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DNA Fingerprinting and Genetic Diversity Analysis of some Egyptian Rice Genotypes

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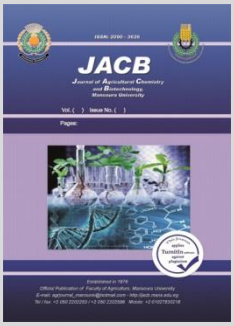

ABSTRACT

A new rice variety takes about 12 years to release, the number of available distinct morphological features is very small, due to the continual pressure of releasing new varieties. The objectives of this study were: to estimate the genetic variation of Egyptian japonica, indica, and indica/japonica genotypes, and to identify, DNA fingerprinting with employing simple sequence repeat (SSR) markers to develop DNA barcode. This study includes 15 rice genotypes that were genetically evaluated using 32 microsatellites. DNA barcodes as well as the unique pattern of SSR polymorphism were produced. A total of 116 alleles was detected and the number of alleles ranged from 2 to 5, with an average of 3.625 alleles per locus. The polymorphism information content (PIC) introduced a mean of 0.566 and there was a positive correlation between the highest value of PIC and the highest number of detected alleles by SSRs. The principal coordinate analysis, specifically, PC1 and PC2, explained 54.72% and 12.96% of the total variability, respectively, for the 32 SSR. One set of multiplex assay with five markers each (RM307, RM317, RM470, RM412 and RM242) was developed for all the 15 rice genotypes. The findings revealed that increasing genetic diversity in the national rice breeding programme requires introducing germplasm from other places with various genetic backgrounds. Furthermore, the identification, DNA barcode approach and certification of genotypes utilizing microsatellite markers could be a decent supplement to existing agro-morphological features when genotypes are firmly related.

***Keywords*:** Genetic variation, DNA barcode, SSR, identification and variety certification

INTRODUCTION

Rice (Oryza sativa L.) is an important cereal crop that feeds more than one third of the world's population (Sarangi *et al.*, 2019). It is an indispensable food crop in the world and grows in temperate and tropical regions of the world. This means that more than half of the world's population depends on the production and supply of rice, which ensures food and nutrition security (Singh *et al.*, 2019). Because land is limited and unlikely to expand, the adoption of high-yielding and enhanced varieties is the only way to increase rice productivity. However, the success of these high-yielding and improved varieties depends to a large extent on the availability of high-quality seeds with better genetic purity requirements, that is essential to complaining the genetic benefits accumulated through plant breeding efforts (Agarwal *et al.*, 1999).

In addition, the characteristics of genetic populations and varieties are mandatory for clearly identifying genetically very close varieties, protecting intellectual property rights and granting plant breeders rights, except to prevent unauthorized commercial use of seeds (Bora *et al.*, 2016). Consequently, preserving seed purity continues to be an important element of crop improvement for seed production and reproduction. For breeding programs, identifying and certifying varieties and determining their relationship is important as a way to defend their quality and royalties. Recognition through morphological traits has constantly been one of the first tools to discover types and species (IRRI, 1965). Thus far, morphological characterization has been powerful in figuring out genetic material; but, this approach is inefficient for differentiation of commercial varieties (Rahman *et al.*, 2009), due to the fact the morphological traits of commercial varieties are greater consistent and the quantity of available morphological capabilities could be very small (Islam *et al.*, 2011). The morphological descriptors generally used for species identity are selective to environmental factors and might not completely meet the requirements. In addition, since it is necessary to record the morphological observations of the entire crop growing season, more time is required. Due to the masking effect of G X E interaction, breed recognition based on the morphological descriptor process is empirical and sometimes illusory (Bora *et al.*, 2016). Past research on rice has facilitated the development of hundreds of microsatellite markers and genetic maps containing so many markers for recording and characterization. SSR molecular markers are simple, fast, reproducible, abundance and high polymorphism detection rate (Wang *et al.*, 2014). DNA fingerprinting offers an efficient and accurate approach for the identity of rice varieties. the development of DNA fingerprints is of extremely good importance for rice breeding, identity, local species variety monitoring and seed management (Song *et al.*, 2016). The United States, Japan, and South Korea have constructed DNA fingerprints of rice varieties. The SSR-based "DNA barcode" is a completely unique SSR polymorphism pattern that allows to discover varieties with excessive accuracy, that is stable and not affected by the environment, epistatic interactions and pleiotropic results (Satturu *et al.*, 2018). The SSR multiple panel presents a powerful tool for rice genetic evaluation and a

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quick and effective choice for rice germplasm characterization (Pessoa-Filho *et al.*, 2007).

Therefore, the purpose of this study is to: 1) determine the genetic diversity of some Egyptian japonica, indica, and indica/japonica genotypes as well as an exotic rice genotype, and 2) identify DNA fingerprinting with employing simple sequence repeat (SSR) markers to develop DNA barcode approach for the studied genotypes.

MATERIALS AND METHODS

Plant material:

Fifteen rice genotypes have been used within the current study, which correspond to most of the presently distributed Egyptian commercial cultivars and some promising lines, which includes the temperate japonica, indica, and indica/japonica genotypes, in addition to an exotic indica genotype. The seeds were provided by the Egyptian Rice Germplasm Unit (ERGU), at the Rice Research Department, Field Crops Research Institute, Agricultural Research Center, Sakha, Egypt. The name, pedigree and characterization of the studied genotypes are shown in Table 1. All possible data collected during the 2019 and 2020 rice growing seasons from the experimental field which located at Sakha Research Station, Kafr El-Sheikh, Egypt.

Table 1. Characteristics of the studied rice genotypes and their pedigree and type

Genotypes	Pedigree	Type	DH	PH	PL	PT	FG	1000-GW	GY	RL	RTH	RN
Giza 177	Giza171/Yamji No.1//Pi No.4	J	91.5	104.4	21.5	15.5	148.5	28.6	37.5	24.5	0.64	240.6
Giza 178	Giza175/Milyang49	I/J	102.2	98.2	21.02	22.15	180.24	21.7	42.02	33.4	0.71	290.2
Giza 179	GZ6296-12-1-2-1/GZ1368-S-5-4	I/J	85.32	101.15	22.05	22.5	190.3	22.78	42.8	35.5	0.75	340.8
Sakha 101	Giza176/Milyang79	J	109.45	92.65	22.6	21.05	159.15	29.12	40.3	25.8	0.62	238.4
Sakha 102	GZ4096-7-1/GZ4120-2-5-2	J	92.2	107.2	23.45	17.1	150.54	28.9	38.75	22.65	0.6	225.7
Sakha 104	GZ4096-8-1/GZ4100-9	J	102.5	110.25	22.75	20.56	160.58	28.67	40.8	26.75	0.69	326.2
Sakha 105	GZ5581/GZ4316	J	91.5	102.6	22.25	17.85	140.61	29.1	37.7	23.25	0.58	245.4
Sakha 106	Giza177/Hexi30	J	93.5	106.5	23.25	17.6	152.3	29.2	38.2	24.2	0.63	266.2
Sakha 107	Giza177/BL1	J	98.5	108.5	22.78	18.1	145.6	27.5	37.9	27.4	0.7	295.6
SKC2015	GZ6214/Giza177	J	91.2	96.5	22.58	18.1	138.4	28.3	36.9	25.65	0.64	273.4
GZ9730-1-1-1-1	Giza159/Milyang23	J	99.2	95.92	23.15	19.7	164.5	28.5	40.04	24.02	0.67	268.3
GZ9626-2-1-3-2	GZ7185-7-4-3-1/Nam Jing 70272	J	94.5	99.6	21.2	19.2	162.25	26.7	38.9	25.8	0.66	291.4
GZ6296-12-1-2-1	AC1225/Hun Lien Yu 202	I/J	97.83	95.3	23.2	19.7	170.38	24.4	40.7	30.61	0.72	315.7
IET1444	TN 1 / CO 29	I	98.82	110.54	24.02	21.2	175.87	24.5	40.5	31.8	0.74	410.2
GZ1368-S-5-4	IR 1615-31 / BG 94-2349	I	108.1	112.52	24.5	23.1	168.9	25.5	41.85	29.23	0.68	325.4
LSD at 0.05			1.02	1.25	0.9	1.18	4.85	0.77	1.18	0.91	0.01	5.05
LSD at 0.01			1.62	1.68	1.15	1.58	6.52	1.03	1.58	1.43	0.03	7.24

Japonica: (J), Indica (I); Indica / Japonica (I/J); DH, days to 50% heading; PH, plant height; PL, panicle length; PT, productive tillers/plant; FG, filled grains/panicle; 1000 GW, 1000-grain weight; GY, Grain yield/plant; RL, root length; RTH, root thickness and RN, root numbers.

Genomic DNA Extraction

In petri dishes, breeder seed of fifteen rice genotypes was allowed to germinate. Leaf samples were taken from single plants of 21-day-old seedlings, and genomic DNA was extracted using Murray and Thompson's CTAB (Cetyl Tri Methyl Ammonium Bromide) method (1980).

PCR Conditions and Allele Size Determination

A set of 40 primers distributed among 6 chromosomes (chr. 4, 5, 6, 7, 8 and 9) have been initially screened for selection and 32 out of 40, displaying higher degrees of polymorphism have been selected (Table 2). The thirty- two primers have been used for genetic characterization of the fifteen studied genotypes, those primer sequences were received from the Gramene database (http://www.gramene.org/ markers). PCR amplification was carried out in 20 ml reaction mixtures, each containing 20 ng of template DNA, 0.13 mM of each primer, 250 mM of each dNTPs, 1X PCR buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl, 2 mM MgCl2, 1% Triton X-100, 1% BSA) and 1 U of Taq DNA polymerase. An MJ Research (PTC-100 TM 96V) thermocycler was used along with the following PCR profile: an initial denaturation step of 3 min at 94° C, followed by 30 cycles of 30 sec. at 94° C, 45 sec. at 48-65° C according to used primer, 1 min at 72° C, and a final extension at 72° C for 5 min. The PCR products were resolved on a 3% agarose gel followed by silver staining following the standard protocol (Temnykh *et al.*, 2000). After air drying, silver-stained gels were scanned to obtain digital images. The migration of the amplified band relative to the 50 bp DNA ladder was used to assess the allele size of each SSR locus (Invitrogen Corp., CA, USA). The sizes of the amplified fragments were then measured using the Bio-Rad Molecular Imager Gel Doc XR System and a 50 bp DNA ladder (NEB) as a size standard.

Microsatellite Markers and DNA Bar Coding/Finger Printing

The 32 primers were chosen for this study because of their capacity to differentiate the 15 genotypes under investigation as well as their readability within the banding pattern. They were also employed to generate DNA fingerprinting and develop single-tube multiplex assays. By sorting the allele size data from the smallest to the largest, DNA barcodes were created from the allelic variation data. Out of 32 markers, five multiplex marker assays were established for the studied rice genotypes. These five primers

namely; RM 307, RM 317, RM 470, RM 412 and RM 242 were closely linked to QTLs controlling yield attributes, abiotic stress and root traits and these could be responsible for differentiation among the studied genotypes. All five markers had been previously mapped to chromosomes 4, 6 and 9 on the Cornell Rice SSR 2001 map (Temnykh *et al.,* 2001) as presented at Table 2. DNA barcodes have been created for every of those multiplex assays to offer a visible illustration of the information that aids inside the accurate identity of varieties.

Table 2. Some major published QTLs detected in rice chromosomes, 4, 6 and 9 which are responsible for differentiation among the studied genotypes (Gramene data base [a]).

No.	Marker name	Chr No.	Published symbol	Trait symbol	Trait name	Trait category
1	RM307	4	QPl4	PNLG	panicle length	Anatomy
			qCTS4-1	COLDTL	cold tolerance	Abiotic stress
			ph4.1	PTHT	plant height	Vigor
			QDg4a	CHLCN	chlorophyll content	Biochemical
2	RM317	4	qRTL4-2	RTLG	root length	Anatomy
			QSnp4a	SPKNB	spikelet number	Yield
			qCTS4-2	COLDTL	cold tolerance	Abiotic stress
			qSD-4	SDDOR	seed dormancy	Vigor
			qby4.1	TBIOMYLD	total biomass yield	Yield
			qpn4.2	PNNB	panicle number	Yield
			qFER-4	SPKFRT	spikelet fertility	Sterility
			qYI-4	GRYLD	grain yield	Yield
			qPL-4	PNLG	panicle length	Anatomy
			QDg4b	CHLCN	chlorophyll content	Biochemical
			Qrga4	LFSNS	leaf senescence	Development
			qCTS4-2	LFRLTL	leaf rolloing tolerance	Abiotic stress
			qCTS4-3	LFYTL	leaf yellowing tolerance	Abiotic stress
			ph4.1	PTHT	plant height	Vigor
3	RM470	4	qRTL4-1	RTLG	root length	Anatomy
			qNOP-4	PNNB	panicle number	Yield
			qNOS-4-1	SPKNB	spikelet number	Yield
			ph4.1	PTHT	plant height	Vigor
4	RM412	6	RDW	RTDWT	root dry weight	Vigor
			BI	RTBR	root branching	Anatomy
5	RM242	9	qRTT9-1	RTTH	root thickness	Anatomy
			qALSRL-9	RTLG	root length	Anatomy
			qRA9-1	RTACT	root activity	Vigor
			qSW9-1	SDWT	seed weight	Yield
			yd9	GRYLD	grain yield	Yield
			qFL9-1	LFAR	leaf area	Anatomy
			qGW9-1	TGRWT	1000-grain weight	Yield
			qCTB9	COLDTL	cold tolerance	Abiotic stress
			gpy-9	GRNPTLYLDFQ	green plantlet yield frequency	Vigor
			pl9	PNLG	panicle length	Anatomy
			spp9.1	SPKNB	spikelet number	Yield

[*] **available on http://www.gramene.org/**

Data analysis:

The polymorphic alleles detected through the 32 simplified SSRs have been analyzed using the power Marker program v.3.25 (Lui and Muse, 2005), which allowed the calculation of the subsequent diversity parameters: allele number, common alleles frequency, unique alleles, genetic diversity (He), and polymorphism information content (%) of rice microsatellites. on the way to convert information into genetic distance, the Roger coefficient was used to degree genetic similarity between pairs, which became described because the square root for the only supplement of the similarity (√ 1-S). As a grouping approach, metric multidimensional scaling (MDS) turned into used to analyze the distance matrix. The unweighted pair group technique with arithmetic mean (UPGMA) method became employed in a hierarchical clustering technique (Weir, 1990). The data turned into statistically analyzed the usage of the information Gen version 2013 FCA statistics program (Balzarini and Di Rienzo, 2013).

RESULTS AND DISCUSSION

Genetic diversity parameters for studied genotypes

The assessment of genetic diversity is a crucial aspect in issue of the characterization and conservation of germplasm. The received results from the evaluation of genetic diversity based on the DNA level might be applied to create efficient breeding programs aimed at increase the genetic bases of commercially cultivated varieties. Of the whole 40 SSRs evaluated, 32 were able to genetically differentiate the 15 rice genotypes. The choice of these SSRs depended on their reproducibility, multi-allelism, and sharpness of their bands. Eighty percent of the amplified primers in this study were successful in detecting genetic variations. From the data presented in Table 3 it could notice that, a total of 116 alleles, with a mean of 3.625 alleles per locus, were detected in the 15 rice genotypes that were analyzed by 32 polymorphic SSR primers. The most polymorphic loci were RM3471 (5 alleles), RM480 (5 alleles), RM1328 (5 alleles) and RM 5535 (5 alleles). The highest polymorphic allele frequency was 0.667 produced by ten SSR markers under this study such as RM307, RM317 and RM470 and the lowest allele frequency was 0.067 produced by 11 SSR primers. For all 32 SSR primers, the general average allele frequency was 0.585. The set of microsatellites utilized in the study displayed a lower mean of alleles (3.625) than the 4.4 alleles detailed by Bonow *et al.,* (2009). El-Refaee *et al.,* (2011) studied genetic diversity in relation to agronomic parameters and biotic stresses in different

Egyptian rice genotypes using SSR markers. They revealed that the average number of detected alleles was 6.08 per locus with 12 SSRs on 22 Egyptian genotypes included japonica, indica and indica/japonica types. In general, the number of alleles found was smaller than that reported in the literature.

In some genotypes, a total of 19 distinct alleles were discovered, with unique alleles defined as those with a frequency of less than or equal to 0.067 Because polymorphic microsatellites markers produce varied banding patterns depending on genotype, those that show different bands could be used to identify a specific variety, such as RM7631 on chromosome 8, which indicated a unique allele for Giza 179. (figure 1-A). In Giza 179, RM518 displayed an allele (figure 1-B) that was identical to the allele identified in its closed parental genotypes, which are considered the most tolerant commercial genotypes under abiotic stress conditions (Giza 178, GZ6296 and GZ1368). RM 470, which located on chromosome 4, was found to be able to distinguish between the japonica, indica/japonica, and indica of studied rice genotypes (Fig 1-C). In future research, this information will be valuable in differentiating rice subspecies.

The genetic diversity mean was 0.608 for all the analyzed loci, while the highest genetic diversity was detected by RM1328 (0.769). The mean PIC value was 0.566, which demonstrated the proper polymorphism information content (PIC) for the whole sample, since, the greatest PICs were found for the most informative SSRs (Table 3). PIC is a useful measure of the polymorphism level of the genotypes under study; values approaching 1 suggest a high degree of genetic diversity and are related with a large number of alleles, whilst values less than 0.5 indicate a low polymorphism level. Results showed a mean PIC of 0.566, which indicates that most of the materials evaluated were more less quite similar. Another rice study showed a higher PIC value (0.86) with 7 SSRs on Indian rice varieties (Rahman *et al.*, 2010) and 0.73 using 12 SSR on 192 Brazilian rice varieties (Brondani *et al.*, 2006). Due to the excessive selection stress and selection procedure utilized in breeding programs, genetic material is known to be more uniform. In intense instances, the repeated use of parental genotypes has contributed even extra to decreasing the variation of genetic materials. Given this situation, it is essential to introduce germplasm from other areas with diverse genetic backgrounds into the national rice breeding program to enhance genetic diversity.

Table 3. Set of evaluated microsatellite primers for identity and certification of some Egyptian rice genotypes and their basic features

No.	Marker	Chr.	Exp. Pro. size	Repeat Motif	No. of alleles	Major alleles frequency	Minor alleles frequency	unique band	Genetic Diversity	PIC
1	RM252	4	190	(CT)19	4	0.533	0.133	0	0.653	0.640
2	RM307	4	170	(AT)14 (GT)21	4	0.667	0.067	2	0.547	0.507
3	RM317	4	155	(GC)4 (GT)18	3	0.667	0.133	0	0.524	0.498
4	RM335	4	110	(CTT)25	4	0.333	0.133	0	0.747	0.720
5	RM470	4	105	(CTT)14	4	0.667	0.067	1	0.542	0.516
6	RM518	4	170	(TC)15	4	0.333	0.133	0	0.751	0.729
7	RM1388	4	220	(AG)46	4	0.533	0.067	1	0.644	0.622
8	RM3471	4	140	(CT)21	5	0.600	0.067	2	0.613	0.596
9	RM3536	4	130	(GA)12	3	0.667	0.067	1	0.511	0.480
10	RM3708	4	160	(GA)15	4	0.600	0.067	1	0.600	0.578
11	RM17377	4	160	(AG)25	4	0.667	0.067	1	0.551	0.516
12	RM8213	4	175	(TC)10	4	0.533	0.133	0	0.676	0.640
13	RM169	5	155	(GA)12	4	0.667	0.067	1	0.538	0.516
14	RM249	5	120	(AG)5A2 (AG)14	4	0.600	0.067	1	0.627	0.600
15	RM440	5	170	(CTT)22	4	0.533	0.133	0	0.662	0.640
16	RM480	5	215	(AC)30	5	0.600	0.067	3	0.640	0.587
17	RM340	6	160	(CTT)8T3 (CTT)14	2	0.667	0.333	0	0.484	0.444
18	RM412	6	190	(GA)22	2	0.667	0.333	0	0.516	0.444
19	RM541	6	150	(TC)16	3	0.667	0.133	0	0.609	0.498
20	RM6467	6	110	(GCC)8	3	0.667	0.133	0	0.569	0.498
21	RM19620	6	160	(GTG)7	4	0.533	0.067	1	0.702	0.622
22	RM427	7	185	(TG)11	3	0.667	0.133	0	0.542	0.498
23	RM447	8	110	(CTT)8	4	0.600	0.067	1	0.640	0.578
24	RM556	8	105	(CCAG)6	2	0.667	0.333	0	0.516	0.444
25	RM6976	8	150	(TTC)15	2	0.667	0.333	0	0.480	0.444
26	RM7631	8	120	(TTCT)6	3	0.667	0.067	1	0.520	0.480
27	OSR28	9	170	(AGA)n	3	0.333	0.333	0	0.720	0.667
28	RM105	9	130	(CCT)6	4	0.600	0.067	1	0.649	0.578
29	RM242	9	210	(CT)26	4	0.533	0.133	0	0.680	0.640
30	RM1328	9	200	(AG)20	5	0.400	0.133	0	0.769	0.747
31	RM3912	9	205	GT)22	3	0.667	0.133	0	0.529	0.498
32	RM5535	9	150	(TG)13	5	0.533	0.067	1	0.698	0.649
			Total		116	18.733	4.267	19	19.449	18.111
			Average		3.625	0.585	0.133	0.594	0.608	0.566

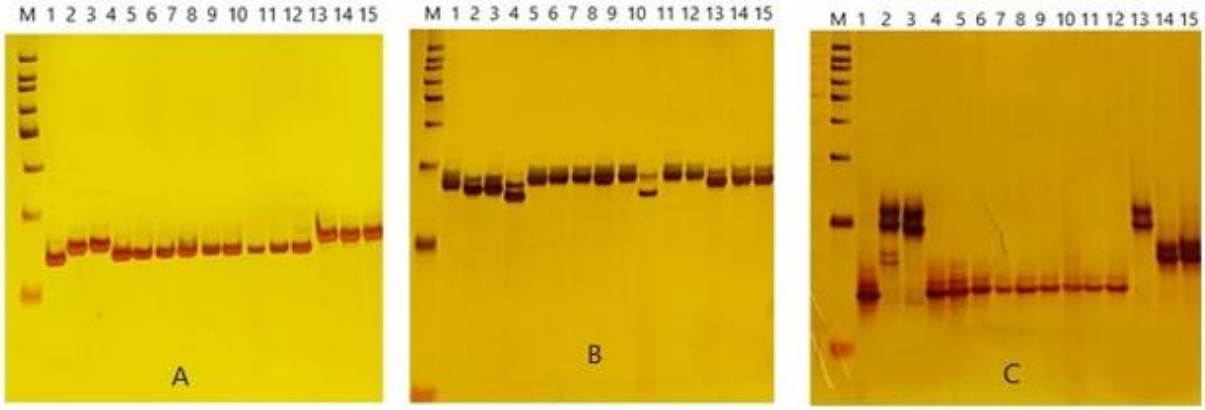

Figure 1. Banding pattern detected in 15 studied rice genotypes with RM7631(A), RM518 (B) and RM470 (C).

Clustering of genotypes based on genetic distances

The UPGMA approach produced a dendrogram with a cophenetic correlation index of 0.93, indicating that dendrogram distances match the actual situation of the sample of genotypes analyzed (Figure 2). The dendrogram separated all the 15 studied genotypes into two major clusters with a general mean of 0.747. First cluster included five rice genotypes (three indica/japonica, one indica local and one indica exotic genotypes). Second major cluster contained the remain ten japonica genotypes and was further divided into two main sub-clusters. This fact, confirms that there are large groups in O. sativa, indica, and japonica. The indica subspecies has a different agro-climatic domestication center than japonica subspecies (Huang et al., 2012). This additionally helps the concept that microsatellites are likely effective when differentiating japonica and indica types (Zhu *et al.*, 2012). Among the two main sub-clusters of japonicas genotypes, first sub-cluster formed with only one genotype (Giza 177) and the second main sub-cluster contained the remaining nine local japonica genotypes. The indica genotypes major cluster was further divided into two main sub-clusters. Among the two main sub-cluster consisted of Giza179 and GZ 6296 because of they are genetically close (offspring), this explains why they were clustered together. Meanwhile, the second sub-cluster was divided into two group, Giza 178 indica/japonica came separately and the other two indica genotypes (GZ1368 and IET1444) came together cause of their genetic background. Those results agreed with the ones found by Kumari *et al.*, (2011), wherein microsatellites had been in a position to distinguish Indica types and Japonica rice.

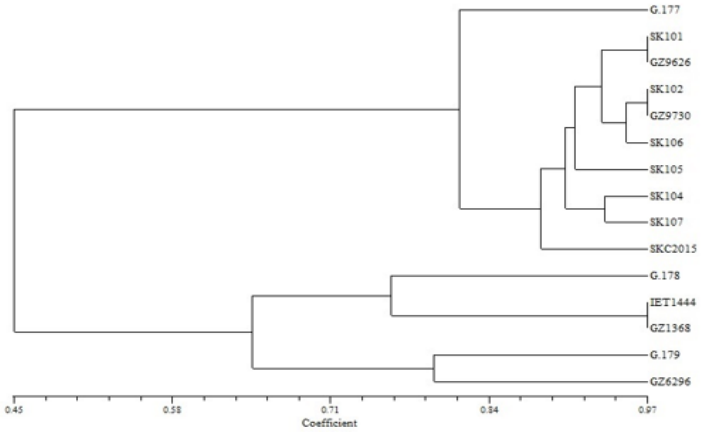

Figure 2. Dendrogram of 15 studied rice genotypes based on SSR analysis. (Roger coefficient).

A metric multidimensional scaling (MDS) analysis was performed using the Roger coefficient to visualize genetic relationships among studied varieties (Figure 3). For these molecular markers, the two-dimensional spatial placement of studied genotypes accounts for 67.68 % of total variability. The principal coordinate analysis, specifically, PC1 and PC2, explained 54.72% and 12.96% of the total variability, respectively, for the 32 SSR. PC1 showed the separation between indica / japonica Egyptian genotypes as well as the exotic indica one and the others Egyptian japonica genotypes. The group on the right consisted of Egyptian commercial indica / japonia varieties and indica genotypes defined as highly yielding with more productive tillers and spikletes per plant as well as more roots number and long roots adapted to the abiotic stress comparing with the other japonica genotypes (Figure 3). The group on the right consisted of two parts, one on the top and the other on the bottom, the upper one consisted of two indica genotypes that are so close with each other based on their morphological performance. The part located on the bottom consisted of two indica/japonica genotypes (Giza179 and GZ 6296) which are offspring. The other ten Egyptian japonica genotypes were evenly distributed along the PC2. Far away from this japonica group was the japonica genotype 'Giza177', which exhibit a different molecular profile, since Giza177 is the most sensitive Egyptian japonica variety for abiotic stress and with low panicles and spikelets. These findings are in agreement with those noted by Viviana Becerra et al., (2015), they used molecular markers to investigate the genetic diversity, identification, and certification of 16 temperate japonica

Chilean rice varieties, along with an indica genotype as an out-group.

Consequently, obtaining a deep understanding of the genetic diversity among the commercial Egyptian rice has extremely good importance for the exploitation of new germplasm sources and could make actual use of the excellent parents in the national rice breeding program.

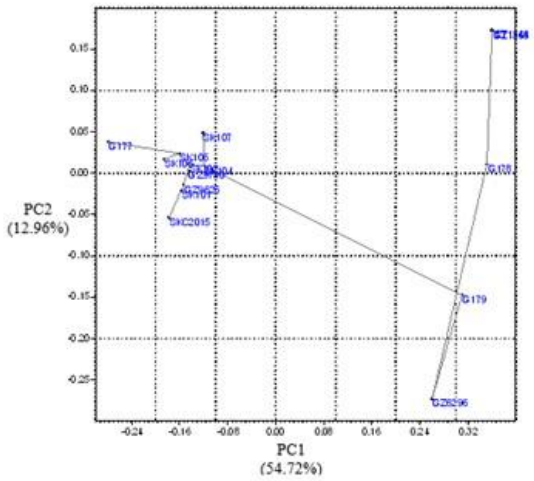

Figure 3. Metric multidimensional scaling (MDS) analysis grouping on 15 rice genotypes analyzed using 32 SSR markers (Roger coefficient)

DNA Molecular Barcodes

DNA fingerprinting, which allows high-precision identification of plant genotypes, was introduced by Peter Gill et al., (1985) earlier to describe barcode-like DNA fragment patterns generated by multi-site probes after electrophoretic separation of genomic DNA fragments. By isolating the allele size from each SSR locus, and then sorting the allele size data from lowest to highest, the allelic variations of 32 SSR markers for 15 rice genotypes are transformed into a DNA barcode profiles. These allele size bars are then drawn to a linear scale for all of the analyzed genotypes (Figure 4). This barcode representation can be used to create a fingerprint profile for each variety that is unique to it. To facilitate identification, DNA barcodes have also been developed for all multiplex assays. Therefore, a single tube marker assays for multiplexing was developed, which can be very useful in seed certification programs, especially in Egypt. Satturu *et al.*, (2018) successfully produced DNA barcodes utilizing SSR marker data for a similar study.

Traditionally, morphological characteristics have been used to assess seed purity, which is labor-intensive, time-consuming, and space demanding, and generally does not allow clear identification of genotypes (Sripathy *et al.*, 2012). In addition, more and more crop varieties with shared gene pool make it difficult to identify varieties based on seed characteristics alone. In today's high-throughput setting, the cost of materials and consumables for PCR testing has dropped significantly, making it economically feasible and cheap A set of multiple assays was developed for all 15 rice genotypes, each with five markers (RM307, RM317, RM470, RM412 and RM242). Similar multiplex assays were previously established by Vemireddy *et al.*, (2014) and used to identify and quantify adulteration in Basmati rice. Details (identity code, code key and DNA barcode) of the multiplex assay are given in Figure 4 and Tables 4 and 5. It was possible to identify each of the varieties using this multiplex based on the band positions on the gel. From this set of multiplex markers, we could differentiate Giza 179 from other similar-looking varieties by running markers using the isolated DNA of the sample seed. For example, if the bands are at 120, 160, 190 and 225 base pairs, then we confirm it as Giza 179, and if the bands are at 115, 150, 190 and 215 base pairs, we identify it as Giza177. Similarly, for Giza 178, the five bands will be at 115, 145, 160, 190 and 240 base pairs.

Figure 4. Microsatellites-based DNA barcodes for 15 studied rice genotypes

MW	115bp	120bp	125bp	130bp	135bp	140bp	145bp	150bp	155bp	160bp	165bp	170bp	175bp	180bp
G.177	I			II		III		III		II	I	IIIII	II	II
G.178		I	I		I	I	IIII			III	II	III	III	II
G.179		II		I		III			I	II	II	I	IIIIII	II
SK101	I			I	I	II		II	I	IIIII		IIIIII		I
SK102	I			I	I	II		II	I	IIII		IIIIII		II
SK104	I			I	I	II		II	I	IIII		IIIII		II
SK105	I			I	I	III		I	II	III		IIIIII		II
SK106	I			I	I	III		II	I	III		IIIIII	I	I
SK107	I			I	I	III		II	I	IIII		IIII		II
SKC2015	I			I	I	III		III		IIIII		IIIII		I
GZ9730	I			I	I	II		II	I	IIII		IIIIII	I	I
GZ9626	I			I	I	II		II	I	IIII		IIIIII	I	I
GZ6296		II		I		I	II		I	II	I	II	III	IIIIII
IET1444		I	I		I	II	III		I	I	II	III	III	III
GZ1368		I	II		I	II	III		I	I	II	III	III	III

MW	185bp	190bp	195bp	200bp	205bp	210bp	215bp	220bp	225bp	230bp	235bp	240bp	260bp
G.177		III	I			III	I	II				I	
G.178	II	III	I	I	I			I		I	I	I	
G.179	II	III	I	I		I			I		I		I
SK101		III	I			IIII	I	I	II				
SK102		IIII	I			III	I	I	I	I			
SK104	I	II	I	I		IIII	I	I	I	I			
SK105		III	I	I		IIII		I	I	I			
SK106		IIII	I	I		III	I	I		I			
SK107	I	III	I	I		III	I	I		I	I		
SKC2015		III	I			IIII	I	II	I				
GZ9730		IIII	I			II	I	II	I	I			
GZ9626		III	I			IIII	II	I	I				
GZ6296	I	IIII			II	I			I		I		I
IET1444	II	III		I	I			I		I	I	I	
GZ1368	I	III		I	I	I				I	I	I	

The most frequent seed complaints are on mixing of Giza 179 with other similar genetically close genotypes (more adapted to abiotic stresses and yielding ability comparing with other japonica genotypes). Based on the ability of SSR markers used in this study to distinguish the varieties. It is clear that by using this set of multiplex markers in case of the two parental genotypes of Giza 179 (GZ6296 and GZ1368) were shown different bands from each other and from its parents as well. If the bands are at 120, 145, 160, 190 and 225 base pairs, its belong to GZ6296. In the same text, if the bands are at 120, 145, 160,190 and 240 base pairs, its belong to GZ1368.

Ultimately, the DNA profiles which received with the 32 SSRs might be taken into consideration as an appropriate genetic fingerprint of the commercial rice varieties to complement agro-morphological information.

The goal of developing these multiplex marker assays is to create a method that makes cultivar identification simple and precise in a single tube simple PCR at a low cost, can provide unique banding patterns specific to each variety, and can work even in a lab with basic molecular testing facilities. However, further PCR data, by repeating the markers with more varieties/seed lots of the same varieties, are required to confirm the results. If multiplex assays produced with a larger number of markers cover all chromosomes and are confirmed on different seed lots from different sources, this form of variety identification will act as a supplement to ‘ ‘grow out tests" in the future.

Table 4. Identity code for the 15 studied rice genotypes

Genotype	RM307	RM317	RM470	RM412	RM242
G.177	B	E	A	A	A
G.178	A	c	A	A	C
G.179	E	A	B	A	B
SK101	B	A	A	A	A
SK102	B	A	A	A	A
SK104	B	A	A	A	A
SK105	B	A	A	A	B
SK106	B	A	A	A	A
SK107	B	A	A	A	A
SKC2015	B	A	A	A	A
GZ9730	B	A	A	A	A
GZ9626	B	A	A	A	A
GZ6296	A	A	B	A	B
IET1444	A	A	B	A	C
GZ1368	A	A	B	A	C

Table 5. Code Key for the five SSR markers used in the multiplex assays in this study

Allele Code	RM307	RM317	RM470	RM412	RM242
A	145	160	115	190	215
B	150	165	120	210	225
C	175	170	135		240
D	180		140		260
E					

CONCLUSION

The results obtained from this study provided some useful implications about a deep understanding of the genetic diversity of the main rice varieties in Egypt. The polymorphism level detected by SSR markers is generally medium to low among Egyptian commercial rice varieties. This is supported by the diversity parameters, such as the number of alleles (3.625) and polymorphism information content (PIC, 0.566). The highest PIC was positively associated with the highest number of alleles detected by SSRs. In light of this, it is critical to continue introducing new rice germplasm from different regions to increase the Rice Breeding Program’s genetic base. Genetic clustering of varieties using SSR with Roger coefficient determined the separation of japonica and indica rice genotypes. This clustering became additionally associated with grain yield attributes and abiotic stresses for that reason; varieties were clustered in line with their performance and origin. a set of SSRs became described to permit the identification and certification of O. sativa varieties, which include temperate japonica indica and indica/japonica genotypes. DNA barcodes developed for the most distributed Egyptian variety, may also be supplemented as DNA fingerprints in Plant variety protection after confirming the range of variation within each of the varieties.

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تحليل البصمة الوراثية والتنوع الوراثى لبعض الطرز الوراثية المصرية من الارز

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يستغرق انتاج صنف جديد من الارز ما يقرب من ١٢ عاما وعدد الصفات المورفولوجية المميزة المتاحة قليلة جدا بسبب الضغط المستمر من اطلاق اصناف جديدة. ولذلك كانت اهداف الدراسة الحالية كالتالى: ١- دراسة التباين الوراثى للطرز الوراثية اليابانية والهندية واليابانية الهندية من اصناف الارز المصرية .٢- تحديد البصمة الوراثية وكذلك العلاقات الوراثية بين الطرز الوراثية المختلفة تحت الدراسة باستخدام المعلمات الجزيئية. تم دراسة تحليل التباين الوراثى ل١٥ تركيب وراثى من الارز بعضهم ذو درجة عالية من القرابة الوراثية باستخدام ٣٢ معلم جزيئي. قد اظهرت المعلمات الجزيئية ال٣٢ المستخدمة قدرة على التمييز بين الخمسة عشر تركيب وراثى المستخدم فى هذة الدراسة. تراوح عدد الاليلات المتحصل عليها ما بين ٢ الى ٥ بمتوسط ٣,٦٢٥ اليلا لكل معلم جزيئي بينما كانت قيمة متوسط محتوى المعلومات لتعدد الاشكال المظهرية (٠,٥٦٦) ولقد ارتبطت قيم محتوى المعلومات لتعدد الاشكال المظهرية ارتباطا موجبا مع الارقام العالية من الاليلات المتحصل عليها باستخدام المعلمات الجزيئية. اظهر تحليل المكونات الاساسية لكلا من PC1 و PC2 قيما تقدر ب ٥٤,٧٢% و ١٢,٩٦% على التوالى من قيم التباين الكلى بواسطة المعلمات الجزيئية الاثنى والثلاثون المستخدمة فى الدراسة . تم اعداد تقنية تحليل الانبوبة الاحادية المتعددة وكذلك شفرة الحامض النووى باستخدام البيانات المتاحة والمتحصل عليها من نتائج ال٣٢ معلم جزيئي على الخمسة عشر طرازوراثى من الارز تحت الدراسة لخمسة معلمات جزيئية هى RM307, RM317, RM470, RM412 و RM242. واوضحت النتائج انه من الاهمية بمكان استقدام اصول وراثية جديدة من مناطق اخرى ذات خلفيات وراثية متنوعة بهدف تعزيز وزيادة التنوع الوراثى فى برنامج تربية الارز المحلى . كما اشارت النتائج ان تعريف الاصناف واعتمادها بناءا على نتائج المعلمات الجزيئية وتحليل نتائج البصمة الوراثية يمكن ان يعتبر داعما جيدا وفعالا للتوصيف المورفولوجى للاصناف عندما تكون الاصناف ذات درجة عالية من التشابهه و القرابة الوراثية.