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

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**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 MHE 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 Lucja Bokszczanin, Warsaw University of Life Sciences, Poland.

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

**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
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 plateaux 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 codominant 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.

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.

| 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 Forward | Ta (°C) | Sequence Reverse |
|----|--------------|-----------------|---------|------------------|
| 1  | BARC-003     | TTCCCTGTCTTTCTAA | 60      | TTTTTTTTT        |
|    |              | GCGAACT CCGAACATTTTAT |       |                  |
| 2  | BARC-004     | GCG TGT TTG TGT CGT TCT A | 64      | CAC CAC ACA TGC CAC CTT CTT T |
|    |              | CAT CCG TCT AAT TGT CAA TGT A |       |                  |
| 3  | BARC-012     | CGA CAG AGT GAT CAC CCA AAT ATA A | 61      | CAT CGG TCT AAT TGT CAA TGT A |
|    |              | CAT CGG TCT AAT TGT CAA TGT A |       |                  |
| 4  | BARC-048     | GCG AGC TGC AGA GGT CCA TC | 64      | GCG TTA GTC TTC TTG GTC AAT CAC |
|    |              | GCG TTA GTC TTC TTG GTC AAT CAC |       |                  |
| 5  | BARC-052     | GCG CCA TCC ATC AAC CGT CAT CGT CAT A | 67      | GCG AGG AAG GCG GCC ACC AGA ATG A |
|    |              | GCG CCA TCC ATC AAC CGT CAT CGT CAT A |       |                  |
| 6  | BARC-066     | CGC GAT CGA TCT CCC GGT TTG CT | 65      | GCG AGG AAG GCG GCC ACC AGA ATG A |
|    |              | GCG AGG AAG GCG GCC ACC AGA ATG A |       |                  |
| 7  | BARC-072     | CGT CCT CCC CCT TTC CTC AAT CTA CTC TC | 65      | GGG AAG AGG ACC AAG GCC ACT A |
|    |              | CGT CCT CCC ATC GTC TCA TCA |       |                  |
| 8  | BARC-074     | GCG CTT GCC CCT TCA GGG GAG | 60      | CGC GGG AGA ACC ACC AGT GAC AGA GC |
|    |              | CGC GGG AGA ACC ACC AGT GAC AGA GC |       |                  |
| 9  | BARC-078     | CTC CCC GGT CAA GTT TAA TCT CT | 64      | GCG ACA TGG GAA TTT CAG AAG TGC CTA A |
|    |              | GCG ACA TGG GAA TTT CAG AAG TGC CTA A |       |                  |
| 10 | BARC-079     | GCG TTG GAA AGG AGG TAA TG T TGT TAG ATA G | 64      | GCG TTG ACA AGT TTG GGA GGT CA |
|    |              | GCG TTG 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 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 GSij = 2Nij / (Ni + N j), where Ni and N j represent the total number of bands present in cultivars/landraces i and j, respectively, and Nij 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 landraces 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                    | 85.2         | 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 | 0.64 | 0.64 | 0.50 | 0.59 | 0.44 | 0.55 | 0.57 | 0.77 | 0.54 |
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 sup-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/marker 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 indistinguishing 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.

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