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Evaluation of barley genotypes under terminal heat stress at grain filling period using polyethylene tunnels

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ABSTRACT

Global warming threatens cereal production especially in arid and semi-arid regions. In particular, heat stress at post-heading stage causes considerable yield reduction due to the stress at critical stage, *i.e.,* anthesis and grain filling. The objective of this study was to explore barley genotypes to terminal heat stress for identifying tolerant genotypes. In addition, to evaluate the ability of polyethylene tunnels to distinguish the tolerant and sensitive genotypes. For this purpose, fifteen barley genotypes were assessed under polyethylene-covered tunnels (heat-stressed) as well as in the open field adjacent to the tunnels (non-stressed). The analysis of variance exhibited highly significant differences between temperature treatments and among the genotypes for all investigated traits. The obtained results showed that the genotypes; G1, G2, G4, G9 and G13 presented the highest grain yield and contributing traits under both conditions. Moreover, four tolerance indices were used to identify the heat tolerant and sensitive genotypes, *i.e.*, mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI) and yield index (YI). The highest indices were assigned for G2, G4 and G1, respectively. In contrast, the lowest values were given by G11, G15, G6, G5, G14 and G12. Besides, remain genotypes exhibited intermediate values. Furthermore, cluster analysis was performed based on grain yield and heat tolerance indices. The genotypes were classified into three groups; A, B and C including; 3, 6 and 6 genotypes, respectively. Group A included the genotypes which had the highest grain yield and heat tolerance indices. Followed by group B included the genotypes that had intermediate values. Instead, group C presented the genotypes which had the lowest values. Correlation coefficients between heat tolerance indices and evaluated traits were calculated. It was noticed significant positive correlations between all estimated indices and all studied traits under both conditions except number of spikes per square meter.

Keywords: Barley genotypes, terminal heat stress, polyethylene tunnel, heat tolerance indices, yield contributing traits

Introduction

Barley (*Hordeum vulgare* L.) is one of the important cereal crops in the world. It ranks fourth in terms of cultivated area and total production after wheat, maize and rice (FAOSTAT, 2015). It is mainly used for animal and poultry feeding, also for malt and some uses in the pharmaceutical industry (Biel and Jacyno, 2013). In addition, it contains about 7% β-glucan, which is essential fiber that has many health benefits (Oscarsson *et al*., 1996). Therefore, lately there is great interest for using barley in human consumption due to its nutritional benefits (Biel and Jacyno, 2013). Barley cultivated area in the world in 2013 was 49.8 million hectares produced 143 million tons. Whereas, Egypt was involved in this value with 79000 hectares produced 131890 tons (FAOSTAT, 2015).

Recent climate change gained the attention of plant breeders due to its disastrous effect on crop production. Principally, obvious increasing in temperature (global warming) which negatively affect production of field crops (El-Beltagy and Madkour, 2012; Lipiec *et al*., 2013). In particular, terminal heat stress at post-heading stage causes considerable yield reduction due to stress at critical stage, *i.e.,* anthesis and grain filling (Wardlaw and Moncur, 1995; Rehman *et al*., 2009). At flowering, it causes negative effect on pollen fertility and seed setting which lead to low grain number per spike (Ferris *et al*., 1998). Furthermore, during grain filling period it shortens the period of grain filling and reduce individual grain weight (Zhao *et al*., 2007; Dias and Lidon, 2009; Kaur and Behl, 2010).

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Climate models predict rising of global temperature 2.0 to 4.5°C than the present (IPCC 2007). Therefore, screening the genetic potentiality under temperature regimes to identify tolerant and sensitive genotypes is very important. Which is effective for overcoming heat stress and helps to improve crop productivity under global climatic changes. Since, the genotypes display different ability to produce acceptable yield under heat stress.

Polyethylene-covered tunnel is a useful tool to explore cereal genotypes to heat stress and it was used by previous researchers as Sinsawat *et al*. (2004); Rehman *et al*. (2009); Farooq *et al*. (2011). In respect of identifying tolerant genotypes, there are many indices could be used based on grain yield under heat-stressed and non-stressed conditions, such mean productivity (MP), geometric mean productivity (GMP), and yield index (YI), (Hossain *et al*., 1990; Fernandez, 1992; Gavuzzi *et al*., 1997). The objective of this study was to explore fifteen barley genotypes to terminal heat stress to identify heat tolerant genotypes, which could be exploited in future breeding program. In addition, to evaluate the ability of polyethylene tunnels to distinguish between tolerant and sensitive genotypes to heat stress.

Materials and Methods

Description of Experimental Site

Two field experiments were performed at El-Khattara Research Station, Faculty of Agriculture, Zagazig University, Egypt (30°41′N, 31°51′E) during winter sowing seasons of 2013-2014 and 2014-2015. Trials were sown on 21 November in both seasons. Based on the soil analysis of experimental site, it is characterized as sandy soil (94% sand) and the other physical and chemical characteristics are presented in Table 1. In addition, meteorological data was recorded at station close to the experimental site are presented in Table 2.

Plant Material and Experimental Design

Fifteen spring barley genotypes were evaluated under two temperature treatments, the genotypes are presented in Table 3. The experimental design was spilt-plot, temperature treatments were laid out in main plots and genotypes were randomized in the sub-plots, in three replications. Each plot consisted of six rows 0.2 m apart, 1-m long. Recommended rates of the fertilizers were applied; 30 kg P_2O_5/ha (15.5% P_2O_5), 100 kg K/ha potassium sulfate (48% K_2SO_4) and 180 kg N/ha ammonium nitrate (33% N). The other recommended agronomic practices for barley production in the region were applied.

Temperature treatments

The genotypes were sown in two sets; one was covered with polyethylene tunnels (heat-stressed) and the other was sown in the open field adjacent to the tunnel (non-stressed). Covering with polyethylene tunnels was after heading to increase the temperature during grain filling period. Temperature was recorded frequently and it was inside the tunnel more than the outside by 4-7°C.

Data recorded

Number of spikes was counted at maturity stage in 0.5 m^2. Additionally, five spikes randomly were chosen from the central rows to measure grain number per spike, thousand-grain weight (g) and spike grain weight (g). Furthermore, grain yield (kg/ha) and aboveground dry matter (kg/ha) were determined from square meter and converted to kilograms/ha.

Data analysis

The analysis of variance (ANOVA) was performed to investigate the significance of studied factors; temperature treatments (T), genotypes (G), and their interaction effect for all studied traits.

Least significant difference (LSD) values were estimated at the 5% probability level (Steel *et al.*, 1997). Besides, the tolerance indices were calculated using the following equations:

Mean productivity $MP = \frac{Ys+Yp}{2}$ (Hossain *et al.*, 1990)

Geometric mean productivity $GMP = \sqrt{(Ys \times Yp)}$ (Fernandez, 1992)

Stress tolerance index $STI = \frac{Ys \times Yp}{(\bar{Y}p)^2}$ (Fernandez, 1992)

Yield index $YI = \frac{Ys}{\bar{Y}s}$ (Gavuzzi *et al.*, 1997)

Where Ys is yield under heat-stressed conditions, Yp is yield of the genotype under non-stressed, $\bar{Y}s$ is the average of all genotypes under heat-stressed conditions and $\bar{Y}p$ Average of all genotypes under non-stressed conditions.

Moreover, correlation coefficients between tolerance indices and the evaluated traits were calculated.

Table 1: Physical and chemical properties of the experimental soil

Soil properties	Value
Soil particles distribution	
Sand (%)	94.18
Silt (%)	4.35
Clay (%)	1.47
Soil texture	Sandy
Calcium carbonate ($CaCO_3$, g kg^{-1})	6.80
Organic matter (g kg^{-1})	6.30
pH	8.07
Electrical conductivity EC, dsm^{-1})	0.64
Soluble cations and anions (mmolc L^{-1}) *	
Calcium (Ca^{++})	1.67
Magnesium (Mg^{++})	0.95
Sodium (Na^{+})	2.43
Potassium(K^{+})	1.37
Bicarbonate (HCO_3^{-})	2.17
Chlorine (Cl^{-})	2.68
Sulphate ($SO_4^{=}$)	1.54
Available nutrient (mg kg^{-1} soil)	
Nitrogen (N)	30.52
Phosphorus (P)	5.49
Potassium (K)	79.34

Table 2: Average of minimum and maximum temperatures and total precipitation during the two growing seasons in the experimental site

Month	2013-2014			2014-2015		
	T_{min}	T_{max}	Precipitation (mm)	T_{min}	T_{max}	Precipitation (mm)
December	9.0	19.5	12.1	10.1	22.0	9.1
January	9.1	20.3	9.8	7.9	18.4	12.8
February	9.0	21.3	11.4	8.6	19.9	13.4
March	11.3	24.3	7.3	11.5	24.5	7.6
April	14.0	29.0	0.0	12.2	27.1	0.0
May	9.0	19.5	12.1	10.1	22.0	9.1

Table 3: Codes, pedigree, origin and registration year of the used barley genotypes

Genotype	Codes	Pedigree	Origin	Year of release
Giza 123	G1	*Giza 117 / FAO 86*	Egypt	1988
Giza 124	G2	*Giza 117/Bahteem 52// Giza 118/FAO 86*	Egypt	1992
Giza 125	G3	*Giza 117/Bahteem 52// Giza 118/FAO 86 (*Sister line to Giza 124)	Egypt	1995
Giza 126	G4	*Baladi Bahteem/S D729-Por12762-BC*	Egypt	1995
Giza 129	G5	*Deir Alla 106/Cel//As 46/A ths*2*	ICARDA	2001
Giza 130	G6	*Comp.cross 229 // Bce Mr / DZ 02391 / 3 / Deir Alla 106*	ICARDA	2001
Giza 131	G7	*CM67-B/CENTENO-/3/ROW906.73/4/GLORA-BAR/COMEB/5/FA/CON-BAR/6/LINO*	ICARDA	2001
Giza 132	G8	*Rihane-O5 // AS 46 / Aths*2" Aths / Lignee 686*	ICARDA	2006
Giza 133	G9	*Carbo/Gustoe*	ICARDA	2011
Giza 134	G10	*Alando-01/4/W12291/3/Api/CM67//L2966-69*	ICARDA	2011
Giza 135	G11	*ZARZA/BERMEJO/DS4931//GLORIA-BAR/COPLA/3SEN/5AYAROSA"*	ICARDA	2011
Giza 136	G12	*PLAISANT/7/CLN-B/4/S.P-B/LIGNEE640/3/S.P-B//GLORIA-BAR/COME-B/5/FALCON-BAR/6/LINO CLN-B/A/S.P-B/LIGNEE640/3/S.P-B//GLORIA-BAR/COME-B/5/FALCON-BAR/6/LINO"*	Egypt	2011
Giza 2000	G13	*Giza 117 / Bahtim 52 // Giza 118 / FAO 86/3/ Baladi 16/ Gem. (Giza 121)*	Egypt	2000
CHK 9	G14	*Aths/Lignee86//ACSAD68*	ICARDA	-
CHK 39	G15	*Alanda-02/4/Arizona5908/Aths//Asse/3/F208-74/5/Alanda/3/CI088*	ICARDA	-

Results and Discussion

Analysis of Variance

The combined analysis of variance for temperature treatments, genotypes, years and their interactions is shown in Table 4. Highly significant differences were observed between temperature treatments and among genotypes for all evaluated traits.

Table 4: Mean squares of studied traits for 15 barley genotypes under two heat treatments over two growing seasons

Source of variation	**d.f.**	**NS/m²**		**GNS**		**TGW**		**SGW**		**GY**		**AGDM**	
Treatment (T)	1	252435	**	423.6	*	1326.3	*	6.83	*	8189090	*	66446018	**
Genotype (G)	14	3040	**	130.2	**	20.61	**	0.31	**	3706423	**	15293940	**
T×G	14	2000	**	13.4	**	7.11	**	0.94	**	454255	**	2548712	**
Year (Y)	1	1894	*	12.2	*	899.5	**	0.59	NS	41805	*	91108	*
T×Y	1	13987	**	464.6	**	368.1	**	0.001	NS	23225	NS	17110058	**
G×Y	14	2239	**	16.1	**	25.57	**	0.06	NS	912581	**	6227594	**
T×G×Y	14	589	*	27.6	**	2.67	NS	0.05	NS	465827	**	4484104	**

NS: Not significant, * P < 0.05, ** P < 0.01
NS/m^2 is number of spikes per m^2, GNS is grain number per spike, TGW is thousand-grain weight (g), SGW is spike grain weight (g), GY is grain yield (kg/ha.) and AGDM is aboveground dry matter (kg/ha.).

Which indicated that the used polyethylene-covered tunnels presented sufficient temperature to screen heat tolerant barley genotypes. Additionally, it revealed to presence of genetic variability in the used genotypes. On the other hand, the mean squares values of temperature treatments and genotypes displayed that the investigated traits were more affected by temperature than the genotypes. Besides, the interaction between genotypes and temperature treatments was highly significant for all studied traits. Which demonstrated different responses of barley genotypes under temperature treatments. The significant difference between the two years of study was due to the weather conditions (Table 2). Similar results were reported by Ferris *et al*. (1998); Chen *et al*. (2000); Sinsawat *et al*. (2004); Rehman *et al*. (2009); Farooq *et al*. (2011). Since, they found significant differences between temperature treatments as well as among the genotypes and their interaction. Moreover, they reported that the polyethylene tunnels could present sufficient heat stress to evaluated cereal genotypes.

Mean Performance

Heat stress adversely affected number of spikes per square meter and the genotypes performed differently under temperature treatments. Number of spikes varied from 272 to 374 spikes under heat-stressed conditions while, from 315 to 404 spikes under non-stressed conditions. The genotypes; G1, G3, G14 and G5 recorded the highest number of spikes under stressed conditions, whereas, G2, G10, G14 and G1 exhibited the highest number under non-stressed conditions. On the contrary, the fewest number was assigned for G11, G8, G6 and G9 under stressed conditions while, G11, G4, G12 and G8 under non-stressed conditions (Fig.1, A). Moreover, grain number per spike was significantly affected by temperature treatments, it ranged between 28.5 to 42.8 grains under stressed conditions and between 30.8 to 43.9 grains under non-stressed conditions. The highest grain number was obtained by G4, G2, G1, G8 and G9 under both conditions. Conversely, G15, G14, G5, G10, G13 and G12 presented the lowest grain number under both conditions (Fig.1, B).

Furthermore, thousand-grain weight was significantly differed between temperature treatments and genotypes. It ranged between 32.8 to 40.6 g under stressed conditions and between 36.8 to 43.3 g under non-stressed conditions. The heaviest grain index was assigned for; G4, G13, G9 and G8 under stressed conditions while, G13, G4, G1 and G3 under non-stressed conditions. In comparison, G6, G5, G7, G11 and G14 gave the lightest thousand-grain weight under stressed conditions while, G6, G14, G5 and G12 under non-stressed conditions (Fig.2, A). Additionally, spike grain weight was varied significantly between temperature treatments and genotypes. It ranged from 1.1 to 1.7 g under stressed conditions and from 1.3 to 2.0 g under non-stressed conditions. The highest values were obtained by G4, G8, G1 and G9 under stressed conditions while, G4, G1, G2 and G9 under non-stressed conditions. In contrast, the lowest values were recorded by G14, G15, G5 and G6 under both conditions (Fig.2, B).

Likewise, grain yield significantly decreased under heat stressed conditions, it ranged between 2960 to 4832 kg/ha under stressed conditions and between 3422 to 5353 kg/ha under non-stressed conditions. The genotypes; G1, G2, G4, G9, G13 and G7 had the highest grain yield under both conditions. Instead, the genotypes; G11, G6, G5, G15, G12 and G14 presented the lowest grain yield under both conditions (Fig.3, A). Similarly, aboveground dry matter was significantly affected by heat stress, it ranged between 6800 to 10800 kg/ha under stressed conditions and between 7703 to 13440 kg/ha under non-stressed conditions. The genotypes; G2, G9, G1, G13, G4 and G15 displayed the highest aboveground dry matter under both conditions. On the contrary, the genotypes; G14, G3, G11, G7 and G6 had the lowest aboveground dry matter under both conditions (Fig.3, B). These results are in consonance with previous finding of Gavuzzi *et al*. (1997); Passarella *et al*. (2002); Rehman *et al*. (2009); Farooq *et al*. (2011); Hossain *et al*. (2012); Faralli *et al*. (2015) and Jedmowski *et al*. (2015). Since, they stated that the heat stress at critical stage as grain filling period leads to considerable reduction in grain yield and contributing traits. Moreover, reported that the genotypes perform differently under heat stress conditions.

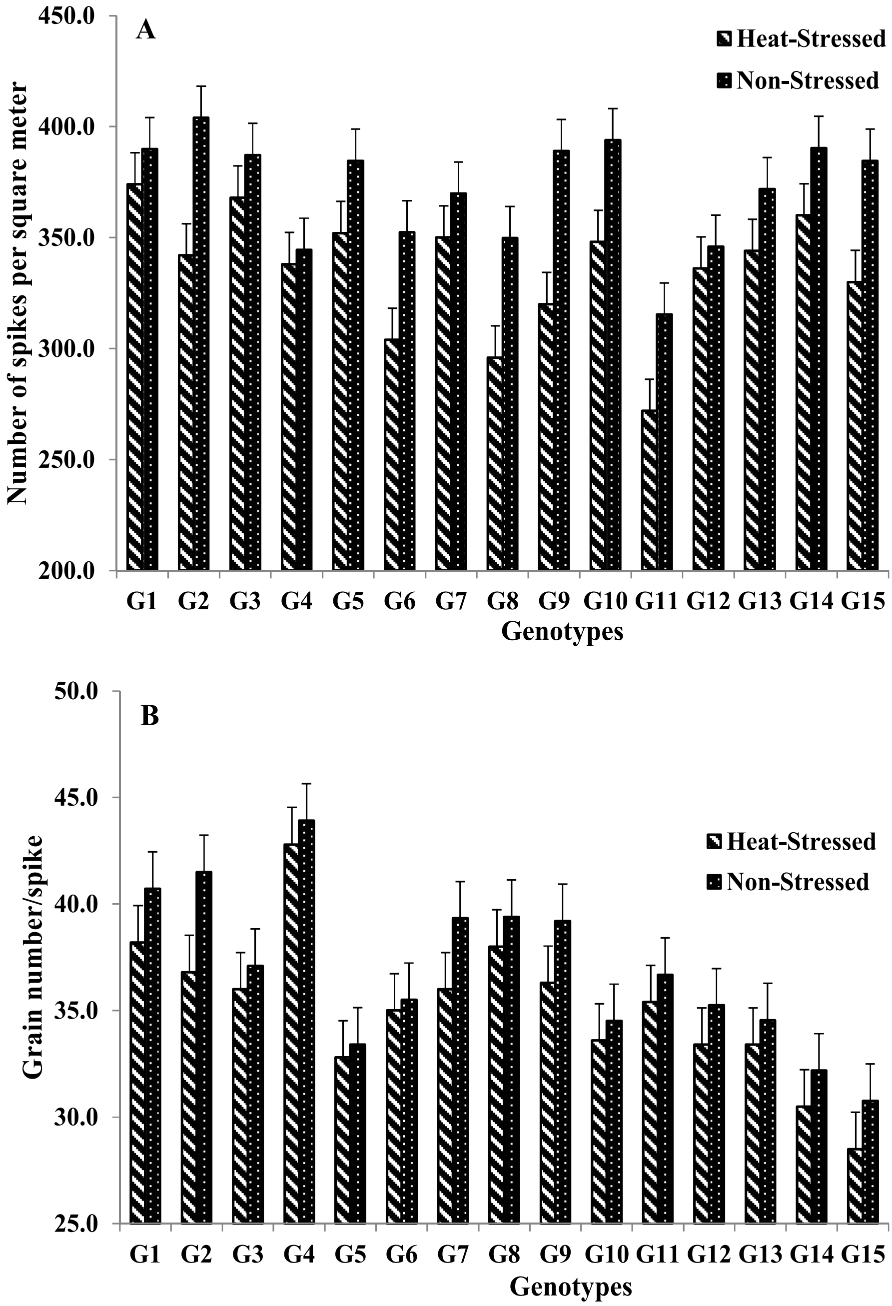

Fig. 1: Impact of heat treatments on number of spikes per square meter (A) and grain number per spike (B) for the 15 barley genotypes. The bars on the top of the columns represent the LSD for mean value comparison.

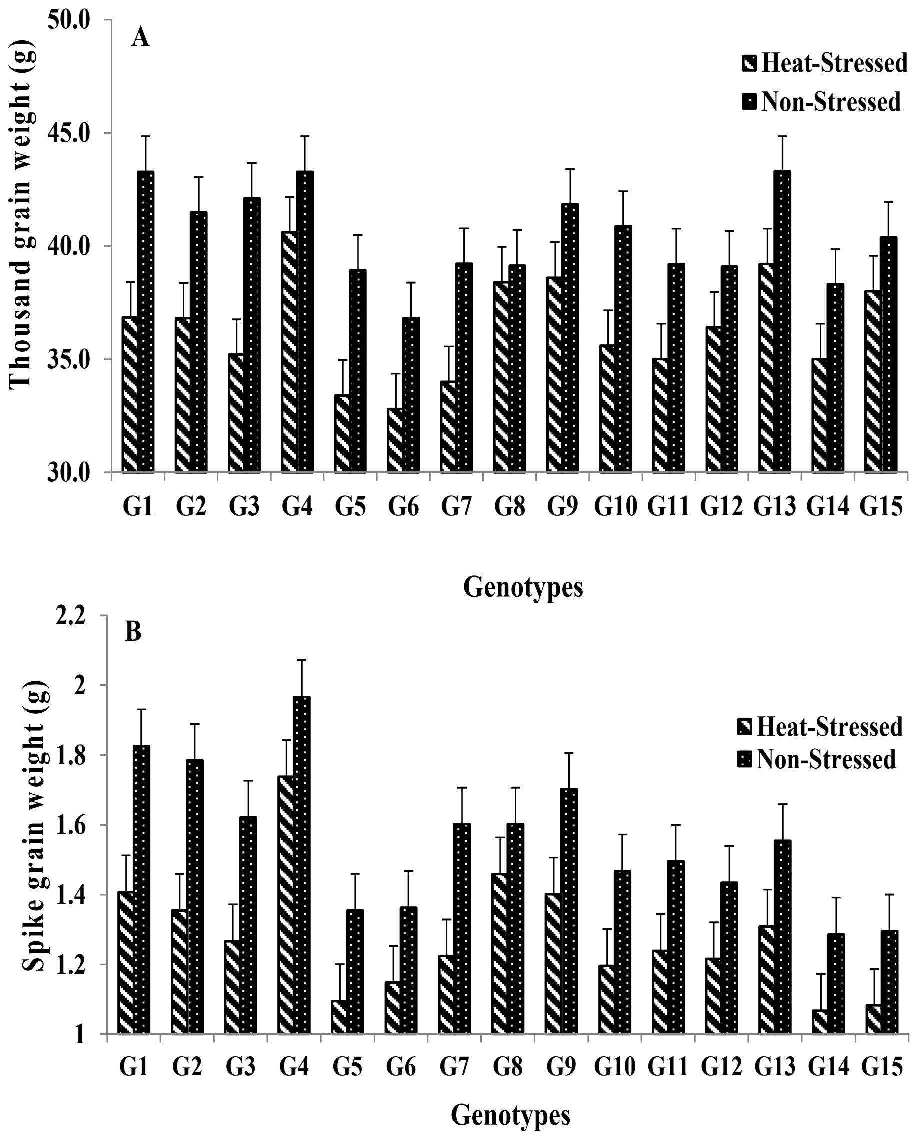

Fig. 2: Impact of heat treatments on thousand-grain weight (A) and spike grain weight (B) for the 15 barley genotypes. The bars on the top of the columns represent the LSD for mean value comparison.

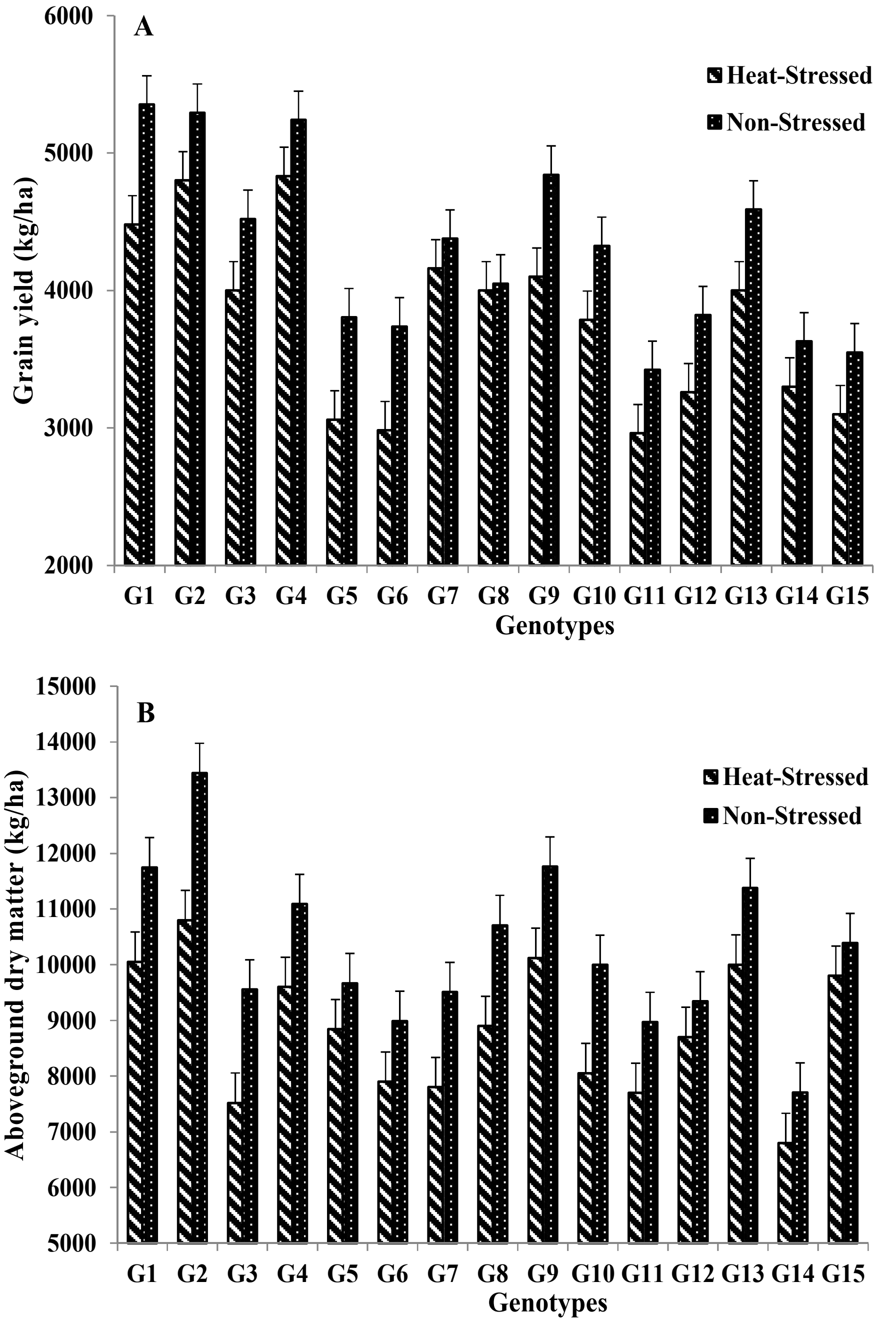

Fig. 3: Impact of heat treatments on grain yield (A) and aboveground dry matter (B) for the 15 barley genotypes. The bars on the top of the columns represent the LSD for mean value comparison.

Heat tolerance indices and cluster analysis

Four tolerance indices MP, GMP, STI and YI were used to identify the tolerant and sensitive genotypes. These indices were estimated based on grain yield under heat-stressed and non-stressed conditions (Table 5). The highest indices were recorded by G2 followed by G4 and G1. Therefore, these genotypes could be considered as tolerant to heat stress. Moreover, they could be exploited in breeding programs for developing tolerant barley genotypes to heat stress at critical growth stage. On the other hand, the lowest values were given by G11, G15, G6, G5, G14 and G12. Accordingly, these genotypes could be considered sensitive ones. Besides, remain genotypes exhibited intermediate values.

Furthermore, cluster analysis was performed based on grain yield and tolerance indices. The genotypes were classified into three groups; A, B and C including; 3, 6 and 6 genotypes, respectively (Fig. 4). Where, group A included G2, G4 and G1, which had the highest grain yield and tolerance indices. Therefore, they are considered heat tolerant genotypes. Followed by group B comprised G8, G10, G3, G7, G13 and G9, that had intermediate values. Instead, group C presented G6, G15, G5, G14, G12 and G11, which had the lowest values, therefore they are considered sensitive ones. These results are in line with the findings of Modhej *et al*. (2005); Bavei *et al*. (2011); Sharma *et al*. (2013) Khan and Kabir (2014).

Table 5: Heat tolerance indices for the studied genotypes under stressed and non-stressed conditions (averaged over the two growing seasons)

Genotype	Code	Yp	Ys	MP	GMP	STI	YI
Giza 123	**G1**	5353	4480	4917	4897	1.29	1.18
Giza 124	**G2**	5292	4800	5046	5040	1.37	1.27
Giza 125	**G3**	4521	4000	4261	4253	0.98	1.06
Giza 126	**G4**	5241	4832	5037	5033	1.37	1.28
Giza 129	**G5**	3805	3060	3433	3412	0.63	0.81
Giza 130	**G6**	3738	2982	3360	3339	0.60	0.79
Giza 131	**G7**	4378	4160	4269	4267	0.98	1.10
Giza 132	**G8**	4051	4000	4025	4025	0.87	1.06
Giza 133	**G9**	4842	4100	4471	4456	1.07	1.08
Giza 134	**G10**	4323	3786	4055	4046	0.88	1.00
Giza 135	**G11**	3422	2960	3191	3183	0.55	0.78
Giza 136	**G12**	3820	3260	3540	3529	0.67	0.86
Giza 2000	**G13**	4590	4000	4295	4285	0.99	1.06
CHK 9	**G14**	3631	3300	3465	3461	0.65	0.87
CHK 39	**G15**	3549	3100	3325	3317	0.59	0.82

Yp: Grain yield under non-stressed conditions, Ys: Grain yield under heat-stressed conditions, MP: Mean productivity, GMP: Geometric mean productivity, STI: Stress tolerance index, YI: Yield index

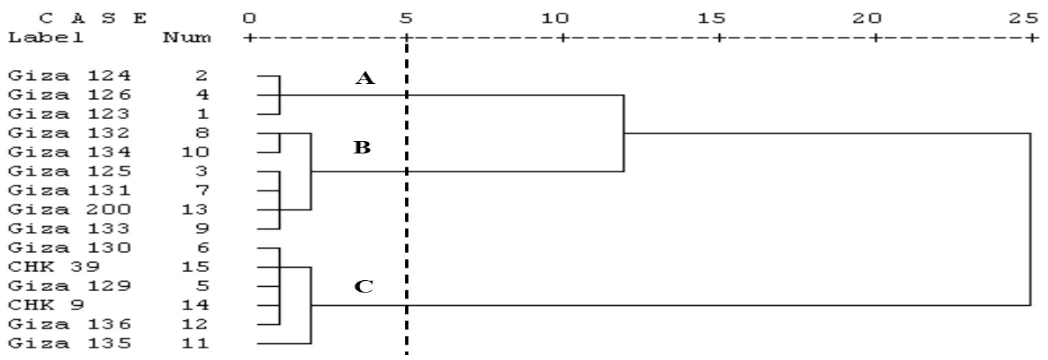

Fig. 4: Dendrogram using average linkage between groups showing classification of 15 barley genotypes based on grain yield and heat tolerance indices, with cutting distance five.

Correlation between investigated traits and heat tolerance indices

Correlation coefficients were calculated between tolerance indices and evaluated traits under heat-stressed and non-stress conditions (Table 6). It was noticed significant positive correlations between all estimated indices and all studied traits under both conditions except number of spikes per square meter (it presented positive but not significant correlation). Which indicated that these indices are suitable for discriminating the best genotypes under both conditions and identifying heat tolerant ones. Similar results were found in Zarei *et al.* (2007); Gholipouri *et al.* (2009); Sharafi *et al.*, (2011); Khokhar and da Silva, (2012); Zare, (2012) and Mehri (2015).

Table 6: Correlation coefficients between studied traits under stressed and non-stressed conditions and heat tolerance indices

Trait	Environment	MP	GMP	STI	YI
NS/m²	Heat-stressed	0.42 NS	0.42 NS	0.40 NS	0.38 NS
NS/m²	Non-stressed	0.35 NS	0.35 NS	0.34 NS	0.31 NS
GNS	Heat-stressed	0.72 **	0.72 **	0.73 **	0.73 **
GNS	Non-stressed	0.82 **	0.82 **	0.83 **	0.83 **
TGW	Heat-stressed	0.54 *	0.54 *	0.54 *	0.56 *
TGW	Non-stressed	0.78 **	0.78 **	0.78 **	0.74 **
SGW	Heat-stressed	0.77 **	0.77 **	0.78 **	0.79 **
SGW	Non-stressed	0.92 **	0.92 **	0.93 **	0.91 **
GY	Heat-stressed	0.99 **	0.99 **	0.98 **	1.00 **
GY	Non-stressed	0.99 **	0.98 **	0.99 **	0.94 **
AGDM	Heat-stressed	0.59 *	0.59 *	0.60 *	0.54 *
AGDM	Non-stressed	0.78 **	0.78 **	0.79 **	0.75 **

NS/m² is number of spikes per m², GNS is grain number per spike, TGW is thousand-grain weight, SGW is spike grain weight, GY is grain yield and AGDM is aboveground dry matter

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