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Phenotypic and genotypic diversity analysis of some Egyptian barley cultivars (*Hordeum vulgare* L.) under different heat stress conditions

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ABSTRACT

Global food security has been disturbed by global climatic changes and increasing human population around the world. Heat stress is one of most imperative abiotic stress factors that limit barley productivity and production. Herein, three different field screening locations were carried out at Sakha, Mallawi and New-valley research stations, to identify the phenotypic and the genotypic diversity of ten Egyptian barley cultivars during two consecutive seasons 2019/2020 and 2020/2021 under different temperatures degrees. phenotypic diversity was evaluated by using some agro-physiological and grain quality characters which were contributing to yield under heat stress. Genotypic diversity was evaluated by using Sequence related amplified polymorphism (SRAP) markers as molecular identification. The results showed that high temperature enhancement all the cultivars to accelerate flowering by an average (11.39 %) and induce proline content, catalases, peroxidase enzyme active, crude protein content to increasing by average values (79.95, 40.74, 76.42 and 8.20%) respectively. However, it had a negative effect on the remaining characters. Giza 124, Giza 134, Giza 138, and Giza 132 gave high mean performance values of all measured characters being considered as heat tolerance cultivars. Ten SRAP combination primers were used where the percentage of polymorphism for each primer combination varied from 50.0% (me2+em5) to 83.3% (me5+em1). The highest polymorphism information content (PIC) was related to primer me5+em1 (0.399), indicating that this primer is highly informative to be used the barley genetic diversity for heat stress tolerance. A cluster heatmap showed that the ten barley cultivars were clustered into two main clusters, each cluster includes the most closed cultivars together due to their response to heat stress, which could be used as a source in future barley breeding programs for heat stress.

Keywords: *Hordeum vulgare*, Agro- physiological traits, grain quality, SRAP markers, PCA-Biplot and cluster heatmap

INTRODUCTION

Global climate change and human population increase had a significant effect on agricultural production (Badr *et al.,* 2018; Zartash *et al.,* 2020). Heat stress is one of most important climate negative change factors, as there is a global increase in the average temperature by 1.8-4°C in the 21st century (De Hertog, 2023), causing a significant yield loss (Wu *et al.,* 2021). In Egypt temperature differs from low and worm in coastal area to hot in Upper region (Elbasiouny *et al.,* 2017; Hamed *et al.,* 2018).

Barley (*Hordeum vulgare* L.) is well adapted to abiotic and is found to be moderately tolerant to drought stress (Gürel *et al.,* 2016). In Egypt, barley is a major winter crop cultivated in old and newly reclaimed lands that suffer from a lack of irrigation and low soil fertility (Mariey *et al.,* 2023)

Heat stress is a multifaceted phenomenon, which affects growth and development (Mondal *et al.* 2013 and Wu *et al.,* 2021). Photosynthesis is the main trait influenced by heat stress (Mathur *et al.,* 2014 and Aneja *et al.,* 2022). Heat stress decreases the grain development phenomenon which depends on grain filling rate and grain duration which is highly sensitive (Sharma *et al.,* 2019). Due to heat stress, there was an increase in total soluble sugar and protein content (Asthir and Bhatia, 2014). Hence, improving barley for heat tolerance is needs to understand the genetic and physiological variation progressions to produce new cultivars not having a high tolerance to heat stress and also had high yield. Hence heat stress can occur at any growth stage depending on the region, therefore any growth stage should be carefully considered. (Sallam *et al.,* 2018, Dawood *et al.,* 2020, Wu *et al.,* 2021; Aneja *et al.,* 2022).

Therefore, understanding the genetic diversity among cultivars will help to ensure that the breeding program has the genetic diversity to improve biotic and abiotic stresses tolerance by crossing genetically diverse parents having desirable characters (Mariey *et al.,* 2021). DNA markers are influential tools for evaluating genetic discrepancy, that the DNA content of a cell cannot be prejudiced by the environmental conditions, stages of plant development, or type of origin, which has important behavior on the consistency of the results (Ismail *et al.,* 2016; Mariey *et al.,* 2021). Sequence related amplified polymorphism (SRAP) is a PCR based marker system as described by (Li, and Quiros, 2001).

SRAP markers were advanced as it is simple, discloses numerous, co-dominant, targets open reading frames (ORFs) as a functional gene that makes it efficiently used in marker assist selection (MAS) and allows easy isolation of bands for sequencing (Wang *et al.,* 2009). Moreover, SRAP marker is an influential technique in assessment of genetic variability, showing high degree of reproducibility, discriminatory power, high polymorphism, plentiful information, larger reproducible, simple, established and more helpful than the other DNA markers (Yang *et al.,* 2010). SRAP markers were successfully used in determining the genetic diversity and relationships among in crops such as barley for abiotic stress such as salinity and drought (Ahmed *et al.,* 2021; Mariey, 2018; 2021 & 2022). For heat stress, SRAP also have been successfully used to measuring the genetic diversity and relationships in many cereal crops such as wheat (Said *et al.,* 2015) and maize (Anwer *et al.,* 2021). Until now, there is no many reports on determining the genetic diversity of Egyptian barley cultivars and or genotypes by SRAP markers for heat stress.

The association between molecular markers and phenotypic evaluation remains one of significant aspects to examine the genetic role of tolerance by guess the genomic regions that touch plant's response will be useful as a comprehensive evaluation in breeding programs for environmental stress (Mohamed, *et al.,* 2021; Mariey *et al.,* 2022 & 2023).

The present study aimed to investigate the phenotypic and genotypic diversity of ten Egyptian barley cultivars using some agro-physiological, grain quality traits and classify them using SRAP molecular level to provide the genetic information of them to use in the future breeding programs for heat stress in Egypt.

MATERIAL AND METHODS

2.1. *Barley plant materials*

The grains of ten barley cultivars were kindly provided by Sakha Barley Dep., Sakha Agricultural Research Station, Field Crops Research Institute, ARC, Egypt, were used in this study their names and pedigree shown in (Table 1).

Table 1: Name, type, row and pedigree of ten barley cultivars used in this study

No.	Name	Pedigree
1	**Giza 124**	Giza 117/Bahteem 52// Giza 118/FAO 86
2	**Giza 130**	Comp.cross"229//Bco.Mr./DZ02391/3/Deir Alla 106
3	**Giza 131**	CM67B/CENTENO//CAMB/3/ROW906.73/4/GLORIABAR/ COME-B/5/FALCON BAR/6/LINO
4	**Giza 132**	Rihane-05//AS 46/Aths*2Athe/ Lignee 686
5	**Giza 133**	Carbo/Gustoe
6	**Giza 134**	Alanda-01/4/W12291/3Api/CM67//L2966-69
7	**Giza 135**	ZARZA/BERMEJO/4/DS4931//GLORIABAR/COPAL/3/SEN/5/AYAROS
8	**Giza 136**	Plaisant/7/Cln-B/Ligee640/3/S.P-B//Gloriaar/ Come B/5/Falconbar/6/Linocln-B/A/S.P/Lignee640/3/S.P-B//Gloria-Bar/Come B/5/Falconbar/6/Lino
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 experimental description

2.2.1. Field experimental sites

A field experiments were performed in three different heat stress locations i.e. 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", Mallawi station, locating in Minya governorate with Latitude: 27° 43' 53.04" N Longitude: 30° 50' 29.94" E. and EL-Dakhla, Oasis station, locating in new valley research governorate with Latitude: 25° 30' 59.99" N and Longitude: 29° 09' 60.00" E, during two winter sowing seasons of 2019/2020 and 2020/2021 to study the effect of heat stress on ten barley cultivars yield production, quality and green compositions.

2.2.2. Field experimental design

The ten cultivars were planted in a randomized complete block design (RCBD) with three replicates using plot area was 3.6 m^2 for each plot, to evaluate the related agro-physiological, grain quality traits 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; i.e. 0-15 cm and 15-30 cm. The physical and chemical analysis of different experimental sites were presented (Table 2)

Table 2. The average of physical and chemical properties for soil samples from the field experiments sites during two growing seasons 2019/2020 and 2020/2021

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. Field experimental area meteorological data

The average month maximum and minimum temperatures (°C) and relative humidity (RH.,%), are recorded for Sakha (T_1), Mallawi (T_2) and New Valley (T_3) during two growing winter seasons 2019/2020 and 2020/2021 as shown in (Table 3).

Table3: Mean values of the experimental area meteorological during the two-growing seasons 2019/2020 and 2020/2021

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 2019/2020	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 2020/2021	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**	**11.2**	**19.34**	**84.06**	**52.23**	**40.5**

2.3. Measured Characteristics:

2.3.1. Physiological parameters

At heading stage, the concentration of the different pigment fractions i.e. total pigment (mg/g. fresh weight) as well as (chlorophyll a and chlorophyll b content and carotenoids) was extracted in 85% acetone from fresh leaf sample according to the method of (Metzner *et al.,* 1965), proline content (mg/g fresh weight) was evaluated following the method described by (Bates et al, 1973). The Total soluble protein (mg/g.dry weight) was estimated quantitatively in the borate buffer extract using the method described by (Bradford, 1976) and the antioxidant enzymes (µM/min/g FW), i.e. catalase and peroxidase were assayed according to (Kato and Shimizu ,1987).

2.3.2. Agronomical parameters

At the heading stage days to heading were recorded, 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, number of tillers m^{-2}, number of grains $spike^{-1}$, and grain yield was determined using the full plot area (3.6 m^{-2}).

2.3.3. Grain quality traits:

After harvest, grain samples were cleaned and grounded to fine powder to determine crude protein and total carbohydrates content, according to **AOAC (2000**)

2.4. Molecular Marker Analysis

2.4.1. DNA extraction and SRAP - PCR Reaction

Genomic DNA from 15 barley cultivars fresh leaves was extracted using CTAB method according to (Doyle and Doyle**,** 1990**).** The concentrations of extracted DNA were measured using nano drop (ND-1000 Spectrophotometer).

PCR cycling was carried out using the following program; 94°C for 4 min for initial denaturation, followed by five cycles comprising for 1 min denaturation at 94 °C, 1 min annealing at 35°C, and 30 s of elongation at 72 °C. In the following 30 cycles, denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 72 °C for 30 s were carried out, ending with an elongation step for 10 min at 72 °C. Ten SRAP primer combinations were used their names and sequencing are listed in (Table 4). The PCR products were separated by electrophoresis using 1.5% agarose gels in 1X TAE Buffer against 100 bp DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and visualized under UV light, then photographed on gel documentation.

Table 4. Ten SRAP primer combinations their names and sequences

no	Name	primer sequences	Name	primer sequences
1	me6	F: TGA GTC CAA ACC GGA CA	em3	R:GACTGCGTACGAATTAAT
2	me2	F: TGAGTCCAAACCGGAGC	em6	R:GACTGCGTACGAATTGAC
3	me2	F: TGAGTCCAAACCGGAGC	em1	R: GACTGCGTACGAATTAAT
4	me1	F: TGAGTCCAAACCGGATA	em5	R: GACTGCGTACGAATTTGC
5	me4	F:TGAGTCCAAACCGGAGC	em4	R:GACTGCGTACGAATTTGC
6	me4	F:TGAGTCCAAACCGGAGC	em2	R: GACTGCGTACGAATTTGC
7	me5	F:GAGTCCAAACCGGAAG	em3	R:GACTGCGTACGAATTAAT
8	me5	F:GAGTCCAAACCGGAAG	em2	R: GACTGCGTACGAATTTGC
9	me5	F: GAGTCCAAACCGGAAG	em1	R: GACTGCGTACGAATTAAT
10	me2	F: TGAGTCCAAACCGGAGC	em5	R: GACTGCGTACGAATTTGC

2.5. Data analysis

2.5.1. Phenotypic data analysis

The results from the two seasons were homogeneity and statistically analyzed as the completely randomized block design (RCBD) model using the SPSS software. There is no significant interaction was found between year and treatment, thus, results were pooled across years (Bartlett, 1937). Fischer's protected least significant difference (LSD) at the 5% level of significance was used for treatment means. Pearson's correlation test was performed using the SPSS 22.0 version (SPPS Inc., Chicago, IL) to determine the relationship between every two studied traits Visualizing clustering of multivariate data was used to construct heatmaps clusters according to (Metsalu *et al.,* 2015).

2.5.2. SRAP marker analysis

The amplified bands from SRAP were scored as a binary data under the heading of total scorable fragments which was determined for each cultivar. The data were used to estimate the genetic similarity on the basis of number of shared amplification products according to (Nei, and Li ,1979). Polymorphism information content (PIC) values were done to distinguish between cultivars for each primer according (Anderson *et al.,* 1993). Cluster analysis was performed to produce a denderogram using un-weighted pair-group method with arithmetical average (UPGMA) using PAST program adapted by (Hammer *et al.,* 2001).

RESULTS

3.1. Temperature stress under different field conditions

Grain filling and anthesis stages of barley growth were difference affect by the temperature degrees in March and April months, there were differ among the maximum and minimum temperatures in March and April 2020 and 2021 under Sakha (T_1), Mallawi (T_2) and New Valley (T_3) Station, Egypt during two growing winter seasons 2019/2020 and 2020/2021 as shown in (Table 3 & Fig 1).

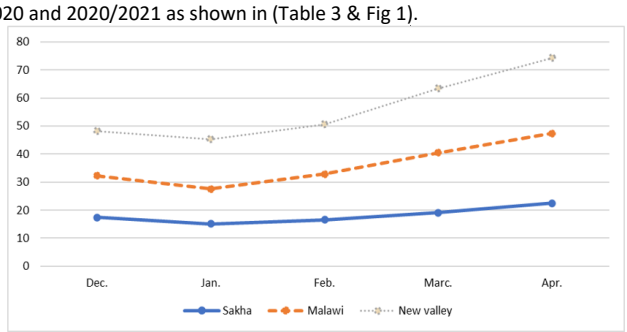

Fig 1. Average month temperature among Sakah , Malawi and New valley station

3.2. Phenotypic diversity changes due to heat stress

3.2.1. Influence of different temperatures degrees on the phenotypic traits

The ANOVA analysis of all phenotypic studied traits including physiological parameters such as total pigment content (TPC), proline content (PC), total soluble protein (TSP), catalase enzyme activity (CAT) and peroxidase enzyme activity (POD), agronomical traits including, days to heading (HD), plant height (PH), number of tillers m^{-2} (TM), number of grain $spike^{-1}$ (NGS^{-1}), and grain yield (GY) and grain quality like curd protein content (CPC) and total carbohydrates content (TCC) indicated at significant statistical effect ($P < 0.05$) by different temperatures degrees under the three locations Sakah (T_1), Mallawi (T_2) and NewVally (T_3), cultivars (C), and years (Y) as shown in (Table 5). A significant two-way interaction between cultivars and temperatures (C X T) were observed for all studied phenotypic traits. While, the two-way interaction between temperatures x years (T X Y) and years x cultivars (C X Y) were significant across all traits expect the catalase enzyme activity (CAT) and peroxidase enzyme activity (POD), number of tillers m^{-2} (TM), number of grain $spike^{-1}$ (NGS^{-1}), curd protein content (CPC) and total carbohydrates content (TCC) there were not significant. Also, the combined ANOVA indicated significant effect for three-ways interaction (G X T X Y) across all traits, expect for catalase enzyme activity (CAT) and peroxidase enzyme activity (POD) , number of tillers m^{-2} (TM), number of grain $spike^{-1}$ (NGS^{-1}), curd protein content (CPC) and total pigment content (TPC) which were not significant.

The results indicate that high temperatures at Mallawi and New Valley (T_2 and T_3) caused a significant decrease in all measured traits, while caused a significant increase in proline content (PC), catalase enzyme activity (CAT), curd protein content (CPC) and total carbohydrates content (TCC) as compared with normal temperature at Sakha station (T_1). Likewise, increasing temperature in Mallawi and New Valley (T_2 and T_3) induced all cultivar to heading early more than Sakha station (T_1).

For barley cultivars, the results showed varied significant among all the Egyptian barley cultivars based on their average mean performances of all studied traits. The results showed that Giza 124, Giza 132, Giza 134 and Giza 138 showed high average values for all studied under the high temperatures degrees than other cultivars, while Giza 130 and Giza 131 had low average values which they were more affected by heat stress as shown in (Table 5 and Fig. 2).

Table 5. Effects of years, different temperature degrees (three location) and barley cultivars on physiological, agronomic, grain quality and their interactions during two growing seasons 2019/2020 and 2020/2021

Parameters	Physiological Parameters					Morphological Parameters					Grain quality Parameters	
	TPC	PC	TSP	CAT	POD	HD	PH	TM	NGS^{-1}	GY	CPC %	TCC
Years												
2019/20	9.26	1.85	10.95	0.08	0.16	84.08	97.07	378.48	56.72	17.81	10.77	72.0
2020/21	9.43	1.84	10.85	0.098	0.267	83.77	98.33	378.36	55.73	17.50	10.89	72.26
Temperature Degrees												
(Sakha) T1	11.521	1.338	12.269	0.054	0.123	89.67	109.05	488.83	66.77	18.278	11.10	72.72
(Malawi) T2	9.249	1.800	11.191	0.064	0.166	86.15	103.13	438.01	62.08	16.801	11.62	71.22
(New valley) T3	7.055	2.405	8.845	0.076	0.217	79.47	86.83	259.23	44.52	12.978	12.01	70.12
Barley cultivars												
Giza 124	11.270	1.937	11.125	0.068	0.196	84.67	96.11	396.25	56.78	16.75	10.525	70.66
Giza 130	6.849	1.300	10.476	0.057	0.134	84.44	96.89	256.19	52.11	14.53	10.786	71.54
Giza 131	7.835	1.542	9.604	0.054	0.138	86.17	83.67	427.67	56.00	13.56	12.945	71.30
Giza 132	10.289	2.159	10.046	0.071	0.175	85.78	93.56	373.44	53.39	15.86	10.921	71.42
Giza 133	8.158	1.908	10.495	0.065	0.156	84.46	92.70	373.01	55.43	17.75	11.520	72.65
Giza 134	10.740	2.135	11.330	0.069	0.182	86.11	96.61	307.89	57.33	17.04	10.456	73.06
Giza 135	9.383	1.877	10.655	0.066	0.198	83.06	95.89	259.17	49.44	15.24	13.543	72.82
Giza 136	8.587	1.575	11.343	0.066	0.150	86.83	102.17	359.89	61.00	15.28	12.167	73.59
Giza 137	8.776	1.800	11.188	0.065	0.156	80.72	105.22	438.78	55.00	16.80	11.162	74.09
Giza 138	10.862	2.239	11.419	0.069	0.202	80.22	106.61	453.44	62.33	17.40	11.742	73.57
ANOVA analysis												
Years (Y)	**	*	**	**	**NS**	*	**	*	**NS**	**	**NS**	**
Cultivars (C)	**	*	**	**	*	**	**	**	**	**	*	**
Temperature (T)	**	*	**	*	*	**	**	**	**	**	*	**
LSD (0.05)												
Years (Y)	0.067	0.011	0.071	0.017	NS	0.361	0.791	4.54	NS	1.066	NS	0.069
Cultivars (C)	0.151	0.026	0.157	0.038	0.009	0.808	1.77	10.15	3.34	2.38	0.144	0.156
Temperature (T)	0.082	0.014	0.086	0.021	0.005	0.442	0.969	5,56	1.831	1.306	0.079	0.085
Interaction												
C X Y	**	**	**	NS	NS	**	**	NS	NS	**	NS	NS
TX Y	**	**	**	NS	NS	**	**	NS	NS	NS	NS	NS
C X T	**	**	**	**	**	**	**	**	**	**	**	**
CX T X Y	**	**	**	NS	NS	**	**	NS	NS	**	NS	NS

Which Ns, * and ** non-significant and significant at the 0.05 and 0.01 levels of probability, respectively, which (TPC): total pigment content, (PC): proline content, (TSP): total soluble protein, (CAT): catalase enzyme activity , (POD) : peroxidase enzyme activity, (HD): days to heading, (PH): plant height, (TM): number of tillers m^{-2} , (NGS^{-1}): number of grain $spike^{-1}$, (GY): grain yield , (CPC) : grain curd protein content and (TCC) : total carbohydrates content

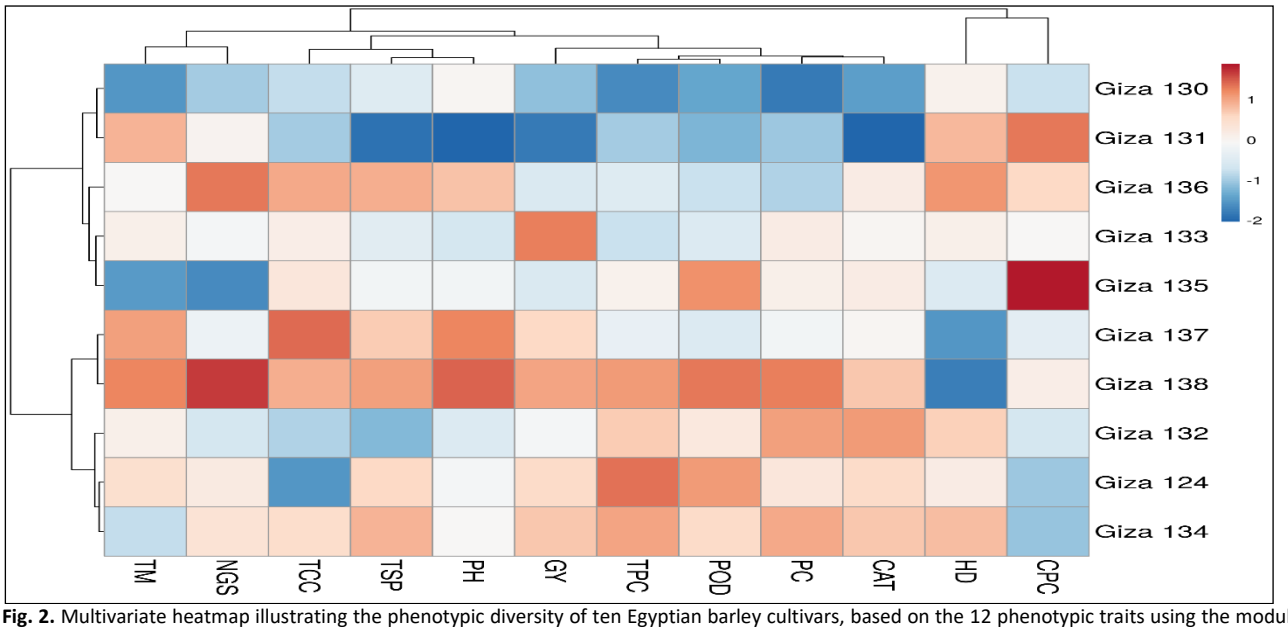

Fig. 2. Multivariate heatmap illustrating the phenotypic diversity of ten Egyptian barley cultivars, based on the 12 phenotypic traits using the module of a heatmap of ClustVis, which (TPC): total pigment content, (PC): proline content, (TSP): total soluble protein, (CAT): catalase enzyme activity , (POD) : peroxidase enzyme activity, (HD): days to heading, (PH): plant height, (TM): number of tillers m^{-2} , (NGS^{-1}): number of grain $spike^{-1}$, (GY): grain yield , (CPC) : grain curd protein content and (TCC) : total carbohydrates content

3.2.2. The relative effect of all phenotypic studied traits due to heat stress

The relative changes reduction or increase due heat stress on the physiological traits, were presented in (Figure 3). The results showed that the heat stress produced reduction for total pigment content (TPC),) under Mallawi T2 and new Valley T3 by average values (19.72 and 38.76 %) respectively and for total soluble protein (TSP) by average values (8.79 and 27.91 %) under T2 and T3 respectively.

While, relative changes increase due heat stress were observed for proline content (PC), catalase enzyme activity (CAT) and peroxidase enzyme activity (POD), by values (79.95, 40.74 and 76.42, respectively) under high temperature at new Valley Station (T3) as camper with low temperature at Sakha T1 station.

With respect to morphological traits, heat stress activated a reduction in all traits ranged from lowest average reduction in plant height by (5.43 and 10.40%) to highest average reduction in number of tillers by (20.38 and 51.19 %) under (T_2 and T_3) respectively as camper to T_1. As for the relative changes due heat stress on grain yield the results showed that there was reduction due to heat stress by average values (8.06 and 29.00%) under Mallawi and New Valley location respectively. However, heat stress induced all cultivars to heading earlier by an average (3.93 and 11.39 %) respectively as shown in (Fig. 3).

Also, the relative changes increasing due heat stress were observed for curd protein content (CPC) with an average value were (4.68, 8.20%), while reduction total carbohydrates content TCC with an average value (2.06 and 3.57 %) under Mallawi and New Valley location respectively as camper by low temperature at Sakha (T1) as shown in (Fig. 3).

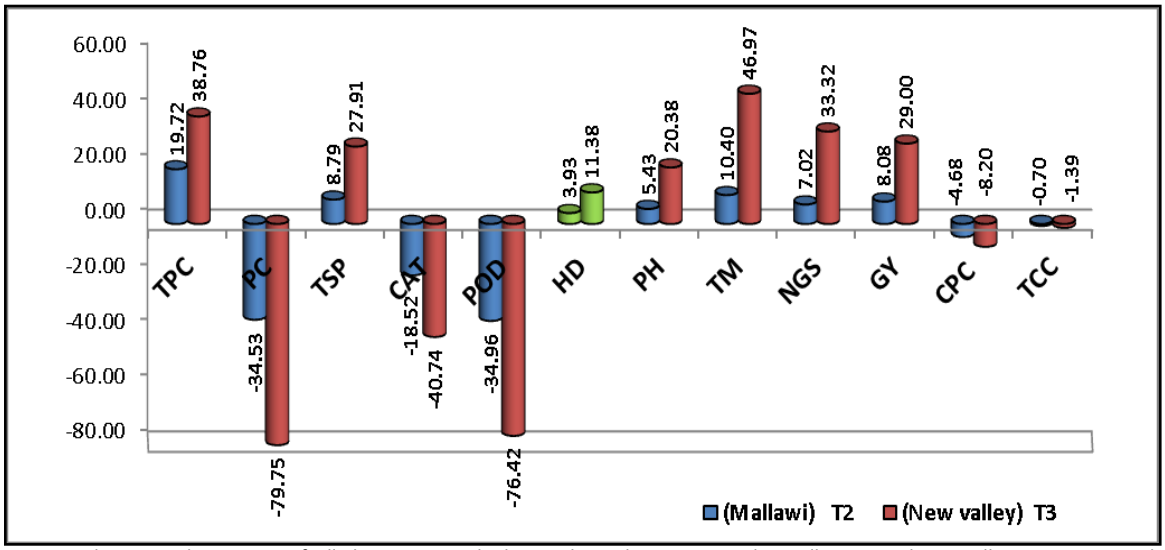

Fig. 3. The average reduction and increasing of all phenotypic studied traits due to heat stress under Mallawi T2 and New valley T3 as compere by T1 at Sakha station , which (TPC): total pigment content, (PC): proline content, (TSP): total soluble protein, (CAT): catalase enzyme activity , (POD) : peroxidase enzyme activity, (HD): days to heading, (PH): plant height, (TM): number of tillers m^{-2} , (NGS^{-1}): number of grain $spike^{-1}$, (GY): grain yield , (CPC) : grain curd protein content and (TCC) : total carbohydrates content which the green refer to inducing heading days

3.2.3. Phenotypic Correlation coefficients

Pearson correlation coefficient among all studied phenotypic traits (Figure 4) were done to understand the relationships among all studied traits under Sakha location (Figure 4 &C), the results showed that the correlation among grain yield and total soluble protein (TSP), catalase enzyme activity (CAT) , days to heading (HD) , number of tillers m^{-2} (TM), and curd protein content (CPC) were significantly negative while under Mallawi (Figure 4 &A) the significantly negative correlation were found between grain yield and catalase enzyme activity and days to heading while under New Valley ((Figure 4 &B) the significantly negative correlation were found among grain yield and catalase enzyme activity and days to heading , Also grain yield (GY) high positive and significant correlation on all phenotypic traits under Sakah , Mallawi and New Valley stations as shown in Fig.4

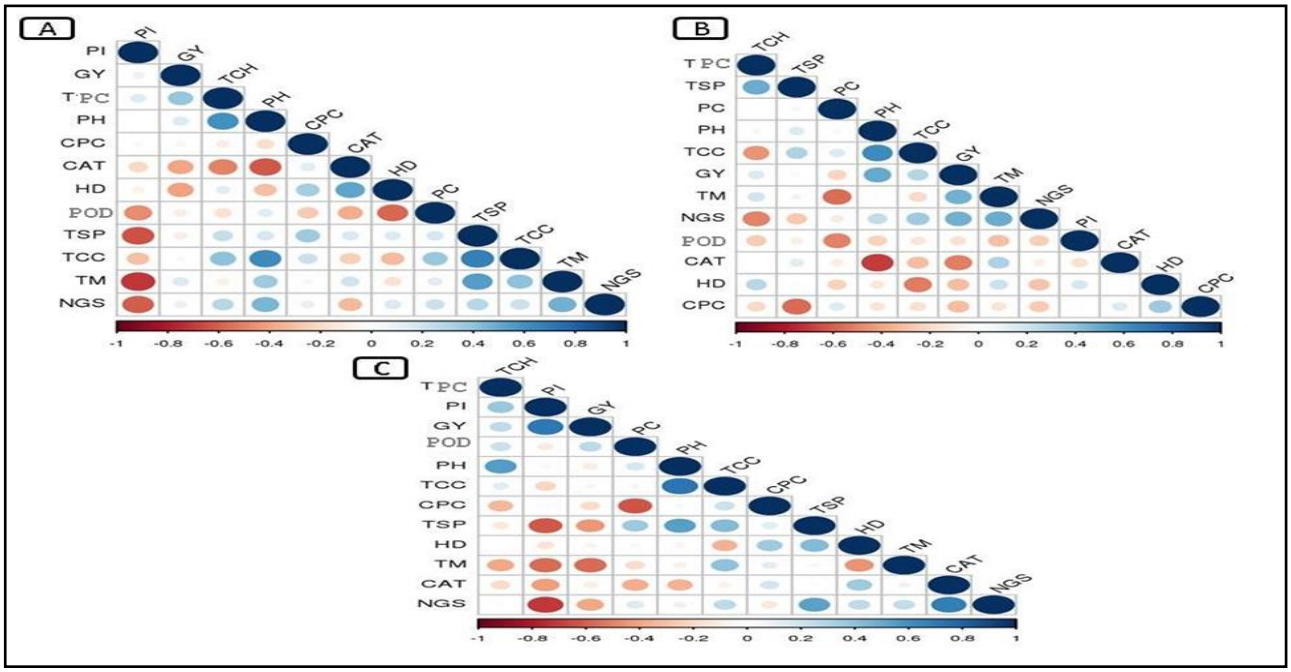

Fig. 4. Pearson correlation coefficient heatmap under A: Mallawi ,B: New Valley, C: Sakha stations correlation coefficient among grain yield (GY) and total pigment content (TPC), proline content (PC), total soluble protein (TSP), catalase enzyme activity (CAT), peroxidase enzyme activity (POD), days to heading (HD), plant height (PH), number of tillers m^{-2} (TM), number of grain spike-1 (NGS^{-1}), grain yield (GY) and grain quality ,curd protein content (CPC), total carbohydrates content (TCC)

3.3. Genotypic diversity changes due to heat stress

3.3.1. Amplification results of SRAP-PCR marker analysis

The SRAP marker was used to evaluate the genetic polymorphism among ten barley cultivars and attempt to find linked marker with heat tolerance. Data in Table (5) showed that the total band were 92 bands. Number of polymorphic bands for used primers was 63 band ranged from six bands in (me6+em3) to twelve bands in (me5+em1) with an average (6.3%) per primer combination.

The percentage of polymorphism for each primer combination varied from 50.0 % (me2+em5) to 83.3% (me5+em1) with average 67.89%. Polymorphic information content (PIC) was evaluated to assess the genetic diversity of ten selected primers combination. The highest PIC was 0.399%, which was related to primer combination me5+em1.

The primer combination me5+em1 was highly informative which had higher values of markers efficiency such as total number of bands TNB, number of polymorphic bands NPP, polymorphic polymorphism percentage PPP%, polymorphism information content PIC, diversity index DI, Marker Index MI and Discriminating power DP were (12,10, 83.3%, 0.399, 0.484, 0.491 and 0.632) respectively that could be as a useful primer set to confirm the genetic differences among barley cultivars for heat tolerant as shown in (Table 6 &Fig. 5).

Table 6. SRAP primer profiles for ten barley cultivars

No.	Primer Name	TNP	NPP	PPP %	PIC	DI	MI	DP
1	me6+ em3	6	4	66.1	0.381	0.131	0.141	0.147
2	me2+em6	9	7	77.7	0.315	0.351	0.391	0.464
3	me1+em5	8	6	75.0	0.314	0.355	0.365	0.424
4	me2+em5	10	5	50.0	0.285	0.322	0.32	0.361
5	me2+em1	7	4	57.1	0.335	0.464	0.461	0.593
6	me4+em4	8	5	62.5	0.365	0.312	0.302	0.337
7	me5+em2	11	7	63.6	0.285	0.282	0.231	0.617
8	me5+em3	11	8	72.7	0.361	0.425	0.490	0.52
9	me5+em1	12	10	83.3	0.399	0.484	0.491	0.632
10	me4+em2	10	7	70.7	0.332	0.373	0.284	0.436
Average		**9.2**	**6.3**	**67.87**	**0.337**	**0.3499**	**0.3476**	**0.4529**
Total		**92**	**63**	**678.7**	**3.372**	**3.499**	**3.476**	**4.529**

Which TNB: total number of bands; NPP, number of polymorphic bands, PPP%: polymorphic polymorphism percentage (%); PIC: polymorphism information content, DI: diversity index , MI : Marker Index and DP: Discriminating power

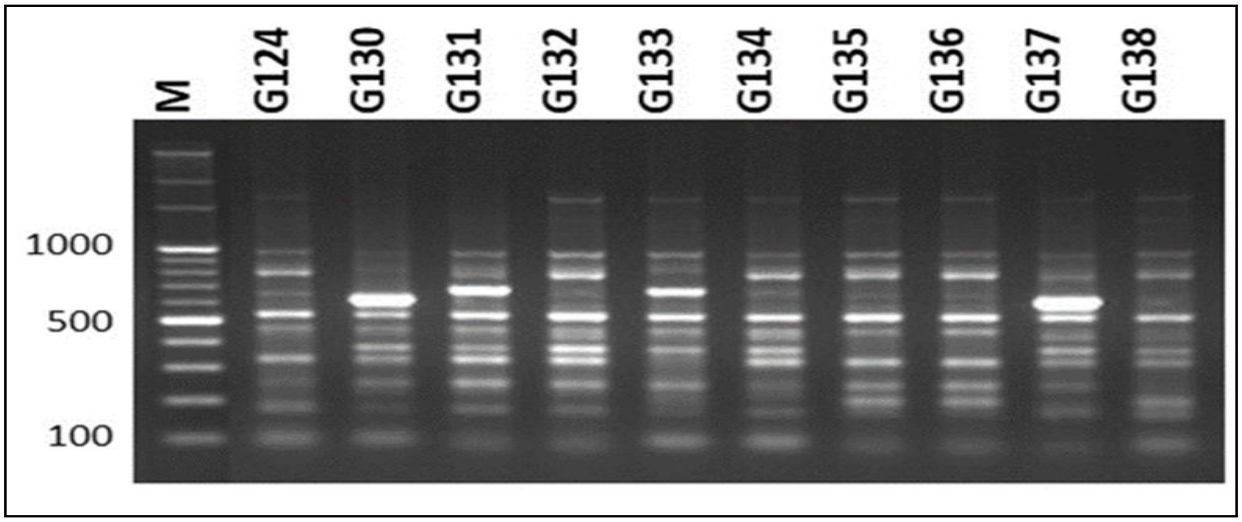

Fig. 5. Agarose gel electrophoresis of SRAP primer (em5+em1) amplification products of different ten barley cultivars.

3.3.2. Cluster analysis

The dendrogram of SRAP markers was constructed using Jaccard's genetic similarity coefficient was outlined by UPGMA method were assessed to illustration the genetic relationships among the 10 barley cultivars through the all studied SRAP primers were showed in (Fig. 6) had clustered all 10 studied barley cultivars into main clusters include the closest cultivars together. Cluster I consisted the heat tolerant cultivars (Giza 124, Giza 134, Giza 133, Giza 132 and Giza 138). Cluster II, consisted the heat sensitive (Giza 130 and Giza 131). Cluster III, consisted the heat moderate cultivars (Giza 137, Giza 135 and Giza 136).

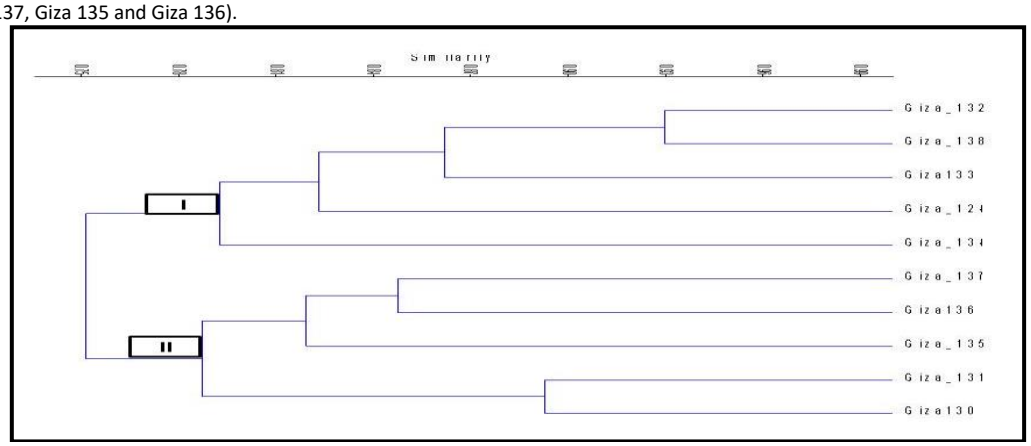

Fig. 6. Dendrogram showing clustering pattern of all 10 barley cultivars using 10 SRAP primer combinations markers.

3.3.3. Genetics Similarity correlation matrix (GS)

The Genetics Similarity correlation matrix (GS) was performed to study the genetics relationships among barley cultivars based on Jaccard's similarity coefficient was shown in (Fig. 7) . Highest GS value was (0.93 %) was observed between both Giza132 and Giza 138 cultivars followed by (0.89) was observed between Giza 133 and Giza138 as a highly heat tolerances cultivars. Similarly, high GS was found between and Giza 131 and Giza 130 was 0.88 as a heat sensitive cultivar as shown in (Figure 7).

	Giza124	Giza137	Giza131	Giza132	Giza133	Giza134	Giza135	Giza136	Giza130	Giza138
Giza124	1.00									
Giza137	0.71	1.00								
Giza131	0.70	0.80	1.00							
Giza132	0.83	0.77	0.80	1.00						
Giza133	0.78	0.75	0.78	0.84	1.00					
Giza134	0.79	0.74	0.76	0.83	0.75	1.00				
Giza135	0.69	0.84	0.78	0.81	0.76	0.72	1.00			
Giza136	0.67	0.85	0.75	0.76	0.77	0.70	0.80	1.00		
Giza130	0.71	0.83	0.88	0.80	0.75	0.77	0.75	0.82	1.00	
Giza138	0.86	0.77	0.80	0.93	0.89	0.80	0.78	0.73	0.77	1.00

Fig.7. Genetics Similarity correlation matrix for 10 barley using 10 SRAP marker

3.4. Association analysis between the phenotypic and SRAP data

3.4.1. Principal component analysis PCA

PCA- Bi-plot was used to study the differences and relatives between genotypes with respect to phenotypic and genotypic data under temperatures environment salinity environment (GE). PCA- Biplot analysis were presented in horizontal axis using 12 phenotypic traits and 10 SRAP primers to selected the trend of relationship and to characterized barley cultivars due to heat stress. The first and two principal components reported for 82.9% (PCA1= 64.4 % + PCA2 =18.5 %) of the total changeability were clearly presented in (Fig. 8). The ten barley cultivars were divided into four groups; group comprised all heat tolerance cultivars (Giza 132, Giza 134, Giza138 and Giza 124), were influenced by the molecular primers and almost of phenotypic traits which located in the right side (positive) of the horizontal axis according to their positive effect correlation with them under heat stress. While (Giza 130 and Giza 131) as a sensitive heat stress cultivars were located in left side (negative) of the horizontal axis according to their negative effect correlations with most other traits.

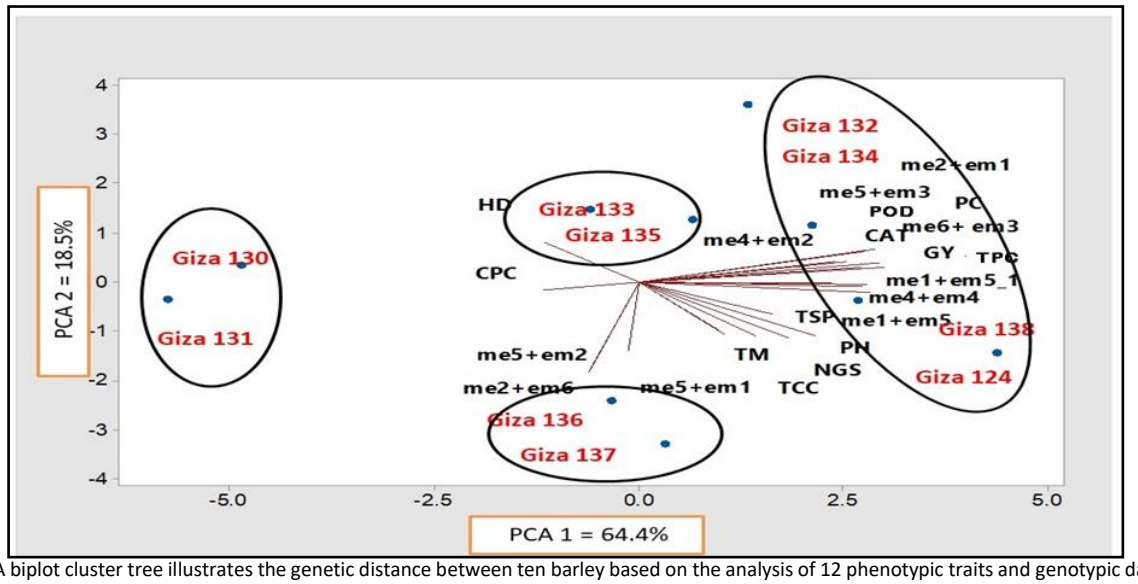

Fig. 8. PCA biplot cluster tree illustrates the genetic distance between ten barley based on the analysis of 12 phenotypic traits and genotypic data using 10 SRAP primers , which (TPC): total pigment content, (PC): proline content, (TSP): total soluble protein, (CAT): catalase enzyme activity, (POD) : peroxidase enzyme activity, (HD): days to heading, (PH): plant height, (TM): number of tillers m^{-2} , (NGS^{-1}): number of grain $spike^{-1}$, (GY): grain yield, (CPC) : grain curd protein content and (TCC) : total carbohydrates content

3.4.2. Multivariate heatmap cluster

Multivariate heatmap cluster constructed using Euclidean distance and average linkage by R software based on 12 phenotypic traits and 10 SRAP markers were presented in (Fig. 9) which showing the differences between the phenotypic data clusters and the molecular data clusters as well as their interaction. Row dendrograms show that the ten barley cluster were clustered into two clusters, first cluster include the heat sensitive cultivars (Giza 130 and Giza 131), second cluster divided into sub cluster, first sub consisted of heat tolerant cultivars (Giza 138, Giza 132, Giza 124 and Giza 134) and the second sub include the moderated heat tolerance (Giza 137, Giza 136, Giza 135 and Giza 133). It also clearly demonstrates the effects of each field trait on the cultivars, along with the effects of each initiator molecule on the Egyptian barley cultivars

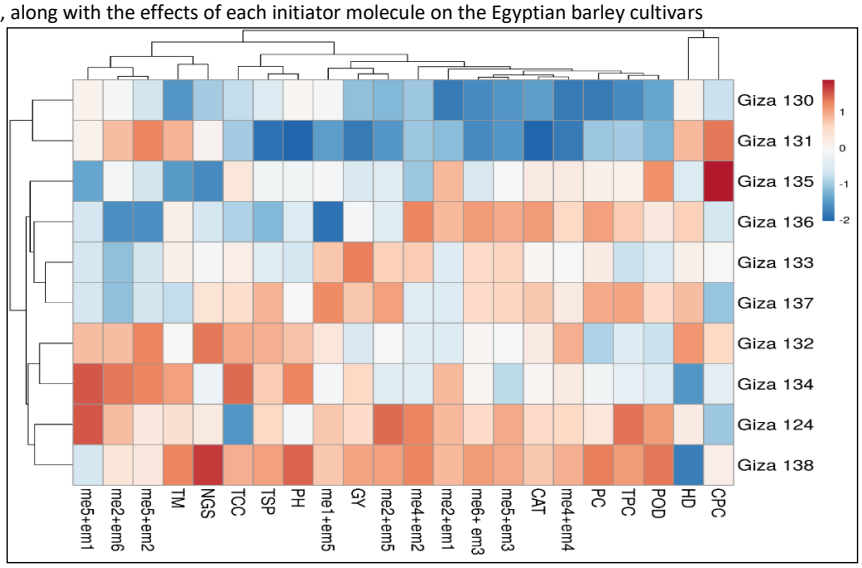

Fig. 9. Multivariate heatmap cluster illustrating the genetic diversity of ten Egyptian barley cultivars, based on the 10 SRAP primers and 12 morphological traits using the module of a heatmap of ClustVis , which (TPC): total pigment content, (PC): proline content, (TSP): total soluble protein, (CAT): catalase enzyme activity, (POD) : peroxidase enzyme activity, (HD): days to heading, (PH): plant height, (TM): number of tillers m^{-2} , (NGS^{-1}): number of grain spike , (GY): grain yield, (CPC) : grain curd protein content and (TCC) : total carbohydrates content

DISCUSSION

In arid and semi-arid regions global climatic change and worming threatens cereal production. Heat stress at post-heading stage causes significant yield decline due to the stress at anthesis and grain filling stages causing decline in yield partial sterility (Sallam *et al.,* 2018 and Dawood *et al.,* 2020). It is expected global warming that has a generally negative impact on plant growth because the high temperatures have a damaging effect on plant development (Zartash *et al.,* 2020; Wu *et al.,* 2021; De Hertog., 2023).

4.1. Phenotypic diversity changes due to heat stress

In this study, twelve phenotypic traits were evaluated under three different temperatures degrees at three different geographic area in Sakha, Mallawi and New valley different stations, which the results showed that the heat stress at anthesis and grain filling stage leads to decline in barley yield and other related traits. For physiological traits heat stress produced reduction for as total pigment content (TPC), total soluble protein (TSP), while increase proline content (PC), catalase enzyme activity (CAT) and peroxidase enzyme activity (POD) by average values (79.95, 40.74 and 76.42) respectively under high temperature at new Valley Station as camper by low temperature at Sakha T1 station. These results are in agreement with (Ur Rehman *et al.,* 2009; Farooq *et al.,* 2011; Jedmowski *et al.,* 2015). Since, they reported that heat stress at grain filling stage leads to decline in barley yield and other related traits. Moreover, (Wu *et al.,* 2021; Aneja *et al .,* 2022) they stated that the plants reaction to heat stress were different at different stages .

other studies determined that heat stress damages plant growth and development in plants (Poudel & Poudel, 2020 and Wu *et al.,* 2021) Mondini *et al.* (2014) found a significant decline of days to anthesis and n days to maturity traits due to heat stress conditions. It may have occurred basically because of life cycle became short because high temperature related to late planting.

With respect to morphological traits, heat stress activated a reduction in all traits ranged from lowest average reduction in plant height (PH), by (5.43 and 10.40%) to highest average reduction in number of tillers m^{-2} (TM) by (20.38 and 51.19 %) under (T_2 and T_3) respectively as camper by T_1. 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 Mallawi and New Valley location respectively. However, heat stress induced all cultivars to flower earlier by an average (3.93 and 11.39 %) respectively as shown in (Fig. 2). Dawood *et al.,* (2020) investigated a group of 60 Egyptian spring barley genotypes that had been subjected to heat stress testing in the field. Several features were scored to assess changes in yield-related traits and grain reserve metrics as markers of heat tolerance.

Also, the relative changes increasing due heat values stress were observed for curd protein content (CPC) while reduction were observed for total carbohydrates content (TCC) under Mallawi and New Valley location respectively as camper by low temperature at Sakha. The decline of barley grain yield and production under heat-stressed conditions occurred due to the alternation in the plant-water relationship occurred because of the high temperature (Qaseem *et al.,* 2019), and other related traits in wheat (Riaz-ud-Din *et al.,* 2010; Farooq *et al.,* 2011) and barley (Oshino *et al.,* 2011).

4.2. Genotypic diversity changes due to heat stress

Assessment of genetic diversity using DNA markers is one of the primary and important steps in barley breeding. SRAP marker is a powerful technique for the assessment of genetic variation because it has shown a high degree of reproducibility and discriminatory power, as well as a high polymorphism rate. Genetic diversity of ten Egyptian barley cultivars was evaluated using ten SRAP primer combinations which gave 92 fragments. High polymorphic rate and PIC value suggested a high level of heterogeneity among studied barley cultivars. These results agree with those obtained (Said *et al.,* 2015, Mariey *et al.,* 2018, 2021 and 2022). Since SRAP marker to evaluate the genetic variations among barley and they suggested that SRAP technology is useful for genetic diversity and relationship analyses, marker assisted selection and genetic map construction in barley.

Accordingly, it is confirmed that the SRAP marker could be efficiently used to assess genetic variation among Egyptian barley and their ability for tolerance to heat stress, which the dendrogram of SRAP markers had clustered all the Egyptian cultivars in to two groups each group include the most closed cultivars together according their response to heat stress. (Said *et al.,* 2015 and Anwer *et al.,* 2021).

4.3. Association analysis of phenotypic and genotypic data

The comparison information of genetic diversity and phenotypic evaluations one of important factors to understand and investigate in germplasm collections or breeding material helps the breeders to plan their programs for specific environments using targeted traits and molecular markers (Mohamed, *et al.,* 2021, Mariey *et al.,* 2022; Mariey *et al.,* 2023) They reported that both PCA and heatmap cluster analysis had used successfully in understanding the information of phenotypic evaluations and genetic diversity of the barley genotypes as an important factors using to helps the breeders to but good plan for their programs for specific environments using targeted traits and molecular markers.

CONCLUSIONS

This present study determined that medium and high heat stress had negative effects on grain yield, grain quality and the physiological behavior of the ten barley cultivars which were grown under three different heat stress environmental conditions. SRAP markers have generated clear patterns with high polymorphism, this polymorphism was adequate to distinguish all cultivars, to discriminate the heat tolerant and heat sensitive cultivars Irrespective of the differences of the molecular level, the ten barley cultivars revealed similarities in terms of field performance under the heat environment, Giza 124, Giza 133. Giza 134 and Giza 138 as heat tolerance were closely distributed in a genetic tree based on the molecular explanation. These differences enable the breeders to use the tolerant at cultivars as a good parent in heat stress breeding programmers in Egypt due to increase the farmer's income.

FUTURE OUTWORK

As the results from our data, we could use the tolerance genotypes in future researcher as good parent to get tolerant promising lines for heat stress through using them in breeding programmers. Also, future studies should focus on studying the effect of salinity on theses promising lines using comprehensive set of Agro-morph-physio-chemical parameters coupled with molecular marker, multivariable and economic analysis to assess the genetic relationships and classified new Barley genotypes for their ability for tolerance to heat stress in breeding programs to produce suitable cultivars at normal and heat stress condition.

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Conflict of Interest: The authors declare no conflict of interest

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تحليل التنوع الوراثى و المظهرى لبعض أصناف الشعير المصري (*Hordeum vulgare* L.) تحت ظروف الإجهاد الحراري

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التغيرات المناخية العالمية وزيادة عدد السكان يؤدى الى اضراب الأمن الغذائي العالمي في جميع أنحاء العالم. الإجهاد الحراري هو أحد عوامل الإجهاد الغيرحيويه التي تحد من إنتاجية الشعير. تم تنفيذ تجربه حقلية في ثلاث مناطق مختلفة وهي محطات بحوث سخا وملوي والوادي الجديد ، للتعرف على التنوع الظاهري والتنوع الوراثي لعشرة تراكيب وراثية من الشعير المصري خلال موسمين متتاليين 2020/2019 و 2021/2020 تحت درجات حرارة مختلفة. تم استخدام اثني عشر صفة حقلية و فسيولوجية والتى لها دور مساهم في إنتاج المحصول كدراسة التنوع المظهري تحت الإجهاد الحراري. تم تقييم التنوع الوراثي باستخدام علامات علاقات التتابع لتعدد الأشكال المبلمرة(SRAP). أظهرت النتائج أن درجات الحرارة العالية شجعت جميع التراكيب الوراثية الى التبكير بمعدل (11.39%) كما ادت الى استحداث البرولين و الكتاليز و البروكسيدز ومحتوى البروتين بمعدل زياده قدرها (79.95 ، 40.74 ، 76.42و 8.20%)، إلا أنه كان له تأثير سلبي على بقيه الصفات الزراعية والفسيولوجية المدروسة. حيث اعطت كلا من الاصناف جيزه 124و جيزة 133 ، وجيزة 134 ، وجيزة 138 ، وجيزة 132 قيم عالية لجميع الصفات المقاسة ، والتي تعتبر تراكيبًا وراثية لتحمل الحرارة وتم استخدام عشرة بادئات لتقييم الاختلاف الوراثي بين الأصناف. وكان متوسط متعدد الأشكال من كل زوج من البرايمرات تراوح من 50٪ للبريمر (me2+em5)، الى 83.3% للبريمر me5+em1. سجل البريمر me5 + em1 أعلى (PIC) بنسبة(0.399). توضح **خريطة التمثيل اللونى العنقودى** أن التراكيب الوراثيه العشرة للشعير قد تم تجميعها في مجموعتين رئيسيتين ، كل مجموعة تتضمن التراكيب الوراثيه الأكثر قرابة معًا بسبب استجابتها للإجهاد الحراري ، والتي يمكن استخدامها كمصدر لبرامج تربية الشعير المستقبلية للإجهاد الحراري كخطوة مهمة للحصول على تراكيب وراثية جديدة ذات قدرة عالية على تحمل الحرارة وانتاج محصول عالى.

الكلمات المفتاحية: الشعير-الصفات الفسيولوجية المحصولية- جودة الحبوب- علامات علاقات التتابع لتعدد الأشكال المبلمرة - تحليل المكون االاساسى الثنائ- خريطة التمثيل اللونى العنقودى