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Assessment of Heat Tolerance in Some Wheat Species and Interspecific Hybrids

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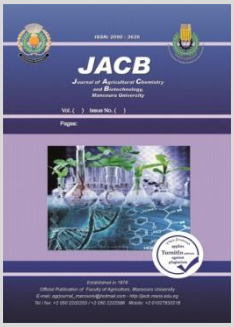

ABSTRACT

Twelve accessions including two diploid, four tetraploid and six hexaploid genotypes were used in this study to assess heat tolerance in wheat. All possible crosses were made and the resulted hybrids with their parents were subsequently evaluated for heat tolerance in two sowing dates. Evaluation was based on 1000 grain weight, grain yield per plant, cell membrane thermostability and tetrazolium chloride reduction. Results revealed significant variability among wheat genotypes and hybrids in all evaluated traits under favorable and heat stress conditions. However, tetraploid genotypes showed the highest performance under heat stress followed by hexaploid and diploid genotypes, respectively. According to results of heat tolerance index, one tolerant and one susceptible parent from each ploidy level along with 6 related interspecific hybrids were chosen to be involved in molecular analysis. Target region amplification polymorphism (TRAP) markers were used based on fixed primers of heat shock protein genes. TRAP clearly confirmed the morpho-physiological findings and generated a dendrogram which was quietly like that of morpho-physiological data. TRAP was able to generate one and five specific bands for diploid and hexaploid genotypes respectively, while no specific bands were generated for tetraploid. In addition, the different genomes showed some shared bands between each other revealing their relationship. Interestingly, two specific bands for tolerant genotypes of different ploidy level were generated which were absent in all susceptible genotypes. Findings herein are of high importance and could help in successive breeding programs for wheat improvement.

Keywords: *Triticum*, Heat shock protein, PCR, CMS, TTC

INTRODUCTION

Wheat is an important cereal that is used as an important product for human consumption in most areas of the world. The genus *Triticum* is grouped into diploids (2n=2×=14), tetraploids (2n=4×=28) and hexaploids (2n=6×=42). *Triticum aestivum*, the common bread wheat, contains three different but genetically related genomes (A, B and D) with a total genomic size of $1.7x10^{10}$ base pairs, illustrating the complex nature of wheat genome (Abd El-Fatah *et al.*, 2017).

The main losses in wheat production are due more to abiotic stresses such as drought, salinity and high temperatures than to biotic stresses. Therefore, understanding the effects of these stresses becomes indispensable for wheat breeding programs that have relied mainly on genetic variations present in the wheat genome through conventional breeding. (Abhinandan *et al.*, 2018). Temperature is involved in determining growth, heading, flowering and wheat production for that it is one of the important factors in plant growth (Heo *et al.*, 2020). With the growing worry about global warming and rising earth temperatures, it is very important to know the proteins that supply thermo-tolerance to crop plants. One such gene family involved in heat stress tolerance is heat shock protein (HSP) family (Kumar *et al.*, 2020). HSPs play in plants a wide variety of roles including stress signal transduction, protecting and repairing damaged proteins and membranes, protecting photosynthesis, and regulating cellular redox state (Asthir *et al.*, 2015). HSPs are classified based on their molecular weight, which ranges from 10 to 200 kDa. There are six major subfamilies of HSPs, namely HSP100, HSP90, HSP70, HSP60, HSP40, and small HSP (Kumar *et al.*, 2020).

High temperature affects yield decrease by about 8.0 and 2.6% for corn and wheat, respectively for every Celsius degree of increase in global average temperature (Zhao *et al.*, 2017). In addition, the photosynthetic product translocation rate to different plant parts is reduced under high temperature stress caused by decrease in membrane stability (Farooq *et al.*, 2011). Moderate but prolonged time of heat stress outcome to gradual senescence, while intensive heat stress for a short time leads to denaturation and aggregation of proteins, resulting in plant death (Hasanuzzaman *et al.*, 2013).

Many physiological processes in plants are often measured as a stander of heat tolerance phenotypes. The electrolyte leakage is an index of reduction of cell membrane thermostability (CMS) and reverberate the performance of wheat genotypes subjected to in vitro heat shock (Farooq *et al.*, 2011). Furthermore, the reduction of tetrazolium triphenyl chloride is one of the physiological evaluation assays for heat stress. It is considered as an index of respiratory enzyme inactivation or mitochondrial dysfunction reflecting the relative level of cell viability (Tewolde *et al.*, 2006 and Mirza *et al.*, 2013).

Genetic diversity evaluation at molecular level gave an important overview to understand the genome variability among species. Thus, molecular analysis of different related species would help in successive breeding approaches. Among several molecular markers available in detecting

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genetic diversity at molecular level, TRAPs (target region amplification polymorphism) which are based on PCR amplification of DNA regions related to coding sequences, open a new route in the assessment of genetic relationship and diversity among different wheat species, providing information to understand and exploit polymorphisms that are mainly associated with functional regions of the genome. TRAP marker is an efficient method for studying wheat genomes for polymorphism and to detect marker loci at large scale and has been shown to be reliable and reproducible in wheat (Liu *et al.,* 2005; Chen *et al.,* 2006; Li *et al.,* 2006; Gadaleta *et al.,* 2010). The TRAP technique it appears to be very efficient relative to other systems where it is a relatively high-throughput PCR-based marker system. The objectives of the study were to evaluate wheat genotypes derived from different species (i.e. diploid, tetraploid and hexaploid) based on physio-morphological traits related to heat tolerance and to determine the association of heat tolerance with the different genomes molecularly.

MATERIALS AND METHODS

Plant materials

The initial plant materials used in the present study consisted of two genotypes of wild diploid wheat *Triticum monococcum* L. (P_1: diploid-01 and P_2: diploid-02), four genotypes of tetraploid durum wheat *Triticum turgidum* L. var. *durum* Desf. (P_3: Ciccico, P_4: Bani-Suef-05, P_5: Svevo and P_6: Sohag-03) and six genotypes of hexaploid bread wheat *Triticum aestivum* L. (P_7: Kuwaiti-01, P_8: Pavon F-076, P_9: Gemmiza-09, P_{10}: Line_1×15, P_{11}: Sakha-08 and P_{12}: Line_6). Subsequently, two parents of each ploidy level and six interspecific hybrids were selected for molecular evaluation based on heat tolerance index, those were: P_1 (tolerant), P_2 (susceptible), P_3 (susceptible), P_6 (tolerant), P_8 (susceptible), P_{11} (tolerant), (P_1×P_6 susceptible), (P_1×P_3 susceptible), (P_1×P_{11} tolerant), (P_2×P_8 susceptible), (P_3×P_{11} tolerant) and (P_6×P_{11} tolerant).

Field experiment:

The field experiments of the present study were carried out at the Experimental Farm of the Faculty of Agriculture, Assiut University, Assiut, Egypt.

Traits measurements:

All possible crosses were achieved (2016-2017) and the resulted hybrids with their parents were subsequently evaluated under favorable and heat stress conditions (2017-2018). After the maturity stage, grain yield per plant (g) and 1000- kernel weight (g) were recorded for each individual plant grown under favorable (1st sowing date: 25 November) and heat stress (2nd sowing date: 30 December) conditions.

***Cell membrane thermostability (CMS) assay*:**

The CMS assay was carried out according to the method described by (Fokar *et al.,* 1998). At the beginning of the flowering stage, flag leaf samples were taken from five individual plants of each genotype per replicate at the beginning of flowering.

***Tetrazolium chloride (TTC) reduction assay*:**

Flag leaf samples for the TTC reduction assay were taken from five individual plants of each genotype per replicate at the beginning of the flowering stage. The level of acquired high temperature tolerance was determined by measuring the percent reduction from TTC to formazan using (Ibrahim and Quick, 2001).

Molecular analysis

DNA extraction:

Total DNA was extracted form high (tolerant) and low responsive (susceptible) genotypes under heat stress of parents and their intraspecific hybrids according to morpho-physiological parameters. The protocol of Youssef *et al.,* (2015) for plant DNA isolation was used. DNA concentration and purity were determined using spectrophotometer and gel electrophoresis.

***Target Region Amplification Polymorphism (TRAP) analysis*:**

Ten TRAP primer combinations were used, consisted of three fixed forward primers related to heat shock proteins genes, i.e. HSP-03 (GQ280382, 70kDa), HSP-05 (LC383647, N1-506) and HSP-10 (GQ240792, Hsp90.3-A1), in combination with five arbitrary reverse primers (EM-1, EM-2, EM-3, EM-4 and EM-7) selected form the sequence related amplified polymorphism (SRAP) reverse primers list. Primer codes and sequences are shown in Table (1). The method of Li and Quiros (2001) was followed for TRAP PCR with some modifications. Each 20-µL amplification reaction consisted of 2 µL of 10X PCR buffer, 0.8 µL of 50 mM $MgCl_2$, 1.6 µL of 10 µM of each forward and reverse primer, 2.5 µL of 2 mM dNTPs, 25 ng template DNA, and 0.25 µL of 5U Taq-DNA polymerase (Invitrogen). PCR conditions were performed as follows: an initial cycle at 94°C for 2 min; 5 cycles of 94°C for 30 s, 35°C for 30 s, and 72°C for 1 min; an additional 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 5 min. PCR products were separated on 2.5% agarose gel and visualized by ethidium bromide staining.

Table 1. Primer sequences and codes used in the molecular analysis.

Codes	HSP Sequence (5` → 3`)	Em Sequence (5` → 3`)
HSP-03/Em-01	ACCTTCTTCTCCGTCAAGCG	GACTGCGTACGAATTAAT
HSP-03/Em-04	ACCTTCTTCTCCGTCAAGCG	GACTGCGTACGAATTTGA
HSP-03/Em-07	ACCTTCTTCTCCGTCAAGCG	GACTGCGTACGAATTATG
HSP-05/Em-01	CAAGAAGGGGCAGCTCAAGA	GACTGCGTACGAATTAAT
HSP-05/Em-02	CAAGAAGGGGCAGCTCAAGA	GACTGCGTACGAATTTGC
HSP-05/Em-03	CAAGAAGGGGCAGCTCAAGA	GACTGCGTACGAATTGAC
HSP-10/Em-01	ACTCGTGATACGTCTGGGGA	GACTGCGTACGAATTAAT
HSP-10/Em-02	ACTCGTGATACGTCTGGGGA	GACTGCGTACGAATTTGC
HSP-10/Em-07	ACTCGTGATACGTCTGGGGA	GACTGCGTACGAATTATG

Statistical data analysis:

All morphological and physiological measurements were taken under a complete randomized design with three replicates. Analysis of variance was performed using MSTATC software (Nissen, 1984). Percentages of crossability among tested wheat genotypes were compared using *t*-test. Morphological and physiological data were used for cluster analysis based on Euclidean coefficient using NTSys tool. For molecular data analysis a binary data matrix recording the presence (1) or the absence (0) of bands was

made. Only strong and readable bands were detected and used in the analysis. Some polymorphism measures were calculated including, percentage of polymorphism, polymorphism information content (PIC), primer resolving power (Rp), diversity index (DI) and marker index (MI). Estimated similarities among wheat parents and hybrids based on Jaccard's coefficient (Jaccard, 1908) were used to perform cluster analysis using unweighted pair group method with arithmetic means (UPGMA) by NTSys software.

RESULTS AND DISCUSSION

Results

Twelve wheat genotypes from different levels of ploidy (i.e. 2 diploid, 4 tetraploid and 6 hexaploid) were preliminarily used as parents in this study. All possible crosses were performed and the resulted hybrids with their parents were subsequently evaluated under favorable and heat stress conditions. The percentage of crossabilty among parents of different level of ploidy was estimated (Table 2). Results showed that the percentage of crossability increased significantly (*t*-test, $P<0.05$) within the same cross when the parent of higher level of ploidy being used as mother parent. Subsequently, six parents (two from each level of ploidy) and six related interspecific hybrids were selected according to heat tolerance based on heat tolerance index of the 1000 grain weight. These 12 genotypes were furtherly used for molecular analysis.

Table 2. Averages of percentage of crossability among wheat genotypes.

Male	Female	Crossability (%)	Mean
Diploid	Tetraploid	70.60 ± 7.24	62.82
Tetraploid	Diploid	55.04 ± 4.95	
Diploid	Hexaploid	47.80 ± 4.77	39.44
Hexaploid	Diploid	31.07 ± 7.15	
Tetraploid	Hexaploid	69.42 ± 3.85	60.44
Hexaploid	Tetraploid	51.46 ± 4.60	

Values represent means ± standard errors

Morphological evaluation:

1000 grain weight and grain yield per plant:

Vegetative traits (i.e. 1000 grain weight and grain yield per plant) were measured in each category of parents of the same ploidy level and the corresponding hybrids as an average. Results revealed significant variability among wheat genotypes and hybrids in all evaluated traits under both favorable and heat stress conditions. 1000 grain weight ranged from 32.18 to 55.79g for diploid (D) and tetraploid × hexaploid (TH) crosses in favorable environment, respectively. While, it ranged from 16.56 to 41.28g for D and T wheat under heat stress, respectively. The hexaploid and tetraploid wheat genotypes showed the lowest reduction percentage for 1000 grain weight (20.83 and 23.36%, respectively) followed by diploid × tetraploid (DT) crosses (24.42%). The hexaploid and tetraploid wheat genotypes showed the highest heat tolerance index for 1000 grain weight (0.79 and 0.77, respectively) followed by DT crosses (0.76) (Table 3).

Grain yield per plant ranged from 33.64 to 104.38g for D and T wheat respectively in favorable conditions. Meanwhile, under heat stress the grain yield per plant ranged from 4.32 to 15.22g for DT crosses and T wheat, respectively. The DT crosses and H genotypes showed the lowest reduction percentage for grain yield per plant (61.53 and 80.82%, respectively) followed by DH (81.91%). Furthermore, the DT crosses and H genotypes showed the highest heat tolerance index for grain yield per plant (0.38 and 0.19, respectively) followed by DH (0.18).

Table 3. Mean of 1000 grain weight and grain yield per plant for D, T, H, DT, DH and TH genotypes under favorable (F) and heat stress (H) conditions as well as percentage reduction (%R) and heat tolerance index (HTI).

Genotypes	1000 grain weight				Grain yield per plant			
	F	H	%R	HTI	F	H	%R	HTI
D	32.18	16.56	48.54	0.51	33.64	4.45	86.77	0.13
T	53.86	41.28	23.36	0.77	104.38	15.22	85.42	0.15
H	48.92	38.73	20.83	0.79	44.68	8.57	80.82	0.19
DT	50.29	38.01	24.42	0.76	27.76	10.68	61.53	0.38
DH	40.76	28.07	31.13	0.69	23.88	4.32	81.91	0.18
TH	55.79	38.61	30.79	0.69	51.93	7.43	85.69	0.14
$LSD_{0.01}$	1.04	1.10			3.47	0.68		
$LSD_{0.05}$	0.67	0.71			2.23	0.44		

D, diploid wheat, T, tetraploid wheat and H, hexaploid wheat. DT, crosses D x T, DH, crosses D x H and TH, crosses T x H.

Means of 1000 grain weight and grain yield per plant for diploid, tetraploid and hexaploid wheat genotypes as well as DT, DH and TH inter-specific crosses under heat stress condition are shown in Table (3). Results refer to the highest 1000 grain weight was observed in tetraploid wheat which was closely followed by hexaploid, TH and DT genotypes (41.28, 38.73, 38.61and 38.01, respectively). However, the grain yield per plant were higher in tetraploid than other wheat genotypes. Data of 1000 grain weight and grain yield for the 12 selected genotypes are shown in Table (4).

Physiological evaluation:

Cell membrane thermostability and tetrazolium chloride reduction:

The mean percentages of cell membrane thermostability (CMS) and tetrazolium chloride reduction (TTC) for diploid (D), tetraploid (T) and hexaploid (H) wheat genotypes as well as crosses DT, DH and TH under heat stress are present in Figure (1). The highest mean of CMS % was obtained for tetraploid genotypes (46.70%) and followed by hexaploid (42.36%), likewise the highest mean of CMS % was observed for the inter-specific crosses TH (44.58%) and inter-specific cross DT (40.92%). Similarly, the highest mean of - TTC % reduction was observed for tetraploid wheat genotypes (43.62%) and followed by hexaploid (40.91%), also the highest mean of TTC % reduction was obtained for the inter-specific cross TH (40.56%) and inter-specific cross DT (38.93%). Data of CMS and TTC for the 12 selected genotypes are shown in Table (4).

Table 4. Morphological and physiological performance of the 12 selected wheat genotypes under heat stress.

Genotype	Status	Grain yield	1000 grain weight	CMS	TTC
Diploid-01 (P1)	Tolerant	4.66	15.00	39.57	39.84
Diploid-02 (P2)	Susceptible	4.27	12.86	32.80	30.39
Ciccico (P3)	Susceptible	8.29	36.78	34.37	36.48
Sohag-03 (P6)	Tolerant	9.47	43.39	64.10	53.46
Pavon-F76 (P8)	Susceptible	6.24	31.13	29.64	35.52
Sakha-80 (P11)	Tolerant	7.08	50.20	77.26	57.27
P1xP6	Tolerant	11.50	39.79	48.90	40.12
P1xP3	Susceptible	4.60	36.95	39.98	36.07
P1xP11	Tolerant	5.38	31.17	55.45	40.69
P2xP8	Susceptible	1.87	22.33	22.75	30.27
P3xP11	Tolerant	13.08	44.63	60.39	43.01
P6xP11	Tolerant	14.99	38.25	44.45	46.59

Figure 1. The % mean of CMS and TTC reduction for D (diploid wheat), T (tetraploid wheat) and H (hexaploid wheat) as well as DT (crosses D x T), DH (crosses D x H) and TH (crosses T x H) under heat stress condition. Different letters in each trait are significantly different according to Duncan's multiple range test at P=0.05.

Mean squares of grain yield per plant and1000 grain weight under favorable and heat stress conditions as well as the CMS % and TTC % reduction under heat stress (**Table 5**) revealed highly significant differences ($P < 0.01$) among genotypes, providing evidence for the presence of genetic variability regarding these traits. Highly significant differences ($P < 0.01$) were found between D and T, between D and H and between T and H wheat genotypes for grain yield per plant and 1000 grain weight under favorable and heat stress and CMS % and TTC % reduction under heat stress conditions.

The analysis of variance for CMS %, TTC % reduction, grain yield per plant and 1000 grain weight among inter-specific crosses is presented in Table (6). Highly significant differences ($P < 0.01$) were found among genotypes for all studied traits, similarly high significant differences were obtained between DT and DH, between DH and TH and between DT and TH inter-specific crosses for CMS %, TTC % reduction, grain yield per plant and 1000 grain weight under heat stress condition.

Table 5. Mean squares for grain yield per plant and 1000 grain weight under favorable and heat stress conditions as well as CMS and TTC under heat stress.

S.O.V	df	Favorable		Heat stress			
		G. yield	1000 GW	CMS	TTC	G. yield	1000 GW
				D vs T			
Rep	2	551.02*	3.99	64.79**	56.31**	0.59	7.56
Gen.	12	5411.21**	349.51**	439.61**	209.64**	122.76**	404.71**
D vs T	1	17564.8**	3256.23**	470.56**	446.32**	803.27**	4230.15**
Among D	2	6314.81**	300.78**	80.94**	68.98**	0.11	65.93**
Among T	9	3859.99**	37.37**	515.88**	214.60**	74.41**	54.94**
Error	24	196.23	1.95	1.20	0.91	2.54	2.13
				D vs H			
Rep	2	112.78	1.52	102.49**	96.37**	2.01	26.85*
Gen.	23	1243.59**	331.88**	487.86**	217.13**	33.75**	304.37**
D vs H	1	685.00**	2207.92**	120.28**	222.65**	133.41**	3868.35**
Among D	2	6314.81**	300.78**	80.94**	68.98**	0.11	65.93**
Among H	20	764.40**	241.19**	546.93**	231.67**	32.13**	150.02**
Error	46	190.49	1.7	0.8	0.67	5.76	6.66
				T vs H			
Rep	2	19.69**	0.46	142.67**	132.14**	3.94	9.16
Gen.	30	4081.72**	188.56**	532.12**	223.81**	73.75**	120.92**
T vs H	1	72423.6**	496.58**	382.14**	149.47**	900.24**	132.71**
Among T	9	3859.99**	37.37**	515.88**	214.60**	74.41**	54.94**
Among H	20	764.40**	241.19**	546.93**	231.67**	32.13**	150.02**
Error	60	200.55	1.38	1.04	0.84	4.99	5.98

D, diploid wheat, T, tetraploid wheat and H, hexaploid wheat, * and ** indicate significance at 0.05 and 0.01 levels of probability, respectively.

Morpho-physiological data of the 12 selected genotypes (Table 4) were used to perform cluster analysis based on Euclidean coefficient. Dendrogram showed clear relationship among genotypes mainly based on heat tolerance (Fig. 2). In this regard, the dendrogram divided into two main clusters. The first main cluster included all susceptible parents (P2, P3 and P8) and hybrids (1x3 and 2x8) along with the tolerant diploid parent (Diploid-01) which was joined with the other diploid parent (Diploid-02). Meanwhile, the second main cluster was consisted of tolerant tetraploid and hexaploid parents (P6 and P11) with all tolerant hybrids (1x6, 1x11, 3x11 and 6x11).

Molecular analysis

Target region amplification polymorphism (TRAP) was used to evaluate the wheat genotypes of different levels of ploidy as well as their interspecific hybrids for some heat shock protein (HSP) genes. Out of 15 primer combinations used, 9 were selected and used in analysis based on polymorphism and band clearness. A total of 91 bands were generated by six parents and their six hybrids. Out of these bands, 30 were polymorphic among wheat genotypes and hybrids. The averaged percentage of polymorphism obtained by TRAP primers was 32.97%, ranged from 12.50 (HSP-05/Em2 and HSP-10/Em7) to 62.50% (HSP-10/Em1). Other genetic diversity measures were calculated, the highest value of polymorphism information content (PIC) was 0.28 shown by HSP-10/Em2 combination, while the lowest PIC was 0.05 generated by HSP-03/Em7 with an average of 0.14 (Table 7). The averaged resolving power of TRAP primers was 2.13, ranged from 0.83 (HSP-03/Em7 and HSP-10/Em7) to 4.00 (HSP-10/Em2). The highest value of diversity index and marker index were 0.98 and 1.39 while the lowest values were 0.31 and 0.06, with an average of 0.94 and 0.56 respectively (Table 7).

Table 6. Mean squares for grain yield per plant and 1000 grain weight under favorable and heat stress conditions as well as CMS and TTC under heat stress.

S.O.V	df	Favorable		Heat stress			
		G. yield	1000 GW	CMS	TTC	G. yield	1000 Kw
				DT vs DH			
Rep.	2	25.38	3.15	75.63**	71.0**	3.99	3.27
Gen.	10	220.40**	122.4**	815.9**	257.0**	43.40**	109.00**
DT vs DH	1	98.60**	594.40**	199.8**	170.9**	264.70**	647.20**
Among DT	7	238.00**	88.10**	872.6**	312.4**	20.30**	42.00**
Among DH	2	219.80**	6.5	925.3**	106.3**	13.60**	74.10**
Error	20	8.4	3.1	1.2	0.7	3.4	6
				DH vs TH			
Rep.	2	34.83*	15.12	108.84**	92.83**	0.04	24.46*
Gen.	17	1230.20**	494.43**	391.36**	151.07**	38.13**	162.39**
DH vs TH	1	5900.95**	1694.92**	633.40**	340.48**	72.35**	834.20**
Among DH	2	219.77**	6.54	925.32**	106.29**	13.63**	74.12**
Among TH	14	1040.92**	478.39**	297.80**	143.93**	39.18**	127.01**
Error	34	33.3	14.39	1.18	0.94	5.04	5.68
				DT vs TH			
Rep.	2	91.96*	19.08	152.90**	134.74**	2.05	37.55*0
Gen.	22	1153.68**	354.00**	476.72**	192.88**	38.93**	94.45**
DT vs TH	1	9142.41**	474.02**	210.23**	41.51**	165.62**	5.69
Among DT	7	237.96**	88.09**	872.64**	312.40**	20.34**	42.02**
Among TH	14	1040.92**	478.39**	297.80**	143.93**	39.18**	127.01**
Error	44	27.96	12.16	1.22	1.02	5.34	2.85

DT, crosses D x T, DH, crosses D x H and TH, crosses T x H. , * and ** indicate significance at 0.05 and 0.01 levels of probability, respectively.

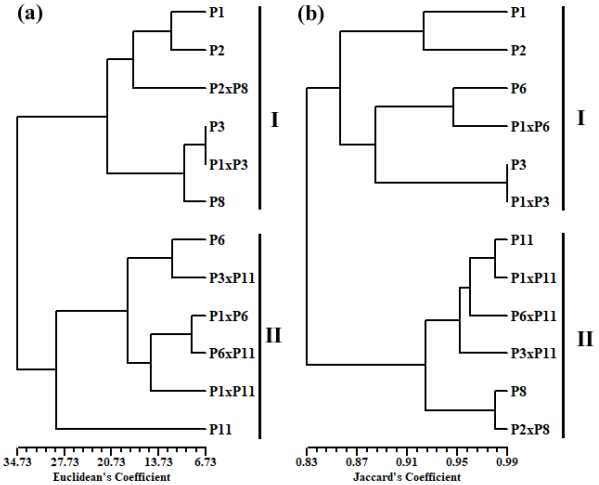

Figure 2.UPGMA-dendrograms showing relationship among wheat genotypes based on (a) morpho-physiological data and (b) TRAP molecular markers.

Wheat parents differed in their ability of amplification exposed by different number of bands generated in TRAP profiles. In this regards, 76, 81 and 84 total bands were generated by bulked samples of D, T and H parents. Moreover, the number of polymorphic bands between tolerant and susceptible genotypes within each group was different. Diploid plants showed 7 bands (9.21% polymorphism), while tetraploid and hexaploid plants showed 11 (13.58% polymorphism) and 6 (7.14% polymorphism) polymorphic bands, respectively between tolerant and susceptible parents (Table 7). In addition, wheat genotypes showed some shared bands between groups of different ploidy level, which appeared in both parents of each group. There were, two shared bands between diploid and tetraploid parents, one shared band between diploid and hexaploid parents and four bands were shared between tetraploid and hexaploid parents (Table 7). Furthermore, diploid parents showed one specific band and hexaploid parents showed five specific bands, which were absent in the other groups. While no specific bands were shown by tetraploid parents (Table 7). TRAP primers were able to generate two bands specific for heat tolerant parents and hybrids. Those were, one band generated by HSP-03/Em-1 with 275 bp size and the other band generated by HSP-05/Em-3 with 350 bp size. The two specific bands were presented only in genotypes showed heat tolerance while they were absent in other susceptible genotypes (Table 7). However, no specific bands were generated for susceptible genotypes.

Similarities among wheat genotypes from different levels of ploidy and their corresponding hybrids were used to perform cluster analysis based on Jaccard's coefficient and UPGMA dendrogram method. Grouping of wheat samples was mainly based on ploidy level and heat tolerance. In this regard, samples were divided into two main clusters; the first cluster has two sub-clusters. Diploid parents were grouped in the first sub-cluster, while the second sub-cluster has two separated branches of tetraploid parents (Sohag-03 and Ciccico) along with their corresponding hybrids of the diploid parent (Diploid-01). Meanwhile, the second main cluster consisted of the two hexaploid parents (Sakha-8 and Pavon-76) separately with their hybrids of diploid and tetraploid parents according to heat tolerance (Fig. 2). UPGMA-dendrogram showed high values of bootstrapping which reflect good stability of sample relationship. Molecular based-dendrogram was quite similar to that based on morpho-physiological data, especially in some sub-clusters e.g. P11 and its related hybrids, P3 with P1xP3 and the sub-cluster of the two diploid parents. However, in the molecular based-dendrogram the grouping was affected mainly by ploidy level followed by the performance of genotypes under heat stress, unlike that based on morpho-physiological data which was mainly due to heat tolerance. Figure (3) showed the TRAP profiles generated by 10 primer combinations

Table 7. Survey of TRAP analysis showing total number of bands, polymorphic, specific and shared bands along with genetic diversity measures calculated for wheat genotypes.

Primer	TNB	NPB	%P	TNB			Specific			Polymorphic			Unique		Shared bands			Diversity measures			
				D	T	H	D	T	H	D	T	H	TL	S	DT	DH	TH	PIC	Rp	DI	MI
HSP-03/Em1	9	4	44.44	8	9	8	0	0	0	2	1	1	1	0	1	0	1	0.17	2.50	0.96	0.69
HSP-03/Em4	14	3	21.43	13	11	14	0	0	1	1	0	1	0	0	0	1	0	0.10	2.33	0.98	0.30
HSP-03/Em7	12	2	16.67	11	11	11	1	0	0	0	0	0	0	0	0	0	1	0.05	0.83	0.50	0.11
HSP-05/Em1	8	2	25.00	8	7	8	0	0	0	2	0	2	0	0	0	0	0	0.11	1.33	0.66	0.22
HSP-05/Em2	8	1	12.50	7	7	8	0	0	1	0	0	0	0	0	0	0	0	0.06	1.00	0.35	0.06
HSP-05/Em3	15	7	46.67	13	15	13	0	0	0	1	7	1	1	0	0	0	0	0.15	3.00	1.56	1.04
HSP-10/Em1	8	5	62.50	4	7	6	0	0	1	1	1	0	0	0	0	0	1	0.24	3.33	1.42	1.20
HSP-10/Em2	9	5	55.56	4	6	9	0	0	2	0	2	1	0	0	0	0	1	0.28	4.00	1.74	1.39
HSP-10/Em7	8	1	12.50	8	8	7	0	0	0	0	0	0	0	0	1	0	0	0.06	0.83	0.31	0.06
Total	91	30	32.97	76	81	84	1	0	5	7	11	6	2	0	2	1	4	0.14	2.13	0.94	0.56

TNB: total number of bands, NPB: number of polymorphic bands, %P: percentage of polymorphism, D: diploid, T: tetraploid, H: hexaploid, TL: tolerance, S: susceptibility, DT: diploid × tetraploid, DH: diploid × hexaploid, TH: tetraploid × hexaploid, PIC: polymorphism information content, Rp: primer resolving power, DI: diversity index and MI: marker index. Primers codes are related to Table (2).

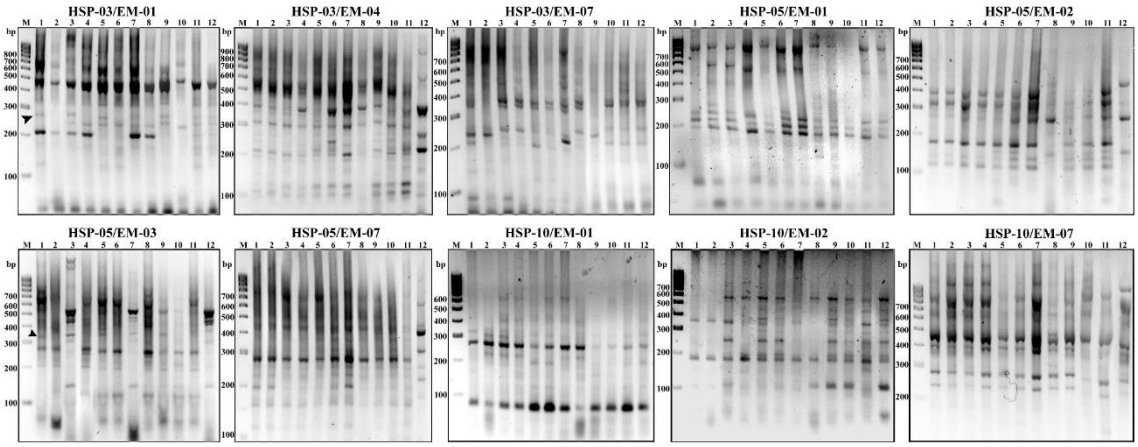

Figure 3. TRAP profiles generated by 10 primer combinations among 12 wheat genotypes based on heat shock protein (HSP) genes primers. Arrows showed specific bands for tolerant wheat genotypes.

Discussion

Heat stress is one of the major abiotic stresses which affect growth and development of plant species. In wheat, heat affected morphological and physiological behaviors (Mondal *et al.*, 2013; Iqbal *et al.*, 2017), caused reduction in grain formation and yield (Challinor *et al.*, 2014) and leads to reduce photosynthetic capacity (Almeselmani *et al.*, 2012; Ashraf and Harris 2013).

In the present study, crossability was high in crosses of diploid × tetraploid (DT) and tetraploid × hexaploid (TH) than that of diploid × hexaploid (DH). This indicates that the near level of ploidy in parents of a cross is an efficient factor determining crossability percentage. Moreover, according to crossability findings in the present study, it was recommended to use the parent of high level of ploidy as a mother to increase crossability percentage and to obtain the highest number of fertile F_1 progeny from an interspecific cross. These results agree with those of Kihara (1982). However, Wang *et al.*, (2005) have reported that successful crosses were by combining tetraploid wheat genotypes as maternal parents and hexaploid genotypes as the paternal parents which produced the best percentage of seeds number in hybrids.

Results of the current study indicate that tetraploid and hexaploid genotypes exposed their heat tolerance more than that of diploid genotypes. This was reflected by showing higher 1000 grain weight and grain yield per plant than diploid plants under stress. However, diploid plants showed higher values of these traits than both tetraploid and hexaploid genotypes under favorable conditions. This information indicated that genotypes used in the study with high level of ploidy may contained heat tolerance mechanisms more efficiently than those of diploid plants. In accordance with these results, Hakim *et al.*, (2012) found that grain yield and 1000 grain weight of wheat genotypes decreased when exposed to late heat stress due to high temperature. Evidently, stress may cause reduction in grain-filling during grain-filling stage (Wardlaw and Moncur 1995), thus the reduction in the starch content causes yield losses (Balla *et al.* 2011). Matching with results of the present study, Karamanos *et al.*, (2008) explained that durum wheat having better yields than bread wheat under stressful conditions. They reported that the most adaptability varieties with environment were the one that showed the highly performed in grain yield/plant and 1000 grain weight under heat stress.

Cell membrane thermostability (CMS) and tetrazolium chloride (TTC) reduction are heat tolerance mechanisms operating at the cellular level (Gupta *et al.*, 2010). Cell membranes, as sites of many biological activities, play a key role in heat-induced wheat damage. On the other hand, level of reduction of TTC to formazan is an effective technique for quantifying tolerance acquired at high temperatures in wheat cultivars and that's because it quantifies cell viability by a spectrophotometric assay of red formazan and determines mitochondrial electron transport activity (Porter *et al.*, 1995). Physiological results of CMS and TTC assays in the present study confirmed findings of

morphological evaluation. Thus, tetraploid wheat species showed the highest heat-tolerance based on CMS and TTC assays when compared to diploid and hexaploid wheat species. These results were matched with those of Dias *et al.*, (2011). The combination of heat-tolerant × heat-tolerant, susceptible parents revealed that the parents Sakha-8 and Sohag-3 may have common genes for heat tolerance for the characters under study. The inhibition of membrane-bound enzymes might be responsible for maintaining chemical gradients in the cell under heat stress, which causes ion leakage from plasma membranes and lead to loss of membrane integrity (Reynolds *et al.,* 2001). Results of the present study in general indicted that the tetraploid wheat genotypes were the highest heat tolerant than other genotypes, while the hexaploid came in second place, as it appeared more heat tolerant than the diploid wheat.

Molecular analysis in this study was performed using TRAP molecular markers based on specific primers for heat shock protein (HSP) genes. TRAPs belong to molecular markers which are based on PCR amplification of DNA regions related to coding sequences. These markers open a new path in the genetic relationship and diversity assessment, providing information to understand and exploit polymorphisms that are mainly associated with functional regions of the genome (Hu and Vick 2003). Three primers related to HSPs were used in combination with five arbitrary primers of SRAP marker. TRAP profiles confirmed the morphological and physiological results. In this regard, the total number of TRAP bands was higher in hexaploid and tetraploid plants than diploid, matching with genome ploidy level and level of tolerance. Moreover, the percentage of polymorphism between the two parents of each ploidy level was higher in tetraploid followed by diploid and lower percentage was found with hexaploid parents. This reflected the amount of genetic variation between each two parents of the same ploidy level which affected the degree of tolerance for heat stress.

TRAP primers were able to generate two specific bands for heat tolerant parents and hybrids generated by HSP-03/Em5 and HSP-05/Em3. These primers were designed from HSP genes (GQ280382.1 70kDa and LC383647.1 N1-506 gene) related to *Triticum aestivum* according to NCBI databases. However, the bands are most probably located in A genome, since all genotypes of different ploidy levels successfully generated the tolerance-specific bands. The two specific bands were presented only in genotypes showed heat tolerance, while they were absent in all other susceptible genotypes

TRAP has been utilized previously in several molecular approaches in wheat, including genetic diversity assessment, screening for abiotic stress, genetic mapping and association with physiological traits. In this regard, TRAP markers have been used to determine the genetic diversity in 6 durum wheat genotypes (Al-Doss *et al.,* 2010). TRAP analysis was efficient to reveal 40.0% polymorphism among genotypes. Additionally, Moustafa *et al.,* (2014) used TRAP and SRAP markers linked to QTL for drought tolerance, they found that results of both markers were valuable and could be further used in breeding programs for drought tolerance in wheat. Combining dendrograms of TRAP with other molecular and morpho-physiological data gives better grouping and clear relationships among durum (Al-Doss *et al.,* 2011) and doubled haploid wheat genotypes (Barakat *et al.,* 2013). Moreover, TRAP was highly efficient in genetic mapping, generating a large number of markers scattered across the genome (Menzo *et al.,* 2013). The association of TRAP markers with some physiological traits (e.g. leaf chlorophyll content, flag leaf senescence, and cell membrane thermostability) was reported as an indicator for drought tolerance genes in wheat (Saleh *et al.,* 2014). Recently, Abd El-Fatah *et al.,* (2017) used TRAP to distinguish between bread and durum wheat genotypes, a highly significant positive correlation was found between TRAP and morphological data. Some TRAP fragments were unique and specific for bread wheat. Authors reported that TRAP-1 and TRAP-7 primers were able to distinguish the durum wheat from the bread wheat genotypes.

CONCLUSION

Morpho-physiological evaluation for heat tolerance in wheat performed in this study was highly efficient to represent the variability among wheat genotypes derived from different ploidy levels. Interspecific hybrids resulted from crosses of different responsive genotypes, clearly showed their inherited performance when they were evaluated against heat stress. In general, tetraploid genotypes used in the present study showed higher degree of heat tolerance than the other two groups. TRAP markers based on fixed primers of HSP genes used in this study were valuable to show an excellent relationship among tolerant and susceptible parents of different ploidy levels as well as their respective interspecific hybrids. This relationship was mainly based on ploidy and variability in genes of heat shock proteins examined by TRAP. The two unique bands specific for tolerant genotypes are very important, which could be recovered and sequenced to be used as specific markers for heat tolerance in wheat.

REFERENCES

Abd El-Fatah, A., El-Rawy, M. A. E., El-Aref, H. M., Tagian, A. S., and El-Sanousy, S. A. (2017). Study of Genetic Diversity using Morphological Traits and Target Region Amplified Polymorphism (TRAP) Marker in wheat. Assiut Journal of Agriculture Science 48 (1):98-119.

Abhinandan, K., Skori, L., Stanic, M., Hickerson, N., Jamshed, M., and Samuel, M. A. (2018). Abiotic stress signaling in wheat–an inclusive overview of hormonal interactions during abiotic stress responses in wheat. Frontiers in plant science 24(9):734.

Al-Doss, A. A., Elshafei, A. A., Moustafa, K. A., Saleh, M., and Barakat, M. N. (2011). Comparative analysis of diversity based on morphoagronomic traits and molecular markers in durum wheat under heat stress. African Journal of Biotechnology 10(19):3671-3681.

Al-Doss, A. A., Saleh, M., Moustafa, K. A., Elshafei, A. A., and Barakat, M. N. (2010). Grain yield stability and molecular characterization of durum wheat genotypes under heat stress conditions. African Journal of Agricultural Research 5(22):3065-3074.

Almeselmani, M., Deshmukh, P. S., and Chinnusamy, V. (2012). Effects of prolonged high temperature stress on respiration, photosynthesis and gene expression in wheat (Triticum aestivum L.) varieties differing in their thermotolerance. Plant stress 6(1):25-32.

Ashraf, M. H. P. J. C., and Harris, P. J. (2013). Photosynthesis under stressful environments: an overview . Photosynthetica 51(2):163-190.

Asthir, B. (2015). Protective mechanisms of heat tolerance in crop plants. Journal of Plant Interactions 10(1): 202-210.

Balla, K., Rakszegi, M., Li, Z., Bekes, F., Bencze, S., and Veisz, O. (2011). Quality of winter wheat in relation to heat and drought shock after anthesis. Czech Journal of Food Sciences 29(2):117-128.

Barakat, M. N., Al-Doss, A. A., Elshafei, A. A., Ghazy, A. I., and Moustafa, K. A. (2013). Assessment of genetic diversity among wheat doubled haploid plants using TRAP markers and morpho-agronomic traits. Australian Journal of Crop Science 7(1):104.

Challinor, A. J., Watson, J., Lobell, D. B., Howden, S. M., Smith, D. R., and Chhetri, N. (2014). A meta-analysis of crop yield under climate change and adaptation. Nature Climate Change 4(4):287-291.

Chen, J., Hu, J., Vick, B. A., and Jan, C. C. (2006). Molecular mapping of a nuclear male-sterility gene in sunflower (Helianthus annuus L.) using TRAP and SSR markers. Theoretical and applied genetics 113(1):122-127.

Dias, A. S., Semedo, J., Ramalho, J. C., and Lidon, F. C. (2011). Bread and durum wheat under heat stress: a comparative study on the photosynthetic performance. Journal of Agronomy and Crop Science 197(1):50-56.

Farooq, M., Bramley, H., Palta, J. A., and Siddique, K. H. (2011). Heat stress in wheat during reproductive and grain-filling phases. Critical Reviews in Plant Sciences 30(6):491-507.

Fokar, M., Nguyen, H. T., and Blum, A. (1998). Heat tolerance in spring wheat. I. Estimating cellular thermotolerance and its heritability. Euphytica *104*(1):1-8.

Gadaleta, A., Mastrangelo, A. M., Russo, M. A., Giove, S. L., D'onofrio, O., Mango, T., ... and Cifarelli, R. A. (2010). Development and characterization of EST-derived SSRs from a 'totipotent'cDNA library of durum wheat. Plant breeding 129(6):715-717.

Gupta, S., Kaur, S., Sehgal, S., Sharma, A., Chhuneja, P., and Bains, N. S. (2010). Genotypic variation for cellular thermotolerance in Aegilops tauschii Coss., the D genome progenitor of wheat. Euphytica 175(3):373-381.

Hakim, M. A., Hossain, A., da Silva, J. A. T., Zvolinsky, V. P., and Khan, M. M. (2012). Protein and starch content of 20 wheat (Triticum aestivum L.) genotypes exposed to high temperature under late sowing conditions. Journal of Scientific Research 4(2): 477-477.

Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R., and Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. International Journal of Molecular Sciences 14(5):9643-9684.

Heo, J. H., Seong, H. J., Yang, W. H., and Jung, W. (2020). Growth Responses and Differences in Gene Expression depending on Cultivation Temperature between Alternative type Wheat Varieties. Journal of Crop Science and Biotechnology 23(1):47-55.

Hu, J., and Vick, B. A. (2003). Target region amplification polymorphism: a novel marker technique for plant genotyping. Plant Molecular Biology Reporter 21(3):289-294.

Ibrahim, A. M., and Quick, J. S. (2001). Heritability of heat tolerance in winter and spring wheat. Crop Science 41(5):1401-1405.

Iqbal, M., Raja, N. I., Yasmeen, F., Hussain, M., Ejaz, M., and Shah, M. A. (2017). Impacts of heat stress on wheat: a critical review. Advances in Crop Science and Technology 5: 251.

Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44: 223-270.

Karamanos, A. J., Livanos, G., Economou, G., and Papastavrou, A. (2008). Evaluating yield responses to water stress and drought resistance mechanisms of 20 local bread and durum wheat landraces. Agricultural University of Athens Press**,** Athens.

Kihara, H. (1982) Wheat studies retrospect and prospects. Kodansha Ltd, Tokyo

Kumar, A., Sharma, S., Chunduri, V., Kaur, A., Kaur, S., Malhotra, N., ... and Sonah, H. (2020). Genome-wide Identification and Characterization of Heat Shock Protein Family Reveals Role in Development and Stress Conditions in *Triticum aestivum L*. Scientific Reports 10(1):1-12.

Li, G. Q., Li, Z. F., Yang, W. Y., Zhang, Y., He, Z. H., Xu, S. C., ... and Xia, X. C. (2006). Molecular mapping of stripe rust resistance gene YrCH42 in Chinese wheat cultivar Chuanmai 42 and its allelism with Yr24 and Yr26. Theoretical and applied genetics 112(8):1434-1440.

Li, G., and Quiros, C. F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theoretical and applied genetics 103(2-3):455-461.

Liu, Z. H., Anderson, J. A., Hu, J., Friesen, T. L., Rasmussen, J. B., and Faris, J. D. (2005). A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theoretical and Applied Genetics 111(4):782-794.

Menzo, V., Giancaspro, A., Giove, S., Nigro, D., Zacheo, S., Colasuonno, P., ... and Gadaleta, A. (2013). TRAP molecular markers as a system for saturation of the genetic map of durum wheat. Euphytica 194(2): 151-160

.

Mirza, H., Kamrun N, MdMA, Rajib R and Masayuki F. (2013). Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. International Journal of Molecular Sciences 14(5):9643-9684

Mondal, S., Singh, R. P., Crossa, J., Huerta-Espino, J., Sharma, I., Chatrath, R., ... and Kalappanavar, I. K. (2013). Earliness in wheat: a key to adaptation under terminal and continual high temperature stress in South Asia. Field Crops Rresearch 151:19-26.

Moustafa, K. A., Saleh, M., Al-Doss, A. A., Elshafei, A. A., Salem, A. K., Al-Qurainy, F. H., and Barakat, M. N. (2014). Identification of TRAP and SRAP markers linked with yield components under drought stress in wheat (*Triticum aestivum* L.). Plant Omics 7(4):253.

Nissen, O. (1984). MSTAT. A microcomputer program for statistical analyses of experiments and surveys. In 10. General Meeting of the European Grassland Federation, Aas (Norway), 26-30 Jun 1984. Norwegian State Agricultural Research Stations.

Porter, D. R., Nguyen, H. T., and Burke, J. J. (1995). Genetic control of acquired high temperature tolerance in winter wheat. Euphytica 83(2):153-157.

Reynolds, M. P., Ortiz-Monasterio, J.L., and McNab, A. (2001). Application of physiology in wheat breeding. Cimmyt.

Saleh, M. S., Al-Doss, A. A., Elshafei, A. A., Moustafa, K. A., Al-Qurainy, F. H., and Barakat, M. N. (2014). Identification of new TRAP markers linked to chlorophyll content, leaf senescence, and cell membrane stability in water-stressed wheat. Biologia Plantarum 58(1):64-70.

Tewolde, H., Fernandez, C. J., and Erickson, C. A. (2006). Wheat cultivars adapted to post-heading high temperature stress. Journal of Agronomy and Crop Science 192(2):111-120.

Wang, H., Liu, D., Yan, Z., Wei, Y., and Zheng, Y. (2005). Cytological characteristics of F~ 2 hybrids between Triticum aestivum L. and T. durum Desf. with reference to wheat breeding. Journal of Applied Genetics 46(4):365.

Wardlaw, I. F., and Moncur, L. J. F. P. B. (1995). The response of wheat to high temperature following anthesis. I. The rate and duration of kernel filling. Functional Plant Biology 22(3):391-397.

Youssef, M., Valdez-Ojeda, R., Ku-Cauich, J. R., and Escobedo-GraciaMedrano, R. M. (2015). Enhanced protocol for isolation of plant genomic DNA. Journal of Agriculture and Environmental Sciences 4(2):172-180.

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D. B., Huang, Y., ... and Durand, J. L. (2017). Temperature increase reduces global yields of major crops in four independent estimates. Proceedings of the National Academy of Sciences 114(35):9326-9331.

تقدير تحمل الحرارة في بعض الأنواع والهجن النوعية للقمح

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تم في هذه الدراسة استخدام 12 تركيب وراثي من القمح تشتمل على تركيبين ثنائي وأربعة تراكيب رباعية وست تراكيب سداسية المجموعة الكروموسومية. وتم اجراء كافة التهجينات الممكنة، حيث تم تقييم الآباء والهجن النوعية الناتجة لتحمل الاجهاد الحراري في ميعادين زراعة. اعتمد التقييم على بعض الصفات المورفولوجية والفسيولوجية مثل وزن الألف حبة، محصول الحبوب لكل نبات، الثبات الحراري للغشاء الخلوي وصفة اختزال كلوريد التترازيليوم. أظهرت النتائج اختلافاً معنوياً بين التراكيب الوراثية والهجن النوعية للقمح في جميع الصفات المدروسة تحت كلاً من الظروف المثلى وظروف الاجهاد الحراري. وبالرغم من ذلك، أظهرت التراكيب رباعية المجموعة الكروموسومية الأداء الأعلى تحت ظروف الاجهاد تتبعها التراكيب السداسية ثم الثنائية، على التوالي. بناءً على نتائج دليل تحمل الحرارة، تم اختيار تركيب وراثي متحمل وآخر حساس كآباء من كل مجموعة وراثية وأيضاً ست هجن نوعية لإجراء التحليل الجزيئي. حيث تم استخدام الواسم الجزيئي TRAP المعتمد على بادئات خاصة بجينات بروتينات الصدمة الحرارية للقمح. وأكدت نتائج الواسم TRAP ما تم الحصول عليه من نتائج على المستوى المورفولوجي والفسيولوجي. حيث كان هناك اختلافاً في درجة التنوع الوراثي داخل كل مجموعة تعدد كروموسومي من القمح وانعكس ذلك في نسبة تعدد الأشكال الناتجة من كل مجموعة بناءً على نتائج الـ TRAP. كما تمكن الواسم من إظهار عدد حزمة واحدة خاصة بالنباتات ذات التركيب الثنائي، بالإضافة لعدد خمس حزم خاصة بالنباتات سداسية المجموعة بينما لم تتكون أي حزم خاصة بالنباتات الرباعية. بالإضافة لذلك، أظهرت مجاميع القمح المختلفة بعض الحزم المشتركة بين كل مجموعتين، فيما يوضح علاقة الجينومات المشتركة. ومن الجدير بالذكر، أنه تكوَّن عدد حزمتين خاصتين بالتراكيب الوراثية والهجن النوعية المتحملة للحرارة، حيث كانتا غائبتين في جميع النباتات الحساسة. وتعتبر النتائج المتحصل عليها في هذه الدراسة ذات أهمية بالغة، حيث يمكن الاستفادة منها في برامج التربية المستقبلية لتحسين القمح.