

Molecular Assessment of Genetic Diversity among Egyptian Landraces of Wheat (*Triticum aestivum* L.) Using Microsatellite Markers

Ahmed Medhat Mohamed Al-Naggar^{1*}, Mohamed Abd El-Maboud Abd El-Shafi¹, Mohamed Helmy El-Shal² and Ali Hassan Anany²

¹*Department of Agronomy, Faculty of Agriculture, Cairo University, Giza, Egypt.*

²*National Gene Bank, Agricultural Research Centre, Giza, Egypt.*

Authors' contributions

This work was carried out in collaboration among all authors. Author AMMAN designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MAEMAES and MHES managed the literature searches. Author AHA managed the experimental process and performed data analyses. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2020/v3i430094

Editor(s):

(1) Dr. Carlos Henrique dos Anjos dos Santos, Instituto Nacional de Pesquisas da Amazônia, Brazil.

Reviewers:

(1) Manoj Kumar Yadav, SVP University of Agriculture & Technology, India.

(2) Kamila Łucja Bokszczyńska, Warsaw University of Life Sciences, Poland.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/56398>

Received 02 March 2020

Accepted 08 May 2020

Published 20 May 2020

Original Research Article

ABSTRACT

To increase the genetic progress in wheat (*Triticum aestivum* L.) yield, breeders search for germplasm of high genetic diversity, one of them is the landraces. The present study aimed at evaluating genetic diversity of 20 Egyptian wheat landraces and two cultivars using microsatellite markers (SSRs). Ten SSR markers amplified a total of 27 alleles in the set of 22 wheat accessions, of which 23 alleles (85.2%) were polymorphic. The majority of the markers showed high polymorphism information content (PIC) values (0.67-0.94), indicating the diverse nature of the wheat accessions and/or highly informative SSR markers used in this study. The genotyping data of the SSR markers were used to assess genetic variation in the wheat accessions by dendrogram. The highest genetic distance was found between G21 (Sakha 64; an Egyptian cultivar) and the landrace accession No. 9120 (G11). These two genotypes could be used as parents in a hybridization program followed by selection in the segregating generations, to identify some

*Corresponding author: E-mail: ahmedmedhatalnaggar@gmail.com;

transgressive segregates of higher grain yield than both parents. The clustering assigned the wheat genotypes into four groups based on SSR markers. The results showed that the studied SSR markers, provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat landraces. The analyzed wheat landraces showed a good level of genetic diversity at the molecular level. Molecular variation evaluated in this study of wheat landraces can be useful in traditional and molecular breeding programs.

Keywords: Landraces; molecular diversity; SSRs; PIC; UPGMA; dendrogram.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most commonly cultivated cereal crop in most parts of the world. It is used in the form of flour that provides one fifth of the global required calories. In 2017, the harvested area of wheat in Egypt was 1,342,805 ha, the annual consumption of wheat grains was about 19 million tons, while the local production was about 8.8 million tons with an average grain yield of 6.55 t/ha [1]. Therefore, the gap between annual local production and consumption is about 10.2 million tons. This gap could be narrowed by increasing local production of wheat, which could be achieved through the development of new high yielding cultivars. Yield plateaus of wheat were reported in Egypt [2] as well as in some European countries [3]. However, a significant increase in wheat yield will be required if demand from the growing human population, is to be met. The challenge in Egypt for wheat breeders is to increase the rate of genetic gain in yield at a rate not lower than the rate of growing human population.

For wheat breeders, to increase the genetic progress in yield, they search for germplasm of high genetic diversity, one of them is the landraces. A wheat landrace was defined by [4] as a traditional variety with a good tolerance to biotic and abiotic stresses. It has high stability, but shows moderate yield under poor environment. It is generally thought that during the process of wheat domestication, new adaptive traits suitable for the new environments were selected [5]. Probably traits such as easy harvest, large seeds, non-shattering plants were considered as main aims of the ancient farmers [6], or flowering time to fit with the prevailing environmental conditions of the region [7]. Many other characteristics had also been selected by farmers, such as plant height, number and weight of spikes and grains [5]. Wheat landraces cultivated in the Saharan oases have been subjected during centuries to drought, heat and salinity and are expected to have developed

tolerance to these stresses; most landraces may have been introduced from Egypt, possibly during wet climatic episodes [8].

Lack of information as well as the potential interest of wheat landraces for wheat improvement would deserve further studies. Marker-based diversity analysis of wheat landraces might permit to precise their classification and confirm their origin. The estimated genetic diversity has great importance for optimal utilization and conservation of germplasm for plant breeding and other activities [9]. So, it is necessary to investigate the genetic diversity in wheat germplasm in order to broaden the genetic variation for future breeding and genetic resource conservation program.

Knowledge of genetic diversity among adapted cultivars or elite breeding materials has a significant impact on the improvement of crop plants and this information has been successfully used for efficient germplasm management and genotype selection for different breeding purposes [10]. It was generally achieved in the past through the use of phenotypic, cytogenetic, and biochemical attributes, including isozyme patterns [11].

Recently, molecular markers are used to estimate genetic diversity. Molecular marker analyses overcome many of the limitations of morphological traits [12]. Simple sequence repeats (SSRs) are common, informative molecular markers used for genetic diversity studies because of their simplicity, high levels of polymorphism [13], high reproducibility, and co-dominant inheritance patterns [14]. These markers are chromosome-specific (often amplifying a single locus with multiple alleles), can be evenly distributed along different chromosomes [14] and can be used by researchers to tag useful genes. Numerous wheat SSR markers are available and many have been mapped to specific chromosome arms [14,15]. Consequently, SSR markers are excellent markers for genetic diversity analyses

and genotype identification in self-pollinated species such as wheat [16]. SSR markers have been used to estimate genetic diversity in wheat germplasm [13], elite lines [17-20] cultivars [11,21,22] and landraces [23-26].

The objectives of the present study were: (i) to characterize a subset of 20 Egyptian wheat (*Triticum aestivum* L.) landraces and two cultivars for their genetic diversity using SSR markers, (ii) to measure the genetic distance among these accessions using UPGMA cluster analysis.

2. MATERIALS AND METHODS

2.1 Plant Materials

Seeds of 20 bread wheat (*Triticum aestivum* L.) Egyptian landraces, obtained from the National Gene Bank, Agricultural Research Center (ARC), Egypt and seeds of Sakha 64 (an Egyptian cultivar) obtained from Wheat Research Department, Field Crops Research Institute, ARC and seeds of Yakora Kogo (a drought tolerant variety) obtained from CIMMYT (Table 1) were used in the present investigation.

2.2 SSR-Marker Analysis

The present SSR work was carried out in the laboratory of the National Gene Bank of the Agricultural Research Center, Giza, Egypt. Chemicals and kits used were obtained from Qiagen Company and Sigma (Santa Clarita, CA, USA). Ten simple sequence repeats (SSR) primer pairs were used to characterize the wheat samples at the DNA level (Table 2). The SSR primer information and chromosome location can be found on the U.S. Wheat and Barley Scab Initiative website https://scabusa.org/pdfs/BARC_SSRs_011101.html.

Genomic DNA was extracted separately from a randomly selected sample of eight plants representing each landrace or cultivar and bulked at equal quantities. Leaf samples were collected and stored immediately in (-80°C) ultra-freezer until the DNA extraction was carried out. The DNA was extracted using the Qiagen DNeasy plant mini kit (Cat No. 69104) according to the manual instructions. DNA quality was determined visually on 0.8% agarose gel. The DNA concentration was adjusted to 50 ng/μl by adding TE buffer.

Table 1. Wheat landraces and check varieties used in this study

Genotype no.	Accession no. in NGB	Landrace/ cultivar	Country of origin	Governorate
G 1	9226	Landrace	Egypt	Giza
G 2	9227	Landrace	Egypt	Giza
G 3	9234	Landrace	Egypt	Giza
G 4	9235	Landrace	Egypt	Giza
G 5	9236	Landrace	Egypt	Giza
G 6	9311	Landrace	Egypt	Giza
G 7	9331	Landrace	Egypt	Giza
G 8	9373	Landrace	Egypt	Giza
G 9	9361	Landrace	Egypt	Giza
G 10	9144	Landrace	Egypt	Giza
G 11	9120	Landrace	Egypt	Giza
G 12	9266	Landrace	Egypt	Giza
G 13	9286	Landrace	Egypt	Qalyubia
G 14	9287	Landrace	Egypt	Qalyubia
G 15	9222	Landrace	Egypt	Qalyubia
G 16	9290	Landrace	Egypt	Dakahlia
G 17	9150	Landrace	Egypt	Monufia
G 18	9293	Landrace	Egypt	Beheira
G 19	9243	Landrace	Egypt	Sharqia
G 20	9110	Landrace	Egypt	Gharbia
G 21	YakoraKojo	DT-Variety	CIMMYT/Mexico	-
G 22	Sakha 64	Cultivar	Egypt	-

DT=Drought tolerant, NGB=National Gene Bank, CIMMYT=International Maize and Wheat Improvement Center

Table 2. SSR marker name, sequence and annealing temperature (Ta) of the microsatellite markers used

No	Marker name	Sequence	Ta (°C)	
1	BARC-003	Forward	TTCCCTGTCTTTCTAATTTTTTTT	60
		Reverse	GCGAACT CCCGAACATTTTTAT	
2	BARC-004	Forward	GCG TGT TTG TGT CTG CGT TCT A	64
		Reverse	CAC CAC ACA TGC CAC CTT CTT T	
3	BARC-012	Forward	CGA CAG AGT GAT CAC CCA AAT ATA A	61
		Reverse	CAT CGG TCT AAT TGT CAA TGT A	
4	BARC-048	Forward	GCG AGC TGC AGA GGT CCA TC	64
		Reverse	GCG TTA GTC TTC TTG GTC AAT CAC	
5	BARC-052	Forward	GCG CCA TCC ATC AAC CGT CAT CGT CAT A	67
		Reverse	GCG AGG AAG GCG GCC ACC AGA ATG A	
6	BARC-066	Forward	CGC GAT CGA TCT CCC GGT TTG CT	65
		Reverse	GGG AAG AGG ACC AAG GCC ACT A	
7	BARC-072	Forward	CGT CCT CCC CCT CTC AAT CTA CTC TC	65
		Reverse	CGT CCC TCC ATC GTC TCA TCA	
8	BARC-074	Forward	GCG CTT GCC CCT TCA GGC GAG	60
		Reverse	CGC GGG AGA ACC ACC AGT GAC AGA GC	
9	BARC-078	Forward	CTC CCC GGT CAA GTT TAA TCT CT	64
		Reverse	GCG ACA TGG GAA TTT CAG AAG TGC CTA A	
10	BARC-079	Forward	GCG TTG GAA AGG AGG TAA TGT TAG ATA G	64
		Reverse	TGG GTT ACA AGT TTG GGA GGT CA	

The PCR reaction was carried out in a 0.2 ml thin-wall PCR tube. Amplification of the microsatellite markers was performed in a MyCycler–BioRad® thermo cycler machine. The thermocycling profile was as follows: An initial primary denaturation cycle at 94°C for 3 min; then: 35 cycles each comprised of: A denaturation step at 94°C for 15 sec, an annealing step for 30 sec and an extension step at 72°C for 1 min. The final extension step was extended to 10 min at 72°C. The amplification products were stored at 4°C.

The PCR products were checked by electrophoresis in 2% agarose gel (Sigma, USA) in 1X TBE running buffer containing ethidium bromide at 100 volts. SSR products were visualized on UV trans illuminator, and photographed using a Gel Documentation System (Alpha Innotech). The PCR products were separated on 8% polyacrylamide gels, (Serva 4X, Germany) according to the methodology described by Sambrook et al. [27]. Acrylamide solution (8%) was prepared. The glass plates were washed thoroughly with ddH₂O and detergent several times. The plates were then wiped with ethanol soaked tissues. The two glass plates were left to dry in a clean place away from the dust. Two spacers of 1.5 mm thickness were placed between the two glass plates like a sandwich. The two plates were assembled and clamped with clamps. The gel was poured between the two plates and the

comb was inserted in its place then left at room temperature for around 60 min until polymerization of the gel. At the end of the polymerization, the comb was removed gently. Then, the gel was placed in the electrophoresis apparatus (Amersham 16 cm x 18 cm, Ruby SE 600) and the whole system was assembled. The 1X TBE running buffer was added. The microsatellite PCR-products (5 µl) were loaded on the gel simultaneously with a DNA ladder. The electrophoretic separation was run at 120 volts for 2 hrs. The glass plates were gently detached keeping the polyacrylamide gel adhered to the lower one. The gel and its attached glass plate were gently submerged in the staining solution (0.5 µg / ml ethidium bromide in ddH₂O). After staining for 30 minutes at room temperature, the gel was removed, visualized and photographed on the Gel Documentation system (Alpha Innotech). Double distilled water was added up to 1000 ml and pH was adjusted to 8.3. To obtain 1X TBE buffer, the 10X solution was diluted to bring the concentration.

2.3 Statistical Analysis

The SSR banding pattern was scored as present (1) or absent (0) for each primer pair and cultivar/landrace combination. Scored SSR products included monomorphic markers, but only polymorphic bands were considered in the genetic analysis. A binary matrix was used to

estimate the genetic similarities (GS) between pairs of cultivars/landraces, by employing Nei and Li coefficient [28]. Genetic similarity between two genotypes within one locus was calculated using the formula $GS_{ij} = 2N_{ij} / (N_i + N_j)$, where N_i and N_j represent the total number of bands present in cultivars/landraces i and j , respectively, and N_{ij} refers to the total number of bands common to the same cultivars/landraces. Thus, GS_{ij} reflects the proportion of bands in common between two parents and may range from 0 (no common bands) to 1 (identical profiles for two bands). Genetic distance matrices were used to cluster cultivars/landraces using Agglomerative Hierarchical Clustering (AHC) analysis based on UPGMA and the results were used to construct dendrogram. All analyses of genetic diversity based on SSRs were performed by the XLSTAT [29] computer software program.

3. RESULTS

3.1 SSR Polymorphism

Ten SSR primers were screened for their ability to produce polymorphic patterns across the 22 wheat genotypes. They were repeatable and produced high resolution bands for all the genotypes and were selected for evaluation of genetic diversity in the accessions (Table 3 and Fig. 1). The ten SSR primers amplified a total of 27 bands in the set of 22 wheat landraces and cultivars, of which 23 bands showed polymorphism and 4 bands were monomorphic. Number of bands varied from two (five primers, namely BARC003, BARC048, BARC066, BARC078, BARC079) to four (two primers; namely BARC012 and BARC074). The percentage of polymorphic bands ranged

between 0 and 100 with an average of 85.2%. Mean numbers of bands and polymorphic bands per primer were 2.7 and 2.3, respectively (Table 3). The PIC values for the ten primers varied from 0.0 to 0.94 with an average of 0.67. Eight out of ten primers showed PIC values between 0.67 and 0.94. The lowest and PIC indices were recorded for primer BARC012 and BARC074, and the highest PIC indices were recorded for primer BARC004 (Table 3).

3.2 Genetic Similarity Coefficients Based on SSR

The genetic similarity binary coefficients based on SSR markers among the 22 wheat genotypes, ranged from 0.43 to 1.00 with an average of 0.71 (Table 4). Similarity binary distances showed that G2 (Accession No. 9227) and G13 (Accession No. 9286) were the most similar genotypes, the pair of genotypes G3 (Accession No. 9234) and G9 (Accession No. 9361) and the pair of genotypes G12 (Accession No. 9262) and G19 (Accession No. 9243), since they showed the highest similarity coefficient (1.00); so these pairs of genotypes are genetically the most related landraces. On the other hand, the most unrelated genotypes based on SSR data; i.e. those showed the lowest similarity coefficient (0.43-0.44), were the pair of genotypes G11 (Accession No. 9120) and G21 (YakoraKojo) (the well-known drought tolerant variety) followed by the pair of genotypes G22 (Sakha 64) and G3 (Accession No. 9234) and the pair of genotypes G22 (Sakha 64) and G9 (Accession No. 9361) and the pair of genotypes G22 (Sakha 64) and G17 (Accession No. 9150); they are the most unrelated land races in this experiment.

Table 3. Simple sequence repeats (SSR) primer combinations, total number of bands, number and percentage of polymorphic bands, average PIC per primer combination for 22 wheat genotypes

Markers	MW (bp)	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism (%)	PIC
BARC003	160-169	2	0	2	100	0.69
BARC004	151-181	3	0	3	100	0.94
BARC012	156-230	4	0	4	100	0.92
BARC048	171-192	2	2	0	0	0.00
BARC052	111-120	3	0	3	100	0.84
BARC066	106-136	2	2	0	0	0.00
BARC072	163-173	3	0	3	100	0.93
BARC074	153-178	4	0	4	100	0.88
BARC078	152-162	2	0	2	100	0.67
BARC079	148-162	2	0	2	100	0.80
Total	106-230	27	4	23		6.67
Average		2.7	0.4	2.3	85.2	0.67

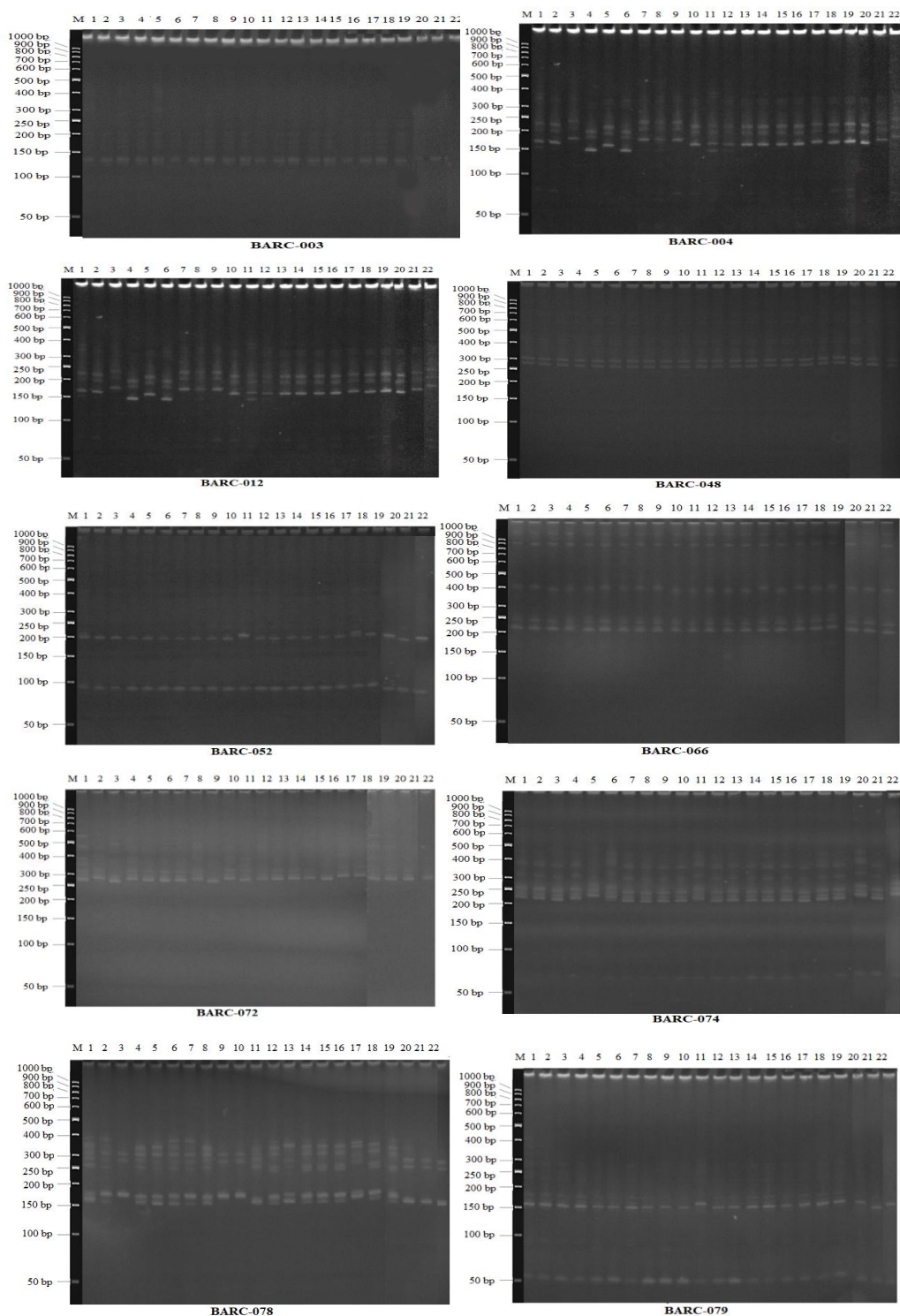


Fig. 1. Banding patterns of 22 wheat genotypes amplified with the SSR primers BARC-003, BARC-004, BARC-012, BARC-048, BARC-052, BARC-066, BARC-072, BARC-074, BARC-078 and BARC-079, M: 100bp DNA ladder, Lanes from 1 to 20: Landraces from No.1 to No. 20, Lane 21: Yakora, Lane 22: Sakha 64

Table 4. Genetic similarity coefficients based on SSR analysis among 22 bread wheat genotypes

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21
G1	1																				
G2	0.90	1																			
G3	0.60	0.69	1																		
G4	0.90	0.93	0.69	1																	
G5	0.71	0.80	0.76	0.73	1																
G6	0.71	0.67	0.83	0.73	0.80	1															
G7	0.65	0.73	0.97	0.73	0.80	0.87	1														
G8	0.81	0.71	0.80	0.77	0.77	0.84	0.84	1													
G9	0.60	0.69	1.00	0.69	0.76	0.83	0.97	0.80	1												
G10	0.87	0.97	0.71	0.90	0.76	0.62	0.69	0.67	0.71	1											
G11	0.77	0.67	0.48	0.67	0.47	0.60	0.53	0.58	0.48	0.62	1										
G12	0.84	0.80	0.76	0.73	0.87	0.80	0.80	0.90	0.76	0.76	0.60	1									
G13	0.90	1.00	0.69	0.93	0.80	0.67	0.73	0.71	0.69	0.97	0.67	0.80	1								
G14	0.90	0.87	0.69	0.80	0.80	0.73	0.73	0.84	0.69	0.83	0.67	0.93	0.87	1							
G15	0.77	0.87	0.83	0.80	0.93	0.80	0.87	0.84	0.83	0.83	0.53	0.93	0.87	0.87	1						
G16	0.87	0.83	0.71	0.76	0.83	0.76	0.76	0.87	0.71	0.79	0.62	0.97	0.83	0.97	0.90	1					
G17	0.80	0.90	0.71	0.83	0.69	0.62	0.69	0.60	0.71	0.93	0.62	0.69	0.90	0.76	0.76	0.71	1				
G18	0.88	0.84	0.60	0.77	0.65	0.65	0.65	0.69	0.60	0.80	0.77	0.77	0.84	0.84	0.71	0.80	0.87	1			
G19	0.84	0.80	0.76	0.73	0.87	0.80	0.80	0.90	0.76	0.76	0.60	1.00	0.80	0.93	0.93	0.97	0.69	0.77	1		
G20	0.76	0.71	0.52	0.64	0.71	0.64	0.57	0.69	0.52	0.67	0.71	0.79	0.71	0.79	0.71	0.74	0.59	0.69	0.79	1	
G21	0.55	0.64	0.74	0.64	0.79	0.71	0.79	0.76	0.74	0.59	0.43	0.71	0.64	0.64	0.79	0.67	0.52	0.48	0.71	0.62	1
G22	0.62	0.57	0.44	0.57	0.57	0.50	0.50	0.62	0.44	0.52	0.57	0.57	0.57	0.64	0.50	0.59	0.44	0.55	0.57	0.77	0.54

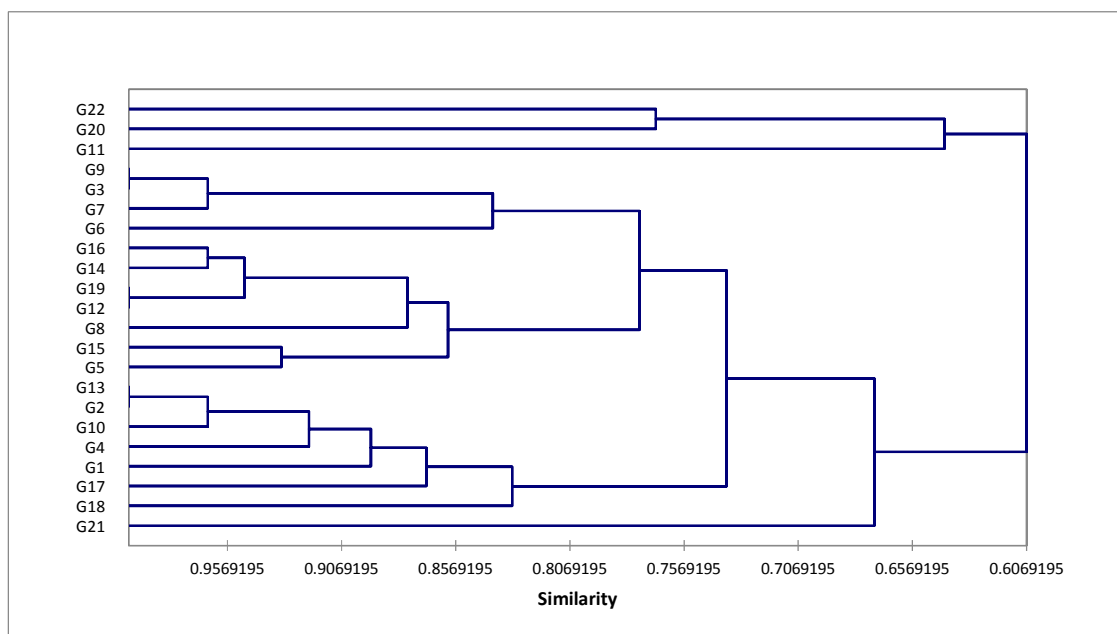


Fig. 2. Dendrogram of 22 bread wheat landraces and cultivars using SSR markers as per the average method of clustering

3.3 Genotype Identification by Unique SSR Markers

The SSR assay permitted the identification of one wheat genotype by unique positive and negative markers. The genotype G22 (the Egyptian cultivar Sakha 64) was characterized by one unique positive marker amplified by the primer BARC003 (160 bp) and one negative unique marker amplified by the primer BARC (169 bp).

3.4 Cluster Analysis Based on SSR Data

The clustering pattern of the studied wheat landraces and cultivars generated from the SSR data using complete linkage method is shown in Fig. 2. The analysis assigned the genotypes into four groups. Group 1 included only one genotype (G21) (Yakora Kojo). Group II included seven genotypes and was separated into two sub-groups; the 1st sub-group included four genotypes (G18, G17, G1, G4), and the second sub-group included three genotypes (G10, G2, G13); the two genotypes G2 and G13 were closely related.

The 3rd group comprised seven genotypes divided into four classes; the 1st class include two genotypes (G16 and G14), the 2nd class include two genotypes (G19 and G12), the third class

included one genotype (G8) and the 4th class included two genotypes (G5 and G15). The fourth group included four genotypes divided into two sub-groups; the 1st sub-group included three genotypes (G9, G3, G7) and the 2nd sub-group included one genotype (G6). The fifth group comprised three genotypes divided into two sub-groups; the 1st sub-group contained two genotypes (G20 and G22; the Egyptian cultivar Sakha 64) and the 2nd sub-group contained only one genotype (G11).

4. DISCUSSION

The potential interest as well as lack of information on wheat landraces for wheat improvement would deserve more investigations. Genetic diversity analysis of wheat landraces might permit to precise their classification and confirm their origin. The estimated genetic diversity of wheat landraces has great importance for optimal utilization of germplasm for plant breeding and other activities [9]. So, it is necessary to study the genetic diversity in wheat landraces in order to broaden the genetic variation of available germplasm for future breeding and conservation programs [10]. It is generally achieved through the use of morphologic traits, biochemical and molecular techniques.

In the present study, SSR markers have been used successfully to estimate genetic diversity in wheat landraces [23-26]. Ten SSR markers in our study revealed a high level of genetic diversity among the wheat accessions. The markers detected 2.3 polymorphic alleles per marker with an average polymorphism of 85.2%. Variable efficiencies of different marker systems for detecting DNA polymorphism in wheat have been reported. Joshi and Nguyen [30] observed 1.8 polymorphic alleles per RAPD marker among 15 wheat cultivars, while SSRs with 6.2 alleles/maker were more polymorphic. Nagaoka and Ogihara [31] detected 3.7 polymorphisms per ISSR marker, while Carvalho et al. [32] reported 12.9 polymorphic alleles per marker using 18ISSR markers in 48 wheat accessions. We detected a high level of polymorphism among the wheat landraces and cultivars using SSRs, indicating high efficiency of the marker technique to reveal genetic diversity in the case of wheat. Markers based on more infrequent tetranucleotide SSRs amplified few alleles in rice [33], while detected more polymorphism in Dent and Popcorn [34].

SSRs seems to be randomly distributed in the genome, and (GA) dinucleotide repeats are most abundant in plant species [35]. In the present study, the PIC values differed between 0.0 (markers BARC048 and BARC066) and 0.94 (marker BARC004) with an average of 0.67. The majority of the markers (8 out of 10) showed PIC values higher than the average (0.67-0.94) (Table 3).

The high values of PIC for the SSR markers could be attributed to the diverse nature of the wheat accessions and/or highly informative SSR markers used in this study. The PIC index has been used extensively in many genetic diversity studies [18,36].

Among pairs of genotypes, the most unrelated (diverged) pair of genotypes based on SSR analysis, Sakha 64 (the Egyptian commercial cultivar) and the landrace accession No. 9120 (G11) could be recommended to the Egyptian Wheat Breeding Program to be used as parents in a hybridization program followed by selection in the segregating generations, as an attempt to identify some transgressive segregates of higher grain yield and drought tolerance than their parents, since both of the two parents are widely related and showed high grain yield. Previous studies using SSR markers revealed high levels

of polymorphism. In a previous study [37], using a panel of 192 durum wheat genotypes (mainly Mediterranean landraces) genotyped with 44 SSR markers, found expected heterozygosity of 0.71. Similar results with SSRs have been reported in bread wheat [38-41]. The polymorphism of SSR loci detected in our study was consistent with data obtained in a previous study [42]. The simple sequence repeats (SSRs) represent the most suitable marker system in wheat [43] and have been successfully used to characterize genetic diversity in advanced wheat breeding materials [44], wheat landraces [23-26] and wheat cultivars [10,21,22].

The markers showed high PIC values of which confirm that SSR markers used in our study were highly informative, because PIC values higher than 0.50 indicate high polymorphism. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at specific locus [45].

Unique markers are defined as alleles that specifically identify an accession from the other by their presence or absence. The alleles that are present in one accession but not found in the others are termed positive unique markers (PUM), in contrast with the negative unique markers (NUM), which are absent in a specific genotype. These alleles could be used for genotype identification [2,19,20,46,47].

The number of alleles and the percentages of polymorphism found in this study are suitable for estimating genetic diversity when compared with other species that used SSR markers [2,47]. Using SSR analysis, we were able to identify two unique alleles associated with wheat genotypes. Further experiments need to be achieved to determine the linkage between the SSR markers used in the present study and gene(s) of wheat genotypes. The present results support the idea that SSR analysis can provide fast detection of SSR markers linked to wheat genotypes. These markers would help inbreeding programs of wheat.

Grouping of wheat genotypes *via* SSR markers into different clusters have relevance to the future breeding programs [22,48,49]. In the present study, The SSR analysis classified the landraces and cultivars into four groups. It was observed that the genotype G21 (Yakora Rojo); globally known as drought tolerant variety (obtained from CIMMYT) occupied one whole

group and was separated from the remaining genotypes (Fig. 2). The relatively related genotypes to this variety, based on SSR markers are G5, G7 and G15 with a genetic similarity of 79% (Table 4). On the contrary, the most the unrelated pair of genotypes based on SSR analysis was Sakha 64 (the Egyptian commercial cultivar) and the landrace accession No. 9120 (G11). The results of the present study clearly revealed significant molecular diversity of the Egyptian wheat landraces and cultivars. Therefore, these promising landraces could be potentially utilized for the introgression of adaptive traits, which may be found in extreme environments [50-53]. The distribution of landraces/cultivars into groups based on SSR analysis should quicken the usefulness of these data to wheat breeders [54,55].

5. CONCLUSION

Knowledge of the level of genetic diversity among landrace accessions is prerequisite for germplasm conservation and breeding programs. Landraces can be considered as likely sources of putatively lost variability and may provide new genes or alleles, which could be introgressed into modern varieties by hybridization. Our data showed substantial variation in microsatellite DNA polymorphisms among local bread wheat germplasm. Our study recommends that the landrace accession No. 9120 (G11) could be crossed to G21 (Sakha 64; an Egyptian cultivar) followed by selection in the segregating generations, to identify some transgressive segregates of higher grain yield than both parents. The information on genetic diversity of local landraces is very useful for better management of Egyptian bread wheat gene pool and genetic enhancement of cultivars in bread wheat breeding programs. Further investigations should be undertaken for collection, conservation, characterization and utilization of Egyptian bread wheat germplasm.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAOSTAT. Food and Agriculture Organization, Statistical Division; 2020. Available:<http://www.fao.org/faostat/en/#data/QC> (Accessed on 16 February 2020)
2. Al-Naggar AMM, Al-Azab KF, Sobieh SES, Atta MMM. Morphological and SSR assessment of putative drought tolerant M3 and F3 families of wheat. *British Biotechnology Journal*. 2015;6(4):174-190. Available:<https://doi.org/10.9734/BBJ/2015/15898>
3. Grassini P, Eskridge KM, Cassman KG. Distinguishing between yield advances and yield plateaus in historical crop production trends. *Nat. Commun*. 2013;4:1–11. Available:<https://doi.org/10.1038/ncomms3918>
4. Zeven AC. Landraces: A review of definitions and classifications. *Euphytica*. 1998;104:127–39. Available:<https://doi.org/10.1023/A:1018683119237>
5. Peng JH, Sun D, Nevo E. Domestication evolution, genetics and genomics in wheat. *Mol. Breed*. 2011;28:281–301. Available:<https://doi.org/10.1007/s11032-011-9608-4>
6. Fuller DQ. Contrasting patterns in crop domestication and domestication rates: Recent archaeobotanical insights from the old world. *Ann. Bot*. 2007;100:903–24. Available:<https://doi.org/10.1093/aob/mcm048>
7. Cockram J, Norris C, O’Sullivan DM. PCR-based markers diagnostic for spring and winter seasonal growth habit in barley. *Crop Sci*. 2009;49:403–10. Available:<https://doi.org/10.2135/cropsci2008.07.0398>
8. Zaharieva M, Ayana NG, Hakimi AAI, Misra SC, Monneveux P. Cultivated emmer wheat (*Triticum dicoccom Schrank*), an old crop with promising future: A review. *Genet Resour Crop Evol*. 2010;57:937–62. Available:<https://doi.org/10.1007/s10722-010-9572-6>
9. Uddin MS, Boerner A. Genetic diversity in hexaploid and tetraploid wheat genotypes using microsatellite markers. *Plant Tissue*

- Culture and Biotechnology. 2008;18(1):65-73.
Available:<https://doi.org/10.3329/ptcb.v18i1.3267>
10. Fufa H, Baenziger PS, Beecher BS, Dweikat I, Graybosch RA, Eskridge KM. Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. *Euphytica*. 2005;145:133–46.
Available:<https://doi.org/10.1007/s10681-005-0626-3>
 11. Al-Ashkar I, Alderfasi A, Ben Romdhane W, Seleiman MF, El-Said Rania A, Al-Doss A. Morphological and genetic diversity within salt tolerance detection in eighteen wheat genotypes. *Plants*. 2020;9(3):287.
Available:<https://doi.org/10.3390/plants9030287>
 12. Gupta PK, Varshney RK, Sharma PC, Ramech B. Molecular markers and their applications in wheat breeding. *Plant Breed*. 1999;118:369-390.
Available:<https://doi.org/10.1046/j.1439-0523.1999.00401.x>
 13. Huang XQ, Börner A, Röder MS, Ganai MW. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics*. 2002;105(5):699-707.
Available:[https://doi.org/10.1016/0168-9452\(93\)90038-2](https://doi.org/10.1016/0168-9452(93)90038-2)
 14. Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P. A microsatellite map of wheat. *Genetics*. 1998;149:2007–23. PubMed CAS Google Scholar
 15. Bryan GJ, Stephenson P, Collins A, Kirby J, Smith JB, Gale MD. Low levels of DNA sequence variation among adapted genotypes of hexaploid wheat. *Theor. Appl. Genet*. 1999;99:192–8.
Available:<https://doi.org/10.1007/s001220051224>
 16. Domini P, Law JR, Koebner RMD, Reeves JC, Cooke RJ. Temporal trends in the diversity of UK wheat. *Theor. Appl. Genet*. 2000;100:912–917 CrossRef Google Scholar
 17. Kamel AK, Al-Naggar AMM, Safwat G, Diab AA, Hussein MH. Molecular characterization of some Egyptian bread wheat genotypes. *Arab J. Biotech*. 2011;14(1):113–124.
 18. Najaphy A, Parchin RA, Farshadfar E. Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines. *Aust J Crop Sci*. 2012;6:326–32.
Available:<https://search.informit.com.au/documentSummary;dn=054770544732860;res=IELHSS>
 19. Al-Naggar AMM, Al-Azab KF, Sobieh SES, Atta MMM. Molecular analysis of new drought tolerant segregants selected from F2 populations of bread wheat crosses. *World Research Journal of Agronomy*. 2013;3(1):58-69.
Available:<http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000009>
 20. Al-Naggar AMM, Sobieh SES, Atta MMM, Al-Azab KF. Unique SSR markers for drought tolerance in newly-developed bread wheat mutants. *World Research Journal of Agronomy*. 2013;2(1):015-025.
Available:https://bioinfopublication.org/files/articles/2_1_2_WRJAG.pdf
 21. Bohn M, Utz HF, Melchinger AE. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. *Crop Sci*. 1999;39:228–37.
Available:<https://doi.org/10.2135/cropsci1999.0011183X003900010035x>
 22. Sharma V, Vaishali PK, Yadav MK, Pooran C. Assessment of genetic diversity among twenty Indian wheat (*Triticum aestivum* L.) cultivars using simple sequence repeat (SSR) markers. *Int J Curr Microbiol App Sci*. 2018;7(03):1708-1717.
Available:<https://doi.org/10.20546/ijcmas703.202>
 23. Lopes MS, Dreisigacker S, Peña RJ, Sukumaran S, Reynolds MP. Genetic characterization of the wheat association mapping initiative (WAMI) panel for dissection of complex traits in spring wheat. *Theor. Appl. Genet*. 2015;128:453–64.
Available:<https://doi.org/10.1007/s00122-014-2444-2>
 24. Gurcan K, Demirel F, Tekin M, Demirel S, Akar T. Molecular and agro-morphological characterization of ancient wheat landraces of Turkey. *BMC Plant Biol*. 2017;17(Suppl 1): 171.
Available:<https://doi.org/10.1186/s12870-017-1133-0>
 25. Al Khateeb W, Al Shalabi A, Schroeder D, Musallam I. Phenotypic and molecular variation in drought tolerance of Jordanian durum wheat (*Triticum durum* Desf.) landraces. *Physiol Mol Biol Plants*. 2017;23:311–9.

- Available:<https://doi.org/10.1007/s12298-017-0434-y>
26. Asmamaw M, Keneni G, Tesfaye K. Genetic diversity of Ethiopian durum wheat (*Triticum durum* Desf.) landrace collections as revealed by SSR markers. *Adv Crop Sci Technol.* 2019;07:1–9. Available:<https://doi.org/10.4172/2329-8863.1000413>
 27. Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: A laboratory manual.* 1989 No. Ed. 2. Cold Spring Harbor Laboratory Press, USA. 1989;1546.
 28. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci. USA.* 1979;76:5269–73. Available:<https://doi.org/10.1073/pnas.76.10.5269>
 29. Addinsoft. *XLSTAT statistical and data analysis solution.* Boston, USA; 2014. Available:<http://www.xlstat.com>
 30. Joshi CP, Nguyen HT. RAPD (Random amplified polymorphic DNA) analysis based intervarietal genetic relationships among hexaploid wheats. *Plant Sci.* 1993;93:95–103. Available:[https://doi.org/10.1016/0168-9452\(93\)90038-2](https://doi.org/10.1016/0168-9452(93)90038-2)
 31. Nagaoka T, Ogiwara Y. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor. Appl. Genet.* 1997;94:597–602. Available:<https://doi.org/10.1007/s001220050456>
 32. Carvalho A, Lima-Brito J, Maças B, Guedes-Pinto H. Genetic diversity and variation among botanical varieties of old Portuguese wheat cultivars revealed by ISSR assays. *Biochem. Genet.* 2009;47:276–94. Available:<https://doi.org/10.1007/s10528-009-9227-5>
 33. Blair MW, McCouch SR, Panaud O. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 1999;98:780–92. Available:<https://doi.org/10.1007/s001220051135>
 34. Kantety RV, Zeng X, Bennetzen JL, Zehr BE. Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Mol. Breed.* 1995;1:365–73. Available:<https://doi.org/10.1007/BF01248414>
 35. Steinkellner H, Lexer C, Turetschek E, Glössl J. Conservation of (GA)(n) microsatellite loci between *Quercus* species. *Mol. Ecol.* 1997;6:1189–94. Available:<https://doi.org/10.1046/j.1365-294X.1997.00288.x>
 36. Thudi M, Manthena R, Wani SP, Tatikonda L, Hoisington DA, Varshney RK. Analysis of genetic diversity in pongamia [*Pongamia pinnata* (L) Pierre] using AFLP markers. *J. Plant Biochem. Biotechnol.* 2010;19:209–16. Available:<https://doi.org/10.1007/bf03263342>
 37. Soriano JM, Villegas D, Aranzana MJ, Garcia del Moral LF, Royo C. Genetic structure of modern durum wheat cultivars and Mediterranean landraces matches with their agronomic performance. *PLoS ONE.* 2016;11(8):e0160983. Available:<https://doi.org/10.1371/journal.pone.0160983> PMID: 27513751
 38. Zhang D, Bai G, Zhu C, Yu J, Carver BF. Genetic diversity, population structure, and linkage disequilibrium in U.S. Elite winter wheat. *Plant Genome J.* 2010;3:117. Available:<https://doi.org/10.3835/plantgenome2010.03.0004>
 39. Hao C, Wang L, Zhang X, You G, Dong Y, Jia J. Genetic diversity in Chinese modern wheat varieties revealed by microsatellite markers. *Sci China, Ser C Life Sci.* 2006;49:218–26. Available:<https://doi.org/10.1007/s11427-006-0218-z>
 40. Chen X, Min D, Yasir TA, Hu YG. Genetic diversity, population structure and linkage disequilibrium in Elite Chinese winter wheat investigated with SSR markers. *PLoS One.* 2012;7:1–9. Available:<https://doi.org/10.1371/journal.pone.0044510>
 41. Rufo R, Alvaro F, Royo C, Soriano JM. From landraces to improved cultivars: Assessment of genetic diversity and population structure of Mediterranean wheat using SNP markers. *PLoS One.* 2019;14:e0219867. Available:<https://doi.org/10.1371/journal.pone.0219867>
 42. Royo CV, Rharrabti DY, Blanco RM, Moral VG, del LF. Grain filling of durum wheat

- under Mediterranean conditions. *Cereal Res. Commun.* 1998;34:1021–8.
DOI: 10.1556/CRC.34.2006.2-3.233
43. Hammer K, Filatenko AA, Korzun V. Microsatellite markers - A new tool for distinguishing diploid wheat species. *Genet. Resour. Crop Evol.* 2000;47:497–505.
Available:https://doi.org/10.1023/A:1008761928959
44. Dreisigacker S, Zhang P, Warburton ML, Van Ginkel M, Hoisington D, Bohn M. SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different mega environments. *Crop Sci.* 2004;44:381–8.
Available:https://doi.org/10.2135/cropsci2004.3810
45. DeWoody JA, Honeycutt RL, Skow LC. Microsatellite markers in white-tailed deer. *J. Hered.* 1995;86:317–9.
Available:https://doi.org/10.1093/oxfordjournals.jhered.a111593
46. Al-Naggar AMM, Hussein HAA, Hussien EHA, Sabry SRS, Gad Kh IM. Genetic diversity and bulked segregant analysis for earliness in bread wheat. *Arab J. of Biotech.* 2010;13(2):157-172.
47. Al-Naggar AMM, Abd El-Salam RM, Badran AEE, El-Moghazi Mai MA. Molecular differentiation of five quinoa (*Chenopodium quinoa* Willd.) genotypes using Inter-Simple Sequence Repeat (ISSR) markers. *Biotechnology Journal International.* 2017;20(1):1-12.
DOI: 10.9734/BJI/2017/37053
Available:https://www.researchgate.net/.../320935203
48. Saghai-Marooof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. USA.* 1984;81:8014–8.
Available:https://doi.org/10.1073/pnas.81.24.8014
49. Al-Ashkar I, Alderfasi A, Ben Romdhane W, Seleiman MF, El-Said RA, Al-Doss A. Morphological and genetic diversity within salt tolerance detection in eighteen wheat genotypes. *Plants.* 2020;9(287):1-21.
DOI: 10.3390/plants9030287
50. Gharib MAH, Salem AH, Ali MMA, Mansour E, Qabil N. Genetic variation and interrelationships among agronomic traits in Egyptian bread wheat landraces and local cultivars. *Zagazig J. Agric. Res.* 2019;46(6A).
51. Ali Y, Khan MA, Hussain M, Atiq M, Ahmad JN. An assessment of the genetic diversity in selected wheat lines using molecular markers and PCA-based cluster analysis. *Applied Ecology and Environmental Research.* 2019;17(1):931-950.
Available:http://dx.doi.org/10.15666/aeer/1701_931950
52. Al-Naggar AMM, Abd El-Shafi MAE, El-Shal MH, Anany AH. Evaluation of Egyptian wheat landraces (*Triticum aestivum* L.) for drought tolerance, agronomic, grain yield and quality traits. *Plant Archives.* 2020;20(Supplement 1):3487-3504.
53. Al-Naggar AMM, Abd El-Shafi MAE, El-Shal MH, Anany AH. Selection criteria and selection environment for drought tolerance of Egyptian wheat (*Triticum aestivum* L.) landraces. *Annual Research & Review in Biology.* 2020;35(2):25-40.
54. Eid M. Validation of SSR molecular markers linked to drought tolerant in some wheat cultivars. *J. Plant Breed. Genet.* 2018;06(03):95-109.
55. Al-Tamimi AJT, Al-Janabi AS. Genetic diversity among bread wheat genotypes using RAPD and SSR markers. *SABRAO Journal of Breeding and Genetics.* 2019;51(3):325-339.

© 2020 Al-Naggar et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/56398>