

Assessment of Genetic Diversity for Some Egyptian Wheat Varieties based on Morphological Characters and SSR Markers

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ABSTRACT

The genetic diversity estimates can be useful in important genotypes selection for plant breeders. Eight durum and three bread wheat cultivars were used to study and compare morphological traits with molecular study using SSR technique based genetic diversity estimates. Significant and highly significant differences among wheat genotypes were observed for all traits except flag leaf attitude and outer glume pubescence. Highly significant differences were obtained between durum and bread wheat genotypes for most studied traits. Moderate to low broad-sense heritability estimates were obtained for morphological studied traits. Positive and high significant correlation was found for ear density with foliage color ($r= 0.51$), waxiness of flag leaf sheath ($r= 0.57$), waxiness of peduncle ($r= 0.60$) and peduncle length ($r= 0.53$), whereas a negative correlation was found between ear density and plant height ($r= -0.52$, $P<0.01$). However, waxiness of flag leaf sheath was positively correlated with ear waxiness ($r= 0.53$, $P<0.01$), waxiness of peduncle ($r= 0.56$, $P<0.01$), and Peduncle length ($r= 0.43$, $P<0.05$). The higher polymorphism (90.63%) was found in the B genome than in the A genome (81.97%). Single marker analysis showed that 11 SSR markers were significantly associated with phenotypic traits, including Xgwm111-2B associated with waxiness of peduncle. A significant but low correlation ($r = 0.25$) was found between the dissimilarity matrix generated from the phenotypic data and that obtained from the SSR markers, suggesting that the characterization based on agro-morphological traits and SSR markers will be a useful tool to the breeders for selection of genotypes with appropriate.

KEYWORDS: Durum wheat, Bread wheat, SSR markers, Waxiness, Polymorphism.

1. INTRODUCTION

Wheat is the most widely grown crop in the world, adapted to a wide range of environments, and considered as a staple source of nutrients for around 40% of the world's population (Giraldo *et al.*, 2019). wheat is the most important grain crop in Egypt and plays a vital role in Egypt's economy as a strategic crop. In Egypt, There is a large gap between the production of this crop and its consumption. Therefore, Egypt remains the largest wheat importer in the world, where wheat imports for the 2019/20 marketing year were estimated at 12.5 million tons, about 15% above the average of the last five years (FAOSTAT, 2020). So, it is important to improve this crop to overcome this problem.

The genetic diversity among plant species offers prospects for the improvement of plant characteristics, and it is an important source for any successful breeding program. Therefore, assessment of the genetic diversity is necessary for the effective exploitation of genetic resources in breeding programs (Khan *et al.*, 2015). Moreover, differences in the genetic components of the

traits can be applied as a new source of variation in other breeding programs for wheat improvement (Khodadadi *et al.*, 2011). Determination of the genetic diversity and the relationships among genotypes is also of great importance for identifying the appropriate parents in breeding programs (Ghasemi *et al.*, 2019).

The methods for detecting and assessing the genetic diversity among genotypes have extended from the analysis of discrete morphological to biochemical and molecular traits (Khaled *et al.*, 2015). Characterization of wheat genotypes based on qualitative and quantitative agro-morphological traits could be helpful in constructing breeding populations and implementing selection strategies (Aghaee *et al.*, 2010). Furthermore, assessment of the genetic variability using molecular markers has been used for understanding the genomic constitution in plants genome and categorizing genes responsible for important traits (Khan *et al.*, 2015). Therefore, the genetic diversity in wheat was successfully assessed using morphological traits (EL-Rawy and Youssef,

2014, Hassan, 2016) and molecular markers (Salem *et al.*, 2015, Hassan, 2016).

Although, morphological traits can be used for assessing the genetic diversity, they are often influenced by the environment (Hassan, 2016). Therefore, the use of molecular markers for assessment of the genetic diversity is receiving much attention from wheat breeders (Salem *et al.*, 2015). In addition, molecular markers provided the opportunity for determining inter and intra-species genetic relationships (Gostimsky *et al.* 2005). Therefore, characterization based on DNA polymorphism using molecular markers is more efficient and accurate. Numerous PCR-based molecular markers were developed to assess the genetic diversity among different genotypes. These marker systems are different in their technical principles as well as the amount of polymorphism (Powell *et al.* 1995).

Microsatellites or simple sequence repeats (SSRs) are characterized by a high level of polymorphism, chromosome-specific, multiallelic and distributed over the genome. These characteristics allow SSR markers to discriminate among cultivars and even among closely related wheat breeding lines (Mantovani *et al.*, 2008; Salem *et al.*, 2015). Therefore, SSR markers have been long used for several purposes including genome mapping, physical mapping, gene tagging and genetic diversity estimates (Wang *et al.*, 2007). Several hundred SSRs have been developed for the A, B, and D genomes of wheat (Mantovani *et al.*, 2008), and used in the association analysis and linkage-based studies for mapping genes or quantitative trait loci (QTL) controlling important traits (Neale and Savolainen 2004).

Clustering genotypes based on similar characteristics could provide valuable information for selecting the better performing lines in breeding programs. Thus, cluster analysis has been widely used for assessment of the genetic diversity and grouping wheat genotypes based on phenotypic data and molecular markers (El-Rawy and Hassan, 2014; Arain *et al.*, 2018; Jamali *et al.*, 2020).

The aims of the present study were to evaluate eight durum and three bread wheat cultivars for several morphological traits; identify molecular markers associated with studied traits to be used in breeding programs and assess the genetic diversity among the tested plant genotypes based on the results of phenotypic traits and SSR markers.

2. MATERIALS AND METHODS

2.1. Plant materials and morphological characters

In the present study, eight Egyptian durum (*Triticum durum* Desf.) and three bread (*T. aestivum* L.) wheat cultivars was chosen for studying the morphological characters as shown in Table (1). The field experiment was conducted at the Experimental Farm of Faculty of Agriculture, Assiut University, Egypt during two growing seasons of 2017/18 and 2018/19.

Seeds of the tested plant genotypes were cultivated on normal sowing dates. The field experiment was designed as a randomized complete block design (RCBD) with three replications. The experimental plot consisted of 6 rows, 20 cm apart, and 3.5 m long (plot area = 4.2 M²). The cultivars under investigation were phenotyped using some morphological characters used for distinctness, uniformity and stability (DUS). The morphological descriptors were 17 characters. Scoring values for each state of selected descriptors were given discrete number value to generate numerical dataset (Table 2).

2.2. Simple sequence repeats (SSRs) technique

The simple sequence repeats (SSRs) technique was carried out at the Department of Genetics, Faculty of Agriculture, Assiut University, Egypt. Twenty one wheat microsatellites or SSR primer pairs were selected and used for screening the studied genotypes (Table 3). The A, B and D wheat genomes were covered by a primer pair for each chromosome. The total genomic DNA of each cultivar was extracted by Cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980).

Twenty one Primer sequences were used and performed by GrainGenes Database (<http://wheat.pw.usda.gov>) in the present study. The PCR conditions and its thermal cycle were carried out according to a SensoQuest LabCycler (SensoQuest GmbH, Göttingen, Germany). The PCR products were separated on 2.5% agarose gels in 0.5× TBE buffer. A 100bp DNA Ladder was used to estimate the molecular size of the amplified DNA fragments. The percentage of polymorphism obtained by each marker was calculated by dividing the number of polymorphic bands with the total number of amplified bands. The polymorphic information content (PIC) was calculated for each marker using the formula described by Roldan-Ruiz *et al.* (2000) to investigate the suitability of each marker and assess the genetic diversity among the studied plant genotypes. Also, Marker index (MI) and Resolving power (Rp) of each RAPD primer were

Table 1. Names, code and pedigree of eight durum and three bread wheat cultivars.

	Name	Code	Pedigree
Durum wheat	Bani Sueif 1	G1	JORI69(SIB)/(SIB)ANHINGA/(SIB)FLAMINGO
	Bani Sueif 4	G2	RoK”S”/Mexi75/a”S”//Ruff”S”/FG”S”/3/Mexi 75
	Bani Sueif 5	G3	DIPPER-2/ BUCHEN-3
	Bani Sueif 6	G4	BOOMER-21/BUSCA-3
	Bani Sueif 7	G5	CBC509CHILE//sooty_9/RASCON_37/9/USDA595/3/D67.3/RABI //CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
	Sohag 3	G6	MEXICALI-75/MAGHREBI-72//S-179/DURUM-6
	Sohag 4	G7	Ajaia-16//Hora/Jor/3/Gan/4/Zar/5/Souk-7/6/Stot//Altar84/aLD
	Sohag 5	G8	TRN//21563/AA/3/BD2080/4/BD2339/5/RASCON37/Tarro 2//RASCON 3/6/Auk/Gull//Green
Bread wheat	Gemmeiza7	G9	CMH74 A. 630/5x//Seri 82/3/Agent CGM 4611-2GM-3GM-1GM-0GM
	Gemmeiza9	G10	Ald”S”/Huac”S”//CMH74A.630/5x CGM4583-5GM-1GM-0GM
	Misr 1	G11	OASIS/SKAUZ//4*BCN/3/2*PASTOR. CMSS00Y01881T -050M-030Y -030M-030WGY-33M0Y--0EGY

calculated according to Powell *et al.*, (1996) and Prevost & Wilkinson (1999), respectively. .

2.3. Statistical analysis for morphological traits and SSR data

The differences between means were tested by Fisher's Least Significant Difference (LSD) at 0.05 and 0.01 levels of probability. Combined analysis of variance was used to test the significance of differences among genotypes (G) and years (E), and the significance of G×E interaction for each trait. The broad-sense heritability (h^2_B) of a trait was computed using the formula described by Nyquist (1991). The phenotypic correlations among the studied traits were measured by Pearson's correlation coefficients.

Cluster analysis of wheat genotypes based phenotypic data was conducted using Standardized Euclidean distance matrix with the unweighted pair group method based on arithmetic averages (UPGMA) by MVSP version 3.22 software (Kovach Computing Services). The genetic distance matrix based on SSR markers was conducted and UPGMA-dendrogram was performed according to Nei and Li's coefficient using MVSP version 3.22. In order to investigate the association between the SSR markers and the studied traits, single marker analysis using linear regression was conducted by Microsoft Excel.

3. RESULTS

The mean performance of the studied genotypes for Growth habit (GH), Auricles coloration pigment (CLAR), Flag leaf attitude (FLAT), Waxiness of flag leaf sheath (WSH), Waxiness of peduncle (W. Ped), Ear shape in profile (Ear Shape), Ear orientation (Ear Orint), Foliage color (FCL), Plant height (PLHT), Ear waxiness (W. Ear), Flag leaf width (FLW), Flag leaf length (FLL), Leaf blade waxiness (WBL), Peduncle length (Ped. L), Ear density (Ear. Dens), Hairs of auricles (HRAR), Outer glume pubescence (Out. Gl. Pub) during the two seasons is presented in Table (4). Means of GH, FCL, CLAR, WSH, Ped L and Ear Den in durum wheat (3.46, 4.92, 4.58, 6.38, 6.08 and 7.21, respectively) were significantly higher than those obtained for bread wheat (2.33, 3.00, 2.78, 4.89, 4.56 and 5.11, respectively). Unlike, means HRAR, FLW and PLHT in bread wheat (5.56, 8.78 and 6.22, respectively) were significantly higher than those observed for durum wheat (4.29, 7.67, and 5.13, respectively). While, nonsignificant differences were found between durum and bread wheat genotypes for the remaining traits. Out of eight durum wheat genotypes tested, the means of FCL, Ped. L and Out.Gl.Pub in G1, G2, G3, G4 and G5 genotypes (Bani Sueif) were higher than those of G6, G7 and G8 genotypes (Sohag) by 47.38, 24.11 and 34.19%, respectively. Unlike, the means of CLAR were greater in G6, G7 and G8 sohang genotypes than those found in

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G1, G2, G3, G4 and G5 Bani Sueif genotypes by
45.11%.

Table 2. Morphological characters and their numerical scores in 11 Egyptian wheat cultivars.

Characters	Abbrev.	Descriptors	Characters	Abbrev.	Descriptors
Growth habit	GH	1 Erect 3 Semi-erect 5 Intermediate 7 Semi- spreading 9 prostrate	Flag leaf width	FLW	1 Narrow (<1.5) 5 Medium (1.5-2) 9 Broad (>2.0)
Foliage color	FCL	1 Pale green 5 Green 9 dark green	Flag leaf length	FLL	1 Short (<20) 5 Medium (20-30) 9 Long (>30)
Hairs of auricles	HRAR	3 Absent 5 Medium 7 Strong	Peduncle length	Ped. L	1 Short (< 30) 5 Medium (30-50) 9 Long (>50)
Auricles coloration pigment	CLAR	1 Absent 5 Medium 9 Strong	Plant height	PLHT	1 Very short (<75 cm) 3 Short (75.1-90 cm) 5 Medium (90.1 – 105 cm) 7 Long (105.1 -120 cm) 9 Very Long (>120 cm)
Flag leaf attitude	FLAT	1 Erect 3 Semi-erect 5 drooping	Outer glume pubescence	Out. Gl. Pub	3 Absent 5 Medium 7 Strong
Waxiness of flag leaf sheath	WSH	1 Absent 3 Weak 5 Medium 7 Strong 9 Very strong	Ear density	Ear. Dens	1 Very lax 3 Lax 5 Medium 7 Dense 9 Very dense
Leaf blade waxiness	WBL	1 Absent 3 Weak 5 Medium 7 Strong 9 Very strong	Ear orientation	Ear Orint	3 Erect 5 Semi-erect 7 Dropping
Ear waxiness	W. Ear	1 Absent 3 Weak 5 Medium 7 Strong 9 Very strong	Ear shape in profile	Ear SH	1 Tapering 3 Parallel sided 5 Semi-clavate 7 Clavate 9 Fusiform
Waxiness of peduncle	W. Ped	1 Absent 3 Weak 5 Medium 7 Strong 9 Very strong			

Table 3. Names, chromosomal location (CL), sequences, and annealing temperature (An.) of SSR markers used in the study.

Marker	CL	Forward primer	Reverse primer	An.
Xgwm33	1A	5' GGAGTCACACTTGTTTGTGCA 3'	5' CACTGCACACCTAACTACCTGC 3'	60 C°
Xgwm95	2A	5' GATCAAACACACACCCCTCC 3'	5' AATGCAAAGTGAAAAACCCG 3'	60 C°
Xgwm155	3A	5' CAATCATTCCCCCTCCC 3'	5' AATCATTGGAAATCCATATGCC 3'	60 C°
Xgwm160	4A	5' TTCAATTCAGTCTTGGCTTGG 3'	5' CTGCAGGAAAAAAGTACACCC 3'	55 C°
Xgwm186	5A	5' GCAGAGCCTGGTTCAAAAAG 3'	5' CGCCTCTAGCGAGAGCTATG 3'	60 C°
Xgwm459	6A	5' ATGGAGTGGTCACACTTTGAA 3'	5' AGCTTCTCTGACCAACTTCTCG 3'	55 C°
Xgwm63	7A	5' TCGACCTGATCGCCCCTA 3'	5' CGCCCTGGGTGATGAATAGT 3'	60 C°
Xgwm18	1B	5' GGTTGCTGAAGAACCTTATTTAGG 3'	5' TGGCGCCATGATTGCATTATCTTC 3'	50 C°
Xgwm111	2B	5' GTTGCACGACCTACAAAGCA 3'	5' ATCGCTCACTCACTATCGGG 3'	55 C°
Xgwm389	3B	5' ATCATGTCGATCTCCTTGACG 3'	5' TGCCATGCACATTAGCAGAT 3'	60 C°
Xgwm513	4B	5' ATCCGTAGCACCTACTGGTCA 3'	5' GGTCTGTTCATGCCACATTG 3'	60 C°
Xgwm408	5B	5' TCGATTTATTTGGGCCACTG 3'	5' GTATAATTCGTTACAGCACGC 3'	55 C°
Xgwm626	6B	5' GATCTAAAATGTTATTTTCTCTC 3'	5' TGAATATCAGCTAAACGTGT 3'	50 C°
Xgwm577	7B	5' ATGGCATAATTTGGTGAAATTG 3'	5' TGTTTCAAGCCCAACTTCTATT 3'	55 C°
Xgwm458	1D	5' TTCGCAATGTTGATTTGGC 3'	5' TTCGCAATGTTGATTTGGC 3'	60 C°
Xgwm261	2D	5' CTCCTGTACGCCTAAGGC 3'	5' CTCGCGCTACTAGCCATTG 3'	55 C°
Xgwm3	3D	5' AATATCGCATCACTATCCCA 3'	5' AATATCGCATCACTATCCCA 3'	55 C°
Xgwm165	4D	5' TGCAGTGGTCAGATGTTTCC 3'	5' CTTTCTTTTTCAGATTGCGCC 3'	60 C°
Xgwm190	5D	5' GTGCTTGCTGAGCTATGAGTC 3'	5' GTGCCACGTGGTACCTTTG 3'	60 C°
Xgwm325	6D	5' TTTCTTCTGTCGTTCTTCTCCC 3'	5' TTTTACGCGTCAACGACG 3'	60 C°
Xgwm437	7D	5' GATCAAGACTTTTGTATCTCTC 3'	5' GATGTCCAACAGTTAGCTTA 3'	50 C°

Table 4. Two seasons means of studied traits for durum and bread wheat genotypes.

Traits	Genotypes												LSD (0.05)	LSD (0.01)	
	Durum wheat						Bread wheat								
	Bani Sueif 1	Bani Sueif 4	Bani Sueif 5	Bani Sueif 6	Bani Sueif 7	Sohag 3	Sohag 4	Sohag 5	Mean	Gemmiza 7	Gemmiza 9	Misr 1			Mean
GH	3.00	1.67	5.33	2.33	4.67	3.67	3.33	3.67	3.46	2.00	2.67	2.33	2.33	1.16	1.54
FCL	3.00	5.67	6.33	7.00	6.33	4.33	3.67	3.00	4.92	3.67	3.67	1.67	3.00	1.60	2.14
HRAR	4.33	3.33	3.67	5.33	5.00	3.33	5.00	4.33	4.29	5.33	6.33	5.00	5.56	0.91	1.22
CLAR	1.67	3.67	5.00	5.00	4.33	5.00	7.00	5.00	4.58	1.67	4.33	2.33	2.78	1.45	1.93
FLAT	2.00	1.67	1.67	2.33	2.67	2.00	1.67	2.00	2.00	2.67	2.00	1.67	2.11	0.90	1.21
WSH	4.00	6.33	6.33	7.33	7.33	6.67	7.00	6.00	6.38	4.67	4.33	5.67	4.89	0.96	1.28
WBL	3.33	5.33	4.00	5.00	6.00	4.00	6.00	5.67	4.92	5.33	3.67	4.33	4.44	1.14	1.52
W. Ear	4.33	6.33	4.33	7.00	6.33	4.33	7.00	5.33	5.63	5.00	5.33	5.00	5.11	0.89	1.19
W. Ped	5.00	5.00	6.67	7.33	6.00	4.67	6.33	6.00	5.88	4.33	5.00	6.33	5.22	0.79	1.06
FLW	5.67	9.00	9.00	7.00	7.67	9.00	8.33	5.67	7.67	9.00	9.00	8.33	8.78	1.16	1.55
FLL	7.00	8.33	5.67	5.67	5.00	8.33	8.33	5.00	6.67	7.00	5.00	8.33	6.78	1.30	1.74
Ped. L	5.00	6.33	7.00	6.33	7.67	6.33	5.00	5.00	6.08	3.67	5.00	5.00	4.56	1.02	1.37
PLHT	5.33	5.33	5.00	5.33	5.33	4.67	5.00	5.00	5.13	6.33	6.67	5.67	6.22	0.70	0.93
Out. Gl. Pub.	5.67	5.00	5.33	4.00	5.33	4.33	4.00	3.00	4.58	5.00	4.33	4.67	4.67	1.14	1.53
Ear Den	6.67	6.33	7.00	8.00	8.00	6.67	7.33	7.67	7.21	4.33	6.33	4.67	5.11	0.83	1.11
Ear Orint	3.33	6.33	5.00	4.67	5.00	5.67	5.33	5.67	5.13	6.33	4.00	5.00	5.11	1.14	1.52
Ear Sh	3.33	2.00	4.00	4.00	4.33	4.33	2.33	4.67	3.63	1.00	3.00	5.00	3.00	0.99	1.32

Growth habit (GH), Auricles coloration pigment (CLAR), Flag leaf attitude (FLAT), Waxiness of flag leaf sheath (WSH), Waxiness of peduncle (W. Ped), Ear shape in profile (Ear Shape), Ear orientation (Ear Orint), Foliage color (FCL), Plant height (PLHT), Ear waxiness (W. Ear), Flag leaf width (FLW), Flag leaf length (FLL), Leaf blade waxiness (WBL), Peduncle length (Ped. L), Ear density (Ear. Dens), Hairs of auricles (HRAR), Outer glume pubescence (Out. Gl. Pub).

3.1. Analysis of variance and heritability estimates

The combined analysis of variance (Table 5) revealed highly significant differences among the two growing seasons (S) for GH, FCL, HRAR, WBL, W. Ped and Ear Den. Significant ($P<0.05$) or highly significant differences ($P<0.01$) among wheat genotypes (G) were observed for all the traits except FLAT and Out.Gl.Pub. Highly significant ($P<0.01$) differences were obtained between durum and bread wheat genotypes for GH, FCL, HRAR, CLAR, WSH, FLW, Ped. L, PLHT and Ear Den., while significant

($P<0.05$) differences were obtained for W. Ped. Highly significant $G \times S$ interactions ($P<0.01$) were observed for HRAR, CLAR, W. Ear, W. Ped and Ped. L, whereas significant interactions $G \times S$ ($P<0.05$) were obtained for GH and Out.Gl.Pub. Moderate broad-sense heritability estimates were obtained for WSH (0.45), FLW (0.52), and FLL (0.47), Ear Den (0.50) and Ear Sh (0.52), whereas low heritability was found for GH (0.17), FCL (0.29), HRAR (0.14), CLAR (0.14), FLAT (0.01), WBL (0.15), W. Ear (0.05), W. Ped (0.25), Ped. L (0.08), PLHT (0.23), Out.Gl.Pub (0.01) and Ear Orint (0.17) (Table 5).

Table 5. The combined analysis of variance and broad-sense heritability for the studied traits.

S. O. V.	df	Mean squares								
		GH	FCL	HRAR	CLAR	FLAT	WSH	WBL	W. Ear	W. Ped
Seasons	1	21.88**	29.33**	8.73**	8.73	3.88	3.88	7.33*	0.24	7.33**
R./Seasons	4	0.06	10.79*	0.73	2.55	0.42	2.24	0.97	2.06	6.79**
Genotypes	10	7.65**	17.31**	5.26**	16.15**	0.86	8.33**	5.50*	6.32**	5.39**
Durum vs Bread	1	16.57**	48.09**	20.91**	42.68**	0.16	28.91**	2.92	3.46	5.58*
Among durum	7	8.37**	15.57**	3.70**	13.67**	0.76	6.94**	6.24**	8.46**	5.13**
Among Bread	2	0.67	8.00	2.89	11.56*	1.56	2.89	4.22	0.22	6.22*
S x G	10	4.28*	6.40	3.39**	10.86**	0.81	1.61	3.20	5.44**	2.53**
Error	40	1.93	3.45	1.13	2.81	1.09	1.24	1.77	1.06	0.85
Heritability (B.S.)	-	0.17	0.29	0.14	0.14	0.01	0.45	0.15	0.05	0.25

S. O. V.	df	Mean squares							
		FLW	FLL	Ped. L	PLHT	Out.Gl.Pub.	Ear Den	Ear Orint	Ear Sh
Seasons	1	6.06	3.88	2.18	0.97	2.97	19.64**	3.88	4.91
R./Seasons	4	3.15	3.88	1.21	0.61	0.79	3.09*	2.61	2.91
Genotypes	10	10.33**	12.99**	8.00**	2.15**	3.58	8.86**	4.97*	9.37**
Durum vs Bread	1	16.16**	0.16	30.56**	15.76**	0.09	57.58**	0.01	5.11
Among Durum	7	12.19**	13.71**	6.05**	0.37	4.90*	2.46*	4.75*	5.80**
Among Bread	2	0.89	16.89**	3.56	1.56	0.67	6.89**	8.22*	24.00**
S x G	10	0.73	1.75	6.45**	0.84	3.77*	1.64	2.55	1.18
Error	40	1.82	2.28	1.48	0.67	1.85	0.96	1.74	1.31
Heritability (B.S.)	-	0.52	0.47	0.08	0.23	0.01	0.5	0.17	0.52

*, ** Significant differences at $P < 0.05$ and $P < 0.01$, respectively.

3.2. Correlation coefficients among traits

Correlations analysis among the studied traits (Table 6) showed that Ear Den was positively correlated with FCL ($r= 0.51$, $P<0.01$), WSH ($r= 0.57$, $P<0.01$), W. Ped ($r= 0.60$, $P<0.01$) and Ped. L ($r= 0.53$, $P<0.01$). However, a negative correlation was found between Ear Den and PLHT ($r= -0.52$, $P<0.01$). Significant and positive correlation was found between GH and CLAR ($r= 0.43$, $P<0.05$), whereas significant and negative correlation was obtained between GH and FLL ($r= -0.44$, $P<0.05$). Meantime, WSH was positively correlated with W. Ear ($r= 0.53$, $P<0.01$), W. Ped ($r= 0.56$, $P<0.01$), and Ped. L ($r= 0.43$, $P<0.05$), but, it was negatively correlated with PLHT (-0.53 ,

$P<0.01$). Positive and highly significant correlations were found between FCL with WSH ($r= 0.55$, $P<0.01$) and Ped. L ($r= 0.54$, $P<0.01$). Similarly, positive correlations were found between HRAR with FLAT ($r= 0.41$, $P<0.05$) and PLHT ($r= 0.68$, $P<0.01$).

3.3. SSR markers data analysis

Out of 21 SSR primer pairs used for screening eight durum and three bread wheat genotypes, a total number of 115 bands were generated, which ranged from 1 band for Xgwm165-4D, Xgwm437-7D and Xgwm325-6D to 13 bands for Xgwm155-3A, with an average of 5.48 bands per primer. Of 115 bands generated, 97 bands were polymorphic with an average of 4.62 bands per primer. The lowest

Table 6. Correlation coefficients among traits studied for two season's average

Traits	GH	FCL	HRAR	CLAR	F.LAT	WSH	WBL	W. Ear	W. Ped	FLW	FLL	Ped. L	PLHT	Out.Gl.P ub	Ear Den	Ear Orint	Ear Sh
GH	1.00																
FCL	0.11	1.00															
HRAR	-0.13	-0.27	1.00														
CLAR	0.43*	-0.02	-0.04	1.00													
FLAT	0.06	0.14	0.41*	-0.29	1.00												
WSH	0.21	0.55**	-0.33	0.45*	-	1.00											
WBL	-0.02	0.02	0.06	0.31	0.24	0.35	1.00										
W. Ear	0.01	0.40	-0.08	0.35	0.01	0.53**	0.54**	1.00									
W. Ped	0.03	0.36	-0.04	0.13	-	0.56**	-0.01	0.35	1.00								
FLW	-0.19	0.19	-0.11	0.04	0.26	0.16	-0.04	0.01	-0.11	1.00							
FLL	-	-0.21	-0.39	-0.17	-	0.09	0.00	0.03	-0.03	0.39	1.00						
Ped. L	0.23	0.54**	-0.18	0.20	0.10	0.43*	-0.24	0.02	0.41*	0.02	-	1.00					
PLHT	-0.31	-0.25	0.68**	-0.21	0.34	-	0.06	0.04	-0.25	0.17	-	-	1.00				
Out.Gl.Pub	0.01	0.00	-0.27	-0.22	-	-0.18	-0.17	-	-0.32	0.20	0.06	-0.11	-0.10	1.00			
Ear Den	0.18	0.51**	-0.30	0.40	-	0.57**	0.01	0.36	0.60**	-0.25	-	0.53**	-	-	1.00		
Ear Orint	-0.25	0.24	-0.21	-0.09	0.04	0.40	0.36	0.17	0.05	0.47**	0.36	-0.07	-0.12	0.27	0.06	1.00	
Ear Sh	0.15	0.00	-0.14	0.11	-	0.31	-0.11	-	0.47**	-0.24	-	0.30	-0.29	-	0.37	-	1.00

*, ** Significant differences at P < 0.05 and P < 0.01, respectively.

polymorphism (0%) was observed with Xgwm33-1A and Xgwm325-6D, whereas the highest polymorphism (100%) was produced by ten SSRs, with 84.35% averaged polymorphism. The polymorphism information content (PIC) values ranged from 0 for Xgwm33-1A and Xgwm325-6D to 0.48 for Xgwm160-4A, with an average of 0.26. The highest MI value (3.96) was obtained for Xgwm95 and the lowest MI value (0.0) was observed in Xgwm33-1A and Xgwm325-6D (Table 7).

Among seven SSR primer pairs represented the A genome, a total number of 61 bands were generated, with an average of 8.71 bands per primer. A high polymorphism (81.97%) was found for the A genome with 50 polymorphic bands with an average of 7.14 per primer. In addition, out of seven SSR primer pairs represented the B genome, a total of 32 DNA bands were detected, with an average of 4.57 per primer. A total of 29 polymorphic bands were obtained (90.63% polymorphism), with an average of 4.14 per primer. Otherwise, out of seven SSR primer pairs represented the D genome, 22 bands were generated, with an

average of 3.14 per primer. Of which, 18 polymorphic bands were obtained (81.82% polymorphism), with an average of 2.57 per primer.

Table 7. Number of total bands, polymorphic bands, PIC, MI and RP for each SSR primer.

Primer	TAB	NPB	POL	PIC	MI	RP
Xgwm33-1A	3.00	0.00	00.00	0.00	0.00	0.00
Xgwm95-2A	11.00	11.00	100.00	0.36	3.96	6.00
Xgwm155-3A	13.00	7.00	53.84	0.39	2.73	6.43
Xgwm160-4A	2.00	2.00	100.00	0.48	0.96	2.63
Xgwm186-5A	11.00	11.00	100.00	0.21	2.31	4.30
Xgwm459-6A	10.00	9.00	90.00	0.28	2.52	4.18
Xgwm63-7A	11.00	10.00	90.09	0.34	3.40	8.00
total	61	50	81.97	-	-	-
Xgwm18-1B	2.00	2.00	100.00	0.28	0.56	3.27
Xgwm111-2B	2.00	1.00	50.00	0.17	0.17	1.81
Xgwm389-3B	4.00	4.00	100.00	0.26	1.04	1.24
Xgwm513-4B	5.00	5.00	100.00	0.31	1.55	1.64
Xgwm408-5B	6.00	5.00	83.33	0.38	1.90	6.90
Xgwm626-6B	7.00	7.00	100.00	0.26	1.82	4.00
Xgwm577-7B	6.00	5.00	83.33	0.19	0.95	3.36
total	32	29	90.63	-	-	-
Xgwm458-1D	3.00	2.00	66.67	0.40	0.80	1.27
Xgwm261-2D	2.00	1.00	50.00	0.17	0.17	0.18
Xgwm3-3D	4.00	3.00	75.00	0.40	1.20	3.45
Xgwm165-4D	1.00	1.00	100.00	0.17	0.17	3.45
Xgwm190-5D	10.00	10.00	100.00	0.26	2.60	3.64
Xgwm325-6D	1.00	0.00	00.00	0.00	0.00	0.00
Xgwm437-7D	1.00	1.00	100.00	0.30	0.30	0.36
total	22	18	81.81	-	-	-
Grand total	115	97	84.35	-	-	-
Average	5.48	4.62	78.57	0.261	1.41	3.25

Total amplified bands (TAB), No. of Polymorphic bands (NPB), % of Polymorphism (POL) Polymorphic information Content (PIC), Marker index (MI), Resolving power (RP)

Based on single marker analysis and among 21 SSR markers analysed, 11 were significantly associated with phenotypic traits (Table 8). Three markers (Xgwm18-1B, Xgwm459-6B and Xgwm160-4A) were associated with Ear Sh, with R² values of 38.81, 51.29 and 37.26%, respectively. Two markers (Xgwm437-7D and Xgwm577-7B) were associated with PLHT (R²= 79.11 and 37.68 %, respectively). Six markers, each of which was associated with a single trait, i.e. Xgwm408-5B with HRAR, Xgwm389-3B with FLW, Xgwm111-2B with W. Ped, Xgwm190-5A with FCL, Xgwm95-2A with Ear Den and Xgwm186-5D with CLAR, with R² values of 46.23, 82.79, 58.09, 62.82, 63.77 and 53.39 %, respectively (Fig 1).

SSR markers analysis showed that four SSR markers generated four specific bands which were able to distinguish durum from bread wheat genotypes. A specific band generated by Xgwm155-3A (120 bp) was detected only in durum wheat genotypes, while three bands generated by Xgwm513-4B (128 bp),

Xgwm458-1D (121 bp) and Xgwm186-5A (298 bp) were present in bread wheat but absent in durum wheat genotypes. Two unique bands generated by Xgwm408-5B and Xgwm458-1D (365 and 232 bp) were present in all durum and bread wheat genotypes but, it was absent only in the durum G1, G2, G3, G4 and G5 Bani Sueif wheat genotype. Two unique bands generated by Xgwm95-2A and Xgwm437-7D (431 and 105 bp) were present in G9 and G10 of bread wheat genotypes but, it was absent in the all durum and bread wheat genotypes. A single specific band generated with Xgwm63-7A primer (510 pb) was present only in G6, G7 and G8 (Sohag) of durum wheat genotypes (Table 9).

The dendrogram constructed based on phenotypic distance matrix obtained using morphological traits showed that all studied cultivars were divided into two main clusters. The first main

Table 8. SSR markers associated with morphological characters based on single marker regression.

Trait	Marker	Specific band (bp)	P value	R ²
Ear Sh	Xgwm160-4A	117	0.046	37.26
	Xgwm459-6A	265	0.013	51.29
	Xgwm18-1B	350	0.041	38.81
HRAR	Xgwm408-5B	111	0.021	46.23
FLW	Xgwm389-3B	122	0.001	82.79
W. Ped	Xgwm111-2B	117	0.006	58.09
FCL	Xgwm190-5D	210	0.004	62.82
Ear Den.	Xgwm95-2A	163	0.047	63.77
PLHT	Xgwm577-7B	944	0.044	37.68
	Xgwm437-7D	105	0.001	79.11
CLAR	Xgwm186-5A	626	0.011	53.39

R², (the coefficient of determination) indicates the percentage of phenotypic variation explained by the marker.

Table 9. Unique DNA bands generated by some SSR markers.

Genotypes	Positive	Negative
Durum wheat	Xgwm155-3A (120 bp)	Xgwm513-4B (128 bp), Xgwm458-1D (121 bp) Xgwm186-5A (298 bp)
Bread	Xgwm513-4B (128 bp), Xgwm458-1D (121 bp) Xgwm186-5A (298 bp)	Xgwm155-3A (120 bp)
G1, G2, G3, G4 and G5 (Bani Sueif)	-	Xgwm408-5B (365bp) Xgwm458-1D (232bp)
G6, G7 and G8 (Sohag)	Xgwm63-7A (510pb)	-
Gemmiza	Xgwm95-7D (431bp) Xgwm437-2A (105bp)	-

cluster contained all durum wheat genotypes except G1 and the seconded main cluster were contained all bread wheat genotypes with G1 (Fig.2). Meanwhile, the dendrogram constructed based on similarity matrix obtained by SSR markers showed that the studied cultivars were grouped into two main clusters. The first main cluster contained G6 and G7 with all bread wheat

genotypes and the seconded main cluster contained G8 with G1, G2, G3, G4 and G5 (Bani Suif) genotypes, which all belong to durum wheat. A significant and positive correlation ($r= 0.25$, $p<0.05$) was found between the matrices obtained by phenotypic data and molecular SSR markers (Fig. 3).

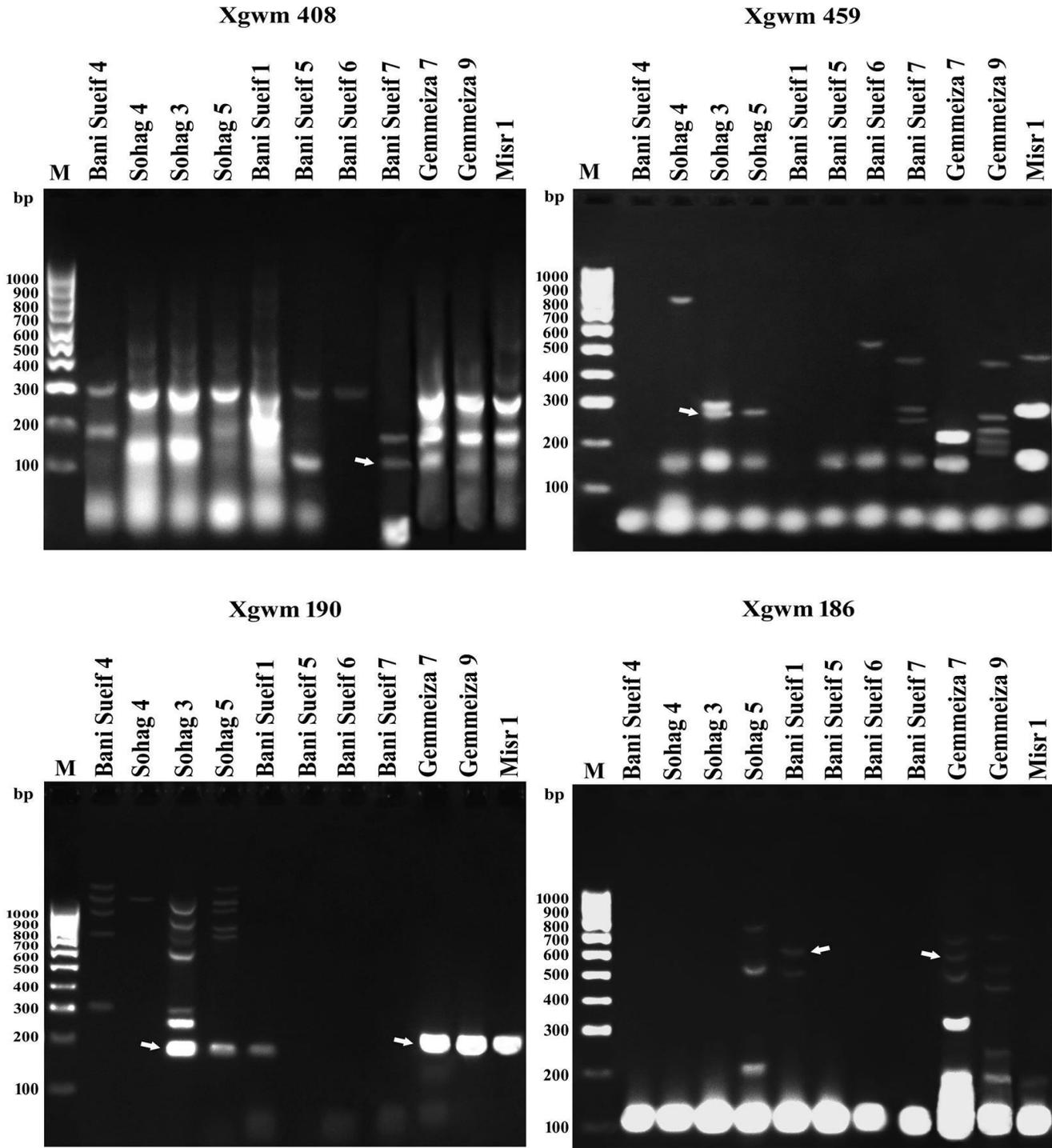


Fig 1. PCR amplification patterns obtained using Xgwm 408, Xgwm 459, Xgwm 190 and Xgwm 186 markers in the studied cultivars. M: A 100bp DNA ladder

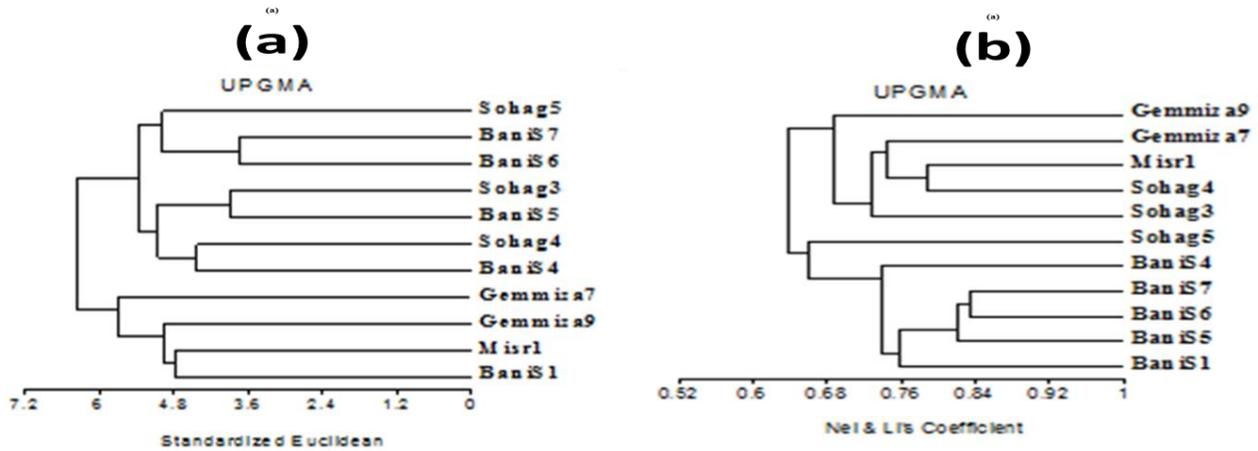


Fig 2. Dendrogram of genetic similarities using SSR data (a), Dendrogram of genetic distance using morphological data (b).

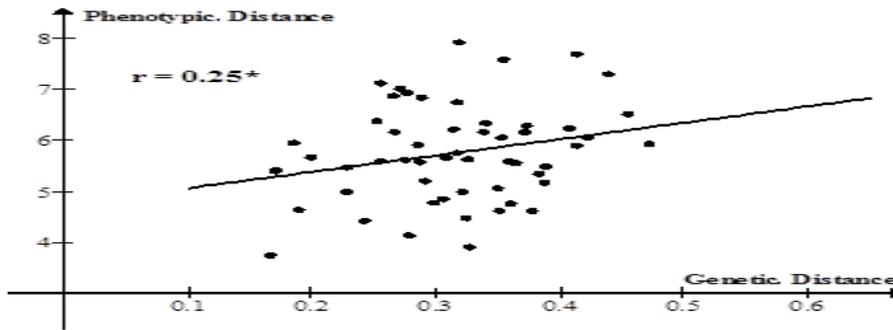


Fig 3. Correlation between genetic and phenotypic distance.

4. Discussion

The present study was carried out to assess the genetic diversity among eight durum and three bread wheat genotypes based on 17 morphological traits and 21 SSR markers. Genetic variation between the local durum and bread wheat cultivars was found in most of the studied morpho-physiological traits and molecular markers. Significant and highly significant differences were obtained among the genotypes for all the traits except FLAT and Out.Gl.Pub. Highly significant differences were obtained between durum and bread wheat genotypes for GH, FCL, HRAR, CLAR, WSH, FLW, Ped. L, PLHT and Ear Den, while nonsignificant differences were observed for FLAT, WBL, W. Ear, FLL, Out.Gl.Pub, Ear Orint and Ear Sh. These agro-morphological features could be useful in exploring and selecting plant material for breeding purposes (Malik *et al.*, 2014). Hassan *et al.* (2016) reported that the grain yield was higher in durum wheat

than bread wheat while the plant height was higher in bread wheat.

Interestingly in the present study, mean values of all wax traits studied in durum wheat were greater in magnitude than those of bread wheat. In this regard, Clarke *et al.* (1993) found an association between visual ratings of glaucousness and epicuticular wax quantity in durum and bread wheat genotypes, and they found that the wax quantity was 44% greater for glaucous than those for nonglauous durum genotypes, and 32% greater for glaucous than for nonglauous bread wheat genotypes. They also found that wax quantity of the flag leaf blade, sheath, and spike was greater for durum than for bread wheat genotypes. Willick *et al.* (2018) observed that greater wax crystal density on the adaxial and abaxial surfaces of the drought-tolerant flag leaves corresponded to higher harvest indices.

Moderate broad-sense heritability estimates were obtained for WSH (0.45), FLW (0.52), FLL

(0.47), Ear Den (0.50) and Ear Sh (0.52), whereas low heritability was found for GH (0.17), FCL (0.29), HRAR (0.14), CLAR (0.14), WBL (0.15), W. Ped (0.25), PLHT (0.23) and Ear Orint (0.17). Moderate to high heritability were recorded for Spike Length (46.03%), flag leaf area (41.39%), 1000-grain weight (58.58%), relative water contents (58.89%) (Ijaz and Smiullah 2013). High heritability and genetic advance estimates were reported for flag leaf area (Saleem *et al.* 2016).

Positive and highly significant correlations were observed between WSH with W. Ear and W. Ped also, between W. Ear and WBL. Positive and highly significant correlations found obtained between FCL with WSH, Ped. L and Ear Den. Similarly, positive and significant correlations were found between HRAR with FLAT and PLHT. These results were in agreement with Feltaous (2019) where all wax characteristics studied in different parts of the plant were positively correlated each other. Significant positive correlations were observed between peduncle wax state with leaf-blade wax, leaf-sheath wax and ear wax by Malik *et al.* (2013). Glaucousness is one of the most eye-catching traits and has been long used as a morphological marker in wheat genetic studies for ~80 years. Its adaptive value in improving crop tolerance to drought and heat was recognized in 1980s (Tsunewaki *et al.* 1999).

Molecular marker analysis showed that the polymorphism obtained using 21 SSR markers ranged from 0 to 100 with an average of 84.35%. The PIC values ranged from 0 to 0.48 with an average of 0.26 per marker. The highest MI value (3.96) was obtained by the Xgwm95. Prasad *et al.* (2000) reported that one to 13 alleles per locus in 55 wheat genotypes with 20 SSR markers, with a PIC value ranging from 0.21 to 0.90, with an of average of 0.68. Mardi *et al.* (2011) indicated that 2 to 10 alleles per locus using 19 SSR markers were found with 122 durum wheat genotypes. These results supported that SSR markers are effective for estimating the genetic diversity as previously reported by several investigations (Hassan 2016).

In the present study, although, the A genome contained the highest mean number of alleles (8.71 per marker) followed by the B genome (4.57 per marker) however, a high polymorphism was found only that in the B genome (90.63%) followed by the A genome (81.97%). In accordance, Chao *et al.* (2007) reported that a high polymorphism among wheat genotypes in the B genome followed by D and A genomes. In addition, microsatellite distribution was abundance on chromosomes of the B genome followed by chromosomes of the A and D genomes (Jaiswal *et al.* 2017). Chen and Li (2007) found that the ranking of

average locus diversity per genome was D>B>A in synthetic hexaploid wheat genotypes also, they reported that the D genome contained the highest mean number of alleles (6.32) followed by A and B genomes (6.13 and 5.94, respectively). The highest mean PIC was recorded in the A genome (0.7540), followed by the D genome (0.7482) and the B genome (0.7361) as reported by Wang *et al.* (2013). In the present study, low number of alleles for the D genome was found due to the low number of used genotypes containing the D genome.

Single marker analysis showed that 11 SSR markers were significantly associated with phenotypic traits. The markers Xgwm18-1B, Xgwm459-6B and Xgwm160-4A, were associated with Ear shape, Xgwm95-2A was associated with Ear Density, and Xgwm437-7D and Xgwm577-7B were found to be associated with PLHT. Sheoran *et al.* (2019) found strong co-localized loci for glume pubescence, spike length, plant height, and awn color located on chromosome 1B in wheat. Wei *et al.* (2010) identified a few stable plant height QTLs on chromosomes 2A, 2B, 2D, 3B, 4B, 5A, 5D, 7B, and 7D. A QTL located on chromosome 3B was associated with increased biomass, grain number and grain weight following heat stress in bread wheat (Thomelin *et al.* 2019). Five SSR markers, each of which was associated with a single trait, i.e. Xgwm408-5B with HRAR, Xgwm389-3B with FLW, Xgwm190-5A with FCL, Xgwm95-2A with Ear. Dens and Xgwm186-5D with Auricles CLAR. Keller *et al.* (1999) identified eight QTLs for flag leaf width on chromosomes 1A, 1B, 2A, 3B, 5A, 5B and 6A, which account for 59.5% of the phenotypic variations. In durum wheat, QTLs for flag leaf angle, length, and width were mapped to chromosomes 2A, 3B, 5B, 7A, and 2B by Isidro *et al.* (2012). In addition, Fan *et al.* (2015) identified 38 additive QTLs for flag leaf width, flag leaf length and flag leaf area on 12 wheat chromosomes, explaining 3.96–27.68 % of the phenotypic variations. Liu *et al.* (2018) found 23 putative QTLs for flag leaf length, width, area, and flag leaf angle on chromosomes 1B, 2B, 3A, 3D, 4B, 5A, 6B, 7B, and 7D. Twenty stable QTLs were identified for flag leaf morphology and could be potentially useful for genetic improvement of drought tolerance in wheat through QTL pyramiding (Yang *et al.* 2016).

In the present study, some of the most important traits in wheat were studied, namely ear waxiness, waxiness of flag leaf sheath, leaf blade waxiness and waxiness of peduncle. In this regard, it has been reported that the wheat leaf, stem and, in some cases, spike surfaces are coated with cuticular waxes,

which confers a glaucousness characteristic (Jensen and Driscoll 1962). The outermost wax layer functions as a barrier between plants and their environment, in defending plants against the biotic and abiotic stresses, such as drought, phytophagous insects, pathogens, solar radiation, and freezing temperatures (Jenks and Ashworth 1999). The wax on wheat leaves and stems is mainly controlled by two sets of genes: glaucousness loci (W1 and W2) and non-glaucousness loci (Iw1 and Iw2) (Ping *et al.* 2015). The wax production genes W1 and W2 contribute while Iw1 and Iw2 inhibit the glaucousness (Bi *et al.* 2017). In the present study, the SSR marker Xgwm111-2B was found to be associated with waxiness of peduncle with R² value of 58.09%. Wu *et al.* (2013) reported that the W1 gene was located on the chromosome arm 2BS between markers Xgwm210 and Xbarc35, Eleven Iw1 and eight Iw2 linked expressed sequence tag (EST) markers were developed and mapped on the distal regions of chromosomes 2BS and 2DS, respectively.

In the present study, a significant with low correlation ($r = 0.25$) was found between the dissimilarity matrix generated from the phenotypic data and that obtained from the molecular data, indicating that the SSR markers were able to bind to effective regions in the genome. However, the SSR markers did not adequately sample the genomic regions that were relevant for the phenotypic differentiation of the studied cultivars. Dendrogram constructed based on similarity matrix obtained from SSR markers, the studied cultivars were clustered into two main clusters. The first main cluster contained all bread wheat genotypes and the second main cluster contained most of the durum wheat genotypes. The same result was obtained from dendrogram constructed based on phenotypic data. These findings supported that SSR markers and morphological traits were found to be useful for the assessment of genetic diversity in wheat. Benin *et al.* (2012) found significant correlations, ranged from low (0.45) to moderate (0.67), between the distance measures based on AFLP markers and hybrid performance in spring wheat. Al-Ashkar *et al.* (2020) found a significant correlation between the morphological and genetic distances ($r = 0.51$, $p < 0.0001$) in wheat under salinity stress conditions. The cluster analysis based on SSR markers showed correlation with the grouping of particular genotypes based on agro-morphological characters (Zarkti *et al.* 2010), suggesting that the characterization based on agro-morphological traits and SSR markers will be a useful tool to the breeders to choose genotypes with appropriate. Very weak correlations between morphologic and molecular data were also reported by

Cupic *et al.* (2009). No correlations between phenotypic and molecular data were found Petrovic *et al.* (2017), implying that both types of data should be used for genetic diversity estimates in order to cover wider variability between tested cultivars.

In conclusion, the phenotypic data and molecular markers were effective in estimating the genetic variability between wheat cultivars. The study indicated the presence of abundant genetic variability among some of the important Egyptian cultivars. Significant positive correlation found between the phenotypic and genotypic distance indicated that SSR markers were able to bind to effective regions in the genome. Single marker analysis revealed that eleven markers were associated with phenotypic traits which can be useful for markers-assisted breeding in the tested wheat genotypes. However, additional markers analyses are still required to validate their effectiveness in wheat breeding programs.

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الملخص العربي

تقييم التنوع الوراثي لبعض أصناف القمح المصري بناءً على الخصائص المورفولوجية والواسمات الجزيئية (SSR marker)

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يمكن أن تكون تقديرات التنوع الوراثي مفيدة في اختيار الطرز الجينية المهمة لمربي النباتات. في هذه الدراسة تم استخدام ثمانية أصناف من قمح المكرونة وثلاثة أصناف من قمح الخبز لدراسة ومقارنة الصفات المورفولوجية مع الدراسة الجزيئية باستخدام تقديرات التنوع الجيني المعتمدة على تقنية SSR marker. لوحظت فروق معنوية وعالية المعنوية بين الطرز الوراثية للقمح لجميع الصفات باستثناء وضع ورقة العلم وظهور الزغب الخارجي للعصافات. تم الحصول على فروق معنوية عالية بين الطرز الوراثية لقمح المكرونة و قمح الخبز لمعظم الصفات المدروسة. تراوحت تقديرات المكافئ الوراثي بالمعنى الواسع بين منخفضة الي متوسطة لجميع الصفات المورفولوجية المدروسة. تم العثور على ارتباط موجب وعالي المعنوية بين إكتناظ السنبله مع كل من لون واخضرار الاوراق ($r = 0.51$) والشمع الموجود علي نصل ورقة العلم ($r = 0.57$) والشمع الموجود علي السويقة ($r = 0.60$) وطول السويقة ($r = 0.53$) ، بينما تم العثور على ارتباط سالب بين إكتناظ السنبله وطول النبات ($r = -0.52$ ، $P < 0.01$). بالاضافة الي ذلك ، ارتبطت شمعة نصل ورقة العلم ارتباطاً إيجابياً بالشمع الوجود علي السنبله ($r = 0.53$ ، $P < 0.01$) وشمع السويقة ($r = 0.56$ ، $P < 0.01$) وطول السويقة ($r = 0.43$ ، $P < 0.05$). تم العثور على تعدد الأشكال المظهرية الأعلى (٩٠,٦٣%) في الجينوم B مقارنة بالجينوم A الذي بلغ (81.97%). أظهر تحليل الوسمات الجزيئية المفردة أن ١١ باديء من معلمات SSR كانت مرتبطة ارتباطاً وثيقاً بالسمات المظهرية ، بما في ذلك Xgwm111-2B الذي ارتبط بالشمع الموجود علي السويقة. تم العثور على ارتباط مهم ومعنوي ولكن منخفض ($r = 0.25$) بين مصفوفة الاختلافات الناتجة عن بيانات الصفات المظهرية وتلك التي تم الحصول عليها من مصفوفة معلمات SSR ، مما يشير إلى أن التوصيف القائم على الصفات المورفولوجية ومعلمات SSR ستكون أداة مفيدة للمربين لاختيار الأنماط الجينية المناسبة.