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Assessment of Genetic Diversity in Bread Wheat Genotypes Based on Heat Tolerance and SSR Markers

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Abstract

Ten bread wheat genotypes (*Triticum aestivum* L.) were evaluated for heat tolerance under normal and late sowing dates during 2014/2015 and 2015/2016 seasons. Four agronomic traits, i.e. grain yield per plant (GYP), 1000-kernel weight (TKW), Spike length (SL) and plant height (PH) were evaluated. The genetic diversity was assessed among genotypes based on phenotypic data and thirteen simple sequence repeats (SSR) markers, representatives of nine wheat chromosomes. Heat stress under the late sowing date was quite strong resulting in 30.4, 14.6, 14.7 and 28.8% average reduction for GYP, TKW, SL and PH, respectively. GYP showed a negative and significant correlation (r= -0.66, P<0.05) with heat susceptibility index (HSI) under heat stress. By using 13 SSR markers, a total of 125 DNA fragments were generated, with an average of 9.6 bands per marker. The level of polymorphism (%P) ranged from 25% for the marker Xgwm497-1A to 85.7% for Xwmc273-7A, with an average of 60.8%. The highest polymorphism information content (PIC) value (0.36) was also recorded for Xwmc273-7A, while the lowest PIC (0.11) was found with Xwmc398-6A, with an average of 0.23. A highly significant correlation (r= 0.872, P<0.01) was found between %P and PIC values. Cluster analysis based on phenotypic data classified the ten genotypes into two groups, of which the group-1 genotypes (Line-1, Line-2 and Line-3) showed high tolerance to heat stress by exhibiting lowest HSI values. However, cluster analysis based on SSR markers generated two clusters, where cluster-I contained five genotypes (Line-1, Line-2, Line-3, Debeira and EL-Nilein) tolerant to heat stress, indicating the efficiency of SSR markers in discriminating wheat genotypes. Moreover, four SSRs generated some unique bands or specific for some tolerant genotypes, that could be used as markers associated with heat tolerance in wheat. However, additional markers analysis is still required to validate their usefulness in breading programs.

Keywords: *Heat stress, genetic diversity, SSR, cluster analysis. Triticum aestivum L.*

Introduction

Wheat is the staple food of about 35% world population and most preferred cereals in the world (Kumar *et al.,* 2016). Heat stress is one of the most important abiotic factors caused yield reduction in many wheat growing regions of the world including the Mediterranean region like Egypt. Therefore, development of heat tolerant genotypes is of major concern in wheat breeding programs. For studies on adaptation of crop plants to stress condition due to climate change, there is a need to exploit the available biodiversity in crop genotypes growing in diverse environments (Bhargava and Sawant, 2013). Moreover, it is necessary to investigate the genetic diversity in wheat germplasm in order to broaden the genetic variation in future wheat

breeding (Huang *et al.,* 2002). Wheat gene pools, resulting from the crossing of various cultivars, lines, and landraces followed by successive selfing, are considered as an important source of genes related to vital traits including abiotic stress tolerance (EL-Rawy and Youssef, 2014). Although, morphological traits can be used for assessing genetic diversity, they are often influenced by the environment. Therefore, the use of molecular markers for the assessment of genetic diversity is receiving much attention from molecular geneticists and wheat breeders (Huang *et al.,* 2002; Salem *et al.,* 2015). Compared with other molecular markers, microsatellites or simple sequence repeats (SSRs) are characterized by a high level of polymorphism, multiallelic, chromosome-specific and evenly distributed over the genome, that allow to discriminate among cultivars and even among closely related wheat breeding lines as well as among different countries (Maccaferri *et al.*, 2007; Mantovani *et al.*, 2008; Salem *et al.,* 2015). Moreover, Condit and Hubbell (1991) reported the first study of SSRs in plants. Since then, SSRs have been widely used for all important crop plants. Several hundred SSR primer pairs have been developed for all the three genomes of wheat (Mantovani *et al.*, 2008) and have been used for a variety of purposes, including genome mapping, physical mapping, gene tagging, and genetic diversity estimates (Wang *et al.*, 2007).

Genetic associations of various molecular markers including SSRs with heat tolerance have been reported in wheat (Barakat *et al.,* 2012; Talukder *et al.,* 2014; Sun *et al*., 2015). Moreover, quantitative and molecular characterization of heat tolerance in hexaploid wheat has also been reported (Yang *et al.,* 2002). Therefore, integrating biotechnological tools with conventional breeding techniques will help to develop wheat varieties with better grain yield under heat stress during reproductive and grain-filling phases (Farooq *et al.,* 2011). Genetic variation may exist within the wheat genotypes for heat tolerance, and it could be important for evaluation of local and exotic germplasm for heat tolerance (Khan *et al.,* 2015). Consequently, the present study aimed to assess the genetic diversity among ten bread wheat genotypes (*Triticum aestivum* L.) evaluated under normal and heat stress conditions, based on agronomic traits and SSR markers.

Materials and Methods

The plant material and field evaluation

The plant material utilized in the present study consisted of ten bread wheat genotypes (*Triticum aestivum* L.); variable in their performance under heat stress conditions (Table 1). Out of the ten genotypes tested, four selected advanced lines (Line-1, 2, 3 and 6) were developed at the Department of Genetics, Faculty of Agriculture, Assiut University, Egypt. The field evaluation was carried out at the Experimental Farm of the Faculty of Agriculture at Assiut University during two successive seasons of 2014/2015 and 2015/2016. Seeds of tested lines and varieties were planted on 1st December as a normal sowing date and 10th January as a late sowing date (heat stress condition). The field

trials were organized as a randomized complete blocked design (RCBD) with three replications. Each genotype was represented in each block by a row of 10 plants spaced 30 cm apart within rows set 30 cm from each other. After the maturity stage, grain yield per plant (g), 1000-kernel weight (g), spike length (cm) and plant height (cm) were recorded for each genotype.

Phenotypic data analysis

Significance of differences among means was compared using Fisher's Least Significant Difference (LSD) Test. The coefficient of variation (CV%) of different traits was calculated according to the formula suggested by Burton (1952). To test the significance of differences among the genotypes (G) and environments (E) and the significance of G×E interaction for each trait, the phenotypic data were statistically analyzed using a combined analyis of variance across environemnts. The broad-sense heritability (h^2_B) of a trait was computed by using the expected value of variance and the formula described by Nyquist (1991). Pearson's correlation coefficients among the traits evaluated under normal and heat stress conditions over two years were estimated. Cluster analysis was done using four agronomic traits based on the Euclidean distance coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) by NTSYS-pc version 2.20 (Applied Biostatics Inc.).

Heat susceptibility index (HSI):

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

$$HSI = \frac{1-\left(Ys/Yp\right)}{SI}$$

The stress intensity $SI = 1-\left(\bar{Y}s/\bar{Y}p\right)$

Where, Ys and Yp represent grain yield of a genotype under stress and favorable conditions, respectively; $\bar{Y}s$ and $\bar{Y}p$ represent mean grain yield of all genotypes under stress and favorable conditions, respectively.

Molecular markers analysis

DNA extraction and quantification

Total genomic DNA from the ten wheat genotypes was extracted according to the cetyltrimethylammonium bromide (CTAB) method for plant tissues (Murray and Thompson, 1980) with some modifications. DNA quality and concentration were determined using a spectrophotometer according to Stulnig and Amberger (1994) and Khirshyat 1.0 microprogram (Youssef, 2012).

SSR-PCR amplification

Thirteen wheat microsatellites or SSR primer sets were selected and used for screening the studied wheat genotypes. Primers sequences and PCR conditions were obtained by GrainGenes Database for *Triticeae* and *Avena* (http://wheat.pw.usda.gov). PCR amplifications were performed in a SensoQuest LabCycler (SensoQuest GmbH, Göttingen, Germany). PCR products were separated on 2.5% agarose gels in 0.5× TBE buffer. A 100bp HyperLadder™ was used to estimate the size of each amplified DNA fragment. Putative

polymorphisms were detected for each marker separately.

Molecular data analysis

A binary data matrix indicating the presence (1) or the absence (0) of bands was made from the SSR profile. Only strong, reproducible, and clearly distinguished bands were scored and differences in band intensity were not considered. The percentage of polymorphism was calculated by dividing the number of polymorphic bands with the total number of amplified bands. To analyze the suitability of the markers to assess the genetic diversity, the polymorphic information content (PIC) was calculated for each marker using the formula described by (Roldan-Ruiz *et al.,* 2000) as:

$$PIC_i = 2f_i\,(1 - f_i)$$

Where, PIC_i is the polymorphic information content of the locus i, (f_i) is the frequency of the amplified fragments and ($1 - f_i$) is the frequency of non-amplified fragments. The frequency was calculated as the ratio between the number of amplified fragments at each locus and the total number of genotypes (excluding missing data). The PIC of a marker was calculated using the average PIC value from all loci of the marker.

The genetic similarities among the tested genotypes were computed and UPGMA-dendrogram was performed according to Jaccard's coefficient of similarity using NTSYS-pc version 2.20 (Applied Biostatics Inc.). Mantel test (Mantel, 1967) was performed to estimate the correlation between the distances matrices conducted based on phenotypic data using Euclidean's coefficient and SSR markers according to Jaccard's coefficient.

Results

Performance of wheat genotypes

The mean values of grain yield/plant (GYP), 1000-kernel weight (TKW), spike length (SL) and plant height (PH) under different environmental conditions are found in Table 2. The results revealed that wheat genotypes varied from each other in one or more traits under normal (1st sowing date, E_1) and heat stress (2nd sowing date, E_2) conditions. Concerning to the average over all the genotypes tested, GYP was reduced from 56.0 g (E_1) to 39.0 g (E_2) in 2014/2015 season and from 54.1 g (E_1) to 37.7 g (E_2) in 2015/2016. Meantime, TKW was reduced from 46.8 g (E_1) to 41.1 g (E_2) in 2014/2015 and from 45.6 g (E_1) to 37.9 g (E_2) in 2015/2016. The highest GYP in E_1 was recorded for Line-3 (64.6 g) in 2014/2015 and Gemmiza-7 (61.7 g) in 2015/2016. However, the highest GYP under heat stress condition (E_2) was recorded for Line-3 (47.4 and 46.1 g) in 2014/2015 and 2015/2016 seasons, respectively. Although, Gemmiza-7 showed the highest TKW (56.5 g) under normal condition (E_1) in 2014/2015, otherwise, Sakha-8 showed the highest TKW (47.6 and 45.1 g) under stress condition (E_2) in 2014/2015 and 2015/2016 seasons, respectively. Constantly, Line-3 showed the highest SL at E_1 (22.3 and 23.0 cm) and E_2 (18.9 and 16.4 cm) in 2014/2015 and 2015/2016 seasons, respectively. Similarly, the highest PH in E_1 (155.1 and 149.2cm) and E_2 (118.2 and 113.2 cm) was recorded

for Line-3 in 2014/2015 and 2015/2016, respectively (Table 2).

The combined analysis of variance (Table 3) revealed highly significant differences (P<0.01) among tested genotypes for all the traits studied, providing an evidence for the presence of genetic variability among genotypes and thus validated further genetic analyses of the traits. The genotype x environment (G x E) interactions were also highly significant (P<0.01) for all the traits, supporting that heat tolerance is a complex trait that shows a high level of G x E interaction.

Apparently, heat stress in E_2, over the mean of the two years, was quite strong resulting in 30.4, 14.6, 14.7 and 28.8% average reduction in GYP, TKW, SL and PH, respectively. The highest GYP reduction (45.6%) was recorded for Gemmiza-7, while the lowest (20.6%) was found in Line-2. The reduction in TKW ranged from 4.6% for El-Nilein to 29.5% for Gemmiza-7. The highest reduction for SL (22.2%) and PH (33.5%) was observed in Line-3 and Line-2, respectively, whereas the lowest reduction in SL (5.5%) and PH (24.0%) was found in EL-Nilein and Line-3, respectively (Fig. 1). This result revealed that the genetic makeup for those genotypes respond differently under heat stress.

Heat susceptibility index (HSI)

Clearly, wheat genotypes evaluated in this study showed significant differences in their GYP under normal and heat stress conditions. Over the two growing seasons, the mean GYP ranged from 45.50 g for Line-1 to 61.30 g for Lira-Sa-92 in E_1, and from 32.21 g for Misr-2 to 46.72 g for Line-3 in E_2 (Table 4). The HSI was calculated based on GYP under normal (E_1) and heat stress (E_2) conditions. Out of ten genotypes tested, seven showed HSI values <1, thereby they were considered as heat tolerant genotypes. Moreover, three genotypes (Line-2, Line-1 and Line-3) with low HSI values (0.68, 0.71 and 0.78), respectively, were considered as high tolerant and three genotypes (EL-Nilein, Sakha-8 and Lira-Sa-92) with moderate HSI values (0.85, 0.87 and 0.88) were considered as moderate tolerant.

Correlation coefficients

Phenotypic correlation coefficients between each pair of the traits studied as well as with HSI are presented in Table 5. GYP in E_1 was positively correlated with TKW in E_1 (r= 0.79, P<0.01) and E_2 (r= 0.74, P<0.05). While, GYP in E_2 was only positively correlated with TKW in E_2 (r= 0.69, P<0.05). A negative and significant correlation coefficient (r= -0.66, P<0.05) was found between GYP in E_2 and HSI.

Cluster analysis based on phenotypic data

Dendrograms obtained by cluster analysis of dissimilarities among wheat genotypes using Euclidean's coefficients are presented in Fig. 2. Cluster analysis classified ten tested genotypes into two groups with three and seven genotypes for cluster-I and cluster-II, respectively. The group-1 genotypes (Line-1, 2 and 3) contained the high tolerant genotypes having the lowest HSI values. However, the other tolerant and susceptible genotypes were grouped together in cluster-II. The first cluster was split into two sub-clusters, where

Line-1 and Line-2 were gathered in a sub-cluster, whereas Line-3 was placed alone in the second sub-cluster (Fig. 2).

SSR markers analysis

In the present study, thirteen SSR primer pairs were selected among wheat genome database in order to assess the genetic diversity among ten bread wheat genotypes. The 13 SSR markers generated a total number of 125 bands ranged from 4 for Xgwm566 to16 for Xgwm339, with an average of 9.6 bands per marker. The amplified DNA fragments ranged in size from 59 bp for Xgwm356 marker located on 2A chromosome to 893 bp for Xwmc398 marker located on 6B (Table 6 and Fig. 3). Of 125 bands amplified, 76 (60.8%) were polymorphic with an average of 5.8 polymorphic bands per marker. The lowest percentage of polymorphism (25.0%) was obtained with Xgwm497, whereas the highest polymorphism (85.7%) was produced with Xwmc273. PIC values ranged from 0.11 for Xwmc398 marker (located on 6B chromosome) to 0.36 for Xwmc273 marker (located on 7A), with an average of 0.23 per marker (Table 6). A highly significant correlation (r= 0.894, $P<0.01$) was found between the total number of bands and the number of polymorphic bands, whereas nonsignificant correlation was found between the total number of bands with the percentage of polymorphism (%P) and PIC values. However, the number of polymorphic bands was significantly correlated with %P (r= 0.595, $P<0.05$), while nonsignificant correlation was found between the number of polymorphic bands and PIC values. A highly significant correlation (r= 0.872, $P<0.01$) was found between %P and PIC values.

DNA amplification patterns obtained using some SSR markers used in the study are presented in Fig. 3. The Xgwm339 marker located on 2A generated a unique band (360 bp) presented only in Misr-2, and another unique band (595 bp) for Lira-Sa-92. Three unique bands (109, 643, 721 bp) generated by Xbarc121 located on 7A were also found only in Misr-2, while the Xwmc398 marker located on 6B generated a unique band (165 bp) for Line-1. Moreover, some SSR markers generated specific bands for some heat tolerant genotypes identified based on HSI values. A specific band (123 bp) generated by Xbarc121-7A was presented only in the high tolerant wheat genotypes (Line-1 and Line-2). Furthermore, the marker Xgwm339-2A generated a specific band (467 bp) for Line-1 and El-Nilein, and Xgwm497-1A amplified a specific band (303 bp) for the tolerant genotypes Line-1, Line-2 and El-Nilein (Fig. 3).

Cluster analysis based on SSR markers

Cluster analysis of similarities using Jaccard's coefficients based on SSR markers data classified the ten bread wheat genotypes evenly into two groups (Fig. 4). Cluster-I contained five of the high tolerant genotypes, however cluster-II contained two tolerant and three susceptible genotypes. Two sub-clusters were formed within cluster-I, of which the most tolerant genotypes (Line-1, Line-2, Line-3) were grouped in a sub-cluster, and the other sub-cluster contained Debeira and EL-Nilein,

where both of them are originally from Sudan.

Comparing distances matrices

Genetic distances (dissimilarity) calculated between each two of the ten wheat genotypes using Euclidean's coefficient (phenotypic data), and Jaccard's coefficient (SSR markers data) are found in Table 7. The genetic distances based on phenotypic data ranged from 0.192 (Debeira and Misr-2) to 0.922 (Line-3 and Debeira), with an average of 0.422. While, the genetic distances based on SSR markers ranged from 0.165 (Line-1 and Line-2) to 0.442 (El-Nilein and Misr-2), with an average of 0.317. The Mantel test revealed that, there was nonsignificant correlation between the genetic distances based on phenotypic data and the genetic distances based on SSR markers (r= 0.217, $P> 0.05$). Moreover, there was a highly significant difference between their means ($P< 0.01$, $t= 4.09$).

Discussion

Apparently, heat stress caused a significant reduction (30.4%) in GYP. The highest reduction (45.6%) was recorded for Gemmiza-7, while the lowest (20.6%) was found in Line-2. Generally all genotypes produced low grain yields when sown at 10th January and significantly different from those sown on 1st December. This finding demonstrated that wheat yields decrease when planting time is delayed that may be attributed to shortened crop development with low dry matter. In accordance, Suleiman *et al.* (2014) indicated that late sowing shortened the development phases of wheat and adversely affected the grain development and thus the grain yield. In the present study, out of ten genotypes tested, seven showed HSI values <1, thereby they were considered as heat tolerant genotypes. Moreover, a negative and significant correlation coefficient ($r= -0.66$, $P<0.05$) was found between GYP under heat stress and HSI values, supporting that HSI could be used for screening tolerant wheat genotypes under stress conditions. The stress susceptibility index (SSI) was firstly proposed by Fischer and Maurer (1978) and was used by Dhyani *et al.* (2013) and Khajuria *et al.* (2016) to evaluate bread wheat genotypes under heat stress. Thus, Line-2, Line-1 and Line-3 with low HSI values (0.68, 0.71 and 0.78), respectively, could be considered as the highest tolerant genotypes to be used as parents for improving heat tolerance in wheat breeding programs. However, further evaluation of these genotypes across multiple locations and years is required to confirm their stability for developing improved verities.

The 13 SSRs produced a total of 125 bands ranged from 4 for Xgwm566 to16 for Xgwm339, with an average of 9.6 bands per marker. Of 125 DNA fragments amplified, 76 (60.8%) were polymorphic with an average of 5.8 polymorphic bands per marker. The lowest polymorphism (25.0%) was obtained with Xgwm497, whereas the highest polymorphism (85.7%) was produced with Xwmc273. Similarly, different numbers of bands have been detected previously in wheat using microsatellite markers, ranged from 3.9 to 18.1 bands per marker (Prasad *et al.,* 2000; Roder *et al.,* 2002; Huang *et al.,* 2002; Khlestkina *et al.,* 2004; Akfirat

and Uncuoglu, 2013; Salem *et al.,* 2015). Such variation in the number of bands amplified by different primer sets is attributable to several factors including primer structure and number of annealing sites in the genome (Kernode *et al.,* 1993). Obviously, polymorphic bands revealed differences among genotypes would be used to examine and establish systematic relationships among genotypes as reported by Hadrys *et al.* (1992). In the current study, PIC values ranged from 0.11 for Xwmc398 to 0.36 for Xwmc273, with an average of 0.23 per marker, indicating that some SSRs were found to be highly informative markers. The difference in PIC values obtained here among SSR markers could be attributed to the variation in allele frequency of different loci. In this regard, Naghavi *et al.* (2007) suggested that multiple allelism is very common in SSR markers and they are able to produce different alleles in one locus. Similarly, many studies have reported remarkable differences in allelic diversity among various microsatellite loci (Ravi *et al*., 2003; Ram *et al.,* 2007).

In the present study, four SSR markers (Xgwm339-2A, Xgwm497-1A, Xwmc398-6B and Xbarc121-7A) generated some unique bands or specific for some tolerant genotypes, that could be used as markers associated with heat tolerance in wheat. However, these markers should be validated by testing their effectiveness in determining the target phenotype in independent populations and different genetic backgrounds (Collins *et al.*, 2003; Collard *et al.*, 2005). Several investigations reported that SSR markers associated with heat tolerance in wheat are located on different wheat chromosomes (Barakat *et al.,* 2012; Talukder *et al.,* 2014; Sun *et al*., 2015).

Cluster analysis based on phenotypic data classified the ten wheat genotypes into two clusters, where three heat tolerant genotypes (Line-1, Line-2 and Line-3) were grouped together in cluster-1. In comparison, grouping according to SSRs markers have resulted also in two clusters, of which cluster-1 contained five tolerant genotypes (Line-1, Line-2, Line-3, Debeira and EL-Nilein), indicating the efficiency of SSR markers in discriminating wheat genotypes based on heat tolerance. However, other tolerant genotypes were distributed in cluster-2, suggesting that different genetic loci could be involved in the inheritance of heat tolerance in these genotypes. Huang *et al.* (2002) and Al-Khanjari *et al.* (2007) reported that not all accessions originating from the same region clustered in the same group, indicating that the genetic diversity of hexaploid wheat is not completely related to geographic distribution. However, cluster analysis has been widely used for description of genetic diversity and grouping based on similar characteristics under stress condition (Tabatabaei, 2013; El-Rawy and Hassan, 2014). Furthermore, genetic diversity in wheat was assessed using morphological traits (Salem *et al.,* 2008, Sonmezoglu *et al.,* 2012; EL-Rawy and Youssef, 2014) and SSR markers (Salem *et al.,* 2008; Sonmezoglu *et al.,* 2012; Salem *et al.,* 2015).

The genetic distances based on phenotypic data ranged from 0.192

(Debeira and Misr-2) to 0.922 (Line-3 and Debeira), with an average of 0.422. While, the genetic distances based on SSR markers ranged from 0.165 (Line-1 and Line-2) to 0.442 (El-Nilein and Misr-2), with an average of 0.317. These results showed relatively high genetic distances based on phenotypic data, comparing to genetic distances based on SSR markers. Powell *et al.* (1996) reported that several factors might affect the estimates of genetic relationships between individuals i.e., number of markers used, distribution of markers in the genome and the nature of evolutionary mechanisms underlying the variation measured. Moreover, several authors reported a large genetic similarity in wheat (Chen *et al.,* 1994; Martin *et al.,* 1995; Barbosa-Neto *et al.,* 1996; Bohn *et al.,* 1999). Obviously, the information about genetic similarity will be helpful to avoid any chance of elite germplasm becoming genetically uniform and endangering long term productivity gains during breeding programs. Cultivars with the most distinct DNA profiles were likely to contain the greatest number of novel genes (Chauhan *et al.,* 2015).

In conclusion, both phenotypic and molecular data were able to assess the genetic variation and identify some genotypes tolerant to heat stress. However, SSR markers would be more informative for estimating the genetic diversity as well as DNA fingerprinting of wheat genotypes. SSR markers were also able to generate some unique and specific bands for some genotypes tolerant to heat stress, suggesting that these bands could be used in advanced work for improving heat tolerance in wheat. Integrating phenotypic and molecular data for estimating the genetic variation could help to develop wheat varieties with better grain yield under abiotic stress conditions.

Table 1. Name, pedigree and origin of bread wheat genotypes used in the study.

No.	Name	Pedigree	Origin	Year
1	Line-1	Selected for heat tolerance at Genetics Dept., Assiut University	Egypt	-
2	Line-2	Selected for heat tolerance at Genetics Dept., Assiut University	Egypt	-
3	Line-3	Selected for long spike at Genetics Dept., Assiut University	Egypt	-
4	Debeira	HD2160/5/TOB/CNO67//BB/3/NAI60*2//TT/SN64/4/HD1954	Sudan	1982
5	El-Nilein	S948.A1/7*SANTA ELENA	Sudan	1992
6	Misr-2	SKAUZ/BAV 92 CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y-0S	Egypt	2011
7	Gemmiza-7	CMH74A.630/SX//SERI82/3/AGENT GM4611-2GM-3GM-1GM-0GM	Egypt	1999
8	Sakha-8	CNO67//SN64/KLRE/3/8156 INDUS/NORTENO "S" PK3418-6S-0S-0S	Egypt	1976
9	Line-6	Selected for long spike at Genetics Dept., Assiut University	Egypt	-
10	Lira-Sa-92	KVZ/TRM//PTM/ANA	ICARDA	1992

Table 2. Performance of bread wheat genotypes under 1st and 2nd sowing date in 2014/2015 and 2015/2016 seasons.

Trait	GYP				TKW				SL				PH			
Year	2014/15		2015/16		2014/15		2015/16		2014/15		2015/16		2014/15		2015/16	
Sowing Date	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Line-1	48.7	36.9	42.3	34.5	39.7	33.6	35.4	32.5	14.3	13.2	15.0	11.3	136.2	102.1	127.0	89.5
Line-2	51.6	40.8	49.6	39.5	44.6	42.9	41.0	34.1	14.6	12.6	14.8	11.8	148.6	104.0	145.0	91.3
Line-3	64.6	47.4	57.8	46.1	45.1	43.9	43.6	36.9	22.3	18.9	23.0	16.4	155.1	118.2	149.2	113.2
Debeira	45.1	35.6	48.5	31.5	41.9	36.0	39.4	34.5	14.4	13.1	14.3	13.7	103.3	79.1	104.5	78.5
El-Nilein	52.7	40.9	54.9	38.8	42.1	41.4	46.0	42.7	11.8	11.6	11.5	10.5	118.9	84.1	119.0	79.0
Misr-2	55.8	32.6	55.2	31.8	40.2	37.8	44.6	36.6	12.1	11.4	12.4	10.7	110.0	85.2	109.5	72.5
Gemmiza-7	59.8	32.7	61.7	33.4	56.5	41.3	54.4	37.0	16.0	13.4	15.5	12.7	114.3	79.2	113.5	75.2
Sakha-8	61.2	44.1	55.6	42.0	52.7	47.6	55.1	45.1	9.1	8.6	9.4	8.6	109.2	75.1	106.5	82.3
Line-6	58.2	34.6	55.1	33.8	52.7	40.4	46.0	36.7	21.1	18.7	20.5	14.6	123.4	91.1	121.0	74.0
Lira-Sa-92	62.1	44.2	60.5	45.5	52.9	45.7	50.7	43.2	10.0	9.2	10.5	8.9	110.2	87.9	118.0	77.5
Mean	**56.0**	**39.0**	**54.1**	**37.7**	**46.8**	**41.1**	**45.6**	**37.9**	**14.6**	**13.1**	**14.7**	**11.9**	**122.9**	**90.6**	**121.3**	**83.3**
CV%	11.3	13.4	10.8	14.5	13.3	10.5	13.9	11.2	29.8	26.2	29.1	20.9	14.5	15.0	12.6	14.7
$LSD_{(0.05)}$	5.5	4.6	5.1	4.8	5.4	3.8	5.6	3.7	3.8	3.0	3.7	2.2	15.6	11.9	13.4	10.7

GYP: grain yield per plant (g), TKW: thousand kernel weight (g), SL: spike length (cm) and PH: plant height (cm).

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

Source of variation	d.f	Traits			
		GYP	TKW	SL	PH
Environment (E)	3	2816.2**	509.7**	55.0**	12649.2**
Replicates within E	8	27.1*	11.9	0.9	10.4
Genotypes (G)	9	267.7**	262.1**	147.9**	2408.1**
G x E	27	42.3**	28.3**	3.8**	80.0**
Error	72	12.9	10.6	0.6	19.0
σ^2_G		18.78	19.48	12.00	194.01
σ^2_E		12.93	10.63	0.57	19.01
σ^2_{GE}		9.79	5.90	1.08	20.31
$h^2_{(B)}$		**0.45**	**0.54**	**0.88**	**0.83**

*, ** Significant differences at P< 0.05 and P< 0.01, respectively. $h^2_{(B)} = \sigma^2_G / \sigma^2_P$, the phenotypic variance (σ^2_P)= $\sigma^2_G+\sigma^2_E+\sigma^2_{GE}$, where σ^2_G= the variance of genetic effect, σ^2_E= the environmental variance and $\sigma^2_{GE=}$ the variance of G × E interactions. GYP: grain yield per plant (g), TKW: thousand kernel weight (g), SL: spike length (cm) and PH: plant height (cm).

Table 4. Ranking the tested bread wheat genotypes based on heat susceptibility index (HSI).

Genotype	2014/2015			2015/2016			Average			Rank	Tolerance
	Yp	Ys	HSI	Yp	Ys	HSI	Yp	Ys	HSI		
Line-1	48.66	36.90	0.80	42.34	34.53	0.61	45.50	35.72	0.71	**2**	**T**
Line-2	51.58	40.76	0.69	49.56	39.52	0.67	50.57	40.14	0.68	**1**	**T**
Line-3	64.61	47.37	0.88	57.83	46.07	0.67	61.22	46.72	0.78	**3**	**T**
Debeira	45.12	35.57	0.70	48.47	31.53	1.15	46.79	33.55	0.93	**7**	**T**
El-Nilein	52.72	40.95	0.74	54.87	38.81	0.96	53.80	39.88	0.85	**4**	**T**
Misr-2	55.80	32.60	1.37	55.21	31.83	1.40	55.50	32.21	1.38	**9**	**S**
Gemmiza-7	59.79	32.70	1.49	61.70	33.40	1.51	60.75	33.05	1.50	**10**	**S**
Sakha-8	61.16	44.08	0.92	55.58	41.96	0.81	58.37	43.02	0.87	**5**	**T**
Line-6	58.15	34.56	1.34	55.09	33.83	1.27	56.62	34.20	1.30	**8**	**S**
Lira-Sa-92	62.07	44.19	0.95	60.52	45.53	0.82	61.30	44.86	0.88	**6**	**T**
Mean	**55.97**	**38.97**	**0.99**	**54.12**	**37.70**	**0.99**	**55.04**	**38.33**	**0.99**	-	-
CV%	11.28	13.42	30.45	10.82	14.53	33.08	10.56	13.82	29.83	-	-

Yp and Ys: GYP under normal and heat stress conditions, respectively. HSI= heat susceptibility index.
T: tolerant genotype, S: susceptible genotype (HSI < 1 = tolerant; HSI > 1 = susceptible).

Table 5. Correlation coefficients between each pair of agronomic traits and HSI under normal (E_1) and heat stress (E_2) conditions.

Traits		GYP		TKW		SL		PH		HSI
Sowing Date		E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	
GYP	E_1	1	0.45	0.79**	0.74*	0.10	0.02	-0.02	0.00	0.38
	E_2		1	0.20	0.69*	-0.03	-0.11	0.45	0.55	-0.66*
TKW	E_1			1	0.73*	-0.15	-0.21	-0.33	-0.39	0.45
	E_2				1	-0.37	-0.43	-0.16	-0.15	-0.08
SL	E_1					1	0.99**	0.61	0.62	0.11
	E_2						1	0.53	0.55	0.14
PH	E_1							1	0.93**	-0.50
	E_2								1	-0.58

*, ** Significant differences at P< 0.05 and P< 0.01, respectively. GYP: grain yield per plant (g), TKW: thousand kernel weight (g), SL: spike length (cm) and PH: plant height (cm). HSI= heat susceptibility index.

Table 6. Polymorphism detected among 10 bread wheat genotypes using 13 SSR markers used in the study.

Marker Name	CL	Sequence (5' - 3')	FR	TB	PB	%P	PIC
Xgwm291	5A	F: CATCCCTACGCCACTCTGC R: AATGGTATCTATTCCGACCCG	107-821	5	2	40.0	0.18
Xgwm294	2A	F: GGATTGGAGTTAAGAGAGAACCG R: GCAGAGTGATCAATGCCAGA	101-698	15	9	60.0	0.28
Xgwm339	2A	F: AATTTTCTTCCTCACTTATT R: AAACGAACAACCACTCAATC	61-595	16	11	68.8	0.24
Xgwm356	2A	F: AGCGTTCTTGGGAATTAGAGA R: CCAATCAGCCTGCAACAAC	59-549	14	10	71.4	0.28
Xgwm484	2D	F: ACATCGCTCTTCACAAACC R: AGTTCCGGTCATGGCTAGG	166-811	9	4	44.4	0.15
Xgwm497	1A	F: GTAGTGAAGACAAGGGCATT R: CCGAAAGTTGGGTGATATAC	73-537	8	2	25.0	0.12
Xgwm566	3B	F: TCTGTCTACCCATGGGATTTG R: CTGGCTTCGAGGTAAGCAAC	105-684	4	3	75.0	0.29
Xgwm577	7B	F: ATGGCATAATTTGGTGAAATTG R: TGTTTCAAGCCCAACTTCTATT	66-343	6	4	66.7	0.28
Xwmc273	7A	F: AGTTATGTATTCTCTCGAGCCTG R: GGTAACCACTAGAGTATGTCCTT	146-842	7	6	85.7	0.36
Xwmc398	6B	F: GGAGATTGACCGAGTGGAT R: CGTGAGAGCGGTTCTTTG	143-893	10	4	40.0	0.11
Xwmc596	7A	F: TCAGCAACAAACATGCTCGG R: CCCGTGTAGGCGGTAGCTCTT	209-780	9	5	55.6	0.23
Xbarc113	6A	F: GCGCACAACAACGGACACTTAACAATT R:GGGACTCATTTAGCTTCTACTCGCCATTA	77-391	8	5	62.5	0.22
Xbarc121	7A	F: ACTGATCAGCAATGTCAACTGAA R: CCGGTGTCTTTCCTAACGCTATG	68-721	14	11	78.6	0.22
Total			-	125	76	-	-
Average			-	9.6	5.8	60.8	0.23

Cl: Chromosol location of a marker, FR: fragment range (bp), TB: number of total bands, PB: number of polymorphic bands, %P: percentage of polymorphism and PIC: polymorphic information content.

Table 7. Genetic distances calculated among bread wheat genotypes based on phenotypic data (above diagonal) and SSR markers (down diagonal).

Genotypes	Line-1	Line-2	Line-3	Debeira	El-Nilein	Misr-2	Gemmiza-7	Sakha-8	Line-6	Lira-Sa-92
Line-1	-	0.270	0.530	0.483	0.362	0.441	0.514	0.580	0.358	0.502
Line-2	0.165	-	0.356	0.683	0.476	0.609	0.608	0.677	0.452	0.569
Line-3	0.220	0.268	-	0.922	0.720	0.850	0.819	0.870	0.672	0.762
Debeira	0.247	0.295	0.317	-	0.288	0.192	0.331	0.356	0.369	0.387
El-Nilein	0.233	0.312	0.293	0.224	-	0.200	0.252	0.263	0.241	0.209
Misr-2	0.434	0.408	0.402	0.407	0.442	-	0.234	0.306	0.272	0.285
Gemmiza-7	0.341	0.326	0.349	0.375	0.337	0.278	-	0.251	0.224	0.241
Sakha-8	0.388	0.379	0.337	0.325	0.387	0.221	0.184	-	0.389	0.197
Line-6	0.344	0.354	0.338	0.386	0.398	0.344	0.273	0.292	-	0.303
Lira-Sa-92	0.358	0.314	0.333	0.303	0.378	0.359	0.183	0.190	0.237	-

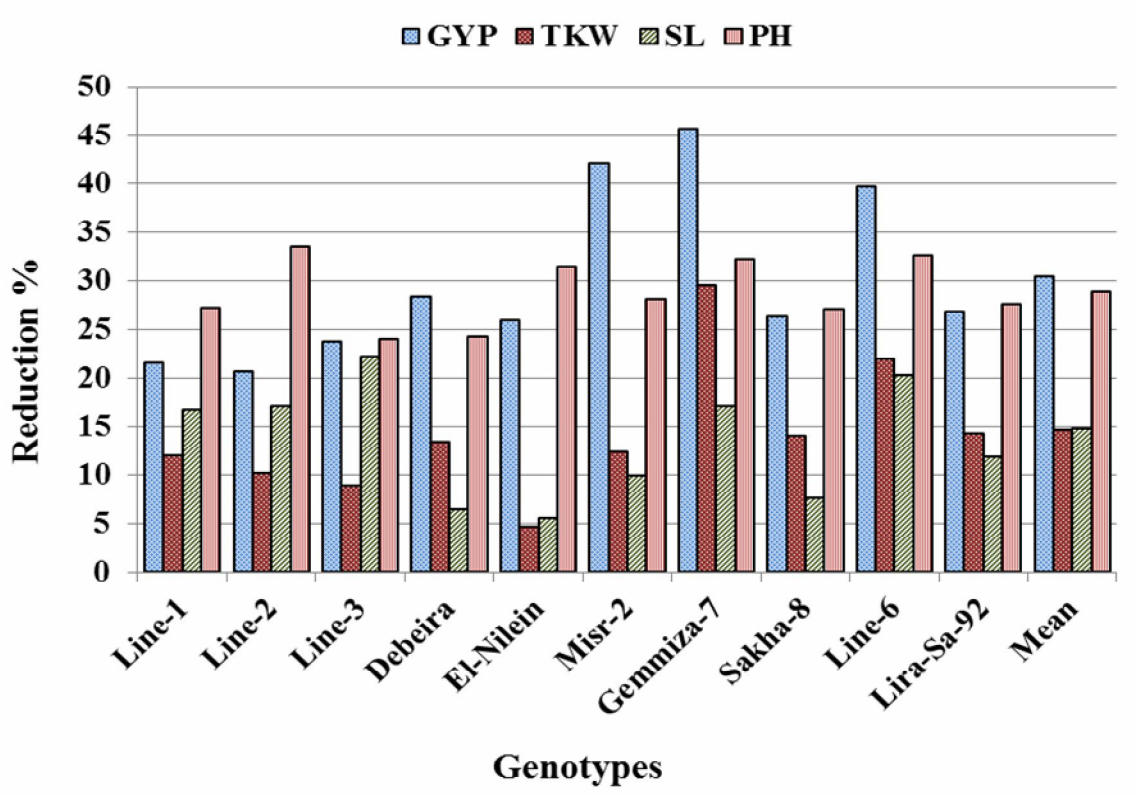

Fig. 1. Reduction percentage in agronomic traits for bread wheat genotypes resulting by heat stress. GYP: grain yield per plant (g), TKW: thousand kernel weight (g), SL: spike length (cm) and PH: plant height (cm).

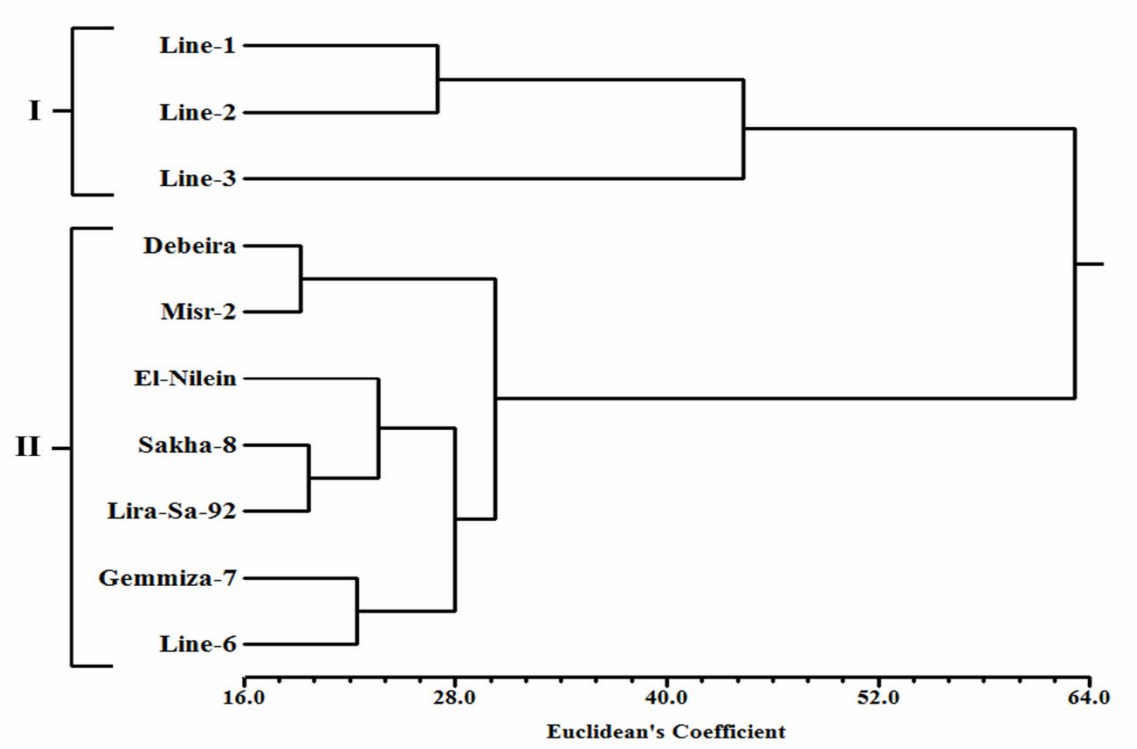

Fig. 2. UPGMA-Dendrogram of genetic dissimilarities among tested bread wheat genotypes using four agronomic traits based on Euclidean's coefficient.

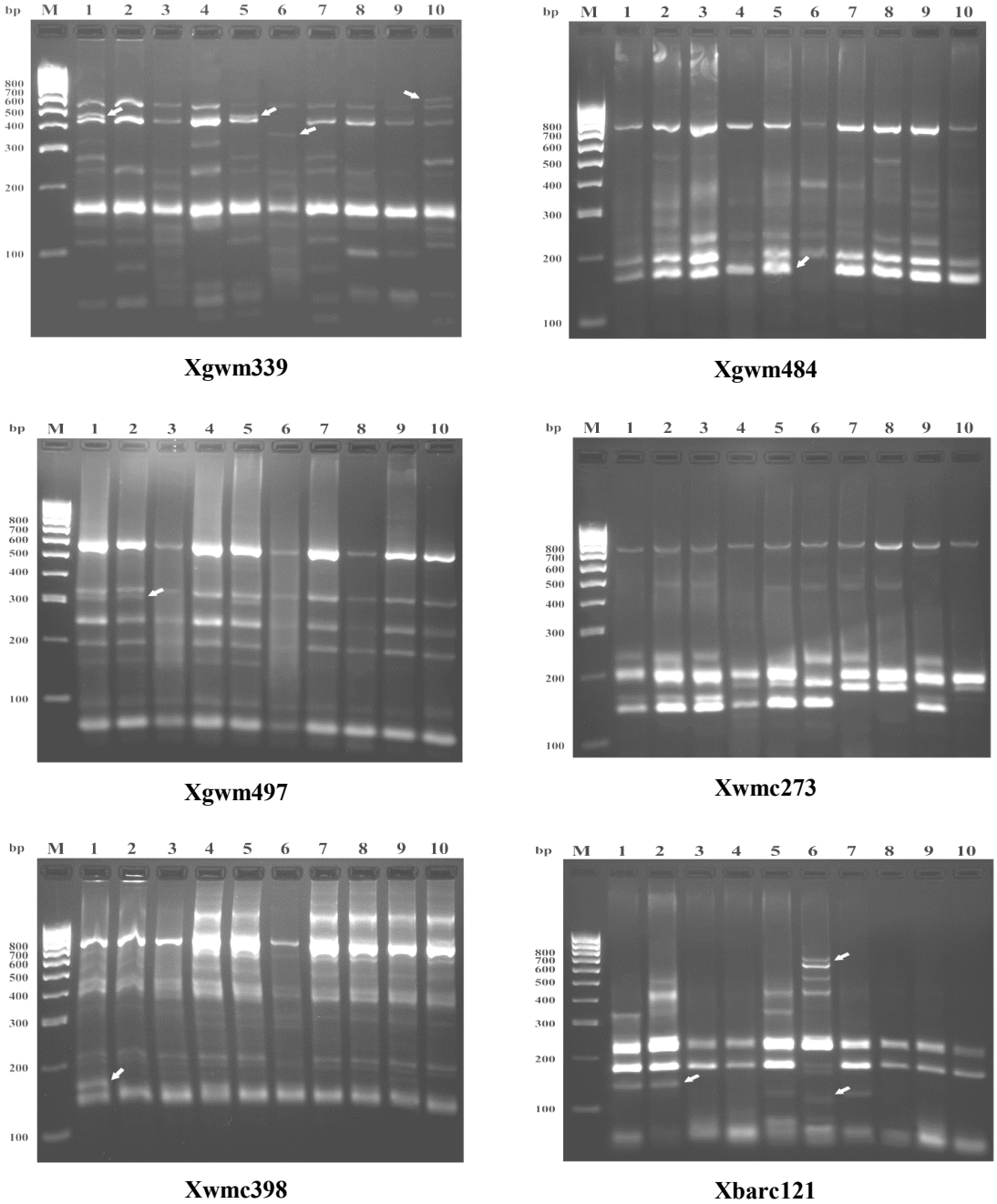

Fig. 3. DNA amplification patterns obtained using Xgwm339, Xgwm484, Xgwm497, Xwmc273, Xwmc398 and Xbarc121 markers for ten bread wheat genotypes; 1: Line-1, 2: Line-2, 3: Line-3, 4: Debeira, 5: El-Nilein, 6: Misr-2, 7: Gemmiza-7, 8: Sakha-8, 9: Line-6 and 10: Lira-Sa-92. M: the 100 bp DNA ladder. Arrows indicate unique bands or specific for some genotypes.

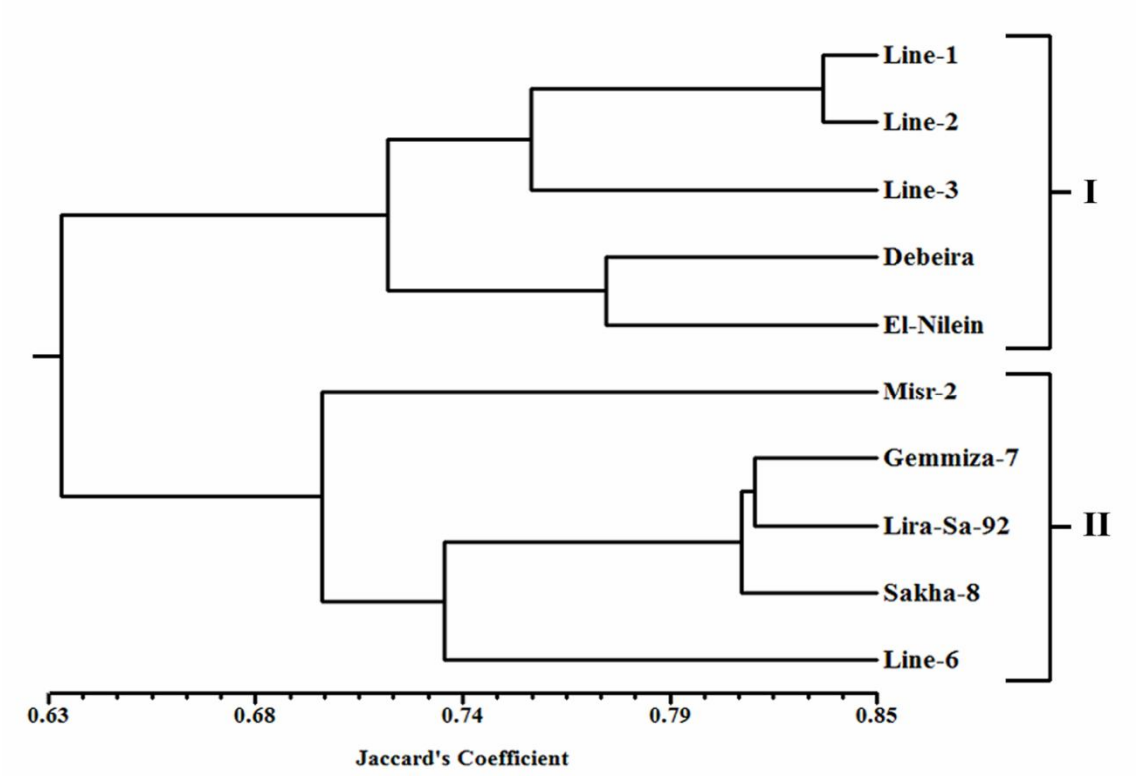

Fig. 4. UPGMA-Dendrogram of genetic similarities among tested bread wheat genotypes using thirteen SSR markers based on Jaccard's coefficient.

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تقدير التنوع الوراثي في طرز وراثية من قمح الخبز اعتماداً على التحمل الحراري وواسمات SSR

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الملخص

تم تقييم عشرة طرز وراثية من قمح الخبز في ميعادي زراعة قياسي ومتأخر خلال موسمي ٢٠١٤/٢٠١٥ و ٢٠١٥/٢٠١٦. تم تقييم أربعة صفات محصولية هي محصول الحبوب للنبات الواحد ووزن الألف حبة وطول السنبلة وطول النبات. تم تقدير التنوع الوراثي بين الطرز الوراثية تحت الدراسة اعتماداً على البيانات المظهرية وثلاثة عشرة واسمة SSR تمثل تسعة من كروموسومات القمح. أظهرت النتائج أن الاجهاد الحراري كان قوياً في ميعاد الزراعة المتأخر مؤدياً إلى متوسط انخفاض مقداره ٣٠,٤ ، ١٤,٦ ، ١٤,٧ ، ٢٨,٨% في صفات محصول الحبوب للنبات ووزن الألف حبة وطول السنبلة وطول النبات، على التوالي. كما اظهر محصول الحبوب للنبات ارتباطاً سالباً معنوياً (r= -0.66, P<0.05) مع دليل الحساسية للحرارة تحت ظروف الاجهاد الحراري. وباستخدام ١٣ واسم SSR، تم الحصول على ١٢٥ حزمة DNA بمتوسط قدره ٩,٦ حزمة لكل واسم. تراوحت النسبة المئوية لتعدد الطرز من ٢٥% بالنسبة للواسم Xgwm497-1A إلى ٨٥,٧% للواسم Xwmc273-7A، بمتوسط قدره ٦٠,٨%. سجلت أيضاً أعلى قيمة للمحتوى المعلوماتي لتعدد الطرز (٠,٣٦) للواسم Xwmc273-7A، بينما أقل قيمة (٠,١١) وجدت مع الواسم Xwmc398-6A، بمتوسط قدره ٠,٢٣. ولوحظ وجود ارتباطاً موجباً معنوي جداً (r= 0.872, P<0.01) بين النسبة المئوية لتعدد الطرز (P%) والمحتوى المعلوماتي لتعدد الطرز (PIC). أظهر التحليل العنقودي اعتماداً على البيانات المظهرية توزيع العشرة طرز وراثية إلى مجموعتين، حيث اظهرت الطرز الوراثية للمجموعة الأولى (سلالة-١ ، سلالة-٢ ، سلالة-٣) أعلى تحمل للاجهاد الحراري وأقل قيم لدليل الحساسية للحرارة. كما أدى التحليل العنقودي باستخدام واسمات SSR إلى تكوين مجموعتين، حيث احتوت المجموعة الأولي على خمسة طرز وراثية متحملة للاجهاد الحراري (سلالة-١ ، سلالة-٢ ، سلالة-٣ ، ديبيرا ، النيلين) ، مما يدل على كفاءة واسمات الـ SSR في تمييز الطرز الوراثية للقمح. علاوة على ذلك، فإن أربعة واسمات SSR اظهرت بعض الحزم الفريدة أو الخاصة ببعض الطرز الوراثية المتحملة، والتي يمكن استخدامها كواسمات مرتبطة بالتحمل الحراري في القمح. ومع ذلك، لا تزال هناك حاجة لتحليل واسمات اضافية للتحقق من فائدتها في برامج التربية.