

GENETIC DIVERSITY OF OLD AND MODERN EGYPTIAN RICE (*Oryza sativa* L.) USING MICROSATELLITE MARKERS

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ABSTRACT: A set of 27 genotypes was analyzed using 22 SSR markers, determining 22 loci located on 12 chromosomes. In total, 91 and 66 alleles were detected with an average of 4.14 and 3.00 in both old and modern Egyptian rice genotypes, respectively. Average of genetic diversity value was 0.610 in old genotypes, while in modern genotypes was 0.575. Compared to old genotypes, the modern genotypes showed the lowest number of alleles (66) for the rice genome. Regarding to average of genetic richness, old genotypes showed higher number of alleles per locus 7.58. The highest value of genetic diversity (0.610) was obtained in old genotype. As a whole, homoeologous group 7 possessed the highest average of allele numbers, while group 12 was the lowest values for both modern and old genotypes. A significant correlation coefficient between gene diversity and the number of alleles for SSRs markers and it was high, $r = 0.639$, and $r = 0.749$, ($P < 0.01$), for old and modern genotypes, respectively. However, the correlation coefficient between gene diversity and the number of alleles for homoeologous groups was high, $r = 0.398$ and $r = 0.502$, ($P < 0.01$), for old and modern genotypes, respectively. Cluster analysis was conducted based on SSRs data to group the rice genotypes and to construct a dendrogram at GS value of 0.29. The present study indicated the presence of high diversity in Egyptian rice genotypes.

Key words: Gene diversity- Microsatellite markers - Old and modern Egyptian rice Rice (*Oryza sativa* L.).

INTRODUCTION

Rice (*Oryza sativa* L.) is the second and strategic important cereal crop in Egypt. Prior to national rice breeding programs, cultivated rice represented essentially *japonica*, *indica* and *indica/japonica* types. Since the initiation of rice breeding program in Egypt, new cultivars were developed by 1) selection from local populations, 2) introduction of new varieties and 3) crossing and selection for yield and its components (Badawi, 1999 and Badawi, 2002. Current breeding objectives are to improve productivity of rice through increased resistance to biotic and abiotic stresses and to develop varieties with good milling and cooking quality properties and a high nutritional value (Guimaraes, 2009). The development of such varieties requires a

continuous supply of a source of desirable genes and/or gene complexes. The sources of such genes could be i) rice varieties which have not been used very intensively but have a higher general adaptation, ii) landraces, iii) wild relatives and iv) weedy species (Bressegello 2013). One prerequisite for efficient utilization of the plant material is a good knowledge about the genetic diversity, within rice germplasm Bressegello (2013).

Molecular markers that reveal polymorphism at the DNA level have been shown to be a very powerful tool for estimation of genetic diversity and genotype characterization. In this regard, microsatellites, due to their multiallelic nature, and easy and accessible laboratory

protocols, have been extensively used in several crops (Gupta and Varshney, 2000).

Genetic diversity in rice was characterized using morphological traits (Patra and Dhua, 2003, Fukuoka *et al.*, 2006) and DNA based markers such as random amplified polymorphic (RAPD) (Arshad *et al.*, 2011), amplified fragment length polymorphism (AFLP) (Zhu *et al.*, 1998), restriction fragment length polymorphism (RFLP) (Zhang *et al.*, 1992) or microsatellite (Ashfaq and Khan 2012; Babu *et al.*, 2014).

Rice microsatellite markers (RM) (McCouch *et al.* 2001; 2002) are known to be abundant, co-dominant, multi-allelic, and can be reliably used to analyze both *indica* and *japonica* germplasm, as well as groups of AA genome *Oryza* species, and are relatively easy to implement (Chen *et al.*, 2002; Ni *et al.*, 2002). The objective of this

study was to i) assess the genetic diversity within old and modern rice (*Oryza sativa* L.) genotypes cultivated in Egypt using SSR markers and ii) consider whether old Egyptian genotypes could be a potential source for improving genetic diversity in modern rice breeding in Egypt.

MATERIALS AND METHODS

Plant material

In total, 27 (19 old and 8 modern) diverse rice (*Oryza sativa* L.) genotypes released from 1917 to 1998, originary from Egypt were used in this study. Grains of all Egyptian genotypes were obtained from the Agricultural Research Center (ARC), Giza, Egypt. A List of the rice genotypes, accession number, source of seeds, subspecies group, phase of released group and group type is presented in Table 1.

Table (1): List of Egyptian rice genotypes released by the Rice Research Department, ARC, Giza, Egypt during the last eighty years.

No	Genotype Name	Accession Number	Source of seeds	Subspecies group	Phase Group	Group type
1	Yabani	PI 431091	USDA	<i>Japonica</i>	Phase I (Before 1917-1952)	Old group
2	Sabini	PI 226307	USDA	<i>Japonica</i>	Phase I (Before 1917-1952)	Old group
3	Agamy	PI 391858	USDA	<i>Japonica</i>	Phase I (Before 1917-1952)	Old group
4	Sultani	PI 18920	USDA	<i>Japonica</i>	Phase I (Before 1917-1952)	Old group
5	Montakei	PI 431090	USDA	<i>Japonica</i>	Phase I (Before 1917-1952)	Old group
6	Nabatat Asmar	PI 233083	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
7	Arabi	PI 329135	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
8	Yabani Montahab 7	GSOR 310163	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
9	Yabani Pearl	PI 400126	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
10	Yabani M 47	PI 233881	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
11	Agami Montakheb 1	PI 439120	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
12	Nahda	PI 422207	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
13	Egyptian 2716	PI 388442	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
14	Egypt 1	PI 431158	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
15	Egypt 2	PI 431159	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
16	Egypt 3	PI 431160	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
17	Egypt 4	PI 431161	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
18	Egypt 5	PI 431162	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
19	Egypt 6	PI 431163	USDA	<i>Japonica</i>	Phase II (1953-1974)	Old group
20	Giza 14	PI 233879	USDA	<i>Japonica</i>	Phase III (1975-1985)	Modern group
21	Giza 170	PI 439132	USDA	<i>Japonica</i>	Phase III (1975-1985)	Modern group
22	Giza 171	PI 442134	USDA	<i>Japonica</i>	Phase III (1975-1985)	Modern group
23	Giza 172	PI 408379	USDA	<i>Japonica</i>	Phase III (1975-1985)	Modern group
24	Giza 177	-----	ARC	<i>Japonica</i>	Phase IV (1986-1998)	Modern group

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25	Giza 178	-----	ARC	<i>Indica/ Japonica</i>	Phase IV (1986-1998)	Modern group
26	Giza 180	PI 439135	USDA	<i>Indica</i>	Phase IV (1986-1998)	Modern group
27	Giza 181	-----	ARC	<i>Indica</i>	Phase IV (1986-1998)	Modern group

DNA extraction

Total genomic DNA was extracted from young leaves of 8-weeks-old seedlings growing under greenhouse at Faculty of Agriculture Minoufiya University (2012 rice growing season) for 5 seedlings per genotype. Only one replication was sampled DNA extraction. DNA extraction was performed according to Plaschke *et al.* (1995).

PCR reaction contained 50-100 ng template DNA, 250 nM forward primer, 250 nM reverse primer, 0.2 mM dNTPs, 2.5 µl PCR buffer (10X), 1.5 mM MgCl, 1U *Taq* DNA polymerase in a total volume of 25 µl. Amplifications were carried out using the

following programs: 5 min at 94°C followed by 35 cycles of 1 min 94°C, 1 min 50°C or 55°C or 60°C according to primer annealing temperature and 2 min at 72°C, with a final extension of 5 min at 72°C as described by Wu and Tanksley (1993). The amplification products were resolved on 10% polyacrylamide denaturing gels (PAGE).

SSR analysis

Twenty-two SSR markers were used to assay genetic variation for all genotypes, based on the Rice Genes Database (<http://gramene.org>). Detailed information of the SSR markers is given in Table 2.

Table (2): Characteristics of 22 rice markers, their chromosomal location, motif, annealing temperature, repeated category and fragment size.

No	SSR markers	Chromosomal location	Motif	Annealing Tm (°C)	Repeat category	Expected Fragment size (bp)
1	<i>Rm 5</i>	1	(GA)14	55	di	84
2	<i>Rm 6</i>	2	(AG)16	55	di	163
3	<i>Rm 11</i>	7	(GA)17	55	di	115
4	<i>Rm 22</i>	3	(GA)22	55	di	194
5	<i>Rm 55</i>	3	(GA)17	55	di	213
6	<i>Rm 118</i>	7	(GA)8	60	di	106
7	<i>Rm 133</i>	6	(CT)8	60	di	224
8	<i>Rm 144</i>	11	(ATT)11	55	tri	208
9	<i>Rm 151</i>	1	(TA)23	55	di	197
10	<i>Rm 154</i>	2	(GA)21	60	di	106
11	<i>Rm 161</i>	5	(AG)20	60	di	116
12	<i>Rm 162</i>	6	(AC)20	60	di	130
13	<i>Rm 215</i>	9	(CT)16	55	di	126
14	<i>Rm 271</i>	10	(GA)15	55	di	65
15	<i>Rm 277</i>	12	(GA)11	55	di	108
16	<i>Rm 285</i>	9	(CT)797	55	di	205
17	<i>Rm 307</i>	4	(AT)14(GT)21	55	complex	104
18	<i>Rm 408</i>	8	(CT)13	55	di	109
19	<i>Rm 413</i>	5	(AG)11	50	di	65

20	<i>Rm 433</i>	8	(AG)13	50	di	215
21	<i>Rm 474</i>	10	(AT)13	55	di	195
22	<i>Rm 552</i>	11	(TAT)13	55	tri	153

Data collection and diversity

Gels were scored as binary data matrix. The presence (1) and absence (0) of alleles for each microsatellites marker were recorded for each variety. Gene diversity was calculated according to formula of Nei (1973) using the equation,

$$PIC = 1 - \sum_{i=1}^k P_i^2$$

where *k* is the total number of alleles detected for a locus of a marker and *P_i* is the frequency of the *i*th allele in the set of twenty-seven Egyptian rice genotypes investigated. Anderson *et al.* (1993) indicated that gene diversity is essentially the same as the polymorphism information content (PIC) as used by Botstein *et al.* (1980).

Genetic similarity estimation and cluster analysis

The data were analyzed using the SIMQUAL (Similarity for Qualitative Data) routine to generate Dice similarity coefficient (Dice 1945). The similarity coefficient were used to construct a dendrogram using Unweighted Pair Group Method with Arithmetic Average (UPGMA) algorithm using the Numerical Taxonomy and Multivariate Analysis System (NTSYS), version 2.1 (Rohlf 2002).

Results

Characteristics of SSR markers

Twenty-two microsatellite markers were used to test the genetic diversity of 27 Egyptian rice. All microsatellite markers allowed identifying DNA fragments amplified polymorphic patterns from genomic DNA extracts of rice genotypes, yielding a polymorphism rate of 100% (Table 3). In total, 91 and 66 microsatellite alleles were detected in both old and modern genotypes, respectively (Table 3). The number of alleles per locus ranged from 2 to 7 with an average of 4.14 alleles per locus for old

genotypes, while the number of alleles per locus ranged from 2 to 5 with an average of 3.00 alleles per locus for modern rice genotypes (Table 3).

The correlation coefficient between gene diversity and the number of alleles for SSRs markers was high, *r* = 0.639, and *r* = 0.749, (*P* < 0.01), for old and modern genotypes, respectively. The linear relationship between them is shown in Fig. 1. However, the correlation coefficient between gene diversity and the number of alleles for homoeologous groups was high *r* = 0.398 and *r* = 0.502, (*P* < 0.01), for old and modern genotypes, respectively. The linear relationship between them is shown in Fig. 2.

Genetic diversity

Gene diversity for the microsatellite loci varied from 0.475 for locus RM55 to 0.795 for locus RM5 with an average of 0.610 for old Egyptian rice genotypes. However, for modern rice genotypes, gene diversity varied from 0.245 for locus RM307 to 0.735 for loci RM6, RM11 and RM413 with an average of 0.575 Table (4).

Compared to old genotypes, the modern genotypes showed the lowest number of alleles (66) for the rice genome AA. Regarding to average genetic richness, the old genotypes showed higher number of alleles per locus 7.58 than that in the modern genotypes Table (4). As for genetic diversity, the old genotypes showed higher genetic diversity 0.610 than that in the modern genotypes (Table 4).

Genetic diversity of the 12 homoeologous groups

As a whole, homoeologous group 7 possessed the highest average of allelic numbers (11) alleles/loci, while group 12 was the lowest values for both modern and old genotypes (2) alleles/loci. The order from the highest to the lowest for old genotypes was 11 for group 7, 10 for group (5=6=10)>

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(9) for group 3, 8 for group (1=2), (5) for group (4=9=11) , (3) for group 8 and (2) for group 12. While, for modern group was 9 (group 7) ,7 (group 2, 3, 5&10) , 6 (group 6) , 5 (group 1) , 4 (group 9 = 11) , 3 group 8 and 2 group (4& 12). Regarding to the average of genetic richness, the old varieties had the highest values than the modern genotypes for all homoeologous groups. In addition, the average genetic richness from 1st to 12th homoeologous group for the modern genotypes ranged from 2 to 5.50.

So, group 7 still hold the highest genetic richness and group 12 was the lowest in both modern and old genotypes (Table 4). With regards to PIC, the highest value was 0.722 for group 10 in old genotypes, however, 0.715 for group 7 in modern genotypes. However, the lowest PIC values was 0.480 (group 1) and 0.245 (group 4) for both old and modern Egyptian rice genotypes, respectively.

Table (3): Characteristics of SSR markers used with the chromosomal location, allele size range, number of alleles per locus and gene diversity calculated over a set of 27 old and modern Egyptian rice

Locus	chromosomal location	Allele size range (bp)		Number of alleles		Gene diversity	
		Min	Max	Old	Modern	Old	Modern
<i>Rm 5</i>	1	106	124	6	3	0.795	0.571
<i>Rm 6</i>	2	144	162	3	4	0.605	0.735
<i>Rm 11</i>	7	120	144	7	5	0.753	0.735
<i>Rm 22</i>	3	186	202	5	4	0.725	0.694
<i>Rm 55</i>	3	210	218	4	3	0.475	0.612
<i>Rm 118</i>	7	150	162	4	4	0.525	0.694
<i>Rm 133</i>	6	227	236	4	3	0.625	0.612
<i>Rm 144</i>	11	218	238	2	2	0.495	0.490
<i>Rm 151</i>	1	78	88	2	2	0.480	0.408
<i>Rm 154</i>	2	176	198	5	3	0.693	0.571
<i>Rm 161</i>	5	165	185	4	2	0.694	0.490
<i>Rm 162</i>	6	203	243	6	3	0.680	0.571
<i>Rm 215</i>	9	148	152	2	2	0.495	0.490
<i>Rm 271</i>	10	98	118	4	3	0.695	0.612
<i>Rm 277</i>	12	114	122	2	2	0.488	0.490
<i>Rm 285</i>	9	198	205	3	2	0.515	0.490
<i>Rm 307</i>	4	122	174	5	2	0.700	0.245
<i>Rm 408</i>	8	125	133	3	3	0.535	0.653
<i>Rm 413</i>	5	80	106	6	5	0.710	0.735
<i>Rm 433</i>	8	210	214	5	3	0.485	0.653
<i>Rm 474</i>	10	226	264	6	4	0.748	0.694

<i>Rm 552</i>	11	174	186	3	2	0.505	0.408
Total				91	66	-----	-----
Mean				4.14	3.00	0.610	0.575

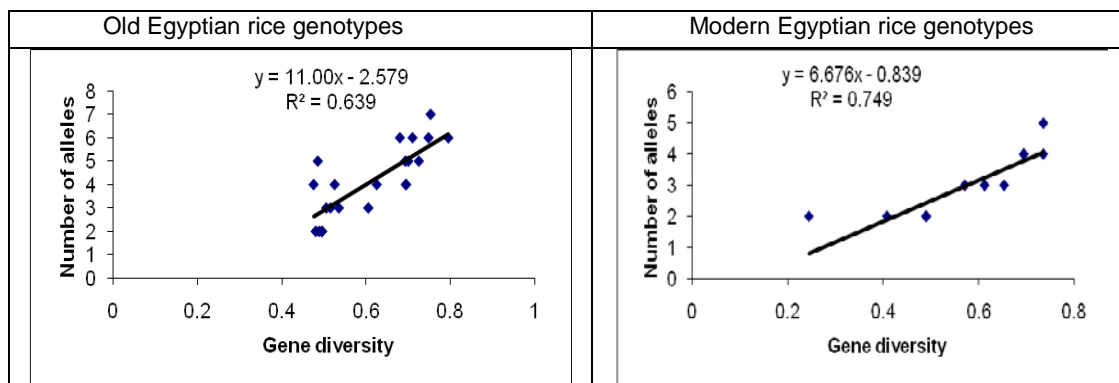


Fig. 1: Correlation between gene diversity and the number of alleles over 22 SSR loci in total of 27 old (left) and modern (right) rice genotypes.

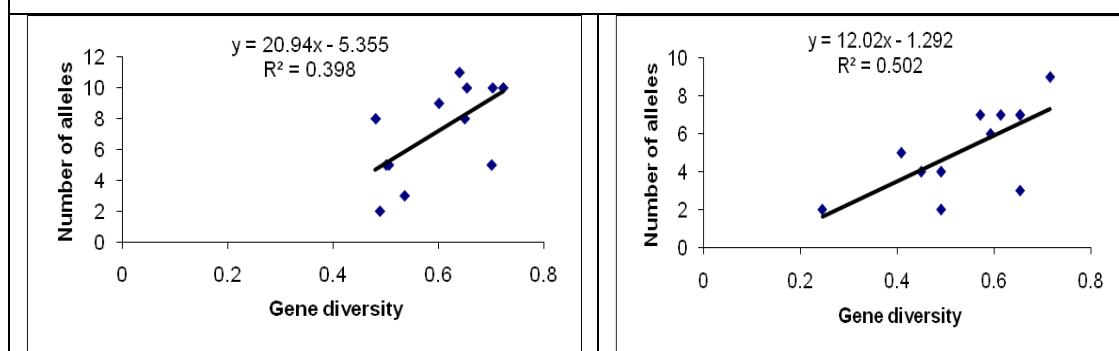


Fig. 2: Correlation between gene diversity and the number of alleles over 12 homoeologous groups in total of 27 old (left) and modern (right) rice genotypes.

Cluster analysis

A dendrogram derived from UPGMA cluster analysis based on the genetic similarity (GS) coefficient matrix for all the genotypes analysed was constructed (Fig.3). All the genotypes could be distinguished. The GS coefficients ranged from 0.03 to 0.40. The largest genetic distance was observed between the two clustered genotypes Yabani Montakhab 7 and Giza 170 and the rest of the 27 Egyptian genotypes analysed. The smallest genetic distance was detected between the genotypes Egypt 1 and Egypt 4 (Fig.3).

Clustering of the genotypes according to their released yield programs periods was not observed. Five groups can be distinguished by truncating the dendrogram at GS value 0.29. The major group detected (group A), consists of thirteen genotypes and includes the Egyptian rice genotypes Nabatat Asmer, Agami Montakheb 1, Yabani 47, Giza 172, Giza 171, Yabani Montakhab 7, Giza 170, Arabi, Nahda, Sabini, Egypt 6, Sultani and Egyptian 2716. Another group (group B) contains three genotypes and includes Yabani pearl, Giza 181 and Giza 178. Group C consists of two genotypes Giza 177 and Egypt 2. Group D includes

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eight genotypes Giza 14, Agamy, Montakei, Yabani, Egypt 1, Egypt 4, Egypt 5 and Egypt 3. Interestingly, the rice genotypes Giza 180

is not clustered to any other varieties/cultivars and forms a separate group (group E).

Table 4: Genetic diversity between the old and modern genotypes in genome and homoeologous chromosome groups.

Location	Number of loci checked	Number of alleles		Average genetic richness		Gene diversity	
		Old genotypes	Modern genotypes	Old genotypes	Modern genotypes	Old genotypes	Modern genotypes
Genome							
A	21	91	66	7.58	5.50	0.610	0.575
Homoeologous chromosome Group							
1		8	5	4.00	2.50	0.480	0.408
2		8	7	4.00	3.50	0.649	0.571
3		9	7	4.50	3.50	0.600	0.653
4		5	2	5.00	2.00	0.700	0.245
5		10	7	5.00	3.50	0.702	0.613
6		10	6	5.00	3.00	0.653	0.592
7		11	9	5.50	4.50	0.639	0.715
8		3	3	3.00	3.00	0.535	0.653
9		5	4	2.50	2.00	0.505	0.490
10		10	7	5.00	3.50	0.722	0.653
11		5	4	2.50	2.00	0.500	0.449
12		2	2	2.00	2.00	0.488	0.490

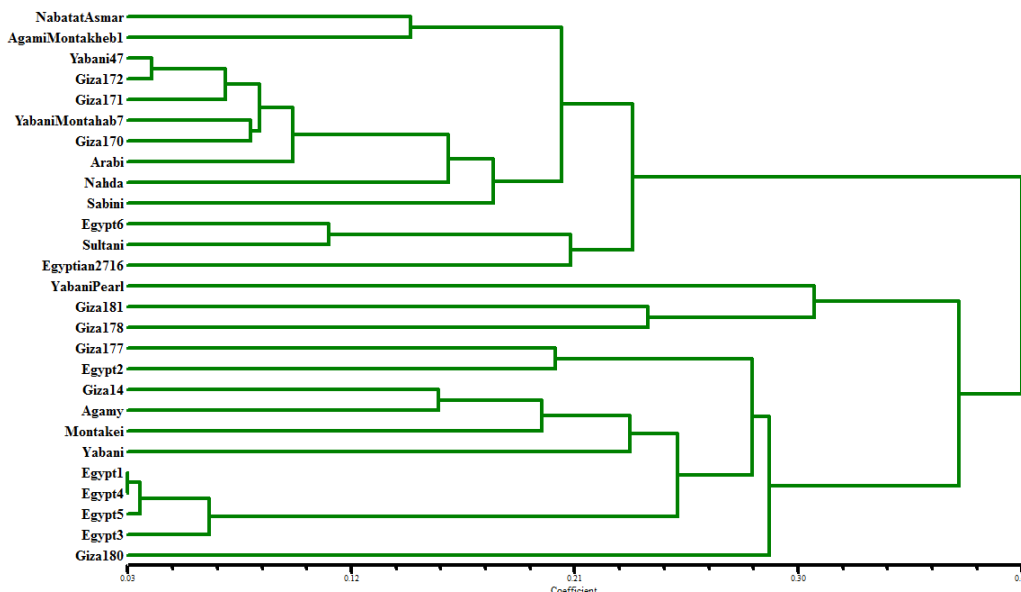


Fig. 3: Dendrogram reflecting genetic similarity between 27 Egyptian rice, based on the analysis of 22 microsatellite loci.

Discussion

Rice microsatellite markers are locus-specific and are, therefore, generally considered to be single-locus markers, which gives them an advantage over other molecular markers, i.e., RFLP and RAPD. Since microsatellite primers are locus specific, only one specific locus was expected to be amplified by each marker, and it was unexpected that this one marker amplified two loci. In several earlier studies also, more than one locus per microsatellite primer pair was detected or mapped in rice (McCouch *et al.*, 2001, 2002). The microsatellite loci are also multiallelic and the alleles co-dominant, thus suggesting their relative superiority in detecting DNA polymorphism (Temnykh *et al.*, 2000). The alleles with higher frequencies might be selected and kept for increase grain yield per plant. For example, the rice microsatellite marker *RM 5*, which mapped on chromosome 1 in *O. rufipogon* was associated with an 18% increase in grain yield per plant (Xiao *et al.*, 1996), produced 6 main alleles that are widespread in Egyptian rice genotypes. This could be explained by the selection for these different alleles in Egyptian genotypes.

In the present study, a total number of alleles (91 and 66) were detected with an average number of alleles per locus (4.14 and 3.00) for both old and modern genotypes, respectively. Different number of alleles has been detected in rice using microsatellite markers. Yang *et al.* (1994) found 9.3 average allele numbers per locus in 238 landrace cultivars. While, Ni *et al.* (2002) investigated with 111 microsatellites a set of 38 *O. sativa* originating from different countries and thereby recorded an average allele number of 6.8. Yu *et al.* (2003) reported 6.3 average allele numbers per locus. Jain *et al.* (2004) found average 7.8 alleles per locus in 69 accessions of Basmati landrace and other *japonica* and *indica* cultivars. Whereas, Zeng *et al.* (2004) detected an averages of 4.9 alleles per locus from 25 microsatellite loci among 33 rice genotypes. Giarrocco *et al.* (2007) detected an average of 8.4 alleles per markers from 26 simple sequence repeat (SSR) markers in sixty-nine accessions from Argentine. Lapitan *et al.* (2007) detected an average of 5.9 alleles per locus in 8 *japonica* and 16 *indica* cultivars. Ghaley *et al.* (2012) reported average of 7.7 alleles per markers from 27 simple sequence repeat (SSR) markers in 352 Bhutan landraces. Das *et al.*

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(2013) detected an average of 7.9 alleles per marker from 23 rice microsatellites in 91 rice accessions. The average allele number obtained in the present investigation of Egyptian rice was 4.14 and 3.00 for both old and modern rice genotypes, respectively. A comparison of the results obtained in the present study with those published earlier indicates that the average number of alleles per locus recorded during the present study was lower than most previous studies, but it was comparable with Zeng *et al.* (2004) results, which detected 4.9 alleles per locus among 33 rice genotypes.

Rice microsatellite markers showed an average PIC value of 0.610 and 0.575 for both old and modern rice genotypes, respectively, which confirms that rice microsatellite markers are highly informative. Botstein *et al.* (1980) investigated that PIC value > 0.5 is considered as being highly informative marker while $0.5 > \text{PIC} > 0.25$ is just informative marker, while $\text{PIC} \leq 0.25$ is a slightly informative marker. Gene diversity obtained in the present investigation was comparable with previous results on genetic diversity of rice using microsatellite analysis. Yang *et al.* (1994) reported an average PIC value of 0.58 in 238 landrace cultivars. Ni *et al.* (2002) reported an average gene diversity (PIC) of 0.621 in 38 *O. sativa* originating from different countries. Jain *et al.* (2004) reported an average PIC value of 0.61 in 69 accessions of Basmati landrace and other *japonica* and *indica* cultivars. Giarocco *et al.* (2007) found an average PIC value of 0.69 in 68 accessions from Argentina. Ghaley *et al.* (2012) reported an average PIC value of 0.61 in 352 Bhutan landraces. Das *et al.* (2013) reported an average PIC value of 0.746 in 91 rice accessions. This indicates that the markers were highly informative. The value of gene diversity increased with the number of alleles at a given locus. There was significant correlation between gene diversity and the number of alleles ($r = 0.639$ and $r = 0.749$, $P < 0.01$) for old and modern genotypes, respectively. Therefore, the number of alleles can be used for the evaluation of genetic diversity. The data obtained in present investigation was agree

with those of Huang *et al.* (2002), Salem *et al.* (2010), Salem *et al.* (2015) who reported that the PIC value was correlated with the number of alleles, and did not agree with those of Prasad *et al.* (2000).

The number of alleles was different for individual rice genome, (91 and 66) in old and modern rice genotypes, respectively. The number of SSR alleles located on genome may reflect its greater variability sustained during evolution (Feldman 2001). There were large differences in the average genetic richness for both old and modern rice genotypes. This indicated that there were more key genes/QTLs controlling important agronomic characteristics and modern breeding provided much higher selective pressures due to breeding program. Among the 12 homoeologous groups, the genetic diversity of group 12 was much lower for both old and modern Egyptian rice genotypes. Therefore, it was estimated that breeding might have brought much higher selection pressure to genes conveyed by this group. This was consistent with the opinions of Börner *et al.* (2002) and Salem *et al.* (2015).

The cluster analysis discriminated all the Egyptian rice genotypes in the present study, although its coming from different yield progress periods during the past eighty years (1917-1998) in Egypt. However, it did not group the genotypes according to their yield progress periods, breeding program or geographic location. Huang *et al.* (2002) and Salem *et al.* (2015) reported that not all accessions originating from the same region clustered in the same group, indicating that the genetic diversity is not completely related to geographic distribution. Similar results were obtained by Khlestkina *et al.* (2004). The results obtained provide new information about the relationships between the Egyptian rice genotypes analyzed. They are useful for variety identification and for their utilization in further rice breeding program.

REFERENCES

- Anderson, J.A., G.A. Churchill, J.E. Antrique, S.D. Tanksley and M.E. Sorrels (1993). Optimising parental selection for

- genetic linkage maps. *Genome* 36: 181-188.
- Arshad, S., A. Iqbal, S. Nawaz and N. Ahmed (2011). Genetic diversity studies of coarse and fine rice using RAPD markers. *Front Agric China* 5: 129-134.
- Ashfaq, M. and AS. Khan (2012). Genetic diversity in basmati rice (*Oryza sativa* L.) germplasm as revealed by microsatellite (SSR) markers. *Russ J Genet* 48: 53-62.
- Babu, B.K., V. Meena, V. Agarwal and PK. Agrawal (2014). Population structure and genetic diversity analysis of Indian and exotic rice (*Oryza sativa* L.) accessions using SSR markers. *Mol Biol Rep* 41: 4329-4339.
- Badawi, AT. (1999). Policy of rice varieties in Egypt. *Egypt J Plant Breed* 3: 29-51.
- Badawi, AT., M.A. Maximos and IR. Aidy (2002). Rice improvement in Egypt during 85 years (1917-2001). In: *Rice in Egypt*. Ministry of Agriculture and Land Reclamation. ARC, Egypt. pp.1-24.
- Babal, MS. (1989). Rice varietal improvement in Egypt, In: *Rice farming systems new directions*, pp. 91-102. Proceedings of an International Symposium, 31 January- 3 February, 1987, Rice Research and Training Center, Sakha, Egypt, Publisher: International Rice Research Institute (IRRI).
- Börner, A., E. Schumann, A. Fürste, H. Cöster, B. Leithold, M.S. Röder and W.E. Weber (2002). Mapping of quantitative trait loci for agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). *Theor Appl Genet* 105: 921-936.
- Botstein, D., R.L. White, M. Skolnick and R.W. Davis (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am J Hum Genet* 32: 413-331.
- Breseghello, F. (2013). Traditional and modern plant breeding methods with examples in rice (*Oryza sativa* L.). *J. Agric. Food Chem* 61: 8277-8286.
- Chen, X., Y.G. Cho, S.R. McCouch (2002). Sequence divergence of rice microsatellites in *Oryza* and other plant species. *Mol Gen Genom* 268: 331-343.
- Das, B., S. Sengupta, S.K. Parida, B. Roy, M. Ghosh, M. Prasad and TK. Ghose (2013). Genetic diversity and population structure of rice landraces from eastern and north eastern states of India. *BMC Genet* 14: 71-85.
- Dice, L.R. (1945). Measures of the amount of ecologic association between species. *Ecology* 26: 297-302.
- Feldman, M. (2001). Origin of cultivated wheat. In: Bonjean AP, Angus WJ (eds). *The World wheat book. A history of wheat breeding*. Intercept, Paris, pp 3-56.
- Fukuoka, S., T.D. Suu, K. Ebanna and L.N. Trinh (2006). Diversity in phenotypic profiles in landraces populations of Vietnamese rice: a case study of agronomic characters for conserving crop genetic diversity on farm. *Genet Resour Crop Evol* 53: 753-761.
- Ghaley, B.B., L.G. Christiansen and S.B. Andersen (2012). Genetic diversity in blast resistance of Bhutan rice landraces. *Euphytica* 184: 119-130.
- Giarocco, L.E., M.A. Marassi and GL. Salerno (2007). Assessment of the genetic diversity in Argentine rice cultivars with SSR markers. *Crop Sci* 47: 853-860.
- Guimaraes, EP. (2009). "Rice breeding", In: Carena MJ, Ed, *Cereals, The banks and the Italian economy*, Springer, New York, pp. 99-126.
- Gupta, P.K. and R.K. Varshney (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113: 163-185.
- Huang, X.Q., A. Börner, M.S. Röder and M.W. Ganai (2002). Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theor Appl Genet* 105: 699-707.
- Jain, S., R.K. Jain and S.R. McCouch (2004). Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently labeled microsatellite markers. *Theor Appl Genet* 109: 965-977.
- Khlestkina, E.K., M.S. Röder, T.T. Efremova, A. Börner and VK. Shumny (2004). The genetic diversity of old and modern

Genetic diversity of old and modern Egyptian rice (*Oryza sativa* L.).....

- Siberian varieties of common spring wheat as determined by microsatellite markers. *Plant Breed* 123: 122-127.
- Lapitan, V.C., D.S. Brar, T. Abe and E.D. Redona (2007). Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. *Breed Sci* 57: 263-270.
- McCouch, S.R., S. Temnykh, A. Lukashova, J. Coburn, G. Declerck, S. Cartinhour, S. Harrington, M. Thomson, E. Septiningsi, M. Semon, P. Moncada and L. Jiming (2001). Microsatellite markers in rice: Abundance, diversity and applications. In: *Rice Genetics IV* IRRI, Manila, Philippines. pp: 117-135.
- McCouch, S.R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing, Q. Zhang, I. Kono, M. Yano, R. Fjellstrom, G. DeClerck, D. Schneider, S. Cartinhour, D. Ware and L. Stein (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Res* 9: 199-20.
- Momtaz, A. (1989). Research and management strategies for increased rice production in Egypt. In: *Rice farming systems new directions*, pp. 55-70. Proceedings of an International Symposium, 31 January- 3 February, 1987, Rice Research and Training Center, Sakha, Egypt, Publisher: International Rice Research Institute (IRRI).
- Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA* 70: 3321-3323.
- Ni, J., P.M. Colowit and D.J. Mackill (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Sci* 42: 601-607.
- Patra, B.C. and S.R. Dhua (2003). Agromorphological diversity scenario in upland rice germplasm of Jeypore tract. *Genet Resour Crop Evol* 50: 825-828.
- Plaschke, J., M.W. Ganal and M.S. Röder (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theor Appl Genet* 91: 1001-1007.
- Prasad, M., R.K. Varshney, J.K. Roy, H.S. Balyan and PK. Gupta (2000). The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. *Theor Appl Genet* 100: 584-592.
- Rolf, F.J. (2002). NTSYS-pc Numerical taxonomy and multivariate analysis system, Version 2.1, Applied Biostatistics, New York.
- Salem, K.F.M., M.S. Röder and A. Börner (2015). Assessing genetic diversity of Egyptian hexaploid wheat (*Triticum aestivum* L.) using microsatellite markers. *Genet Resour Crop Evol* 62: 377-385.
- Salem, K.F.M., R.K. Varshney, M.S. Röder and A. Börner (2010). EST-SSR based estimates on functional genetic variation in a barley (*Hordeum vulgare* L.) collection from Egypt. *Genet Resour Crop Evol* 57: 515-525.
- Temnykh, S., W.D. Park, N. Ayres, S. Cartinhour, N. Hauck, I. Lipovich, Y.G. Cho, T. Ishii and SR. McCouch (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor Appl Genet* 100: 697-712.
- Wu, K.S. and SD. Tanksley (1993). Abundance, polymorphism and genetic mapping of microsatellites in rice. *Mol Gen Genet* 241: 225-235.
- Xiao, J., J. Li, L. Yuan, S.R. McCouch and S.D. Tankley (1996). Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. *Theor Appl Genet* 92: 637-643.
- Yang, G.P., M.A.S. Maroof, C.G. Xu, Q.F. Zhang and RM. Biyashev (1994). Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Mol Gen Genet* 245: 187-194.
- Yu, S.B., W.J. Xu, C.H.M. Vijayakumar, J. Ali, B.Y. Fu, J.L. Xu, Y.Z. Jiang, R. Marghirang, J. Domingo, C. Aquino, S.S. Virmani and Z.K. Li (2003). Molecular diversity and multilocus organization of the parental lines used in the international rice molecular breeding program. *Theor Appl Genet* 108: 131-140.
- Zeng, L., T.R. Kwon, X. Liu, C. Wilson, C.M. Grieve and GB. Gregorio (2004). Genetic

diversity analysed by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptation to saline soils. Plant Sci 166: 1275-1285.
Zhang, Q., M.A. Saghai-Marooif, T.Y. Lu and BZ. Shen (1992). Genetic diversity and differentiation of indica and japonica rice

detected by RFLP analysis. Theor Appl Genet 83: 495-499.
Zhu, J., M.D. Gale, S. Quarrie, M.T. Jackson and GJ. Bryan (1998). AFLP markers for the study of rice biodiversity. Theor Appl Genet 96: 602-611.

التنوع الوراثي لأصناف الأرز المصريه القديمه والحديثه باستخدام المعلم الجزئي الميكروستاليت

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الملخص العربى

يعد استخدام المعلم الجزئ الميكروستاليت (Microsatellite) هام جدا لتقدير التنوع الوراثي فى أصناف الأرز المصريه القديمه والجديده، وكان الهدف من هذه الدراسه هو:-

1) تقييم التنوع الوراثي لأصناف الأرز القديم والحديث المزروعه في مصر باستخدام المعلم الجزئ الميكروستاليت (Microsatellite).

٢) تحديد ما إذا كانت الأصناف القديمه مصدرا محتملا لتحسين التنوع الوراثي في تربية الأرز الحديث في مصر. استخدم لتنفيذ هذا البحث اثنين وعشرون معلم جزئ ميكروستاليت لدراسه التنوع الوراثي لسبعه وعشرون صنف أرز قديم وجديد حسب سنوات أستنباط الأصناف وطرحها للزراعه. وفيما يلي ملخص لأهم النتائج :

١- كان إجمالي عدد الأليلات (٩١) ، (٦٦) أليل وراثي بمتوسط (٤.١٤) ، (٣.٠٠) أليل لكل موقع وراثي فى كل من أصناف الأرز القديمه والحديثه على التوالي.

٢- كانت متوسطات قيم PIC لكل من الأصناف القديمه والحديثه هي ٠,٦١٠ ، ٠,٥٧٥ على التوالي.

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٣- كان أعلى عدد للأليلات على الكروموسومات ٧ ، ١٠ ، ٥ ، ٦ على التوالي بالنسبة للأصناف الحديثة مقارنة بالأصناف القديمة.

٤- بالنسبة لمتوسط الثراء الوراثي ، وجد ان اعلى قيمة هي ٥,٥٠ في الأصناف الحديثة مقارنة ٧,٥٨ بالأصناف القديمة.

٥- وجد أن أعلى قيم للإختلاف الوراثي في أصناف الأرز الحديثة هي ٠,٧١٥ على الكروموسوم السابع.

٦- اشتملت نتائج الدندوجرام على إنعزال الأصناف محل الدراسة خمسة مجموعات رئيسيه.