |
| Strength and weaknesses of selected genotypes, $\omega_{ij} = \frac{\sqrt{D_{ij}^2}}{\sum_{j=1}^f \sqrt{D_{ij}^2}}$                                   | (5)          |           |

The key purpose of calculating MGIDI was selecting the top-ranked genotype (Eq. (1)) from among the candidates. Before the computation of MGIDI, it was essential to define ideotypes and rescale the data (Eq. (2)). Then, the steps involved factor analysis (Eq. (3)) and their loadings (Eq. (4)), which was done based on a varimax rotation technique suggested by Kaiser [32]. The objective of this multi-trait selection approach was to enhance the selection process in terms of all traits except for leaf broadness (LFB), at an extremely stringent selection intensity of 15%. Finally, the relative strengths and weaknesses of the selected genotypes (Eq. (5)) were identified using factor loadings.

The correlation coefficients among quantitative traits were analyzed and depicted in a network plot using the ‘tidyverse’ and ‘corr’ libraries of R software [28]. The significance of correlations was visualized using ‘metan’. A heatmap incorporating phenotypic and genotypic relative values was created using the ‘Complex Heatmap’ package. Principal component analysis (PCA) was performed using the library ‘MASS’, ‘factoextra’, ‘tidyverse’, ‘ggplot2’, ‘FactoMineR’ using function ‘prcomp’ and ‘fviz’ of R software. A PCA biplot was visualized using the package ‘ggbiplot’ [33] where the first two PCs were plotted.

### 3 Results

#### 3.1 ANOVA of Quantitative Traits

The analysis of variance (ANOVA) confirmed extremely significant variation amongst the quantitative traits for treatments (heat) and genotypes. The interaction between treatments and genotypes also significantly affected the traits of the genotypes studied (Table 2).

#### 3.2 Frequency Distribution and Descriptive Statistics of the Traits

All traits conformed to a normal distribution, except for some that were left- or right-skewed (Figs. 2 and 3).

**Table 2:** ANOVA for the yield and yield contributing traits of 50 barley genotypes studied under both control and heat stress.

| Traits | Mean Sum of Squares (MSS) |               |            |                      |
|--------|---------------------------|---------------|------------|----------------------|
|        | Replication               | Treatment     | Genotype   | Treatment × Genotype |
| PLH    | 0.13 ns                   | 5966.14***    | 458.03***  | 16.74***             |
| TDM    | 0.60 ns                   | 2587.27***    | 158.71***  | 7.09***              |
| NT     | 25.95***                  | 10.31**       | 7.12***    | 0.09 ns              |
| NSP    | 22.79***                  | 9.003**       | 6.06***    | 0.15 ns              |
| SL     | 0.96 ns                   | 941.70***     | 30.85***   | 5.77***              |
| NGS    | 25.22***                  | 3407.90***    | 39.71***   | 13.41***             |
| THGW   | 59.03***                  | 790,534.59*** | 7536.0***  | 4722.17***           |
| YIELD  | 16.47***                  | 110,669.12*** | 1674.70*** | 264.93***            |
| LA     | 5.50**                    | 17,737.98***  | 224.40***  | 9.62***              |
| SPAD   | 4.27*                     | 1248.54***    | 1062.01*** | 6.61***              |

Note: \*\*\* = significant at  $p < 0.001$ , \*\* = significant at  $p < 0.01$ , \* = significant at  $p < 0.05$ , ns = Non-significant, PLH = Plant height (cm), TDM = Total biomass accumulation/plant (g), NT = Number of tillers/plant, NSP = Number of spikes/plant, SL = Spike length (cm), NGS = Number of grains/spike, THGW = Thousand grain weight (g), YIELD = Grain yield/plant (g), LA = Leaf area/plant (cm<sup>2</sup>), SPAD = Soil Plant Analysis Development.

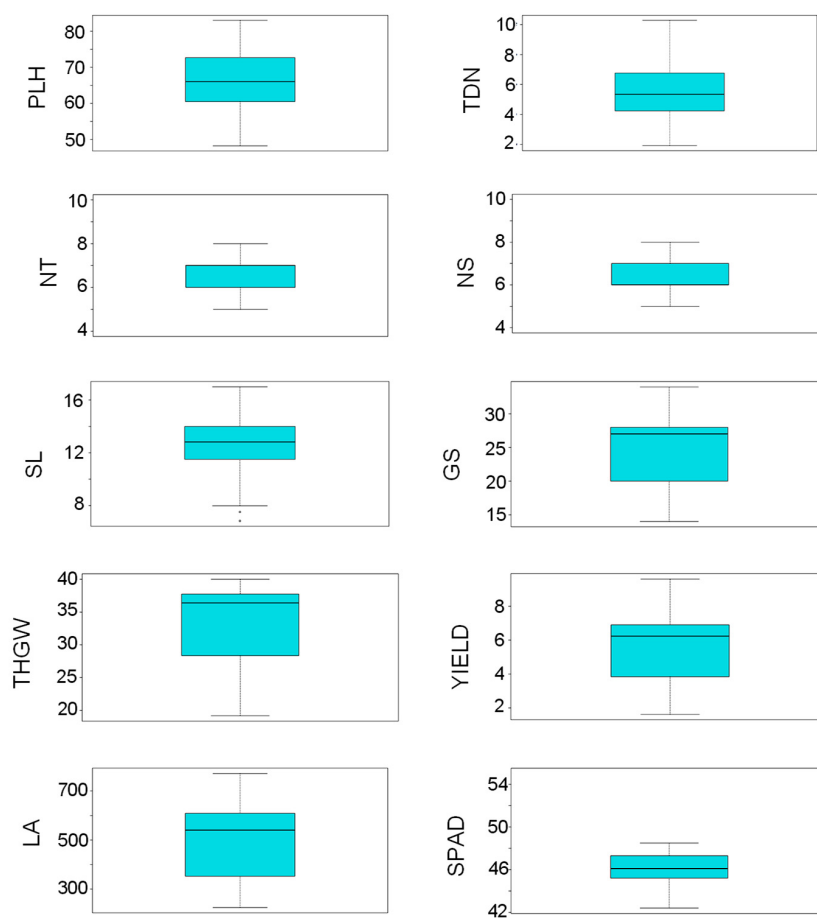
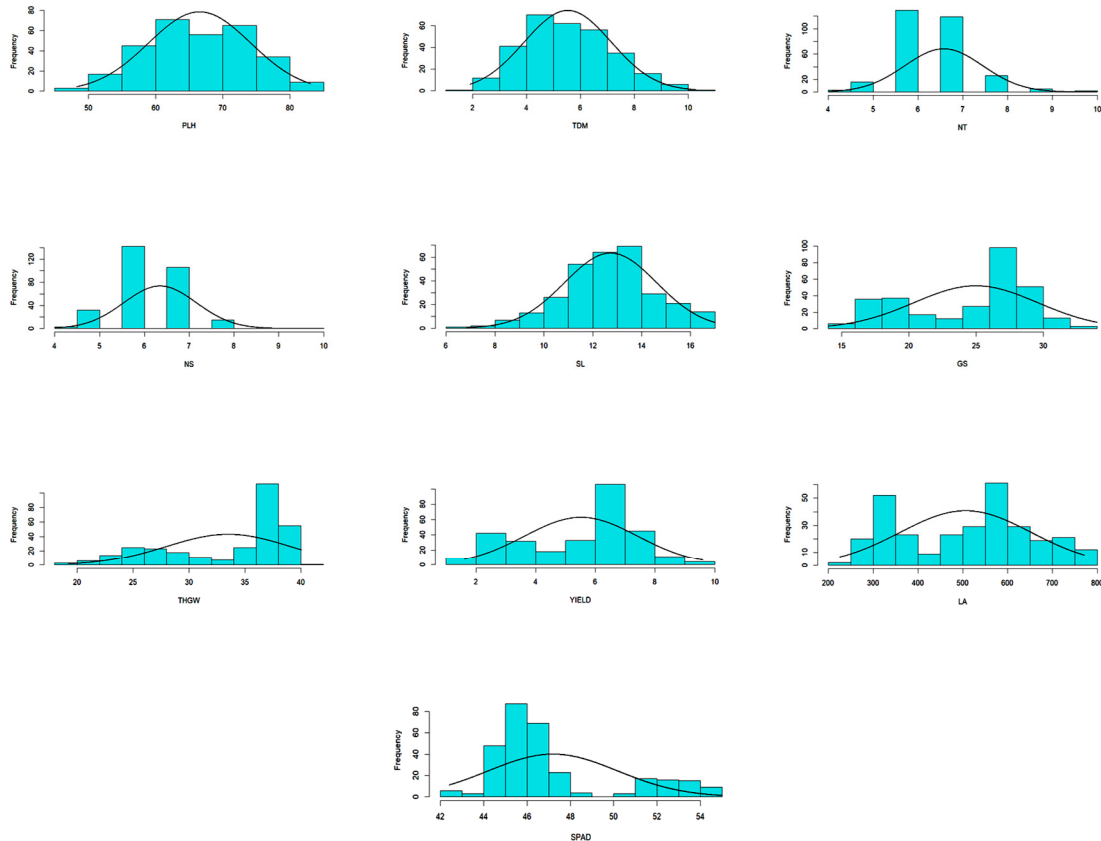
**Figure 2:** Boxplots showed the pattern of the measured traits for 50 barley genotypes under pooled observations. PLH = Plant height (cm), TDM = Total biomass accumulation/plant (g), NT = Number of tillers/plant, NS = Number of spikes/plant, SL = Spike length (cm), GS = Number of grains/spike, THGW = Thousand grain weight (g), YIELD = Grain yield/plant (g), LA = Leaf area/plant (cm<sup>2</sup>). SPAD = Soil Plant Analysis Development.



Table 3 shows descriptive statistics for the measured morpho-physiological traits, such as mean, standard deviation (SD), minimum (Min), and maximum (Max). A large range of variability was observed in all the phenotypic values. PLH varied from 48.23 to 83.00. THGW values ranged from 19.18 to 40.02, YIELD from 1.62 to 9.60, LA from 224.90 to 771.20, and SPAD from 42.40 to 55.00. TDM values ranged from 1.91 to 10.29, NT values from 4.00 to 10.00, NS values from 4.00 to 10.00, SL values from 6.83 to 17.00, GS values from 14.00 to 43.00 (Fig. 3).



**Figure 3:** The frequency distribution of the 50 barley genotypes according to quantitative attributes under pooled observations. PLH = Plant height (cm), TDM = Total biomass accumulation/plant (g), NT = Number of tillers/plant, NS = Number of spikes/plant, SL = Spike length (cm), GS = Number of grains/spike, THGW = Thousand grain weight (g), YIELD = Grain yield/plant (g), LA = Leaf area/plant (cm<sup>2</sup>), SPAD = Soil Plant Analysis Development.

Across the whole dataset, the CVs indicated different levels of relative dispersion within each trait, ranging from 0.24% for THGW to 9.39% for NS. Traits with lower CVs, such as PLH, THGW, YIELD, LA, and SPAD, showed less variability, reflecting higher consistency in their values across the dataset. In contrast, traits with higher CVs, including NT, NS, TDM, SL, and GS, exhibited greater relative dispersion, indicating a wider range of values throughout the sample (Table 2).

The following Table 4 shows the computed values of various genetic parameters such as the phenotypic variance, genotypic variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in the broad sense, and genetic advance expected in relation to all the ten tested traits. TDM showed the highest values of both GCV (35.20%) and PCV (35.53%), followed by YIELD and LA. Extreme scoring values of PCV and GCV show that there is a high level of genetic control on these specific

traits. The traits with medium PCV and GCV include PLH, NT, NS, SL, GS, and THGW, whereas the trait with the lowest PCV and GCV is SPAD.

**Table 3:** Descriptive data on quantitative features of 50 barley genotypes.

| Traits | Mean   | Minimum | Maximum | Standard Deviation | CV (%) |
|--------|--------|---------|---------|--------------------|--------|
| PLH    | 66.55  | 48.24   | 83.00   | 7.62               | 11.45  |
| TDM    | 5.53   | 1.91    | 10.29   | 1.62               | 29.29  |
| NT     | 6.57   | 4.00    | 10.00   | 0.87               | 13.24  |
| NSP    | 6.35   | 4.00    | 10.00   | 0.81               | 12.76  |
| SL     | 12.73  | 6.83    | 17.00   | 1.89               | 14.85  |
| NGS    | 24.98  | 14.00   | 34.00   | 4.62               | 18.49  |
| THGW   | 33.52  | 19.1    | 40.02   | 5.56               | 16.59  |
| YIELD  | 5.52   | 1.62    | 9.60    | 1.90               | 13.42  |
| LA     | 506.20 | 224.90  | 771.20  | 147.17             | 29.07  |
| SPAD   | 47.19  | 42.40   | 55.00   | 2.99               | 6.34   |

Note: PLH = Plant height (cm), TDM = Total biomass accumulation/plant (g), NT = Number of tillers/plant, NSP = Number of spikes/plant, SL = Spike length (cm), NGS = Number of grains/spike, THGW = Thousand grain weight (g), YIELD = Grain yield/plant (g), LA = Leaf area/plant (cm<sup>2</sup>), SPAD = Soil Plant Analysis Development.

**Table 4:** Genetic variability and statistical parameter estimation for yield components in 50 barley genotypes.

| Traits | GV        | EV     | PV        | GCV (%) | PCV (%) | H (%)  | GA     | GAM   |
|--------|-----------|--------|-----------|---------|---------|--------|--------|-------|
| PLH    | 89.93     | 0.60   | 90.53     | 14.25   | 14.30   | 99.34  | 19.34  | 29.06 |
| TDM    | 3.79      | 0.07   | 3.86      | 35.20   | 35.53   | 98.14  | 3.90   | 70.50 |
| NT     | 0.76      | 0.37   | 1.14      | 13.29   | 16.22   | 67.12  | 0.99   | 15.06 |
| NSP    | 0.60      | 0.36   | 0.96      | 12.21   | 15.40   | 62.81  | 0.79   | 12.52 |
| SL     | 3.63      | 0.36   | 3.99      | 14.97   | 15.69   | 90.98  | 3.41   | 26.75 |
| NGS    | 13.17     | 1.00   | 14.17     | 14.53   | 15.07   | 92.94  | 6.70   | 26.81 |
| THGW   | 16.73     | 0.00   | 16.73     | 12.20   | 12.20   | 100.00 | 8.43   | 25.14 |
| YIELD  | 2.94      | 0.01   | 2.95      | 31.04   | 31.10   | 99.66  | 3.51   | 63.63 |
| LA     | 16,395.33 | 220.00 | 16,615.33 | 25.30   | 25.46   | 98.68  | 258.55 | 51.08 |
| SPAD   | 17.67     | 0.05   | 17.72     | 8.91    | 8.92    | 99.72  | 8.62   | 18.27 |

Note: PLH = Plant height (cm), TDM = Total biomass accumulation/plant (g), NT = Number of tillers/plant, NSP = Number of spikes/plant, SL = Spike length (cm), NGS = Number of grains/spike, THGW = Thousand grain weight (g), YIELD = Grain yield/plant (g), LA = Leaf area/plant (cm<sup>2</sup>), SPAD = Soil Plant Analysis Development.

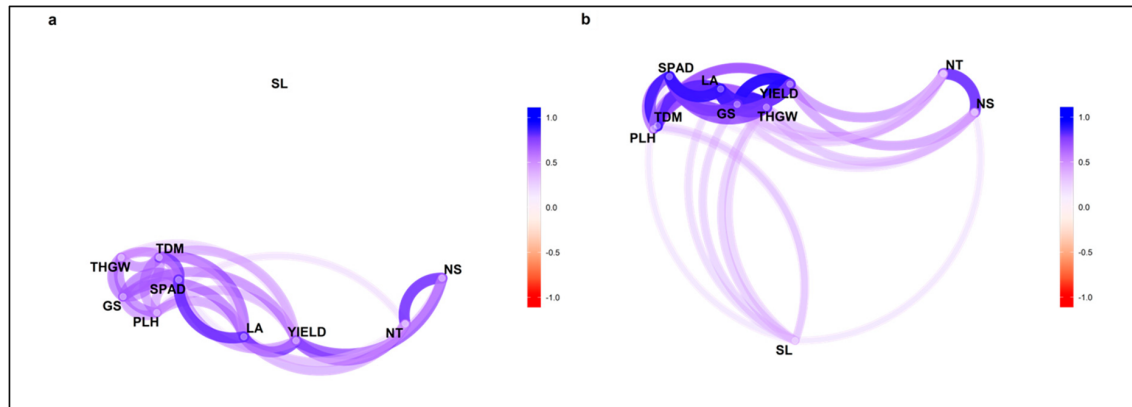
Genetic variability components, specifically the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and genetic advance as a percentage of mean (GAM) were categorized as low (<10%), moderate (10–20%), or high (>20%). Meanwhile, broad-sense heritability (H) was established as low (0–30%), moderate (31–60%), and high (≥61%).

### 3.3 Correlation Coefficients among Various Traits of Barley Genotypes under Control and Heat Stress

The correlation between various traits of 50 barley genotypes under control and heat stress was observed as positive and significant (Fig. 4). Three clusters of the studied traits under both control and heat stress were identified in the network plot (Fig. 4). While NS and NT clustered together under control conditions, PLH, GS, TDM, THGW, and SPAD were clustered together and had significant positive relationships. SL situated alone away from the other two traits cluster and exhibited no correlations with the traits studied. No significant correlation was found between NS, NT, and PLH. In heat stress conditions, PLH, TDM, SPAD, LA, GS, THGW, and YIELD were situated together and exhibited strong positive correlations, whereas NS and NT clustered together. SL was positioned alone, away from the other two trait clusters, and



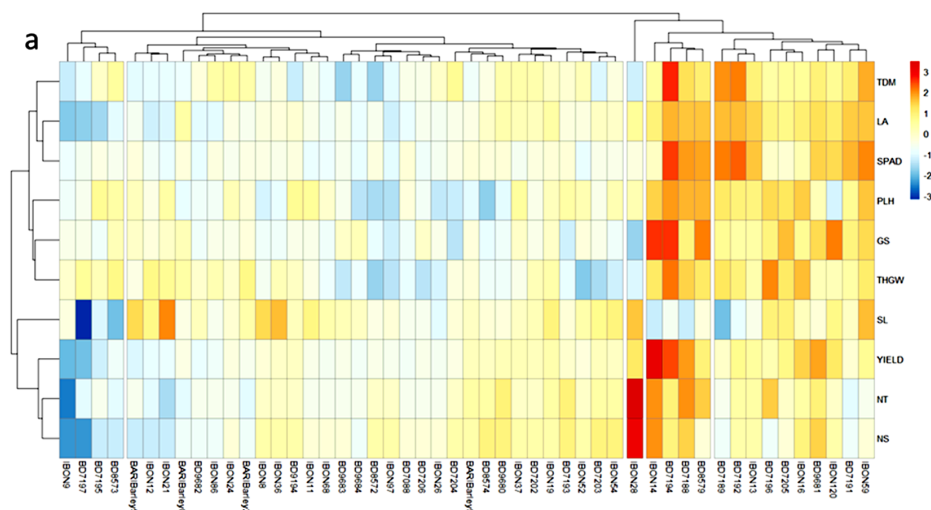
showed weaker positive correlations with the traits studied. No significant correlation was found between NS and PLH.



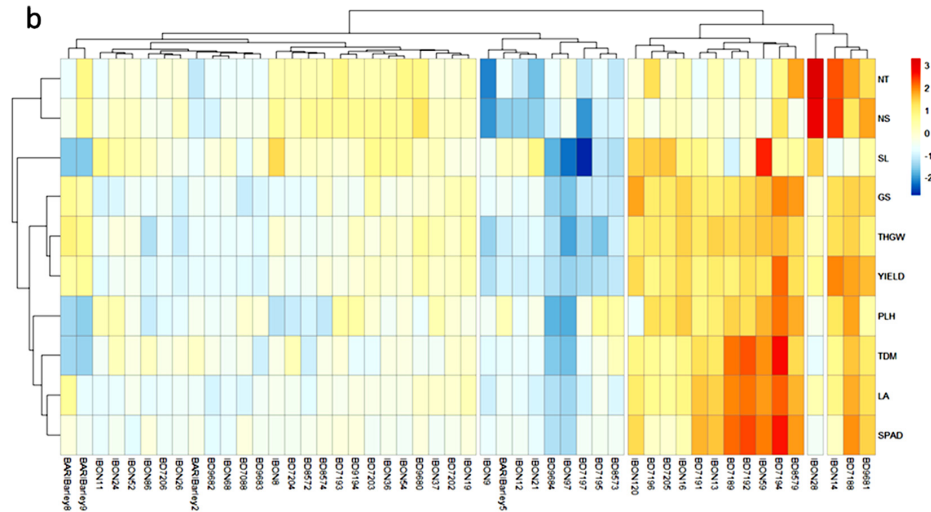
**Figure 4:** Network plot for correlation coefficients among various traits of 50 barley genotypes under control (a) and heat stress (b). PHT = Plant height (cm), TDM = Total biomass accumulation/plant (g), LA = Leaf area/plant (cm<sup>2</sup>), NT = Number of tillers/plants, SL = Spike length (cm), NS = Number of spikes/plants, GS = Number of grains/spikes, THGW = Thousand grain weight (g) and YIELD = Grain yield/plant (g), SPAD = Soil Plant Analysis Development.

### 3.4 Heatmap for the Chromatic Presentation of the Performance of the Genotypes under Control and Heat Conditions

The heatmap independently clustered agronomic traits and genotypes based on data from control and heat stress conditions (Fig. 5a,b). Under control conditions, genotypes BD7194, BD7188, IBON 14, and BD8579 exhibited higher performance across the agronomic traits (Fig. 5a). BD7189 and BD7192 showed moderate performance, while IBON9 and BD7197 had the lowest performance. Under heat stress, BD7194, BD7188, IBON 14, and BD8579 displayed the highest tolerance according to the measured traits (Fig. 5b). BD7189 and BD7192 showed moderate tolerance, whereas IBON97, BD9684, and BARI Barley 9 showed the lowest tolerance.



**Figure 5: Cont.**



**Figure 5:** Heatmap for the chromatic presentation of the 50 barley genotypes performance under control (a) heat (b) condition with 10 morpho-physiological traits. Bootstrap-based (1000 samples) cluster validation demonstrated moderate to high stability of genotype groupings under both control and heat-stress conditions. Jaccard similarity coefficients ranged from 0.607 to 0.897 under control conditions and from 0.762 to 0.893 under heat stress, indicating that clustering patterns were generally reproducible, with stronger cluster stability observed under heat stress. PHT = Plant height (cm), TDM = Total biomass accumulation/plant (g), LA = Leaf area/plant (cm<sup>2</sup>), NT = Number of tillers/plant, SL = Spike length (cm), NS = Number of spikes/plant, GS = Number of grains/spike, THGW = Thousand grain weight (g) and YIELD = Grain yield/plant (g), SPAD = Soil Plant Analysis Development.

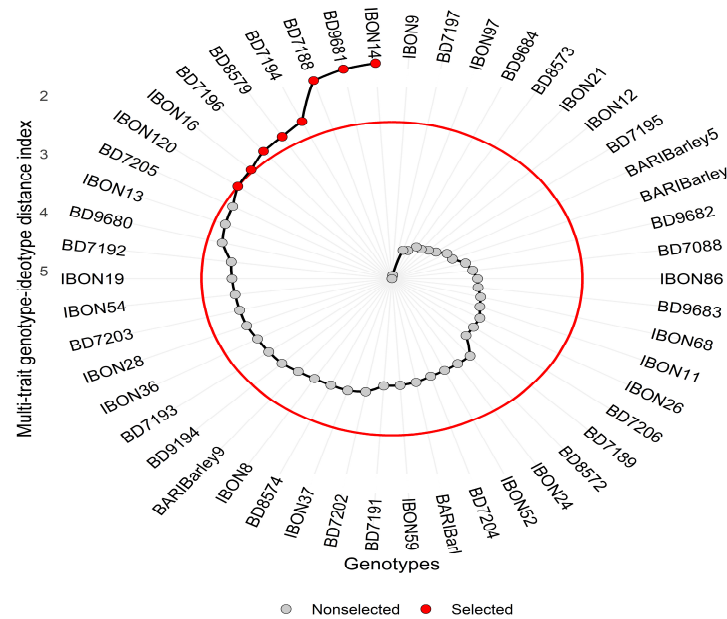
### 3.5 Multi-Trait Genotype-Ideotypes Distance Index (MGIDI)

The most superior lines in barley for all the examined traits were selected based on the MGIDI index. As a result, eight cultivars were selected: IBON14, BD9681, BD7188, BD7194, BD8579, BD7196, IBON16, and IBON120 (Fig. 6). The functional characteristics (strengths and weaknesses) of the selected genotypes are shown in Fig. 7 and were categorized using factor analysis (Table 5). Traits with higher factor loadings were considered strengths, whereas those with lower loadings were considered weaknesses of the selected genotypes. The average uniqueness of the traits studied was 0.18; the highest uniqueness was obtained for THGW, which was 0.72, while SPAD and NT had the lowest unique scores, which were 0.05 (Table 5).

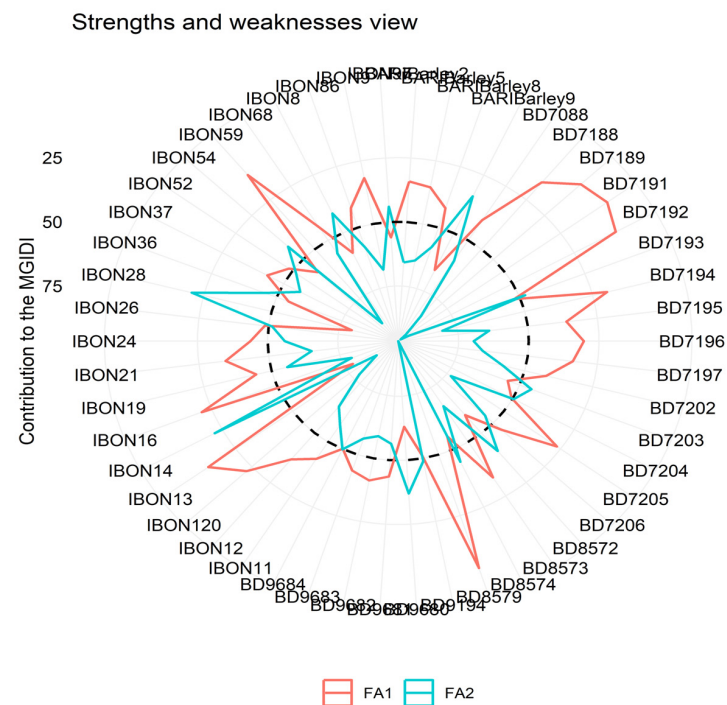
**Table 5:** Factor analysis and factor loadings of studied traits under heat stress for MGIDI.

| VAR     | FA1   | FA2   | Uniquenesses |
|---------|-------|-------|--------------|
| PLH     | -0.84 | -0.05 | 0.29         |
| TDM     | -0.93 | -0.07 | 0.13         |
| SL      | -0.17 | -0.92 | 0.12         |
| GS      | -0.1  | -0.95 | 0.08         |
| THGW    | -0.41 | -0.34 | 0.72         |
| YIELD   | -0.87 | -0.37 | 0.11         |
| LA      | -0.82 | -0.47 | 0.11         |
| SPAD    | -0.79 | -0.57 | 0.05         |
| NT      | -0.93 | -0.29 | 0.05         |
| NS      | -0.94 | -0.12 | 0.1          |
| Average | -     | -     | 0.18         |

Note: PLH = Plant height (cm), TDM = Total biomass accumulation/plant (g), SL = Spike length (cm), GS = Number of grains/spike, THGW = Thousand grain weight (g) and YIELD = Grain yield/plant (g), LA = Leaf area/plant (cm<sup>2</sup>), NT = Number of tillers/plant, NS = Number of spikes/plant, SPAD = Soil Plant Analysis Development.



**Figure 6:** 50 barley genotypes ranking and selected genotypes through MGIDI considering 15% selection intensity.

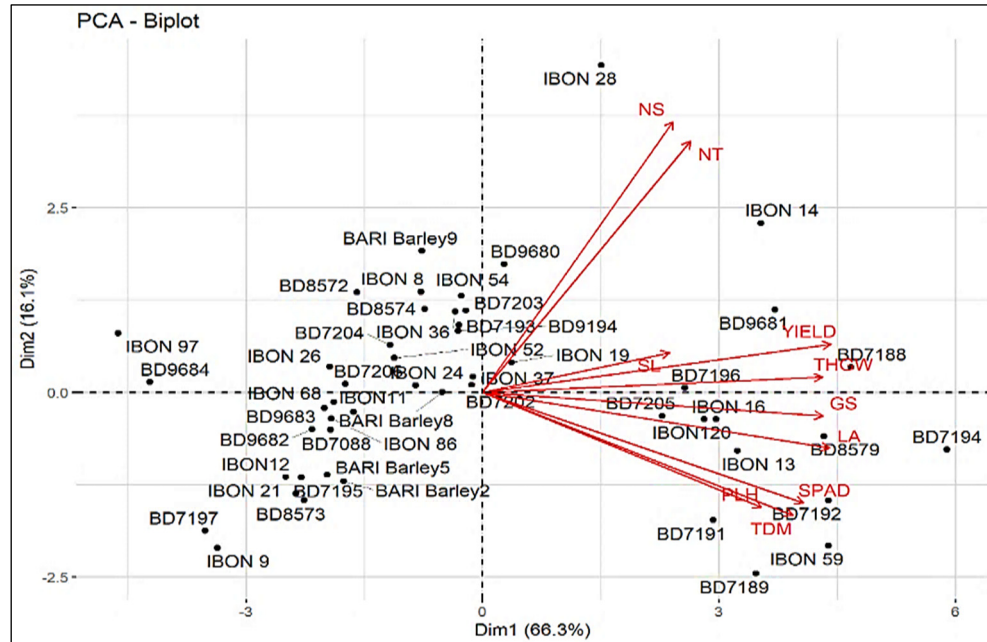


**Figure 7:** Phenotypic Profiles: strengths and weaknesses of evaluated genotypes.

### 3.6 PCA-Based Ordination of the Performance of the Genotypes

Principal component analysis (PCA) identified 10 components (Fig. 8), with the first two explaining 82.4% of the variation. LA and YIELD contributed most to these PCs. THGW, GS, and LA were strongly linked with YIELD, while PLH, SPAD, and TDM were also correlated. Genotypes BD7194, BD7188, and BD8579 clustered together, whereas IBON97 and BD9684 were in the opposite quadrant. BD7194, BD7188, BD9681,

and IBON14 showed higher YIELD under heat stress due to favorable trait combinations (Fig. 8). Bootstrap validation of PCA demonstrated high stability of the extracted components. The standard deviations of the explained variance across 1000 bootstrap resamples ranged from 0.031 for PC1 and 0.029 for PC2 to less than 0.001 for the later components, indicating that the PCA structure was highly reproducible.



**Figure 8:** Biplot ordination mapping yield components and production traits for fifty barley (*Hordeum vulgare* L.) lines subjected to heat stress. PLH = Plant height (cm), TDM = Total biomass accumulation/plant (g), NT = Number of tillers/plant, NS = Number of spikes/plant, SL = Spike length (cm), GS = Number of grains/spike, THGW = Thousand grain weight (g), YIELD = Grain yield/plant (g), LA = Leaf area/plant (cm<sup>2</sup>), SPAD = Soil Plant Analysis Development.

### 3.7 Selection of Genotypes Based on Stress Tolerance Indices

Different heat indexing parameters like Tolerance Index (TOL), Mean Productivity (MP), Geometric Mean Productivity (GMP), Stress Susceptibility Index (SSI), Yield Stability Index (YSI), Yield Index (YI), Stress Tolerance Index (STI) and Relative Yield (RY) were studied for fifty barley genotypes. A considerable variation among the genotypes was observed regarding different heat indexing parameters (Table 6). In the parameter tolerance index (TOL), the lowest difference between yields in both conditions was observed for the BD7189 followed by BD7192, BD7191 and BD7194, but the highest difference belonged to the genotype IBON 97 followed by BD9684, BD8573 and BD9683. The highest MP value was found in genotypes IBON 14, BD7194, BD7188, BD9681, IBON 16 and IBON120. While the genotype BD7197, IBON 9, BD7195 and IBON 97 showed the lowest MP. More or less, similar trend was observed in case of GMP. Similarly, the highest GMP value was found in IBON 14, BD7194, BD7188, BD9681, IBON 16 and IBON120 and the lowest was found in BD7197 followed by IBON 9, IBON 97 and BD7195. According to Fischer and Maurer's (1978) parameter, SSI, the genotypes BD7194 followed by, BD7192, BD7189 and IBON 59 were in the lowest value, whereas the genotypes of IBON 97 followed by BD9684 and BD7195 with highest SSI values. According to yield stability index (YSI) the highest value was found in BD7194, BD7192, BD7189, IBON 59 and BD7188 and the lowest was found in IBON 97, BD9684 and BD7195 and yield index (YI) showed almost similar trend like YSI. Under heat stress conditions, the genotypes IBON 14, BD7194, BD7188, BD9681, IBON 16

and IBON120 showed higher values of stress tolerance index though BD7197, IBON 9 and IBON 97 were produced lowest values. Relative yield (RY) was highest in genotype BD7194 followed by BD7192, BD7189 and IBON 59. The lowest RY value was found in IBON 97, BD9684, BD7195 and BD8573.

**Table 6:** Heat tolerance indices of forty barley genotypes under two temperature regimes.

| Genotypes    | Yp   | Ys   | TOL  | MP   | GMP  | SSI  | YSI  | YI   | STI  | RY    |
|--------------|------|------|------|------|------|------|------|------|------|-------|
| IBON 14      | 9.55 | 7.32 | 2.24 | 8.44 | 8.36 | 0.58 | 0.77 | 1.77 | 1.46 | 76.56 |
| BD7194       | 8.95 | 7.67 | 1.28 | 8.31 | 8.29 | 0.36 | 0.86 | 1.86 | 1.44 | 85.65 |
| BD7188       | 8.53 | 7.00 | 1.53 | 7.76 | 7.72 | 0.45 | 0.82 | 1.69 | 1.25 | 82.09 |
| BD9681       | 8.43 | 6.76 | 1.67 | 7.60 | 7.55 | 0.49 | 0.80 | 1.64 | 1.19 | 80.22 |
| IBON 16      | 8.10 | 6.31 | 1.78 | 7.20 | 7.15 | 0.55 | 0.78 | 1.53 | 1.07 | 77.96 |
| IBON120      | 7.92 | 6.35 | 1.58 | 7.14 | 7.09 | 0.49 | 0.80 | 1.54 | 1.05 | 80.09 |
| BD8579       | 7.72 | 6.29 | 1.44 | 7.01 | 6.97 | 0.46 | 0.81 | 1.52 | 1.02 | 81.39 |
| BD7192       | 7.50 | 6.26 | 1.23 | 6.88 | 6.85 | 0.41 | 0.84 | 1.52 | 0.98 | 83.52 |
| IBON 59      | 7.46 | 6.17 | 1.29 | 6.82 | 6.79 | 0.43 | 0.83 | 1.49 | 0.96 | 82.67 |
| BD7205       | 7.56 | 5.83 | 1.73 | 6.70 | 6.64 | 0.57 | 0.77 | 1.41 | 0.92 | 77.12 |
| IBON 13      | 7.35 | 5.92 | 1.43 | 6.64 | 6.60 | 0.48 | 0.81 | 1.43 | 0.91 | 80.56 |
| IBON 28      | 7.92 | 5.28 | 2.64 | 6.60 | 6.46 | 0.83 | 0.67 | 1.28 | 0.88 | 66.61 |
| BD7189       | 6.87 | 5.71 | 1.16 | 6.29 | 6.26 | 0.42 | 0.83 | 1.38 | 0.82 | 83.17 |
| BD7196       | 7.09 | 5.48 | 1.60 | 6.29 | 6.23 | 0.56 | 0.77 | 1.33 | 0.81 | 77.41 |
| BD7191       | 6.79 | 5.52 | 1.27 | 6.16 | 6.12 | 0.46 | 0.81 | 1.34 | 0.79 | 81.34 |
| BARI Barley9 | 7.00 | 5.13 | 1.87 | 6.07 | 5.99 | 0.66 | 0.73 | 1.24 | 0.75 | 73.32 |
| IBON 19      | 7.20 | 4.64 | 2.56 | 5.92 | 5.78 | 0.88 | 0.64 | 1.12 | 0.70 | 64.44 |
| BD9680       | 7.07 | 4.71 | 2.35 | 5.89 | 5.77 | 0.83 | 0.67 | 1.14 | 0.70 | 66.71 |
| BARI Barley8 | 6.56 | 4.96 | 1.60 | 5.76 | 5.71 | 0.61 | 0.76 | 1.20 | 0.68 | 75.57 |
| BD7203       | 6.99 | 4.24 | 2.74 | 5.61 | 5.44 | 0.98 | 0.61 | 1.03 | 0.62 | 60.71 |
| BD7202       | 6.99 | 4.23 | 2.76 | 5.61 | 5.44 | 0.98 | 0.61 | 1.03 | 0.62 | 60.52 |
| IBON 37      | 7.04 | 4.18 | 2.85 | 5.61 | 5.42 | 1.01 | 0.59 | 1.01 | 0.62 | 59.44 |
| IBON 36      | 6.90 | 3.98 | 2.92 | 5.44 | 5.24 | 1.05 | 0.58 | 0.96 | 0.58 | 57.70 |
| IBON 54      | 6.97 | 3.88 | 3.10 | 5.43 | 5.20 | 1.10 | 0.56 | 0.94 | 0.57 | 55.60 |
| BD8574       | 6.97 | 3.87 | 3.11 | 5.42 | 5.19 | 1.11 | 0.55 | 0.94 | 0.56 | 55.45 |
| BD9194       | 6.97 | 3.87 | 3.10 | 5.42 | 5.19 | 1.11 | 0.55 | 0.94 | 0.56 | 55.48 |
| BD7193       | 6.85 | 3.56 | 3.29 | 5.21 | 4.94 | 1.19 | 0.52 | 0.86 | 0.51 | 51.99 |
| IBON 8       | 6.75 | 3.55 | 3.20 | 5.15 | 4.90 | 1.18 | 0.53 | 0.86 | 0.50 | 52.62 |
| IBON 52      | 6.68 | 3.42 | 3.26 | 5.05 | 4.78 | 1.21 | 0.51 | 0.83 | 0.48 | 51.18 |
| IBON 24      | 6.59 | 3.21 | 3.39 | 4.90 | 4.60 | 1.28 | 0.49 | 0.78 | 0.44 | 48.60 |
| BD8572       | 6.48 | 3.20 | 3.28 | 4.84 | 4.55 | 1.26 | 0.49 | 0.78 | 0.43 | 49.42 |
| BD7204       | 6.56 | 3.16 | 3.40 | 4.86 | 4.55 | 1.29 | 0.48 | 0.77 | 0.43 | 48.15 |
| BD7206       | 6.50 | 3.01 | 3.50 | 4.75 | 4.42 | 1.34 | 0.46 | 0.73 | 0.41 | 46.23 |
| IBON 68      | 6.38 | 2.97 | 3.41 | 4.67 | 4.35 | 1.33 | 0.47 | 0.72 | 0.40 | 46.57 |
| BARI Barley2 | 6.47 | 2.86 | 3.61 | 4.66 | 4.30 | 1.39 | 0.44 | 0.69 | 0.39 | 44.15 |
| IBON11       | 6.52 | 2.78 | 3.74 | 4.65 | 4.26 | 1.43 | 0.43 | 0.67 | 0.38 | 42.66 |
| BD9683       | 6.51 | 2.68 | 3.82 | 4.60 | 4.18 | 1.46 | 0.41 | 0.65 | 0.37 | 41.27 |
| BD9682       | 6.17 | 2.79 | 3.39 | 4.48 | 4.15 | 1.36 | 0.45 | 0.67 | 0.36 | 45.14 |
| BD7088       | 6.35 | 2.69 | 3.66 | 4.52 | 4.13 | 1.43 | 0.42 | 0.65 | 0.36 | 42.34 |
| IBON 26      | 6.40 | 2.66 | 3.74 | 4.53 | 4.12 | 1.45 | 0.42 | 0.64 | 0.36 | 41.51 |
| IBON 86      | 6.30 | 2.62 | 3.69 | 4.46 | 4.06 | 1.45 | 0.42 | 0.63 | 0.35 | 41.51 |
| IBON 21      | 6.16 | 2.57 | 3.59 | 4.37 | 3.98 | 1.45 | 0.42 | 0.62 | 0.33 | 41.73 |
| BARI Barley5 | 5.99 | 2.49 | 3.50 | 4.24 | 3.87 | 1.45 | 0.42 | 0.60 | 0.31 | 41.64 |
| IBON12       | 6.12 | 2.42 | 3.70 | 4.27 | 3.85 | 1.50 | 0.40 | 0.59 | 0.31 | 39.51 |
| BD8573       | 6.27 | 2.27 | 3.99 | 4.27 | 3.77 | 1.58 | 0.36 | 0.55 | 0.30 | 36.30 |
| BD9684       | 6.34 | 1.99 | 4.35 | 4.17 | 3.55 | 1.70 | 0.31 | 0.48 | 0.26 | 31.42 |
| BD7195       | 5.87 | 2.07 | 3.80 | 3.97 | 3.49 | 1.61 | 0.35 | 0.50 | 0.25 | 35.29 |
| IBON 97      | 6.45 | 1.73 | 4.72 | 4.09 | 3.34 | 1.82 | 0.27 | 0.42 | 0.23 | 26.87 |
| IBON 9       | 5.21 | 2.04 | 3.16 | 3.63 | 3.26 | 1.51 | 0.39 | 0.50 | 0.22 | 39.25 |
| BD7197       | 5.30 | 1.95 | 3.35 | 3.62 | 3.21 | 1.57 | 0.37 | 0.47 | 0.22 | 36.81 |

## 4 Discussion

### 4.1 Impact of Heat Stress on Morpho-Physiological and Yield Traits in Barley

Heat stress significantly affected major agronomic traits such as LA, GS, YIELD, and THGW, showing that rising temperatures adversely influenced barley growth and reproduction. Previous studies indicated that the reproductive phase was particularly sensitive to heat, with pollen sterility, impaired fertilization, and reduced kernel set contributing to lower productivity [34]. The analysis of variance (ANOVA) test revealed a high level of variation among the 50 barley genotypes with respect to all yield-related and morpho-physiological traits in both control and heat-stressed conditions, which clearly shows that there is an extensive genetic background present in the germplasm population [35]. Furthermore, the occurrence of significant genotype  $\times$  treatment interactions for multiple critical traits indicates that the lines responded divergently to elevated temperatures. This differential performance underlines the existence of exploitable genetic variation essential for breeding heat-resilient cultivars [36].

### 4.2 Genetic Variation and Trait Stability under Heat Stress in Barley

Descriptive and genetic parameter analyses revealed considerable phenotypic variation among barley genotypes [37]. Among the traits, YIELD and THGW exhibited substantial variation, reflecting differences in reproductive and grain-filling potential. High GCV and PCV, along with high heritability and GAM, were observed for TDM, YIELD, and LA, indicating that these traits were predominantly governed by genetic factors and could be effectively improved through selection. This was supported by Gebregergs and Mekbib [38], who reported similar patterns for grain yield in sorghum. Traits such as PLH, NT, SL, NS, and THGW exhibited moderate GCV and PCV, suggesting that both genetic and environmental factors contributed to their expression. In contrast, SPAD showed low GCV and PCV, reflecting the relative stability of chlorophyll content across genotypes under heat stress. This stability may have enabled certain genotypes to maintain photosynthetic capacity and physiological performance under high temperatures [10].

### 4.3 Trait Associations and Their Contribution to Yield under Heat Stress

Correlation analysis under heat stress associated with strongly positive among traits such as PLH, TDM, SPAD, LA, GS, THGW, and YIELD in barley, related to physiological and morphological responses that supported productivity under stress [39–41]. Similar interrelationships were observed in maize and wheat, where higher LA and SPAD content showed a positive relationship with assimilating production and translocation to the developing grains [42–44]. Our study also suggested that PLH and TDM had greater effects on THGW, GS, and YIELD, which aligned with the findings of Shirdelmoghanloo et al. [16]. The independent position of SL in the correlation network indicated its limited contribution to yield variability under heat, demonstrating that spike fertility and grain-filling rate, rather than spike length, were more critical for yield determination under stress [39].

### 4.4 Heatmap-Based Identification of Heat-Tolerant and Susceptible Genotypes

The heatmap-based clustering clearly discriminated against tolerant and susceptible genotypes based on multi-trait performance [45]. Genotypes such as BD7194, BD7188, IBON14, and BD8579 consistently maintained superior performance in agronomic traits under heat stress, suggesting the presence of adaptive mechanisms such as osmotic adjustment, efficient stress dissipation, and stable reproductive functioning [46]. In contrast, genotypes IBON97, BD9684, and BARI barley 9 exhibited severe reductions in yield and related traits, confirming their susceptibility to high temperature [47].



#### 4.5 Multi-Trait Evaluation and Strengths-Weaknesses of Barley Genotypes

The multi-trait genotype-ideotype distance index (MGIDI) proved to be highly effective in identifying genotypes with superior and balanced performance across traits [48], selecting BDIBON14, BD9681, BD7188, BD7194, BD8579, BD7196, IBON16, and IBON120 as elite lines. The integration of multiple traits within the MGIDI framework captured genotype adaptability and trade-offs more effectively than single-trait selection approaches [49]. The strengths and weaknesses analysis derived from factor loadings indicated that THGW contributed the most to genotype stability, likely due to its high variability and strong association with overall performance [50], while the low uniqueness values for SPAD and NT confirmed the robustness of the factor model and the reliability of the multivariate selection approach because these traits were well explained by the common factors [51].

#### 4.6 PCA and Genotype-by-Trait Biplot of Barley under Heat Stress

The PCA and genotype-by-trait biplot revealed substantial morpho-physiological and yield variation among barley genotypes under heat stress [52]. LA and YIELD were the most influential traits, highlighting their importance in heat adaptation. Positive correlations between NT and NS, and between YIELD, TGW, and GS, suggested coordinated resource allocation toward reproductive structures. PLH, SPAD, and TDM formed a separate cluster, reflecting biomass accumulation. Genotypes BD7194, BD7188, BD9681, and IBON14 combined favorable trait expression with superior YIELD, while other genotypes clustered differently, indicating diverse adaptive strategies. Similarly, Sachin et al. [53] reported that PCA revealed variation in productivity and heat stress tolerance traits among barley genotypes.

### 5 Conclusion

Exploring barley genotypes for heat tolerance is essential to address the growing challenges posed by climate change. Trait-based evaluation under heat conditions is important for identifying genotypes with desirable agronomic characteristics. In this experiment, 50 barley genotypes were screened to identify heat-tolerant lines. Based on the studied traits under heat stress, genotypes IBON14, BD7188, BD9681, BD8579, BD7194, IBON16, IBON120, IBON13, and BD7192 were found to be tolerant. The ability to select superior genotypes with desired features was further enhanced with advanced statistical approaches such as PCA, heatmap clustering, and MGIDI. These findings contribute to the development of barley varieties capable of withstanding heat stress. Further molecular and biochemical studies are needed to elucidate the mechanisms underlying heat tolerance. As global temperatures continue to rise and existing cultivars show limited variability in heat tolerance, developing reliable screening methods for heat-tolerant germplasm remains crucial. The tolerant genotypes identified in this study could serve as valuable parental lines or be released as new variety(s) to enhance heat tolerance ability of barley after revealing the advanced gene expression.

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