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MEANS OF BASIC GENERATION AND BULKED SEGREGANT ANALYSIS FOR HEAT TOLERANCE IN BREAD WHEAT (*Triticum aestivum* L.)

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ABSTRACT

In the present study, two bread wheat crosses were subjected to generations means analysis in order to determine the types of gene effects controlling grain yield per plant and 1000 kernel weight under favorable and heat stress under filed conditions, as well as tetrazolium chloride (TTC) reduction, chlorophyll a and b content at seedling stage. The A, B, C, and D scaling tests for grain yield per plant, 1000-KW and chlorophyll a and b content in the two crosses at both environments indicated the non adequacy of the simple additive-dominance model of inheritance, except for 1000-KW in cross 2 under heat stress condition and TTC in both crosses. The additive effects were found to be significantly ($P \leq 0.05$) moderate for all studied traits under the two environmental conditions, except for grain yield per plant in cross 1 at favorable environment. Meanwhile, the dominant parameter (h) was significant ($P \leq 0.05$) for all traits in the two crosses under the two environmental conditions as well as seedling traits, except for TTC in cross 1 and 1000-KW under favorable condition in cross 2. Grain yield per plant predominantly controlled by dominance gene effect (h) and the magnitude of dominance effect was higher than additive gene effects (d), while the dominance effects were higher than the additive effects for 1000-KW in cross 1 under both environmental conditions. The broad and narrow-sense heritabilities for 1000-KW in cross 2 under heat stress condition were high in magnitude being 0.85 and 0.66, respectively. For chlorophyll a and b content and TTC, the dominance effect (h) was higher in magnitude than the additive gene effect (d). The narrow-sense heritability for TTC was higher in magnitude of 0.59 and 0.63 in crosses 1 and 2, respectively. These results indicated that TTC reduction and chlorophyll content are powerful indicators for screening wheat genotypes for heat tolerance. Out of eight SSR markers used to identify those which associated with heat tolerance using bulked segregant analysis (BSA), the Xgwm566 marker was found to be associated with Chlorophyll a content, whereas the Xwmc603 and Xgwm456 markers were associated with TTC, indicating that these markers would be considered as markers associated with heat tolerance in wheat and could be used for selecting heat tolerance genotypes in wheat breeding programs.

Keywords: Bread wheat, heat tolerance, tetrazolium chloride (TTC) reduction, chlorophyll content, SSR markers and bulked segregant analysis (BSA).

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop all over the world. Heat stress causes 10-15% reduction in wheat grain yield in wheat due mainly to reduced single grain weight (Wardlaw and Wrigly 1994). Plants response to high temperature is very complex, it involves many physiological, morphological and biochemical process. In general, high temperature stress induces many changes such as enzymes inactivation in chloroplast, protein denaturation, photosynthetic inactivation, high rate of respiration and membrane injury. These changes cause a significant reduction in wheat production (Howarth 2005). High temperature stress is one of the major threats to crop production worldwide particularly at the reproductive stage (Hall 2001). Global mean temperature may rise up to 0.3 °C per decade (Jones *et al*. 1999) reaching an approximately 1 °C to 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 is in the range of 18–24 °C and even short periods of about 5 to 6 days of exposure to temperatures of 28–32 °C may cause 20 percent decrease in yield (Mullarkey and Jones 2000). All plant processes are sensitive to heat stress because high temperatures accelerate senescence, reduce the duration of viable leaf area and diminish photosynthetic activities. Due to high temperatures severe cellular injury may occur within a short period, leading to a collapse of cellular organization, but at moderately high temperatures, injuries may occur after long-term exposures (Farooq *et al*. 2011). High temperature stress in plants also causes oxidative damage to respirational process which is based on the fact that the tetrazolium salt is reduced to formazan by dehydrogenases complex 1 respiratory enzymes. Moreover, the efficiency of the red formazan formation depends on the activity of cytochrome oxidase which determines the aerobic state (Towil and Mazur 1974; Rich *et al*. 2001). TTC reduction has been widely used in the viability assay of plant tissues exposed to high temperature, where genotypic differences in thermotolerance were also evaluated in different plant tissues (Chen *et al*. 1982; Fokar *et al*. 1998; Gupta *et al*. 2010). In higher plants, Chl *a* and Chl *b* are the most important forms of chlorophyll which are essential for the oxygenic conversion of light energy into stored chemical energy that powers the biosphere (Richardson *et al*. 2002). Chlorophyll degradation is accelerated at high temperature although chlorophyll a:b ratio remained unchanged, but when chlorophyll a+b contents were calculated both on the basis of leaf area and dry weight were decreased by 15% after heat treatment. It has been well documented that high temperature stress accelerates plant senescence (Bhullar and Jenner 1983). Such a situation is well associated with loss of photosynthetic activity, which is mainly caused by the reduction in chlorophyll and RuBP carboxylase activity (AI Khatib and Paulsen 1984). chlorophyll synthesis is sensitive to heat stress and is a good indicator of heat stress injury. Thus, heat is a key type of abiotic

constraint responsible for accumulation of reactive oxygen spices, which are detrimental to plant cells causing damage to valuable biomolecules like DNA, proteins, lipids, membrane etc. (Yuan *et al*. 2011).

Generation mean analysis provides information on the relative importance of additive effects, dominance deviations, and effects due to non allelic genetic interactions. It also determines genotypic values of the individuals and, consequently, mean genotypic values of families and generations. Generation mean analysis is a simple but useful technique for estimating gene effects of polygenic trait, and to estimate epistatic gene actions such as additive x additive, dominance x dominance and additive x dominance effects (Bayoumi *et al*. 2008 and Amin 2013).

Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity among and within species or populations. It has been shown that different markers might reveal different classes of variation (Powell *et al*. 1996 and Russell *et al*. 1997). The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques such as random amplified of polymorphic DNA (RAPD), simple sequence repeats (SSR or microsatellite), sequence tagged sites (STS), inter-simple sequence repeat polymorphic DNA (ISSR) and so on (Akkaya *et al*. 1992; Nagaoka and Ogihara 1997; Saiki *et al*. 1988 and Zietkiewicz *et al*. 1994). 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). This study aimed to: i) determine the types of gene effects controlling tetrazolium chloride (TTC) reduction , chlorophyll a and b content, grain yield and 1000 kernel weight under favorable and heat stress conditions; ii) identify SSR markers associated with TTC reduction and chlorophyll a content, as heat tolerance indicators, using bulked segregant anaylsis (BSA).

MATERIALS AND METHODS

Genetic material and field trials:

Field trials of the present study were carried out at the Experimental Farm of the Faculty of Agriculture, Assiut University, Assiut, Egypt. Based on the prior screening tests for cell membrane thermostabilty, four parental lines were selected to be used in this study, namely WT7 and WT9 (P_1 heat tolerant in cross 1 and 2) and WS10 and WS5 (P_2 heat sensitive in cross 1 and 2), respectively. In 2011/2012 season, two crosses were done among the four parents to produce F_1 hybrids. In 2012/2013 season, some F_1 plants of each cross were backcrossed to their respective parents to produce the backcross 1 (BC_1) and backcross 2 (BC_2). At the same time, some other F_1 plants were selfed to produce the F_2 generation. Moreover, crosses were done to produce more F_1 hybrids seeds. In the 2013/2014 season, the six populations, i.e., P_1, P_2, F_1, F_2, BC_1 and BC_2 of the two crosses were sown in two experiments in two sowing dates, (Nov. 25 as favorable and Dec. 25 as late sowing = heat stress) in a randomized complete blocks design with three replicates. Each replicate consisted of a single row containing 10 seeds from each parent and F_1 bybrid, three rows each containing 10 seeds (a total of 30 seeds) for each backcross and six rows each containing 10 seeds (a total of 60 seeds) for each F_2 population. Rows were 3.0 m long, 30 cm apart and 30 cm spaces between plants. At the maturity stage, phenotypic data for 1000 kernel weight (1000-KW g) and grain yield per plant (g) were recorded for each row using five random individual plants.

Tetrazolium chloride (TTC) reduction and chlorophyll a, b content (Chl a,b)

Both TTC and Chl a, b measurements were measured on individual plants bases. From each cross, 15 plants were taken from each parent and F_1; 45 plants from each backcross and 90 plants were taken from each F_2 population. All plants were grown in Petri dishes at 25°C in the laboratory and evaluated for TTC reduction using the method of Ibrahim and Quick (2001). The level of acquired high temperature tolerance was determined by measuring the percentage reduction of TTC to formazan using the following formula:

TTC = ODh / ODc × 100

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

In order to estimate chlorophyll content in the two crosses, 12-day-old seedlings were acclimated for 24 hours at 39°C in an incubator. After acclimation, leaf samples (0.1 g) were cut into discs of uniform size and taken in spectrophotometric tubes containing 1 ml of 95% ethanol, and then submerged for 12 hours at 25 °C in an incubator according to Gosavi (2014) with some modifications. Chlorophyll a and b content were determined using Lichtenthaler and Buschmann (2001) formulae as follows:

Chla = 13.36×A664 – 5.19×A648

Chlb = 27.43×A648 – 8.12×A664

Where, Chla means chlorophyll *a* [µg/ml], Chlb means chlorophyll b [µg/ml], A664 means absorbance at wavelength 664 nm, A648 means absorbance at wavelength 648 nm

Scaling tests

In order to test the presence of non-allelic gene interactions in the two crosses, the basic six generations were subjected to A, B, C, and D scaling tests using Hayman (1958) formulae as follow:

$$A = 2\overline{BC_1} - \overline{P}_1 - \overline{F}_1$$

$$V_A = 4V_{(\overline{BC}_1)} + V_{(\overline{P}_1)} + V_{(\overline{F}_1)}$$

$$B = 2\overline{BC_2} - \overline{P}_2 - \overline{F}_1$$

$$V_B = 4V_{(\overline{BC}_2)} + V_{(\overline{P}_2)} + V_{(\overline{F}_1)})$$

$$C = 4\overline{F}_2 - 2\overline{F}_1 - \overline{P}_1 - \overline{P}_2$$

$$V_C = 16V_{(\overline{F}_2)} + 4V_{(\overline{F}_1)} + V_{(\overline{P}_1)} + V_{(\overline{P}_2)}$$

$$D = 2\overline{F}_2 - \overline{BC}_1 - \overline{BC}_2$$

$$V_D = 4V_{(\overline{F}_2)} + 4V_{(\overline{BC}_1)} + V_{(\overline{BC}2)}$$

The total phenotypic variance (V_P) for each trait in the two crosses was calculated as follows: $Vp = V_G + V_E$, where V_G and V_E represent the genetic and environmental variance, respectively. The environmental variance (V_E) was calculated as $V_E = 1/3 (V_{P1} + V_{P2} + V_{F1})$, while the genetic variance (V_G) was partitioned into additive (D) and dominance (H) components and they were calculated using the following formulae:

$D = 2V_{F2} - (V_{BC2} + V_{BC2})$ $H = 4(V_{F2} - 1/2\ VD - E)$.

The genetic components of variance were then used to compute narrow-sense (h^2_n) and broad-sense (h^2_b) heritability. The average degree of dominance was calculated as follows: Average degree of dominance = $(H/D)^{0.5}$.

Estimation of gene effects

For estimating the main effects i.e m, d and h, assuming the adequacy of additive-dominance model, the formulae outlined by Jinks and Jones (1958) were used, as follow:

Mean

$$m = \tfrac{1}{2}\overline{P}_1 + \tfrac{1}{2}\overline{P}_2 + 4\overline{F}_2 - 2\overline{BC}_1 - 2\overline{BC}_2$$

Additive effect $[d] = \tfrac{1}{2}\overline{P}_1 - \tfrac{1}{2}\overline{P}_2$

Dominanceeffect

$$[h] = 6\overline{BC}_1 + 6\overline{BC}_2 - 8\overline{F}_2 - \overline{F}_1 - 1.5\overline{P}_1 - 1.5\overline{P}_2$$

Whereas in the presence of non-allelic gene interaction, the analysis proceeded to compute the interaction types involved using the six generations model of Jinks and Jones (1958) was as follows:

m (mean) = $\overline{F}_2$

[d] (additive effect) = $\overline{BC}_1 - \overline{BC}_2$

[h] (dominance effect) = $\overline{F}_1 - 4\overline{F}_2 - \tfrac{1}{2}\overline{P}_1 - \tfrac{1}{2}\overline{P}_2 + 2\overline{BC}_1 + 2\overline{BC}_2$

[i] (additive X additive type of gene interaction) = $2\overline{BC}_1 + 2\overline{BC}_2 - 4\overline{F}_2$

[j] (additive X dominance type of gene interaction) = $\overline{BC}_1 - \tfrac{1}{2}\overline{P}_1 - \overline{BC}_2 + \tfrac{1}{2}\overline{P}_2$

[l] (dominance X dominance type of gene interaction) = $\overline{P}_1 + \overline{P}_2 + 2\overline{F}_1 + 4\overline{F}_2 - 4\overline{BC}_1 - 4\overline{BC}_2$

The significance of a genetic component was tested using the "*t*" test where:

$$\pm t = \frac{\text{The effect}}{\text{Standard error of the effect}}$$

Statistical and genetic analyses

Analysis of variance (ANOVA) was carried out for the seedling traits using MSTAT-C statistical program (Nissen 1984). The mean squares were used to estimate genotypic and phenotypic variance according to Johnson et al. (1955).

Bulked segregant analysis (BSA)

In order to identify molecular markers associated with heat tolerance in specific genomic regions, the F_2 populations of the two crosses were subjected to BSA (Quarrie *et al* 1999) with eight simple sequence repeat (SSR) markers. TTC reduction was used as a heat tolerance related trait in cross 2, whereas Chl. a content was used in cross 1. The highest 10 and the lowest 10 genotypes selected from each of the F_2 populations were used to construct two DNA bulks for BSA. DNA extraction from the remaining tissues of the two parents and selected F_2 plants was carried out according to the cetyltrimethylammonium bromide (CTAB) method for isolation of total genomic DNA from plants with some modifications (Murray and Thompson 1980). Aliquots of DNA from the two extreme groups of 10 high and 10 low genotypes were mixed to produce high and low DNA bulks for BSA. SSR primers were, then, screened on the parents and the two bulk DNA samples, from which some primer combinations revealed bands that were polymorphic, not only among parental genotypes, but also between the pair of the bulk DNA.

Molecular markers analysis

High and low DNA bulks and their respective parents were screened for differences using eight simple sequence repeat (SSR) markers, namely Xgwm456, Xgwm566, Xwmc596, Xwmc603, Xgwm339, Xgwm493, Xwmc398 and ERAD. Primers sequences and PCR conditions 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 56-61 °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 gel 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 and were recorded as **Younes (2009).** Putative polymorphisms were detected for each marker separately.

RESULTS AND DISCUSSION

The genotypes performance

Means of grain yield per plant and 1000-KW for P_1, P_2, F_1, F_2, BC_1 and BC_2 generations of the two crosses evaluated under two environmental conditions, as well as seedling traits i.e Ch a, Chl b and TTC are given in Tables 1. The distributions of F_2 segregates of the two crosses for chlorophyll a content in cross 1 and TTC reduction in cross 2 at seedling traits are illustrated

in Figs. 1 and 2. The distributions were continuous and approached normality indicating that both traits are under the control of polygenes of quantitative type of inheritance for both of them. The analysis of variance indicated that there were significant ($P \leq 0.01$) differences among different genotypes for all the studied traits under the two environmental conditions as well as seedling traits in the two crosses as presented in Table 2.

The results indicated that tetrazolium chloride (TTC) reduction in both crosses was significantly ($P \leq 0.05$) different between P_1 and P_2 as seen in Table 1. The F_1 mean exceeded the midparent but was not significantly different from P_1, indicating that gene(s) controlling acquired thermotolerance as measured by TTC cell viability assay are dominant. In both crosses, the mean of BC_1 was found to be intermediate between the means of its respective F_1 and P_1. However, BC_1 was not significantly different from the P_1. Similarly, the mean of BC_2 was insignificantly larger than the mean of its respective P_2, which also indicates the dominant nature of gene action.

Table 1. Mean performance of P_1, P_2, F_1, F_2, BC_1 and BC_2 generations under favorable and heat stress conditions.

Generation	Favorable		Heat stress		Seedling stage		
	Grain yield	KW 1000	Grain yield	KW 1000	Chl. a	Chl. b	TTC
	Cross (1)						
P_1	65.70±1.35	51.92±0.44	60.39±10.8	50.35±0.37	11.68±0.22	7.15±0.17	25.20±0.80
P_2	53.44±1.56	41.32±0.25	53.98±0.84	43.18±0.26	8.13±0.25	5.02±0.13	14.78±0.32
F_1	88.36±1.51	52.52±0.55	45.10±1.03	43.35±0.50	10.42±0.24	6.75±0.30	25.66±1.05
F_2	59.87±1.38	53.28±0.65	51.50±0.87	49.38±0.31	8.76±0.30	5.37±0.17	24.49±0.71
BC_1	66.42±1.63	55.91±0.35	60.29±0.84	47.62±0.37	11.28±0.19	7.08±0.23	24.80±0.19
BC_2	64.80±0.99	54.28±0.54	57.88±0.69	46.67±0.36	7.80±0.34	5.14±0.19	21.41±0.78
$LSD_{(0.05)}$	15.09	7.31	12.2	4.93	2.33	2.56	7.53
Cross (2)							
P_1	93.00±0.78	59.07±0.53	67.02±1.02	50.08±0.35	15.00±0.22	8.39±0.25	57.92±1.10
P_2	64.28±0.67	53.52±0.66	50.64±0.68	46.12±0.55	10.62±0.21	5.75±0.24	24.62±0.96
F_1	47.59±0.71	49.57±77	45.85±0.75	41.67±0.25	12.63±0.09	6.75±0.26	46.66±1.23
F_2	59.38±0.84	50.75±0.62	52.08±0.78	45.57±0.42	8.43±0.30	4.77±015	42.39±0.67
BC_1	68.39±1.14	52.90±0.22	52.63±0.87	45.82±0.48	14.80±0.22	8.17±0.18	50.57±0.79
BC_2	58.38±0.81	49.15±0.66	49.30±1.01	43.52±0.49	9.22±0.13	5.13±0.18	37.39±0.48
$LSD_{(0.05)}$	9.90	8.55	13.63	6.06	2.88	2.42	18.58

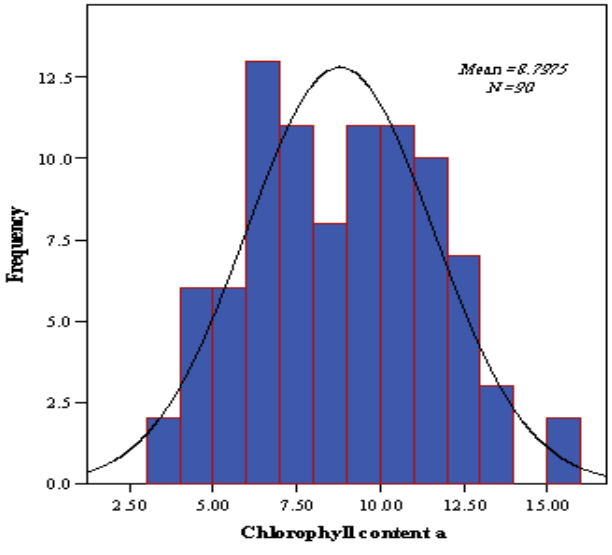

Figure 1. Distributions of F_2 segregates for chlorophyll a content in cross 1.

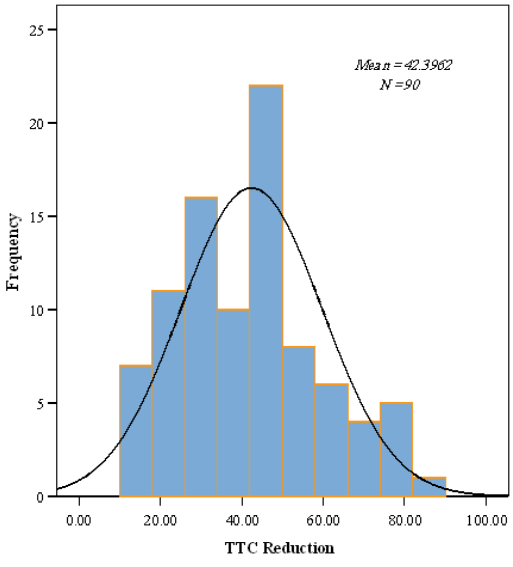

Figure 2. Distributions of F_2 segregates for TTC reduction in cross 2.

Table 2. Analysis of variance and broad sense heritability for grain yield per plant and 1000-KW for the two crosses under favorable and heat stress conditions as well as seedling traits.

S. O. V.	Favorable		Heat stress		Seedling stage		
	Grain yield	KW 1000	Grain yield	KW 1000	Chl. a	Chl. b	TTC
	Cross (1)						
Reps.	135.64**	7.59	0.50	8.16	0.51	1.18	3.02
Genotypes	417.56**	81.14**	105.94**	26.94**	8.42**	3.07*	67.18**
Error	22.94	5.39	14.54	2.45	0.55	0.66	5.71
h^2_b	0.85	0.82	0.68	0.77	0.83	0.55	0.78
Cross (2)							
Reps.	19.49	7.22	17.89	2.34	0.23	0.99	24.94
Genotypes	698.22**	40.17*	162.35**	24.22**	23.62**	7.12**	436.05**
Error	9.88	7.36	18.72	3.70	0.84	0.59	34.47
h^2_b	0.95	0.60	0.72	0.65	0.90	0.79	0.80

***= (P≤ 0.05); ** = (P≤ 0.01)**

Scaling tests

The results of A, B, C and D scaling tests for grain yield per plant, 1000-KW and a and b chlorophyll content were significant for one or more tests in the two crosses at both environments except for 1000-KW in cross 2 under heat stress condition. This indicated non adequacy of the simple additive-dominance model of inheritance as presented in Table 3. These results are in agreement with the results of Amin 2013 and Ahmad *et al*. 2011. Higher order non-allelic gene interactions (epistasis) or linkage was observed for 1000 seed weight and chlorophyll content in sesame by Jatothu *et al*. (2013). Otherwise, Lal *et al*. 2013 found that two crosses showed adequacy of additive-dominance model for grain yield and its components, similarly, the simple additive-dominance model of inheritance was adequacy for TTC in the two crosses. Fokar *et al*. 1998 found that the greatest mean for genetic variation by TTC in wheat could be explained by additive genetic variation. Ibrahim and Quick (2001) reported the importance of additive gene effects in acquired thermal tolerance. While, Dhanda and Munjal (2012) reported that the significant values of GCA and SCA variances indicated the presence of both additive and dominant types of gene action in TTC and Chlorophyll fluorescence.

Types of gene effects and components of variances

The estimates of the means (m) were highly significant for all traits in the two crosses at both environments, as well as, seedling traits (Table 4). In general, the additive effects were found to be significantly (P≤ 0.05 or P≤ 0.01) moderate for all studied traits under the two environmental conditions, except for grain yield per plant in cross 1 at favorable environment. Meanwhile, the dominant parameters (h) were significant (P≤ 0.05 or P≤ 0.01) for all traits in the two crosses under the two environmental conditions, as well as, seedling traits except for TTC in cross 1 and 1000-KW under favorable condition in cross 2. However, the magnitude of additive x additive (i) gene effects and dominance x dominance (l) effects were quite high in comparison with additive x dominance gene effects (j) as presented in Table 4. Grain yield per plant predominantly controlled by dominance gene effect (h) and the magnitude of dominance effect was larger than additive gene effects (d). Similar finding was reported by Shekhawat *et al*. (2006), who reported that recurrent reciprocal selection or bi-parental mating is suitable for the genetic improvement of grain yield in the segregating generations. Dominance gene effects and additive x additive epistsis were also reported to be more important than additive effects and other types of epistatic (Novoselovic *et al*. 2004 and Motawea 2006).

Table 3. The A, B, C and D values of the scaling tests in the two crosses for grain yield per plant and 1000-KW under favorable and heat stress conditions as well as seedling traits.

Value	Favorable		Heat stress		Seedling stage		
	Grain yield	KW 1000	Grain yield	KW 1000	Chl. a	Chl. b	TTC
	Cross (1)						
A	-21.21**±3.72	7.39**±0.99	15.11**±2.12	1.54±0.96	0.45±0.50	0.26±0.57	-1.26±2.24
B	-12.20**±2.78	14.72**±1.23	16.68**±1.91	6.80**±0.91	-2.95**±0.77	-1.48**±0.50	2.38±1.90
C	-56.38**±6.33	14.85**±2.86	1.43±4.21	17.27**±1.66	-5.63**±1.32	-4.17**±0.94	6.64±3.63
D	-11.48**±3.36	-3.63*±1.45	-15.18**±2.05	4.46**±0.81	-1.56*±0.71	-1.47**±0.46	2.76±1.86
Cross (2)							
A	-3.81±2.51	-7.60**±2.16	-2.85**±1.03	-0.91±1.04	1.98**±049	1.21**±0.51	3.77±3.93
B	4.88*±1.89	2.11±2.25	-4.79**±1.65	-0.74±1.16	-4.82**±0.35	-2.24**±0.51	3.40±2.01
C	-14.95**±3.81	-1.02±3.67	-8.71**±3.05	2.75±1.87	-17.16**±1.26	-8.57**±0.86	-6.30±3.43
D	-8.01**±2.19	2.23±2.05	-0.54±1.43	1.80±0.99	-7.16**±0.66	-3.77**±0.40	-3.14±1.70

***= (P≤ 0.05); ** = (P≤ 0.01)**

Table 4. Estimates of the additive and dominance parameters for morpho-physiology traits, the components of variance and heritability in two crosses as well as degree of dominance.

Parameter and components of variance	Favorable		Heat stress		Seedling stage		
	Grain yield	KW 1000	Grain yield	KW 1000	Chl. a	Chl. b	TTC
	Cross (1)						
M	59.87**±1.38	53.28**±0.65	51.50**±0.87	49.38**±0.31	8.76**±0.30	5.37**±0.18	25.52**±3.74
[d]	1.63±1.91	1.63*±0.64	2.41*±1.08	0.96*±0.55	3.48**±0.39	1.93**±0.29	5.21**±0.43
[h]	51.76**±6.90	13.16**±2.96	18.27**±4.27	-12.34**±1.7	3.64*±1.25	3.61**±0.97	-4.26±9.31
[i]	22.97**±6.72	7.26*±2.90	30.35**±4.11	-8.92**±1.61	3.12*±1.42	2.95**±0.91	
[j]	-4.51*±2.17	-3.67**±0.69	-0.79±1.23	-2.63**±0.56	1.70**±0.43	0.87**±0.31	
[l]	10.45±9.94	-29.37**±3.85	-62.14**±6.05	0.58±2.63	-0.62±2.05	-1.72±1.51	
D							9.20
H							53.75
E							38.03
h^2_n							0.59
h^2_b							0.80
D. of dominance.							0.41
Cross (2)							
M	59.38**±0.85	50.75**±0.62	52.08**±0.78	51.69**±2.00	8.43**±0.30	4.77**±0.15	35.00**±3.46
[d]	10.02**±1.40	3.74**±1.69	3.33**±1.33	1.98**±0.33	5.59**±0.25	3.05**±0.26	16.65**±0.62
[h]	-15.03**±4.47	-5.64±2.98	-17.44**±4.13	-14.46**±4.70	14.15**±1.32	7.21**0.85	17.90*±8.61
[i]	16.02**±4.39	1.08±2.85	-4.47±4.11		14.32**±1.31	7.54**±0.79	
[j]	-4.34**±1.49	0.97±0.81	-4.86**±1.47		3.40**±0.29	1.73**±0.31	
[l]	-17.09**±6.76	6.56±4.11	9.96±6.76		-11.48**1.62	-6.51**±1.35	
D				2.43			11.91
H				20.73			48.46
E				12.13			9.92
h^2_n				0.66			0.63
h^2_b				0.85			0.69
D. of dominance.				0.34			0.50

***= (P≤ 0.05); ** = (P≤ 0.01)**

For 1000-KW, the dominance effects were higher than the additive effects in cross 1 under both environmental conditions. In cross 2, the dominance effects and epistatic gene effects were not significant under favorable condition. The adequacy of additive-dominance model for 1000-KW in cross 2 under heat stress condition indicated the importance of both the additive and dominance gene effects for this trait. These results are in agreement with the results of Amin (2013).

The estimates of different components of variance for 1000-KW in cross 2 under heat stress condition exhibited no genetic interactions, as shown in Table 4. The dominance component (H) was greater in magnitude than the additive (D). The broad and narrow-sense heritability values were high in magnitude being 0.85 and 0.66, respectively. The degree of dominance was found to be partial for 1000-KW at heat stress environment.

The importance of the additive gene effects under the favorable condition for 1000-KW in wheat was reported by Mann and Sharma (1995); Saad (1999); Ismail *et al*. (2001) and Shoran *et al*. (2005). Meanwhile, non-additive genetic variance was also reported for 1000-KW (Hassan, 1997; Khalifa *et al*. 1998 and Motawea 2006). Additive and additive x additive interaction were also found to be significant, while dominance and dominance x dominance gene effects were of larger magnitude (Singh *et al*., 1998). High heritability estimates were also observed for 1000-KW by Moghaddam *et al*. (1997) and Shoran *et al*. (2005) which were coupled with high genetic advance indicating that direct selection might be effective. Furthermore, different ranges of narrow-sense heritability (0.22 to 0.47) were reported by Mustafa (1999) and from 0.23 to 0.73 as reported by Novoselovic *et al*. (2004).

Concerning chlorophyll a and b content, the genetic parameters, namely m, (d), (h), (i), (j), and (l) were highly significant (P≤ 0.01) in the two crosses, except for (l) in cross 1 was insignificant. The dominance effect (h) was higher in magnitude than the additive gene effect (d). Significant positive dominance (h), negative dominance x dominance (l) parameters were found in cross 2, indicating the role of duplicate gene action and the evidence of higher order epistasis or linkage. These are in agreement with those reported by Lal, *et al*. (2013) and Jatothu, *et al*. (2013). In the present investigation, there are no traits in the two crosses exhibited complementary type of epistasis, which is also noticed by Rajendrakumar and Raveendran (1999) and Iqbal and Nadeem (2003).

The adequacy of additive-dominance model was appropriate for TTC in both crosses. The dominance component (H) was greater in magnitude than the additive (D). This result agreed with Dhanda and Munjal (2012). The broad-sense heritability values were higher in magnitude being 0.80 and .0.69 in cross 1 and cross 2, respectively. Whereas the narrow-sense heritability for TTC was higher in magnitude (0.59 and 0.63) in crosses 1 and 2, respectively. The degree of dominance for TTC was 0.41 in cross 1 and 0.50 in cross 2, these indicated the presence of partial

dominance in the two crosses. Porter (1989), using a diallel mating design, reported that acquired thermotolerance trait in term of TTC cell viability was controlled by additive genetic effects. In other study, Ibrahim and Quick (2001) found that parent-offspring regression and correlation heritability was intermediate to high (0.50–0.65) for TTC. The results of the present study supported that TTC reduction and chlorophyll content are powerful indicators for screening wheat genotypes for heat tolerance. These are in agreements with the results of Blum (1988); Shanahan *et al*. (1990); Camejo *et al*. (2005); Yildiz and Terzi (2008); Mohammed *et al*. (2014) and El-Rawy (2015), as they reported that genotypes screening based on membrane tests like membrane thermostability (MTS), electrolyte leakage, TTC reduction from leaves subjected to extreme temperatures could be considered as rapid screening methods for selecting heat tolerant genotypes.

SSR markers

Out of eight SSR markers tested, three SSRs (37.5%) namely Xgwm456, Xgwm566 and Xwmc603 located on chromosomes 1D, 3B and 7A, respectively, distinguished tolerant from susceptible bulks as seen in Fig. 3. They generated a total number of 13 bands ranged from 3 (Xgwm566 and Xwmc603) to 7 (Xgwm456) with an average of 4.33 bands per marker. Of the 13 bands amplified with 3 SSRs, 7 bands (53.8%) were polymorphic (Table 5) with an average of 2.33 polymorphic bands per marker. The lowest polymorphism between the two bulks (33.3%) was obtained with Xgwm566 and Xwmc603, whereas the highest polymorphism (71.4%) was produced with Xgwm456 (Table 5). These results suggested that polymorphic bands revealed differences which would be used to examine and establish systematic relationships among genotypes as reported by Hadrys *et al*. (1992).

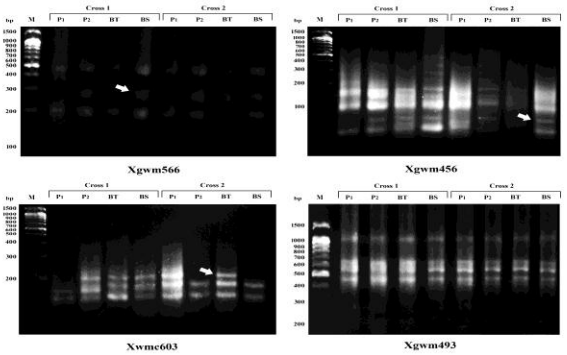

Figure 3. DNA amplification patterns obtained using bulked segregant analysis with 4 SSR markers. M is the 100 bp DNA ladder, BT the tolerant bulk, BS the susceptible bulk. Differences between the two bulks were detected using Xgwm566, Xgwm456 and Xwmc603. Arrows indicate polymorphic bands obtained which distinguished the tolerant from the susceptible bulk.

In cross 2, the SSR primers Xgwm456 was used to identify markers for TTC reduction which generated a single polymorphic fragment at 66 bp. This was only present in the susceptible bulk and WS5 (susceptible parent), while Xwmc603 marker generated a single polymorphic fragment at 103 bp which was only present in the tolerant bulk and WT9 (tolerant parent) (Figure 3). These findings suggested that polymorphic markers could be considered as markers associated with heat tolerance in wheat and could be used for selecting heat tolerance genotypes in wheat breeding programs. In accordance with these, Sun *et al*. (2015) identified 97 marker alleles associated with heat tolerance-related traits. They reported that, these markers might provide important information about heat tolerance genetic pathways, and may be used for molecular assisted breeding to enhance tall fescue performance under heat stress. The quantitative and molecular characterization of heat tolerance in hexaploid wheat has previously been investigated by Yang *et al*. (2002), they reported that two the markers, Xgwm11 and Xgwm293, were linked to grain filling duration (GFD) using quantitative trait loci (QTL) analysis of an F_2 population. Barakat *et al*. (2012) concluded that the R^2 values suggested that Xgwm456-linked QTL account for 33 % of the total phenotypic variation in heat tolerance in the F_2 population for grain filling duration. El-Rawy (2015) found that the SSR markers (Xwmc596 and Xgwm456) were associated with 1000-KW under heat stress and would be used for increasing the frequency of progenies with better performance under heat in a wheat breeding program. Ciuca and Petcu (2009) suggested that Xwmc603 marker, located on chromosome 7A, might be associated with the "*or*" gene for the controller of osmotic adjustment. Moreover, the same authors reported that Xwmc596 and Xwmc603 markers were weakly but significantly associated with cell membrane stability after water stress.

In accordance, several studies developed molecular markers tightly linked to genes or loci controlling complex quantitative traits by BSA (Xu *et al*. 1995; Mackay and Caligari 2000; Altinkuet and Gozukirmizi 2003; Podlich *et al*. 2004; Govindaraj *et al*. 2005; Zhang *et al*. 2009; Barakat *et al*. 2010; Milad *et al*. 2011).

In conclusion, narrow-sense heritability was found to be moderately high in the two crosses for TTC reduction, indicating the possibility to improve heat tolerance in wheat by the selection for high TTC reduction. SSR markers associated with the TTC reduction and chlorophyll a content in the present study, once validated by genotyping the whole F_2 population, could be used in marker assisted-selection for heat tolerance in wheat breeding programs. The present study also supported that bulked segregant analysis (BSA) is a rapid method for development of molecular markers associated with heat tolerance in wheat.

Table 5. Polymorphism detected between tolerant and susceptible bulks by the use of three SSR markers

Marker	Sequence (5'-3')	Fragment range (bp)	No. of bands	No. of polymorphic bands	Polymorphic %
Xgwm456-1D	TCTGAACATTACACAACCCTGA TGCTCTCTCTGAACCTGAAGC	45-165	7	5	71.4
Xgwm566-3B	TCTGTCTACCCATGGGATTTG CTGGCTTCGAGGTAAGCAAC	185-466	3	1	33.3
Wmc603-7A	ACAAACGGTGACAATGCAAGGA CGCCTCTCTCGTAAGCCTCAA	55-103	3	1	33.3
Total	----------	-----	13	7	53.8

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تحليل متوسطات الأجيال الاساسية وضم الإنعزالات المتفارقة للتحمل الحراري في قمح الخبز.

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تهدف هذه الدراسة إلي عرض تحليل متوسطات ستة اجيال في اثنين من الهجن في قمح الخيز وذلك لتحديد طبيعة الفعل الجيني المتحكم في وراثة المحصول ووزن الالف حبة تحت الظروف الملائمة والاجهاد الحراري في الحقل. كما تم دراسة اختزال مادة *tetrazolium chloride (TTC) reduction* وكل من *Chlorophyll content a and b (Chl a,b)* في طور البادرات. أظهر تحليل متوسط الستة اجيال وجود تفاعل جيني في كل الصفات المدروسة ما عدا وزن الالف حبة في الهجين الثاني تحت الاجهاد الحراري واختزال مادة (TTC) في كلا الهجينين. وجدت تأثيرات مضيفة معنوية في كل الصفات المدروسة ماعدا محصول الحبوب للنبات الواحد في الهجين الاول في البيئة الملائمة، كما تحكمت التأثيرات السيادية في كل من صفتي محصول الحبوب للنبات الواحد ووزن الالف حبة بدرجة أعلي من التأثيرات الاضافية، في حين وجدت غير معنوية في كل من اختزال مادة الـ (TTC) في الهجين الاول ووزن الالف حبة في الهجين الثاني في البيئة الملائمة. كانت قيم المكافئ الوراثي بالمعني الواسع والضيق لصفة وزن الالف حبة في الهجين الثاني تحت الاجهاد الحراري مرتفعة وقدرت بـ ٠.٨٥، ٠.٦٦ علي الترتيب. كانت التأثيرات السيادية في كل من الصفات (TTC)، (Chl a,b) اعلي من التأثيرات المضيفة وقدرت قيم المكافئ الوراثي لصفة (TTC) بالمعني الضيق بـ ٠.٥٩ و ٠.٦٣ في الهجين الاول والثاني علي الترتيب.

وجد ان الواسم Xgwm566 من بين ثماني واسمات جزيئية استخدمت لتحديد الواسمات المرتبطة بالتحمل الحراري باستخدام تحليل ضم الانعزالات المتفارقة كان مرتبط بصفة (Chl a) بينما ارتبط الواسم Xwmc603 و الواسم Xgwm456 بصفة اختزال مادة الـ (TTC)، هذه الواسمات الثلاثة يمكن استخدامها في برامج الانتحاب بالواسمات الجزيئية لتحمل درجات الحرارة المرتفعة.