Egypt. J. Plant Breed. 19(3):979 – 994 (2015)

ASSESSMENT OF HEAT TOLERANCE IN BREAD WHEAT USING SOME AGRONOMIC TRAITS AND SRAP MARKERS

A.A. Said[1*], A. Hamada[2], M. Youssef [3]

1- Agron. Depart., Faculty of Agri., Sohag University, Sohag, Egypt
2- Agron. Depart., Faculty of Agri., Assiut University, Assiut, Egypt
3- Genetics Depart., Faculty of Agri., Assiut University, Assiut, Egypt

ABSTRACT

Fifty bread wheat lines were evaluated for heat tolerance and compared to some local cultivars under three sowing dates (November 25th, December 15th and January 5th). Four agronomic traits were evaluated, i.e. No. of spikes per plant, grain yield per plant, 100-kernel weight and harvest index under normal and stress conditions. Analysis of variance showed highly significant variations among the tested lines and demonstrating that the main effect of sowing dates was due to the late date. Grain yield per plant was the most affected trait by heat followed by 100-kernel weight and No. of spikes per plant, while harvest index showed the lowest reduction due to heat stress. Six lines (L1, L11, L16, L34, L37 and L41) showed heat tolerance based on high performance in grain yield/plant by 29.45, 29.75, 27.75, 27.43, 27.37 and 31.90 g, respectively under late sowing conditions as well as low heat sensitivity index. The sequence related amplified polymorphism (SRAP) was able to differentiate between bulked DNA samples of lines with the highest and lowest performance in agronomic traits under heat stress. SRAP generated 2, 1 and 3 bands specific for lines with high performance of No. of spikes per plant, grain yield per plant and harvest index, respectively as well as it showed 5 and 3 bands specific for lines with low performance of grain yield per plant and harvest index, respectively. These specific bands could serve in wheat genotyping and screening, and might be used as SRAP markers associated with heat tolerance in wheat breeding programs.

Keywords: ***Triticum aestivum, Heat stress, SRAP, Grain yield.***

INTRODUCTION

Wheat is one of the most widely grown cereal crops, contributing protein, source of energy and dietary fiber in human nutrition. It is consumed as food, with an average of 53% in the developed world and close to 85% in the developing countries. Wheat production reaches over 718.5 million tons produced worldwide in 2013 (FAO 2014). This accounts for almost one-fifth of global caloric intake.

Heat stress is one of the main abiotic stresses that limit plant biomass production and productivity, especially in tropical and subtropical countries (Boyer 1982). High temperatures at the end of wheat-growing season in Mediterranean climate regions like Egypt are a major abiotic stress affecting yield and its components. The genetic diversity for heat tolerance in wheat has been reported (Al-Khatib and Paulsen 1990 and Joshi *et al* 2007). The photosynthetic process as well is affected under heat stress conditions, especially during grain filling stage (Fischer and Byerlee 1991).

The evaluation of different genotypes and/or lines derived from single seed descent could help in selecting promising genotypes that can thrive on high temperature and subsequently could be used in further breeding programs to improve wheat productivity (Ahmad *et al* 2013). Heat stress tolerance in bread and durum wheat has been evaluated previously by both laboratory experiments, e.g. seedling traits and cell membrane stability (Shafeeq *et al* 2006, Elshafei *et al* 2013 and El-Rawy and Youssef 2014) and field evaluation of agronomic traits (Kumari *et al* 2013). However, abiotic stress evaluation under field conditions gives more authenticity.

Heat tolerance is not controlled by a single 'thermotolerant' gene in cereals. Different components of tolerance determined by different sets of genes are critical for heat tolerance at different stages of the life cycle and in various tissues (Maestri *et al* 2002). Understanding the genetic basis of tolerance to high temperature is important for improving the productivity of wheat (*Triticum aestivum* L.) in regions where the stress occurs (Yang *et al* 2002). Molecular markers associated with tolerance for abiotic stress are one of the most important objectives for wheat breeders. Such markers could be utilized as selectable marker for genotypes screening and/or detecting major genes involved in abiotic stress tolerance which could be used in modern DNA technology. Several molecular markers are being used with wheat for the assessment of genetic variability among genotypes under normal and stress conditions (Eivazi *et al* (2007), Salem *et al* (2008), El Siddig *et al* (2013), Soliman and Hendawy (2013) and El-Mouhammady *et al* (2014)). Among which, the sequence related amplified polymorphism (SRAP) which based on the amplification of the open reading frames (ORFs) has various advantages, such as high reproducibility, more stable, simple and more informative (Li and Quiros 2001). These features made SRAP the best choice for detecting markers associated with heat stress in this study. SRAP has been used with bread and durum wheat previously for genotype identification and evaluation under abiotic stress (Zaefizadeh *et al* (2009), Al-Doss *et al* (2010) and (2011), Elshafei *et al* (2013) and El-Rawy and Youssef (2014)).

The present study was conducted to determine the effects of sowing date and heat stress during grain filling on yield and yield components of new inbred wheat lines using SRAP markers for detecting markers associated with heat stress tolerance in wheat.

MATERIALS AND METHODS

Plant material:

Fifty promising bread wheat lines were evaluated under heat stress. These lines were derived from the two crosses as follow; 24 lines were derived from a cross between a high yielding local variety "Sids-4" with a drought tolerant variety "Tokwie" (South Africa) and 26 lines were derived from a cross between "Sids-4" and "Kasyon/glennson-81" (ICARDA). In addition some local commercial varieties were used for agronomic evaluation comparison; those were Giza-164, Gemmiza-11, Sids-12, Shandawil-1, Masr-1 and Sahel-1.

Phenotypic evaluation

Heat stress evaluation

Seeds of tested lines and varieties were planted on 25th November as favorable sowing date ,15th December as the dead line for wheat sowing in upper Egypt and 5th of January as a late sowing date (stress condition). After maturity, four agronomic traits were evaluated under normal and heat stress. Those were No. of spikes per plant, grain yield per plant (g), 100 kernel weight (g) and harvest index. The experiment was repeated in two seasons 2012-2013 and 2013-2014 at the Experimental Farm of Faculty of Agriculture, Sohag University, Egypt. The trend of temperature (0C) during the two seasons was recorded (Table 1).

Table 1. The trend of temperature (^{0}C) during the two seasons (2012/13and 2013/14).

Season	Months	2012/13			2013/14		
		Average	Min.	Max.	Average	Min.	Max.
HC Air temperature [°C]	Nov.	18.7	5.35	32.05	21.93	7.41	36.44
	Dec.	13.31	2.75	23.86	16.75	2.82	30.67
	Jan.	14.95	5.00	24.9	15.00	5.00	25.00
	Feb.	16.20	4.85	27.54	16.50	6.00	27.00
	Mar.	18.50	7.00	30.00	18.50	7.00	30.00
	Apr.	20.95	8.87	33.02	22.55	8.76	36.33
	May	27.61	16.65	38.57	28.24	13.42	43.05

Heat sensitivity index

Grain yield per plant as the most affected trait during filling stage (Stone 2001) was used for the calculation of heat sensitivity index (HSI). The formula of Fisher and Maurer (1978) was used:

$$HSI = \frac{(\bar{X}_{Y_1} - \bar{X}_{Y_2})}{(\bar{X}_{Y_1} \times HI)}$$

Where, $\bar{X}_{Y_1}$ and $\bar{X}_{Y_2}$ = the grain yield per plant of each genotype under control (1st date) and heat stress (3rd date), respectively, and HI is the heat intensity.

The heat intensity was calculated for the grain yield per plant to measure the effect of heat stress on the trait, using the following formula:

$$HI = 1 - \frac{Y_2}{Y_1}$$

Where Y_1 and Y_2 = the average of all genotypes for the grain yield per plant under control and heat stress, respectively.

Statistical analysis

Randomized complete block design combined over sowing dates and seasons was used for experimental design, with three replications for each planting date. Analysis of variance (ANOVA) was carried out using MSTAT-C statistical program (Nissen 1984).

Molecular analysis

DNA extraction

Total genomic DNA from the fifty lines was extracted at seedling stage using Dellaporta *et al* 1983 method with some modifications (Youssef 2012). DNA concentration and purity were measured using spectrophotometer according to Stulnig and Amberger (1994) and Khirshyat 1.0 micro-program (Youssef 2012). Ten DNA samples of the highest and lowest lines in four agronomic traits evaluated under stress conditions were bulked and used for molecular analysis.

SRAP-PCR amplification

Ten SRAP primer sets were selected and used for the molecular analysis. The core sequence of the forward primer was (5`-TGAGTCCAAACCGG-3`), while for the reverse primer it was (5`-GACTGCGTACGAATT-3`). The selective nucleotides of the ten primer combinations were: Me6-TAG/Em6-GCA, Me7-TTG/Em9-ACG, Me10-TAC/Em8-AGC, Me8-TGT/Em10-TAG, Me9-TCA/Em7-ATG, Me6-TAG/Em7-ATG, Me8-TGT/Em9-ACG, Me7-TTG/Em7-ATG, Me8-TGT/Em8-AGC and Me9-TCA/Em9-ACG. The method of Li and Quiros (2001) was followed for the SRAP marker system. SRAP-PCR products were separated on 8% polyacrylamide gel (PAGE) and visualized by ethidium bromide.

Molecular data analysis

A binary data matrix indicating the presence (1) or the absence (0) of bands was made from SRAP profiles. Only strong, reproducible and clearly distinguished bands were used for the analysis. The number of unique and specific bands for each agronomic trait was registered. The percentage of polymorphism was calculated by dividing the total number of polymorphic bands on the total number of bands.

RESULTS AND DISCUSSION

Field evaluation of heat stress

Four agronomic traits were evaluated under normal and heat stress, in order to assess the heat tolerance and to estimate the variation among 50 wheat lines. Broad ranges were observed for all agronomic traits. A summary of averages, range and mean of 10 highest lines and 10 lowest lines is shown in Table (2).

The analysis of variance showed highly significant differences amongst lines for all agronomic traits evaluated under heat stress (Table 3).

Table 2. Estimation of minimum and maximum values, mean of all lines, mean of highest and lowest 10 lines and mean of check local cultivars.

Trait	Date	Mean	Min	Max	10-L	10-H	M-cv	-/+ %
NSP	25 Nov.	13.25	7.76	16.30	10.28	15.79	11.62	-
	15 Dec.	12.66	7.47	15.83	9.96	14.99	11.28	4.44
	5 Jan.	10.28	7.14	12.03	8.20	11.78	9.53	22.37
GYP	25 Nov.	34.90	20.93	47.54	27.12	43.13	34.97	-
	15 Dec.	31.99	18.10	44.43	24.31	39.74	34.14	8.34
	5 Jan.	21.60	12.54	31.90	15.92	28.18	26.30	38.11
KW	25 Nov.	4.65	4.00	5.32	4.24	5.02	4.85	-
	15 Dec.	4.47	3.86	5.20	4.06	4.87	4.67	3.77
	5 Jan.	3.46	2.80	4.22	3.02	3.99	3.48	25.54
HI	25 Nov.	33.54	27.63	38.90	29.34	37.89	35.69	-
	15 Dec.	31.40	27.02	38.11	27.88	35.47	33.63	6.39
	5 Jan.	29.56	25.18	35.39	25.98	33.52	32.42	11.87

10-L: mean of lowest 10 lines, 10-H: mean of highest 10 lines, M-cv: mean of local cultivars,
-/+%: percentage of decrease due to heat stress, NSP: No. of spikes per plant, GYP: grain yield per plant, KW: 100 kernel weight, HI: harvest index.

Table 3. Analysis of variance and mean squares of the tested lines for agronomic traitsunder heat stress conditions.

Source	DF	No. of spikes/plant	Grain yield/ plant	100-Kernel weight	Harvest index
YEAR	1	138.89 NS	4140.28 **	69.07 **	1410.78 *
DATE	2	777.20 **	19092.66 **	142.75 **	1285.80 *
Late vs the rest	1	1501.25 **	36971.08 **	280.36 **	1805.08 *
Among Normal	1	53.15 NS	1214.24 NS	5.14 NS	766.51 NS
DATE*YEAR	2	13.39 NS	850.53 NS	3.10 NS	52.54 NS
Error A	12	57.31	373.01	7.46	257.52
LINE	55	63.15 **	348.06 **	1.15 **	137.37 **
LINE*YEAR	55	1.60 **	11.53 **	0.15 **	13.71 **
LINE*DATE	110	2.75 **	33.27 **	0.27 **	5.59 **
LINE*DATE*YEAR	110	0.74 **	5.67 **	0.06 **	7.90 **
Error B	660	0.15	1.04	0.01	0.62

NS= none significant
*** and ** indicate significant at 0.05 and 0.01 probability levels respectively.**

Moreover, significant differences were detected for the effect of year and sowing dates and their interaction with lines, while the interaction between year and date was insignificant. In addition, the effect of year was highly significant in all traits except no. of spikes per plant.

The significant effect of sowing date (Table 3) was mainly due to the difference between the first (25^{th} November) and the third date (5^{th} January) where there was insignificant variation between the first and the second sowing date (15th December).

Heat stress affected the studied traits significantly at the third date. In this regard, the grain yield per plant was the most affected trait by heat stress while harvest index was the lowest. Particularly, the reduction due to heat stress was by 4.44 and 22.37% for No. of spikes per plant, 8.34 and 40.68% for yield, 3.77 and 25.54% for 100 kernel weight and 6.39 and 11.87% for harvest index under the second and third dates, respectively compared with the first date (Table 2). Furthermore, the average performance of the highest 10 lines (10-high lines) in all traits under the third date was higher than the highest performance of local cultivars except for harvest index, in which cultivars Gemmiza-11 and Sids-12 showed higher performance. However, some lines showed higher performance than these cultivars in HI, i.e. L34, L38 and L41. The performance of the tested lines and local cultivars under heat stress is shown in Tables 4 and 5.

Among the tested lines four of them (L1, L11, L37 and L41) surpassed in the three traits, No. of spikes per plant, grain yield per plant and harvest index. While two lines (L22 and L42) preceded in the two traits, grain yield per plant and 100-kernel weight. Moreover, five lines (L14, L22, L39, L44 and L47) showed higher performance in 100-kernel weight. However, none of the local cultivars was able to show high performance in more than one trait. As under the third date the highest performance shown by for No. of spikes per plant, yield, 100-kernel weight and harvest index was by Shandawil-1, Sahel-1, Sids-12 and Gemmiza-11, respectively (Tables 4 and 5).

Heat sensitivity index values (Table 5) ranged from 0.59 to 1.54. Six lines (L1, L11, L16, L34, L37 and L41) were relatively heat tolerant (HSI values < 1), and surpassed in grain yield/plant of 29.45, 29.75, 27.75, 27.43, 27.37 and 31.90 g, respectively compared with local commercial varieties under late sowing condition representing that these lines were less affected by terminal heat stress under late sowing conditions.

Molecular analysis of wheat lines under heat

Ten lines were selected for both the highest (10-high) and lowest (10-low) performance in four agronomic traits evaluated under heat stress for molecular comparison. Ten SRAP primer combinations were used to assess the genetic variability between the two bulked DNA of the 10-high and 10-low lines. The number of bands per primer ranged from 9 to 20 with an average of 15 bands. The 10 SRAP primers generated a total of 161, 169, 162 and 166 bands in the comparison of 10-high and 10-low lines in No. of spikes per plant, grain yield per plant, 100-kernel weight and harvest index respectively (Table 6). SRAP was able to differentiate between the 10-high and 10-low bulked-lines by generating several unique bands specific for each, in all tested traits except harvest index (Table 6 and Fig 1).

Table 4. Mean performance of number of spikes/plant and 100-kernal weight.

Genotypes	No. of spikes/plant			100 kernel weight		
	1st date	2nd date	3rd date	1st date	2nd date	3rd date
1	14.87	14.50	12.53	6.22	6.06	4.72
2	12.93	11.89	11.04	6.46	6.36	4.47
3	15.49	14.32	12.16	6.52	6.16	5.01
4	16.07	14.07	10.95	6.75	6.56	5.35
5	13.33	13.17	10.56	5.72	5.49	4.30
6	11.64	11.05	9.66	6.03	5.93	5.22
7	13.47	13.30	10.51	6.84	6.66	5.52
8	14.94	13.02	9.98	5.49	5.90	4.56
9	10.60	10.14	8.46	7.18	7.11	4.59
10	13.37	12.34	7.63	6.48	6.08	5.06
11	16.36	15.45	11.88	6.16	6.13	4.84
12	13.74	11.80	11.01	6.16	6.09	4.91
13	12.91	12.63	10.74	6.80	6.56	4.42
14	16.14	14.42	11.72	6.43	6.19	5.50
15	16.73	14.93	12.37	6.00	5.89	4.66
16	10.93	10.99	9.64	6.06	5.43	4.52
17	17.06	16.06	12.93	6.46	5.78	4.73
18	13.42	12.46	10.68	6.79	6.48	4.73
19	12.91	12.53	11.21	6.34	6.23	3.84
20	10.82	10.25	8.83	6.48	6.02	4.55
21	12.24	11.85	8.21	6.52	6.38	4.46
22	15.66	14.88	11.61	6.52	6.55	5.49
23	16.06	15.27	10.90	6.90	6.56	4.57
24	12.60	12.32	11.01	5.74	5.57	4.80
25	13.49	13.10	9.86	5.58	5.33	4.08
26	17.03	16.30	12.12	6.41	6.23	4.59
27	15.27	14.57	11.76	6.32	6.02	4.87
28	15.30	14.45	11.93	7.29	7.18	5.76

Table 4. Cont.

Genotypes	No. of spikes/plant			100 kernel weight		
	1st date	2nd date	3rd date	1st date	2nd date	3rd date
29	13.03	12.38	10.53	6.04	5.97	4.38
30	15.05	14.56	12.06	6.52	6.67	5.03
31	16.01	15.15	11.36	6.28	6.06	4.09
32	11.57	11.52	10.03	5.70	5.60	4.25
33	14.49	14.07	12.49	6.61	5.82	4.28
34	13.83	13.17	11.46	5.99	5.72	4.37
35	15.24	14.33	11.41	6.35	6.16	5.31
36	12.34	11.33	9.51	6.15	5.90	5.15
37	14.70	14.33	11.90	6.52	6.20	4.50
38	12.53	12.23	10.67	6.56	6.31	4.12
39	16.52	15.80	11.96	6.26	6.58	5.31
40	14.23	14.02	12.38	6.64	6.29	4.51
41	17.01	16.08	12.21	6.32	5.99	4.14
42	15.61	14.50	11.61	6.54	6.34	5.21
43	10.59	10.35	9.18	6.58	6.64	4.34
44	12.29	12.03	9.36	6.83	6.71	5.89
45	9.40	9.18	8.88	6.79	6.64	4.61
46	10.96	11.00	8.54	6.36	6.02	4.57
47	8.13	7.80	7.32	6.42	6.50	5.58
48	12.11	11.92	8.79	6.46	6.17	4.58
49	13.41	13.27	9.62	6.67	6.62	5.46
50	15.44	15.06	11.83	7.04	6.94	4.59
Mean	13.80	13.12	10.70	6.41	6.22	4.77
Giza 168	13.63	13.58	10.86	6.71	6.49	4.86
Gemmiza11	13.00	12.42	11.27	6.43	6.28	4.85
Sids 12	5.38	5.42	4.39	7.28	7.04	5.27
Shanda.1	15.79	15.02	12.08	6.41	6.37	4.68
Masr 1	12.63	12.43	9.98	6.57	6.20	4.18
Sahel 1	12.28	11.70	11.08	6.70	6.30	5.13
$LSD_{0.05}$	0.79			0.11		

Table 5. Mean performance of harvest index, grain yield/plant and HSI.

Genotypes	Harvest index			Grain yield/plant			
	1st date	2nd date	3rd date	1st date	2nd date	3rd date	HSI
1	46.16	46.81	43.19	45.84	41.19	29.45	0.86
2	41.51	41.88	43.11	36.16	32.44	27.29	0.59
3	41.34	41.33	34.89	35.31	31.21	17.10	1.24
4	46.42	46.17	39.22	45.88	41.43	21.57	1.27
5	46.20	46.12	43.93	35.85	31.78	24.02	0.79
6	46.97	47.19	39.43	33.72	31.05	17.09	1.19
7	39.49	39.94	39.80	32.72	31.13	19.90	0.94
8	37.79	38.93	41.87	28.41	24.80	19.97	0.71
9	47.37	48.78	38.97	38.80	36.50	21.02	1.10
10	39.33	38.44	43.43	29.57	25.95	20.57	0.73
11	48.70	48.17	46.95	47.81	43.53	29.75	0.91
12	43.40	43.43	39.54	34.97	29.91	19.90	1.04
13	45.85	46.59	42.08	33.60	31.33	19.72	0.99
14	42.75	42.06	38.28	35.53	32.10	19.93	1.06
15	42.36	42.18	38.35	35.72	31.36	20.76	1.01
16	48.57	49.43	50.67	35.97	33.30	27.75	0.55
17	40.70	38.60	37.09	37.61	31.20	20.75	1.08
18	42.70	43.04	35.61	36.64	34.34	16.78	1.30
19	38.13	39.45	37.11	36.58	34.06	23.52	0.86
20	43.29	44.52	46.08	29.07	27.87	20.50	0.71
21	40.09	39.35	33.97	30.22	27.01	16.10	1.12
22	44.75	42.24	43.34	38.47	33.97	26.64	0.74
23	47.73	47.00	48.30	36.81	33.18	23.04	0.90
24	46.03	45.21	48.36	43.03	38.89	27.12	0.89
25	47.34	45.04	40.49	33.28	28.46	17.50	1.14
26	49.67	49.14	40.34	48.12	43.35	17.24	1.54
27	46.95	44.42	41.20	46.15	39.30	22.02	1.26
28	43.37	45.05	36.88	39.52	37.89	16.62	1.39

Table 5. Cont.

Genotypes	harvest index			Grain yield/ plant			
	1st date	2nd date	3rd date	1st date	2nd date	3rd date	HSI
29	42.13	42.38	42.35	38.25	33.39	24.66	0.85
30	40.76	39.96	39.30	39.84	35.27	22.81	1.03
31	43.79	42.77	41.76	38.42	34.39	21.68	1.05
32	49.48	48.62	44.72	37.69	34.73	19.85	1.14
33	50.78	50.25	43.91	37.16	35.15	19.88	1.12
34	48.95	47.19	51.71	42.37	36.12	27.43	0.85
35	50.78	48.52	42.08	38.80	34.11	19.03	1.22
36	37.07	36.92	42.96	24.20	21.78	16.10	0.81
37	48.34	46.97	45.22	45.90	40.47	27.37	0.97
38	49.77	51.77	47.93	43.74	41.40	25.92	0.98
39	42.15	40.55	37.85	41.12	34.13	19.67	1.25
40	39.36	35.50	38.06	32.60	26.46	17.65	1.10
41	50.84	49.54	49.58	50.25	45.84	31.90	0.88
42	40.98	40.52	44.08	40.22	35.77	27.10	0.78
43	44.22	42.94	44.93	36.18	33.29	24.21	0.80
44	40.16	38.25	43.33	24.68	22.29	16.74	0.77
45	41.58	39.14	34.66	31.49	28.23	16.65	1.13
46	36.60	37.25	43.48	33.30	30.98	21.53	0.85
47	40.14	38.47	35.74	21.74	18.88	12.54	1.02
48	47.08	45.72	47.08	37.47	33.33	22.07	0.99
49	40.43	40.50	40.60	33.86	31.12	18.90	1.06
50	39.28	39.80	41.68	37.65	35.59	22.56	0.96
Mean	43.99	43.48	41.91	36.97	33.22	21.60	
Giza 168	42.89	43.63	45.61	37.40	40.19	27.04	
Gemmiza11	51.27	51.96	45.23	39.28	38.12	24.94	
Sids 12	46.90	47.52	50.15	38.38	39.92	26.78	
Shanda.1	44.31	42.79	45.14	38.63	38.63	26.53	
Masr 1	49.19	49.41	43.50	35.04	38.26	25.21	
Sahel 1	47.45	46.97	45.38	33.46	37.98	27.30	
$LSD_{0.05}$	1.02			0.81			

Table 6. Level of polymorphism and number of specific bands of 10 lines showed highest and lowest performance in the agronomic traits under heat stress.

Primers	Traits																TSB
	No. of spikes per plant				Grain yield per plant				100-kernel Weight				Harvest index				
	TNB	%P	L	H	TNB	%P	L	H	TNB	%P	L	H	TNB	%P	L	H	
1	18	0.00	0	0	20	10.00	2	0	17	0.00	0	0	15	0.00	0	0	2
2	15	13.33	0	2	19	0.00	0	0	16	12.50	0	2	17	0.00	0	0	4
3	14	0.00	0	0	14	0.00	0	0	18	0.00	0	0	14	0.00	0	0	0
4	17	0.00	0	0	17	0.00	0	0	17	0.00	0	0	17	0.00	0	0	0
5	18	0.00	0	0	14	0.00	0	0	17	5.88	0	1	16	0.00	0	0	1
6	19	0.00	0	0	16	0.00	0	0	18	16.67	3	0	20	0.00	0	0	3
7	9	0.00	0	0	13	23.08	2	1	9	0.00	0	0	10	0.00	0	0	3
8	14	0.00	0	0	18	5.56	1	0	19	0.00	0	0	18	0.00	0	0	1
9	17	0.00	0	0	18	0.00	0	0	11	0.00	0	0	19	0.00	0	0	0
10	20	0.00	0	0	20	0.00	0	0	20	0.00	0	0	20	0.00	0	0	0
Total	161	1.24	0	2	169	3.55	5	1	162	3.70	3	3	166	0.00	0	0	14
G-total			2				6				6				0		

TNB: total number of bands, %P: percentage of polymorphism, L: Lines with low performance, H: lines with high performance,TSB: total number of specific bands, G-total: grand total.

Figure captions

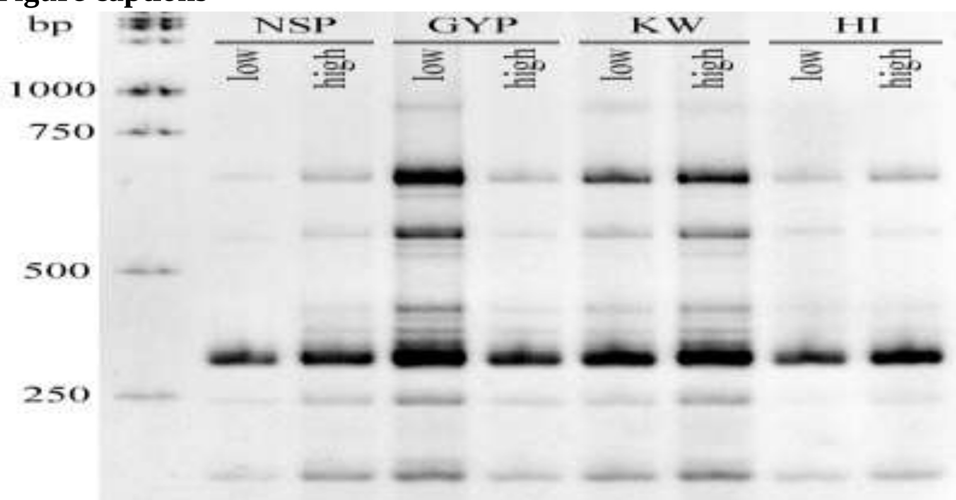

Figure 1. SRAP profile showing the difference between two bulks of 10-high and low performance wheat lines in some traits evaluated under heat stress; NSP: No. of spikes per plant, GYP: grain yield per plant, KW: 100 kernel weight, HI: harvest index, H: bulk of the highest 10 lines and L: bulk of the lowest 10 lines.

A total of 14 bands were unique and specific for the tested lines, of which 2 bands were specific for lines with high No. of spikes per plant. Six polymorphic bands were generated for grain yield per plant of which 5 bands were specific for lines with low yield and one band was for lines of high yield. When lines compared for the 100-kernel weight trait, 6 polymorphic bands were generated, of which three bands were specific for lines with high 100-kernel weight and three bands for lines low in 100-kernel weight. However, no specific bands were generated for lines with high or low harvest index.

The percentage of polymorphism (%P) between 10-high and 10-low lines in the tested traits varied depending on the trait and SRAP primers. The highest %P per primer was 23.08% generated by primer-7 in the grain yield. While the average percentage of polymorphism of all primers ranged from 1.24 to 3.70% for No. of spikes per plant and 100 kernel weight traits, respectively (Table 6).

In this study, the significant variations found among lines indicated the high range of variability in these lines toward heat stress. High temperatures have general effects in developing cereal grains including faster rate of grain development, decrease of kernel weight, shriveled seeds, reduced starch accumulation and alterations of polypeptide and lipid composition (Stone 2001). Similar to our findings, Laghari *et al* (2012) studied the effect of high temperature stress on yield and its components in wheat; they reported that each genetic trait responded differently to high temperature stress. In the present study, grain yield was the most affected trait by heat stress followed by 100-kernel weight and No. of spikes per plant, while the lowest affected trait was harvest index. Moreover, previous studies showed similar percentages of reduction in agronomic traits, e.g. 10-15, 17 and 20.61% in kernel weight (Wardlaw and Wrigley 1994, Blumenthal *et al* 1995 and Modarresi *et al* 2010, respectively), and 40.23 and 46.63% in yield (Hamam 2013 and Modarresi *et al* 2010, respectively).

The main effect of heat stress caused by the third sowing date (5th of January), while there was insignificant effect for the second sowing date (15th of December) as compared with the first date (25th of November). These findings matched with previous studies (Sial *et al* 2005 and Hamam 2013). The effect of high temperature mainly happens during the grain filing period in wheat which generally during March to May (Al-Doss *et al* 2010). Six lines (L1, L11, L16, L34, L37 and L41) gave the low values of heat sensitivity index (HSI values < 1), and the highest grain yield/plant of 29.45, 29.75, 27.75, 27.43, 27.37 and 31.90 g, respectively under late sowing condition. Moreover, these lines were superior in their performance under heat stress than the local cultivars used for comparison in this study, which open the opportunity for the replacement the local cultivars with some of these lines in high temperature environments.

The genetic basis of tolerance could be determined by associating the prevalence of molecular markers with morphological scores to predict DNA genomic regions that harbor a factor influencing the plant's response (Roy *et al* 2011). Among several molecular marker have been applied with wheat, SRAP was conveyed as an effective technique for wheat diversity evaluation due to its advantages including the capacity to reveal relatively more informative bands leading to desirable discrimination ability, combination of reliability and genomic abundance with high levels of polymorphism and targeting coding sequences (Zaefizadeh and Goliev 2009, Dong *et al* 2010, Al-Doss *et al* 2011, Elshafei *et al* 2013 and El-Rawy and Youssef 2014).

Bulking the DNA *of* 10 lines with the highest or lowest performance in each trait reduces the differences among each group and displays mainly the difference in the trait of interest. In this study, SRAP was able to differentiate between the two bulked DNA samples related to the highest and lowest performance lines in all studied traits, except harvest index. The number of specific bands for high or low-bulked DNA in the studied traits was matched with the percentage of reduction caused by heat stress in the agronomic traits. In this regard, yield and 100-kernel weight showed the highest number of specific bands as they also were the most affected traits by heat tolerance. While, harvest index showed the lowest reduction by heat, as well no specific bands were generated for this trait.

Recently, El-Rawy and Youssef (2014) used SRAP to evaluate some bread wheat lines under drought and heat stress using seedling traits. They reported that, SRAP was able to generate some unique and specific bands for drought tolerance; however, in contrary to our findings, no specific bands were generated for heat tolerance in their study. In addition, Moustafa *et al* (2014) reported that, TRAP and SRAP markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to six agronomic traits; (days to heading, plant height, spike number/m^2, kernel number/spike, 1000-kernel weight and grain yield), as indicators for drought tolerance genes in wheat.

In conclusion, the late sowing date (5^{th} of January) used in this study showed the differences among the tested lines by reducing agronomic traits particularly grain yield and 100-kernel weight. Six lines (L1, L11, L16, L34, L37 and L41) produced the highest grain yield under heat stress conditions (HSI values < 1) and were superior to some local cultivars, indicating their heat tolerance that they can be employing in breeding programs for stress environments. SRAP was able to differentiate between bulked lines of high and low performance for agronomic traits by generating several unique and specific bands for each performance. These bands could serve in wheat genotyping and consequence breeding programs as heat tolerance markers.

ACKNOWLEDGMENTS

The authors wish to thank the Regional Councils for Agricultural Research and Extension, Ministry of Agriculture and land reclamation, Egypt for the financial support.

REFERENCES

Ahmad M, G. Shabbir, N.M. Minhas and M.K.N. Shah (2013). Identification of drought tolerant wheat genotypes based on seedling traits. Sarhad J. Agric. 29:21-27

Al-Doss A.A., A.A. Elshafei, K.A. Moustafa, M. Saleh and M.N. Barakat (2011). Comparative analysis of diversity based on morphoagronomic traits and molecular markers in durum wheat under heat stress. African J. Biotech. 10:3671-3681.DOI: 10.5897/AJB10.1993

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

Al-Khatib K., G.M. Paulsen (1990). Photosynthesis and productivity during high temperature stress of wheat genotypes from major world regions. Crop Sci 30:1127–1132

Blumenthal C., F. Bekes, P.W. Gras, E.W.R. Barlow and C.W. Wrigley (1995). Identification of wheat genotypes tolerant to the effects of heat stress on grain quality. Cereal Chemistry 72:539-544

Boyer J.S. (1982). Plant productivity and environment. Science 218:443–448. doi:10.1126/science.218.4571.443

Dellaporta S.L., H. Wood and H.B. Hicks (1983). A plant DNA mini-preparation: version II. Plant Mol Biol Rep 1: 19–21

Dong P., Y. Wei, G. Chen, W. Li, H. Wang, E. Nevo and Y. Zheng (2010). Sequence-related amplified polymorphism (SRAP) of wild emmer wheat (*Triticum dicoccoides*) in Israel and its ecological association. Biochem. Syst. Ecol. 38: 1–11

Eivazi A.R., M.R. Naghavi, M. Hajeidari, S.M. Pirseyedi, M.R. Ghaffari, S.A. Mohammadi, I. Majidi, G.H. Salekedeh and M. Mardi (2007). Assessing wheat (*Triticum aestivum* L.) genetic diversity using quality traits, amplified fragment length polymorphism, simple sequence repeats and proteome analysis. Annals of Applied Biology 152: 81-91 doi:10.1111/j.1744-7348.2007.00201.x

El Siddig M.A., Dweikat I, Baenziger S, El Hussein and Elbasyoni I. (2013). Genetic diversity among Sudanese wheat cultivars as revealed by molecular markers. Middle-East J. Sci. Res. 14:1135-1142

El-Mouhammady A.A., M.R. Rady and E.H. El-Seidy (2014). Assessment of genetic variability for six lines of wheat using physiological traits and molecular markers technique under normal irrigation and water stress conditions. World Appl. Sci. J. 29: 506-516

El-Rawy M.A. and M. Youssef (2014). Evaluation of drought and heat tolerance in wheat based on seedling traits and molecular analysis. J. Crop Sci. Biotech 17(3):183-189. doi:10.1007/s12892-014-0053-x

Elshafei A.A., M. Saleh, A.A. Al-Doss, K.A. Moustafa, F.H. Al-Qurainy and M.N. Barakat (2013). Identification of new SRAP markers linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits in wheat under water-stressed condition. Aust. J. Crop Sci. 7:887-893

FAOSTAT (2014). Food and Agriculture Organization of the United Nations. http://faostat.fao.org

Fischer R.A. and D.B. Byerlee (1991). Trends of wheat production in the warmer areas: major issues and economic considerations. In: Wheat for the Non-traditional Warm Areas. Proceeding conference. CIMMYT, Iguazu, pp 3–27

Fischer R.A. and R. Maurer (1978). Drought resistance in spring wheat cultivars: 1. Grain-yield responses. Aust. J. Agric. Res. 29:897-912

Hamam K.A. (2013). Response of Bread Wheat Genotypes to Heat Stress. Jordan Journal of Agricultural Sciences 9:486-506

Joshi A.K., R. Chand, B. Arun, R.P. Singh and R. Ortiz (2007). Breeding crops for reduced-tillage management in the intensive, rice-wheat systems of South Asia. Euphytica 153(1–2):135–151

Kumari M., R.N. Pudake, V.P. Singh and A.K. Joshi (2013). Association of staygreen trait with canopy temperature depression and yield traits under terminal heat stress in wheat (*Triticum aestivum* L.). Euphytica 190:87–97. doi: 10.1007/s10681-012-0780-3

Laghari K.A., M.A. Sial and M.A. Arain (2012). Effect of high temperature stress on grain yield and yield components of wheat (*Triticum aestivum* L.). Sci Tech and Dev 31 (2):83-90

Li G. and C.F. Quiros (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. Theor. Appl. Genet. 103:455–461

Maestri E., N. Klueva, C. Perrotta, M. Gulli , H.T. Nguyen and N. Marmiroli (2002). Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Molecular Biology 48:667–681

Modarresi M., V. Mohammdi A. Zali and M. Mardi (2010). Response of wheat yield and yield related traits of high temperature. Cereal Research communications 38(1):23-31

Moustafa K.A., M. Saleh, A.A. Al-Doss, A.A. Elshafei, A.K. Salem, F.H. Al-Qurainy and M.N. Barakat (2014). Identification of TRAP and SRAP markers linked with yield components under drought stress in wheat (*Triticum aestivum* L.). Plant Omics Journal 7(4):253-259

Nissen O. (1984). MSTAT. A microcomputer program for statistical analyses of experiments and surveys. In H Riley, AO Skjelvag, eds, The Impact of Climate on Grass Production and Quality. Proc General Meet Eur Grassl Fed, 10th, As, Norway. 26–30 June 1984. Norwegian State Agric. Res. Stations, As, pp.555–559

Roy S.J., E.J. Tucker and M. Tester (2011). Genetic analysis of abiotic stress tolerance in crops. Current Opinion in Plant Biol. 14:232–239

Salem K.F.M., A.M. El-Zanaty and R.M. Esmail (2008). Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatellite markers. World J. Agric. Sci. 4:538-544

Shafeeq S., M. Rahman and Y. Zafar (2006). Genetic variability of different wheat (*Triticum aestivum* L.). Pak. J. Bot. 38:1671-1678

Sial M.A., M.A. Arain, S.K.M.H. Naqvi, M.U. Dahot and N.A. Nizamani (2005). Yield and quality parameters of wheat genotypes as affected by sowing dates and high temperature stress. Pak J Bot 37(3):575-584

Soliman H.I.A. and M.H. Hendawy (2013). Selection for drought tolerance genotypes in durum wheat (*Triticum durum* Desf.) under *in vitro* conditions. Middle-East J. Sci. Res. 14: 69-78

Stone, P. (2001). The effects of heat stress on cereal yield and quality. In: A.S. Basra (Ed.) Crop Responses and Adaptations to Temperature Stress, food products Press, Binghamton, NY, pp. 243–291.

Stulnig T.M. and A. Amberger (1994). Exposing contaminating phenol in nucleic acid preparations. BioTech. 16:403–404
Wardlaw, I.F. and C.W. Wrigley (1994). Heat tolerance in temperate cereals: an overview. Aust. J. Plant Physiol. 21: 695–703.
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
Youssef M. (2012). Khirshyat 1.0: a simple micro-program for some molecular biology protocols. Genes, Genomes and Genomics 6: 102-105
Zaefizadeh M and R. Goliev (2009). Diversity and relationships among durum wheat landraces (subconvars) by SRAP and phenotypic marker polymorphism. Res. J. Biol. Sci. 4:960-966

تقييم التحمل للحرارة فى قمح الخبز باستخدام بعض الصفات المحصولية وواسمات SRAP

علاء على سعيد [١] *، الحسين حماده[٢]، محمد يوسف[٣]

١. قسم المحاصيل – كلية الزراعة – جامعة سوهاج
٢. قسم المحاصيل – كلية الزراعة – جامعة أسيوط
٣. قسم الوراثة – كلية الزراعة – جامعة أسيوط

تم تقييم ٥٠ سلالة من قمح الخبز للتحمل للحرارة و مقارنتهم ببعض الاصناف المحلية تحت ثلاثة مواعيد زراعة (٢٥ نوفمبر، ١٥ ديسمبر و ٥ يناير). استخدمت اربع صفات محصولية وهم عدد السنابل على النبات ، ومحصول الحبوب للنبات الفردى، وزن الـ ١٠٠ حبة و ومعامل الحصاد فى كل من الزراعة العادية وظروف الاجهاد. وقد اظهر تحليل التباين فروق معنوية جدا بين السلالات المدروسة نتيجة لمواعيد الزراعة المتاخرة. وكان محصول الحبوب للنبات الفردى اكثر الصفات تأثرا بالحرارة يتبعة وزن الـ ١٠٠ حبة ثم عدد السنابل للنبات بينما كان معامل الحصاد اقل الصفات تأثرا نتيجة للاجهاد الحرارى. أظهرت ٦ سلالات (س ١، س ١١، س ١٦، س ٣٤، س ٣٧ وس ٤١) تحملا للحرارة عن طريق الاداء العالى فى صفة محصول الحبوب للنبات الفردى تحت ظروف الزراعة المتأخرة وكانت على التوالى (٢٩.٤٥، ٢٩.٧٥، ٢٧.٧٥، ٢٧.٤٣، ٢٧.٣٧ و ٣١.٩٠ جم) بالاضافة الى ذلك كانت لهم اقل قيم فى معامل الحساسية للحرارة. كان للتتابعات واسمات الـ SRAP القدرة على التفرقة بين عينات الحامض النووى المجمعة من السلالات التى كان لها اعلى واقل اداء فى الصفات المحصولية المدروسة تحت ظروف الاجهاد الحرارى. حيث كانت لها القدرة على اظهار ٢، ١ و ٣ حزم خاصة بالسلالات ذات الاداء العالى فى صفات عدد السنابل للنبات ، محصول الحبوب للنبات و معامل الحصاد على التوالى. بالاضافة الى ذلك نجحت ايضا فى اظهار ٥ و ٣ حزم متخصصة للسلالات ذات الاداء المنخفض فى صفتى محصول الحبوب للنبات و معامل الحصاد على التوالى. هذه الحزم المتخصصة قد يكون من الممكن استخدامها فى تمييز سلالات القمح ويمكن ايضا استخدام واسمات الـ SRAP المرتبطة بالتحمل للحرارة فى برامج التربية فى القمح.

المجلة المصرية لتربية النبات ١٩(٣) : ٩٧٩- ٩٩٤ (٢٠١٥)