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Investigation of Multivariable Analysis and Phenotypic Diversity in Various Barley Genotypes Across Different Temperature Conditions

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ABSTRACT

High temperature is one of most domineering abiotic stress influences that border barley production. Herein, three different field experiments at three different locations were carried out at Sakha, Malawi and New-valley research stations, to identify the response of ten barley genotypes to different temperatures degrees using phenotypic diversity and, multivariable analysis during two consecutive seasons 2020/2021 and 2021/2022 under three different temperatures degrees. Heat stress index (HI) activated a reduction in all traits ranged from lowest average reduction in plant height (PH) by (5.43 and 20.37%) to highest average reduction in No. of tires m^2 TM by (14.49 and 40.83 %) under Malawi (T2) and New Valley (T3) locations respectively as camper by Sakha, also high temperature enhancement all the genotypes to quicken flowering and days to maturity by average (7.24 and 8.35 %) under New Valley. Days to heading HD and days to maturity MD exhibited a strong and negative significant relationship with all studied traits. Loading principal component analysis PCA accounted 86.1% of the total variability, which PCA2 clarified 24.2 % of the total variability influenced by HD and MD which placed in the left side (negative). The scatter plot from the PCA analysis shows that the Egyptian barley genotypes—Giza 137, Giza 138, line 5, line 1, and line 3 are distinctly separated from the other genotypes. They cluster on the right side of PCA1, forming a significant group that indicates their potential for heat tolerance. Meanwhile, a cluster heatmap reveals two primary groupings among the ten barley genotypes. Giza 137, Giza 138, line 5, line 1, and line 3 are closely related, likely due to their heat stress resilience, whereas line 2 and line 8 group together, displaying a sensitivity to heat. This differentiation allows us to leverage these genotypes in our future barley breeding programs aimed at enhancing heat tolerance and increasing yield, marking a crucial step toward developing new, robust genotypesز

Keywords: *Hordeum vulgar*, phenotypic diversity, heat stress index HI, PCA, heatmap analysis

1. Introduction

Heat stress is one of most vital climate change influences, which there was a universal will increase the average of temperature by 1.8–4 °C in the 21st century, the increasing will due to a significant yield loss with great dangers for the future global food safety around the worldwide (Mariey *et al.,* 2023a; Horváth *et al.,* 2024). In Egypt temperature changes from low and worm in coastal zone to hot in the Upper area that the winter season is from December to February and the summer season from June to August, so these weather change had negative influence on Egypt agricultural strategy. (Elbasiouny *et al.,* 2017; Mahmoud *et al.,* 2017 and Mariey *et al.,* 2023a).

Barley (*Hordeum vulgare* L.) is a chief cereal crop that has well improved to numerous abiotic stresses in dry areas, it was found to be discreetly tolerant to drought stress, due to it is the restricted amount of water that is available for irrigation (Habib *et al.,* 2021 and Mariey *et al.,* 2022). In Egypt, barley is a main winter crop cultured in old and newly domestic lands that hurt from a dearth of irrigation, low soil fecundity, and salinity of both soil and water. However, there is a lack of

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consciousness of the nutritional role of barley for both humans and animals (Mariey et al., 2023b and Horváth et al., 2024)

Barley growth and productivity are highly influenced by heat stress, which presents a significant challenge under current climatic conditions. Higher temperatures interfere with core metabolic activities, leading to a marked decline in photosynthetic efficiency and impaired grain formation. When heat stress persists, it often triggers premature maturation, smaller kernel sizes, and heightens the crop's vulnerability to various diseases (Mariey *et al.,* 2023b)

The important responsibilities for plant breeders is to growth yield per unit area by evolving high tolerant genotypes to be suitable for sowing in bad area which surfing from abiotic stresses, these tasks could be realized over using effective methods that help the breeders to screening and documentation the response of genotypes for stress. Morphological traits and yielding dated were the greatest suitable assortment principles to evaluation barley response to heat stress under physical field environments. (Mariey *et al.,* 2021, 2023a; Horváth *et al.,* 2024 and Kim *et al.,* 2024).

Multivariate analysis allows researchers to consider multiple factors and their interactions simultaneously. This analytical method is particularly valuable for multi-trait datasets where traditional univariate approaches may fail to capture the underlying structure of the data. By analyzing traits collectively, multivariate techniques can uncover hidden patterns, relationships, and trait clusters that might go unnoticed otherwise One of the most widely used multivariate techniques is Principal Component Analysis (PCA). PCA simplifies the complexity of high-dimensional data by reducing it to a smaller set of uncorrelated variables, known as principal components. These components capture the maximum variance present in the dataset and provide a clear picture of the relationships among traits. Heatmap cluster analysis was employed to visualize patterns and relationships among traits and genotypes. This method excelled in grouping similar traits and categorizing genotypes with comparable responses to varying temperature conditions (Mansour *et al.,* 2021; Mariey *et al.,* 2022 & 2023b; and Kumar *et al.,* 2024).

Consequently, understanding the phenotypic diversity among genotypes will aid to ensure plant breeders to put program to improve biotic and abiotic stresses tolerance by crossing genetically-diverse parents having necessary characters, so using phenotypic diversity is one of the chief and significant steps in breeding programs for abiotic stresses tolerance (Mariey *et al.,* 2021 & 2023 and Horváth *et al.,* 2024).

This research holds crucial implications for barley breeding programs, especially under growing environmental challenges. By employing the phenotypic diversity of ten Egyptian barley genotypes multivariable analysis, PCA, and heatmap clustering, the study identified heat-tolerant genotypes and their critical traits. for future breeding programs aimed at improving barley's resilience to heat, thereby enhancing its productivity under conditions of heat stress.

2. Material and Methods

2.1. Barley plant materials

Ten barley genotypes were kindly provided by Barley Department, Field Crops Research Institute, Agriculture Research Center, ARC, Egypt, were used in this study their names and pedigree shown in (Table 1).

Table 1: Name, and pedigree of ten barley genotypes used in this study

No.	Name	Pedigree
1	**Line 1**	Giza 124/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra,01
2	**Line 2**	BLLU/PETUNIA1//CABUYA/3/Alanda// Lignee527 / Arar
3	**Line 3**	Giza 118/3/Alanda/Hamra//Alanda,01
4	**Line 4**	Rihane03/7/Bda/5/Cr.115/Pro/Bc/3/Api/CM67/4/Giza120/6/Dd/4/Rihane,03
5	**Line 5**	Giza 2000/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra,01
6	**Line 6**	Giza 119/3/Alanda/Hamra//Alanda,01
7	**Line 7**	Giza 117/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra,01
8	**Line 8**	Giza 123/5/Furat 1/4/M,Att,73,337,1/3/Mari/Aths*2//Attiki
9	**Giza 137**	Giza 118 /4/Rhn-03/3/Mr25-//Att//Mari/Aths*3-02
10	**Giza 138**	Acsad1164/3/Mari/Aths*2//M-Att-73-337-1/5/Aths/ lignee686 /3/Deir Alla 106//Sv.Asa/ Attiki /4/Cen/Bglo."S")

2.2. Field investigational description

2.2.1. Field experimental Locations

Three field experimentations were achieved in three dissimilar heat stress sites were growing through two winter sowing seasons of 2020/ 2021 and 2021. /2022 to study the effect of heat stress on ten barley genotypes yield production as shown in Fig 1:

1. Sakha station, locating in the center of the Delta -Kafer EL-Sheik governorate, has an elevation of 8.30 above sea level, with Latitude: 31° 6' 22.75" N" and Longitude: 30° 56' 31.11" E".
2. Malawi station, locating in Minya governorate with Latitude: 27° 43' 53.04" N Longitude: 30° 50' 29.94" E
3. EL-Dakhla, Oasis station, locating in New valley research governorate with Latitude: 25° 30' 59.99" N and Longitude: 29° 09' 60.00" E.

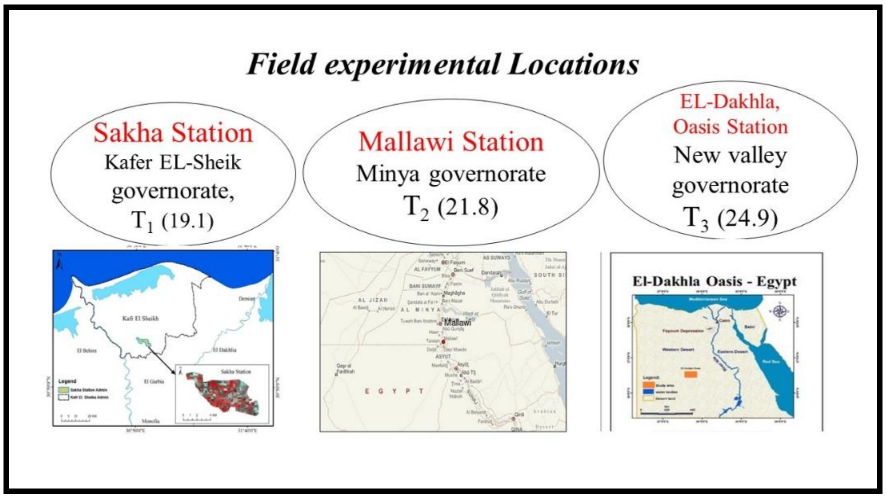

Fig. 1: The maps of field experimental locations

2.2.2. Field experimental design

The ten barley genotypes were growing in (RCBD) randomized complete block design with three replicates using (plot area =3.6 m^2) for each plot, to evaluate the associated traits to grain yield and heat stress index.

2.2.3. Field experimental Soil samples

Soil samples were taken before land preparation in two depths from the soil surface. The physical and chemical analysis of dissimilar investigational locations were presented (Table 2).

Table 2: The average of physical and chemical properties for soil samples from the field experiments locations during two growing seasons 2020/2021 and 2021/2022.

Soil analysis	Sakha station	Mallawi station	New valley station
A: Physical analysis			
Sand (%)	18.94	14.1	67.1
Silt (%)	28.15	43.1	9.0
Clay (%)	51.35	40.2	23.9
Texture	Clayey	Silty caly	Sandy clay loam
B: Chemical analysis			
EC(dSm$^{-1)}$	2.76	1.62	5.78
pH	7.6	7.86	7.85
K^+ meq100^{1} g soil	0.1	0.57	0.58
$CaCO_3^{--}$ meq100^{1} g soil	0	2.21	4.52
SO_4^{--} meq100^{1} g soil	4.95	0.55	-

2.2.4. The Agro- meteorological information

The data of average month maximum and minimum temperatures (°C) and relative humidity (RH., %), were documented for weather station belonging to the Sakha (T_1), Malawi (T_2) and New Valley (T_3) Station, Egypt during two growing winter seasons 2020/2021 and 2021/2022 were shown in (Table 3).

Table 3: The Meteorological of the experimental area during the two-growing seasons of barely 2020/2021 and 2021/2022 under three different locations Sakha, Mallawi and New valley sites

Season	Month	Temperature, C°									Relative humidity, RH %		
		Sakha (T_1) Normal temperature			Mallawi (T_2) Medium temperature			New valley (T_3) High temperature					
		Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Sakha	Malawi	New valley
Season 2020/2021	Dec.	21.4	13.4	17.4	20.7	9.15	14.93	21.9	9.9	15.9	86.9	64.83	54.2
	Jan.	18.4	11.8	15.1	18.7	6.13	12.42	28.0	7.6	17.8	86.7	64.83	54.5
	Feb.	20.4	12.7	16.6	22.7	9.82	16.26	25.7	9.8	17.75	84.6	61.81	41.9
	Marc.	22.6	15.6	**19.1**	28.7	14.2	**21.45**	30.0	13.8	**22.9**	81.1	61.19	32.9
	Apr.	26.0	18.9	22.5	32.53	17.1	24.82	35.5	18.2	26.85	80.0	53.46	25.2
	Seasonal	**21.71**	**14.4**	**18.4**	**24.6**	**11.8**	**17.9**	**28.2**	**11.8**	**20.4**	**83.6**	**61.2**	**39.9**
Season 2021/2022	Dec.	22.9	13.7	18.3	25.0	14.0	19.50	25.3	11.2	18.25	87.7	54.56	51.7
	Jan.	21.0	13.5	17.25	24.5	12.5	18.50	23.1	6.6	14.85	86.7	54.54	44.9
	Feb.	21.5	12.5	17.0	23.5	9.71	16.61	25.2	8.4	16.8	87.5	54.20	43.6
	Marc.	23.8	15.2	**19.5**	29.3	13.9	**21.60**	31.6	14.6	**23.1**	83.8	52.35	35.2
	Apr.	27.6	19.4	23.5	31.0	14.6	22.80	31.9	15.5	23.7	74.6	45.51	27.3
	Seasonal	**23.35**	**14.8**	**19.11**	**26.6**	**12.9**	**19.8**	**27.4**	**11.26**	**19.34**	**84.06**	**52.23**	**40.5**

2.2.5. Measured Characteristics

At the heading stage days to heading were documented, at maturity stage days to maturity were recorded and at the harvest stage ten guarded plants were randomly taken from each plot to measure plant height cm PH, number of tillers m^{-2}, TM number of grains $spike^{-1}$ NGS, and grain yield GY was determined using the full plot area (3.6 m^{-2}).

2.2.6. Multivariable studied analysis

1. **Heat stress index**: The comparative alteration due to heat stress was computed for each trait according to **Bouslama and Schapaugh, (1984**)
2. **Correlation coefficient:** person and matrix were used to study the association between each two deliberate traits were done using Minitab 18.1 statistical software (Minitab Inc., Coventry, UK) and
3. **Principal Component Analysis (PCA**): Loading and scatted plot were attained to study the changes and interrelations between genotypes with respect to measured phenotypic traits using Minitab 18.1 arithmetical software program (Minitab Inc., Coventry, UK)
4. **Heatmaps cluster:** visualizing cluster of multivariate statistics, was used to created heatmaps according to Metsalu *et al.* (2015).

2.7. Data analysis.

The data pertaining to the examined traits over the two seasons were subjected to statistical analysis through ANOVA in a randomized complete block design (RCBD). This approach aimed to evaluate the effects of genotypes, temperature variations, and their interactions on the studied traits. To achieve reliable and precise results, the SAS software program version 9.1 (SAS, 2011was employed, enabling rigorous and professional analysis of the dataset. Mean comparison was conducted using Duncan's multiple range test (Duncan, 1955) at a 95% confidence level, ensuring robust differentiation among mean values and providing essential insights into the traits under investigation.

3. Results and Discussion

3.1 Impact of Varied Temperature Conditions on Selected Traits of Barley Genotypes

The analysis of variance (ANOVA) conducted on various phenotypic traits including days to heading (HD days), days to maturity (DM days), plant height (PH cm), number of tillers per square meter (TM in tillers/m²), number of grains per spike (NGS-1 in grains/spike), thousand kernel weight (TKW in grams), and grain yield (GY in ard/fad)—revealed a statistically significant effect ($P < 0.01$) attributed to different temperature degrees at three locations: Sakha (T1), Malawi (T2), and New Valley (T3). This effect was also influenced by genotypes (G) and years (Y), as detailed in Table 4.

A significant interaction two-way between temperatures degrees and barley genotypes (G X T) were detected for all deliberate characters. While, the two-way interaction between years x temperatures degrees (Y X T) and years x genotypes (Y X G) were significant. across all traits expect the, TM were non- significant. Similarly, the combined ANOVA indicated significant effect for three-ways interaction (G X T X Y) across all traits, expect for TM, which were non-significant.

The results indicate that high temperatures at Malawi and New Valley (T_2 and T_3) caused a significant decrease in all studied characters, while caused a significant increase in HD and MD as compared with normal temperature at Sakha station (T_1). which induced all genotypes to flowering and maturity early in Malawi and New Valley (T_2 and T_3) more than Sakha station (T_1).

Table 4: ANOVA analysis of years, different temperature degrees (three location) and barley genotypes on agronomic, and their interactions during two growing seasons 2020/2021and 2021/2022.

	Days to heading days	Days to maturity days	Plant height cm	No. of tillers /m²	No. of grain spike^{-1}	Thoued kernel weight (g)	Grain Yield ard/fad
Years							
2019/20	85.18	113.37	98.07	379.48	56.72	44.91	17.81
2020/21	84.77	113.26	99.33	378.36	55.73	44.36	17.50
Temperature Degrees							
(Sakha)T1	85.67	118.02	109.05	488.83	66.77	53.24	19.78
(Malawi) T1	82.15	115.81	103.13	418.01	62.08	49.85	17.59
(New valley)T2	79.47	108.17	86.83	289.23	44.52	34.19	14.78
Barley cultivars							
Line 1	80.67	114.00	101.11	396.25	56.78	48.79	19.08
Line 2	84.44	113.72	83.67	256.19	52.11	43.21	13.53
Line 3	80.17	115.22	104.17	427.67	56.00	48.99	19.89
Line 4	83.83	113.67	96.89	359.89	61.00	44.92	18.61
Line 5	81.11	112.67	105.61	407.89	57.33	45.66	19.70
Line 6	84.46	113.82	92.70	373.01	55.43	45.56	17.42
Line 7	83.06	112.39	95.89	373.44	53.39	44.00	18.09
Line 8	85.78	113.44	93.56	259.17	49.44	42.78	14.86
Giza 137	80.72	112.06	102.61	438.78	55.00	41.98	18.96
Giza 138	80.22	112.72	105.22	453.44	62.33	43.18	19.54
ANOVA analysis							
Years (Y)	*	**	*	NS	**	**	**
Cultivars (C)	**	**	**	**	**	**	**
Temperature (T)	**	**	**	**	**	**	**
LSD (0.05)							
Years (Y)	**0.381**	**0.876**	**0.891**	**4.54**	**NS**	**3.45**	**1.166**
Cultivars (C)	**0.808**	**1.123**	**1.79**	**11.15**	**3.34**	**3.54**	**2.18**
Temperature (T)	**0.442**	**0.876**	**0.969**	**5.56**	**1.831**	**1.56**	**1.906**
Interaction							
C X Y	**	**	**	NS	NS	NS	**
TX Y	**	**	**	NS	NS	NS	NS
C X T	**	**	**	**	**	**	**
CX T X Y	**	**	**	NS	NS	**	**

Which Ns, * and ** non-significant and significant at the 0.05 and 0.01 levels of probability, respectively

Correspondingly, the results showed diverse significantly which were found among all the Egyptian barley genotypes according their average of mean performances of all studied traits due to the differ temperature (Table 4), which the results showed that Giza 138, Line 1 line 3, line 5 and Giza 137 high average values for all studied under the high temperatures degrees than other genotypes recognized as greater heat tolerance barley cultivars traits, while Line 2 and Line 8 had low average values which they were more affected by heat stress. Regarding grain yield (GY), the data indicated a significant decrease in GY due to heat stress, particularly at the Malawi and New Valley locations (T2 and T3), compared to the Sakha station (T1), as illustrated in Fig 2. Giza 138 achieved the highest yields of 21.11, 19.03, and 17.47 ard/fad at Sakha, Malawi, and New Valley sites, respectively, followed closely by Giza 137, Line 5, Line 3, and Line 1, all of which also yielded well at these locations. Conversely, Line 2 recorded the lowest GY values of 15.11, 13.33, and 9.47 ard/fad at Sakha, Malawi, and New Valley, respectively. Line 8 also fell short, with GYs of 15.89, 13.47, and 10.14 ard/fad at the same sites, as depicted in Fig 2. These findings are consistent with the studies conducted by Kaseva *et al.* (2023), Mariey *et al.* (2023a), Kim *et al.* (2024), and Horváth *et al.* (2024), which confirmed the detrimental impacts of heat stress significantly diminished various traits related to grain yield. It was observed that the interaction between genotype (G) and environment (E) greatly influenced grain yield across different years for each barley genotype under heat stress conditions. Furthermore, it was noted that high temperatures have indirect negative effects on yield by disrupting the plant's growth cycle and interfering with optimal development processes.

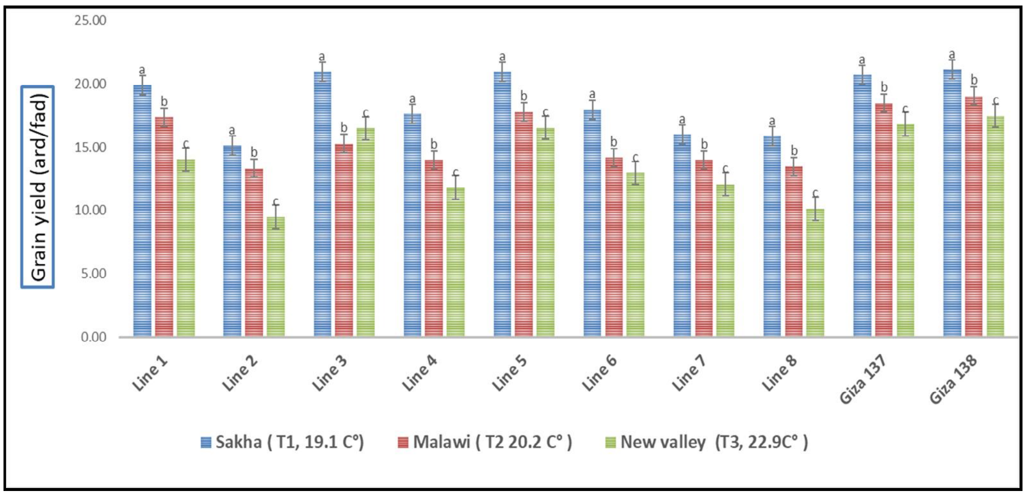

Fig. 2: Effect of different temperatures degrees on grain yield among ten barley genotypes at Sakha, Malawi and New Valley locations

3.2 Multivariable analysis

3.2.1. Heat stress index (HI)

The virtual changes reduction due heat stress (HI) on morphological studied traits, were presented in (Figure 3), the results showed that heat stress activated a reduction in all traits ranged from lowest average reduction in PH by (5.43 and 20.37%) to highest average reduction in TM by (14.49 and 40.83 %) under Malawi (T2) and New Valley (T3) locations respectively as camper by Sakha T_1. About the heat index due heat stress on grain yield the results showed that there a reduction was happened due heat stress by average values. Nevertheless, heat stress induced all genotypes to flowered and maturity earlier by an average (4.11 and 1.87 %) respectively under Malawi (T2) and by an average (7.24 and 8.35 %) under New Valley (T3) location respectively as camper by Sakha T_1as shown in (Figure 3). On behalf of the relative changes due heat stress on grain yield the results showed that there a reduction was happened due heat stress by average values (8.06 and 29.0 %) under Malawi and New Valley location respectively. However, heat stress induced all cultivars to flowered earlier by an average (3.93 and 11.39 %) respectively as shown in (Figure 3). This results were agree with Devi *et al.* (2021); Bhagat *et al.* (2023); Mariey *et al.* (2023a); Kim *et al.* (2024); and Kumar *et al.* (2024) whom, found that barley heat tolerant genotypes were significantly less affected by stress factors than heat sensitive genotypes which heat stress index is an inductor for detect the barley heat tolerant genotypes depends on it grain yielded values.

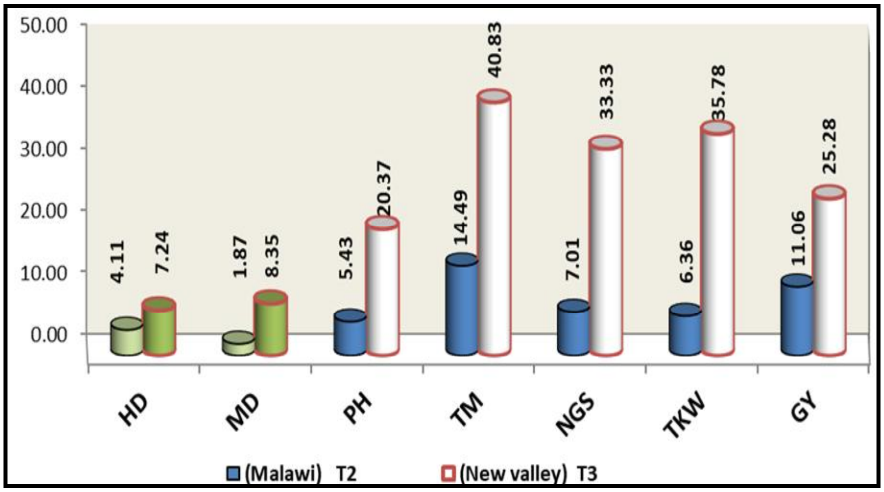

Fig. 3: heat stress index of studied traits under Malawi T2 and New valley T3 as compere by T1 at Sakha station which days to heading (HD) , days to maturity (MD), plant height (PH), number of tillers m^2 (TM), number of grain spike-1 (NGS-1), thousands kernel weight(TKW and grain yield (GY) , which the green refer to inducing flowering and maturity days.

3.3.2 Correlation coefficient

Both Pearson and matrix correlation coefficient was done to recognize the relationships among all studied characters across the three different temperature degrees (three locations) (Figure 4 & 5). Results designated clearly that the correlation coefficients among grain yields GY and PH, TM, TKW and NGS traits were highly positive and significantly correlated. HD exhibited a strong and negative relationship with all studied traits grain yield, and days to maturity MD showed negative relationship with all studied traits expect NGS. These results were in agreements with Mariey *et al.* (2023a); Kim *et al.* (2024) and Horváth *et al.* (2024) whom reported that there was a significant correlation between the heat stress-induced changes in grain-yield related traits.

	HD	MD	PH	TM	TKW	NGS
MD	0.055					
PH	-0.842	-0.148				
TM	-0.878	-0.174	0.872			
TKW	-0.564	-0.039	0.586	0.692		
NGS	-0.395	0.734	0.325	0.301	0.223	
GY	-0.821	-0.068	0.919	0.942	0.712	0.461

Fig. 4: Pearson correlation coefficient heatmap among grain yield (GY) and days to heading (HD) , days to maturity (MD), plant height (PH), number of tillers m^2 (TM) , number of grain spike-1 (NGS-1), thousands kernel weight(TKW) across the three heat stress locations. Correlation key and the scale reads, red box indicted strong negative correlation, green box indicted strong positive correlation, white yellow box mean medium positive correlation, orang box mean medium negative correlation.

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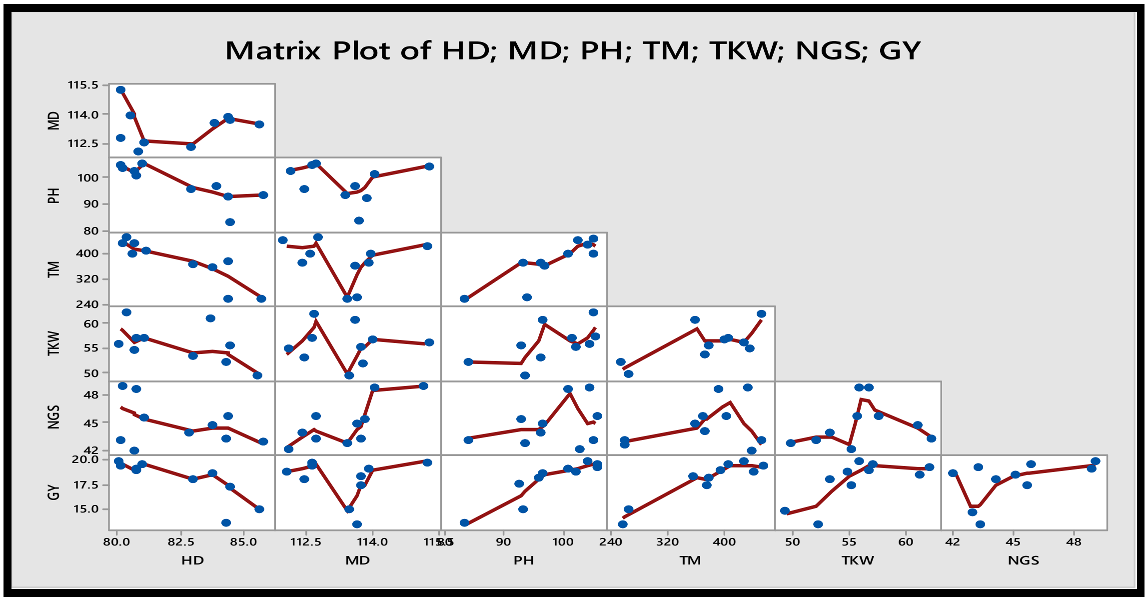

Fig. 5: Matrix plot correlation coefficient heatmap among grain yield (GY) and days to heading (HD), days to maturity (MD), plant height (PH), number of tillers m^2 (TM) , number of grain spike-1 (NGS-1), thousands kernel weight(TKW) across the three heat stress locations.

3.2.3. Principal component analysis (PCA)

3.2.3.1. Loading plot PCA

The loading plot was created using the distance matrix, represented along the horizontal axis to illustrate the relationships among all the examined traits, as shown in Figure 6. The results indicated that principal component analysis (PCA) accounted for 86.1% of the total variability. PCA1 explained 61.9% of this variation, with traits such as plant height (PH), maturity time (MT), number of grains per spike (NGS), thousand kernel weight (TKW), and grain yield (GY) positioned on the positive side (right side) of the horizontal axis. This positioning reflects their significant positive correlations with the other traits investigated. In contrast, PCA2 accounted for 24.2% of the total variability, influenced by heading date (HD) and maturity date (MD), which were located on the left side (negative side) of the horizontal axis, indicating their significant negative associations with the other traits in the study.

.3.2.3.2. PCA scatter plot

The scatter plot of PCA analysis based on all studied traits categorizing all the barley genotypes in four groups as shown in (Figure 6), which PCA analysis indicated that the Egyptian barley genotypes (Giza 137, Giza 138, line no5) and (line 1 and line 3) were separate from the other genotypes and located in the right side with of PCA1 analysis cluster with a significant distance, which they had achievement high average of all studied traits under study that could be documented them as heat tolerance genotypes .line 4 , line 6 and line 7 which were distributed distance from one other in the scatter plot of PCA analysis cluster based in their medium average value of studied traits could be documented them as moderated heat tolerance genotypes. Whereas both of line 2 and 8 genotypes were scattered distance far from the two other groups which located on left side, as selected by cluster analysis of PCA2 affording to their lowest values of all studied traits with high reduction that could recognized as heat sensitive genotypes. The results agree with Mariey *et al.* (2023) and Kumar *et al.* (2024) They confirmed that principal component analysis (PCA) is the most impactful mathematical tool available for simultaneously analyzing multiple variable quantities. This approach enhances the accuracy of genotype assessments in barley under abiotic stress conditions.

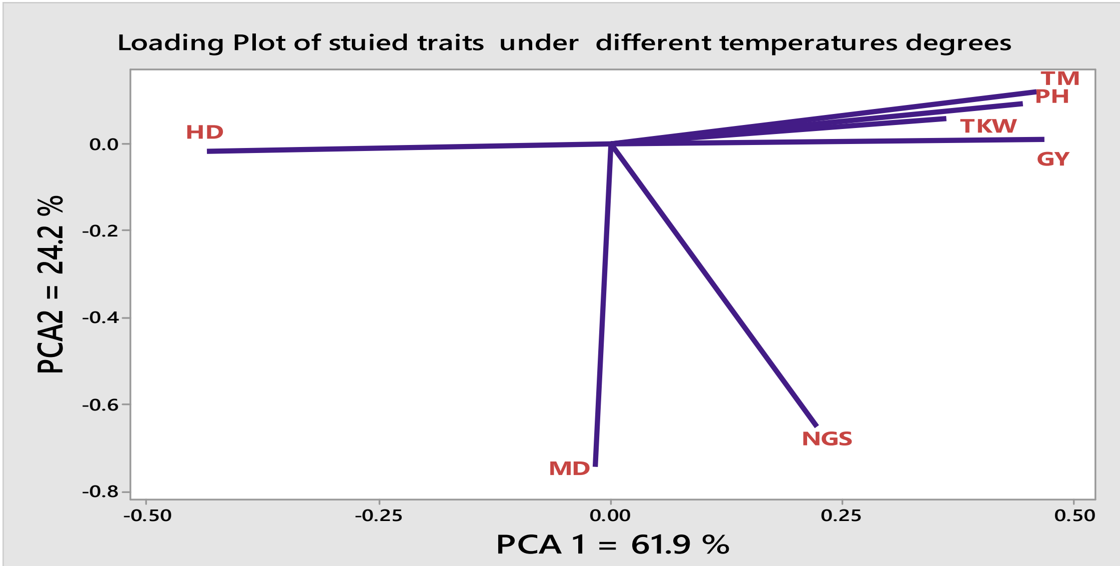

Fig. 6: Loading plot graph, showing the first two principal components (PCA) of the correlation matrix among the studied characters which leaf area index (LAI), chlorophyll fluorescence (Fv/Fm), Total chlorophyll content SPAD, days of heading (HD), plant height (PH) numbers of tiller m^2 (TM), number of grains spike,1 (NGS,1), thousand kernel weight (TKW) and grain yield (GY).

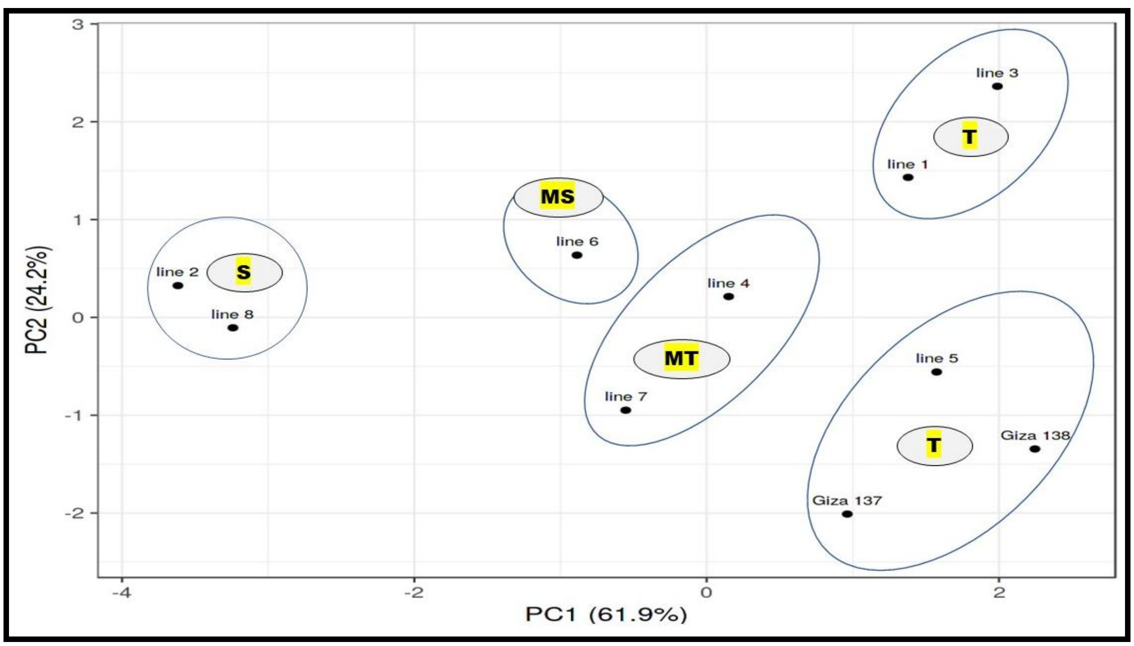

Fig. 7: PCA scatter plot of all the ten barley genotypes based on studied traits

2.2.4. Heatmap Cluster Analysis

The heatmap cluster analysis was performed to examine how various temperatures affect the morphological traits of ten barley genotypes. Using Euclidean distance and average linkage in R software (Figure 7), the analysis grouped all genotypes and traits into two primary dendrograms. The column dendrogram illustrates the morphological traits studied, while the row dendrogram represents the ten barley genotypes, which were organized into two main clusters based on the analysis., The first cluster encompasses both heat tolerance and moderate heat tolerance, which are further divided into sub-clusters. The first sub-cluster features heat-tolerant barley genotypes including Giza 137, Giza 138, line 5, line 1, and line 3. The second sub-cluster is made up of moderate heat-tolerant genotypes, notably line 7. In contrast, the second cluster focuses on heat-sensitive and moderately heat-sensitive groups. This cluster is also divided into sub-clusters, with the first consisting of heat-sensitive barley genotypes like line 1 and line 8. The second sub-cluster includes moderately heat-sensitive genotypes, specifically line 4 and line 6. Our findings align well with the work of Mohamed et al. (2021), Mariey et al. (2022), and Mariey et al. (2023 a&b), who highlighted the effectiveness of heatmap cluster analysis in phenotypic evaluations of barley genotypes. This method proves to be an essential resource for breeders, enabling them to devise informed plans suited to specific environments by concentrating on targeted traits

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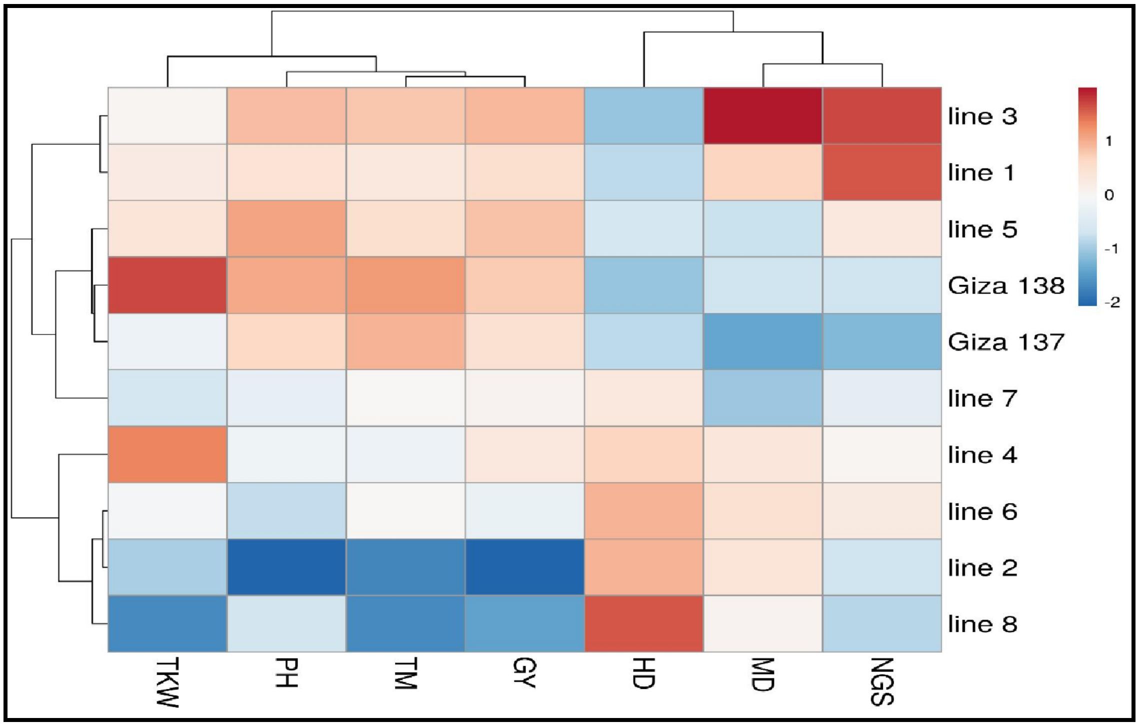

Fig 7: Multivariate heatmap illustrating the phenotypic diversity of ten barley genotypes , based morpho traits using the module of a heatmap of ClustVis, ,days of heading (HD) days to maturity MD, plant height (PH) numbers of tiller m^2 (TM), number of grains spike,1 (NGS,1) , thousand kernel weight (TKW) and grain yield (GY)

4. Conclusions

Ten barley genotypes were cultivated across three distinct field screening locations: Sakha, Malawi, and New Valley research stations. The study examined their responses to three varying temperature levels through phenotypic diversity and multivariable analysis. The results indicated that Giza 138, Giza 137, Line 1, Line 3, and Line 5 could be classified as heat-tolerant genotypes, while Line 2 and Line 8 were identified as heat-sensitive genotypes. These variations provide breeders with the opportunity to utilize the tolerant cultivars as effective maternal lines in heat stress breeding programs in Egypt, thereby enhancing farmers' income.

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