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DIVERGENT PHENOTYPIC SELECTION AND MOLECULAR MARKER ANALYSIS FOR HEAT TOLERANCE IN BREAD WHEAT (*Triticum aestivum* l.)

El-Rawy, M. A.
Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt

ABSTRACT

This study aimed to performed divergent phenotypic selection for 1000-kernel weight (1000-KW) under heat stress in a population of 140 F_8 recombinant inbred lines (RILs) derived from a cross between tolerant and susceptible landraces of bread wheat (*Triticum aestivum* L.). Correlated responses to selection for grain yield per plant, cell membrane thermostability (CMS) and reduction in tetrazolium chloride (TTC) traits were also measured. Grain yield per plant was evaluated in the field at both optimal and late sowing dates, while CMS and TTC were assayed at seedling stage. Highly significant responses to selection were obtained for 1000-KW in both high (9.76%) and low (4.64%) directions. Realized heritability for 1000-KW (h^2= 0.28) was similar to those calculated from the parent-offspring regression (b_{po}= 0.29). Selection for low 1000-KW produced highly significant and positive correlated responses for grain yield per plant (7.95%), CMS (17.24%) and TTC (36.74%), whereas selection performed for high 1000-KW only produced a highly significant and positive correlated response for grain yield per plant (9.74%). Highly significant and positive correlation coefficients were found between 1000-KW with grain yield per plant (r= 0.54, *P*<0.01), CMS (r= 0.31, *P*<0.05) and TTC (r = 0.52, *P*<0.01). Grain yield per plant was also significantly and positively correlated with CMS (r= 0.31, *P*<0.05) and TTC (r= 0.69, *P*<0.01). A highly significant and positive correlation was also observed between CMS and TTC (r= 0.40, *P*<0.01). Out of five simple sequence repeats (SSR) and three inter simple sequence repeats (ISSR) markers used to screen ten RILs selected for 1000-KW, two SSR (Xgwm456 and Xwmc596) and a single ISSR (HB-13) markers were specific for 1000-KW. These markers could be considered as reliable markers for heat tolerance in wheat breeding programs.
Keywords: Bread wheat, Heat tolerance, Selection, CMS, TTC and SSR markers.

INTRODUCTION

High temperature stress is one of the major threats to crop production worldwide, particularly at reproductive and grain-filling stages (Hall 2001). It reduces plant photosynthetic capacity through metabolic limitations and oxidative damage to chloroplasts, with concomitant reductions in dry matter accumulation and grain yield (Farooq *et al*. 2011). Global mean temperature may rise up to 0.3 °C per decade (Jones *et al.* 1999) reaching to approximately 1 °C and 3 °C above the normal temperature by years 2025 and 2100, respectively (Wahid *et al.* 2007). The optimum temperature required for growth and development of wheat ranges from 18 to 24 °C and even short periods (5–6 days) of wheat crops exposure to temperatures of 28 to 32 °C may result up to 20 percent decrease in yield (Mullarkey and Jones

2000). Heat stress at the time of anthesis until ripeness significantly reduces grain yield. Moreover, heat stress (35 °C) that began 10 days after anthesis and continued until ripeness caused a reduction by 78% in grain yield and 63% in kernel number. Besides, it decreased the kernel weight by 29%. It is known that, heat stress that began 15 days after anthesis and continued until complete ripeness caused an effect not only on kernel number but also decrease about 18% on kernel weight (Gibson and Paulson, 1999). Thus, heat stress in wheat is a major factor caused yield reduction in many wheat-growing regions of the world including the Mediterranean regions like Egypt.

Heat tolerance in wheat would be improved by selecting and developing genotypes with heat resistance. Wheat pre-breeding and breeding may be based on secondary traits like membrane stability, photosynthetic rate and grain weight under heat stress. Nonetheless, improvement grain yield under heat stress implies selecting genotypes for grain size and rate of grain filling (Farooq *et al.* 2011). Several techniques have been developed for measuring heat tolerance of crop plants which include cell membrane thermostability (Martineau *et al*. 1979; Sullivan and Ross 1979; Saadalla *et al*. 1990a) and using 2,3,5-triphenyl tetrazolium chloride reduction (Towill and Mazur 1974; Chen *et al*. 1982). Cell membrane thermostability (CMS) is based on the observation of leaf tissue injury caused by high temperature increases membrane permeability and electrolytes diffuse out of the cell into the solution. Relative heat damage is assessed by measuring the amount of electrolyte leakage from injured cells by the electrical conductivity of the solution. Heat hardening of the plant samples is performed prior to measuring CMS by exposure to mild heat stress in order to induce tolerance to high temperature. The cell membranes are thought to be the primary site of direct high temperature injury (Levitt 1980; Blum 1988). Leakage of solutes through the membrane after heat stress has been measured by electrical conductivity and used in many crops including wheat, soybean and vegetables as an index of membrane stability to identify heat tolerant genotypes (Sullivan and Ross 1979; Martineau *et al.* 1979; Saadalla *et al.* 1990b; Shanahan *et al.* 1990). High temperature stress in plants also causes oxidative damage to respirational process which is based on the principles of tetrazolium salt reduction to formazan by dehydrogenase respiratory enzyme thus evaluating the chain of mitochondrial electron transport which represents respirational activity. TTC reduction has been widely used in the viability assay of plant tissues exposed to high temperature. In addition, genotypic differences in thermotolerance were evaluated in different plant tissues (Chen *et al.* 1982; Fokar *et al.* 1998; Gupta *et al.* 2010; Satyavir and Handa, 2012).

Due to the general complexity of abiotic stress tolerance and the difficulty of phenotypic selection for tolerance, marker-assisted selection (MAS) has been considered as an effective approach to disscover and improve plant stress tolerance (Foolad 2005). The use of this approach requires identification of genetic markers associated with genes or quantitative traits loci (QTLs) affecting on plant stress tolerance or individual contributing components. Genetic associations of various molecular markers including microsatellite or simple sequence repeats (SSR) and inter simple

sequence repeats (ISSR) markers with heat tolerance have been reported in wheat (Sofalian *et al.* 2008; Ciuca and Petcu 2009; Barakat *et al.* 2012). Moreover, quantitative and molecular characterization of heat tolerance in hexaploid wheat has also been reported (Yang *et al.* 2002). Therefore, integrating physiology and biotechnological tools with conventional breeding techniques would help to develop wheat varieties with better grain yield under heat stress during reproductive and grain-filling phases (Farooq *et al.* 2011).

In Egypt, a large number of wheat landraces have been preserved, which possessed abundantly genetic diversity, including many heat tolerance genes. Thus, it is of great importance to identify tolerant genetic resources among there landraces and use them in wheat breeding programs aiming to develop improved varieties. In the present study, divergent phenotypic selection for 1000-KW was applied under heat stress to a population of 140 F_8 recombinant inbred lines (RILs) derived from a cross between two local landraces of bread wheat (*Triticum aestivum* L.), quite variable in heat susceptibility index. Objectives of this study were: (1) to estimate the response to selection for 1000-KW under heat stress and to study the correlated responses of grain yield per plant, CMS and TTC traits; (2) to identify molecular markers associated with heat tolerance in bread wheat.

MATERIALS AND METHODS

Genetic material and field trials

The basic genetic material utilized in the present study consisted of 140 F_8 recombinant inbred lines (RILs) derived from a cross between two local landraces of bread wheat (*Triticum aestivum* L.), quite variable in their performance under heat stress conditions. The two parental landraces WA125 (tolerant) and WA120 (susceptible) were chosen from germplasm collected from farmer's fields in stressful area in Upper Egypt in 1993 and maintained since then at the Department of Genetics of Assiut University in Egypt. They were characterized for heat susceptibility index (Omara 1994).

Field traits

Field experiment was carried out at the Experimental Farm of the Faculty of Agriculture, Assiut University, Assiut, Egypt. In the 2011/2012 winter season, seeds of 140 F_8 recombinant inbred lines (RILs) were planted in the field at late sowing date (25 December). Each RIL was represented by a 10-plant/row with rows spaced 30 cm apart and plants within rows set 30 cm from each other. Temperatures during February and March 2013 were provided by Agriculture Meteorological Station at Assiut Governorate which indicated that heat waves occurred with temperature raised above 34 °C for several days (Fig. 1) which coincided with the post flowering stages of plant development. At the maturity stage, twenty spikes were hand harvested randomly from each row (with excluding the plants near the boarders) and 1000-kernel weight (1000-KW) was recorded for each RIL.

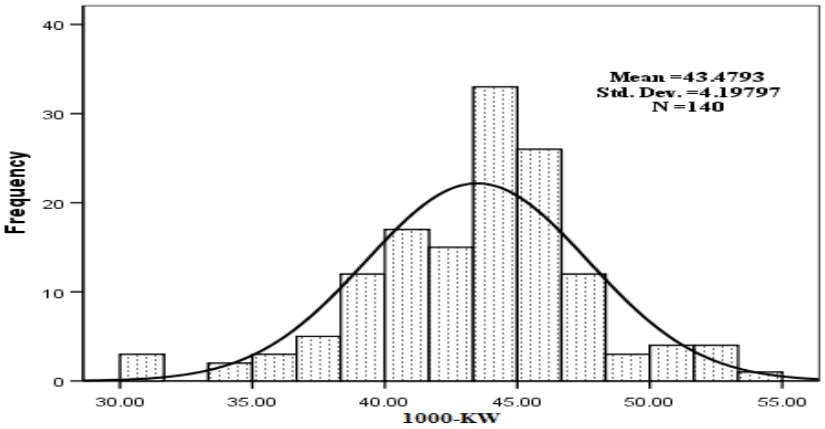

Fig. 1. Frequency distribution of 140 RILs for 1000-KW under heat stress.

Selection procedure

Based on phenotypic data recorded, divergent selection for 1000-KW was applied to the 140 F_8 RILs population. The highest and the lowest five RILs in 1000-KW were selected (an intensity of 3.6%) in the high and low direction. Equal numbers of seeds were pooled from each RIL in order to construct the non-selected (bulk) RILs. In the 2012/2013 winter season, seeds of the five RILs selected for 1000-KW in the high direction and the five RILs selected in the low direction, as well as, the bulk were sown in the clay soil of the Experimental Farm of the Faculty of Agriculture of Assiut University at optimal (25^{th} November) and late (2^{nd} January) sowing dates. The field trials were organized as a randomized complete blocks design (RCBD) with three replications for selected and bulked RILs. Each selected RIL was represented in each block by a row of 10 plants spaced 30 cm apart within rows set at 30 cm from each other, whereas the bulk was represented in each block by three rows. At the maturity stage, grain yield per plant (grams) and 1000-KW (grams) measured were recorded for each RIL on individual plant for each block of the two sowing dates.

Cell membrane thermostability (CMS)

In order to perform cell membrane thermostability (CMS) a laboratory experiment at seedling stage was applied to the RILs selected for 1000-KW in both high and low directions, as well as, the bulk. Observations were recorded from five samples of each RIL grown in Petri dish at 25 $^{\circ}$C at the laboratory in three replications. For acclimation, 10-day-old seedlings were acclimated for 48 hours at 37°C in incubator. Leaf disks (2 cm) were taken from leaf number 4 at 10 day old seedlings (4 leafs stage). For each sample, a 2 cm^2 segment was taken from the middle of the leaf, cut into equal halves, washed three times in distilled water and each half was placed in a 10 ml capped vial containing 1 ml of distilled water (4 leaf segment for each tube). The treatment tubes were heated in a water bath for 1 h at 49°C while control

tubes remained at room temperature. After treatment, 9 ml of distilled water was added to all vials (treatment and control) and incubated at 6°C for 24 h. Vials were then brought to room temperature and solution conductance was measured with a conductivity meter (model WPA CM 35 Linton Cambridge, England). After the measurements were taken, vials were autoclaved for 2 min at 120°C and their conductance was measured again. CMS was calculated as the reciprocal of cell membrane injury following Blum and Ebercon (1981) formula as follows:

$$CMS(\%) = \frac{\left(1 - T_1 / T_2\right)}{\left(1 - C_1 / C_2\right)} x100$$

Where T and C refer to the stress and control samples, respectively; the subscripts 1 and 2 refer to the initial and final conductance readings, respectively.

Reduction in tetrazolium chloride (TTC)

Observations were recorded from five samples of each RIL grown in Petri dish at 25 °C at the laboratory in three replications. For acclimation, 10-day-old seedlings were acclimated for 48 hours at 37°C in incubator. After acclimation, two sets of two leaves (3.5 cm each) per sample were excised, rinsed in distilled water, and placed singly in a test tube with 0.1 ml of distilled water. Out of two sets, the first set was kept at 25 °C for 90 min as control set, and the second set was placed in a water bath at 49 °C for 90 min. Immediately following the 25 °C and 49 °C, 10 ml of TTC solution (0.8% TTC in 0.05 M NaPO4 buffer, pH 7.4,and 0.5 ml/l Tween 20) was added per tube. The tissues were incubated in the TTC solution for 24 h at 25 °C in dark. After incubation, the leaves were removed and rinsed with distilled water, placed individually in separate spectrophotometric tubes containing 2 ml of 95% ethanol, and submerged for 24 h at 25 °C in the dark. The level of acquired high temperature tolerance was determined by measuring the percentage reduction of TTC to formazan using Ibrahim and Quick (2001) formula as follows:

TTC = ODh / ODc × 100.

Where ODh referred to the mean optical density (530 nm) values for the heat-stressed set (49 °C for 90 min), and ODc referred to the mean optical density for the control set (25 °C for 90 min).

Molecular markers analysis

DNA extraction from young and fresh leaves of each RIL selected for 1000-KW in both high and low directions was carried out according to the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980) with some modifications. The highest and lowest five RILs were screened for differences using five simple sequence repeat (SSR) and three inter simple sequence repeat (ISSR) markers. Primers sequences and PCR conditions of five SSR markers (ERAD, Xgwm456, Xgwm566, Xwmc596 and Xwmc603) and three ISSR markers (H12, H13 and H15) were obtained by

the database provided at the web site http://www.graingenes.gov. PCR amplifications were performed in 25 µl reaction mixtures, each containing 50-100 ng of genomic DNA, 1X PCR buffer, 2 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of each primer, and 1 U Taq DNA-polymerase. Amplifications were performed in a Senso Quest Lab Cycler (Senso Quest GmbH, Göttingen, Germany) using the following PCR profile: initial denaturation at 94°C for 3 min, followed by 45 cycles each consisting of 1 min at 94°C, 1 min at 38-61 °C (depending on the suggested annealing temperature), followed by 2 min at 72°C, with a final extension at 72°C for 10 min. PCR products were separated using horizontal gel electrophoresis unit on 2% agarose gels in 0.5 X TBE buffer. A 100 bp DNA ladder was used to estimate the size of each amplified DNA fragment. The gel was run for approximately 2-3 hours using constant voltage of around 80 V and then visualized and photographed under UV light. Putative polymorphisms were detected for each marker separately.

Statistical and genetic analyses

Direct response to selection (R), the difference between the mean phenotypic value of the offspring of the selected parents and the whole of the parental generation before selection, for 1000-KW in both directions and correlated responses to selection for grain yield per plant, CMS and TTC traits were obtained using the analysis tools of Microsoft Excel. Each sowing date was treated as an environment in the subsequent statistical analysis. To test for the significance of differences among the genotypes and the environments and the significance of genotype-by-environment (GxE) interaction, data of five RILs selected for high 1000-KW and five RILs selected in the low direction, as well as, three bulked RILs were statistically analyzed using a combined analyis of variance across envirnments. Pearson's correlation coefficients among different traits related to heat tolerance were also estimated. The significance of correlation was tested against the value of *t*-tabulated. Similarity and cluster analyses based on ISSR and SSR markers, as well as, different traits studied were done using NTSYS pc 2.01 software (Rohlf 1998). Dendrograms were produced according to the unweighted pair-group mean arithmetic method (UPGMA) using NTSYS pc 2.01 Software.

Heritability estimation

Heritability of each traits was estimated by the following two methods:

Realized heritability was calculated as follows:

$$h^2 = \frac{\left[\overline{H}_S - \overline{L}_S\right]}{\left[\overline{H}_B - \overline{L}_B\right]}$$

Where: $\overline{H}_S$ and $\overline{L}_S$ are the average of the F_n RILs selected for a 1000 KW in the high and low directions, respectively. While $\overline{H}_B$ and $\overline{L}_B$ are the average of the F_{n-1} RILs selected for that trait is the two directions (Ibrahim and Quick 2001).

Parent-offspring regression (b_{po}) was determined for 1000- KW by regressing the means of the F_n selected RILs on the means of their corresponding F_{n-1} progenitor plants.

RESULTS AND DISCUSSION

Responses to selection and heritability estimates

The distribution of 140 RILs segregates for 1000-KW under late sowing date illustrated in Fig. 1 was continuous and approached normality, indicating that 1000-KW is under the control of polygenes and amenable to selection. The 1000-KW of the 140 RILs ranged from 30.5 to 55.42 (g) with an average of 43.48 (g). The selection differential in the high direction was 22.03%, whereas the selection differential in the low direction was higher in magnitude than those in the high direction being 30.45% (Table 1).

Table 1. Means of 1000-KW of 140 RILs population estimated under heat stress.

Traits	Population Mean	Mean of the selected F_8 RILs		Selection differential	
		High	Low	High	Low
1000-KW	43.48	53.06	30.24	9.58	13.24

Positive and high significant responses to selection for 1000-KW were obtained in both high and low directions at favorable and heat stress environments (Table 2). The average 1000-KW of RILs selected reduced from 50.81 g under favorable to 43.76 under heat stress conditions (reduction percentage 13.87%). The % response in the high and low directions were symmetrical in favorable environmental. However, asymmetrical responses were obtained under heat stress conditions. A moderate realized heritability value was observed for 1000-KW (h^2= 0.28) and was found to be similar and corresponded to the heritability estimate obtained by the parent-offspring regression (b_{po}= 0.29). The heritability values obtained in this study were lower than heritability estimates reported by Muhammad and Ihsan (2004); Bayoumi and El- Demardash (2008); Amin (2013) and Sallam *et al.* (2014).

Table 2. Response to selection for 1000-KW in high and low directions.

Traits	Environments	Means			% O. Responses		Heritability	
		High	Bulk	Low	High	Low	Realized	b_{po}
1000-KW	Favorable	56.13	50.81	44.17	10.46**	13.06**		
	Heat stress	48.03	43.76	41.73	9.76**	4.64**	0.28	0.29**

Correlated responses to selection and phenotypic correlations

Selection for low 1000-KW under heat stress revealed highly significant and positive correlated responses in grain yield per plant (7.95%), CMS (36.74%) and TTC (17.24%). However, selection for high 1000-KW only produced a highly significant and positive correlated responses in grain yield

per plant being 9.74% (Table 3). This would be due to the selection differentials in the high direction which were lower than those obtained in the low direction.

Table 3. Correlated responses to selection for different traits.

Environments	Traits	Means			% Observed responses	
		High	Bulk	Low	High	Low
Favorable	Grain yield	56.97	57.34	43.60	-0.64	23.97**
Heat stress		45.95	41.87	38.54	9.74**	7.95**
Seedling stage	TTC	55.28	53.59	33.91	3.15	36.74**
	CMS	67.80	66.78	55.27	1.53	17.24**

Cellular thermotolerance in terms of cellular membrane thermostability is often implied as an indication of crop heat tolerance and it is therefore considered as a possible selection criterion for heat tolerance (Blum *et al.* 2001). Regarding the importance of CMS and TTC assays as heat tolerance indices at seedlings stages, Saadalla *et al.* (1990a) concluded that heat tolerance measured at seedling stage would predict thermotolerance of the fully developed plants. Despite the apparent advantages of measuring CMS in seedlings which requires less resources, its main disadvantage is the limited number of plants that would be processed in a controlled environment facility. Genetic variation in membrane thermostability has been inferred from conductometric measurements in various field grown crops, including spring wheat (Muthappa *et al.* 2007). Shanahan *et al.* (1990) obtained a significant increase in yield of spring wheat in hot locations by selection of membrane thermostable lines, as determined by measurements on flag leaves at anthesis. Applying the membrane thermostability test to various crops, a high correlation in between membrane thermostability and grain yield has been reported by various researchers (Thiaw and Hall 2004; Shah *et al.* 2011). Although both CMS and TTC assays gave similar results in measuring heat injury, Fokar *et al.* (1998) concluded that TTC is less predictive of plant performance under heat stress than CMS.

The analysis of variance (Table 4) revealed highly significant differences among RILs selected for 1000-KW under favorable and heat stress conditions.

Table 4. Combined analysis of variance for different traits under favorable and heat stress conditions.

S. O. V.		Environments	Rep./ Env.	Genotypes	G x E	Error	$h^2_{(B)}$
d.f		1	4	12	12	48	
Traits	1000-KW	6.30**	0.03	1.50**	0.54**	0.03	0.45
	Grain yield	1611.8**	11.49	473.14**	222.67**	7.44	0.35

Phenotypic correlation coefficients for all comparisons among the traits studied are presented in Table 5. Highly significant and positive correlation

coefficients were found between 1000-KW with grain yield per plant (r= 0.54, *P*<0.01), CMS (r= 0.31, *P*<0.05) and TTC (r = 0.52, *P*<0.01). Grain yield per plant was also significantly and positively correlated with CMS (r= 0.31, *P*<0.05) and TTC (r= 0.69, *P*<0.01). A highly significant and positive correlation was also observed between CMS and TTC (r= 0.40, *P*<0.01).

These findings are in agreement with those reported by Blum *et al.* (2001) on CMS being correlated with grain yield under heat stress. Similar positive associations between CMS and grain yield under drought and heat stresses were also reported in wheat by Shanahan *et al.* (1990); Blum and Ebercon (1981); Tripathy *et al.* (2000); Ibrahim and Quick (2001); Omara *et al.* (2006 and 2010) and sharma *et al.* (2014). CMS was also positively correlated with TTC under heat stress as reported by Satyavir and Handa (2012).

Table 5. Correlation coefficients (r) among different traits related to heat tolerance.

Traits	TTC	CMS	1000 KW	G yield
TTC	1.00	0.40**	0.52**	0.69**
CMS		1.00	0.31*	0.31*
1000 KW			1.00	0.54**
G. yield				1.00

Molecular markers and cluster analysis

In the present study, five SSR and three ISSR markers were used to study the molecular differences among ten RILs selected for both high and low 1000-KW under heat stress. A total number of 54 DNA fragments were amplified using these markers from all selected RILs with an average of 6.75 bands per marker. The amplified fragments ranged in size from 985 bp for H12 marker to 39 bp for Xgwm566 marker (Table 6 and Fig. 2). The highest number of amplified DNA fragments (10 bands) was obtained using H15 marker, while the lowest number (5 bands) was generated with Xgwm566 and Xwmc596 markers. The RIL L1 selected for low 1000-KW displayed the highest number of DNA fragments (51 bands), while those selected for high 1000- KW (H1) revealed the lowest number of bands (39 bands). Such variation in the number of bands amplified by different primers is attributable to several factors including primer structure and number of annealing sites in the genome (Kernode et al*.* 1993).

Table 6. PCR amplification results and polymorphism between RILs selected for high 1000-KW and RILs selected for low 1000-KW using three ISSR and five SSR primers.

Marker	Sequence (5'-3')	Fragment range (bp)	No. of bands	No. of polymorphic bands	Polymorphic %
ERAD	CAACTCTGAACTATTGCAAAAGTGAACTTT CTGCAATATCGGTGAGTTTCTTGTAGTTAA	45 - 172	6	3	50
Xgwm456-1D	TCTGAACATTACACAACCCTGA TGCTCTCTCTGAACCTGAAGC	81-306	6	2	33.3
Xgwm566-3B	TCTGTCTACCCATGGGATTTG CTGGCTTCGAGGTAAGCAAC	39-134	5	3	60
Wmc596-7A	TCAGCAACAAACATGCTCGG CCCGTGTAGGCGGTAGCTCTT	95-297	5	3	60
Wmc603-7A	ACAAACGGTGACAATGCAAGGA CGCCTCTCTCGTAAGCCTCAAC	67-212	6	3	50
HB-12	CACCACCACGC	294-985	9	0	0
HB-13	GAGGAGGAGGC	69-274	7	3	42.8
HB-15	GTGGTGGTGGC	217-975	10	0	0
Total	-	-	54	17	31.5

Among the eight markers used, six markers detected polymorphism among selected RILs, whereas two markers (HB-12 and HB-15) displayed monomorphic patterns. Out of 54 DNA fragments amplified, 17 bands (31.5%) were polymorphic while the rest (68.5%) were common among the 10 RILs. The highest number of polymorphic bands (3 bands) was amplified by ERAD, Xgwm566, Xwmc596 and Xwmc603 markers, while the HB-12 and HB-15 markers generated the lowest number of polymorphic bands (0 bands). However, the percentages of these polymorphic bands were differed according to the number of bands generated by the tested marker. These results suggested that polymorphic bands revealed differences could be used to examine and establish systematic relationships among genotypes as reported by Hadrys *et al.* (1992).

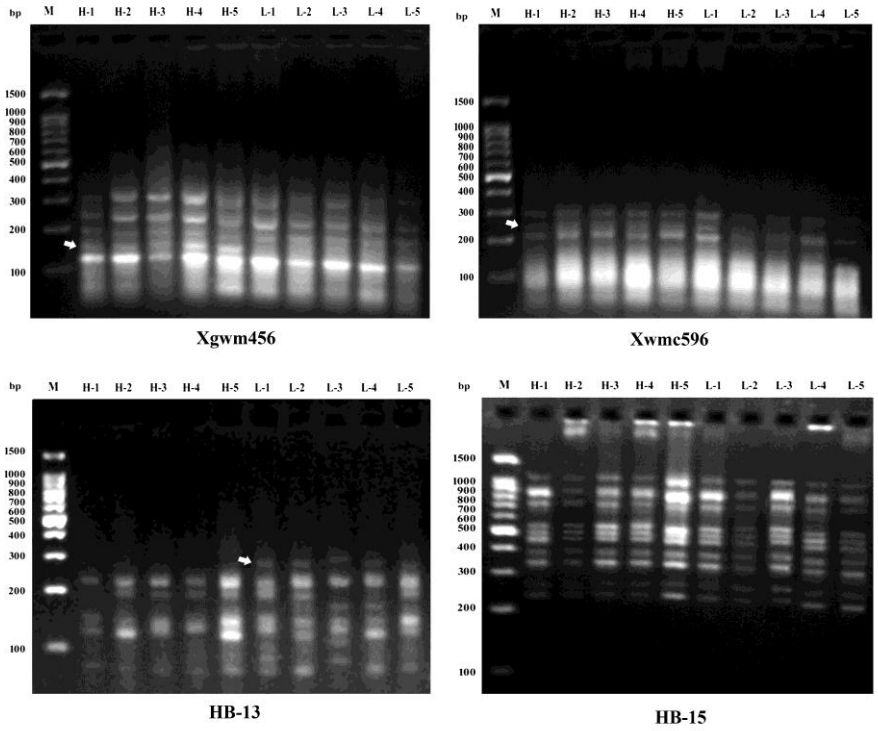

Fig. 2. DNA amplification patterns obtained using two SSR and two ISSR markers. M is the 100bp DNA ladder, H-1 to H-5 indicate RILs selected for high 1000-KW and L-1 to L-5 indicate RILs selected for low 1000-KW. Differences between the two selected groups were detected using Xgwm456, Xwmc596 and HB-13 markers. Arrows indicate polymorphic bands obtained which distinguished selected high from selected low RILs. The profile of HB-15 ISSR marker is presented as an example for a marker showing monomorphic amplification patterns.

DNA amplification patterns obtained using SSR and ISSR markers (Fig. 2) indicated that unique DNA fragments with different sizes were found to be specific for selected high or selected low RILs. It is interesting to note that the 155 bp DNA fragment amplified by the Xgwm456 marker was detected in all RILs selected for high 1000-KW. Similarly, the 245 bp DNA fragment amplified by Xwmc596 marker was detected in all RILs selected for high 1000-KW, but also was found in the RIL L1 which was selected for low 1000-KW. In addition, the 274 bp DNA fragment amplified by the HB-13 marker was detected in all RILs selected for low 1000-KW (Fig. 2). These results suggested that these DNA fragments are unique bands for high or low 1000-KW which could be used as markers specific for 1000-KW as an indicator for heat tolerance in wheat breeding programs. Naghavi *et al.* (2003)

suggested that multiple allelism is very common in SSR markers and they are able to produce different alleles in one locus. Similarly, many studies have reported remarkable differences in allelic diversity among various microsatellite loci (Ravi *et al*. 2007; Ram *et al.* 2007). The markers Xgwm456 and Xgwm566 were assigned to chromosomes 1D and 3B, respectively, and the homoeologous groups of chromosomes 2, 3, 5 and 7 of wheat contain a number of genes that are important for tolerance to abiotic stress (Somers *et al.* 2004; Golabadi *et al.* 2011). Barakat *et al.* (2012) concluded that the R^2 values suggested that Xgwm456-linked QTL and Xgwm566-linked QTL account 33 and 64% of the total phenotypic variation, respectively, in heat tolerance in the F_2 population for grain filling duration. An association was reported by Banica *et al.* (2008) between membrane stability and genetic differences in the capacity of osmotic adjustment expressed in pollen grains, suggested that Xwmc596 and Xwmc603 markers might be associated with the "*or*" gene for the controller of osmotic adjustment. Moreover, Ciuca and Petcu (2009) reported that Xwmc596 and Xwmc603 markers are weakly but significantly associated with cell membrane stability after water stress. In addition, ISSR markers, which were used to assess genetic diversity among common wheat, and progeny of recurrent selection, can differentiate wheat cultivars or lines that selected from the same cross combination (Du *et al.* 2002). Extensive DNA polymorphism has been reported using ISSR markers in several other crops plants (Blair *et al.* 1999; Sofalian *et al.* 2008; Kantety *et al.* 1995; Hou et al. 2005). However, Sharma *et al.* (2014) reported that the ISSR marker were useful for characterizing genetic relatedness but could not distinguish the level of heat tolerance.

In general, molecular markers have been proved to be an important way to increase selection efficiency and there are good prospects for marker-assisted selection in improving drought responses in wheat (Quarrie *et al.* 2003). Due to difficulty of managing of heat tolerance through conventional phenotypic selection and the presence of several QTLs for a single target trait with complex inheritance, the selection of target traits can be achieved indirectly using molecular markers that are closely inked to underlying genes or that have been developed from the actual gene sequences. QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress have been reported (Mason *et al.* 2010). Therefore, the use of correlation and co-segregation analysis, and molecular marker techniques in genetic stocks with different degrees of heat tolerance are promising approaches to dissect.

Similarity and Cluster analysis

Cluster analysis was performed to categorize the 10 RILs selected in both directions for genetic variation in heat tolerance based on the 1000-KW, grain yield per plant, CMS and TTC traits. The dendrogram constructed based on these phenotypic traits classified the 10 RILs into two groups or clusters (Fig. 3). Cluster I consisted of all the five RILs selected for low 1000-KW (L1, L2, L3, L4 and L5), while the five RILs selected for high 1000-KW (H1, H2, H3, H4 and H5) were grouped in the Cluster II.

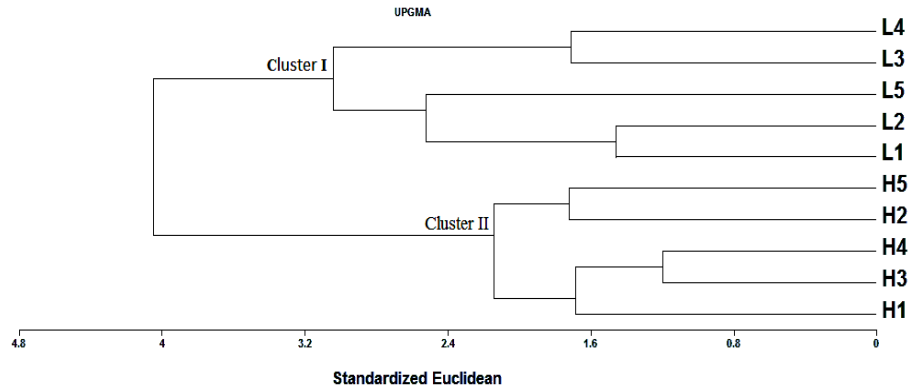

Fig 3. Dendrogram of measured traits for selected 10 RILs by using UPGMA method.

The dendrogram tree among the ten RILs which were selected for high and low 1000-KW resulting from the UPGMA clustering values using ISSR and SSR markers is presented in Fig. 4. The analysis was basically based on the number of markers that were different between any given pair of RILs. The combined dendrogram obtained using SSR and ISSR markers clearly indicated two clusters. Four out of five RILs selected for low 1000-KW (L2, L3, L4 and L5) were grouped in cluster I. However, the cluster II collected four out of the five RILs selected for high 1000-KW (H2, H3, H4, and H5) side by side with a single RIL selected for low 1000-KW (L1) within genetic similarity ranged from 0.98 to 0.91. The H3 and H4 were clustered together firstly within a genetic similarity of 0.98 and then with H5 and H2 within a common genetic similarity of 0.95 followed by L1 within a common genetic similarity of 0.91.

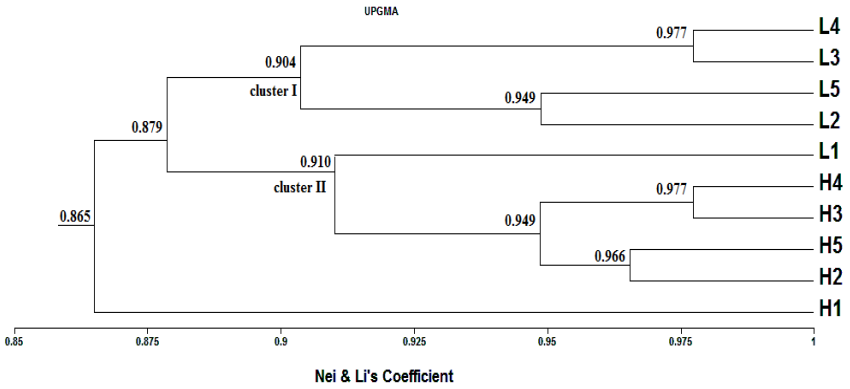

Fig 4. Dendrogram constructed from similarity coefficients showing the clustering selected 10 RILs using ISSR and SSR markers

The results presented here in reflected that these four out of five RILs selected for high 1000-KW (H2, H3, H4, and H5) were closely related. Moreover, the dendrogram reveled that the high 1000-KW RILs were clustered together in the lower side of the dendrogram while those with low 1000-KW clustered in the upper side. These results indicated that there is discrimination features between RILs selected for high 1000-KW and RILs selected for low 1000-KW at the molecular level based on SSR and ISSR-markers. Therefore, SSR and ISSR markers identified in the study would be used as markers associated with 1000-KW as an indicator for heat tolerance. Moreover, RILs selected for high 1000-KW would be consider as the mot tolerant genotypes to be used as parents for developing improved varsities in wheat breeding programs. In agreements with these results, cluster analysis has been widely used for description of genetic diversity and grouping based on similar characteristics under stress condition (Golabadi *et al.* 2006; Golestani and Pakniat 2007; Mohammadi *et al.* 2011; Tabatabaei 2013; El-rawy and Hassan, 2014).

In conclusion, Positive and high significant response to selection for 1000-KW and correlation coefficients obtained between 1000-KW with grain yield, CMS and TTC under heat stress indicated the importance of these traits as heat tolerance indictors in wheat. Molecular markers identified in the present study would be used as markers specific for 1000-KW and reliable markers for heat tolerance. Moreover, RILs selected for high 1000-KW would be characterized as the most tolerant genotypes for developing improved varieties in wheat breeding programs.

REFERENCES

Amin, I.A. (2013). Genetic Behaviour of Some Agronomic Traits in Two Durum Wheat Crosses under Heat Stress. Alex. J. Agric. Res. 58 (1) 53-66.

Banica, C.; E. Petcu; A. Giura and N. N. Săulescu (2008). Relationship between genetic differences in the capacity of osmotic adjustment and other physiological measures of drought resistance in winter wheat (Triticum aestivum L.). Romanian Agricultural Research, 25:7-12.

Barakat, M.N; A. Al-Doss; A.A. Elshafei; A.K.A. Moustafa (2012). Bulked segregant analysis to detect quantitative trait loci (QTL) related to heat tolerance at grain filling rate in wheat using simple sequence repeat (SSR) markers. Afr. J. Biotechnol. 11: 12436-12442.

Bayoumi, Y.T. and I.S. El-Demardash. (2008). Influence of nitrogen application on grain yield and end use quality in segregating generations of bread wheat (Triticum aestivum L). Afr. J. Biochem. Res. 2: 132-140.

Blair, M.W.; O. Panaud and S.R. McCouch (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (Oryza sativa L.). Theor. Appl. Genet. 98: 780-792.

Blum, A and A. Ebercon (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21: 43-47.

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Inc. Boca Raton.

Blum, A.; S. Torch N. Lambeng; M. Nissou; A.L. Benabid; R. Sadoul and J.M. Verna (2001). Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. Prog Neurobiol 65:135–172

Chen, H.H.; Z.Y. Shen and P.H. Li (1982). Adaptability of crop plants to high temperature stress. Crop Sci. 22: 719–725.

Ciuca, M. and E. Petcu (2009). SSR markers associated with membrane stability in wheat *(Triticum aestivum* L*.)*. Romanian Agric. Res. 26: 21-24.

Du, J.K.; Y.Y. Yao; Z.F. Ni; H.R. Peng and O.X. Sun (2002). Genetic diversity revealed by ISSR molecular marker in common wheat, spelt, compactum and progeny of recurrent selection. Yi Chuan Xue Bao. 29:445-52.

EL-Rawy, M.A. and M.I. Hassan (2014). A diallel analysis of drought toleranc indices at seedling stage in bread wheat (Triticum aestivum L.), Plant Breeding and Biotechnology. 2: (3), 276-288.

Farooq, M.; K.H.M. Siddique; H. Rehman; T. Aziz; A. Wahid and D. Lee (2011). Rice direct seeding experiences and challenges. Soil Till. Res., 111: 87–98.

Fokar, M.; A. Blum and H.T. Nguyen (1998). Heat tolerance in spring wheat. II. Grain filling. Euphytica. 104: 9–15.

Foolad, M.R. (2005). Breeding for abiotic stress tolerances in tomato. In: Ashraf M, Harris, PJC (Eds.), Abiotic Stresses: Plant Resistance Through Breeding and Molecular Approaches. The Haworth Press Inc., New York, USA, pp. 613–684.

Gibson, L.R.; G.M. Paulsen (1999). Yield components of wheat grown under high temperature stress during reproductive growth. Crop Sci., 39: 1841-1846.

Golabadi, M.; A. Arzani and S.A.M. Maibody (2006). Assessment of drought tolerance in segregating populations in durum wheat. Afr. J. Agric. Res. 5: 162-171.

Golabadi, M.; A. Arzani; B.E.S. Tabatabaei; S.M. Maibody and S.A. Mohammadi (2011). Identification of microsatellite markers linked with yield components under drought stress at terminal growth stages in durum wheat. Euphytica 177: 207-221.

Golestani, M.; and H. Pakniat (2007). Evaluation of drought tolerance indices in sesame lines. J. Sci, Technol. Agric. Nat. Resour. 41: 141-149.

Gupta, S.; S. Kaur; S. Sehgal; A. Sharma and P. Chhuneja (2010). Genotypic variation for cellular thermotolerance in Aegilops tauschii Coss. The D genome progenator of wheat. Euphytica 175: 373–381.

Hadrys, H.; M. Balick and B. Schierwater (1992). Applications of random amplified polymorphic DNA(RAPD) in molecular ecology. Mol. Ecol. 1:55-63.

Hall, A.E. (2001). Crop responses to environment. CRC Press LLC, Boca Raton, Florida.

Hou, Y.C.; Z.H. Ya, Y.M. Wei and Y.I. Zheng (2005). Genetic diversity in barley from west China based on RAPD and ISSR analysis. Barley Genet. Newslett. 35: 9-12.

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

Jones, P.D.; M. New; D.E. Parker; S. Mortin and I.G.Rigor (1999). Surface area temperature and its change over the past 150 years. Rev. Geophys. 37: 173–199.

Kantety, R.V.; X. Zeng; J.L. Bennetzen and B.E. Zehr (1995). Assessment of genetic diversity in dent and popcorn (Zea mays L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. Mol. Breed. 1: 365–373.

Kernode, S.P.R.; R.E. Cannon and J.G. Scandaios (1993).Concentration of primer and template qualitatively affects products in random-amplified polymorphic DNA PCR. Bio techniques 14:362-364.

Levitt J. 1980. Responses of plants to environmental stress. Volume I . 2nd Ed. Academic Press, New York. 497 pages.

Martineau, J.R.; J.E. Specht; J.H. Williams and C.Y. Sullivan (1979). Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. Crop Sci. 19: 75-78.

Mason, R.E.; A. Pacheco; D.B. Hays; S. Mondal; B. Jampala; F.W. Beecher and A.M. Ibrahim (2010). QTL associated with heat susceptibility index in wheat (*Triticum aestivum L*.) under short-term reproductive stage heat stress. Euphytica 174: 423-436.

Mohammadi, M.; R. Karimizadeh and M. Abdipour (2011). Evaluation of drought tolerance in bread wheat genotypes under dryland and supplemental irrigation conditions. Aust. J. Crop Sci. 5: 487-493.

Muhammad, K. and K. Ihsan (2004). Heritability, correlation and path coefficient analysis some metric traits in wheat. Int. J. Agric. Biol. 1: 138-142.

Mullarkey, M. and P. Jones (2000). Isolation and analysis of thermotolerant mutants of wheat. J. Exp. Bot. 51: 139–146.

Murray, M.G. and W.F. Thompson (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8(19): 4321–4325.

Muthappa S.; G. Kumar; V. Srikanthbabu and M. Udayakumar (2007). Assessment of variability in acquired thermotolerance: potential option to study genotypic response and the relevance of stress genes. Journal of Plant Physiology . 164(2):111-125.

Naghavi, M.R.; M. Mardi; S.M. Pirseyedi; M. Kazemi; P. Potki and M.R.Ghafari (2007). Comparison of genetic variation among accessions of Agilops taushii using AFLP and SSR markers. Genet. Resour. Crop Evol. 54: 237-240.

Omara MK. (1994). Collection, maintenance and gene banking of germplasm of barley, berseem clover, maize and sorghum from moisture deficient areas in Upper Egypt. Final Report No. A-5-4, NARP, Egypt.

Omara, M.K.; N.A. Mohamed; E.N. El-Sayed and M.A. El-Rawy (2006). Selection for cell membrane thermostability in bread wheat *(Triticum aestivum* L*.).* Assiut J. Agric. Sci. 37: 61-76.

Omara M.K.; E.M.A. Ibrahim; B.E. Abd El-fatah and M.A. El-Rawy (2010). Selection for cell membrane thermostability and stomatal frequency under drought and heat stress conditions in wheat (*Triticum aestivum* L). Assiut J. Agric. Sci. 41 (Special Issue) (4^{th} conference of young scientists Fac. of Agric. Assiut univ. April., 27 (2010).

Quarrie, S.A.; D. Dodig; S. Pekić; J. Kirby and B. Kobiljski (2003). Prospects for marker-assisted selection of improved drought responses in wheat. Bulgarian J. Plant Physiol., Special Issue, 83–95.

Ram, S.G.; V. Thiruvengadam and K.K. Vinod (2007). Genetic diversity among cultivars, landraces and 42 wild relatives of rice as revealed by microsatellite markers. J. Appl. Genet. 48: 337-345.

Ravi, M.; S. Geethanjali; F. Sameeyafarheen and M. Maheswaran (2003). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR Markers. Euphytica 133: 243-252.

Rohlf, F.J. (1998). NTSYSpc: Numerical Taxonomy and Multivariate Analysis System. Version 2.02. Exeter Software, Setauket, New York.

Saadalla, M.M.; J.S. Quick and J.F. Shanahan (1990a). Heat tolerance in winter wheat. I. Hardening and genetic effects on membrane thermostability. Crop Sci. 30: 1243-1247

Saadalla, M.M.; J.S. Quick and J.F. Shanahan (1990b). Heat tolerance in winter wheat: II. membrane thermostability and field performance. Crop Sci. 30: 1248-1251.

Sallam, A.; E.N. Hamed; M.M. Hashad, and M.K.Omara (2014). Inheritance of stem diameter and its relationship to heat and drought tolerance in wheat (*Triticum aestivum* L.). Journal of Plant Breeding and Crop Sci. 6: 11-23.

Satyavir, S. and D.M. Handa (2012). Heat tolerance in relation to acquired thermotolerance for membrane lipids in bread wheat. Field Crops Res. 135: 30–37.

Shah, M.M.; M. Migliore and D.A. Brown (2011). Differential effects of Kv7 (M-) channels on synaptic integration in distinct subcellular compartments of rat hippocampal pyramidal neurons. J. Physiol. 589: 6029-38.

Shanahan, J.F.; I.B. Edwards; J.S. Quick and J.R. Fenwick (1990). Membrane thermostability and heat tolerance of spring wheat. Crop Sci. 30: 247–251.

Sharma, P; S. Sindhu; S. Manoj; V. Ajay; S.T. Bhudev and I. Sharma (2014). Assessing genetic variation for heat tolerance in synthetic wheat lines using phenotypic data and molecular markers. Aust. J. Crop Sci. 8: 515-522.

Sofalian, O.; N. Chaparzadeh; A. Javanmard and M.S. Hejazi (2008). Study the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers. Int. J. Agric. Biol. 10: 465-468.

Somers, D.J.; P. Isaac and K. Edwards (2004). A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109: 1105-1114.

Sullivan, C.Y. and W.M. Ross (1979). Selecting for drought and heat resistance in grain sorghum. In H. Mussell and R. Staples (ed.) Stress physiology in crop plants. John Wiley and Sons, New York.

Tabatabaei, S.A. (2013). Study relationship of drought tolerance indices in wheat (*Triticum aestivum*) genotypes. Int. J. Biosci. 3: 15-22.

Thiaw, S. and A.E. Hall (2004). Comparison of selection for either leaf-electrolyte-leakage or pod set in enhancing heat tolerance and grain yield of cowpea. Field Crops Res. 86: 239–253.

Towill, L.E. and P. Mazur (1974). Studies on the reduction of 2,3,5-triphenyl tetrazolium chloride as a viability assay for plant tissue culture. Can. J. Bot. 53: 1097-1102.

Tripathy, J.N.; J. Zhang; S. Robin; T.T. Nguyen and H.T. Nguyen (2000). QTLs for cell membrane stability mapped in rice (*Oryza Sativa* L.) under drought stress. Theor. Appl. Genet. 100: 1197-1202.

Wahid, A.; S. Gelani; M. Ashraf and M.R. Foolad (2007). Heat tolerance in plants: an overview. Environ. Exp. Bot. 61:199-223.

Yang, J.; R.G. Sears; B.S. Gill and G.M. Paulsen (2002). Quantitative and Molecular characterization of heat tolerance in hexaploid wheat. Euphytica 126: 275–282.

الإنتخاب ثنائي الاتجاه والتحليل الجزيئي للتحمل الحراري في قمح الخبز

محمود ابوالسعود الراوي
قسم الوراثة – كلية الزراعة – جامعة اسيوط – جمهورية مصر العربية

يهدف هذا البحث إلي الإنتخاب ثنائي الاتجاه لصفة وزن الالف حبة تحت الإجهاد الحراري في عشيرة مكونة من 140 سلالة تحت التلقيح الذاتي إلي الجيل الثامن والمشتقة من التهجين بين سلالة حساسة وأخري مقاومة من سلالات قمح الخبز, قيست الإستجابة المتلازمة لكل من الصفات التالية: محصول الحبوب للنبات الواحد, الثبات الحراري للغشاء الخلوي *(CMS)* وإختزال مادة *tetrazolium chloride (TTC).*

تم تقدير محصول الحبوب للنبات الواحد في الظروف الملائمة في الحقل وتحت ظروف الإجهاد الحراري بينما تم قياس كل من الـ *CMS* وإختزال مادة الـ *TTC* في طور البادرة. نتج عن الإنتخاب لوزن الألف حبة إستجابة معنوية جدا في كلا الإتجاهين وقدرت في الإتجاه العالي بـ 9.76% والمنخفض بـ 4.64% والمكافئي الوراثي المحقق لوزن الألف حبة (0.28) والذي تشابه مع المحسوب من إنحدار النسل علي متوسط الأبوين (0.29), الإنتخاب لوزن الألف حبة المخفض نتج عنه إستجابة متلازمة موجبة ومعنوية في كل من محصول الحبوب للنبات الواحد (7.95%) والـ *CMS* (17.24%) وإختزال مادة الـ *TTC* (36.74%) بينما الإنتخاب لوزن الالف حبة العالي نتج عنة إستجابة متلازمة موجبة ومعنوية فقط في محصول الحبوب للنبات الواحد (9.74%). كما وجد إرتباط معنوي بين وزن الألف حبة وكل من الصفات التالية محصول الحبوب للنبات الواحد (0.54) والـ *CMS* (0.31) وإختزال الـ TTC (0.52), كما أرتبط محصول الحبوب للنبات الواحد مع كل من الـ *CMS* (0.31) و الـ *TTC* (0.69), وكذلك وجد ايضا إرتباط موجب ومعنوي جدا بين الـ *CMS* و الـ *TTC* (0.40). إستخدمت ثمانية واسمات جزيئية، خمس واسمات *SSR* وثلاث واسمات *ISSR* للكشف الجزيئي علي العشر سلالات المنتخبة حيث تم تحديد واسمين جزيئين من *SSR* وواسم من *ISSR* خاصين بوزن الألف حبة يمكن إستخدامهما كعلامات يمكن الإعتماد عليها في برامج التربية للتحمل الحراري في القمح.