



## **FIELD EVALUATION AND MOLECULAR ANALYSIS OF SOME BREAD WHEAT GAMETOCLONES AND SOMACLONES UNDER NATURAL HEAT STRESS**

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Received: 14 August (2016)

Accepted: 4 October (2016)

### **Abstract**

Extra new wheat cultivars will be required to face the challenges which caused by climate change and unusual increasing of temperature in Egypt. Therefore, in this study, fourteen wheat (*Triticum aestivum* L.) gametoclone and somaclones in addition to one check variety (Giza 168) were evaluated for their ability to heat stress tolerance and genetic variability, via field performance and inter simple sequence repeat (ISSR) molecular marker analysis. Five agronomic characters were evaluated for heat tolerance under normal and late sowing (stress) conditions. The results showed high significant differences among genotypes in both normal and heat stress conditions. High temperature decreased most of traits in most of the genotypes. Based on heat tolerance index, the genotypes were distributed into, high, moderate and low tolerant genotypes. The nine primers of ISSR markers amplified a total 71 bands out of them 33 were polymorphic (with 43.9% of polymorphism). The similarity values ranged from 0.77 to 1.00. The dendrogram separated the genotypes in two main clusters, high and moderate heat tolerant genotypes were distributed among the clusters, while the low heat tolerant genotypes restricted in one group. Superiority of some genotypes under heat stress conditions suggests that gametoclonal and

somaclonal variation could be beneficial tool for genetic improvement of heat stress tolerance in wheat.

**Key words:** gametoclonal variation, somaclonal variation, genetic improvement, ISSR, field trials, dendrogram, polymorphism.

### Introduction

Wheat (*Triticum aestivum* L.) is one of the most imperative and extensively cultivated crops in the world. It is playing a dominant source of energy and proteins for the world populations. In Egypt, wheat is the most important cereal crops which cover more than 2.387 million feddan (~ 1 million hectare) with an annual production of 8.4 and 8.79 million tons (FAO, 2011 and 2012, respectively), that supply only 45-50 % of the fast growing population demand. Thus wheat occupies a unique position in the Egyptian agricultural economy, there is essential needed to improve wheat productivity to meet our food requirements. Using classical breeding methods hampered by several limitations, therefore, new biotechnology ways open new facilities to overcome these limitations (Ahmed 1992).

Besides biotic stress, temperature is one of plentiful abiotic stress factors which endanger the worldwide wheat productivity (Keresza *et al.*, 2000). Increment of weather temperature is very noted during the last few years especially in Egypt. Therefore, genetic improvement approach is necessary to generate new tolerant genotypes to overcome the harmful effects which results from different stress. Plant tissue culture is one of the most useful tools for induction of genetic variability during plant differentiation

and regeneration which have been termed gametoclonal and somaclonal variations (Larkin and Scowcroft 1981; Filipecki and Malepszy 2006). These variations can be used in scientific breeding programs for development of new crop varieties, having desirable traits (Quraishi *et al.*, 2000; Bairu *et al.*, 2011). Breeding through tissue culture in wheat produced large numbers of superior varieties having higher production, disease resistance and tolerant to harmful climatic conditions (Bajji *et al.*, 2004; Yadav *et al.*, 2004; Gawande *et al.*, 2005; Svabova and Lebeda 2005). Morphological characterization is not enough to study the diversity among gametoclones and somaclones because these characters are under control of environmental changes. Therefore, several PCR based molecular markers have been used to determine of genetic diversity e.g., Simple Sequence Repeats (SSR; Akkaya *et al.*, 1992), Inter Simple Sequence Repeats (ISSR; Zietkiewicz *et al.*, 1994), Amplified Fragment Length Polymorphism (AFLP; Vos *et al.*, 1995) and Randomly Amplified Polymorphic DNA (RAPD; Williams *et al.*, 1990).

In this study, 14 bread wheat gametoclone and somaclones lines as well as check commercial variety were evaluated for their ability to heat stress tolerance and genetic variability via

field evaluation and ISSR molecular marker analysis.

#### MATERIALS AND METHODS

Plant material: Fifteen bread wheat (*Triticum aestivum* L.) genotypes were used in this study (Table 1), one of them Ss5-8 gametoclone line was obtained via regeneration from anther culture of Egyptian Sides 5 commercial hexaploid cultivar (Ali 1998), and 13 somaclones were obtained via regeneration of plants from callus derived from immature inflorescences or immature embryos of 4 commercial hexaploid spring wheats (Ahmed 1992): where 12 somaclones derived

from 3 Egyptian cultivars (Sakha 8, Sakha 69 and Giza 160), and only one somaclone derived from Spanish (Lerma Rojo 64) cultivar (Table 1). The seeds of R4 plants of Ss5-8 gametoclone and R10 plants of somaclones (the 4<sup>th</sup> and 10<sup>th</sup> seleded generation, Chaleff, 1981) were supplied by Prof. Kasem Zaki Ahmed (Department of Genetics, Faculty of Agriculture, Minia University, El-Minia, Egypt). The fifteenth wheat genotype used in this study was the most important commercial cultivar in Egypt "Giza 168" which used as control (check variety) in these experiments (Table 1).

Table (1): Original commercial cultivars and it is derived gametoclone and somaclones of the 4 Egyptian and one Spanish spring bread wheat (*Triticum aestivum* L.) cultivars used in this study.

Original cultivars	Code	Number of evaluated somaclones	Number of evaluated gametoclones	Code of studied somaclones
Sides 5	Ss5	-	1	Ss5-8
Sakha 8	S8	9	-	S8-4, S8-56, S8-7, S8-8, S8-10, S8-14, S8-18, S8-19 and S8-20
Sakha 69	S69	2	-	S69-1, and S69-4
Lerma Rojo 64	LR	1	-	LR-2
Giza 160	G160	1	-	G163-1

Field trials: The field experiment was conducted during two growing seasons (2012/2013 & 2013/2014), at the Experimental Farm of Faculty of Agriculture, South Valley University, Qena, Egypt. The cultivation was carried out in two sowing dates for each season, 26<sup>th</sup> of November as normal condition sowing date and in

26<sup>th</sup> of December which was considered as heat stress condition. Experimental layout was a split plot design with three replications. Each replication consisted of 15 plots. Each plot was 3 m long, 20 cm apart and 15 cm between hills within row. All farming applications were performed as recommended. The salinity (ECe) of

soil and irrigation water was 9.98 and 7.94 ds m<sup>-1</sup>, respectively. Weather data included maximum and minimum temperature and relative humidity

measured in each season as recorded by Meteorological station, Qena, Egypt are shown in Table (2).

Table (2): Weather data at Qena, Egypt, from November to May (2012-2014) during execution of the experiments (Meteorological station, Qena, Egypt).

Months	Average temperature (C°)				Relative humidity			
	2012/2013		2013/2014		2012/2013		2013/2014	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
November	29.6	16.3	28.2	14.1	57.4	23.5	61.6	24.6
December	23.4	9.8	23.4	8.8	74.0	28.5	72.5	29.5
January	22.9	9.5	23.8	8.6	64.5	23.7	68.9	25.3
February	26.5	11	26.1	10.7	53.3	16.0	58.3	17.1
March	32	15.2	29.7	15.1	45.5	11.9	50.5	14.4
April	33.3	17.1	35.9	20.1	36.3	8.2	33.3	7.4
May	39.1	23.8	38.4	23.1	27.3	7.3	28.6	7.8

At harvest time (1<sup>st</sup> and 15<sup>th</sup> of April for normal and late sowing, respectively), for each sowing date, ten random plants were taken from each plot (genotype) to estimate, plant height (cm), spike length (cm), number of spikelets per spike. In addition, all plants of each plot were harvested and grain yield (gm) per plot and 1000-kernel weight was estimated. Data of wheat grain yield were used to estimate heat tolerance measurements as following: the heat tolerance (HT) and the heat susceptibility (HSI) were obtained via using the following formulas (Sharma *et al.*, 2014):

$$TOL = x_p - x_s \text{ and } HSI = [1 - (x_s/x_p)] / [1 - (X_s/X_p)] \text{ respectively,}$$

Where, TOL: tolerance; x<sub>p</sub>: and x<sub>s</sub>: are the trait value of the genotype under non-stress and stress conditions, respectively.

X<sub>s</sub>: is mean values of the trait of all the genotypes under stress conditions but X<sub>p</sub>: under non-stress conditions.

of April for normal and late sowing, Genotypes classified according Relative Performance (P) (Abo-Elwafa and Bakeit 1999).

$$P = (YS/YP)/R, \text{ Where, } R = (\bar{Y}S/\bar{Y}P),$$

YS= Yield potential under stress conditions. YP= Yield potential under normal conditions.

$\bar{Y}S$  and  $\bar{Y}P$ = yield of all genotypes in the stress and normal conditions, respectively.

Statistical analysis: Analysis of variance (ANOVA) according to Gomez and Gomez (1984) for a split plot design was performed. Mean comparisons were performed using Least Significant Differences (L.S.D) and Revised Least Significant Differences (Revised L.S.D.) according to El-Rawi and Khalafalla (1980).

**Molecular characterization:**

DNA extraction: Genomic DNA was extracted from fresh leaves by using a modified version of CTAB

method as done as in Ben El Maati *et al.*, (2004).

PCR amplification and electrophoresis: nine Primers of ISSR markers (UBC 840, UBC 834, UBC 846, UBC 807, UBC 808, UBC 810, UBC 811, UBC 814, UBC 823) were used in this study (EZBiolab-USA). PCR amplification reaction was achieved as described by Ben El Maati *et al.*, (2004). PCR products were separated on 1.5% agarose gels, then visualized by staining with ethidium bromide. The detected bands were scored as 1 (present) and 0 (absent). Genetic similarity was estimated using Nei-Li's similarity index (Nei and Li *et al.*, 1979). A dendrogram was constructed on the basis of the similarity matrix data by unweighted pair group method with arithmetic average (UPGMA), cluster analysis was achieved using the software MEGA program.

## RESULTS AND DISCUSSION

### A. Field traits evaluation:

Combined analysis of variance for plant height, spike length, number of spikelets per spike, 1000-kernel weight and grain yield are presented in Table (3). The recorded variation among R5 & R6 gametoclones, R11 & R12 somaclones and Giza 168 check cultivar revealed that the effect of years or generations were non-significant in three studied traits (plant height, spikelets per spike and 1000-kernel weight), while spike length and grain yield were significant and highly significant, respectively. For sowing date was highly significant effect on

all of studied traits, except spike length displayed non-significant effect. Moreover all genotypes exhibited highly significant effect on all traits, reflecting presence of differences in performance of these 15 genotypes.

The mean performance of the all genotypes over the two seasons in all tested traits (Table 4), for the plant height, in normal sowing date the mean values varied from 69.00 for both S8-18 and S8-20 to 101.35 cm for Giza 168. On other hand, in late sowing date (unfavorable conditions) the mean values were decreased for all genotypes, the range was between 57.15 for S8-20 and 66.27 cm for S8-19. Obviously, the plant high varied significantly among the genotypes under heat stress condition, even lines have been derived from the same parents, suggesting the presence of genetic variation among them. Plant height trait clearly reduced in all genotypes under heat stress condition. Agrawal *et al.*, (2014) and El-Nakhlawy *et al.*, (2015) also reported significant reduction in wheat plant height by delay of planting time.

Concerning spike characteristics, spike length varied among genotypes in normal sowing and ranged from 7.88 (S8-7) to 11.83 cm (S69-1) while was ranged from 9.03 (S8-20) to 11.70 cm (S69-1) for late sowing date. Therefore, S69-1 was the best somaclone in both normal and late sowing dates, with approximately similar values in both of them. In generally, among genotypes the spike length of three genotypes (Giza 186, S8-8 and G160-1) was decreased due

to delayed sowing. While, in six genotypes (S8-4, S8-10, S8-14, S8-20, S69-1 and LR-2) did not affected, but in rest genotypes the values were increased. Number of spikelets per spike in normal sowing date ranged from 16.67 (S8-18, S8-20) to 21.67 (S69-1) and ranged from 14.67 (S8-8) to 21.50 (S69-4) under late sowing date. It's significantly reduced in Giza 168, S8-8, S8-20, S69-1, LR-2 and G160-1 under late sowing date. In contrast, two somaclones (S8-18 and S69-4) were increased. Nevertheless, most of somaclones were not changed. These results are in accordance with these found by Sial *et al.*, (2005) and El-Nakhlawy *et al.*, (2015), who reported, in response to stress condition, spike length and number of spikelet per spike fluctuated among genotypes.

About 1000-grain weight, under normal sowing, its values ranged from 30.8 (LR-2) to 57.3 gram (S8-6), while in late sowing date, most of tested genotypes reduced except S8-10, S8-20 and LR-2 genotypes were not changed. Its values ranged from 17.65 (S69-1) to 39.92 g (S8-10). Grain yield of the genotypes was significantly varied among them in normal sowing date and decreased with delay in sowing date. In normal sowing date, grain yield was ranged between 341.75g (S8-18) to 628.25g (S8-19). Grain yield at late sowing date was ranged from 173.25 to 397.50 for S8-14 and S8-10 somaclone, respectively. According to both traits i.e. 1000-kernel weight and grain yield similar results were obtained by Aslani and

Mehrvar (2012), Dhyani *et al.*, (2013) and El-Nakhlawy *et al.*, (2015). In general, it was similar with our founding wheat yield, its components and grain quality have been found to be affected by terminal heat stress during growth and development (Subhan 2004; Sial *et al.*, 2005 and El-Nakhlawy *et al.*, 2015). Moreover, performance of the genotypes varied according planting date, this would attributed to the genetic background of each genotype, where genotypes with heat tolerant genetic background performed better than susceptible genotypes (Alghabari *et al.*, 2014).

By comparing one gametoclone and 13 somaclones with the check Giza168 variety in normal sowing date, gametoclone and somaclones did not show better performance in plant height, spike length and number of spikelets per spike. While Ss5-8 gametoclone as well as some of somaclones were better than Giza 168 for the other studied traits. On the other hand, under stress sowing date somaclones displayed better performance in all traits comparing with the check Giza168 variety.

#### **B. Heat tolerance evaluation:**

The summary information of heat tolerance measurement was presented in Table (5). All genotypes classified into three groups, high heat tolerance (HHT) group which involved the genotypes displayed lower values for tolerance index (65-100.5), heat sensitivity index (0.389-0.489) and % reduction of yield (19.1-24), while had the higher values in relative performance (1.490-1.586). Moderate

heat tolerance (MHT) group included genotypes exhibited moderate values in all previous parameters. Low heat tolerance (LHT) group included genotypes which had higher values for all of tolerance index (256.25-393.5), heat sensitivity index (1.217-1.285) and % reduction of yield (59.66-62.97), while had lower values in relative performance (0.73-0.79). However, these results showed a match with reduction percentage of grain yield under the comparison of normal and stress condition. In fact, using of stress tolerance index through comparing normal and late sown time was more useful parameter for detection of more heat tolerant genotypes having high yield (Khodarahmpouret *et al.*, 2011).

**C. ISSR marker analysis:**

To study the genetic diversity among all 15 tested genotypes, nine ISSR primers have been used in amplification of various numbers of DNA fragments from genomic DNA (Fig. 1). The average number of

detected bands was 7.8 bands per primer with size ranged from approximately 160 bp to 1030 bp (Table 6). UBC834 and UBC846 primers gave both the maximum number of bands (11 bands) and the highest percentage of polymorphism (72.79 %) for each. While the minimum bands was 4 bands obtained by UBC823 primer. Out of the total 71 bands, 33 were polymorphic, with an average of 3.6 polymorphic bands per primer. The percentage of polymorphism ranged from 20% to 72.79% with an average of 43.9% (Table 6). Actually, as consistent with our founding, in general wheat has low level of polymorphism comparing with other cereals (Chao *et al.*, 1989 and Lui *et al.*, 1990). However, many research groups have been reported different levels of polymorphism across wheat genotypes in several studies (Motawei *et al.*, 2007; Abouzied 2011; Sharma *et al.*, 2014; Khaled *et al.*, 2015 and Kassem 2016).

Table (3): Mean squares for all 5 investigated field traits of 15 bread wheat genotypes in response to heat stress.

S.O.V	df	Plant height	Spike length	Number of Spikelets per spike	1000-kernel weight	Grain yield
Reps	2					
Years (Y)	1	8.45	7.9*	2.69	694.63	4722.49**
Error a	2	6.44	0.13	1.09	68.46	37.29
Sowing date (D)	1	11719.26**	8.49	27.22**	7242.82**	2376127.64**
Y x D	1	31.55	14.17*	17.42*	72.42	110012.95**
Error b	4	28.90	1.12	1.16	39.35	3015.84
Genotypes (G)	14	228.62**	13.34**	20.86**	241.13**	20953.20**
G x Y	14	48.11**	1.51**	3.62**	86.97**	7167.58**
G x D	14	247.85**	3.55**	13.18**	294.26**	24516.73**
G x Y x D	14	57.08**	1.55**	7.95**	70.25**	4528.58**
Error c	112	7.14	0.16	0.87	7.35	1319.99

\* and \*\* indicate significant at 5% and 1% levels of probability, respectively

Table (4): Means performance over two seasons of fifteen bred wheat genotypes (one Ss5-8 gametoclone, 13 somaclones and Giza 168 check variety) for all investigated traits under normal and heat stress conditions.

Character	Plant height (cm.)			Spike length (cm.)			No. of Spikelets/spike			1000-kernel weight (g.)			Grain yield (g.)		
	Normal	Heat stress	Dif.	Normal	Heat stress	Dif.	Normal	Heat stress	Dif.	Normal	Heat stress	Dif.	Normal	Heat stress	Dif.
Giza 168	101.35	59.48	41.87	11.53	10.92	0.61	21.33	15.33	6	37.92	24.68	13.24	452.25	250.75	201.5
Ss5-8	73.60	63.55	10.05	10.50	11.32	-0.82	17.67	17.83	-0.16	54.70	26.87	27.83	411.75	225.50	186.25
S8-4	70.52	62.97	7.55	8.38	9.18	-0.8	17.50	17.33	0.17	39.87	31.58	8.29	502.75	188.25	314.5
S8-6	75.08	63.45	11.63	8.63	9.75	-1.12	17.83	16.83	1	57.30	27.18	30.12	493.75	281.75	212
S8-7	73.67	60.93	12.74	7.88	10.45	-2.57	18.00	18.00	0	40.60	23.23	17.37	460.25	231.00	229.25
S8-8	87.05	62.15	24.9	11.17	9.63	1.54	17.17	14.67	2.5	33.60	28.88	4.72	500.00	194.00	306
S8-10	74.70	61.53	13.17	9.03	9.18	-0.15	17.83	18.00	-0.17	36.67	39.92	-3.25	498.00	397.50	100.5
S8-14	75.40	59.27	16.13	11.68	11.68	0	19.83	19.33	0.5	38.12	22.73	15.39	429.50	173.25	256.25
S8-18	69.00	63.53	5.47	8.45	10.25	-1.8	16.67	19.00	-2.33	51.95	30.87	21.08	341.75	276.50	65.25
S8-19	79.60	66.27	13.33	9.25	10.45	-1.2	17.67	18.67	-1	43.37	26.52	16.85	628.25	234.75	393.5
S8-20	69.00	57.15	11.85	9.08	9.03	0.05	16.67	15.00	1.67	35.08	32.63	2.45	456.25	215.50	240.75
S69-1	78.35	61.52	16.83	11.83	11.70	0.13	21.67	19.17	2.5	36.88	17.65	19.23	566.00	209.58	356.42
S69-4	74.12	60.23	13.89	10.07	11.53	-1.46	19.17	21.50	-2.33	42.27	29.32	12.95	527.25	212.58	314.67
LR-2	88.85	61.72	27.13	11.00	10.68	0.32	18.00	16.83	1.17	30.80	33.17	-2.37	349.25	265.42	83.83
G160-1	76.63	61.18	15.45	11.63	10.88	0.75	19.50	17.33	2.17	36.67	28.25	8.42	464.00	252.83	211.17
Revised L.S.D for genotypes at	0.05	2.73		0.40			0.96			2.78			37.76		
L.S.D for dates at	0.01	3.56		0.52			1.25			3.63			49.30		
L.S.D for dates at	0.05	3.56		0.60			1.07			3.91			45.53		
L.S.D for dates at	0.01	5.16		0.88			1.41			5.63			63.18		

Dif. Indicate difference in trait performance between normal and stress condition



Table (5): Heat tolerance measurements of grain yield for fourteen bread wheat genotypes (one Ss5-8 gametoclone, 13 somaclones) comparing with Giza 168 check variety.

Genotypes	Tolerance index	Heat sensitivity index (HSI)	Relative performance (P)	% reduction of trait	Tolerance
Giza 168	201.5	0.909	1.087	44.56	M
Ss5-8	186.25	0.923	1.074	45.23	M
S8-4	314.5	1.276	0.734	62.56	L
S8-6	212.0	0.876	1.119	42.94	M
S8-7	229.25	1.016	0.984	49.81	M
S8-8	306.0	1.248	0.761	61.2	L
S8-10	100.5	0.411	1.565	20.18	H
S8-14	256.25	1.217	0.791	59.66	L
S8-18	65.25	0.389	1.586	19.1	H
S8-19	393.5	1.278	0.733	62.6	L
S8-20	240.75	1.076	0.926	52.77	M
S69-1	356.42	1.285	0.726	62.97	L
S69-4	314.67	1.217	0.791	59.68	L
LR-2	83.83	0.489	1.490	24.0	H
G160-1	211.17	0.928	1.068	45.5	M

The relationships among the 15 genotypes based on ISSR analysis were estimated by the UPGMA cluster analysis of genetic similarity matrices, cluster analysis was performed based on the Nei-Li's similarity coefficient matrices and showed that the highest similarity value (1.00) was recorded between S69-1 and S69-4 line and the lowest value (0.77) was found between Giza168 and S8-6 line (Table 7).

The dendrogram of genetic distant among the 15 tested genotypes separated them into two main clusters; the first one included only the check variety (Giza168) which had moderate heat tolerance. while the second cluster divided into two sub-clusters, the first sub-cluster contained LR-2 somaclone line with HHT, the second sub-cluster

subdivided into two main groups, the first one included Sides 5-8 gametoclone had MHT, the other group divided into seven sub-groups, one of them consisted of two HHT lines (S8-10 and S8-18), three others included four MHT lines (G160-1) and (S8-20) and (S8-6 and S8-7) respectively and the last three sub-groups included the rest genotypes which had LHT (Fig. 2). Non extent of similarity range and close relationship which found between the most of genotypes is due to they were derived from the same parents. Furthermore, the two lines which derived from Sakha 69 and the nine derived lines from Sakha 8 were combined in one group.

Table (6): Polymorphism obtained by nine ISSR primers in fifteen bred wheat genotypes (one Ss5-8 gametoclone, 13 somaclones and Giza 168 check variety).

Primers	Primer sequence	Range of fragment size bp	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism %
UBC 840	(GA) <sub>8</sub> YT	240-600	10	7	3	30.0
UBC 834	(CA) <sub>8</sub> TT	245-1030	11	3	8	72.79
UBC 846	(CA) <sub>8</sub> RT	208-990	11	3	8	72.79
UBC 807	(AG) <sub>8</sub> T	190-740	7	5	2	28.0
UBC 808	(AG) <sub>8</sub> C	160-595	8	6	2	25.0
UBC 810	(GA) <sub>8</sub> T	230-720	5	4	1	20.0
UBC 811	(GA) <sub>8</sub> C	210-810	8	6	2	25.0
UBC 814	(CT) <sub>8</sub> A	430-940	7	2	5	71.4
UBC 823	(TC) <sub>8</sub> C	320-635	4	2	2	50.0
Total		160-1030	71	38	33	
Average			7.8	4.2	3.6	43.9

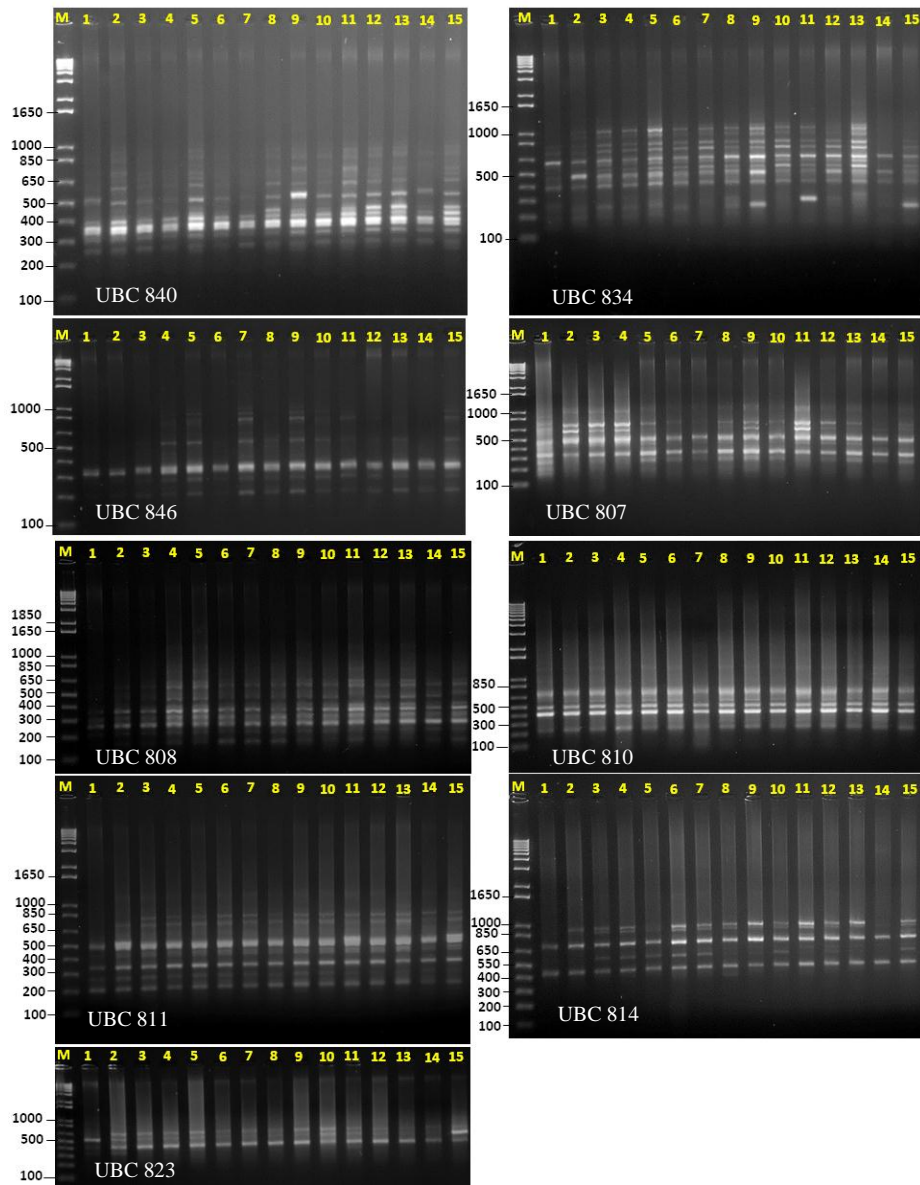


Fig. (1): ISSR-PCR amplified fragments produced by nine primers for 15 bread wheat genotypes (1, Giza 168; 2, Ss5-8; 3, S8-4; 4, S8-6; 5, S8-7; 6, S8-8; 7, S8-10; 8, S8-14; 9, S8-18; 10, S8-19; 11, S8-20; 12, S69-1; 13, S69-4; 14, LR-2; 15, G160-1). M, 1kbp DNA marker.

Table (7): The similarity index among the fifteen bred wheat genotypes (one Ss5-8 gametoclone, 13 somaclones and Giza 168 check variety) based on ISSR

Genotypes	Giza-168	Ss5-8	S8-4	S8-6	S8-7	S8-8	S8-10	S8-14	S8-18	S8-19	S8-20	S69-1	S69-4	LR-2	G160-1
Giza 168	1.00														
Ss5-8	<b>0.86</b>	1.00													
S8-4	0.82	0.91	1.00												
S8-6	0.77	0.88	0.94	1.00											
S8-7	0.78	0.85	0.94	0.97	1.00										
S8-8	0.82	0.93	0.98	0.93	0.92	1.00									
S8-10	0.79	0.84	0.91	0.93	0.96	0.91	1.00								
S8-14	0.81	0.88	0.94	0.91	0.92	0.94	0.95	1.00							
S8-18	0.79	0.84	0.92	0.94	0.96	0.90	0.96	0.92	1.00						
S8-19	0.78	0.87	0.96	0.96	0.95	0.94	0.93	0.94	0.94	1.00					
S8-20	0.78	0.85	0.90	0.92	0.90	0.88	0.90	0.90	0.94	0.94	1.00				
S69-1	0.78	0.87	0.95	0.94	0.93	0.93	0.92	0.95	0.93	0.97	0.94	1.00			
S69-4	0.78	0.87	0.95	0.94	0.93	0.93	0.92	0.95	0.93	0.97	0.94	1.00	1.00		
LR-2	0.82	0.84	0.87	0.82	0.84	0.87	0.83	0.88	0.86	0.86	0.83	0.89	0.89	1.00	
G160-1	0.80	0.82	0.87	0.89	0.92	0.87	0.91	0.88	0.90	0.89	0.90	0.88	0.88	0.86	1.00

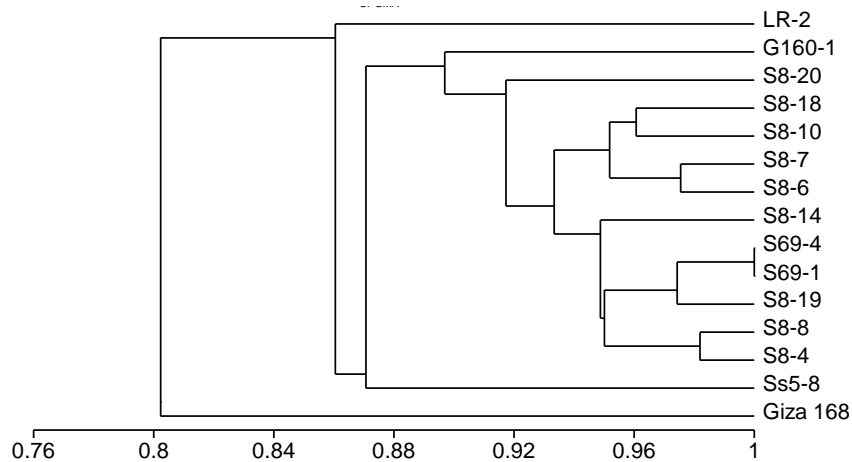


Fig. (2): The dendrogram of genetic distances among all 15 tested genotypes using UPGMA cluster analysis of Nei-Li's similarity coefficient based on ISSR markers.

These results are in harmony with finding by Malik *et al.*, (2010) and Abou-Deif *et al.*, (2013) who reported that ISSR marker able to combine cultivars together according their origin genetic background. Moreover the two HHT lines were grouped in one sub-group and the six LHT genotypes were joined together in three sub-groups, as similar with results of Sharma *et al.*, (2014) and Katakpara *et al.*, (2016). Who observed that ISSR distinguished HHT genotypes and LHT genotypes in individual separated groups.

However, the superiority of some gametoclone/somaclones for most studied yield traits especially under heat stress climate is confirming the possibility of wheat improvement through somaclonal variation. Furthermore, the superior

gametoclone/somaclones can be used as parents in the breeding programs for enhancing heat tolerance in the wheat genotypes. Whereas, breeding through gametoclonal and somaclonal variations have produced many new plant lines. In wheat large numbers of superior lines were produced, having higher production, disease-resistant and tolerant to harmful climatic conditions (Bajji *et al.*, 2004; Yadav *et al.*, 2004; Ahmed *et al.*, 2005; Gawande *et al.*, 2005; Svabova and Lebeda, 2005).

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الملخص العربي

التقييم الحقلّي والتحليل الجزيئي لبعض سلالات قمح الخبز الجاميضية و الجسدية تحت ظروف الإجهاد الحراري الطبيعي

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إنتاج أصناف قمح جديدة من الضروريات الملحة لمواجهة التحديات الناجمة عن تغير المناخ والارتفاع غير المعهود في درجة الحرارة في مصر. لذلك في هذه الدراسة تم تقييم أربعة عشر سلالة جاميضية (**gametoclone**) و جسدية (**somaclones**) من قمح الخبز (*Triticum aestivum* L.) مقارنة بالصنف التجاري (جيزة 168) ككونترول لمدي قدرتها على تحمل الاجهاد الحراري وكذلك التباين الوراثي بينهم عن طريق التقييم الحقلّي و تحليل الواسمات الجزيئية (**ISSR**). حيث تم التقييم لخمس صفات محصوليه كمؤشر لتحمل الحرارة تحت ظروف كلا من ميعاد الزراعة الطبيعي و ميعاد متأخر للزراعة (الإجهاد الحراري). وأظهرت النتائج وجود فروق معنوية عالية بين التركيب الوراثية في كل من الظروف العادية و الإجهاد الحراري. حيث أدى ارتفاع درجة الحرارة الي انخفاض معظم الصفات في معظم التركيب الوراثية. واستنادا إلي مؤشر تحمل الحرارة تم توزيع جميع التركيب الوراثية إلى تراكيب أكثر تحملا للحرارة و تراكيب ذات قدرة متوسطة علي تحمل الحرارة و تراكيب أقل تحملا للحرارة. باستخدام الواسم الجزيئي **ISSR** تم الحصول علي 71 حزمة من الـ **DNA** كان من بينها عدد 33 حزمة متعددة الأشكال المظهرية (بنسبة 43.9%) وتراوحت قيمة التشابه بين التركيب الوراثية من 0.77 الي 1.00. و لقد قسم التحليل العنقودي وقياس القرابة الوراثية كل التركيب الوراثية إلى عنقودين رئيسيين توزعت خلالهما كلا من التركيب الأكثر تحملا ومتوسطة التحمل للحرارة. بينما تجمعت التركيب الأقل تحملا في مجموعة واحدة رئيسية. تفوقت السلالة الجاميضية أو بعض السلالات الجسدية تحت ظروف الاجهاد الحراري مقارنة بالصنف التجاري يشير الي أنه يمكن ان تكون تلك السلالات الناتجة من تقنية زراعة الخلايا النباتية مصدرا جيدا للتحسين الوراثي لانتاج تراكيب وراثية أكثر تحملا للحرارة.