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GENOTYPING AND PHENOTYPING FOR SOME BREAD WHEAT GENOTYPES UNDER TERMINAL HEAT STRESS

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ABSTRACT

The present investigation was conducted at Shandaweel Agricultural Research Station, Agricultural Research Center, Egypt during two winter seasons to assess 12 bread wheat genotypes under normal and late sowing dates by using agronomic data and SSR markers. The studied genotypes were tested in a RCBD experiment for each sowing date; normal was on 25th November while late sowing date (heat stress) was on 25th December during 2017/2018 and 2018/2019 seasons. The results indicated highly significant differences among genotypes, sowing dates, and seasons for all traits. Significant or highly significant differences were found for the interactions between genotype x sowing date, genotype x seasons, sowing date x season, and genotype x sowing date x season for most of the studied traits. Based on heat susceptibility index the genotypes were classified into three groups i.e. tolerant, moderate tolerant, and sensitive. The molecular marker analysis showed high values of polymorphism information content (PIC) and marker index (MI). A positive and significant association was found between grain filling period and Xgwm293 marker with 87 bp specific band (r = 0.58*), as well as between grain filling rate and Xgwm557 marker with 437 bp specific band (r = 0.67*). These markers are suggested as candidate linked markers with the particular traits; they can be used in marker assisted selection (MAS). Specific fragments were unique to some genotypes. Although, some of these fragments were found to be linked with some of the studied traits, the others were not. The dendrograms successfully grouped the studied genotypes based on their performance under sowing dates as well as based on SSR patterns. The current study revealed that there were some high yielding genotypes tolerant to terminal heat stress. These genotypes can be utilized either in terminal heat stress affected regions or in breeding programs as a heat tolerance source.

Key words: ***Agronomic traits, Molecular markers, Sowing dates, Grain filling period***.

INTRODUCTION

Wheat is the most important grain worldwide based on its acreage and ranked the second after maize when it comes to the total production values. The world total wheat planted area and total production are 218.54 million hectares and 771.72 million metric tons in 2017, respectively (FAOSTAT 2017). The scenario is the same in Egypt where wheat is the most important cereal crop in the winter growing season, where its cultivated area in 2017 was 1.34 million hectares (equal to 3.20 million feddan; fed. = 2.38 hectares) producing 8.80 million tons (FAOSTAT 2017). There is a huge gap between production and consumption; this gap can be filled by international imports from foreign countries which in turn cost the government a very large amount of hard currencies. For all these aspects wheat is considered a strategic, staple food crop and took a very special attention from Egyptian government because it affects directly on

Egypt's economy. The optimum temperature for wheat growth stages ranges from 15 to 30 °C, however, wheat can be grown near optimally up or down few degrees *i.e.* 20-32 °C (Wheeler 2012). Heat stress terminology is known as rise in air temperature beyond the optimum temperature for growth stages to be sufficient to cause injury or irreversible damages. Heat stress is a major and a very serious constraint limiting grain yield and has negative effects on wheat production (Asseng *et al* 2015). Heat stress became more severe and more frequent as a consequent of climate change impacts. Climate changes, which rise from increased CO_2 in the atmosphere, cause heat waves worldwide leading to significant yield losses with great risks for future global food security (Deepak *et al* 2019).

Elevated air temperature during the grain filling period or post anthesis (reproductive stages) is known as terminal heat stress. Terminal heat stress (≥ 32 °C) causes reduction in starch content, grain quality, and grain weight which is negatively reflected on grain yield (Gupta *et al* 2015). Moreover, the day/night temperatures play a crucial role in heat stress scenario, where higher temperature than optimal, ranging from 18/13 and 21/16 diminished period of grain filling (Dias and Lidon 2009). There are many investigations and extensive work on terminal heat stress because of its serious affects on grain weight, grain number, protein content, starch composition, and grain yield. This reduction under severe terminal heat stress may be up to 47% and 67% in yield and 1000-kernel weight, respectively (Joshi *et al* 2016). It has been shown that there are two types of terminal heat stress; the first type of terminal heat stress is heat shock which happens suddenly, extreme high temperature for a short period of 3-5 days. While the second type is chronic heat stress which is means high maximum temperature for longer time at grain filling period (Talukder *et al* 2014).

Thermo tolerance is a complex trait results in a number of mechanisms, controlled by many genes which in turn alter some pathways to express the tolerance. Because of the complexity of the heat tolerance inheritance, DNA molecular markers present a helpful tool to be used in plant breeding programs. Molecular markers linked to terminal heat tolerance related traits are prerequisite in identifying quantitative trait loci (QTL) related to these traits to be used in marker assisted selection (MAS) in plant breeding programs. Recently many investigations were carried out on identifying QTLs associated with traits related to terminal heat tolerance *e.g.* Paliwal *et al* (2012), Pandey *et al* (2014) and Sharma (2016).

The objectives of this study were: (1) Assessment of some bread wheat genotypes, under normal and terminal heat stress conditions, to determine their capability to tolerate terminal heat stress. (2) Detection of some molecular markers linked to some important agronomic traits, related to heat stress conditions, to be utilized as marker assisted selection (MAS) in breeding programs.

MATERIALS AND METHODS

Plant materials

The present study was conducted at Shandaweel Agricultural Research Station, Agricultural Research Center, Egypt, during the two successive growing seasons 2017/2018 and 2018/2019. Twelve bread wheat genotypes were included in the present study (Table 1). The twelve bread wheat genotypes were planted at two sowing dates. The normal sowing date was planted on 25th November and the late sowing date (terminal heat stress) was planted on 25th December. The experimental design for each sowing date was in randomized complete block design (RCBD) with three replications. Each plot consists of 6 rows spaced by 20 cm and of 3.5 m long with a total area of 4.2 m^2.

Table 1. The pedigree of the twelve bread wheat genotypes and their origin.

No.	Genotypes	Pedigree	Origin
1	Shandaweel 1	Site / Mo / 4/ Nac / Th. Ac //3* Pvn /3/ Mirlo / Buc	Egypt
2	Line 1	QUAIU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//…	CIMMYT
3	Misr 3	Rohf 07*2/Kiriti	Egypt
4	PBW 343	ND/VG9144//KAL/BB/3/YACO/4/VEE #5	India
5	Baj 1	Waxing/4/sni/Trap#1/3/Kauz+2/Trap//Kauz	India
6	Vorobey	CROc-1/AE.TA(wx-224)//OPTA-M-85/3/Pastor	Mexico
7	Gemmiza 11	Bow"s''/Kvz//7C/ Seri 82 /3/Giza 168/Sakha 61	Egypt
8	Gemmiza 12	OTUS/3/SARA/THB//VEE	Egypt
9	Sids 14	Bows “s”/vee”s”// Bows “s”/TSI/Bani Sewef 1	Egypt
10	Giza 171	Gemmiza 9 / Sakha 93	Egypt
11	Misr 2	SKAUZ/ BAV 92	Egypt
12	Sakha 95	PASTOR//SITE/MO/3/CHEN/AEGILOPS SQARROSA(TAUS)//BCN/4/WBLL1	Egypt

The studied traits included days to heading (DH), days to maturity (DM), grain filling period (GFP), grain filling rate (GFR), plant height (PLH), number of spikes/m^2 (S/M^2), 1000-kernel weight (1000-KW), number of kernels/spike (K/S), biological yield in kg/plot (BY), and grain yield in kg/plot (GY). Heat susceptibility index (HSI) was calculated according to Fischer and Maurer (1978).

Molecular markers

The molecular marker characterization was carried out at Department of Genetics, Faculty of Agriculture, Assiut University, Egypt. Eleven SSR markers were used in this study. Total genomic DNA was extracted from young leaves "two weeks old" for all genotypes under investigation using cetyltrimethyl ammonium bromide procedure (modified CTAB procedure) described by Poresbski *et al* (1997). RNAase was added to DNA for 30 minutes at 37° C to remove RNA. DNA amplification was performed and the amplified fragments were separated in 2% gel. The amplicon was visualized by UV light documentation system. The SSR primers, their sequences, chromosomal location (Chr.), annealing temperature (Ann.), and references are presented in Table (2).

Table 2. The forward and reverse primer sequences, chromosome location (Chr.), annealing temperature (Ann.), and references.

No.	Primer	Primers' sequences	Chr.	Ann.	References
1	Xgwm33	5' GGAGTCACACTTGTTTGTGCA3' 5' CACTGCACACCTAACTACCTGC3'	1A	60 C°	Roder *et al* (1998)
2	Xgwm389	5' ATCATGTCGATCTCCTTGACG3' 5' TGCCATGCACATTAGCAGAT3'	3B	60 C°	Roder *et al* (1998)
3	Xgwm513	5' ATCCGTAGCACCTACTGGTCA3' 5' GGTCTGTTCATGCCACATTG3'	4B	60 C°	Roder *et al* (1998)
4	Xgwm261	5' CTCCCTGTACGCCTAAGGC3' 5' CTCGCGCTACTAGCCATTG3'	2D	55 C°	Roder *et al* (1998)
5	Xgwm557	5' ATGGCATAATTTGGTGAAATTG3' 5' TGTTTCAAGCCCAACTTCTATT3'	7B	55 C°	Roder *et al* (1998)
6	Xcfd29	5' GGTTGTCAGGCAGGATATTTG 3' 5' TATTGATAGATCAGGGCGCA 3'	5D	60 ° C	Guyomarc'h *et al* (2002)
7	Xbarc229	5' GGCCGCTGGGGATTGCTATGAT 3' 5' TCGGGATAAGGCAGACCACAT 3'	1D	58° C	Song *et al* (2005)
8	Xgwm484	5' ACATCGCTCTTCACAAACCC 3' 5' AGTTCCGGTCATGGCTAGG 3'	2D	58 ° C	Roder *et al* (1998)
9	Xgwm293	5' TACTGGTTCACATTGGTGCG 3' 5' TCGCCATCACTCGTTCAAG 3'	5A	55° C	Roder *et al* (1998)
10	Xwmc601	5'ACAGAGGCATATGCAAAGGAGG3' 5'CTTGTCTCTTTATCGAGGGTGG3'	2D	C °	Somers *et al* (2004)
11	Xbarc186	5' GGAGTGTCGAGATGATGTGGAAAC 3' 5' CGCAGACGTCAGCAGCTCGAGAGG 3'	5A	58° C	Song *et al* (2005)

Statistical analysis

All calculations and statistical analysis were performed using SAS v9.3 (2011) statistical software including analysis of variance (ANOVA), single linear regression (Karl Pearson coefficient), and cluster analysis. The analysis of variance (ANOVA) was performed to determine the variances due to genotypes and sowing dates, and seasons. Performance means for the genotypes across the two seasons and over all were calculated. Dissimilarity matrix (Euclidian method) was computed for the all studied traits leading to dendrogram using unweighted pair group method arithmetic mean (UPGMA).

Marker analysis

Variability for each locus was estimated using polymorphism information content value (PIC) for SSR markers according to Anderson *et al* (1993). Marker index (MI) was calculated according to Powell *et al* (1996). Dissimilarity matrix (Dice coefficient) was computed, dendrogram was constructed using unweighted pair group method arithmetic mean (UPGMA). Single marker analysis was done based on simple linear regression (Karl Pearson coefficient) computed to find the association between agronomic traits and molecular markers.

RESULTS AND DISCUSSION

Phenotypic evaluation

The analysis of variance (Table 3) indicated highly significant variances due to genotypes, sowing dates, and seasons for all traits, except 1000-kernel weight trait. The results showed that the differences among genotypes were sufficient to provide a scope to characterize the effect of terminal heat stress. The interactions between genotypes, sowing dates and seasons were significant or highly significant for all the studied traits, except season × sowing date interaction for 1000-kernel weight and season × sowing date × genotype interaction for plant height. These results are in agreement with those obtained by Abd El-Rady and Koubisy (2017) who found high significant variances due to genotypes, seasons, sowing dates, and the interaction between genotypes and sowing dates for days to heading, plant height, No. of spikes/m^2, No. of kernels/spike, 1000-kernel weight, and grain yield.

Table 3. Mean squares from combined analysis of variance for all the studied traits.

SOV	df	D H	D M	GFP	GFR	PLH	S/M^2	K/S	1000-KW	B Y	G Y
Seasons (S)	1	5954.7^{**}	11972^{**}	$1040.^{**}$	5210.4^{**}	14440^{**}	204454^{**}	1495^{**}	16.34^{ns}	131.05^{**}	16.74^{**}
R (S)	4	1.07	0.69	1.70	16.99	12.80	383.91	16.05	5.63	0.33	0.05
Dates (D)	1	2756.3^{**}	12750^{**}	3650^{**}	1310.40^{**}	8836^{**}	258741^{**}	1056^{**}	459.75^{**}	28.15^{**}	16.71^{**}
S * D	1	169.00^{**}	966.20^{**}	327^{**}	5622.50^{**}	1100^{**}	12469^{*}	342.3^{*}	18.56	29.24^{**}	3.67^{**}
Error	4	2.71	2.90	2.76	13.01	7.076	799.80	17.10	3.58	0.14	0.05
Genotypes (G)	11	51.08^{**}	16.84^{**}	37.52^{**}	183.07^{**}	266^{**}	2843.60^{**}	78.73^{**}	15.19^{**}	2.32^{**}	0.40^{**}
S * G	11	16.66^{**}	6.96^{**}	17.35^{**}	47.30^{**}	30.20^{**}	1265.50^{**}	81.31^{**}	22.17^{**}	0.65^{**}	0.10^{**}
D * G	11	23.40^{**}	16.89^{**}	16.16^{**}	37.07^{**}	30.20^{*}	1602^{**}	65.45^{**}	13.75^{**}	1.37^{**}	0.08^{**}
S *D*G	11	21.03^{**}	6.19^{**}	26.02^{**}	43.98^{**}	15.35^{ns}	2608.80^{**}	113.30^{**}	15.99^{**}	0.53^{**}	0.07^{**}
Error	88	1.62	1.61	2.75	14.85	12.430	243.85	14.90	1.60	0.12	0.02
C.V %	--	1.36	0.94	3.95	8.73	3.57	4.20	8.15	2.86	5.75	7.08

Where:

ns, *, and ** are non-significant, significant at 0.05 and 0.01 probability, respectively.

DH = days to heading, DM = days to maturity, GFP = grain filling period, GFR = grain filling rate, PLH = plant height, S/M^2 = number of spikes/m^2, K/S = number of kernels/spike, 1000-KW = 1000- kernels weight, BY= biological yield, GY = grain yield.

Effect of genotypes

The results, presented in table 4, revealed that the response of the studied genotypes varied widely and significantly to both of the sowing dates for all the studied traits. These results declared the importance of assessing the genotypes under different environments to identify the best genetic make up for a particular environment. These findings are in harmony with those obtained by Tawfelis *et al* (2011); where they found that highly significant differences between planting dates and bread wheat genotypes for days to maturity, plant height and other agronomic traits.

Table 4. Performance means across the two seasons for the studied traits.

Treatment	DH	DM	GFP	GFR	PLH	S/M^2	K/S	1000-KW	BY	GY
Sowing dates										
Normal	98.0	145.0	47.0	47.2	106.6	414.6	50.1	45.9	10.10	3.30
Late	89.0	126.0	37.0	41.1	90.9	329.8	44.5	42.4	8.10	2.30
Reduction	9.2	13.1	21.3	12.9	14.7	20.5	11.2	7.8	19.80	30.30
F test	**	**	**	**	**	**	**	**	**	**
Cultivars										
Shandaweel	91.3	137.0	45.7	52.8	93.2	360.8	49.4	41.9	8.96	3.09
Line 1	93.9	136.5	42.6	53.3	105.7	380.4	46.6	45.0	10.21	3.29
Misr 3	91.3	135.1	43.8	47.8	95.5	390.7	49.4	43.5	10.04	2.97
PBW 343	96.1	135.5	39.4	46.3	96.2	351.1	50.9	43.8	8.28	2.46
Baj 1	96.3	136.0	39.7	42.9	89.3	390.1	46.8	43.7	9.39	2.95
Vorobey	93.1	135.0	41.9	48.9	103.3	390.4	43.4	45.7	9.73	2.85
Gemmiza 11	90.4	133.1	42.7	45.2	99.1	370.1	42.5	45.1	8.94	2.67
Gemmiza 12	92.3	133.8	41.5	43.5	98.3	361.8	50.6	45.7	8.72	2.66
Sids 14	96.1	137.1	41.0	44.0	101.5	360.3	45.5	43.6	8.25	2.42
Giza 171	92.4	135.7	43.3	41.5	101.4	381.9	45.9	44.9	9.04	2.58
Misr 2	95.4	136.0	40.6	51.0	102.0	348.5	49.9	43.5	8.78	2.83
Sakha 95	93.6	135.2	41.6	51.4	101.7	380.3	47.2	43.8	8.95	2.97
F test	**	**	**	**	**	**	**	**	**	**
L.S.D 0.05	1.03	1.03	1.34	3.11	2.85	12.62	3.12	1.02	0.28	0.11

Where ** is significant at 0.01 probability.

Performance of the genotypes

Days to heading (DH)

Results in Table 5 revealed that mean values of days to heading were (90.4 and 83.7), (105.4 and 94.5) in the first and second seasons for normal and late sowing dates, respectively. While, the mean number for days to heading across the two seasons for normal and late sowing dates were 97.9 and 89.1 days for normal and late sowing dates, respectively. Overall mean values for days to heading ranged from 90.4 day for the cultivar Gemmiza11 to 96.3 day for the cultivar Baj1 with an average of 93.5 days.

Table 5. Mean number of days to heading for the twelve bread wheat genotypes under two seasons, two sowing dates and overall.

Genotypes	Season 1			Season 2			Over all		
	Date 1	Date 2	$\bar{\bar{X}}$	Date 1	Date 2	$\bar{\bar{X}}$	Date 1	Date 2	Mean
Shandaweel 1	89.0	83.0	86.0	99.7	93.7	96.7	94.4	88.4	91.4
Line 1	90.3	83.7	87.0	106.0	95.7	100.9	98.2	89.7	93.9
Misr 3	87.3	81.7	84.5	101.3	95.0	98.2	94.3	88.4	91.3
PBW 343	94.7	87.7	91.2	107.7	94.3	101.0	101.2	91.0	96.1
Baj 1	95.3	87.3	91.3	110.0	92.7	101.4	102.7	90.0	96.3
Vorobey	90.0	83.3	86.7	104.3	94.7	99.5	97.2	89.0	93.1
Gemmiza 11	89.3	80.3	84.8	97.3	94.7	96.0	93.3	87.5	90.4
Gemmiza 12	87.7	82.7	85.2	103.3	95.7	99.5	95.5	89.2	92.4
Sids 14	92.7	86.3	89.5	112.0	93.3	102.7	102.4	89.8	96.1
Giza 171	87.7	83.7	85.7	103.3	95.0	99.2	95.5	89.4	92.4
Misr 2	93.0	85.0	89.0	107.7	96.0	101.9	100.4	90.5	95.4
Sakha 95	87.7	81.0	84.4	112.3	93.3	102.8	100.0	87.2	93.6
Mean	90.4	83.7	87.1	105.4	94.5	100.0	97.9	89.1	93.5
L. S. D. 0.05									
Seasons (S)							0.66	0.57	0.76
Dates (D)			1.26			2.00			0.76
Genotypes (G)	2.18	1.36	1.25	2.38	ns	1.69	1.62	1.39	1.03
S×D									1.08
S×G							2.29	1.97	1.46
D×G			1.77			2.39			1.46
S×D×G									2.06

It is clear that average number of days to heading of the first season was smaller than the same trait in the second season. This difference can be attributed to the rise in temperature of the first season compared to the second season (Figure 1). The results showed that PBW 343 and Sakha 95 had the highest and the lowest number of days to heading under terminal heat stress with 91.0 and 87.2 day, respectively overall the two seasons. Lopes *et al* (2012) found a negative correlation between high days to heading and high yielding genotypes under high temperature environments.

Days to maturity (DM)

Mean number of days to maturity (Table 6) was (133.2 and 119.6), (156.6 and 132.6) day in the first and second seasons for normal and late sowing dates, respectively. While, the mean number of days to maturity across the two seasons for normal and late sowing dates was 144.9 and 126.1 day for normal and late sowing dates, respectively.

Table 6. Means of days to maturity for the twelve bread wheat genotypes under two seasons and two sowing dates and overall.

Genotypes	Season 1			Season 2			Over all		
	Date 1	Date 2	$\overline{X}$	Date 1	Date 2	$\overline{X}$	Date 1	Date 2	Mean
Shandaweel 1	132.0	121.0	126.5	160.7	134.3	147.5	146.4	127.7	137.0
Line 1	134.3	120.0	127.2	158.7	133.0	145.9	146.5	126.5	136.5
Misr 3	132.3	118.7	125.5	157.7	131.7	144.7	145.0	125.2	135.1
PBW 343	135.3	119.3	127.3	155.0	132.3	143.7	145.2	125.8	135.5
Baj 1	135.7	119.3	127.5	156.7	132.3	144.5	146.2	125.8	136.0
Vorobey	133.3	119.3	126.3	155.0	132.3	143.7	144.2	125.8	135.0
Gemmiza1 1	129.0	119.7	124.4	151.0	132.7	141.9	140.0	126.2	133.1
Gemmiza 12	131.0	119.7	125.4	152.0	132.7	142.4	141.5	126.2	133.9
Sids 14	136.0	119.0	127.5	161.3	132.0	146.7	148.7	125.5	137.1
Giza 171	131.7	119.7	125.7	158.3	133.0	145.7	145.0	126.4	135.7
Misr 2	136.0	119.7	127.9	155.7	132.7	144.2	145.9	126.2	136.0
Sakha 95	131.7	119.3	125.5	157.3	132.3	144.8	144.5	125.8	135.2
Mean	133.2	119.6	126.4	156.6	132.6	144.6	144.9	126.1	135.5
L. S. D. 0.05									
Seasons (S)							0.6	0.55	0.8
Dates (D)			1.76			1.7			0.8
Genotypes	2.51	ns	1.7	2.1	ns	1.3	1.6	ns	1.0
S×D									1.1
S×G							2.2	ns	1.5
D×G			2.4			1.8			1.5
S×D×G									2.1

Overall mean number of days to maturity was 135.5 and ranged from 133.1 day for Gemmiza11 to 137.1 day for Sids14. The average number days to maturity in the first season was lower than the second season because the temperatures of the first season were higher than the same time of the second season (Figure 1). Heat stress causes a reduction in reproductive stages which in turn reduces number of days to maturity. Consequently, high number of days to maturity under heat stress conditions is desirable. In this situation, Shandaweel 1 followed by Line 1 are more preferable under late sowing date with an average of 127.7 and 126.5 day across the two seasons, respectively. Lopes *et al* (2012) found positive association between number of days to maturity and grain yield under high temperature environments.

Grain filling period (GFP)

The means of grain filling period (Table 7) were (42.8 and 35.9), (51.2 and 38.1) and (47.0 and 37.0) day in the first season, second season, and across two seasons for normal and late sowing dates, respectively. The average of grain filling period over all ranged from 39.7 day for Baj 1 to 45.7 day for Shandaweel 1 with an average of 42.0 day. The variability between the two seasons in grain filling period can be explained by the high differences between minimum and maximum temperatures during the grain filling period in first and second seasons (Figure 1).

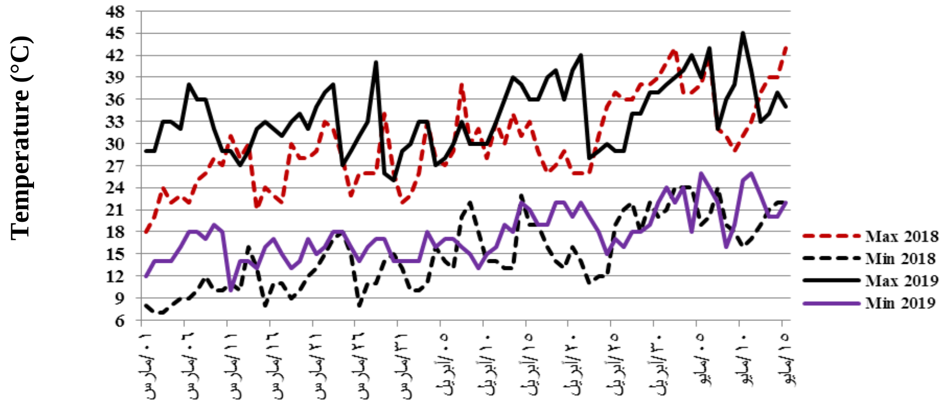


Fig. 1. The average minimum and maximum temperature over the two seasons.

Table 7. Means of grain filing period for the twelve bread wheat genotypes under the two sowing dates, across two seasons, and overall.

Genotypes		Season 1			Season 2			Over all		
		Date 1	Date 2	$\bar{X}$	Date 1	Date 2	$\bar{X}$	Date 1	Date 2	Mean
Shandaweel 1		43.0	38.0	40.5	61.0	40.6	50.8	52.0	39.3	45.7
Line 1		44.0	36.3	40.2	52.7	37.3	45.0	48.4	36.8	42.6
Misr 3		45.0	37.0	41.0	56.4	36.7	46.6	50.7	36.9	43.8
PBW 343		40.6	31.6	36.1	47.3	38.0	42.7	44.0	34.8	39.4
Baj 1		40.4	32.0	36.2	46.7	39.6	43.2	43.6	35.8	39.7
Vorobey		43.3	36.0	39.7	50.7	37.6	44.2	47.0	36.8	41.9
Gemmiza 11		39.7	39.4	39.6	53.7	38.0	45.9	46.7	38.7	42.7
Gemmiza 12		43.3	37.0	40.2	48.7	37.0	42.9	46.0	37.0	41.5
Sids 14		43.3	32.7	38.0	49.3	38.7	44.0	46.3	35.7	41.0
Giza 171		44.0	36.0	40.0	55.0	38.0	46.5	49.5	37.0	43.3
Misr 2		43.0	34.7	38.9	48.0	36.7	42.4	45.5	35.7	40.6
Sakha 95		44.0	38.3	41.2	45.0	39.0	42.0	44.5	38.7	41.6
Mean		42.8	35.9	39.4	51.2	38.1	44.7	47.0	37.0	42.0
L. S. D. 0.05	Seasons (S)							0.81	0.75	0.77
	Dates (D)			2.11			1.10			0.77
	Genotypes	2.79	2.45	1.81	3.03	ns	2.05	1.98	1.84	1.34
	S×D									1.09
	S×G							2.81	2.60	1.90
	D×G			2.56			2.90			1.90
	S×D×G									2.68

The maximum grain filling period across the two seasons, under late sowing, was shown by Shandaweel 1 with an average of 39.3 days. The long grain filling period may give sufficient time for reproductive stages to take place.

Grain filling rate (GFR)

The grain filling rate means (Table 8) were 47.4 and 28.9 g/day in the first season, 46.9 and 53.4 g/day in the second season and 47.2 and 41.1 g/day over the two seasons, for normal and late sowing dates, respectively. Grain filling rate mean overall ranged from 41.5 gm/day up to 53.3 g/day for Giza 171 and Line 1, respectively. High grain filling rate can compensate, roughly, the reduction in grain size and grain weight which will result in high grain yield. Though, the highest grain filling rate under late sowing date was for Line 1 with an average of 48.8 g/day across the two seasons. Based on this concept, Line 1 is expected to have high grain yield among all the studied genotypes under late sowing date across the two seasons (will be discussed later in grain yield trait).

Table 8. Means of grain filing rate (g/day) for the twelve bread wheat genotypes under two seasons, two sowing dates and across the two seasons.

Genotypes	Season 1			Season 2			Over all		
	Date 1	Date 2	$\bar{X}$	Date 1	Date 2	$\bar{X}$	Date 1	Date 2	Mean
Shandaweel 1	52.8	30.6	41.7	43.8	52.4	48.1	48.3	41.5	52.8
Line 1	53.3	37.1	45.2	53.5	60.5	57.0	53.4	48.8	53.3
Misr 3	47.8	26.8	37.3	46.8	59.1	53.0	47.3	42.9	47.8
PBW 343	46.3	29.7	38.0	40.3	48.1	44.2	43.3	38.9	46.3
Baj 1	42.9	36.3	39.6	58.9	56.5	57.7	50.9	46.4	42.9
Vorobey	48.9	22.3	35.6	50.9	55.8	53.4	49.9	39.1	48.9
Gemmiza 11	45.2	23.6	34.4	48.3	47.5	47.9	46.8	35.5	45.2
Gemmiza 12	43.5	28.7	36.1	43.2	55.5	49.4	43.4	42.1	43.5
Sids 14	44.0	23.3	33.6	37.9	44.3	41.1	40.9	33.8	44.0
Giza 171	41.5	27.0	34.2	38.2	52.7	45.4	39.8	39.9	41.5
Misr 2	51.0	29.1	40.1	49.1	54.3	51.7	50.0	41.7	51.0
Sakha 95	51.4	31.8	41.6	52.3	53.8	53.1	51.8	42.8	51.4
Mean	47.4	28.9	38.1	46.9	53.4	50.2	47.2	41.1	47.4
L. S. D. 0.05									
Seasons (S)							ns	2.01	1.67
Dates (D)			2.73			4.39			1.67
Genotypes (G)	5.50	7.59	4.57	6.03	6.79	4.43	3.91	4.91	3.11
S×D									2.36
S×G							5.52	ns	4.41
D×G			6.46			6.26			4.41
S×D×G									6.23

Plant height (PLH)

The means of plant height (Table 9) were 93.8 and 83.6 cm in the first season, 119.3 and 98.1 cm in the second season and 106.6 and 90.9 cm across the two seasons, for normal and late sowing date, respectively.

Overall means of plant height ranged from 89.4 cm in Baj1 up to 105.7cm in Line 1 with an average of 98.7 cm. The highest and lowest average of plant height across the two seasons under late sowing date was recorded in Line 1 and Baj 1 with 97.5 and 80.2 cm, respectively. The reduction in plant height is up to fast phasic change and due to this, vegetative phase becomes short and reproductive come early. Heat stress causes shorter plants, for this reason taller plant under some limits is more desirable, in some cases.

Table 9. Means of plant height (cm) for the twelve bread wheat genotypes under two seasons, two sowing dates and overall.

Genotypes	Season 1			Season 2			Over all		
	Date 1	Date 2	$\bar{X}$	Date 1	Date 2	$\bar{X}$	Date 1	Date 2	Mean
Shandaweel 1	89.0	76.7	82.9	112.3	94.7	103.5	100.7	85.7	93.2
Line 1	102.3	88.3	95.3	125.3	106.7	116.0	113.8	97.5	105.7
Misr 3	86.7	80.0	83.4	117.0	98.3	107.7	101.9	89.2	95.5
PBW 343	95.0	77.3	86.2	119.0	93.3	106.2	107.0	85.3	96.2
Baj 1	85.0	71.7	78.4	112.0	88.7	100.4	98.5	80.2	89.4
Vorobey	97.3	85.7	91.5	125.3	105.0	115.2	111.3	95.4	103.3
Gemmiza1 1	98.3	85.0	91.7	117.0	96.3	106.7	107.7	90.7	99.2
Gemmiza1 2	95.0	85.0	90.0	114.7	98.3	106.5	104.9	91.7	98.3
Sids 14	96.7	85.0	90.9	124.3	100.0	112.2	110.5	92.5	101.5
Giza 171	96.7	93.3	95.0	117.3	98.3	107.8	107.0	95.8	101.4
Misr 2	96.7	90.0	93.4	122.0	99.3	110.7	109.4	94.7	102.0
Sakha 95	96.7	85.7	91.2	125.7	98.7	112.2	111.2	92.2	101.7
Mean	93.8	83.6	88.7	119.3	98.1	108.7	106.6	90.9	98.7
L. S. D. 0.05									
Seasons (S)							1.51	1.83	1.23
Dates (D)			3.13			2.17			1.23
Genotypes	5.67	6.92	4.36	4.85	6.24	3.85	3.69	4.49	2.85
S×D									1.74
S×G							ns	ns	4.03
D×G			6.16			ns			4.03
S×D×G									ns

1000-Kernel weight (1000-KW)

The results (Table 10) revealed that mean of 1000-kernel weight was 46.6 and 42.4 g in the first season, 45.3 and 42.4 g in the second season under normal and late sowing date, respectively.

Table 10. Means of 1000-kernel weight (g) for the twelve bread wheat genotypes under two seasons and two sowing dates and overall.

Genotypes		Season 1			Season 2			Over all		
		Date 1	Date 2	$\bar{X}$	Date 1	Date 2	$\bar{X}$	Date 1	Date 2	Mean
Shandaweel 1		41.9	38.7	40.3	44.5	42.4	43.5	43.2	40.6	41.9
Line 1		46.2	44.3	45.3	45.9	43.7	44.8	46.1	44.0	45.0
Misr 3		44.5	41.1	42.8	44.5	43.8	44.2	44.5	42.5	43.5
PBW 343		45.6	43.9	44.8	44.4	41.1	42.8	45.0	42.5	43.8
Baj 1		49.4	37.6	43.5	44.6	43.1	43.9	47.0	40.4	43.7
Vorobey		49.2	44.5	46.9	45.7	43.4	44.6	47.5	44.0	45.7
Gemmiza 11		49.7	42.9	46.3	45.1	43.5	44.3	47.4	43.2	45.3
Gemmiza 12		49.2	41.9	45.6	49.3	42.5	45.9	49.3	42.2	45.7
Sids 14		44.2	43.2	43.7	46.7	40.1	43.4	45.5	41.7	43.6
Giza 171		49.4	40.9	45.2	45.7	43.3	44.5	47.6	42.1	44.8
Misr 2		44.2	41.2	42.7	45.3	43.1	44.2	44.8	42.2	43.5
Sakha 95		49.4	45.9	47.7	41.3	38.6	40.0	45.4	42.3	43.8
Mean		46.6	42.4	44.5	45.3	42.4	43.9	46.0	42.4	44.2
L. S. D. 0.05	Seasons (S)							0.61	ns	ns
	Dates (D)			2.58			0.85			0.88
	Genotypes (G)	1.21	2.38	1.30	2.66	2.03	1.63	1.50	1.49	1.02
	S×D									ns
	S×G							2.12	2.11	1.44
	D×G			1.84			2.30			1.44
	S×D×G									2.04

While, across the two seasons it was 46.0 and 42.4 g for normal and late sowing date, respectively. The highest 1000-kernel weight under late sowing date across the two seasons was recorded for Line 1 and Vorobey with 44.0 g while the lowest was recorded for Baj 1. Over all means of 1000-kernel weight ranged from 41.9 g for Shandweel1 to 45.7 g for Vorobey and Gemmiza12. High temperature reduces number of days to maturity and grain filling period, consequently shrivelled kernels produced and ultimately affect negatively on 1000-kernel weight as reported by Gupta *et al* (2015).

Number of spikes/m^2 (S/M^2)

Results of number of spikes/m^2 presented in Table (11) showed that mean number of spikes/m^2 was 367.6 and 301.4 in the first season, 461.6 and 358.2 in the second season and 414.6 and 329.8 across the two seasons, for normal and late sowing date, respectively. Across the two seasons highest number of spikes/m^2 under late sowing date was recorded for Misr 3 (358.4) and the lowest was for Misr 2 (285.8). Overall mean for number of spikes/m^2 ranged from 348.6 spikes for Misr 2 to 390.7 spikes for Misr 3. Abd El-Rady and Koubisy (2017) reported that delaying sowing significantly decreased number of spikes/m^2.

Table 11. Means of number of spikes/m2 for twelve bread wheat genotypes under two seasons and two sowing dates and overall.

Genotypes		Season 1			Season 2			Over all		
		Date 1	Date 2	$\bar{X}$	Date 1	Date 2	$\bar{X}$	Date 1	Date 2	Mean
Shandaweel 1		368.0	280.0	324.0	439.9	355.3	397.6	404.0	317.7	360.8
Line 1		386.0	280.0	333.0	475.0	380.7	427.9	430.5	330.4	380.4
Misr 3		368.0	344.0	356.0	478.1	372.7	425.4	423.1	358.4	390.7
PBW 343		390.0	284.0	337.0	403.3	327.0	365.2	396.7	305.5	351.1
Baj 1		378.0	307.3	342.7	503.7	371.3	437.5	440.9	339.3	390.1
Vorobey		398.0	320.0	359.0	455.9	387.7	421.8	427.0	353.9	390.4
Gemmiza 11		342.0	314.0	328.0	517.2	306.7	412.0	429.6	310.4	370.0
Gemmiza 12		340.0	316.0	328.0	451.7	339.5	395.6	395.9	327.8	361.8
Sids 14		361.7	304.0	332.9	428.0	347.6	387.8	394.9	325.8	360.3
Giza 171		358.0	310.0	334.0	458.7	400.9	429.8	408.4	355.5	381.9
Misr 2		361.7	254.0	307.9	460.9	317.6	389.3	411.3	285.8	348.6
Sakha 95		360.0	304.0	332.0	466.0	391.0	428.5	413.0	347.5	380.3
Mean		367.6	301.4	334.5	461.6	358.2	409.9	414.6	329.8	372.2
L. S. D. 0.05	Seasons (S)							7.33	7.50	13.08
	Dates (D)			25.26			31.73			13.08
	Genotypes	21.61	27.74	17.13	30.50	25.09	19.24	17.95	18.37	12.62
	S×D									ns
	S×G							25.38	25.98	17.85
	D×G			24.23			27.21			17.85
	S×D×G									25.24

Number of kernels/spike (K/S)

Results presented in Table (12) showed that mean values of number of kernels/spike were 45.3 and 43.0 kernels in the first season, 54.8 and 46.4 kernels in the second season and 50.1 and 44.7 kernels across the two seasons, for normal and late sowing dates, respectively. Mean number of kernels/spike under late sowing date across the two seasons ranged from 37.9 kernels for Gemmiza 11 to 49.4 for Gemmiza 12. Overall means were

42.6 kernels for Gemmiza11 and 50.9 kernels for Gemmiza12. These results can be attributed to the high temperature during the reproductive stages which can cause pollen sterility and adverse effects on floral organs. Ultimate result of high temperature during reproductive stages is decreasing number of grains/spike.

Table 12. Means of number of kernels/spike for the twelve bread wheat genotypes under two seasons and two sowing dates and overall.

Genotypes		Season 1			Season 2			Over all		
		Date 1	Date 2	$\bar{X}$	Date 1	Date 2	$\bar{X}$	Date 1	Date 2	Mean
Shandaweel1		54.1	36.7	45.4	57.8	48.9	53.4	56.0	42.8	49.4
Line 1		40.8	40.2	40.5	55.4	50.0	52.7	48.1	45.1	46.6
Misr 3		48.5	47.9	48.2	53.7	47.4	50.6	51.1	47.7	49.4
PBW 343		50.1	38.7	44.4	66.4	48.4	57.4	58.3	43.6	50.9
Baj 1		45.1	41.3	43.2	53.0	47.7	50.4	49.1	44.5	46.8
Vorobey		39.8	37.2	38.5	53.6	43.0	48.3	46.7	40.1	43.4
Gemmiza 11		48.5	32.0	40.3	45.9	43.8	44.9	47.2	37.9	42.6
Gemmiza 12		51.5	51.0	51.3	52.0	47.8	49.9	51.8	49.4	50.6
Sids 14		48.0	44.1	46.1	48.2	41.5	44.9	48.1	42.8	45.5
Giza 171		46.7	42.8	44.8	54.6	39.5	47.1	50.7	41.2	45.9
Misr 2		48.5	46.7	47.6	56.4	48.0	52.2	52.5	47.4	49.9
Sakha 95		41.2	40.9	41.1	61.0	45.7	53.4	51.1	43.3	47.2
Mean		45.3	43.0	44.2	54.8	46.4	50.6	50.1	44.7	47.4
L. S. D. 0.05	Seasons (S)							1.53	2.12	1.91
	Dates (D)			ns			4.43			1.91
	Genotypes	3.26	7.19	3.85	7.06	Ns	5.07	3.75	5.19	3.12
	S×D									ns
	S×G							5.30	7.34	4.41
	D×G			5.44			7.17			4.41
	S×D×G									6.24

Biological yield (BY)

The results in Table (13) revealed that mean values of biological yield were 9.0 and 6.32 kg/plot in the first season, 11.18 and 9.94 kg/plot in the second season for normal and late sowing dates, respectively. Across the two seasons biological yield means were 10.09 and 8.13 kg/plot across the two seasons for normal and late sowing date, respectively. The means under late sowing date across the two seasons were 7.17, 9.47 kg/plot for PBW

343 and Line 1, respectively. Over all means ranged from 8.26 kg/plot for Sids14 to 10.21 kg/plot for Line 1 with an average of 9.11 kg/plot.

Table 13. Means of biological yield (kg/plot) for the twelve bread wheat genotypes under two seasons, two sowing dates and overall.

Genotypes	Season 1			Season 2			Over all		
	Date 1	Date 2	$\overline{X}$	Date 1	Date 2	$\overline{X}$	Date 1	Date 2	Mean
Shandaweel1	9.39	5.60	7.50	10.93	9.91	10.42	10.16	7.76	8.96
Line 1	9.65	7.20	8.43	12.25	11.73	11.99	10.95	9.47	10.21
Misr 3	10.10	7.00	8.55	12.38	10.69	11.54	11.24	8.85	10.04
PBW 343	9.00	5.40	7.20	9.78	8.94	9.36	9.39	7.17	8.28
Baj 1	8.15	6.50	7.33	11.75	11.18	11.47	9.95	8.84	9.40
Vorobey	10.40	6.13	8.27	11.88	10.53	11.21	11.14	8.33	9.74
Gemmiza 11	8.15	6.00	7.08	11.73	9.89	10.81	9.94	7.95	8.94
Gemmiza 12	8.00	6.95	7.48	11.00	8.93	9.97	9.50	7.94	8.72
Sids 14	8.75	5.30	7.03	9.86	9.11	9.49	9.31	7.21	8.26
Giza 171	8.80	6.20	7.50	11.05	10.10	10.58	9.93	8.15	9.04
Misr 2	8.75	6.78	7.77	10.43	9.15	9.79	9.59	7.97	8.78
Sakha 95	8.80	6.75	7.78	11.10	9.16	10.13	9.95	7.96	8.95
Mean	9.00	6.32	7.66	11.18	9.94	10.56	10.09	8.13	9.11
L. S. D. 0.05									
Seasons (S)							0.15	0.18	0.18
Dates (D)			0.41			ns			0.18
Genotypes (G)	0.61	0.81	0.49	0.43	0.42	0.29	0.37	0.45	0.28
S×D									0.25
S×G							0.52	ns	0.40
D×G			0.70			0.42			0.40
S×D×G									0.56

Grain yield (GY)

Results of grain yield presented in Table (14) showed that mean values of grain yield/plot were 3.04 and 1.57 kg in the first season, 3.66 and 3.01 kg in the second season. Means across the two seasons were 3.35 and 2.29 kg under normal and late sowing date, respectively. These results indicated that late sowing decreased grain yield; this may be due to the high temperature during grain filling and reducing development of grains which ultimately decreased the grain yield. Means of grain yield across the two seasons under late sowing ranged from 2.01 to 2.71 kg/plot for Sids 14 and Line 1, respectively. Line 1 ranked number one followed by Baj 1 with 2.71 kg/plot and 2.55 kg/plot. Overall means ranged from 2.46 kg/plot for PBW343 to 3.29 kg for Line 1. Not all traits related to heat tolerance will be found in one genotype, but every genotype will have some of these traits, giving some advantages over the other genotypes.

Table 14. Means of grain yield (kg/plot) for the twelve bread wheat genotypes under two seasons, two sowing dates and overall.

	Genotypes	Season 1			Season 2			Over all			HSI
		Date 1	Date 2	$\bar{X}$	Date 1	Date 2	$\bar{X}$	Date 1	Date 2	Mean	
	Shandaweel 1	3.40	1.75	2.58	4.01	3.19	3.60	3.71	2.47	3.09	0.99
	Line 1	3.52	2.02	2.77	4.22	3.39	3.81	3.87	2.71	3.29	0.87
	Misr 3	3.23	1.48	2.36	3.95	3.23	3.59	3.59	2.36	2.97	1.10
	PBW 343	2.82	1.41	2.12	2.86	2.74	2.80	2.84	2.08	2.46	1.01
	Baj 1	2.59	1.74	2.17	4.12	3.36	3.74	3.36	2.55	2.95	0.67
	Vorobey	3.18	1.24	2.21	3.86	3.15	3.51	3.52	2.20	2.86	1.26
	Gemmiza 11	2.69	1.39	2.04	3.89	2.71	3.30	3.29	2.05	2.67	0.98
	Gemmiza 12	2.83	1.59	2.21	3.16	2.88	3.02	3.00	2.24	2.62	0.89
	Sids 14	2.85	1.45	2.15	3.65	2.57	3.11	3.25	2.01	2.63	1.00
	Giza 171	2.73	1.45	2.09	3.15	3.00	3.08	2.94	2.23	2.58	0.95
	Misr 2	3.29	1.51	2.40	3.52	2.98	3.25	3.41	2.25	2.83	1.10
	Sakha 95	3.38	1.83	2.61	3.53	3.14	3.34	3.46	2.49	2.97	0.93
	Mean	3.04	1.57	2.31	3.66	3.01	3.34	3.35	2.29	2.82	
L. S. D. 0.05	Seasons (S)							0.06	0.06	0.10	
	Dates (D)			0.21			0.23			0.10	
	Genotypes	0.21	0.26	0.16	0.23	0.19	0.15	0.15	0.16	0.11	
	S×D									0.14	
	S×G							0.21	ns	0.15	
	D×G			0.23			0.21			0.15	
	S×D×G									0.21	

In this situation, crossing strategies between the genotypes with desirable traits to be combined into one genotype is an acceptable option. On the other hand, cultivating these high yielding genotypes, under heat stress regions will be a good choice; this will depend upon the breeder objectives.

Heat susceptibility index (HIS)

Heat susceptibility index, based on grain yield, for all studied genotypes are presented in Table (14). Tolerant genotypes should have heat susceptibility index of ≤ 0.75 but moderate tolerant genotypes should have HSI of 0.75 ≤1.0, while sensitive genotypes should have HSI >1. The results showed that heat susceptibility index ranged from 0.67 for Baj 1 to 1.26 for Vorobey. Based on these categories, the studied genotypes can be categorized into three groups *i.e.* tolerate, moderately tolerant, and sensitive genotypes. Baj 1 is the most tolerant genotype with HSI = 0.67. While, moderate tolerant group contained Line 1, Gemmiza 12, Sakha 95, Giza 171, Gemmiza 11, and Shandaweel 1 with HSI = 0.87, 0.89, 0.93, 0.95, 0.98, and 0.99, respectively. Finally, the most sensitive genotypes were Sids 14, PBW343, Misr 3, Misr 2, and Vorobey, respectively.

Correlation among the studied traits

The results indicated that there were significant or highly significant correlations (Table 15) among the studied traits.

Table 15. Correlation coefficient among the studied traits under normal (below diagonal) and late (above diagonal) sowing dates for the studied bread wheat genotypes over the two seasons.

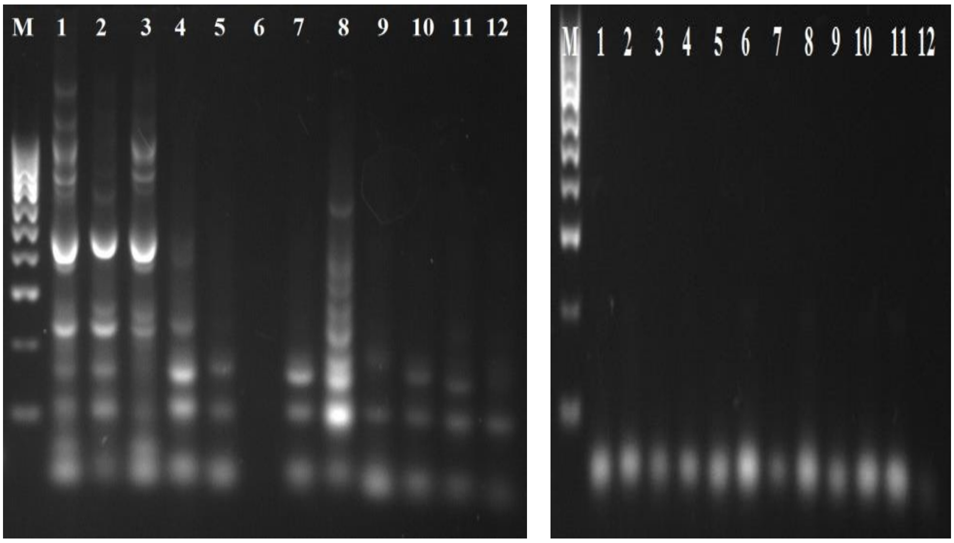
Traits		Under late sowing date									
		Days to heading	Days to maturity	Grain filling period	Grain filling rate	Plant height	1000-kernels weight	No. spikes/m^2	No. kernels/spike	Biological yield	Grain yield
Under normal sowing date	Days to heading	1.00	0.92**	0.16	0.89**	0.69**	-0.02	0.60**	0.30*	0.86**	0.86**
	Days to maturity	0.91**	1.00	0.52**	0.89**	0.74**	0.25*	0.67**	0.26*	0.91**	0.92**
	Grain filling period	0.45**	0.78**	1.00	0.31**	0.37**	0.10	0.39**	0.00	0.45**	0.47**
	Grain filling rate	-0.00	-0.08	-0.17	1.00	0.72**	0.02	0.68**	0.31**	0.95**	0.98**
	Plant height	0.85**	0.91**	0.68**	0.00	1.00	0.17	0.60**	0.26*	0.78**	0.73**
	1000-kernels weight	0.25*	0.27*	0.21	-0.22	-0.22	1.00	-0.23*	-0.29*	0.22	0.24*
	No. spikes/m^2	0.74**	0.82**	0.66**	0.29*	0.78**	-0.21	1.00	0.05	0.75**	0.71**
	No. kernels/spike	0.63**	0.62**	.0.40**	-0.05	0.54**	-0.55**	-0.38**	1.00	0.34**	0.29*
	Biological yield	0.32**	0.52**	0.65**	0.45**	0.49**	0.24*	0.74**	0.19	1.00	0.97**
	Grain yield	0.33**	0.51**	0.60**	0.69**	0.51**	0.32**	0.72**	0.25*	0.85**	1.00

Under normal sowing date, grain yield was significantly or highly significantly correlated with days to heading (0.33), days to maturity (0.51), grain filling period (0.60), grain filling rate (0.69), plant height (0.51), 1000-kernel weight (0.32), number of spikes (0.72), number of kernels/spike (0.25) and biological yield (0.85). On the other hand, under heat stress conditions, grain yield had significant or highly significant correlation with days to heading (0.86), days to maturity (0.92), grain filling period (0.47), grain filling rate (0.98), plant height (0.73), 1000-kernel weight (0.24), number of spikes (0.71), number of kernels/spike (0.29) and biological yield (0.97). Similar results were obtained by Lopes *et al* (2012), who found significant association between grain yield and 1000-kernels weight, days to heading, and plant height.

Genotypic evaluation:

Marker analysis

In the present study the polymorphism level was determined based on 11 microsatellite markers; out of them ten were found to be polymorphic. The marker's patterns were scored as (1) for presence and (0) for absence of the amplicon, only the polymorphic markers are included in the analysis and calculations. The markers' results (Table 16) showed that total number of alleles were 57 polymorphic at 10 loci with an average of 5.7 per locus.



Xcfd29 **Xbarc229**

Fig. 2. The amplified bands in the studied genotypes of Xcfd29 and Xbarc229 primers.

Where: M= marker 100-1000bp, 1= Shandaweel 1, 2= Line 1, 3= Misr 3, 4= PBW 343, 5=, Baj 1 6= Vorobey, 7= Gemmiza 11, 8= Gemmiza 12, 9= Sids 14, 10= Giza 171, 11= Misr 2, 12= Sakha 95.

The number of alleles in this study is more than alleles obtained by Salem et al. 2014 with an average of 3.2 alleles. Out of the 57 bands 45 bands (78.9 %) were found to be polymorphic. The number of alleles (Figure 2) ranged between one in Xbarc229 and Xwmc601 up to 14 alleles in Xcfd29 with an average of (5.7). The variability in number of alleles for these markers showed how much they are polymorphic which is confirmed by the high PIC and MI values for each marker.

PIC and MI indices are used to estimate the polymorphism; these parameters are widely used in this concern. PIC ranged from 0.54 in Xgwm484 up to 0.95 in Xgwm33 and Xgwm389 with an average of 0.8 while, MI ranged from 0.40 for Xgwm261 to 0.89 for Xbarc229 with an average of 0.67 (Table 16).

Table 16. Summary of SSR primer combination characteristics.

Primer	Range of	PB	NPB	No. of alleles	PPB	PIC	MI
Xgwm33	52-235 bp	5	1	6	0.83	0.95	0.79
Xgwm389	47-205 bp	5	2	7	0.71	0.95	0.68
Xgwm513	56-704 bp	6	1	7	0.86	0.85	0.73
Xgwm261	52-198 bp	2	2	4	0.50	0.80	0.40
Xgwm577	67-1111 bp	6	1	7	0.86	0.86	0.74
Xcfd29	51-1571 bp	10	4	14	0.71	0.92	0.66
Xbarc229	73 bp	1	0	1	1.00	0.89	0.89
Xgwm484	55-182 bp	5	0	5	1.00	0.54	0.54
Xgwm293	54-188 bp	4	1	5	0.80	0.83	0.66
Xwmc601	65 bp	1	0	1	1.00	0.66	0.66
Total	----	45	12	57	8.28	8.25	6.75
Mean	----	4.5	1.2	5.7	0.83	0.83	0.67

Where: PB = number of polymorphic bands, NPB = number of non-polymorphic bands, PPB = percentage of polymorphic bands, PIC = polymorphic information content, and MI = marker index.

Single marker analysis using simple linear regression was performed to determine the association between a specific marker/s and particular trait/s under late sowing traits mean over the two seasons. Karl Pearson Coefficient (Table 17) indicated significant or highly significant association; with positive values in some cases and negative values for the others.

Table 17. The linked SSR markers to some agronomic traits based on Karl Pearson coefficient.

Markers	Trait	Band	r	P	R^2	Genotypes Ψ
Xcfd29	D.M	1571 bp	0.80**	0.002	0.46	Shandaweel 1 with 127.5 days
Xgwm389	PLH	192 bp	-0.66*	0.018	0.44	Baj 1 with 80.2 cm
Xgwm293	GFP	87 bp	0.58*	0.046	0.34	Shandaweel 1 with 39.3 days Sakha 95 with 38.7 days Shandaweel 2 with 36.8 days
Xgwm577	GFR	437 bp	0.67*	0.016	0.46	Shandaweel 2 with 48.8 mg/day Shandaweel 1 with 46.4 8 mg/day Gemmiza 12 with 42.1 mg/day
Xgwm389	K/S	128 bp	0.65**	0.022	0.42	Gemmiza 12 with 49.4 kernels/spike Misr 2 with 47.4 kernels/spike
Xgwm577	HSI	437 bp	-0.71**	0.010	0.50	Baj 1 with 0.67 Shandaweel 2 with 0.87 Gemmiza 12 with 0.89
Xcfd29	GY	828 bp	0.59*	0.040	0.34	Shandaweel 2 with 2.71 kg/plot

Where: *, ** are significant and highly significant at 0.05 and 0.01 probability, respectively. P, r, and R^2 are P-value, Kael Pearson, and coefficient of determination, respectively. Ψ are the genotypes with the specific amplified bands along with the mean of these traits under late sowing date over the two seasons, and r is Karl Pearson coefficient.

The usefulness of positive and negative coefficients depends on the trait itself. For example, the positive coefficient is desirable in case of days to maturity, grain filling period, grain filling rate, and grain yield while the negative coefficient is preferable in case of heat susceptibility index. Terminal heat stress reduces days to maturity consequently; shorten grain filling period as well as plant lifetime. For this reason, the number of days to maturity is preferred to be long (till a particular limit) under heat stress. A positive, high, and highly significant association (0.80**) was found between Xcfd29 (1571 bp band) and days to maturity (Shandaweel 1 with mean across the two seasons = 127.5 days). A negative, high, and significant association (r = -0.66*) was found between Xgwm389 (192 bp band) and plant height (Baj 1 with mean across the two seasons = 80.2 cm); where it was not desirable in this case. On the other hand, a positive, high, and significant association (r = 0.58*) was found between Xgwm293 (87 bp band) and grain filling period. In the same context, Xgwm577 with a specific band of 437 bp and grain filling rate (r = 0.67*), Xgwm389 with a specific band of 128 bp and number of kernels/spike (r = 0.65**). The situation is different in case of heat susceptibility index where negative association is desirable, where, a negative, high, and highly significant association (r = 0.71**) was found between Xgwm577 (437 bp) and heat susceptibility index. A positive, high, and significant association (r = 0.59*) was found between Xcfd29 (828 bp) and grain yield.

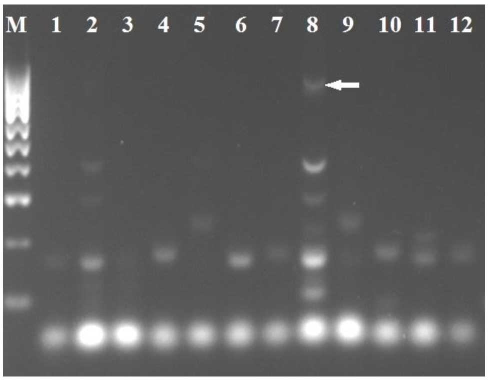
These markers are candidate linked markers for these traits and can be used in marker assisted selection (MAS). These results are in agreement with those obtained by Paliwal *et al* (2012) and Abdelsabour *et al* (2019), who they suggested some markers as candidate linked markers for some important agronomic traits to be used in MAS. The linked SSR markers, associated traits, specific amplified bands, Karl Pearson coefficient (r), and the genotypes with the amplified bands are presented in Table (17).

The primers patterns revealed that there is an amplified band is unique to a specific genotype (Table 18). Some of these bands are associated with some agronomic traits *i.e.* Xcfd29 (1571bp) is unique to Shandaweel and associated with days to maturity. On the other hand, some bands are unique to particular genotypes *e.g.* Xgwm577 (1111 bp) with Gemmiza 12, Xgwm513 (413 bp) with Vorobey (Figure 3).

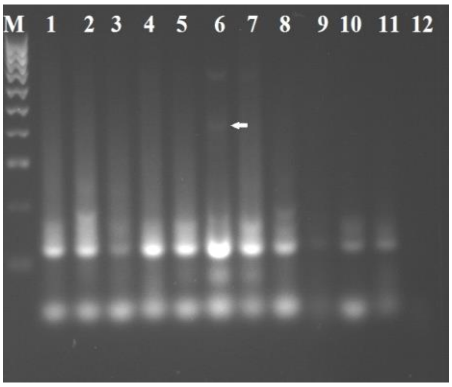
Table 18. Unique bands for SSR marker amplified in some of the studied genotypes.

Genotype	Marker	Specific band/s	Trait
Shandaweel 1	**Xcfd29**	**1571 bp**	**Days to maturity**
PBW 343	**Xgwm33**	**227 bp**	**None of the studied traits**
Baj 1	**Xgwm389**	**192 bp**	**Plant height**
Vorobey	**Xgwm513**	**413 bp**	**Positive association with heat**
Gemmiza 11	**Xgwm33**	**102 bp**	**None of the studied traits**
Gemmiza 12	**Xgwm513** **Xgwm577** **Xcfd29**	**186 bp** **1111 bp** **308 bp**	**None of the studied traits**
Sakha 95	**Xgwm484** **Xgwm293**	**182 bp** **143 bp**	**None of the studied traits**

Although, these markers are not associated with any of the studied traits, they may be associated with traits other than those studied in the current investigation. These specific fragments need further investigation with traits other than the studied traits.



Xgwm 577 (1111 bp)



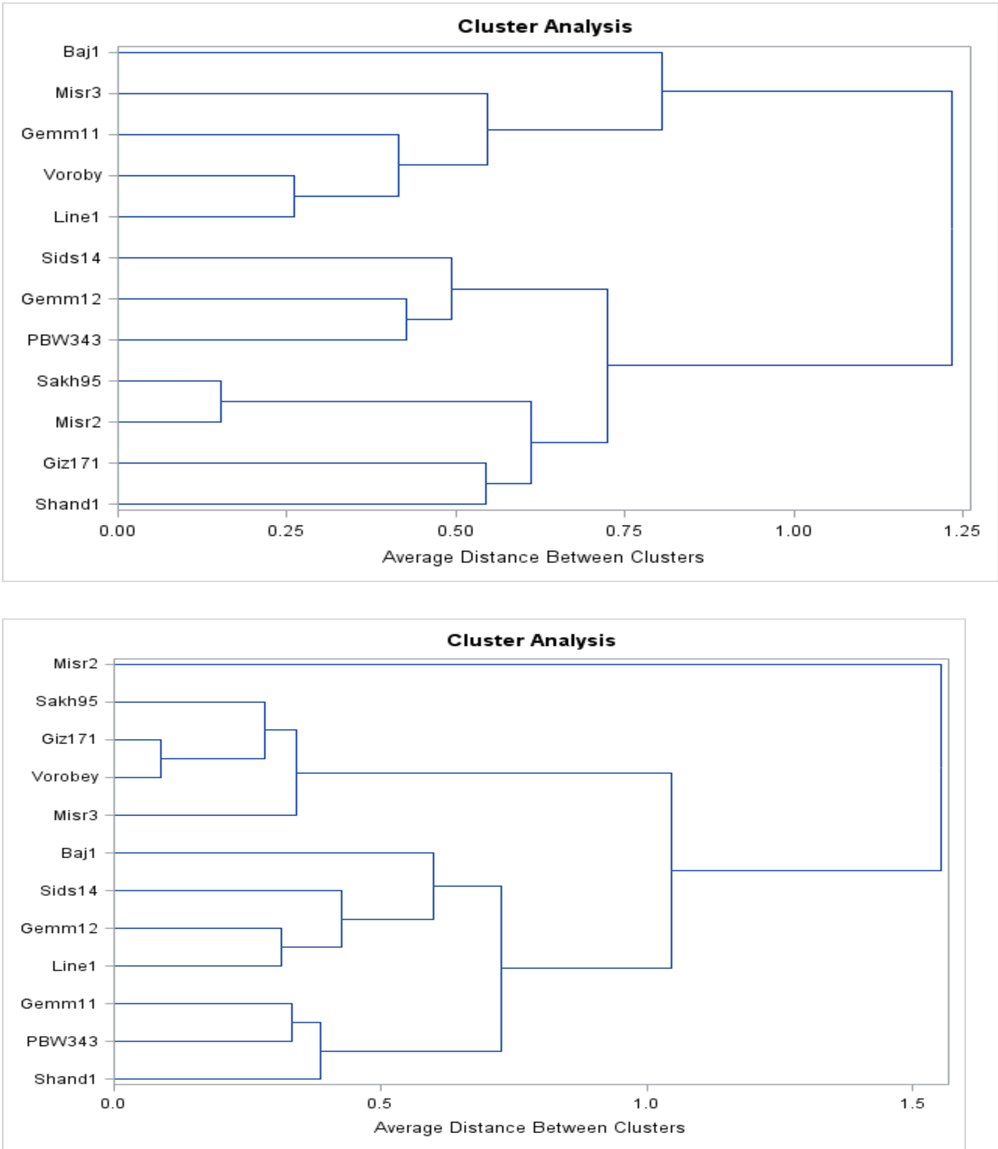
Xgwm513 (413 bp)

Fig. 3. The amplified bands in the studied genotypes by using Xcfd29 and Xbarc229 primers.

Where: M= marker 100-1000bp, 1= Shandaweel 1, 2= Line 1, 3= Misr 3, 4= PBW 343, 5=, Baj 1 6= Vorobey, 7= Gemmiza 11, 8= Gemmiza 12, 9= Sids 14, 10= Giza 171, 11= Misr 2, 12= Sakha 95.

Genetic distance

Dissimilarity matrices for the agronomic traits under normal and late sowing dates conditions were computed (Euclidean coefficient). The dendrogram was constructed for the agronomic traits (Figure 4), using unweighted pair group method with arithmetic mean (UPGMA), to distinguish between the genotypes based on their performance under normal and late sowing dates' conditions. The dendrogram of genetic distance for the studied genotypes, based on their performance under normal sowing date conditions, (Figure 4 a) clustered the genotypes into four forks. The closest genotypes based on agronomic traits under normal conditions were Sakha 95 and Misr 2 while, the most distant were Sakha 95 and Baj 1. On the hand, the dendrogram for genetic distance of the studied genotypes, based on performance under late sowing date condition, (Figure 4 b) clustered the genotypes into four clusters. The closest genotypes based on agronomic traits under terminal heat stress were Giza 171 and Vorobey while the most distant were Giza 171 and Misr 2.



a **b**

Fig. 4. Phylogenetic tree among the studied bread wheat genotypes based on the agronomic traits under normal (a) and late sowing date (b).

In both dendrograms, each cluster contained number of genotypes. The genetic distances in the two dendrograms were quite different in some way; this can be attributed to the performance for the studied genotypes under normal and late sowing dates.

The dissimilarity matrix (Dice coefficient) between the genotypes based on the markers' patterns was computed, consequently, the dendrogram was constructed (figure 5).

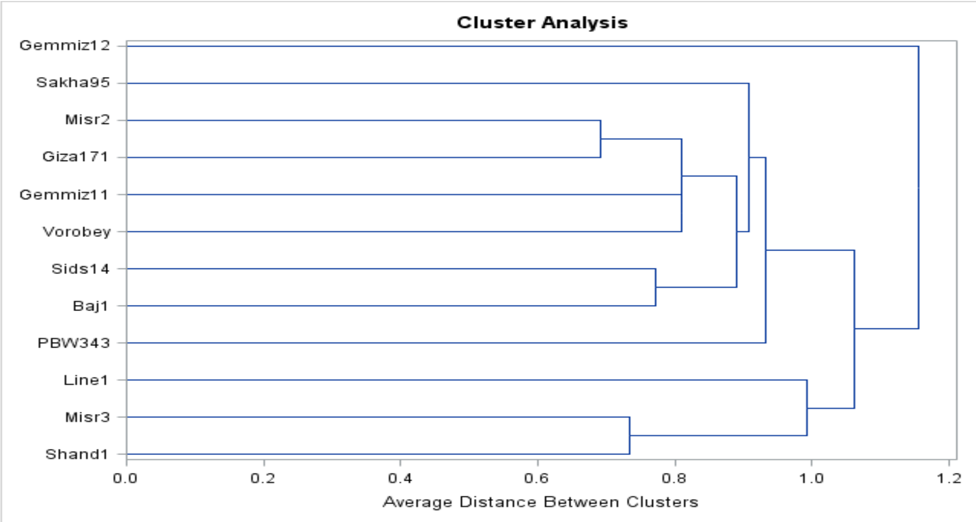


Fig. 5. Phylogenetic tree among the studied bread wheat genotypes based on SSR fragments.

The closest genotypes were Misr 2 and Giza 171 while, the most distant was Gemmiza 12 and Misr 2. The dendrogram grouped the genotypes into seven clusters. Most of the clusters contained one or two genotypes except one cluster contained four genotypes. This dendrogram is branched into many clusters and quite different than the other two dendrograms; because it was constructed based on DNA polymorphism based markers. DNA polymorphism based markers cover a large portion of genome and can give a clear-cut differentiation between so many genotypes. The most distant genotypes can be utilized in breeding programs.

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التقييم الوراثى والمظهرى لبعض التراكيب الوراثية من قمح الخبز تحت ظروف الاجهاد الحرارى فى نهاية الموسم

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تمت هذه الدراسه بمحطة البحوث الزراعية بشندويل – مركز البحوث الزراعيه – مصر خلال موسمى ٢٠١٨/٢٠١٧ و ٢٠١٩/٢٠١٨ لتقييم ١٢ تركيب وراثى من قمح الخبز تحت ميعادى زراعه الميعاد الأول وهو الموصى به وميعاد الزراعه المتأخر وايضاً باستخدام المعلمات الجزيئيه. أظهرت النتائج وجود إختلافات عاليه المعنويه بين التراكيب الوراثيه ومواعيد الزراعه والمواسم لكل الصفات تحت الدراسة. وأيضاً وجود اختلافات معنويه للتفاعلات بين التراكيب الوراثية ومواعيد الزراعه والمواسم الزراعيه لبعض الصفات تحت الدراسه. بناءاً على معامل الحساسيه للحراره لمحصول الحبوب تم تقسيم التراكيب الوراثيه الى ثلاث مجموعات متحمله ومتوسطة التحمل وحساسه للحراره. تم إجراء الجزء الخاص بالمعلمات الجزيئيه بقسم الوراثه – كلية الزراعه – جامعة أسيوط. أوضحت نتائج المعلمات الجزيئيه وجود اختلافات عاليه بين التراكيب الوراثيه عبر عنها بالقيم العاليه لكل من محتوى معلومات التباين polymorphism information content (PIC) و دليل المعلمات الجزيئية Marker Index (MI). اظهر التحليل الفردى للمعلمات الجزيئيه وجود تلازم معنوى او عالى المعنويه وقد يكون موجب أو سالب بين بعض المعلمات الجزيئيه وصفات محصوليه بعينها. على سبيل المثال، وجد تلازم معنوى موجب بين المعلم الجزيئى Xgwm293 وصفة فترة امتلاء الحبوب. كما وجد ايضاً، تلازم معنوى موجب بين المعلم الجزيئى Xgwm577 وصفة معدل امتلاء الحبوب. اظهرت النتائج الى وجود حزم جزيئيه ذات وزن جزيئى معين موجوده فقط ببعض التراكيب الوراثيه دون غيرها. بالرغم من ان بعض من هذه الحزم الجزيئيه مرتبط بصفات محصوليه معينه من الصفات المدروسه الا ان البعض الاخر لم يكن مرتبطاً باى من الصفات المدروسه. تم إجراء التحليل العنقودى للصفات المحصوليه تحت ظروف الميعاد الموصى به وظروف الميعاد المتأخر وايضا للحزم الجزيئيه للمعلمات الجزيئيه، و بناءاً عليه قسمت التراكيب الوراثيه الى مجموعات حسب التشابه الوراثى بينها. اظهرت الدراسه أنه هناك بعض التراكيب الوراثيه عالية المحصوليه متحمله للحراره يمكن الاستفاده منها فى المناطق المتأثره بالحراره العاليه او الاستفاده منها كمصدر للتحمل للحراره فى برامج التربيه. أيضا إمكانية الاستفاده من بعض المعلمات الجزيئيه المستخدمه بهذ الدراسه فى عملية الانتخاب بمساعدة المعلمات الجزيئيه (MAS) والمرتبطه بصفات محصوليه مهمه مرتبطة بتحمل الحراره.

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