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SSR- Based Genetic Diversity Assessment in Tetraploid and Hexaploid Wheat Populations

Hanaa Mahdy Abouzied[1*], Samah M. M. Eldemery[2] and Kamal Fouad Abdellatif[3]

[1]Crop Science Department, Faculty of Agric., Damanhour, Damanhour University, Egypt.
[2]Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minoufiya University, Egypt.
[3]Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minoufiya University, Egypt.

Authors' contributions

The three authors contributed equally in this work. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Molecular analysis for a set of hexaploid (*Triticum aestvium*) and tetraploid (*Triticum durum*) wheat cultivars was investigated by applying 11 SSR primers set. The plant materials consisted of 45 genotypes 15 of which were *Triticum aestivum* and 30 of *T. durum* obtained from four different regions Egypt, Greece, Cyprus and Italy. PCR products were separated on a 6% denaturing polyacrylamide gel electrophoresis and produced a total of 3840 DNA fragments which were used for the molecular analysis. The estimated parameters computed by POPGENE (Version 1.32) within the two population indicated that the Nei's genetic diversity (*H*) was 0.2827, and the Shannon's Information index (*I*) was 0.4533 with standard deviation ± 0.0699 and ± 0.0852 respectively. The analysis of population structure revealed that genetic diversity within populations (*Hs*=0.2761) represented 97.7% of the total genetic diversity (H_T=0.2827). The proportion of the total genetic diversity that was attributed to the population differentiation was low (*Gst*=0.0233) within population. ANOSIM (ANalysis Of Similarities), results showed that *R* was equal to 0.9048 (*P<0.0001*) indicated that all the most similar samples of genotypes are within the same population. The wheat varieties from the four distinct regions were clustered according to SSR data into two main clusters, durum wheat varieties and bread

**Corresponding author: Email: hmmahdy@yahoo.com;*

wheat varieties, the principal coordinate analysis (PCOORDA) validated the results of the dendrogram. This study showed that the two populations still had moderate considerable level of genetic diversity and show little genetic differentiation among them. Understanding genetic variation within and between populations is essential for the establishment of an effective breeding program concerning the intraspecific and interspecific hybridization.

Keywords: ANOSIM; bread wheat; durum wheat; genetic diversity; POPGENE; SSR markers.

1. INTRODUCTION

The genus *Triticum* exists as a polyploid series of diploid, tetraploid, and hexaploid species complexes [1]. Of special cultural and economic importance are the tetraploid durum wheat *T. turgidum* L. and the hexaploid bread wheat (common wheat) *T. aestivum* L. [2]. *T. aestivum* L. is by far the most important staple crop in the world. The economic significance of this crop has attracted attention for a long time and stimulated intense interest in the determination of its ancestral diploid genome donors [3]. Different species belonging to the genus *Triticum* and its congeners are diverse in their phenotypic adaptation to a wide range of environments. They hold a rich pool of genetic heterogeneity viz., resistance to pathogens and pests, drought tolerance, winter hardiness, adaptability to poor soil, and high protein content besides other qualities and yield traits. Furthermore, diploid, tetraploid, and hexaploid wild wheat share one or more genomes with cultivated wheat. They play a major role in wheat improvement through introgression of genes. Specific level utilization, however, depends on the production of successful interspecific hybrids with adequate fertility [4].

The cultivated durum wheat (*Triticum durum* Desf.) is a tetraploid with A and B genomes (AABB). It is not only the main source of semolina for the production of pasta, couscous, burghul and other Mediterranean local end-use products, but also provides many beneficial traits, including resistance, environmental stability, yield potential, and high quality for bread wheat improvement [5,6,7,8]. Owing to the common A and B genomes, durum wheat is easily used to improve bread wheat by interspecific hybridization with homologue chromosome pairing and recombination [9]. Crosses such as (diploid x hexaploid, tetraploid x hexaploid) were practiced by many researchers. In general, *T. aestivum* has been used as the mother plant in inter-generic and inter-specific crossing. Many crosses have been successful, although techniques such as embryo rescue may be required to obtain viable progeny. Differences have been noted in the receptivity of different varieties of *T. aestivum* to accept cross-fertilization by other species such as rye [10].

Wheat exceeds all other cereal grain crops in acreage and production. The genetic variability within cultivated wheat is rapidly diminishing, primarily due to the replacement of the highly variable landraces with pure line varieties [11]. Wheat breeders have been targeting immediate progenitors of wheat (*Triticum* and *Aegilops*) and more distantly related genera (*Secale, Agropyron, Aegilops, Haynaldia, Elymus, Hordeum*, etc.) as supplementary sources to harness beneficial genes [4].

Genetic diversity is the basis for genetic improvement. Knowledge of germplasm diversity has a significant impact on the improvement of crop plants. Due to modern breeding, it has been suggested that genetic diversity in wheat has been increasingly narrowed. Narrow

genetic diversity is a problem in breeding for adaptation to biotic stresses, like diseases, and abiotic stresses, such as drought or salt tolerance. Therefore, it is necessary to investigate the genetic diversity in wheat germplasm in order to broaden the genetic variation in future breeding programme [12].

Molecular markers have been widely used in wheat breeding programs, because they have numerous advantages as compared to morphological markers, including high polymorphism and independence on effects related to environmental conditions and the physiological stage of the plant [13,14,15,16]. The use of molecular markers for the evaluation of genetic diversity is receiving much attention. Many wheat scientists have studied genetic diversity in common wheat using different molecular markers such as RAPDs [17]. RFLPs [18,19], AFLPs [20, 21], STS [22] and ISSRs [23]. Simple sequence repeat (SSR) marker has been used as an ideal molecular marker to investigate the genetic diversity because of its multi-allelic nature, reproducibility, codominant inheritance, high abundance and extensive genome coverage [24] in many crops.

The aim of this study is to analyse the genetic diversity within and among two populations of wheat tetraploid and hexaploid. Analyses were made to estimate the following genetic parameters: observed number of alleles (*Na*), effective allele number (*Ne*), Nei's gene diversity (*He*) and Shannon's index (*I*) [25], genetic diversity within population (*Hs*) and total (H_T), Nei's genetic differentiation index among populations (*Gst*) and gene flow (*Nm*) were calculated using the computer program POPGENE version 1.32 [26,27].

2. MATERIALS AND METHODS

A total of 45 wheat varieties were used, originating from Greece (20 durum wheat varieties and nine bread wheat varieties), Egypt (seven bread wheat varieties), Cyprus (seven durum wheat varieties) and Italy (two durum wheat varieties). Most of the entries have known pedigrees while few of them have unknown background (Table 1).

2.1 DNA Extraction

Genomic DNA was extracted from fresh leaves of wheat genotypes using the Saghai-Maroof et al. [28] CTAB method. RNA was removed from the DNA preparation by adding 10 µl of RNAase (10 mg/ml) and then incubated for 30 min at 37ºC. DNA sample concentration was quantified by using a spectrophotometer (Beckman Du-65). The reagents were obtained from Pharmacia Biotech (Amersham Phar- macia Biotech Limited, UK).

Table 1. Wheat entries used for SSR molecular marker analyses

No	Varieties	Origin	Pedigree
	Durum Wheat (4X)		
1	ATHOS (C)	Greece	HOHENHEIMER/PETIT-QUINQUIN//COTE-D-OR[39] [1790]
2	AGIAS (C	Greece	UNKNOWN
3	ANA (C)	Greece	MEXIKALI- 81/ SANDA
4	ELECTRA (OC)	Greece	S. Capelli/4/LIMNOS//Florence/Arditto/3/Sinai
5	ELECTRA –A	Greece	S. Capelli/4/LIMNOS//Florence/Arditto/3/Sinai
6	ELECTRA –M	Greece	S. Capelli/4/LIMNOS//Florence/Arditto/3/Sinai
7	KALLITHEA (C)	Greece	UNKNOWN
8	LIMNOS (OC)	Greece	Local durum wheat selection for landrace Asprostachi
9	MEXIKALI- 81	Greece	Selection from CIMMYT`s variety Mexicali 75
10	SIFNOS (C)	Greece	UNKNOWN
11	KORNOS	Greece	UNKNOWN
12	MAVRAGHANI HERAKLIO*	Greece	Landrace
13	MAVRAGHANI HERAKLIO –A	Greece	Landrace
14	MAVRAGHANI HERAKLIO –M	Greece	Landrace
15	MYRINA	Greece	UNKNOWN
16	PAPADAKIS (C)	Greece	ATHOS/MEXIKALI- 81//MEXIKALI- 81
17	PONDOS (C)	Greece	UNKNOWN
18	SANDA (OC)	Greece	Selection from irradiated variety METHONI
19	SIMETO (C)	Italy	CAPEITI-8/VALNOVA [1620] [1622] [1623] [1625] [1666]
20	KYPEROUNDA	Cyprus	AMBER
21	KYPEROUNDA –A	Cyprus	AMBER
22	KYPEROUNDA –M	Cyprus	AMBER
23	ARONAS	Cyprus	RAE (RIALE-E) /4 X TC60 // STEWART 63 /3/ AA “S” = CISNE = COCORIT 71
24	MESAORIA	Cyprus	AA “S”/VOLUNTEER = MIA
25	MAKEDONIA	Cyprus	UNKNOWN
26	KARPASIA	Cyprus	PLC “S”/RUFF “S”//GTA “S”/RTTE
27	SELAS (C)	Greece	Selection from the variety Stork "S"
28	SKITI (C)	Greece	Selection of CR "S"/ T.DIC S VERNUM-GLL"S"
29	SKYROS	Greece	UNKNOWN
30	PIETRAFITTA	Italy	Grazia/Isa
	Bread Wheat (6X)		
31	SAKHA 61	Egypt	61 INIA/RL4220//7C-3/YR’’s’’CM15430-2S-5S-0S
32	SAKHA 69	Egypt	INIA/RL4220//7C/YR’’s’’CM15430-2S-1S-0S
33	SAKHA 94	Egypt	OPATA/RAYON//KAUZ
34	GEMMIZA 7	Egypt	7CMH74A-630/SX//SERI82/AGENT
35	GEMMIZA 9	Egypt	ALD’’s’’/HUAC//CMH74A-630/SX
36	GIZA 168	Egypt	MRL/BUC//SERI
37	SIDS 1	Egypt	HD2172/PAVON’’s’’//1158-57/MAGA74’’s’’
38	GEKORA-E	Greece	Individual selection from CIMMYT‘s variety Gecora 70
39	ACHERON	Greece	LOCAL CULTIVAR
40	VERGHINA	Greece	G-38290/YG-3297 (see AIGES)
41	GENOROZO-E	Greece	AUTONOMIA//AQUILA/AUTONOMIA [39] [221] [2289]; AUTONOMIA/MARA, ITA[2494]
42	STRYMONAS	Greece	YAQUI 50/ENANO//KALYANSONA from CIMMYT
43	NESTOS	Greece	Selection from segregated material of INIA 66R//HBGN/DRC
44	ACHELOOS-	Greece	Siete Cerros T-66//Weibulls-Karin/Yt 54B II–7518-3c-2h-1h.
45	ELISAVET (C)	Greece	UNKNOWN

(C) Cultivar, (OC) Old cultivar

2.2 Simple Sequence Repeat (SSR) Analysis

Eleven primer pairs were selected as the most informative ones to carry out the analysis. These SSR primers included six WMSs, three WMCs, and two Xgwms (Table 2). The PCR amplification reactions were performed in a 25 µl volume using 50 ng DNA containing 0.5 µ moles of each primer pair, 100 µM of dNTPs, 5 µl (1X) of Taq polymerase buffer, 1 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (Promega). The SSR reactions were carried out using Touchdown PCR program. The main program was: 7 cycles at 94°C for 1 min, 59°C for 1 min, decreasing 1°C in every cycle, and 72°C for 1 min, followed by 28 cycles at 94°C for 1 min, 52°C for 1 min and 72°C for 1 min. The previous cycles were preceded by a denaturation step at 94°C for 3 minutes. An extension step at 72°C for 5 minutes was added at the end of the program. The PCR products were separated on a 6% denaturing polyacrylamide gel electrophoresis. Denaturing polyacrylamide gel was stained with silver staining kit (Promega Silver Sequence DNA Staining Reagents, No. Q4134) according to the manufacturer's protocol, dried and scanned as a permanent image.

Table 2. Primer sequences used for SSR PCR-reactions

Primer and chromosome position	Sequence[+] (left)	Sequence[+] (right)
WMS52 (3DL)	CTATGAGGCGGAGGTTGAAG	TGCGGTGCTCTTCCATTT
WMS95 (2AS)	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG
WMS218 (3AS)	CGGCAAACGGATATCGAC	AACAGTAACTCTCGCCATAGCC
WMS234 (5BL)	GAGTCCTGATGTGAAGCTGTTG	CTCATTGGGGTGTGTACGTG
WMS297 (7BS)	ATCGTCACGTATTTTGCAATG	TGCGTAAGTCTAGCATTTTCT
WMS375 (4BL)	ATTGGCGACTCTAGCATATACG	GGGATGTCTGTTCCATCTTAGC
Wmc25 (2AS,2BS, 2DS)	TCTGGCCAGGATCAATATTACT	TAAGATACATAGATCCAACACC
Wmc233 (5DS)	ATCTGCTGAGCAGATCGTGGTT	ATCTGCTGAGCAGATCGTGGTT
Wmc256 (6A, 6D)	CCAAATCTTCGAACAAGAACCC	ACCGATCGATGGTGTATACTGA
Xgwm 136 (1AS)	GACAGCACCTTGCCCTTTG	CATCGGCAACATGCTCATC
Xgwm 644 (7BL)	GTGGGTCAAGGCCAAGG	AGGAGTAGCGTGAGGGGC

[*] Y is (C,T) nucleotide bases and R is (A,G) nucleotide bases; [+] All sequences are in 5' to 3' direction

2.3 Statistical Analysis

2.3.1 Genetic diversity parameters

Data of hexaploid (*Triticum aestivum*) and tertaploid wheat (*Triticum durum*) obtained from SSR markers were analyzed using POPGENE version 1.32 [26, 27] assuming Hardy-

Weinberg equilibrium. Samples were grouped into two populations (tetraploid and hexaploid wheat populations). Analyses were observed at two levels (1) within populations (combined populations); and (2) among populations. Different genetic parameters were computed such as: the percent (%) polymorphism, genetic diversity or heterozygosity (*H* = Nei's [29] gene diversity); Shannon's index (*I*) the *Gst* values of genetic differentiation, and gene flow estimation (*Nm*) ($Nm = 0.5 \times (I - Gst) / Gst$) [30] and diversity among populations *Dst*.

2.3.2 Analysis of similarity (ANOSIM)

Analysis of similarities, was computed using using the PAleontological Statistics software (PAST) Version 2.04 [32] and was used to test the significant difference between groups based on distance measurement [31,32]. The one way ANOSIM measures differences between and within pairs of groups and converts these to ranks using the crossed design [31]. The test statistic *R* signifies dissimilarity between groups. The studied genotypes were classified in to three groups, (a) tetrapolid group (b) Egyptian hexaploid group and (c) European hexaploid group the larger the *R* value (up to 1), the more dissimilar the groups are. The distance measure used was Bray-Curtis and computed by permutation of group membership, with 10,000 replicates. Clarke [33,31] proposed the following statistic to measure the differences between the groups, the test statistic *R* is then defined as:

$$R = \frac{\bar{r}_B - \bar{r}_W}{n(n-1)/4}$$

where r_B be the mean rank of all distances between groups, and r_w the mean rank of all distances within groups. Large positive *R* (up to 1) signifies dissimilarity between groups.

2.3.3 UPGMA cluster and principal coordinate analysis (PCOORDA)

An Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis and principal coordinate analysis (PCOORDA) based on Dice similarity index were performed using the PAleontological Statistics software (PAST) Version 2.04 [32]

3. RESULTS AND DISCUSSION

3.1 Genetic Diversity Analysis

In this study, genetic diversity was analysed in forty five different wheat varieties grouped in two populations tetraploid and hexaploid population by applying SSR method. Popgene version 1.31 [27] was used to estimate genetic diversity parameters, a total of 3840 DNA fragments were amplified with 11 SSR primers set. At the genotypes level the percentage of polymorphic loci was 100% for all the loci. SSR markers have been proven to be powerful tools for molecular genetic analysis , genetic diversity and quantitative trait loci (QTL) mapping of wheat cultivars [34,35,36,37,38,39,40,41,42,43,44,45,46,47].

3.1.1 Means within populations

Combined populations of durum and bread wheat genotypes showed that the mean of the observed number of alleles (*Na*) was 2.0000 with standard deviation ± 0.0000, the effective number of alleles (*Ne*) was 1.4065 with standard deviation ± 0.1353, the mean of Nei's

gene diversity (*H*) was 0.2827 ± 0.0699, the Shannon's Index of phenotypic diversity (*I*) was 0.4533 ± 0.0852 (Table 3).

3.1.2 Means among populations

Means at populations level, for tetraploid population, *Na* was 2.0000, *Ne* was 1.4051, *H* was 0.2812, and *I* was 0.4509 with standard deviation± 0.0000, 0.1428, 0.0750 and 0.0924, respectively (Table 3). The hexaploid population showed that the *Na* was 2.0000 , *Ne* was 1.4092, *H* was 0.2827, and *I* was 0.4527 with standard deviation 0.0000, 1.1537, 0.0756 and 0.0922, respectively (Table 3).

Table 3. Means of the observed number of alleles (*Na*), the effective alleles (*Ne*) and the gene diversity (*H*) and Shannon's Information index (*I*) across the 45 wheat varieties level and the populations level

Populations	(*Na*)	(*Ne*)	(*H*)	*(I)*
Tetraploid population (4X)	2.0000±0.0000	1.4051±0.1428	0.2812±0.0750	0.4509±0.0924
Hexaploid population (6X)	2.0000±0.0000	1.4092±0.1537	0.2827±0.0756	0.4527±0.0922
Combined populations	2.0000±0.0000	1.4065±0.1353	0.2827±0.0699	0.4533±0.0852

(± standard deviations)

3.1.3 *Genetic diversity parameters*

Total gene diversity (H_T) for tertaploid, hexaploid population and within the all studied genotypes was 0.2812, 0.2827 and 0.2827 respectively (Table.4). Gene diversity (*Hs*) among populations tertaploid and hexaploid was 0.2756 and 0.2771 respectively, while that within populations was 0.2761. The coefficient of gene differentiation (*Gst*) was 0.0197, 0.0198 and 0.0233 for tetrapolid, hexaploid and within the two populations, respectively (Table 4). *Dst* for teteraploid population and hexaploid population showed same value 0.0056 while within populations was 0.0066. Gene flow (*Nm*) was 24.8393 and 24.7289 for tetrapolid and hexaploid population, respectively (Table 4) and was 20.9256 within the two populations. Results revealed high levels of gene flow which would account for low differentiation between populations. It should be noted that indirect estimates of *Nm* values must be interpreted with caution [48,29] and this data therefore should be viewed as general indicators of the magnitude of genetic exchange. Values of Coefficient of gene differentiation (*Gst*) range from zero to one, with low values indicating that little variation is proportioned among populations (high values denote that a large amount of variation is found among populations [49]. In present study the *Gst*-derived *Nm* value found to be 0.0233 within the two populations (*T. durum* and *T. aestivium*) indicated little differentiation, which means most percentage of the variation existed within populations. *Nm* was found to be high (20.9256) where *Nm* is the number of migrants per generation [50]. Different studies concerning wheat species showed that genetic diversity of domesticated crops is usually reduced compared to wild ancestors [51,52,53,54]. In tetraploid wheat, the population bottleneck that accompanied tetraploid emmer wheat domestication about 10,000 years ago [55] reduced nucleotide diversity by 30 to 50% in the A- and B-genomes, depending on the study and diversity measure used [53,56]. Diversity was further reduced in hexaploid wheat as a consequence of the polyploidy bottleneck resulting from hexaploid wheat speciation [56,57]. Different rates of gene flow from the ancestors of hexaploid wheat, tetraploid wheat for the A- and B-genomes and *Aegilops tauschii* for the D-genome [58,59] resulted in different levels of

diversity in hexaploid wheat genomes [57]. While diversity levels are similar in the A- and B-genomes, it is greatly reduced in the D-genome [56,57]. The D-genome also shows higher levels of LD than the A- and B-genomes [57,60].

Table 4. Means of genetic diversity at each wheat populations level (among tetraploid and hexaploid) and genotypes level (within the combined populations)

Among population	H_T	H_S	Gst	Nm	Dst
Tetraploid population	0.2812± 0.0056	0.2756± 0.0055	0.0197	24.8393	0.0056
Hexaploid population	0.2827±0.0057	0.2771±0.0055	0.0198	24.7289	0.0056
Combined populations	0.2827± 0.0049	0.2761± 0.0047	0.0233	20.9256	0.0066

H_T, Total gene diversity; *Hs*, Gene diversity within populations; *Gst*, Coefficient of gene differentiation (*Gst* =*Dst*/H_T); *Nm*, Gene flow, *Nm*= (1-*Gst*)/*4Gst*; *Dst*, diversity among populations (*Dst* = $H_T - H_S$).

3.2 ANOSIM

The analysis of similarities, results showed that *R* was equal to 0.9048 (*P<0.0001*) the high value of *R* indicated that the overall difference between groups were large and statistically significant, moreover all the most similar samples of genotypes are within the same groups or population. Analysis of similarities (ANOSIM) has been widely used for testing hypotheses about spatial differences and temporal changes in assemblages and particularly for detecting environmental impacts [61].

3.3 Cluster and Principle Coordinate Analysis

Cluster analysis based on Dice similarity index for the present studied wheat materials, created dendrogram which showed a very high goodness of fit of cluster analysis (*r* = 0.944). The wheat varieties from the four distinct regions were clustered according to SSR analysis into two main clusters, durum wheat varieties and bread wheat varieties (Fig. 1). The Egyptian bread wheat varieties were distinctly separated from the Greek's ones, whiles the Greek variety "Strymonas" and "Acheloos" were overlapped with Egyptian bread wheat varieties. The Cypriot wheat varieties (durum wheat) were dispersed in the dendrogram with the Greek and Italian durum wheat varieties (Fig. 1). Comparing two patterns using either similarity or distance measure is of great importance to many statistical pattern analysis problems [62]. The hexaploid wheat classification with cluster analysis generated with Dice's cooefficient in the present study is very similar to that constructed in our previous study based on the analysis of RAPD markers with Jaccard coefficient [63]. The Jaccard and Dice coefficients are very similar, even so that dendrogram topology will not differ. The only difference is in the branch lengths. Usually, there is a slight preference for the Dice coefficient, because this coefficient is the same as the Nei & Li coefficient, known to be the most suitable coefficient to determine genetic relatedness based upon DNA restriction fragment patterns [64]. The two-dimensional (PCOORDA) plot further validated the results of the dendrogram. A principal coordinate analysis was used to visualize the dispersion of genotypes among the tetraploid and hexaploid populatioin. The two-dimensional (PCOORDA) plot (Fig. 2) shows that the first principal coordinate accounts for 36.44% of total variation and separates the tetraploid population from the hexaploid population. The second principal coordinate (9.3095 %) of total variation) separated most individuals bread wheat of the Egyptian region from those of the other European one, but there were some overlap, with certain individuals from the Egyptian region grouping with those from Greek region.

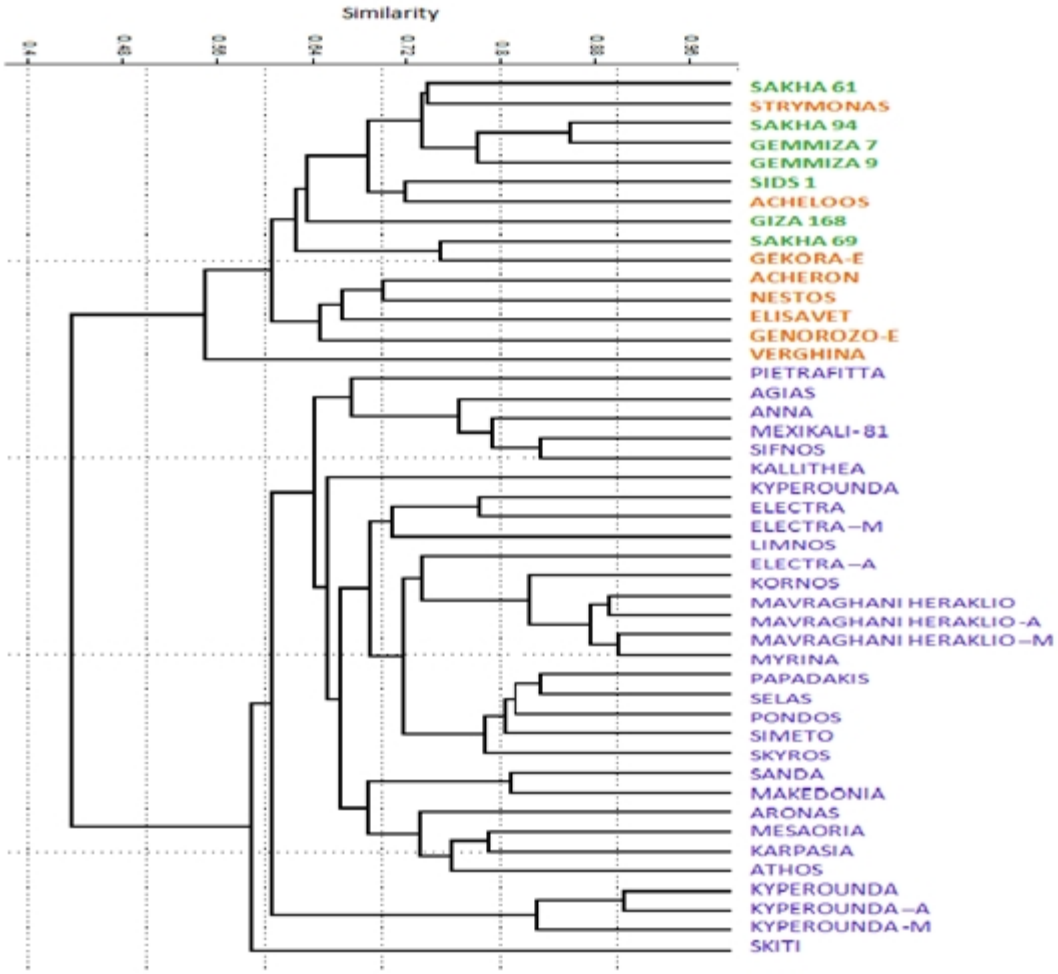

Fig. 1. Dendrogram of 45 wheat genotypes developed from SSR marker data using un-weighted pair group method of arithmetic means (UPGMA)

(Red and green color were referred to hexaploid European and Egyptian wheat genotypes respectively, while the blue one was referred to tetraploid).

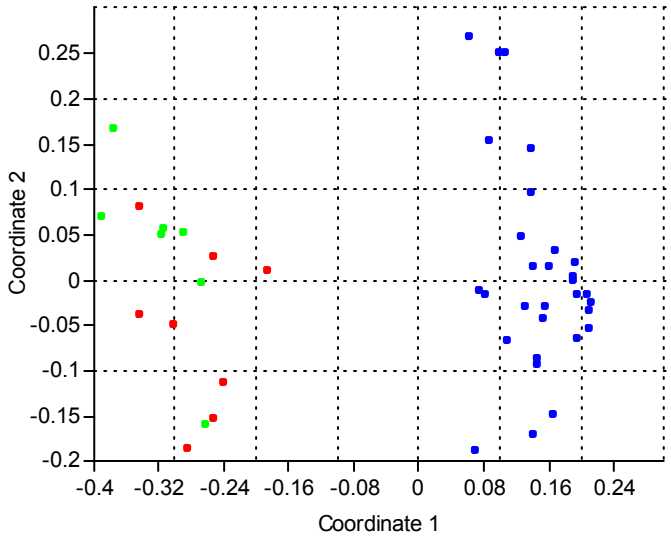

Fig. 2. Scatter plot of the first principal coordinates analyses matrix of SSR marker data

(Red and green color were referred to hexaploid European and Egyptian wheat genotypes respectively while the blue one was referred to tetraploid)

There are various statistics for molecular analysis of the 'genetic distance' between subgroups or populations. Major distance measures include Nei's distance [29,25], Reynold's distance [65]. This study indicates moderate genetic variability in the two populations sets of wheat, the results of Nei's gene diversity (*H*) for tetraploid population was 0.2812 and was 0.2827 for hexaploid population while Shannon's Information index also showed genetic diversity 0.4509 for the tetraploid population and 0.4527 for the hexaploid (Table 3). These results indicated that cultivars from different regions of *Triticum aestivum* and *Triticum durum* still *maintained* a considerable level of genetic diversity. Genetic Diversity of High and Low Molecular Weight Glutenin Subunits in Saharan Bread and Durum Wheat from Algerian Oases were studied by Bellil et al [66] they concluded that Saharan wheats from Algerian oases have extensive allelic variation in HMW-GS and LMW-GS, including new alleles. This indicates that Saharan wheats have a potential value in wheat breeding, and that further studies of their diversity are warranted. Results obtained from a study carried out by [67] on the using of AFLPs to determine phylogenetic relationships and genetic erosion in durum wheat cultivars released in Italy and Spain throughout the 20th century indicated that extent of genetic variability in Italian and Spanish durum wheat seems to have remained quite constant over the last century. They suggested that this constancy should be considered of qualitative relevance, as it indicates that cultivated pool was enriched by material different from the native and locally adapted Mediterranean germplasm, which resulted in a consistent broadening of the genetic background in these countries. The evaluation of the genetic bases and diversity of Egyptian wheat cultivars released during the last 50 years using coefficient of parentage carried out by Basnet et al [68]. They mentioned that the genetic base ranged from very low in pre1960's cultivars such as 'Giza 139' (with only 3 landraces in the background) to very high in modern cultivars such as 'Gemmeiza-7' (with 73 landraces in the background). 'Hindi-62', 'Red Fife', 'Hard Red Calcutta' and 'Akagomughi' were the major ancestors with 6, 5, 4, and 4% of total genetic contribution to the Egyptian wheat gene pool, respectively. Egypt, United

States of America, Kenya and Ukraine were the major source countries with 16, 11, 9 and 7% of total genetic contribution to this gene pool, respectively. Though Marquis-Thatcher germplasm from North America has the greatest influence on overall Egyptian cultivars, Mexican-based sources of dwarfing and high yield, derived from ancestors such as 'Akagomughi' and 'Daruma' and exploited by the International Maize and Wheat Improvement Center (CIMMYT), were very prominent in Egyptian cultivars post 1970's.

4. CONCLUSION

In conclusion, both Nei's diversity indices and Shannon's Index results led to the same conclusion that moderate genetic variability was found within and among populations. Applying the new genetic diversity software for updating and observing new genetic information about one from the most important crop like wheat would help breeders to design their breeding programs concerning durum and bread wheat.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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