SVU-International Journal of Agricultural Sciences
Volume 6 Issue (3) pp.: 155-171, 2024
Print ISSN 2636-3801 | Online ISSN 2636-381X
Doi: 10.21608/svuijas.2024.306534.1382
RESEARCH ARTICLE

Morphological and molecular characterization of some bread wheat (*Tritium aestivum* L.) genotypes

Fouad, H.M.[1], Hanaa S. Bakry [2] and H.A. Soltan [3]*

[1] *Agronomy Department, Faculty of Agriculture, Minia University, 61519 Minia, Egypt.*
[2] *Department of Genetics, Faculty of Agriculture, Minia University, 61519 Minia, Egypt.*
[3] *Central lab. of Organic Agriculture, Agricultural Research Center, 12619 Giza, Egypt.*

Abstract

This investigation was conducted at the Experimental Farm of Agronomy Department and Genetics Department, Faculty of Agriculture, Minia University during two seasons 2021/22 and 2022/23 to characterize 12 Egyptian bread wheat genotypes by morphological and molecular markers. The relative phenotypic diversity index was higher than 0.60 for all the 19 morphological traits**.** Cluster analysis classified the 12 genotypes based on 19 traits into 5 clusters. Cluster 1 comprised six lines of 2, 8, 13, 15, 23 and 34 and their two parents of Sids 4 and Giza 168. Cluster 2, 3, 4 and 5 each of composed one genotype; line 4, line 17, line 31 and Giza 171, respectively. The percentages of polymorphism among nine ISSR primers ranged from 20 to 100% with an overall mean of 75.12±10.88%. Ten unique bands were produced by four ISSR primers (HB, HB08, HB10 and 812). These primers were the highest and a more successful in proofing identity of the bread wheat genotypes. It produced (3, 2, 3 and 2 unique bands, respectively) then it can be used as a marker to distinguish among them. The dendrogram revealed that the ISSR markers were a successful tool in differentiating amongst the 12 bread wheat genotypes due to their genetic background. Finally, these results showed that both of morphological and ISSR markers could be used as important tools for characterizing the studied Egyptian bread wheat genotypes. It might also provide important information that helps breeders to select the right individuals in plant breeding programs.

Keywords: Bread wheat; morphological traits; phenotypic diversity index; ISSRs; cluster analysis.

1. Introduction

Wheat is a strategic cereal crop around the world. It occupies the largest area in the world at 220.61 million hectares representing 15.4% of the total arable land. It comes after corn in production with 789.5 million tons. Egypt cultivated wheat in 1.45 million hectares produced 9.5 million tons while the wheat consumption about 20.00 million tons. According to USDA 2023, the percentage of self-sufficiency was 47.50% and the gap between production and consumption was about 10.50 million tons during the two seasons 2022/2023.

Wheat provides carbohydrates and calories. Wheat contains proteins and nearly 1.2 billion people in the developing countries depend on wheat for protein. The demand of wheat will increase by 60% by 2050. Nonetheless, it is cultivated widely in all countries of the world where adapted to different climatic conditions which suitable for its production and unique property of wheat flour to make a large range of products (Mateo-Sagasta *et al*., 2018; Guin *et al*., 2019).

Genetic purity of wheat cultivar is one of the quality traits required for successful seed production. The introduction of rights of plant breeder resulted in exacting requirements for

***Corresponding author: Hassan A. Soltan**
Email: soloo2525@gmail.com
Received: July 23, 2024; Accepted: September 22, 2024;
Published online: September 27, 2024.

distinctness testing in seed certification of genotypes (Cooke, 1999). This goal could be achieved through using stable international method to identify morphological traits at different growth stages. UPOV use the international descriptors to differentiate among the tested wheat genotypes. It's very important using morphological and agronomic traits for classifying wheat genotypes and studying genetic variability. Hence, wheat breeders using characterization and classification to improve new germplasm (Najaphy *et al*., 2012). New germplasm considered essential source for different desirable genes to improve cultivars of wheat (Ahmadi *et al*., 2012; Mansour *et al*., 2018). Since the parental cultivars with extended genetic variation can crossed together to produce a crosses can exploited in breeding program by selection to improve yield and yield attributes to enhance food production (Sajjad *et al.,* 2018; Abaza *et al.,* 2020; Park *et al.,* 2021; Erdinc *et al.,* 2021).

Agro-morphological characteristics that using for investigating genetic variability of bread wheat germplasm also using to perform the tests of distinctness, uniformity and stability (DUS) presented in the guidelines of the International Union for Protection of New Varieties of Plants (UPOV). DUS tests are routinely carried out during the official process of new plant varieties registration to identify plant varieties and protect intellectual property rights for plant breeders (Rukavina *et al*., 2017; Petrović *et al*., 2017).

Molecular markers have been commonly employed to determine the similarity and purity of different cultivars and estimate the genetic diversity of various crops. Due to, it is unaffected by environmental conditions, and DNA can be examined at any stage of plant growth (Abd El-Moneim *et al.,* 2021).

Molecular markers are known as genetic loci that are easily detected and seen within a population and might be associated with a significant gene or characteristic (Nadeem *et al.,* 2018). ISSRs are as semi-random markers that participate in PCR amplification with a single primer that is complementary to a microsatellite target (Abd El-Moneim, 2020). It is a ubiquitous, reliable, stable, and repeatable technique for assessing genetic diversity among different genotypes of numerous plant species, including *Triticum aestivum* L. (Etminan *et al.,* 2016; Henareh *et al.,* 2016; Bekhit and Salim, 2019; Nosair, 2020; Shaban *et al.,* 2022; Mesfer *et al.,* 2022; Abouseada *et al.,* 2023). This study was conducted to characterize 12 Egyptian bread wheat genotypes based on morphological and molecular attributes.

2. Materials and methods

2.1. Morphological studies

The current study was carried out in the Experimental Farm of Agronomy Department, Faculty of Agriculture, Minia University during two seasons, 2021/2022 and 2022/2023. Twelve bread wheat genotypes included nine recombinant inbreed lines (RILS); L2, L4, L8, L13, L15, L17, L23, L31, L34, their two parents and the check cultivar were used for morphological characterization. Pedigree of the two parental and check cultivar are presented in Table 1.

Table 1. Pedigree of parents and the check bread wheat cultivar.

Cultivar	Giza168 (female)	Sids4 (male)	Giza 171
Pedigree	MIL/BUC// Seri	Maya (S) /Man (S)	Gemmeiza9 / Sakha93

The 12 genotypes were sown in 17th and 20th November of the two seasons 2021/2022 and 2022/2023. A randomized complete blocks design with three replicates was used. The experimental plot was three rows 2 m long, 20 cm apart and 5 cm between plants within row. Nineteen agro-morphological characters were recorded using scales as reported by test

guidelines international Union for the Protection of new Varieties of plants **(**UPOV, 2017) to conduct tests of distinctness, uniformity and stability (DUS) of bread wheat. Ten plants per replicate were taken to record the character then expressed as scales as follows: Seven characters i.e. Days to 50% heading DH was determined as number of days from planting to date of protrude 2 cm from awns of 50% of plants. Plant height PH in cm was registered as distance from soil surface to base of main spike. Spike length SL in cm measured as distance from base to tip of spike. Awn length AL in cm. Beak length of lower glume BLLG in mm. Shoulder width of lower glume SWLG in mm. Spike density SD determined as ratio number of spikelets / spike length.

Analysis of variance of the studied traits was performed using MSTAT-C software. The first seven characters were expressed as five scores; 1, 3, 5, 7 and 9 of their description according to their ranges as in Table 2. The remained characters were registered in different growing stages and given same five scores; 1, 3, 5, 7 and 9 to generate the numerical data set according to the selected descriptor of each character Table 2 and 19 agro-morphological traits were analyzed using the Shannon–Weaver diversity index (H) (Shannon and Weaver, 1949) to calculate phenotypic variation of each trait as follows: $H = -\sum_i^n pi \ln pi$, where *pi* is the genotypes frequency belonging to the i^{th} class, n is the number of phenotypic classes for each trait. This index was standardized by dividing it on *H max = ln* (*n*) to estimate the relative phenotypic diversity index H', H' = H / *H max* using PAST 4.03 software (Hammer, 2001).

Multivariate analysis of the morphological characters performed by analyses of principal component and cluster using PAST 4.03 software on average the two growing seasons of standardization data. Cluster analysis was carried out based on dissimilarity of Euclidean distance by unweighted pair-group method with an arithmetic average UPGMA.

2.2. Molecular studies

Molecular studies were conducted at Molecular Genetics Laboratory, Genetics Department, Faculty of Agriculture, Minia University, Egypt.

2.2.1. Genomic DNA isolation

Total genomic DNA was extracted from 200 mg of the young shoot leaves of twelve bread wheat genotypes as mentioned above. Samples were grinded in liquid nitrogen to fine powder then DNA was extracted using Cornel extraction buffer (500 mM NaCl, 100 mM Tris-HCl, PH 8.0, 50 mM EDTA and 0.84 % SDS). The resuspended DNA was verified with 1% agarose gel electrophoresis. Concentration and purity of extracted DNA were determined by a Nano-spectrophotometer in the Central Lab, Faculty of Agriculture, Minia University.

2.2.2. PCR condition for ISSR analysis

Nine ISSR primers (Table 3) were used to determine the genetic diversity among twelve bread wheat genotypes. PCR amplifications were performed in 25µl reaction volume containing: 12.5µl PCR Master mix, 2µl primer, 5.5µl double distilled water and 5µl of genomic DNA (con.5Nanogram/1µl).

The PCR reactions were performed in a Multigene thermal cycler with one cycle for 4 min at 94°C, 40 cycles for 30 s at 94°C, 45s at 55°C, and for for 2 min at 72°C, followed by a final extension stage for 7 min at 72°C. The PCR products loaded onto 1.5 % agarose gel electrophoresis. Electrophorized DNA samples were stained using Ethidium bromide stain (0.1g Ethidium bromide dissolved in 10 ml 1X TAE buffer). DNA fragment sizes were estimated according to the standard marker of 100-2000 bp ladder resolved in the same gel. Photography was done by using Gel Doc. (GBOX 230V).

Table 2. The numerical scores of the agro-morphological characters based on range and/or description.

Character	Score	1	3	5	7	9
Days of 50% heading DH	Range	≤72.10	72.20-80.30	80.30-88.50	88.50-96.70	≥96.80
	Description	very early	early	medium	late	very late
Plant height in cm PH	Range	≤87.10	87.20-95.30	95.40-103.50	103.60-111.70	≥111.80
	Description	very short	short	medium	long	very long
Spike length in cm SL	Range	≤9.00	10-11	12-13	14-15	≥16
	Description	very short	short	medium	long	very long
Awn length in cm AL	Range	≤3.20	3.30-6.40	6.50-9.60	9.70-12.8	≥12.9
	Description	very short	short	medium	long	very long
Beak length of lower glume in mm BLLG	Range	≤4.7	4.8-6.1	6.2-7.5	7.6-8.9	≥9
	Description	very short	short	medium	long	very long
Shoulder width of lower glume in mm SWLG	Range	≤4.1	4.2-5.70	5.80-7.30	7.40-8.99	≥9
	Description	absent	narrow	medium	broad	very broad
Spike density SD	Range	≤1.7	1.71-1.96	1.97-2.3	2.24-2.49	≥2.50
	Description	very lax	lax	medium	dense	very dense
Growth habit GH	Description	erect	semi erect	intermediate	semi prostrate	prostrate
Frequency of plants with recurved flag leaves FPRFL	Description	absent	low	medium	high	very high
Glaucosity of sheath GS	Description	absent	weak	medium	strong	very strong
Glaucosity of blade GB	Description	absent	weak	medium	strong	very strong
Glaucosity of ear GE	Description	absent	weak	medium	strong	very strong
Glaucosity of neck GN	Description	absent	weak	medium	strong	very strong
Spike shape in profile SSP	Description	tapering	parallel sided	slightly clavate	strongly clavate	fusiform
Area of hairiness on convex surface of apical rachis AHCS	Description	absent	small	medium	large	very large
Shoulder shape of lower glume SSLG	Description	strongly sloping	slightly sloping	horizontal	slightly elevated	strongly elevated
Beak shape of lower glume BSLG	Description	straight	slightly curved	moderately curved	strongly curved	geniculate
Area of hairiness on internal surface of lower glume AHISLG	Description	very small	medium	very large	-	-
Hairiness on external surface of lower glume HESLG	Description	absent	-	-	-	present

Table 3. The nucleotide sequence of the ISSR primers used for specific-PCR analysis

Primer	Name	Nucleotide sequence 5'→3'	Repeat	Nucleotide Numbers
1	HB	5'-CACACACACACAAC -3'	6(CA)AC	14
2	HB08	5'-GAGAGAGAGAGAGG -3'	6(GA)GG	14
3	HB10	5'-GAGAGAGAGAGACC -3'	6(GA)CC	14
4	HB12	5'-CCACCACCAGC-3'	3(CCA)GC	11
5	HB15	5'-GTGGTGGTGGC-3'	3(GTG)GC	11
6	807	5'-AGAGAGAGAGAGAGAGT-3'	8(AG)T	17
7	810	5'-GAGAGAGAGAGAGAGAT-3'	8(GA)T	17
8	812	5'-GAGAGAGAGAGAGAGAA-3'	8(GA)A	17
9	817	5'-CACACACACACACACAA-3'	8(CA)A	17

2.3. Statistical analysis

Gel images detected via PCR-based methods were analyzed using the free software GelAnalyzer3 which is available free on the internet at http://www.geocities.com/egygene (GelAnalyzer Version three, 2007). Molecular sizes of the amplified fragments, its presence (1) or absence (0) through samples, their frequencies through samples, and their polymorphism type either monomorphic or polymorphic as well as the mean of band frequency and the polymorphism percentage for each primer were determined. Data of the similarity matrix were used for cluster analysis by using the software SPSS Ver. 1

3. Results and discussion

Analysis of variance in Table 4 showed significant (P≤0.01) differences of genotypes for all studied characters. Referring, presence genetic variability among the 12 genotypes. Genotype-year interaction variance was insignificant for all studied characters. Indicating these traits were little interacted with year this may be attributed to high uniformity of the genotypes. Feltaous, (2019) showed significant differences between cultivars in most of studied traits. The variance of genotypes x years interaction was significant in some characters and insignificant in case of ear density. Almarri *et al.*, (2023) and Marzario *et al.*, (2023) found significant (P≤0.01) differences between genotypes for most agro-morphological traits. Table (5) showed scores the 19 agro-morphological traits according to their description of the genotypes over two years. Days to 50% heading DH was very early in Sids 4, early for four lines (2, 4, 15, 23), medium early for lines (13, 17, 31, 34) and late in other remained genotypes. For plant height PH, 5 genotypes were short, 6 genotypes were medium and Sids 4 was tallest plant. Concerning spike length SL, the shortest genotype was line 17, while line 34 recorded very long spike. Moreover, 3 genotypes (lines 2, 4 and Giza 171) were medium spike length. The remained genotypes which were long spike. In respect of awn length AL, lines 8, 17, 23, 31 and Sids 4 were short awn, while three genotypes line 4, Giza 168 and Giza 171 were medium awn length, lines 2, 15 and 34 were long awn. Meanwhile, the line 13 recorded the very long awn. Regarding beak length of lower glume BLLG, lines (13, 31, 34 and Sids 4) were short, while, line 2 and Giza 168 were medium peak length.

Moreover, the long peak length recorded for lines 8, 17 and Giza 171. Additionally, three lines 4, 15 and 23 which were very long peak length. For shoulder width of lower glume SWLG, the two lines 17, 31 and Giza 168 were narrow; four lines 4, 13, 15, 34 and Sids 4 were medium shoulder width, while two lines 2 and 23 were broad shoulder. Meanwhile, the very broad shoulder recorded for line 8 and Giza 171. Respecting

spike density SD, five lines 8, 13, 15, 23, 34, Sids 4 and Giza 168 which were lax spike, while the remained five genotypes were dense spike. SD is important morphological trait correlated to grain yield. The wheat breeders selected genotypes with long and compact spikes to increase grains spike^{-1} consequently grain yield (Liu *et al.*, 2020). For growth habit GH, the two parents Sids 4 and Giza 168 were erect, while five lines 2, 8, 13, 17 and 34 were semi erect. Moreover, Giza 171 and three lines 4, 15 and 23 were recorded intermediate growth habit. Only line 31 was semi-prostrate. Regarding frequency of plants with recurved flag leaves FPRFL, two lines 4 and 17 were absent, three lines 2, 13 and 31 were low in FPRFL, three lines 8, 23, 34 and Sids 4 were medium. Meanwhile, line 15 and Giza 168 and Giza 171 were high FPRFL. For glaucosity of sheath GS, only one line 31 was weak, 4 lines 4, 8, 17 and 34 were medium GS, three lines 13, 15, 23, two parent and check cultivars were strong GS. While, line 2 recorded very strong GS. Gaucosity of blade GB behaved the same trend of glaucosity of sheath with exception line 8 showed strong GB. Glaucosity of ear GE, two line 4 and 17 were weak, one line 31 was medium GE, line 15 was strong, while the remained genotypes which were very strong GE. Five genotypes lines 8, 17, 23, Giza 168 and Giza 171 recorded the same score of glaucosity of ear and neck ranged from weak for line 17 to very strong for four remained genotypes. Line 4 was absent GN, moreover, lines 13, 17, 31, 34 and Sids 4 which were weak GN. The traits of glaucosity of blade, sheath, neck and spike were correlated with abiotic stress tolerance as drought, heat to improve yield under stress conditions (Würschum *et al.*, 2020) so it decrease permeability of cuticle, water loss, temperature and reflects sun radiation (Gharib *et al*., 2021).

Table 4. Mean squares of the seven traits for the genotypes over two years.

S.V.	Year	Rep/Y	Genotypes/Y	Genotypes	G x Y	Error/Y
d.f.	1	4	22	11	11	44
DH	0.22	1.89	10.6**	20.53**	0.71	3.28
PH	0.22	1.89	6.77**	12.83**	0.71	2.19
SL	4.01	9.85	6.91**	13.32**	0.50	2.42
AL	1.39	2.89	13.39**	25.87**	0.90	1.80
BLLG	1.39	2.44	12.52**	24.62**	0.42	1.84
SWLG	0.22	4.11	13.23**	25.64**	0.83	2.60
SD	0.89	3.56	12.04**	23.56**	0.53	3.19
GH	1.13	2.79	9.56**	18.43**	0.70	2.64
FPRFL	0.89	2.22	19.34**	38.04**	0.65	2.10
GS	3.56	2.56	9.25**	18.34**	0.16	1.77
GB	0.00	3.78	7.26**	13.68**	0.85	1.96
GE	3.56	2.89	14.13**	27.98**	0.28	1.49
GN	0.89	2.89	24.65**	49.01**	0.28	1.74
SSP	0.06	0.39	9.99**	19.09**	0.90	1.24
AHCS	0.00	7.00	7.79**	14.48**	1.09	2.52
SSLG	0.89	3.06	9.77**	18.77**	0.77	2.21
BSLG	0.50	2.33	7.11**	13.11**	1.11	3.30
AHISLG	1.39	2.22	5.27**	9.87**	0.66	2.65
HESLG	0.06	1.06	17.69**	32.4**	2.96	1.90

DH days of 50% heading, PH plant height, SL spike length, AL Awn length, BLLG Beak length of lower glume, SWLG shoulder width of lower glume, SD spike density, GH growth habit, FPRFL frequency of plants with recurved flag leaves, GS glaucosity of sheath, GB glaucosity of blade, GE glaucosity of ear, GN glaucosity of neck, SPP spike shape in profile, AHCS area of hairiness on convex surface of apical rachis, SSLG shoulder shape of lower glume, BSLG beak shape of lower glume, AHISLG area of hairiness on internal surface of lower glume, HESLG hairiness on external surface of lower glume.

Table 5. The numerical scores of agro-morphological traits of the studied genotypes.

Trait\line	L2	L4	L8	L13	L15	L17	L23	L31	L34	S4	G168	G171
DH	3	3	7	5	3	5	3	5	5	1	7	7
PH	3	3	3	3	5	5	3	5	5	7	5	5
SL	5	5	7	7	7	3	7	7	9	7	7	5
AL	7	5	3	9	7	3	3	3	7	3	5	5
BLLG	5	9	7	3	9	7	9	3	3	3	5	7
SWLG	7	5	9	5	5	3	7	3	5	5	3	9
SD	7	7	3	3	3	7	3	7	3	3	3	7
GH	3	5	3	3	5	3	5	7	3	1	1	5
FPRFL	3	1	5	3	9	1	5	3	5	5	9	9
GS	9	5	5	7	7	5	7	3	5	7	7	7
GB	9	5	7	7	7	5	7	3	5	7	7	7
GE	9	3	9	9	7	3	9	5	9	9	9	9
GN	5	1	9	3	5	3	9	3	3	3	9	9
SSP	5	1	1	1	5	5	1	5	1	1	5	5
AHCS	7	5	5	5	3	5	5	3	5	5	3	1
SSLG	3	3	7	3	3	3	7	3	3	1	1	5
BSLG	3	1	5	3	3	5	5	7	3	3	3	5
AHISLG	1	3	3	5	1	5	1	1	5	1	5	1
HESLG	9	1	9	9	9	9	9	9	9	9	9	1

L: line, S4:Sids4, G168:Giza168, G171:Giza171, DH days of 50% heading, PH plant height, SL spike length, AL Awn length, BLLG Beak length of lower glume, SWLG shoulder width of lower glume, SD spike density, GH growth habit, FPRFL frequency of plants with recurved flag leaves, GS glaucosity of sheath, GB glaucosity of blade, GE glaucosity of ear, GN glaucosity of neck, SPP spike shape in profile, AHCS area of hairiness on convex surface of apical rachis, SSLG shoulder shape of lower glume, BSLG beak shape of lower glume, AHISLG area of hairiness on internal surface of lower glume, HESLG hairiness on external surface of lower glume.

Respecting spike shape in profile SSP, the tapering shape was recorded for lines 4, 8, 13, 23, 34 and Sids 4, meanwhile, slightly clavate shape was shown for the remained six genotypes. For area of hairiness on convex surface of apical rachis AHCS, graded from absent for Giza 171, to small AHCS for lines 15, 31 and Giza 168, to medium AHCS for lines 4, 8, 13, 17, 23, 34 and Sids 4 to large AHCS for line 2. For shoulder shape of lower glume SSLG, two parents Sids 4 and Giza 168 were strongly sloping, seven lines 2, 4, 13, 15, 17, 31 and 34 which were slightly sloping, two lines 8 and 23 were slightly elevated. For beak shape of lower glume BSLG, line 4 showed straight beak, lines 2, 13, 15, 34, Sids 4, Giza 168 were showed slightly curved beak. While, moderately curved beak recorded for lines 8, 17, 23 and Giza 171 moreover, strongly curved beak for line 31. Concerning area of hairiness on internal surface of lower glume AHISLG, six genotypes included lines 2, 15, 23, 31, Sids 4 and Giza 171 which were very small. Two lines 4 and 8 were medium while remained genotypes showed very large area. For hairiness on external surface of lower glume HESLG, all genotypes showed present of HESLG except line 4 and Giza 171 showed absent in this trait.

3.1. Principle components analysis PCA

Traits which cause maximum variation can be known by PCA analysis. Hence, PCA abbreviate a many of variables to a little of variables (traits) caused maximum variation. PCA of the nineteen agro-morphological characters of the twelve bread wheat genotypes were shown in Table 6. PCA extracted 6 PCs had eigenvalues higher than unity which caused 88.41% of the total variation. The first two principle components caused maximum variation by 42.95% include PC_1 (22.43%) and PC_2 (20.52%), followed by PC_3

(16.71%), PC_4 (11.66%), PC_5 (9.71%) and PC_6 (7.39%). Factor loading values of traits indicate its contribution in variation, where highest absolute factor loading value close to unity of traits refers to high contribution in variability of the PC (Fouad, 2020). Hence, the traits contributing in variation of PC_1 were SL (0.61), SD (-0.73), FPRFL (0.60), GS (0.74), GB (0.73) and GE (0.79) (Table 6).

Similarly, in PC_2 the major traits contributing were BLLG (0.60), SWLG (0.54), GH (0.61), GN (0.75), SSLG (0.84), BSLG (0.54) and AHISLG (0.63). Three traits DH (0.60), PH (0.75) and AHCS (0.64) caused the major contribution in variation of PC_3. The three remained traits SSP (0.76), HESLG (0.55) and AL caused maximum variation in PC_4, PC_5 and PC_6, respectively. The traits contributed to the genotypes distinction were beak length of the lower glume, shape of lower glume and length ear of awns (Takac *et al.*, (2019). The relative diversity index (H') reaches its minimum value, which is zero for monomorphic characters. Moreover, the value of this index increases with the degree of polymorphism and reaches a maximum value (1) when all the phenotypic classes present in equal frequencies.

Table 6. Principal component analysis and relative phenotypic diversity index H' for the studied traits.

Character	PC1	PC2	PC3	PC4	PC5	PC6	H'
DH	-0.13	0.07	0.60	0.25	0.42	0.60	0.93
PH	0.03	-0.15	0.75	-0.01	-0.40	-0.18	0.84
SL	0.61	-0.03	0.31	-0.45	-0.34	0.22	0.78
AL	0.41	-0.34	-0.17	0.45	-0.28	0.53	0.91
BLLG	-0.12	0.60	-0.58	0.26	0.10	-0.05	0.98
SWLG	0.51	0.54	-0.34	-0.31	-0.04	0.28	0.94
SD	-0.73	0.16	-0.25	0.35	-0.20	0.11	0.98
GH	-0.42	0.61	-0.01	-0.13	-0.51	0.04	0.89
FPRFL	0.60	0.47	0.46	0.16	-0.22	-0.14	0.98
GS	0.74	-0.02	-0.38	0.46	-0.02	-0.21	0.81
GB	0.73	0.06	-0.51	0.38	0.13	-0.08	0.78
GE	0.79	0.20	0.48	-0.14	0.02	-0.05	0.71
GN	0.44	0.75	0.16	0.27	0.27	-0.08	0.89
SSP	-0.29	0.19	0.29	0.76	-0.03	-0.29	0.99
AHCS	0.10	-0.53	-0.64	-0.40	0.25	-0.01	0.78
SSLG	0.13	0.84	-0.08	-0.27	0.36	0.20	0.81
BSLG	-0.54	0.54	0.29	-0.12	0.50	0.02	0.81
AHISLG	0.13	-0.63	0.27	0.36	0.34	0.39	0.92
HESLG	0.21	-0.42	0.29	-0.15	0.55	-0.48	0.65
Eigenvalues	4.26	3.90	3.18	2.22	1.85	1.40	-
Variance %	22.43	20.52	16.71	11.66	9.71	7.39	-
Cumulative %	22.43	42.95	59.66	71.32	81.03	88.41	-

The relative phenotypic diversity index (H') is shown in Table 6. According to classification Eticha *et al.* (2005) for the diversity index to three classes; high $H' \geq 0.60$, medium $0.40 \leq H' \leq 0.60$ and low $H' \leq 0.40$. All the 19 agro-morphological traits were high polymorphism. Belhadj *et al.* (2015) and Marzario *et al.*, (2023) found high levels of phenotypic diversity of the most studied in UPOV descriptors in both the environments. Attia *et al.* (2015) revealed that morphological traits could be used in characterization the genetic diversity in bread wheat genotypes.

The factor loadings for 19 traits of these first two PCs explained 42.95% of the total variations were plotted on Fig. 1 to display the relationship between the 12 genotypes and their traits. The vectors of trait revealed angles between studied traits, angles < 90° refer to a positive correlation, while angles > 90° refer to a negative correlation. Further, angles near zero° and 180° indicated to

high correlation intensity. Moreover, length of character vector refers to the range of variation caused by this character in PC (Boshev *et al.*, 2016). Accordingly, the studied traits could be classified into 4 groups with positive correlation among them. The first group included SD, SSP, DH, BSLG, GH and BLLG. The second group included SSLG solely and represented the highest vector in length and responsible maximum variation in PC_2. The third group consisted GN, SWLG, FPRFL, GE, GB, GS and SL. The fourth group included AL, PH, HESLG, AHISLG and AHCS. Strongest positive correlations were revealed by acute angles among traits (SD, DH, SSP, BSLG and GH), (SSLG, GN, SWLG and FPRFL), (GE, GB, GS and SL) and (AHCS, PH, AHISLG, HESLG and AL) (Fig. 1).

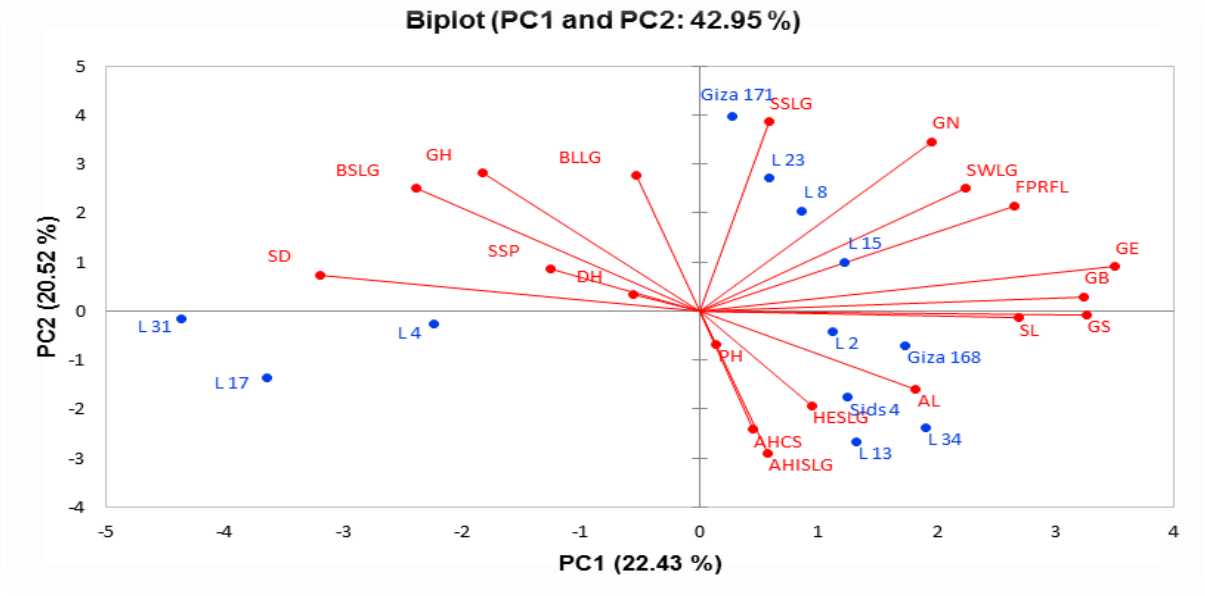

Figure 1. Biplot of PC_1 and PC_2 representing correlation between the 12 genotypes and traits.

Location of the genotype is distance it from the biplot origin which refer to differ the genotype from a "average" genotype located at the biplot origin that has an average level for all traits Yan and Fregeau (2008). Consequently, long vectors of the three lines 23, 31 and 34 indicated they possess high values for one or more studied traits. Furthermore, lines 23 and 34 are considered superior, where located in place with high positive values nearly for all studied traits (Fig. 1).

3.2. Genotypes classification based on morphological traits

Hierarchical clustering analysis classified the 12 genotypes into five clusters (**Fig. 2**). Cluster 1 comprised six lines of 2, 8, 13, 15, 23 and 34 and their two parents of Sids 4 and Giza 168. Cluster 1 characterized by long spike, semi erect growth habit, strong glaucosity for each of sheath, blade, spike, parallel sided shape of spike, medium area of hairiness on each of convex surface of apical rachis and on internal surface of lower glume, present hairiness on external surface of lower glume. Cluster 2, 3, 4 and 5 each of composed one genotype; line 4, line 17, line 31 and Giza 171, respectively. Petrovic *et al.*, (2017) revealed cluster analysis for phenotypic data portioned cultivars in four groups, 1st group contain one cultivar, 2nd group comprised one cultivar, 3rd group contains two cultivars and 4th group divided into sub-clusters the 1st one (five cultivars) and the 2nd one (36 cultivars).

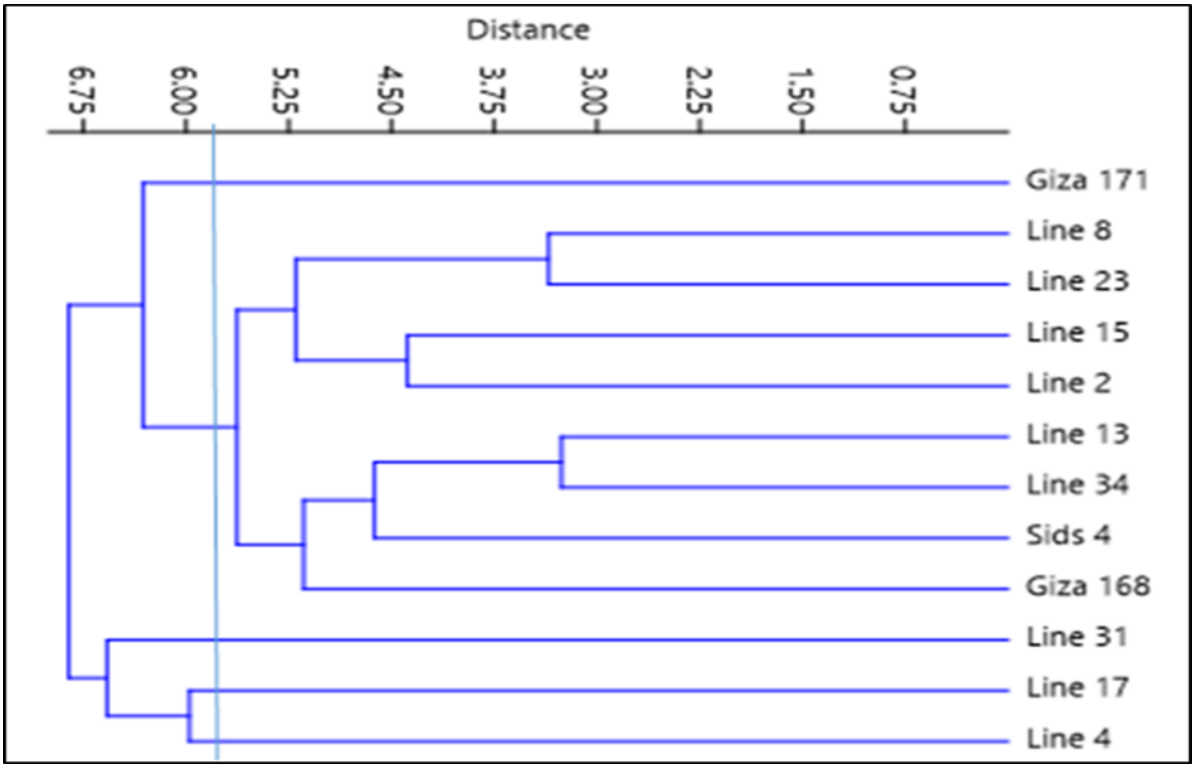

Figure 2. Dendrogram of the distances among 12 wheat genotypes based on morphological traits.

3.3. ISSR ANALYSIS

Nine ISSR primers were used to examine the genetic variability among twelve Egyptian wheat genotypes which include, two parental genotypes (Sids-4 and Giza-168), nine of their offspring's (lines 2, 4, 8, 13, 15, 17, 23, 31 and 34) and Giza-171 as a check genotype. PCR reactions generated a total of 60 amplified bands at size ranged from 156 to 1476bp with an overall mean of 6.67±0.76 Figure (3) and Table (7). Out of the (60) obtained bands 12 were monomorphic with an overall mean of 1.33±0.55 and 48 bands were polymorphic with an overall mean of 5.33±0.99. The percentages of polymorphism among primers ranged from 20 to 100% with an overall mean of 75.12±10.88% (Table 7). Furthermore, Emam *et al.* (2022) and Shaban *et al.* (2022) whose emphasized the efficiency of ISSR markers for evaluating the genetic relationships among various wheat genotypes.

Table 7. Fragment size, total number of polymorphic and unique bands and polymorphism % obtained using nine ISSR primers twelve different wheat genotypes.

Primers	Fragment size (bp)	Monomorphic bands	Unique bands	Polymorphic bands		Total number of bands	Polymorphism (%)
				With Unique	without Unique		
HB	251-1466	3	3	8	5	11	72.73
HB08	270-1476	0	2	6	4	6	100
HB10	259-621	0	3	7	4	7	100
HB12	232-1089	0	0	9	9	9	100
HB15	156-685	4	0	1	1	5	20
807	303-1118	3	0	3	3	6	50
810	221-753	0	0	6	6	6	100
812	234-679	0	2	7	5	7	100
817	244-408	2	0	1	1	3	33.33
Total		12	10	48	38	60	
Mean ± SE		1.33±0.55	1.11±0.45	5.33±0.99	4.22±0.83	6.67±0.76	75.12±10.88

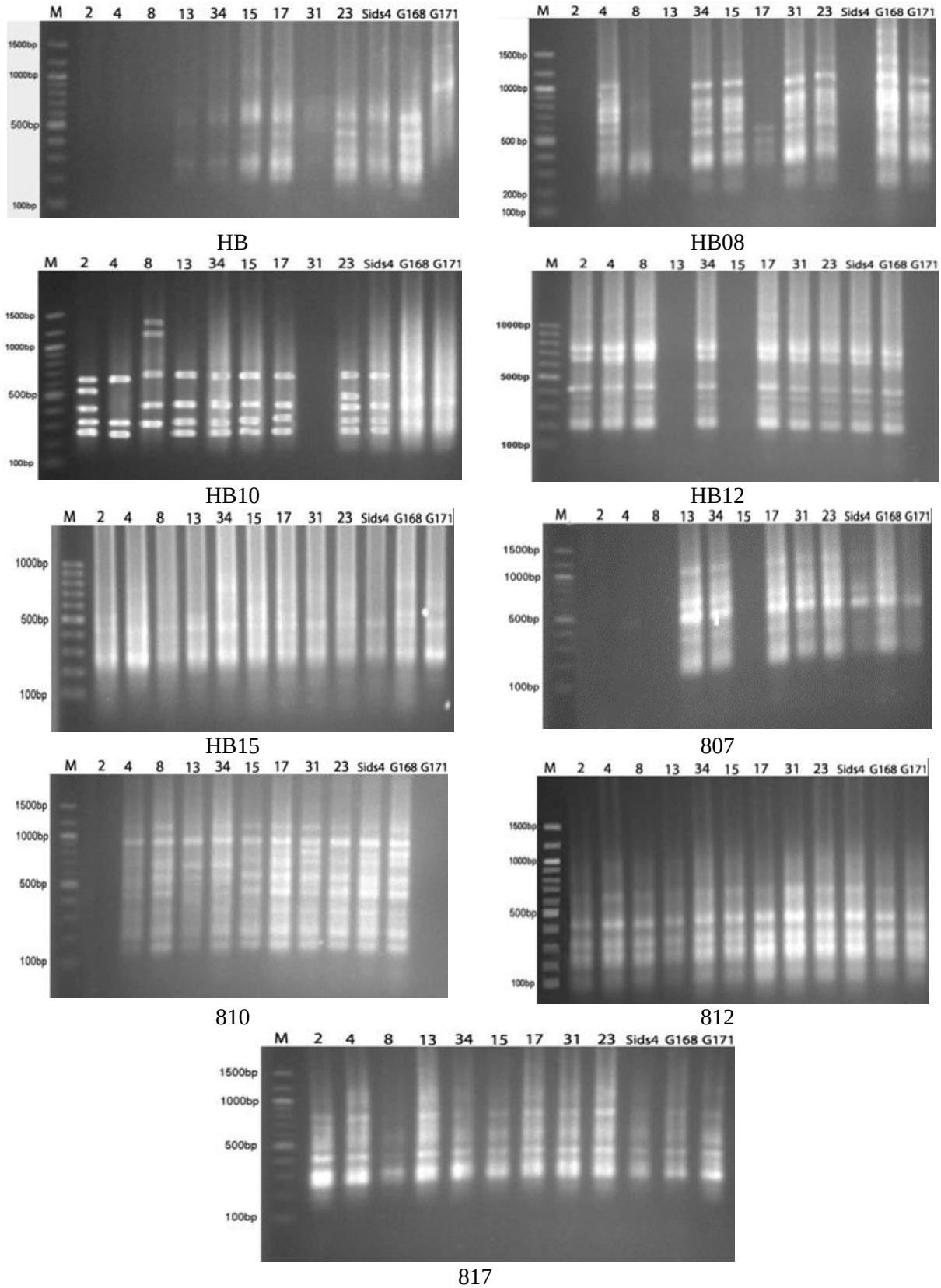

Figure 3. Electrophoretic gel patterns of ISSR DNA products of HB, HB08, HB10, HB12, HB15, 807, 810, 812 and 817 primers, lane1 (M), refer to DNA ladder 100bp, Lanes 2-13 refer to the twelve wheat genotypes.

ISSR amplicons produced by the five primers (HB08, HB10, HB12, 810 and 812) exhibited 100% polymorphism among all tested genotypes while the four other primers (HB, HB15, 807 and 817) showed 72.73, 20, 50 and 33.33% polymorphism, respectively. As shown in Table 7, ten unique bands were produced by four ISSR primers (HB, HB08, HB10 and 812), while the other five primers did not produced any unique bands. The number of amplified fragments produced by any of the ISSR primers depends on primer sequence and the extent of variation of the examined genotype(s). According to the above-mentioned results, it can be concluded that the nine utilized primers generated relatively high polymorphism within the studied bread wheat genotypes. The primers of HB, HB08, HB10 and 812 were the highest and a more successful in proofing identity of the studied bread wheat genotypes. It produced (3, 2, 3 and 2 unique bands, respectively) then it can be used as a marker to distinguish among them. Our results are in agreement with that of Nosair (2020) who analyzed the genetic relationship between six Egyptian wheat cultivars (Masr1, Swiss2, Swiss4, Giza7, Giza9, Giza10 and Sakha94) by using ISSR markers. He found that ISSR markers are useful for genetic diversity analysis of wheat cultivars and provided greater information which may be utilized in plant breeding programs. Furthermore, Emam *et al.* (2022) and Shaban *et al.* (2022) whose emphasized the efficiency of ISSR markers for evaluating the genetic relationships among various wheat genotypes.

The data of ISSR analysis were used to estimate the genetic relationships among the 12 Egyptian bread wheat genotypes through a UPGMA cluster analysis of genetic similarity matrices. Cluster analysis was achieved based on Dice's similarity coefficient matrix (Table 8 and Figure 4). Data showed that some genotypes had high genetic similarity with others, such as Sids-4 and Line-34 (94.8%), Giza-168 and Line-15 (94.4%) and Line -34 with the two Lines 15 and 23 (93.8%). On the contrary, several genotypes showed low genetic similarity, such as Giza-171 and Line-31 (53.9%), Line-17 and Line-2 (60.3%) and Line-13 and Line-8 (63.8%). The similarity values exhibited clearly the major variations among the all studied wheat genotypes.

Table 8. Dice's similarity coefficient matrix within the wheat genotypes based on polymorphism bands of ISSR primers.

Genotypes	2	4	8	13	15	17	23	31	34	S-4	G-168	G-171
2	-											
4	.788	-										
8	.721	.853	-									
13	.633	.703	.638	-								
15	.722	.860	.765	.800	-							
17	.603	.831	.722	.732	.771	-						
23	.722	.930	.790	.725	.891	.867	-					
31	.667	.831	.778	.761	.867	.649	.795	-				
34	.684	.867	.753	.786	.938	.828	.938	.851	-			
S-4	.658	.851	.780	.765	.903	.810	.925	.833	.948	-		
G-168	.754	.867	.795	.779	.944	.800	.899	.850	.925	.889	-	
G-171	.667	.648	.545	.708	.649	.735	.701	.529	.691	.692	.649	-

The dendrogram was constructed using the hierarchical cluster analysis method with the average linkage between pairs from the matrix of Dice (1945) and similarity coefficient values (S) within the twelve studied Egyptian bread wheat genotypes (Figure 4). The obtained dendrogram divided the studied wheat genotypes into two main clusters; the first cluster contained the check genotype (Giza-171), while the second cluster contained the remaining genotypes (2, 4, 8, 13, 15, 17, 23, 31, 34, Sids-4 and Giza-168). The second main cluster is divided into two subgroups; the first one contains the line 2 genotype, while the other contains the rest ten genotypes.

It was observed that the two genotypes Sids-4 and Line-34 had the closest genetic relationship and sharing the same clade. As well as, the two genotypes Giza-168 and Line-15 were also very similar and engaged the same clade. The dendrogram revealed that the ISSR markers were a successful tool in differentiating amongst the 12 bread wheat genotypes due to their genetic background. The obtained results are in agreement with that found by Carvalho *et al.* (2008) whose analyzed 48 bread wheat cultivars of an Old Portuguese collection by using 18 ISSR markers. They reported that cultivars with similarity at genetical level were shared the same main cluster. In this study, ISSR markers yielded a promising finding and grouping, due to their ability to generate specific regions of the genome (Gajera *et al.,* 2010). Consequently, these markers gave more detailed and varied information about the genetic variability of the studied Egyptian wheat genotypes (Rao *et al.,* 2020).

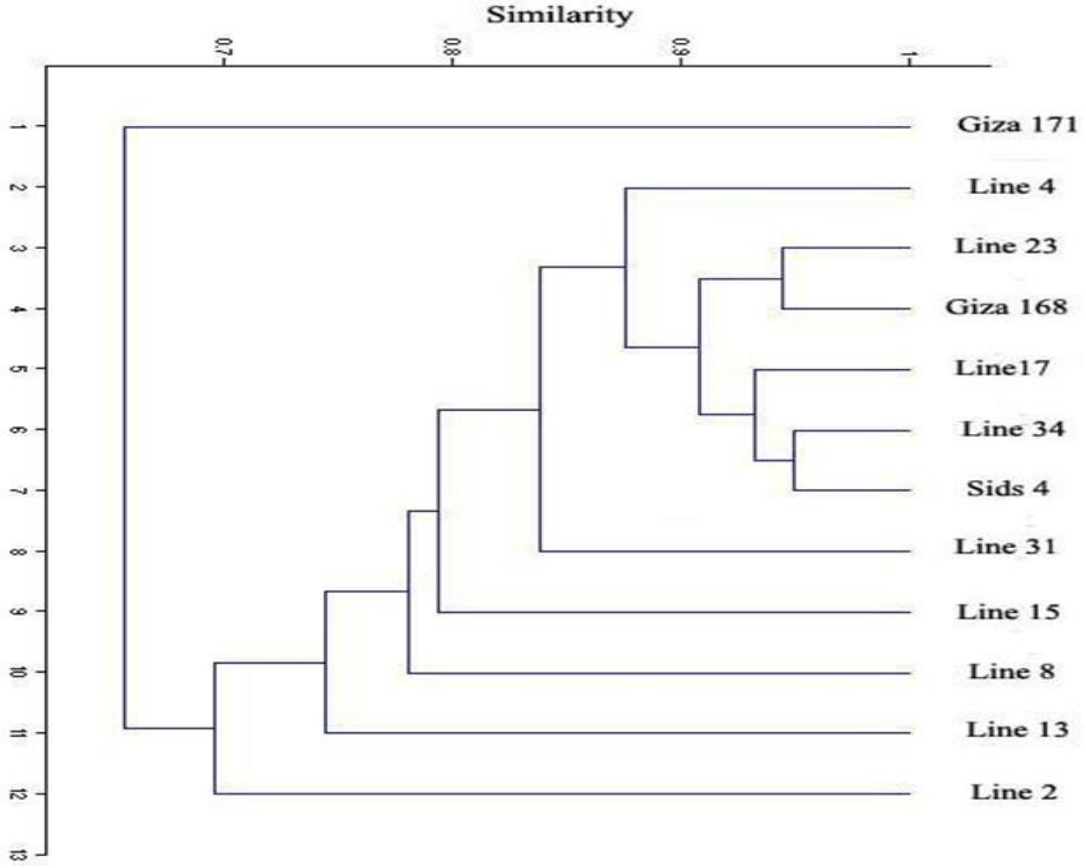

Figure 4. The dendrogram of genetic relationships among twelve bread wheat genotypes based on polymorphism bands of ISSR primers

It's not certain that the morphological traits and DNA markers will produce findings that are nearly identical (Vollmann *et al*., 2005; Mart´nez *et al*., 2005). There are two explanations for the poor association among morphological characters, DNA markers and protein data were proposed by Semagn (2002). The first reason: DNA markers are more comprehensive than morphological markers in covering a greater percentage of the genome, including both coding and noncoding regions. The second reason: The artificial selection applied to morphological markers is greater than that of DNA markers. Martinez *et al.* (2005) thought that examining more morphological traits and DNA markers may enhance the agreement between different methods.

4. conclusion

Finally, these results showed that both of morphological and ISSR markers could be used as important tools for characterizing the studied Egyptian bread wheat genotypes. It might also provide important information that helps breeders to select the right individuals in plant breeding programs.

Authors' Contributions
All authors are contributed in this research
Funding
There is no funding for this research.
Institutional Review Board Statement
All Institutional Review Board Statements are confirmed and approved.
Data Availability Statement
Data presented in this study are available on fair request from the respective author.
Ethics Approval and Consent to Participate
Not applicable
Consent for Publication
Not applicable.
Conflicts of Interest
The authors disclosed no conflict of interest.

5. References

Abaza, G. M. S. M., Awaad, H. A., Attia, Z. M., Abdel-lateif, K. S., Gomaa, M. A., Abaza, S. M. S. M., Mansour, E. (2020). 'Inducing potential mutants in bread wheat using different doses of certain physical and chemical mutagens.', *Plant breeding and biotechnology*, 8(3), pp. 252-264.

Abd El-Moneim, D. (2020). 'Characterization of ISSR and SCoT markers and TaWRKY gene expression in some Egyptian wheat genotypes under drought stress.', *J. Plant Prod. Sci.,* 8, pp. 31–46.

Abd El-Moneim, D., ELsarag E.I.S., Aloufi S., El-Azraq A.M., ALshamrani S.M., Safhi F.A.A., Ibrahim A.A. (2021). 'Quinoa (*Chenopodium quinoa* Willd.): genetic diversity according to ISSR and SCoT markers, relative gene expression, and morpho-physiological variation under salinity stress.', *Plants,* 10:2802.

Abouseada, H. H., Mohamed, A. H., Teleb, S.S., Badr, A., Tantawy, M. E., Ibrahim, S. D., Ellmouni, F. Y., Ibrahim, M. (2023). 'Genetic diversity analysis in wheat cultivars using SCoT and ISSR markers, chloroplast DNA barcoding and grain SEM.', *BMC Plant Biology*, 23(193), pp. 1-15.

Ahmadi, M., Farshadfar, E., Veisi, S. (2012). 'Evaluation of genetic diversity in land races of bread wheat under irrigated and rainfed conditions.', *Inter. J. Agric. and Crop Sci.*, 4(21), pp. 1627-1636.

Almarri, N. B., Alghamdi, S. S., ElShal, M. H., Afzal, M. (2023). 'Estimating genetic diversity among durum wheat (*Triticum durum* desf.) landraces using morphological and SRAP markers.', *J. Saudi Soc. Agric. Sci.*, 22, pp. 273-282.

Attia, A. N. E., Sultan, M. S. A., Badawi, M. A., Alfahdawey, A. A. K. (2015). 'Morphological identification of some wheat varieties and its crosses.', *J. Plant Prod*, 6(6), pp. 889-901.

Bekhit, M. M. M., Salim T. M. S. (2019). 'Cytomolecular genetic diversity assessments of two wheat species grows in

Egypt.', *J. Agric. Chem. and Biotech.,* 10 (12), pp. 269 – 277.

Belhadj, H., Medini, M., Bouhaouel, I., Amara, H. (2015). 'Analysis of the phenotypic diversity of some indigenous accessions of wheatgrass (*Triticum turgidum* ssp. durum Desf.) from southern Tunisia.', *J. New Sci., Agric. and Biotech.,* 24(5), pp. 1115-1125.

Boshev, D., Jankulovska, M., Ivanovska, S., Jankuloski, L. (2016). 'Assessment of winter wheat advanced lines by use of multivariate statistical analyses.', *Genetika,* 48(3), pp. 991-1001.

Carvalho, A., Brito, J. L., Macas, B., Pinto, H. G. (2008). 'Genetic variability analysis of a collection of Old Portuguese bread wheat using ISSRs.', *Options Mediterraneennes. Ser. A, Sem. Medit.,* 81, pp. 35-38.

Cooke, R.J. (1999). 'Modern methods for cultivar verification and transgenic plant challenge.', *Seed Sci. and Tech.,* 27, pp. 669-680.

Dice, L. R. (1945). 'Measures of the amount of ecologic association between species.', *Ecology*, 26, pp. 297-302.

Emam, M.A., Abd EL-Mageed, A.M., Niedbała, G., Sabrey, S.A., Fouad, A.S., Kapiel, T., Piekutowska, M., Mahmoud, S.A. (2022). 'Genetic characterization and agronomic evaluation of drought tolerance in ten Egyptian wheat (*Triticum aestivum* L.) cultivars.', *Agronomy,* 12, pp. 12-17.

Erdinc, C., Ekincialp, A., Turan, S., Kocak,M., Baloch, F. S., Sensoy, S. (2021). 'The first report about genetic diversity analysis among endemic wild rhubarb (*Rheum ribes* L.) populations through iPBS markers.', *Turk. J. Agric. For.*, 45 (6), pp. 784–796.

Eticha, F., Bekele, E., Belay, G., Börner, A. (2005). 'Phenotypic diversity in tetraploid wheats collected from Bale and Wello regions of Ethiopia.', *Plant Genetic Resources,* 3(1), pp. 35-43.

Etminan, A., A. Pour-Aboughadareh, R. Mohammadi, Ahmadi-Rad, A. Noori, Z. Mahdavian and Z. Moradi (2016). 'Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes.', *Biotechnol. Biotechnol. Equip*., 30(6), pp. 1075-1081.

Feltaous, Y. M. (2019). 'Genetic diversity among some Egyptian bread wheat cultivars based on morphological characters and SSR markers.', *Assiut J. Agric. Sci.,* 50(4), pp. 35-50.

Fouad, H. (2020). 'Principal component and cluster analyses to estimate genetic diversity in bread wheat (*Triticum aestivum* L.) genotypes.', *J. Plant Prod.,* 11(4), pp. 325-331.

Gajera, B. B., Kumar, N., Singh, A. S. *et al.,* (2010). 'Assessment of genetic diversity in Castor (*ricinus communis* L.) using RAPD and ISSR markers', *Industrial Crops and Products*, 32(3), pp. 491–498.

GelAnalyzer, Version three (2007). 'Gel Analyzer Ver.3 program software for windows. www.geocities.Com/egygene. gene expression.', *Genome Res.,* 19, pp. 1419–28.

Gharib, M., Qabil, N., Salem, A., Ali, M., Awaad, H., Mansour, E. (2021). 'Characterization of wheat landraces and commercial cultivars based on morphophenological and agronomic traits.', *Cereal Res. Commun.* 49, pp. 149–159.

Guin, K., Sethi, S.K., Arya, R.K. (2019). 'Genetic Studies on *Triticum timopheevi* based cytoplasmic genetic male sterility (CGMS) system in relation to hybrid seed production in wheat (*T. aestivum* L.).', *Ekin J. Crop Breed. Genetics*, 5(2), pp. 103-110.

Hammer, O. (2001). 'PAST: Paleontological statistics software package for education and data analysis.', *Palaeontol electron*, 4, 9.

Henareh, M., Dursun, A., Abdollahi-Mandoulakani, B., Haliloğlu, K. (2016). 'Assessment of genetic diversity in tomato landraces using ISSR markers.', *Genetika,* 48, pp. 25–35.

Liu, H., Ma, J., Tu, Y., Zhu, J., Ding, P., Liu, J., et al. (2020). 'Several stably expressed QTL for spike density of common wheat (*Triticum aestivum*) in multiple environments.', *Plant Breed,* 139, pp. 284–294.

Mansour, E., Moustafa, E.S., Qabil, N., Abdelsalam, A., Wafa, H.A., El Kenawy, A. *et al*. (2018). 'Assessing different barley growth habits under Egyptian conditions for enhancing resilience to climate change.', *Field Crops Res.,* 224, pp. 67–75.

Mart´nez, L., Cavagnaro, P., Masuelli, R. (2005). 'Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varieties using morphological data and AFLP markers.', *Elec. J. Bio. technol.,* 6, pp. 37-45.

Marzario, S., Sica, R., Taranto, F., Fania, F., Esposito, S., De Vita, P., ... Logozzo, G. (2023). 'Phenotypic evolution in durum wheat (*Triticum durum* Desf.) based on SNPs, morphological traits, UPOV descriptors and kernel-related traits.', *Frontiers in Plant Science,* 14, pp. 1-20.

Mateo-Sagasta, J., Zadeh, S. M., and Turral, H. (Eds.). (2018). *'More people, more food, worse water?',* a global review of water pollution from agriculture.', Pp1-41. books.google.com

Mesfer, A., S., Safhi, F.A., Alshaya, D.S., Ibrahim, A.A., Mansour, H., Abd El Moneim, D. (2022). 'Genetic diversity using biochemical, physiological, karyological and molecular markers of *Sesamum indicum* L.', *Front. Genet.,* 13:1035977.

Nadeem, M.A., Nawaz M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H. (2018). 'DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing.', *Biotechnol. Biotechnol. Equip.,* 32(2), pp. 261–285.

Najaphy, A., Parchin, R. A., Farshadfar, E., Farshadfar, E. (2012). 'Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines.', *Australian J. Crop Sci.,* 6(2), pp. 326-332.

Nosair, H. R. (2020). 'Genetic diversity studies on seven Egyptian wheat (*Triticum aestivum* L) cultivars using Scot and ISSR polymorphism markers.', *Taeckholmia,* 40, pp. 143-151.

Park, S., Kumar, P., Shi, A., Mou, B. (2021). 'Population genetics and genomewide association studies provide insights into the influence of selective breeding on genetic variation in lettuce.', *Plant Genome* 14, 20086.

Petrovic, S., Maric, S., Cupic, T., Rebekic, A., Rukavina, I. (2017). 'Assessment of molecular and phenotypic diversity among winter wheat cultivars.', *Genetika,* 49(2), pp. 583-598.

Rao, G. K., Kapadia, C., Patel, N. B., Desai, K. D., Narasimha Murthy, P. N., (2020). 'Genetic diversity analysis of greater yam (*Dioscorea alata* L.) genotypes through RAPD and ISSR markers.', *Biocatalysis and Agricultural Biotechnology*, 23, 101495.

Rukavina, I., Petrovic, S., Cupic, T., Vila, S., Guberac, S., Drenjancevic, L. (2017). 'Genetic variability of wheat germplasm represented in the south Pannonian region.', *Genetika,* 49(3), pp. 831-842.

Sajjad, M., Khan, S. H., Shahzad, M. (2018). 'Patterns of allelic diversity in spring wheat populations by SSR-markers.', *Cytology and Genetics*, 52(2), pp. 155–160

Semagn, K. (2002). 'Genetic relationships among ten endotypes as revealed by a combination of morphological, RAPD and AFLP markers. *Hereditas,* 137, pp. 149-156

Shaban, A. S., Arab, S. A., Basuoni, M. M., Abozahra, M. S., Abdelkawy, A. M., Mohamed, M. M. (2022). 'SCoT, ISSR, and SDS-PAGE investigation of genetic diversity in several Egyptian wheat genotypes under

normal and drought conditions.', *Inter. J. Agronomy*, pp. 1-14.

Shannon, C. E., Weaver, W. (1949). *'The mathematical theory of communication',* (Urbana: University of Illinois Press).

Takac, V., Mikic, S., Mirosavljevic, M., Momcilovic, V., Trkulja, D., *et al.* (2019). 'Characterisation of Serbian durum wheat genotypes based on UPOV-defined characteristics.', *Ratar. Povrt.*, 56(3), pp. 97-102.

UPOV (2017). *'International union for protection of new varieties of plants. Guidelines for the conduct of test for distinctness, uniformity and stability of bread wheat.',* pp. 1-24. https://www.upov.int/edocs/tgdocs/en/tg003.pdf

USDA (2023). *'United States department of Agriculture, Foreign Agricultural Service.',* Global Market Analysis.

Vollmann, J., Grausgruber, H., Stift, G., Dryzhyruk, V., Lelley, T. (2005). 'Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism.', *Plant Breeding*, 124, pp. 446-453.

Würschum, T., Langer, S. M., Longin, C. F. H., Tucker, M. R., Leiser, W. L. (2020). 'Refining the genetic architecture of flag leaf glaucousness in wheat.', *Theor. Appl. Genet.*, 133, pp. 981–991.

Yan, W., Fregeau, J. (2008). 'Breeding line selection based on multiple traits.', *Crop Sci.*, 48, pp. 417-423.