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Multivariate Analysis of Morpho-physiological and Biochemical Traits in Barley Genotypes under Heat Stress

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ABSTRACT

The experiment was conducted during the December–April, 2022–23 and 2023–24 at the Barley Research Area of the Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India to estimate the genetic variation and association among 22 morpho-physiological and biochemical traits in 51 barley (*Hordeum vulgare* L.) genotypes under heat stress conditions using principal component analysis. The trials were carried out in randomized block design with three replications having plot size of 2.5×0.46 cm^2. The results revealed highest coefficients of variation for 1000-grain weight followed by number of grains $spike^{-1}$ and plant height, implies the significance of traits for genetic improvement, while the low variation observed for days to maturity and transpiration. The PCA results revealed that the first eight principal components (PCs) accounted for 71.5% of the total variation. The first principal component (PC1) explained 19.5% of the total variation and also showed strong association with grain yield $plant^{-1}$, biological yield, SPAD, 1000-grain weight, NDVI 1, number of tillers $plant^{1}$, total soluble sugars and photosynthetic rate. The genotypes BH 19-44, BH 1029, BH 946, BH 1039, EIBGN-1, EIBGN-48, Lakhan, NBGSN-1, NBGSN-9 and RD 2907 exhibited strong yield potential under heat stress, highlighting their adaptability to adverse conditions. Further, the association of PC1 and PC2 with productivity and stress tolerance traits, respectively serve as key indicators for breeding selection. Hence, the study reflected substantial genetic variability among the barley genotypes studied and demonstrated the utility of principal component analysis in understanding and evaluating complex traits.

KEYWORDS: Barley, correlation, heat stress, principal components, variation

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1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is a true diploid (2n=2x=14) annual cereal crop belongs to the *Poaceae* family. It has persisted as a major cereal crop during many centuries and in term of world cereal production (Patial et al., 2023). It is fourth important cereal crop after wheat, rice and maize (Huang et al., 2020; To et al., 2020). In India, barley occupied an area of 0.61 mha with production and national productivity of 1.59 mt and 29.30 q ha^{-1}, respectively during the crop season 2023–24 (Anonymous, 2025). It was cultivated on 11420 hectares with a production of 38,660 tons in Haryana state (Anonymous, 2024) which ranks third in average productivity (33.85 q ha^{-1}) after Punjab (38.83 q ha^{-1}) and Rajasthan (36.03 q ha^{-1}). It is commonly used for making breads, soups, stews and many health products, though it is primarily grown as animal fodder and as a source of malt for alcoholic beverages, especially beer (Kumar et al., 2023; Ashok et al., 2024). Because of its hardiness, in many countries, it is considered the only possible rainfed cereal crop under low input and stressful environment (Kumar et al., 20024a; Eticha et al., 2010).

With climate change pushing temperatures higher, especially in the coming decades, heat stress has become a significant challenge for barley crop (Sallam et al., 2018), temperatures above 32°C, particularly during the critical grain-filling and post-heading stages, can cause significant yield losses by shortening the period for assimilate accumulation (Dwivedi et al., 2017; Bahrami et al., 2019). Hence, it became more important to develop resilient barley varieties that can withstand under such extreme environmental conditions. Abiotic stresses frequently cause morphological, physiological, and biochemical alterations that negatively impact the plant growth, productivity and yield, either alone or in combination (Bita and Gerats, 2013; Varshney et al., 2011). Physiological traits such as normalized vegetative index and canopy temperate are very crucial for the assessment of the genotypes under heat stress environment (Sehgal et al., 2018; Soni and Munjal, 2022). The yield potential under heat stressed environment largely influenced by the yield attributing traits and govern the stability of the genotypes under varied climatic conditions (Redhu et al., 2025; Guendouz and Bendada, 2022), however many of the quantitative traits usually possesses difficulty for their assessment (Bouchetat et al., 2020; Patel et al., 2024). Consideration of physiological and biochemical traits along with the yield attributing traits became crucial as they significantly alter the expression of the phenotype and alter the yield realization according to stress level and duration (Redhu et al., 2024; Liaqat et al., 2023; Meng et al., 2023). One of the best ways to identify such resilient varieties is through principal component analysis (PCA). PCA helps to simplify complex data by identifying the key traits that contribute most to genetic diversity, making it easier to identify which genotypes is more heat tolerant. It's an invaluable tool for breeders focused on creating barley varieties that can thrive despite the challenges posed by climate change (Jha et al., 2015; Verma et al., 2016).

This study focused on using principal component analysis to evaluate barley genotypes under heat stress, with the goal of identifying promising candidates for developing heat tolerant varieties. Traits with higher loading factors were analyzed for their correlation with major principal components to identify the most influential traits for the heat stress, helping to secure a more resilient future for barley production.

2. MATERIALS AND METHODS

The experiment was conducted during the December–April, 2022–23 and 2023–24 at the Barley Research Area of the Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. The experimental site was located at an altitude of 215.2 m above sea level, with coordinates of 29°10'N latitude and 75°46'E longitude in the subtropical region of North India. The experimental trials were carried out under heat stress condition that was induced by late sowing, ensuring that the critical grain-filling stages coincided with higher-than-optimal temperatures.

A total of 51 barley genotypes (Table 1) were evaluated in a randomized block design (RBD) with three replications. The varieties namely DWRB 91 and DWRB 64 were used as checks for heat stress conditions. Each genotype was planted in two rows of 2.5 m length each, with 23 cm spacing between them. Both Sowing were completed on 17th December, 2022 and 18th December, 2023 and standard agricultural practices were followed to ensure healthy crop growth. The observations were recorded at different growth stages for 22 traits (Table 2), out of which eleven were yield and its attributing traits; eight physiological traits and three heat stress related biochemical traits.

The recorded data pooled over two years was analyzed using statistical tools OPSTAT (Sheoran et al., 1998) and R Studio (R Core Team, 2023). Principal component analysis (PCA) was used to simplify the data by reducing the number of traits into a few key components that captured most of the variability. The correlation matrix was used to identify patterns in the data and determine which traits contributed most to the observed variability. Components with eigenvalues greater than one were retained, following Kaiser's rule (1958). To refine the analysis further, the varimax rotation method was applied to group traits with significant contributions to specific components, which

Table 1: List of barley genotypes evaluated in the study

Sl. No.	Genotype	Row type	Sl. No.	Genotype	Row type	Sl. No.	Genotype	Row type
1.	BH 1018	2	18.	BH 20-37	2	35.	EIBGN-48 (21-22)	6
2.	BH 1025	2	19.	BH 20-38	2	36.	EIBGN-52 (21-22)	6
3.	BH 1026	2	20.	BH 21-03	6	37.	EIBGN-6 (21-22)	6
4.	BH 1027	2	21.	BH 21-05	2	38.	EIBGN-61 (21-22)	2
5.	BH 1029	6	22.	BH 21-06	6	39.	EIBGN-8 (21-22)	2
6.	BH 1034	2	23.	BH 21-35	6	40.	EIBGN-9 (21-22)	6
7.	BH 1035	2	24.	BH 21-36	6	41.	K-603	6
8.	BH 1036	2	25.	BH 946	6	42.	LAKHAN	6
9.	BH 1038	6	26.	DWRB 64 (c)	6	43.	NBGSN-1 (21-22)	6
10.	BH 1039	6	27.	DWRB 91 (c)	2	44.	NBGSN-13 (21-22)	2
11.	BH 18-29	6	28.	EIBGN-1 (21-22)	6	45.	NBGSN-16 (21-22)	6
12.	BH 19-13	6	29.	EIBGN-17 (21-22)	2	46.	NBGSN-17 (21-22)	6
13.	BH 19-15	2	30.	EIBGN-2 (21-22)	6	47.	NBGSN- 18 (21-22)	2
14.	BH 19-44	6	31.	EIBGN-29 (21-22)	2	48.	NBGSN-9 (21-22)	6
15.	BH 19-52	6	32.	EIBGN-31 (21-22)	2	49.	RD 2794	2
16.	BH 20-04	2	33.	EIBGN-3 (21-22)	6	50.	RD 2849	2
17.	BH 20-08	2	34.	EIBGN-34 (21-22)	2	51.	RD 2907	6

made it possible to clearly identify the traits most important for explaining variability. Correlations between studied traits and key principal components were also calculated to assess their relationships and contributions.

3. RESULTS AND DISCUSSION

3.1. Genetic variability estimates

The estimates of genetic variability for 22 studied traits in 51 barley genotypes were presented in Table 2, highlighted the existence of broad genetic diversity in the barley population. The coefficient of variation (CV) served as a key indicator of the extent of genetic variability and our findings indicated enough variability in most of the traits, which was essential for the success of any breeding program aimed at trait improvement.

In particular, the CV was highest for 1000-grain weight followed by number of grains spike^{-1} and plant height with estimated values of 9.16%, 8.81%, and 8.75%, respectively (Table 2). The high CV for these traits suggested that there was significant genetic variation, offering ample opportunity for selection. Our results of variability aligned with the findings of Kumar et al. (2018), who also observed substantial variation in traits like grain weight and number of grains spike^{-1} among barley genotypes, which are vital contributors to yield.

In contrast, traits namely transpiration rate (0.68%) and days to maturity (0.87%) exhibited low CV values, suggesting that they were more genetically stable. However, despite their lower variability, these traits remain critical for assessing the stress resilience of barley genotypes, especially under heat stress conditions. The low-to-moderate variability observed in most traits signaled that selection based on these traits would be fairly effective under heat stress in isolating superior barley genotypes with high productivity and stress tolerance (Bhagat et al., 2024).

3.2. Principal component analysis (PCA)

To delve deeper into the genetic structure of the barley genotypes and reduce the dimensionality of the data, we performed principal component analysis (PCA) and identified the main sources of variation. The PCA results summarized in Table 3 revealed that the first eight principal components (PCs) accounted for 71.5% of the total variation, underscoring the significance of these components in explaining the variability in the barley genotypes.

The loading of individual traits on the principal components as presented in Table 4 provides additional insight into the relationships between the traits and their contribution to the overall genetic variability. PCA rotates the principal axes in a way that groups similar traits together, facilitating the identification of trait clusters that can be used for selection in breeding programs.

Table 2: Descriptive statistics in barley

Traits	Mean±SEm	Range	Min.	Max.	SD	CV (%)
Days to heading	60.2±0.16	5.17	58.33	63.5	1.11	1.02
Days to maturity	118.24±0.31	9.18	113.17	122.35	2.20	0.87
Plant height (cm)	89.94±1.07	32	74.56	106.56	7.63	8.75
Number of effective tillers plant^{-1}	4.41±0.09	3.22	3.00	6.22	0.67	7.74
Peduncle length (cm)	24.94±0.5	13	19.44	32.44	3.56	7.69
Number of grains spike^{-1}	41.76±1.76	43	20.67	63.67	12.55	8.81
Spike length (cm)	8.14±0.15	4.78	6.50	11.28	1.09	7.01
1000 grain weight (g)	44.48±0.97	29.01	31.84	60.85	6.94	9.16
Biological yield plant^{-1} (g)	35.17±0.91	26.83	23.82	50.65	6.44	6.40
Grain yield plant^{-1} (g)	30.86±0.64	23.08	21.33	44.41	4.57	6.49
Harvest index (%)	10.98±0.3	10.52	7.19	17.71	2.13	5.64
Canopy temperature depression (°C) (CTD)	3.31±0.22	7.64	-0.98	6.66	1.57	1.13
Chlorophyll content (SPAD)	47.8±0.41	11.66	42	53.66	2.96	3.89
Normalized difference vegetation index (NDVI1)	0.81±0.01	0.07	0.77	0.84	0.03	3.86
Normalized difference vegetation index (NDV2)	0.76±01	0.06	0.74	0.80	0.01	2.41
Photosynthetic rate (μmol CO$_2$ m^{-2} s^{-1})	8.37±0.07	1.67	7.65	9.32	0.49	5.25
Relative water content (%)	77.02±0.4	9.49	72.26	81.75	2.83	3.37
Stomatal conductance (mol H$_2$O m^{-2} s^{-1})	0.22±01	0.04	0.2	0.24	0.01	4.86
Transpiration rate (mmol H$_2$O m^{-2} s^{-1})	1.86±0.03	0.84	1.33	2.17	0.19	0.68
Total soluble sugar content (mg g^{-1})	4.47±0.01	0.11	4.43	4.54	0.06	1.78
Proline content (mg g^{-1})	0.51±01	0.01	0.51	0.52	0.02	4.54
Malondialdehyde content (MDA)	0.77±0.01	0.37	0.61	0.98	0.10	1.70

SD: Standard deviation; CV: Coefficient of variation

Table 3: Principal component analysis of studied trait

Principal components	Eigenvalue	Proportion of variability	Cumulative proportion
PC1	4.37	19.5	19.5
PC2	2.93	13.1	32.6
PC3	1.91	8.5	41.1
PC4	1.57	7.0	48.1
PC5	1.52	6.8	54.9
PC6	1.40	6.2	61.1
PC7	1.26	5.6	66.7
PC8	1.07	4.8	71.5

The first principal component (PC1) which explained 19.5% of the total variation, was strongly associated with grain yield plant^{-1}, biological yield, SPAD, 1000-grain weight, NDVI 1, number of tillers plant^{1}, total soluble sugars and photosynthetic rate. These traits were critical components of productivity, and the strong loading of PC1 on these traits indicated that this component represented overall productivity potential. This is consistent with the findings of Kumar et al. (2021), identified high loading of grain yield and 1000-grain weight *etc.* on key principal components of yield in barley. The high contribution of PC1 to the total variability further emphasized the importance of yield-related traits in barley improvement programs.

This association suggested that the studied morphological, physiological and biochemical traits were genetically linked and contributed synergistically to the overall yield potential of the barley genotype. This supported the hypothesis that selection for increased 1000-grain weight, which is known to have a strong positive correlation with grain yield (Yadav et al., 2022), can lead to improved productivity in barley.

The second principal component (PC2) explained 13.1% of the variation and was closely associated with number of grains spike^{-1}. Similarly, stomatal conductance on third; relative water content on fourth component; peduncle length, spike length, harvest index and transpiration rate on PC5 had high loading values. Principal component (PC6)

Table 4: Factor loadings of different traits with respect to different PCs in barley

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
DH	-0.07	0.12	0.49	0.30	-0.01	0.12	-0.03	-0.15
DM	-0.25	-0.01	0.32	0.05	-0.13	-0.18	-0.36	-0.17
PH	0.21	0.26	0.31	-0.07	0.06	-0.02	0.10	-0.21
NTP	0.22	0.04	0.01	-0.10	0.18	-0.42	0.03	-0.37
PDL	0.14	0.26	0.04	0.01	0.33	0.32	-0.36	-0.18
NGS	0.08	0.36	-0.13	0.45	-0.15	-0.16	0.09	-0.04
SL	0.23	0.07	0.25	-0.10	0.34	0.19	-0.14	0.18
TGW	0.27	-0.12	0.17	-0.23	-0.25	0.06	-0.01	0.012
BY	0.35	0.16	0.21	0.10	0.01	-0.05	0.08	0.20
HI	0.15	0.16	-0.37	0.11	0.18	-0.25	-0.04	-0.05
GYP	0.43	0.12	-0.16	0.06	0.09	-0.02	0.08	0.03
CTD	0.24	-0.21	-0.01	-0.30	-0.01	0.17	0.35	-0.04
SPAD	0.29	-0.34	-0.08	-0.02	-0.04	-0.13	-0.10	-0.13
NDVI1	0.26	-0.23	-0.23	0.10	-0.0	0.03	-0.33	-0.10
NDVI2	0.17	0.062	-0.08	0.09	-0.19	0.16	-0.39	0.59
PSR	0.15	-0.10	0.10	0.13	-0.58	0.102	0.03	-0.04
RWC	0.13	-0.23	0.05	0.49	-0.06	-0.17	-0.09	-0.06
STC	0.06	-0.42	0.22	0.13	0.18	0.09	0.04	-0.03
TPR	0.15	-0.30	0.25	0.18	0.28	-0.23	0.10	0.18
TSS	0.20	0.11	0.04	-0.32	-0.27	-0.05	-0.37	-0.28
Proline	0.12	0.26	0.21	-0.14	-0.23	-0.28	0.23	0.27
MDA	-0.13	-0.07	0.11	-0.27	0.08	-0.53	-0.28	0.30

DH: Days to heading; DM: Days to maturity; PH: Plant height (cm); NTP: Number of effective tillers plant^{-1}; PDL: Peduncle length (cm); NGS: Number of grains spike^{-1}; SL: Spike length (cm); TGW: 1000 grain weight (g); BY: Biological yield per plant (g); HI: Harvest index (%); GYP: Grain yield plant^{-1} (g); CTD: Canopy temperature depression (°C); SPAD: Chlorophyll content; NDVI1: Normalized difference vegetation index at vegetative stage: NDVI2: Normalized difference vegetation index at anthesis; PSR: Photosynthetic rate (μmol CO_2 m^{-2} s^{-1}); RWC: Relative water content (%); SMC: Stomatal conductance (mol H_2O m^{-2} s^{-1}); TPR: Transpiration rate (mmol H_2O m^{-2} s^{-1}); TSS: Total soluble sugar content (mg g^{-1}); Proline: Proline content (mg g^{-1}); MDA: Malondialdehyde content

loaded with the malondialdehyde (MDA) while days to maturity and canopy temperature depression were highly loaded on PC7 which accounts for the 5.6% of the total variability. The last major component (PC8) explained the 4.8% of the variation and loaded with the days to heading, plant height, NDVI 2 and proline content. This component reflected the genotype's ability to tolerate heat stresses.

The association of principal components with stress-adaptive traits such as SPAD, proline and Malondialdehyde aligned with research of Zeng et al. (2018), who found that proline content and SPAD value is a key indicator of abiotic stress tolerance in barley, as proline accumulation helped the plant to maintain cellular function under stressed conditions.

The proportion of variance explained by the first few PCs supported the use of PCA for understanding the complex genetic structure of barley, where major sources of variation were captured by a small number of components (eight components). This finding aligned with the study of Yadav et al. (2021), who found that the first five principal components explained more than 90% of the total variation in barley genotypes. Similarly, Sreesaeng et al. (2024) were also found that four key components contributed 72.5% variation in the data. Enyew et al. (2019) study revealed that 49.96% of the total variations was alone contributed by the principal component one (PC1) whereas Jha et al. (2015) also recognized the 60.8% contribution by first three PCs in total variability existed in the genotypes under high temperature stress, emphasizing the effectiveness of PCA in identifying key traits for selection.

3.3. Factor scores and genotype classification

The factor scores, derived from the Anderson-Rubin method, provided a means of classifying the genotypes based on their performance across the principal factors. The distribution of genotypes along the first two principal components (PC1 and PC2), as shown in Figure 1, revealed clear differentiation between high yielding genotypes and those with stress tolerant characteristics.

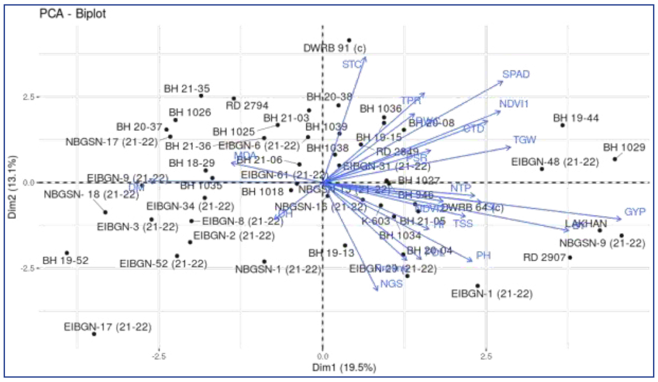

Figure 1: Biplot based on of PC1 and PC2

For instance, genotypes such as BH 1039, BH 1027, EIBGN 48 (21-22) and BH 946 which scored high in PC1, exhibited superior productivity with traits such as grain yield plant^{-1}, 1000-grain weight and total soluble sugars *etc*. These genotypes were promising candidates for breeding programs focused on improving barley yield under favorable conditions. Genotypes like NBGSN-1 (21-22) and EIBGN-34 (21-22) which loaded with high scores in PC2, exhibited strong resilience to heat stress.

Genotypes such as BH 19-44, BH 1029, BH 1039 and EIBGN-31 (21-22) exhibited strong correlations with PC1, indicated that they were promising candidates for selection based on high yield. Meanwhile, genotypes such as BH 21-36 and EIBGN-6, associated with PC2, are well-suited for breeding programs targeting heat stress resilience.

3.4. Correlation between principal components and traits

The correlation analysis presented in Table 5 and Figure 2 highlighted the relationships between principal components and specific traits, providing further insights into the biological processes underlying barley productivity and stress

Table 5: Correlation of principal components with traits studied

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
DH	-0.144	0.208	0.667	0.370	-0.009	0.145	-0.034	-0.155
DM	-0.518	-0.008	0.438	0.062	-0.162	-0.215	-0.402	-0.175
PH	0.444	0.447	0.422	-0.091	0.075	-0.026	0.108	-0.213
NTP	0.452	0.074	0.017	-0.124	0.216	-0.495	0.032	-0.379
PDL	0.293	0.434	0.060	0.006	0.402	0.373	-0.403	-0.188
NGS	0.163	0.613	-0.174	0.559	-0.182	-0.190	0.104	-0.037
SL	0.472	0.112	0.347	-0.124	0.410	0.225	-0.155	0.184
TGW	0.558	-0.201	0.237	-0.279	-0.298	0.071	-0.011	0.013
BY	0.731	0.272	0.283	0.125	0.005	-0.062	0.087	0.205
HI	0.316	0.265	-0.504	0.138	0.224	-0.287	-0.049	-0.047
GYP	0.886	0.208	-0.216	0.081	0.104	-0.023	0.092	0.035
CTD	0.490	-0.348	-0.014	-0.372	-0.017	0.202	0.384	-0.040
SPAD	0.535	-0.574	-0.112	-0.027	-0.049	-0.155	-0.109	-0.136
NDVI1	0.528	-0.404	-0.319	0.124	-0.108	0.037	-0.371	-0.105
NDVI2	0.358	0.105	-0.105	0.111	-0.142	0.185	-0.437	0.603
PSR	0.321	-0.182	0.139	0.159	-0.706	0.119	0.036	-0.044
RWC	0.273	-0.391	0.068	0.604	-0.079	-0.199	-0.104	-0.062
STC	0.127	-0.712	0.304	0.161	0.217	0.103	0.044	-0.027
TPR	0.302	-0.508	0.339	0.228	0.336	-0.273	0.109	0.185
TSS	0.424	0.192	0.056	-0.391	-0.329	-0.063	-0.408	-0.288
Proline	0.250	0.441	0.285	-0.170	-0.279	-0.326	0.256	0.278
MDA	-0.272	-0.112	0.152	-0.338	0.098	-0.623	-0.312	0.304

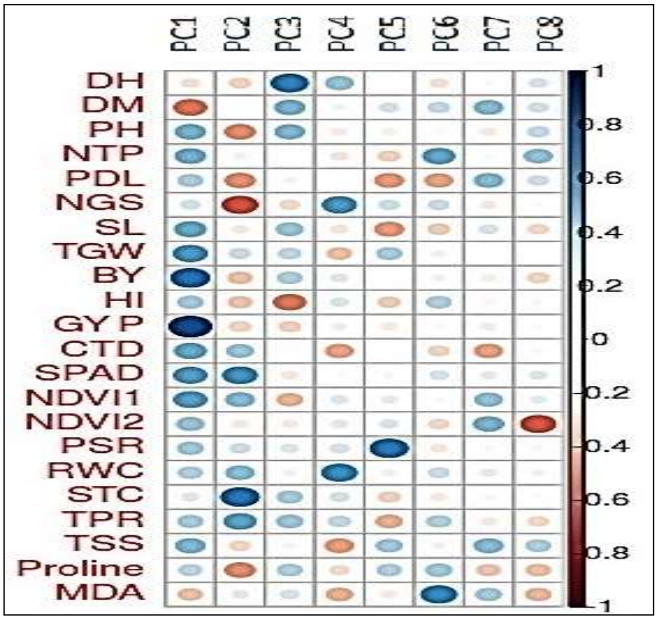

Figure 2: Correlogram of variables vs PCs

adaptation. PC1 and PC2, which were strongly associated with productivity and stress tolerance traits, respectively, served as key indicators for breeding selection.

For example, the positive correlation of proline content and number of grains spike^{-1} with PC2 suggests that these traits play a synergistic role in osmotic adjustment and thermotolerance, crucial for maintaining cellular integrity under stress conditions. The negative correlation between MDA (malondialdehyde), a marker of oxidative stress, and yield-related traits such as grain yield plant^{-1} indicated that oxidative stress negatively impacted the productivity, emphasizing the need for breeding genotypes with reduced oxidative damage under stress conditions (Kumar et al., 2024c).

The associations between remaining major PCs (PC3 to PC8) with biochemical traits and physiological water retention traits such as relative water content, stomatal conductance and transpiration rate, further highlighted the role of water-use efficiency in heat stress adaptation. These traits were crucial for barley's survival in regions experiencing terminal heat stress, as efficient water use and timing of growth stages significantly impacted the overall yield of the barley.

The findings of this study emphasized the importance of integrating both productivity traits and physio-biochemical traits into barley breeding programs, especially in light of climate change and the increasing frequency of heat stress. The integration of PCA into barley breeding strategies can accelerate the development of varieties that were not only high-yielding but also resilient to the stresses that had been becoming more prevalent in global agricultural systems.

4. CONCLUSION

This study revealed significant genetic variability in barley genotypes and PC1 was found the most important as it alone defined the 19.5% of the total variability. Genotypes such as BH 1039, BH 946, BH 1029 and NBGSN-1 demonstrated high productivity, while EIBGN-34, EIBGN-6 and EIBGN 3 exhibited strong resilience to stress. The findings suggested that selecting genotypes BH 19-44, BH 1029, BH 946, BH 1039, EIBGN-1 (21-22), EIBGN-48 (21-22), Lakhan, NBGSN-1, NBGSN-9 and RD 2907 enhanced barley performance under heat stress.

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