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 higthest value of genetic diversity (0.610)was obtained in old genotype . As a whole, homoeologous group 7 possessed the highest average of allelic 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 (Breseghello 2013). One prerequisite for efficient utilization of the plant material is a good knowledge about the genetic diversity, within rice germplasm Breseghello (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

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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	e Rice Research	Department,
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24 Giza 177 ARC Japonica Phase IV (1986-1998) Modern group 25 Giza 178 ARC Indica/ Japonica Modern group 26 Giza 180 PI 439135 USDA Indica Phase IV (1986-1998)	23	Giza 172	PI 408379	USDA	Japonica	Phase III (1975-1985)	Modern group
25 Giza 178 ARC Indica/ Japonica Modern group 26 Giza 180 PI 439135 USDA Indica Phase IV (1986-1998) Modern group	24	Giza 177		ARC	Japonica		
Japonica Phase IV (1986-1998) 26 Giza 180 PI 439135 USDA Indica Phase IV (1986-1998) Modern group	25	Giza 178		ARC	Indica/		
					Japonica		-
27 Giza 181 ARC Indica Phase IV (1986-1998) Modern group	26	Giza 180	PI 439135	USDA	Indica	Phase IV (1986-1998)	Modern group
	27	Giza 181	*****	ARC	Indica		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 2	2 rice	markers,	their	chromosomal	location,	motif,
		annealing tempe	erature	, repe	ated categ	ory an	d fragment size.		

No	SSR markers	Chromosomal location	Motif	Annealing Tm (ºC)	Repeat category	Expected Fragment size (bp)
1	Rm 5	1	(GA)14	55	di	[*] 84
2	Rm 6	2	(AG)16	55	di	163
3	Rm 11	7	(GA)17	55	di	115
4	Rm 22	3	(GA)22	55	di	194
5	Rm 55	3	(GA)17	55	di	213
6	Rm 118	7	(GA)8	60	di	106
7	Rm 133	6	(CT)8	60	di	224
8	Rm 144	11	(ATT)11	55	tri	208
9	Rm 151	1	(TA)23	55	di	197
10	Rm 154	2	(GA)21	60	di	106
11	Rm 161	5	(AG)20	60	di	116
12	Rm 162	6	(AC)20	60	di	130
13	Rm 215	· 9	(CT)16	55	di	126
14	Rm 271	10.	(GA)15	55	di	65
15	Rm 277	12	(GA)11 ·	55	di	108
16	Rm 285	9	(CT)797	55	di	205
17	Rm 307	4	(AT)14(GT)21	55	complex	104
18	Rm 408	8	(CT)13	55	di	109
19	Rm 413	5	(AG)11	50	di	65
20	Rm 433	8	(AG)13	50	di	215
21	Rm 474	10	(AT)13	55	di	195
22	Rm 552	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 aene diversity and the number of alleles for homoeologous groups was high r = 0.398and r= 0.502, (P<0.01), for old and modern respectively. genotypes. 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)> (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 (group2, 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

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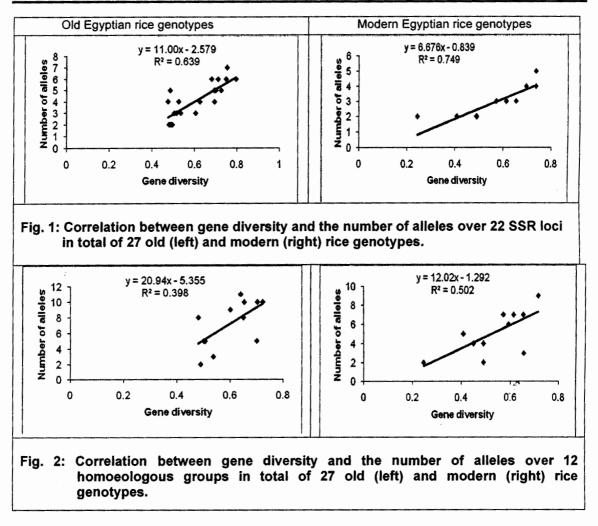
genotypes for all homoeologous groups. In addition, the average genetic richness from 1st to 12th homoeologous group for the modern genotypes ranged from 2 to 5.50. So, group 7 still hold the highest genetic richness and group 12 was the lowest in both modern and old genotypes (Table 4). With regards 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 range		Number of alleles		Gene diversity		
		Min	Max	Old	Modern	Old	Modern	
Rm 5	1	106	124	6	3	0.795	0.571	
Rm 6	2	144	162	3	4	0.605	0.735	
Rm 11	7	120	144	7	5	0.753	0.735	
Rm 22	3	186	202	5	4	0.725	0.694	
Rm 55	3	210	218	4	3	0.475	0.612	
Rm 118	7	150	162	4	4	0.525	0.694	
Rm 133	6	227	236	4	3	0.625	0.612	
Rm 144	11	218	238	2	2	0.495	0.490	
Rm 151	1	78	88	2	2	0.480	0.408	
Rm 154	2	176	198	5	3	0.693	0.571	
Rm 161	5	165	185	4	2	0.694	0.490	
Rm 162	6	203	243	6	3	0.680	0.571	
Rm 215	9	148	152	2	2	0.495	0.490	
Rm 271	10	98	118	4	3	0.695	0.612	
Rm 277	12	114	122	2	2	0.488	0.490	
Rm 285	9	198	205	3	2	0.515	⁻ 0.490	
Rm 307	4	122	174	5	2	0.700	0.245	
Rm 408	8	125	133 .	3	3	0.535	0.653	
Rm 413	5	80	106	6	5	0.710	0.735	
Rm 433	8	210	214	5	3	0.485	0.653	
Rm 474	10	226	264	6	4	0.748	0.694	
Rm 552	11	174	186	3	2	0.505	0.408	
Total				91	66			
Mean				4.14	3.00	0.610	0.575	

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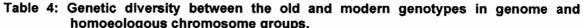
Cluster analysis

A dendrogram derived from UPGMA cluster analysis based on the genetic similarity (GS) coefficient matrix for all the analysed genotypes constructed was (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 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 anv other varieties/cultivars and forms a separate group (group E).

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	homoeologous chromosome groups.									
Location	Number of loci checked	Number	of alleles		e genetic ness	Gene diversity				
		Old	Modern	Old	Modern	Old	Modern			
Ganama		genotypes	genotypes	genotypes	genotypes	genotypes	genotypes			
Genome										
Α	21	91	66	7.58	5.50	0.610	0.575			
Home	peologous	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			



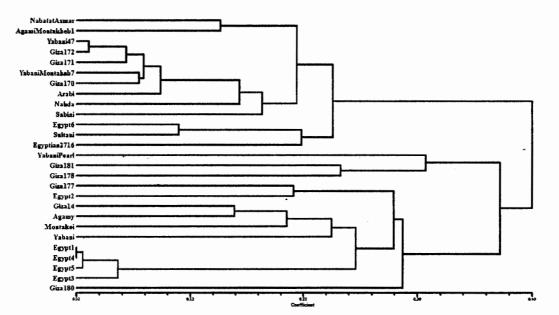


Fig. 3: Dendrogram reflecting genetic similarity between 27 Egyptian rice, based on the analysis of 22 microsatellite loci.

Discussion

Rice microsatellite markers are locusspecific 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 the plant. For example. 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. (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 > PIC > 0.25 is just informative marker, while PIC ≤ 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 (2002) reported an average gene al. 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. Giarrocco 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

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significant correlation between gene diversity and the number of alleles (r = 0.639and 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 pressures due to breeding selective 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.

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التنوع الوراثى لأصناف الأرز المصريه القديمه والحديثه باستخدام المعلم الجزيئي الميكروستاليت

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الملخص العربى

يعد استخدام المعلم الجزئ الميكوستاليت (Microsatelitte) هام جدا لتقدير التنوع الوراثي في أصناف الأرز المصري القديمة والجديدة، وكان الهدف من هذه الدراسة هو :–

- 1) تقييم التنوع الوراثي لأصناف الأرز القديم والحديث المزروعة في مصر باستخدام المعلم الجزئ الميكروستاليت(Microsatelitte).
- ٢) تحديد ما إذا كانت الأصناف القديمة مصدرا محتملا لتحسين التنوع الوراثي في تربية الأرز الحديث في مصر.
 ١ استخدم لتنفيذ هذا البحث اثنين وعشرون معلم جزئ ميكروستاليت لدراسة التنوع الوراثي لسبعه وعشرون صنف
 - أرز قديم وجديد حسب سنوات أستنباط الأصناف وطرحها للزراعة. وفيما يلي ملخص لأهم النتائج :
- ١- كان إجمالي عدد الأليلات (٩١) ، (٦٦) أليل وراثي بمتوسط (٤.١٤) ، (٣٠٠٠) أليل لكل موقع وراثى في كل
 من أصناف الأرز القديمة والحديثة على التوالي.
 - ٢- كانت متوسطات قيم PIC لكل من الأصناف القديمة والحديثة هي ٩,٦١٠ ، ٥٧٥, على التوالي.
- ٣- كان أعلى عدد للأليلات على الكروموسومات ٧ ، ١٠، ٥، ٢ على التوالي بالنسبة للأصناف الحديثة مقارنة بالأصناف القديمة.
- ٤- بالنسبة لمتوسط الثراء الورائى ، وجد ان اعلى قيمة هى ٥,٥٠ فى الأصناف الحديثة مقارنة ٧,٥٨ بالأصناف القديمة.
 - ٥- وجد أن أعلى قيم للإختلاف الوراثي في أصناف الأرز الحديثة هي ٧١٥. على الكرموسوم السابع.
 ٦- اشتملت نتائج الدندوجرام على إنعزال الأصناف محل الدراسه خمسه مجموعات رئيسيه.