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EVALUATION OF GRAIN YIELD IN FIFTY-EIGHT SPRING BREAD WHEAT GENOTYPES GROWN UNDER HEAT STRESS

KHAIR MOHAMMAD YOULDASH[1], CELALEDDIN BARUTCULAR[1*], AYMAN EL SABAGH[1,2*], IREM TOPTAS[1], GOKHAN TAMER KAYAALP[3], AKBAR HOSSAIN[4], HESHAM ALHARBY[5], ATIF BAMAGOOS[5], HIROFUMI SANEOKA[6] AND MUHAMMAD FAROOQ[7,8,9]

[1]*Department of Field Crops, Faculty of Agriculture, University of Çukurova, Adana, Turkey;*
[2]*Department of Agronomy, Faculty of Agriculture, University of Kafrelsheikh, Egypt.*
[3]*Department of Animal Science, Faculty of Agriculture, University of Cukurova, 01330 Adana, Turkey;*
[4]*Bangladesh Wheat and Maize Research Institute, Nashipur, Dinajpur-5200, Bangladesh;*
[5]*Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21577, Saudi Arabia*
[6]*Pant Nutritional Physiology, Graduate School of Biosphere Science, Hiroshima University, Japan*
[7]*Department of Crop Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Al-Khoud-123, Oman;*
[8]*Department of Agronomy, University of Agriculture, Faisalabad-38040, Pakistan;*
[9]*The UWA Institute of Agriculture, The University of Western Australia, Perth WA 6001, Australia.*
[*]*Corresponding authors' email: cebar@cu.edu.tr; ayman.elsabagh@agr.kfs.edu.eg*

Abstract

In Mediterranean region, terminal heat stress in late sown wheat is one of the major constraints to harvest good wheat yield. Therefore, this is desired to identify and develop wheat varieties that can grow under heat stress conditions of Mediterranean environments. In this study, fifty-eight wheat genotypes of diverse origin were planted at optimal (optimal conditions; October 1, 2015) and late sowing (high temperature; March 1, 2016) times at the Agricultural Research Area, Cukurova University, Adana, Turkey. Wheat sown at optimal time had better growth and yield-related traits, which lead to better grain yield. Considering the correlation between various yield-related traits. When planted at optimum time, the highest grain yield and related traits were recorded from the genotype 'Misr-2' that was followed by genotypes 'Shandaweel-1' 'Misr-1', 'Sakha-94' and 'Giza-171', while the genotype 'Shirogane kamugi' produced the lowest grain yield. In late sown (heat stress conditions) crop, grain yield of all tested genotypes was significantly decreased. In this regard, the genotypes 'Norin' and 'Eagle rock' were more sensitive to high temperature, while genotypes 'Misr-1' and 'Misr-2' were tolerant to heat stress and produced the highest yield under late sown conditions. Based on GGE biplot analysis, genotype 'Misr-2' performed better both under optimal and late sown conditions, this was followed by genotypes 'Misr-1' and 'Shandaweel-1'. Biplot analysis indicated that the genotype Misr-2' is highly adaptive to late sown (heat stress) condition, this was followed by the genotypes 'Misr-1' and 'Shandaweel-1'. These genotypes could be used in future breeding programmes aimed to develop heat tolerant wheat genotypes and more appropriate for cultivation under a changing climate. In conclusion, correlation, stepwise regression and biplot analysis could be successfully used for the identification of heat adaptive wheat genotypes. The genotypes 'Misr-2', 'Misr-1' and 'Shandaweel-1' may be recommended for planting under hot environmental conditions and may be included in breeding program aimed to develop heat tolerant varieties for the Mediterranean region.

Key words: Wheat, Heat stress, Mediterranean environment, Grain yield, GGE biplot analysis.

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the leading cereal crops and staple for the masses across the globe (Liu *et al.*, 2017). Durum wheat (*Triticum turgidum* L. cv. durum) occupies the central position in rainfed regions of the Mediterranean environments. It is considered as a strategic crop and has a vital role in the economy and livelihood of third world countries (Abdipur *et al.,* 2013; Abdelaal *et al*., 2018). However, the wheat growth and yield formation are influenced by environmental conditions. In this regard, high temperature stress is the most important factor and strongly affects the crop growth and development, resulting in substantial reduction in the crop yield (Hays *et al.*, 2007; Farooq *et al*., 2011; Moshatati *et al.,* 2012; Ahmed & Hassan, 2015; Barutcular *et al.*,2016; Yildirim *et al*., 2018).

Plant exposure to heat stress significantly effects the growth and yield development, however, the extent of effect depends upon the stage of crop, and the intensity and duration of the stress (Prasad *et al*., 2008; Farooq *et al*., 2011). In sub-tropics, late planted wheat is often exposed to high temperature during reproductive and grain development stages (Gourdji *et al.,* 2013; Hussain *et al.,* 2016). Heat stress is thus one of the major constraints effecting the wheat yield formation in arid and semi-arid sub-tropics (Kosina *et al*., 2007). High temperatures during grain filling stage not only suppresses the grain yield but also deteriorate the grain quality (Kosina *et al.*, 2007; Joshi *et al*., 2007; Farooq *et al*., 2011; Sareen *et al.,* 2012). This demands thorough understanding of the influences of heat stress on yield formation in wheat so that management strategies to cope with the issue may be developed.

This situation demands identification and development of high yielding wheat varieties adaptive less than optimum conditions (Al-Karaki, 2012). Development and/or identification of early maturity genotypes would be effective to avoid the terminal heat stress (Sayre *et al.,* 1995; Farooq *et al.,* 2011). Researchers are engaged in developing strategies to cope with heat stress including suite of management and husbandry practices, and development of heat-tolerant genotypes (Farooq *et al.,* 2011; Hossain & Teixeira da Silva, 2012; Hossain *et al.,* 2012). However, development of heat tolerant varieties is time demanding and long-way option. Therefore, within a breeding program, consecutively heat-tolerant traits are required to find out the appropriate genotypes tolerant to heat stress for sustainable wheat production (Khan & Kabir, 2014). In this regard, GGE biplot analysis can be used to classify high-yielding varieties for heat stress environments (Koutis *et al.*, 2012). The biplot analysis is a suitable method to analyse the interaction of grain yield of a group of genotypes grown under various environments (Frutos *et al.*, 2013).

Integrated use of better crop management practices, like adjusting planting time, heat tolerant genotypes can help harvest fair crop yield even at high temperature (Hossain *et al.,* 2013). This study was, therefore, conducted to evaluate the influence of sowing time, under variable climatic conditions, on the performance of wheat genotypes, optimizing the planting dates for tested wheat genotypes and identification of heat tolerant genotypes adapted to late sown conditions.

Materials and Methods

Experimental location: The field experiment was carried out during 2015-16 at the Experimental Station, University of Cukurova, Balcali, Adana, Turkey. During the wheat growing season, data on maximum, minimum and mean temperature (°C) were recorded and have been given as Fig. 1.

Plant materials and field layout: Fifty-eight spring bread wheat (*Triticum aestivum* L.) genotypes were used as experimental material. Among these, eight were collected from Australia, nine from Egypt, two from International Center for Agricultural Research in the Dry Areas, three from Japan, one from USA, five from Pakistan and thirty were collected from Turkey (Table 1). These fifty-eight wheat genotypes were planted at optimal (October 1, 2015; cool environment; CE) and late (March 1, 2016; warmer environment; WE) sowing times. Wheat genotypes planted at optimal and late sowing times were exposed to optimum and high temperatures conditions, respectively. Sowing dates were arranged in main plots, and sub plots were allocated with different genotypes.

Experimental procedure: Wheat was planted using seed rate of 500 seeds m^{-2}. Plots contained 6.0 m long 8 rows with a row spacing of 0.15 m. Before sowing, phosphorus (60 kg ha^{-1} P_2O_5) was applied as triple super phosphate. Nitrogen was added, as urea, in three split doses (40 + 80 + 40 kg N ha−1) at Zadok's (Zadoks *et al.,* 1974) growth phases (ZGS) 00, 20 and 30.

Data collection: Data on plant biomass (g m^{-2}), plants m^{-2}, spikes m^{-2}, harvest index (%), grain weight (mg), grains m^{-2} (no), grains per spikes, spikelets per spikes, ear length (cm), peduncle length (cm), plant height (cm), flag leaf area (cm^2), flag leaf wide (cm) were collected. For grains per spikes, spikelets per spike, ear length (cm), peduncle length (cm), and plant height (cm), 20 spikes were collected randomly from each plot for each plot. Harvested grain samples were cleaned to record grain weight (mg), grains m^{-2} (no), grains per spike, single grain weight (mg; GW) and grain yield (GY; t ha^{-1}). The biological yield (BY) was estimated as the total ground dry mater of each plot and converted to t ha^{-1}. Harvest index (HI) was determined as a ratio of grain yield to biological yield and was expressed in percentage.

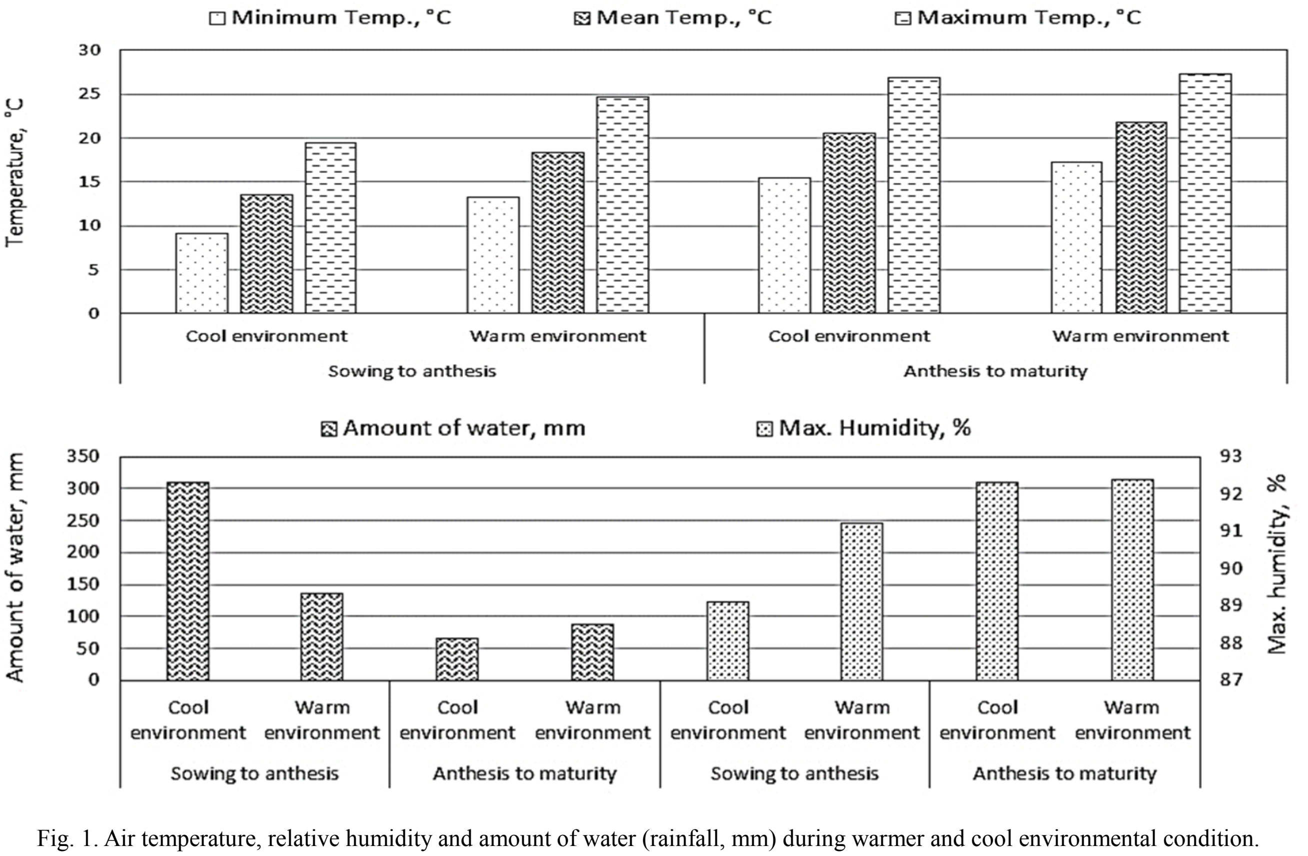

Fig. 1. Air temperature, relative humidity and amount of water (rainfall, mm) during warmer and cool environmental condition.

GGE biplot analysis, calculation of heat resistance index and linear regression analysis: Principal components analysis (GGE biplot) is effective tool for data analysis, with visual displays, from trials conducted in multiple environments, as well as useful for plant breeders and geneticists to study complex genotype and environment interactions (Frutos *et al.,* 2013). Specifically, in the GGE biplot analysis, two-way data from multi-location trials are displayed visually as the G main effect and the GE interaction effect (Gabriel, 1971).

Stress susceptibility Index: Heat stress susceptibility index (HSSI) is determined the stress tolerance in terms of minimize the decrease production that caused by unfavourable and favourable conditions, and was described acceding to (Asana and Williams, 1965; Fisher & Maurer, 1978):

$$HSSI = (1 - Ys / Yp)/(1 - \bar{Ys}/\bar{Yp})$$

where, Ys is the yield of genotypes under warm, Yp is the yield of genotypes under cool environment (CE), $\bar{Ys}$ is the mean yield of all genotypes under heat stress and $\bar{Yp}$ is their mean yield under CE, and $1 - (\bar{Ys}/\bar{Yp})$ is the stress intensity. The CE treatment was maintained under non-stress condition to have a better estimate of optimum environment.

Genotypes tolerant to stress are negatively correlated with HSSI, i.e., genotypes with a low HSSI are more stable in variable stress conditions. The genotype is considered to be highly stress tolerant (HSSI < 0.5), moderately tolerant (0.5> HSSI < 1.0), and intolerant (HSSI > 1.0).

Multiple linear regression and stepwise multiple linear regression: Multiple linear regression and coefficient of determination (R^2) were assessed for each yield attributes (Snedecor & Cochran, 1980). While, stepwise multiple linear regression was done following Draper & Smith (1966).

Data analysis: The experimental data were analysed statistically using Statistical Package for Social Sciences (SPSS) software (version 16, SPSS, Inc, Chicago, IL, USA). Analysis of variance and multiple mean comparisons were done by Student–Newman–Keuls test. Genotypic and traits relations were done by biplot analysis using Statistica program (StatSoft, 2005).

Results and Discussion

In all tested wheat genotypes, grain yield and related traits were significantly influenced by delay in sowing (heat stress) (Table 2). Delay in sowing caused significant reduction in grain yield and related traits (Fig. 2; Table 3). In this regard, under optimal sowing, the highest grain yield and related traits were noted in the genotype 'Misr-2' that was followed by genotypes 'Shandaweel-1', 'Misr-1', 'Sakha-94' and 'Giza-171' while the genotype 'Shirogane kamugi' produced the lowest grain yield. Under late sown conditions (warm environment), the highest grain yield was recorded in genotypes 'Misr-1' and 'Misr-2', while the genotypes 'Norin' and 'Eagle rock' had the lowest grain yield (Fig. 2).

In late sown wheat, the reduction in grain weight, as was observed in this study, may be attributed to the reduction in the period of grain filling due to high temperature stress (Wardlaw, 2002; Farooq *et al*., 2011). Heat stress may also cause early leaf senescence, disturb the rate of photosynthesis and may decrease the rate of assimilate translocation to the developing grains (Farooq *et al*., 2011; Pimentel *et al.,* 2015; Barutcular *et al.,*2017) resulting in reduction in seed size. Heat stress may also cause decrease in pollen viability and poor growth of pollen tube after pollination, whic may cause reduction in number of fertilized flowers (Rehman *et al.,* 2009) resulting in reduction in number of grains and grain yield. Although, the sensitivity of genotypes to heat stress varies with the crop growth stage, wheat growth is suppressed if the crop is exposed to hear stress at vegetative or reproductive stages (Tewolde *et al.,* 2006; Al-Karaki, 2012; Hossain *et al.,* 2018), however, wheat is more sensitive to heat stress at reproductive and grain filling phase (Farooq *et al*., 2011). This is why early maturing genotypes can produce better grain yield under heat stress conditions (Farooq *et al*., 2011; Okechukwu *et al.,* 2016).

The relationship between agronomical parameters and yield under heat stress are given in (Table 3). Most of the traits had significant influence on grain yield. Biomass and grain yield components were positively and more significantly related to grain yield but, spike number per area was negatively and significantly related to grain yield. The relationships between these agronomical traits and grain yield were positive and significant. Similarly, the relationship between peduncle length and kernel weight was significant and strong. A significant positive relationship was also observed between number of kernels per spike and both peduncle length (Table 3). Under the warm environment, spikelet number per spike and spike length were more significant than cool environment. Furthermore, flag leaf traits had no significant difference under the warm environment. In earlier studies, grain filling rate was significantly related with the grain weight (Bruckner & Frohberg, 1987; Nass & Reiser, 1975). However, no correlation was noted between seed weight and heat tolerance (Ismail *et al.,* 2000).

The relationship between agronomical parameters and grain yield under heat stress are given in (Table 4). As expected yield contributing traits, like grain number per spike and grain weight, had strong positive relationship with the grain yield under cool environment (Table 4). While under late sown conditions (warm environment), grain number (per spike), grain weight (mg) and flag leaf area (cm^2) had strong correlation with grain yield (Table 4). Under the cool environment, grain number was the most important trait, while grain weight and heat resistance index were second and third to that at the stepwise regression model. However, under warm environment, grain number per spike (+) was the most important, and grain number (+), grain weight (+) and flag leaf area (-) were followed by that in order at the stepwise regression model (Table 5). The attributes exhibiting direct significant relationship with grain yield could be considered as significant traits combinations to improve yield in wheat through the identifcation under conditions area (Table 5). Yield contributing traits such as grain per spike has influence on the grain yield of wheat whereas rest of the attributes had undesirable impact on yield production (Kumar *et al*., 2017).

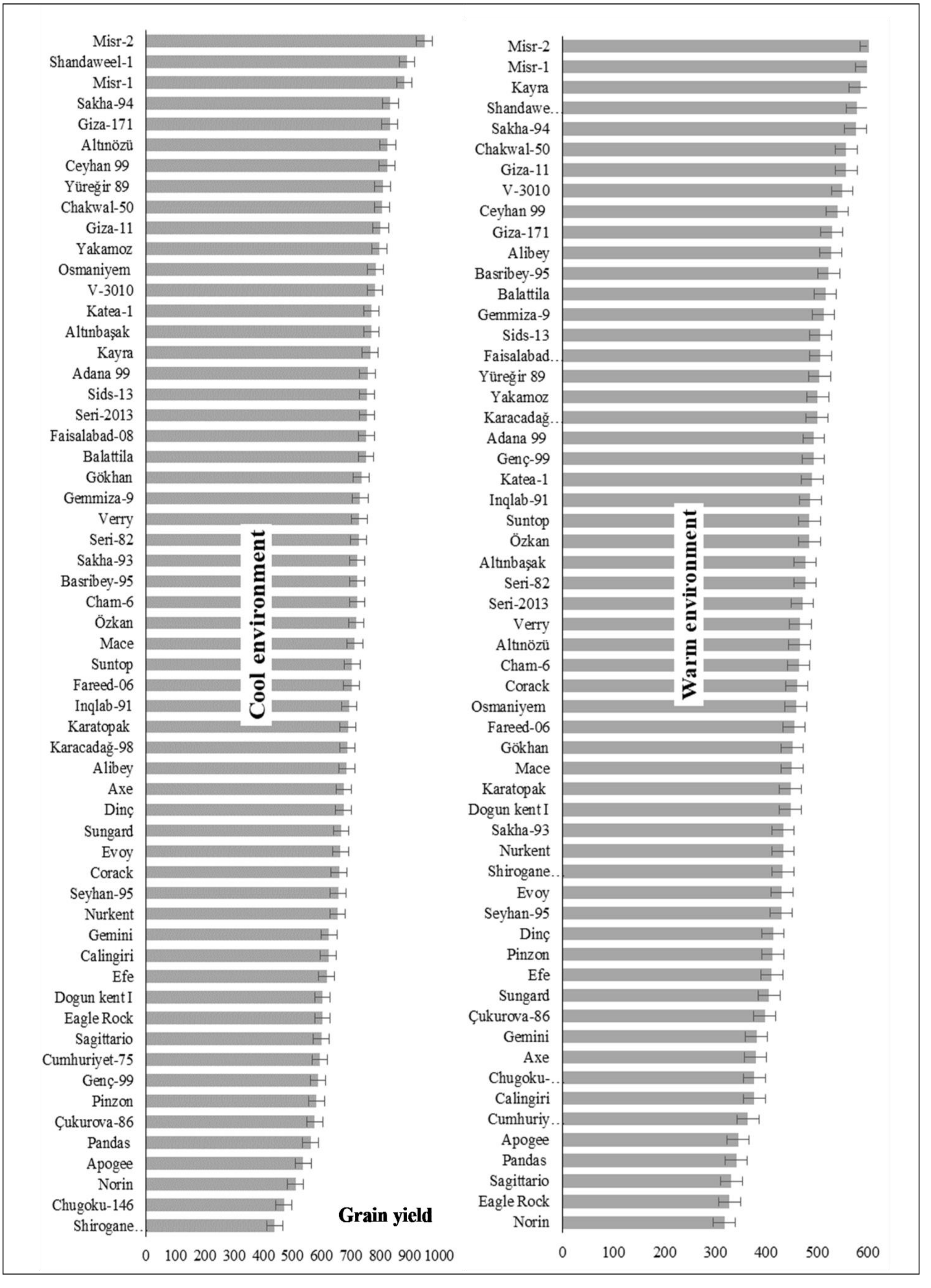

Fig. 2. Grain yield of wheat genotypes grown under optimal (optimum sowing) and late sown conditions (elevated temperature). Bars under optimal and late sown conditions varied significantly at $P = 0.05$ by the Student–Newman–Keuls test. Whereas similar bars indicated that no significant different between the genotypes in both the sowing conditions.

Table 1. Origin of bread wheat genotypes used in the present research.

No.	Cultivars	Origin	No.	Cultivars	Origin
1.	Adana 99	Turkey	30.	Inqlab-91	Pakistan
2.	Alibey	Turkey	31.	Karacadağ-98	Turkey
3.	Altınbaşak	Turkey	32.	Karatopak	Turkey
4.	Altınözü	Turkey	33.	Katea-1	Bulgaria
5.	Apogee	USA	34.	Kayra	Turkey
6.	Axe	Australia	35.	Mace	Australia
7.	Balattila	Turkey	36.	Misr-1	Egypt
8.	Basribey-95	Turkey	37.	Misr-2	Egypt
9.	Calingiri	Australia	38.	Norin	Japan
10.	Ceyhan 99	Turkey	39.	Nurkent	Turkey
11.	Chakwal-50	Pakistan	40.	Osmaniyem	Turkey
12.	Cham-6	ICARDA	41.	Özkan	Turkey
13.	Chugoku-146	Japan	42.	Pandas	Italy
14.	Corack	Australia	43.	Pinzon	Spain
15.	Cumhuriyet-75	Turkey	44.	Sagittario	Turkey
16.	Çukurova-86	Turkey	45.	Sakha-93	Egypt
17.	Dinç	Turkey	46.	Sakha-94	Egypt
18.	Dogankent-I	Turkey	47.	Seri-2013	Turkey
19.	Eagle Rock	Australia	48.	Seri-82	Turkey
20.	Efe	Turkey	49.	Seyhan-95	Turkey
21.	Evoy	Australia	50.	Shandaweel-1	Egypt
22.	Faisalabad-08	Pakistan	51.	Shirogane Kamugi	Japan
23.	Fareed-06	Pakistan	52.	Sids-13	Egypt
24.	Gemini	Italy	53.	Sungard	Australia
25.	Gemmiza-9	Egypt	54.	Suntop	Australia
26.	Genç-99	Turkey	55.	V-3010	Pakistan
27.	Giza-11	Egypt	56.	Verry	ICARDA
28.	Giza-171	Egypt	57.	Yakamoz	Turkey
29.	Gökhan	Turkey	58.	Yüreğir-89	Turkey

Table 2. Analysis of variance (ANOVA) of 58 bread wheat cultivars (C) under the cool and warm environments (E).

Parameters	CV %	Environment (E)	Cultivars (C)	E × C
Biomass (g m^{-2})	9.7	*	***	***
Grain yield (g m^{-2})	7.2	***	***	***
Harvest index (%)	9.9	**	***	***
Grain weight (mg)	5.9	**	***	**
Grains (m^{-2})	8.8	***	***	***
Grains (per spike)	12.1	***	***	***
Spikelets (per spike)	7.4	**	***	*
Plants (m^{-2})	10.6	*	***	**
Spikes (m^{-2})	12.2	**	***	***
Spike length (cm)	8.5	ns	***	ns
Peduncle length (cm)	9.1	*	***	ns
Plant height (cm)	5.1	ns	***	**
Flag leaf area (cm^2)	11.5	**	***	*
Flag leaf wide (cm)	6.9	**	***	**

*: $p<0.05$; ** : $p<0.01$; *** : $p<0.0001$

Table 3. Mean performance of agronomic traits of fifty eight bread wheat cultivars under the cool and warm environments.

Parameters	Cool environment	Warm environment	Heat effect (%)	*P* levels
Biomass (g m^{-2})	1891.0	1732.0	-8.4	*
Grain yield (g m^{-2})	704.0	465.0	-33.9	***
Harvest index (%)	37.5	27.0	-28.0	**
Grain weight (mg)	36.5	35.0	-4.3	**
Grains (m^{-2})	19372.0	13388.0	-30.9	***
Grains (per spike)	29.5	22.0	-25.3	***
Spikelets (per spike)	19.9	17.2	-13.1	**
Plants (m^{-2})	404.0	388.0	-4.0	*
Spikes (m^{-2})	676.0	622.0	-8.0	**
Spike length (cm)	9.30	9.69	4.2	ns
Peduncle length (cm)	37.3	32.3	-13.5	*
Plant height (cm)	90.9	83.6	-8.0	ns
Flag leaf area (cm^2)	37.7	29.6	-21.6	**
Flag leaf wide (cm)	1.77	1.64	-7.3	**

Table 4. Regression of mean grain yield with the corresponding mean agronomic traits of bread wheat cultivars as the independent variables under cool and warm environments.

Traits	Cool environment			Warm environment		
	Slope	Intercept	R^2	Slope	Intercept	R^2
Biomas, g m^{-2}	0.2752 ± 0.046	183.34 ± 86.73	0.395***	0.2173 ± 0.035	88.995 ± 61.60	0.403***
Harvest index, %	14.674 ± 2.570	152.88 ± 97.36	0.367***	13.473 ± 2.222	101.29 ± 60.49	0.396***
Grain weight, mg	12.405 ± 3.340	250.51 ± 122.95	0.197***	7.1214 ± 2.831	216.42 ± 99.37	0.101*
Grains, m^2	0.0289 ± 0.003	143.78 ± 65.98	0.567***	0.028 ± 0.003	90.073 ± 36.69	0.656***
Grains (per spike)	12.974 ± 1.801	320.72 ± 54.06	0.481***	13.981 ± 1.194	157.19 ± 26.80	0.710***
Spikelets (per spike)	9.3623 ± 14.570	517.90 ± 289.55	0.0073	16.374 ± 7.265	183.05 ± 125.58	0.083*
Plants, m^2	0.7352 ± 0.285	406.39 ± 115.88	0.106*	0.669 ± 0.230	205.48 ± 89.95	0.131**
Spikes, m^2	-0.2248 ± 0.124	855.66 ± 84.58	0.0558	-0.3319 ± 0.105	671.66 ± 65.64	0.152**
Spike lenght, cm	26.327 ± 14.129	458.94 ± 132.04	0.0584	22.439 ± 9.158	247.88 ± 89.20	0.097*
Peduncle lenght, cm	10.112 ± 3.039	326.48 ± 114.05	0.165**	7.2752 ± 1.945	230.62 ± 63.30	0.200***
Plant height, cm	4.4834 ± 1.390	296.28 ± 126.96	0.157**	3.7698 ± 0.875	150.22 ± 73.62	0.249***
Flag leaf area, cm^2	5.0579 ± 2.007	512.91 ± 76.83	0.102*	0.7371 ± 1.612	443.57 ± 48.57	0.0037
Flag leaf wide, cm	137.330 ± 76.060	461.02 ± 135.09	0.055	12.452 ± 58.060	444.95 ± 95.63	0.0008
Stress susceptibility index	137.44 ± 62.804	567.45 ± 63.64	0.079*	-104.174 ± 42.873	568.67 ± 43.44	0.095*

*. ** and *** Denotes significance at $p<0.05$, $p<0.01$ and $p<0.001$, respectively

Table 5. Entered variables of stepwise regression between grain yield and traits as the independent variables under cool and warm environments.

Traits	Slope	Intercept	R^2
Cool environment			
Grain number, m^{-2}	0.036 ± 0.000	-143.785 ± 65.985	0.567***
Grain weight, mg	19.007 ± 0.343	-668.879 ± 17.255	0.992***
Stress susceptibility index	-12.499 ± 5.927	-667.902 ± 16.745	0.993***
Warm environment			
Grain number, per spike	13.981 ± 1.194	157.189 ± 26.803	0.710***
Grain number, m^{-2}	0.014 ± 0.004	87.555 ± 30.302	0.770***
Grain weight, mg	13.902 ± 0.375	-479.175 ± 16.408	0.991***
Flag leaf area, cm^2	-0.698 ± 0.185	-459.175 ± 15.585	0.993***

*. ** and *** Denotes significance at p<0.05, p<0.01 and p<0.001, respectively

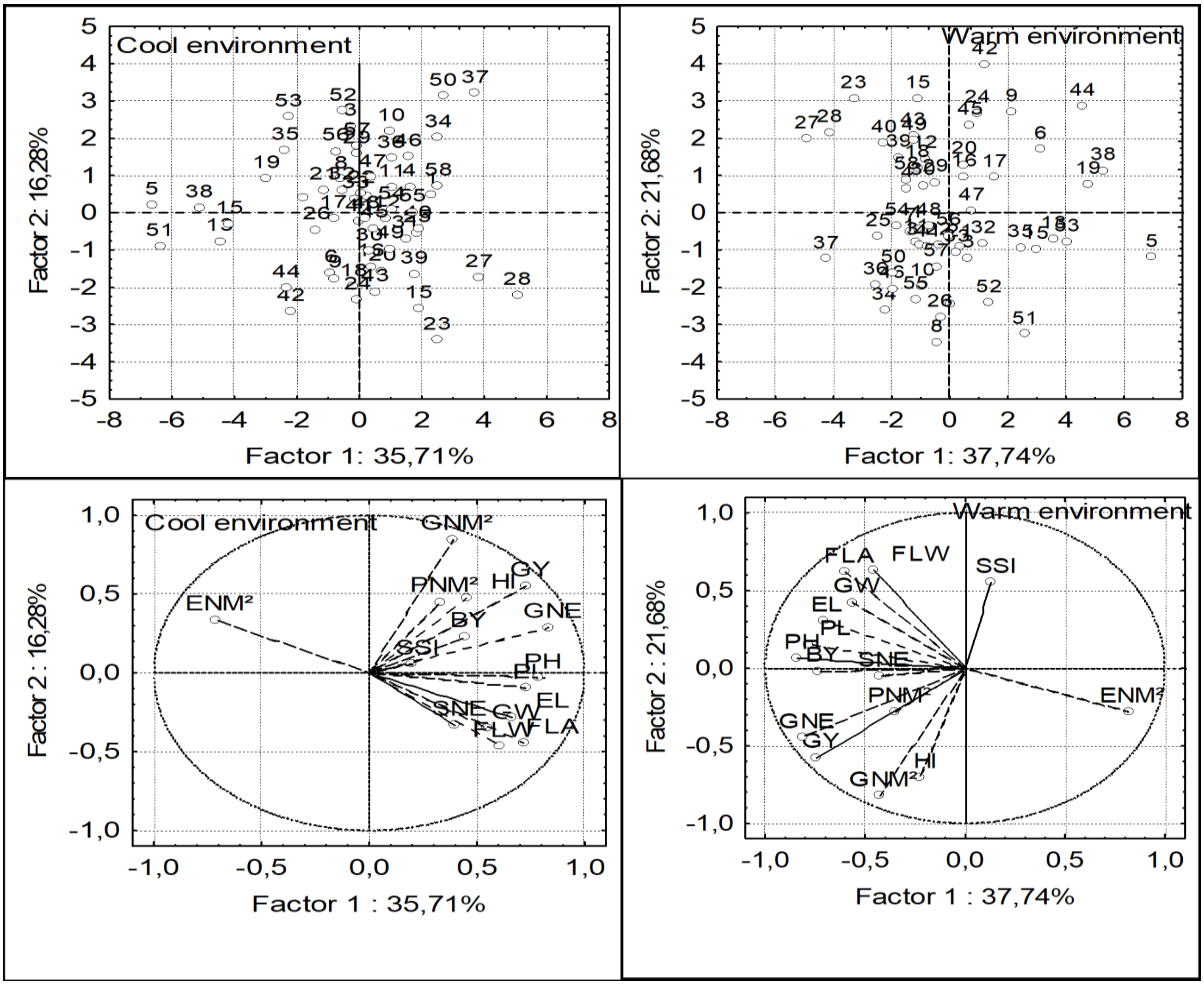

Fig. 3. The first two principal components among wheat genotypes (1, 2, 3 …58) and agronomic traits. BY, biologic yield; GY, grain yield; HI, harvest index; GW, grain weight; GNM², grains m^{-2}; GNE, grains per spike; SEN, spikelets per spike; PNM², plants m^{-2}; ENM², spikes m^{-2}; EL, spike length; PL, peduncle length; PH, plant height; FLA, Flag leaf area; FLW, flag leaf wide; SSI, stress susceptibility index.

The biplot analysis is a suitable method to analysis the interaction between wheat yield properties and wheat genotypes growing under different environments. In this research, yield traits of genotypes were explained by the Principal Component Analysis (PCA) to construct the zone with regard to yield trait expression. Hence, explanation was varied to environment (Fig. 3).

The tested genotypes might be arranged into some groups based on their performances under stress and non-stress conditions. Principal Component Analysis PC1 and PC2 explained 51.99% and 59.42% of total variance for cool and warm conditions respectively. The genotypes ‘Misr-2’ (37) produced higher yield under cool environment than other genotypes, as well as, it is achieved high value in grain yield by the positive effects of the grain number per area (GNM²). While under warm environment genotypes ‘Misr-2’ was in the positive sense of the grain yield vectors indicating that it performed well owing to positive effects of the grain number per spike (Fig. 3).

Genotypes tolerant to stress are negatively associated with HSS, i.e., genotypes with a low HSSI are more stable in variable stress conditions. The genotype is considered to be highly stress tolerant (HSSI < 0.5), moderately tolerant (0.5> HSSI < 1.0), and intolerant (HSSI > 1.0). Genotypic response to heat stress varied from 0.05 to 1.34 (Fig. 4). High temperature stress reduced the grain in genotypes ‘Altınözü’, ‘Axe’, ‘Eagle Rock’, ‘Osmaniyem’ and ‘Sagittatio’. While, genotypes ‘Alibey’, ‘Genç99’ and ‘Kayra’ were resistant to heat stress. Similarly, genotypes ‘Shirogane Kamugi’ and ‘Chukogu-146’ were more resistant to heat stress due to early maturity (Fig. 4). A higher HSSI value indicates relatively greater sensitivity to a given stress condition. Thus for wheat grown under stress condition, a lower HSSI is desired. Selection based on HSSI guides for the selection of genotypes with low grain yield (GY) under non-stress and high GY under stress conditions (Anwar *et al.,* 2011; Ali & El-Sadek, 2016). Similar to stress susceptible index (SSI), genotypes with low tolerance index (TOL) are relatively more stable under stress-prone environments (Hossain & Teixeira da Silva, 2012; Hossain *et al.,* 2012). Wheat genotypes with higher SSI and TOL values were sensitive to stress and with lower value of SSI and TOL were tolerant to stress (Ankit *et al*., 2013; Singh *et al.,* 2015).

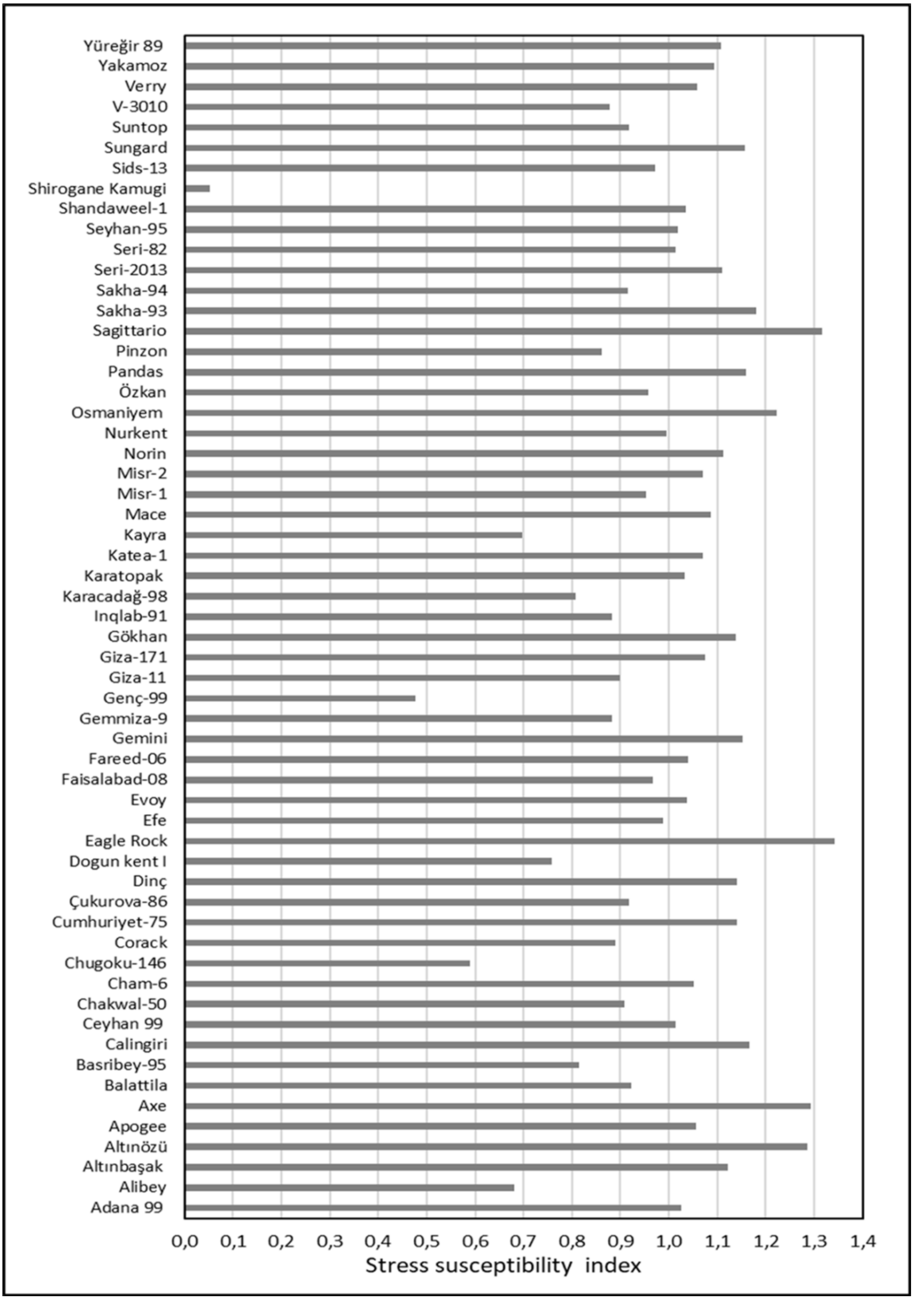

Fig. 4. Heat susceptibility index of fifty-eight wheat genotypes grown under warm environment (heat stress).

Conclusion

Wheat sown at optimum (cool environment) time produced better grain yield, in all tested genotypes, than the late sown crop. Under cool environment (timely sowing), the genotype 'Misr-2' produced the highest grain yield that was followed by genotypes 'shandaweel-1' 'Misr-1', 'Sakha-94' and 'Giza-171'. In case of late sown crop (heat stress condition), genotypes, 'Misr-1' and 'Misr-2' produced the highest grain yield. Based on GGE biplot analysis, genotypes 'Misr-2', 'Misr-1' and 'Shandaweel-1' performed better under both cool and warm conditions. Therefore, genotypes, 'Misr-2', 'Misr-1' and 'Shandaweel-1' may be recommended planting under late sown conditions, and may be included for future breeding program to develop heat tolerant varieties for the Mediterranean region.

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