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Genetic Diversity Analysis by SSR Markers Linked to Major QTLs of Cooking and Eating Quality Traits in Rice (*Oryza sativa* L.)

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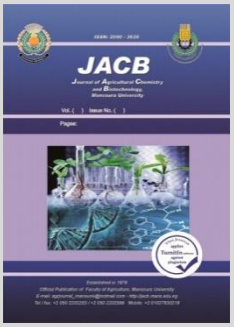

ABSTRACT

Improvement of cooking and eating quality traits is one of the main target of many rice breeding program. The utilization of molecular markers to study genetic variation in specific regions for genome of rice is a useful index which may be used in rice breeding programs to use marker assisted selection. Eight microsatellite markers (SSR) closely related to major QTLs controlling three grain quality traits (amylose content, gelatinization temperature and gel consistency) were used to group 40 rice genotypes in this study. The 40 genotypes tested were divided into six clusters based on their genetic distance. Cluster IV has the most genotypes (16), followed by cluster III with eight genotypes, and cluster V with only two genotypes. Cluster V and VI had the maximum inter-cluster distance (35.627), whereas cluster III and VI had the lowest (5.897). There were a total of 39 polymorphic alleles, with an average of 4.875 alleles per locus. The number of alleles per locus ranged from 3 in RM276 to 6 in both of RM204 and RM340. The 40 genotypes investigated were sorted into six groups by cluster analysis using the UPGMA method, which differentiated the Egyptian genotypes with good cooking and eating quality from the others. The Egyptian japonica genotypes were classified with low amylose content, low gelatinization temperature and soft gel consistency as good grain quality. SSR markers related to QTLs controlling grain cooking and eating traits were found to be an effective technique for marker assisted selection to assess rice grain quality.

Keywords: *genetic diversity; grain quality; Cluster analysis; marker assisted selection; rice.*

INTRODUCTION

Rice is a well-known global crop that provides food for the majority of the world's population. Rice has a high carbohydrate content, a low fats content, and is high in proteins, vitamins, and minerals (Khush, 2005). As people's living standards have grown in recent years, they have begun to demand exceptional rice with good eating and cooking quality features and differing preferences across different areas. Rice breeders must improve grain quality to fulfil consumer demand for specific types of high-quality rice as well as possible market demand for a variety of commercial uses (Lokesh *et al.*,2017).

Grain quality is an example of physical, chemical, cooking and nutritional quality properties (Verma *et al.*,2015). Moreover, as amylose content (AC) is the key characteristic that significantly contributes to the eating and cooking quality of rice (Fernando *et al.*,2015). Rice cooked in a low-temperature environment is sticky and squishy; when the temperature rises, the rice tightens up (Kennedy and Burlingam, 2003). Rice breeders have been concerned about regulating AC because it is crucial for the texture and appearance of rice. Understanding the genetic bases of such traits is crucial to promote the development of new rice genotypes with good cooking and eating quality (Sabouri, 2009). In varietal development, the gelatinization temperature (GT) is used as a cooking time indicator (Cuevas *et al.*, 2010). It's a cost-effective quality indicator because choosing genotypes with shorter cooking times can save money on fuel (Fitzgerald *et al.*,2009).

Cooking and eating quality traits are difficult to improve due to their polygenic inheritance and interactions with the environment, (Ordonez *et al.*,2010). Rice varieties with outstanding cooking and eating quality have been delayed because to the genetic complexity of cooking and eating quality, as well as the difficulties of properly evaluating cooking and eating quality at early breeding generations (Lestari *et al.*,2009).

Understanding the genetic basis of complicated quantitative characteristics like rice eating quality has been made easier thanks to molecular marker technology (Khanin Pathak *et al.*,2015). SSR molecular markers have been employed to compare specific regions for genome of rice (Kumar *et al.*,2011) and to assess the quality of rice genotypes (Kibria *et al.*,2009). Because of their ability to detect large levels of allelic variability, SSRs have been frequently used to identify genetic diversity and investigate genetic structure in rice species (Garris *et al.*,2005). Also, traits that contribute to genetic variation must be identified (Singh, 2014), as this is the basis for classification and clustering of rice genotypes. According to Lapitan *et al.*, (2007), there is information about the genetic diversity of certain regions of the rice genome, it is very useful for the application of gene mapping and marker-assisted selection (MAS) in breeding programs.

Several studies have shown QTLs for rice grain quality in various populations. So far, molecular mapping studies have conclusively demonstrated that numerous QTLs have a significant impact on chromosome 6, which is responsible for differences in cooking and eating quality. The Waxy gene (wx), which is found on chromosome 6's

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short arm, encodes a granule-bound starch synthase (GBSS), which is required for rice amylose synthesis (Tan *et al.*,1999; Fan *et al.*,2005). The alkali degeneration locus (alk), which codes for the soluble starch synthase IIa (SSIIa) isoform, regulates the GT of rice flour (Bao *et al.*,2006). Shu *et al.*, (2006) discovered that GT characteristics are controlled by two separate loci, the first of which was previously identified by Tan *et al.*, (1999) and corresponded to the gene alk2(t) with a genetic distance of 3.93 cM from the wx gene, and the second of which was identified at the alk locus region and linked to SSR marker RM276. QTLs for gel consistency (GC) are linked with wx locus (Narjes Tabkhkar *et al.*,2012).

As a result, we applied eight SSR markers which were linked to major QTLs of AC, GC and GT (located on the short arm of chromosome 6) to assess the genetic variability of 40 rice genotypes with different starch physicochemical traits in the present study. The objectives of this investigation were:1) to estimate the genetic diversity in a set of genotypes through some grain quality traits, 2) to study the capability of these SSR markers for recognizing genotypes with various cooking and eating traits and to propose the most informative markers for marker assisted selection.

MATERIALS AND METHODS

Plant materials:

The present study comprised 40 rice genotypes, collected from Egyptian Rice Germplasm Unit (ERGU) at rice research department, Sakha, Kafr El-Sheikh, Egypt.

Methods:

This research was conducted in the rice research department (experimental farm and laboratory) during the 2020 rice growing season. The following traits were measured on 10 random plants collected from the middle row of the test plot: grain length (mm), grain shape (L/W ratio), grain elongation (%), amylose content (%), gelatinization temperature (GT) and gel consistency (GC). According to Chang and Bardenas (1965), using a "micrometer" to estimate the average of 10 well-shaped brown grains (after shelling) from the bottom to the top of these grains. The grain shape (GSh) is expressed as the ratio between the length and width of the grain.

The samples are used for study the cooking, eating and processing characteristics of its flour upon cooking. Grain elongation trait was measured as the following formula:

$$\text{Grain Elongation \%} = \frac{\text{Grain avg. length b.c.} - \text{Grain avg. length a.c.}}{\text{Grain avg. length b.c.}} \times 100$$

Whereas: b.c: before cooking a.c: after cooking

The water uptake and cooking time are determined by the gelatinization temperature (GT). The temperature at which aqueous starch granules start to grow irreversibly is known as GT. Gelatinization temperature is measured using an alkali test, which uses the following scale to spread values: 1=No effect, 2-3= high and high intermediate GT (75-90C°); 4-5= Intermediate GT (70-74C°) and 6-7= Low GT (55-69C°). According to the methods reported by Juliano (1971), Little et al. (1958) and Cagampang et al. (1973) respectively, the grain quality traits, namely amylose content, gelatinization temperature and gel consistency were estimated.

Genomic DNA extraction

40 leaf samples of rice genotypes were collected from individual plants of 21-day-old seedlings, and genomic DNA was isolated by the CTAB (Cetyl Tri Methyl Ammonium Bromide) method of Murray and Thompson (1980). The quality of DNA is determined by agarose gel electrophoresis and quantified with a spectrophotometer.

Microsatellite markers and PCR amplification

Eight; SSR markers RM204, RM584, RM111, RM527, RM19620, RM276, RM528, RM340 and RM412 linked with two genes, *wx* and *alk*, were used in this study. All markers had been previously mapped to chromosome 6 on the Cornell Rice SSR 2001 map (Temnykh *et al.*,2001) as presented at Table 1. These markers were closely linked to QTLs controlling AC, GC and GT. DNA samples were amplified in 10 µl reaction volumes containing of 2 µl template DNA (5 ng), 5 µl ddH_2O, 1 µl PCR buffer (10X), 0.48 µl $MgCl_2$ (50 mM), 0.6 µl dNTPs (2 mM), 0.4 µl of each primer (60 ng) and 0.12 µl of Taq DNA Polymerase (5 U/µl). PCR was carried out in a thermal cycler (Perkin–Elmer-Gene Amp PCR System 9700, USA) to the cycle profile: Initial denaturation at 94°C for 4 min, 40 cycles of 1 min denaturation at 94°C, 30 sec annealing at 55°C or 61°C (depending on the marker used) and 1 min extension at 72°C, and then 4 min at 72°C for the final extension.

Table 1. Some details of documented QTLs in rice chromosome 6 that are responsible for variations in cooking and eating quality traits (Gramene data base[a]).

No.	SSR marker	Trait name	QTL	Parents	Previous studies
1	RM111	GT	qASV6-1	Zhenshan 97/IRAT109	Yan *et al.* (2001)
2	RM204	GT	qLWR-6	CNHZAU Zh97/Ming63	Zhou *et al*. (2003)
3	RM584	AC	ac6b	Zhenshan97 x H94	Fan *et al*. (2005)
		GC	gc6c	Zhenshan 97/IRAT109	Yan *et al.* (2001)
4	RM527	GC	gc6b	Zhenshan97 x H94	Fan *et al*. (2005)
5	RM276	GT	asv6c	Zhenshan97 x H94	Fan *et al*. (2005)
		GT	-	Huangyu B/II32 B	Shu *et al*. (2006)
6	RM528	GT	qPKV-6	IR64/Azu DH	Bao *et al*. (2002)
7	RM340	GC	-	IR64/IRG105	Shu *et al*. (2006)
8	RM412	GC	qCSV-6-2	IR64/Azu DH	Bao *et al*. (2002)
		GC	qSBV-6	IR64/Azu DH	Bao *et al*. (2002)
		GT	asv6b	Zhenshan97 x H94	Fan *et al*. (2005)

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

Data analysis

The binary coding method was used to evaluate polymorphic products from SSR-PCR tests qualitatively, with '1' indicating the presence of a band and '0' indicating the absence of a band. Constructing a dendrogram using the UPGMA method, the SIMQUAL subprogram in NTSYS-pc software ver. 2.02e (Rohlf, 1998) was used to determine the simple matching similarity coefficient (Sokal and Michener, 1958). The POPGENE programme version 1.32 was used to

calculate genetic polymorphism values and the effective number of alleles (Yeh and Boyle 1997). The allelic frequency observed in the genotypes analyzed by Nei (1973) method was used to calculate polymorphic information content (PIC) values for each marker using the following equation:

$$PIC_i = 1 - \sum_{J=1}^{n} P^2_{ij}$$

Where; ***Pij*** **is the frequency of *j*th allele for *i*th marker and n is the number of observed alleles in the studied population.**

Statistical analysis:

The obtained data of grain appearance and grain quality traits were subjected to statistical analyses using SPSS (version 15.0) software. According to Rao (1952), Genetic diversity was calculated using Mohalanobis's distance (D2) method. Tocher's Method (Rao, 1952) was used to determine genotype clustering.

RESULTS AND DISCUSION

Analysis of variance

Data in Table 2 showed that there were significant differences among the 40 rice genotypes for the studied traits. Data represent the presence of high measure of genetic variability among the studied genotypes, with respect to all the studied traits. Therefore, further comparison between genotypes are valid.

Table 2.Analysis of variance and mean squares for all studied grain quality traits

Sources	df	Grain length	Grain shape	Grain elongation%	Amylose content%	Gelatinization temperature	Gel consistency
Replications	2	0.003	0.001	0.103	0.072	0.058	2.858
Genotypes	39	1.497**	0.817**	244.794**	40.775**	3.451**	1414.622**
Error	78	0.005	0.002	0.875	0.089	0.024	4.251
Total	119	0.494	0.269	80.801	13.423	1.148	466.450
CV%		1.188	2.020	2.381	1.421	2.615	2.753

****: significant at 0.01 probability level**

Mean performance of the studied genotypes

Grain appearance traits:

In Egypt, the majority of farmers and consumers rely on visual qualities to distinguish and evaluate rice varieties. Grain dimension and chalkiness are two visual qualities to look for. Grain dimensions consist of grain length, width and shape. Grain dimensions are the primary quality factor in any breeding program. Mean performance of 40 rice genotypes for six studied grain quality traits are shown in Table 3. For grain length trait, about 19 genotypes are short grained (<5.50 mm) mostly japonica or indica/japonica Egyptian rice genotypes. Sixteen genotypes showed medium grained (5.51-6.6 mm) which mostly belonged to either Indica or Tropical- japonica types. The remaining five genotypes showed long grained (6.61-7.5 mm) which are indica type. Concerning Grain shape trait, sixteen Egyptian japonica genotypes are bold grain shape (1.1-2.0). Meanwhile, the majority of studied genotypes (21 genotype) showed medium grain shape (2.1-3.0) which belonged to either Indica or Tropical-japonica type. The remain three genotypes showed slender shape (> 3.0) are indicas genotypes. These results are in harmony with those obtained by El-Refaee et al., (2018).

Cooking and eating quality traits:

Rice varieties are continually developed and released for human consumption; therefore, breeders must be looking continuously for a domestic demand carefully. The preference for cooking and eating quality differ from place to another. However, the Egyptian consumers prefer cooked rice to be sticky after cooking. Data recorded in Table 3 illustrated that all the japonica and idica/japonica Egyptian genotypes had low amylose content (10-20%), low gelatinization temperature (6-7) and soft gel consistency (61-100mm), respectively except GZ6296-12-1-2-1 had intermediate values for all grain quality traits. Some indicas genotypes showed high amylose content (> 25%) and hard gel consistency (26-40 mm). High volume expansion of milled rice upon cooking is not necessarily a result of grain elongation. All Egyptian rice genotypes elongate between 25-52% of its size. These results are in good agreement with those reported by Abd El-Maksoud et al., (2017).

Distribution of genotypes

Table (4) shows that 40 rice genotypes were disseminated to six groups including diverse number of genotypes. Cluster IV had the most genotypes (16), followed by clusters III, I, VI, and II, which had 8, 5, 5, and 4 genotypes, respectively. Only two genotype were found in the lowest cluster, V.

Intra and inter cluster analysis

Intra and inter clusters distance of 40 genotypes of rice are introduced in Table 5. Cluster III (4.280) had the greatest intra clusters distance, while Cluster VI had the smallest intra clusters distance. In regard of inter clusters distance, the most noteworthy distance was observed between cluster V and VI (35.627) while, the least was found between cluster III and VI (5.897). The genotypes clustered into the same cluster, on the other hand, had the lowest values of inter cluster distance, indicating a close association between them and the lowest degree of divergence from each other. Consequently, no transgressive segregants are predicted from hybridization between genotypes belonging to the same cluster or between genotypes of closed related clusters. Furthermore, the largest inter-cluster distance values indicate the most variability among them. Along these lines, hybridization programs may be constructed so that parental genotypes from different clusters with substantial divergence might be employed to generate appropriate transgressive segregants. These findings are similar to those of Kumar *et al.,* (2013) and El-Refaee *et al.,* (2018).

Thus, it is suggested that hybridization between genotypes belonging to clusters separated by vast inter cluster distances be explored.

Table 3. Characteristics of grain quality for the studied rice varieties.

No.	Genotype	Type	Grain Length(mm)	Grain shape	Grain Elongation(%)	Amylose Content(%)	Gelatinization temperature	Gel consistency(mm)
1	Giza 177	(J)	5.44	1.77	26.96	17.86	6.00	97.67
2	Giza 178	(I/J)	5.07	2.04	39.51	17.02	6.33	99.00
3	Giza 179	(I/J)	5.32	2.11	31.29	19.84	7.00	90.00
4	Sakha 101	(J)	5.48	1.94	38.61	17.57	7.00	94.00
5	Sakha 102	(J)	5.50	1.88	37.12	18.55	6.67	89.33
6	Sakha 104	(J)	5.49	1.86	23.13	18.69	6.00	93.00
7	Sakha 105	(J)	5.63	1.99	47.60	16.71	6.00	99.33
8	Sakha 106	(J)	5.17	1.75	37.14	18.49	6.00	91.33
9	Sakha 107	(J)	4.77	1.75	47.93	16.75	7.00	97.33
10	SKC 2015-1	(J)	4.95	1.75	31.26	19.92	7.00	82.00
11	GZ 9730-1-1-1-1	(J)	6.20	2.18	38.23	16.42	6.67	99.33
12	GZ 9626-2-1-3-2	(J)	5.13	1.83	41.62	17.65	7.00	93.33
13	GZ 6296-12-1-2-1	(I/J)	6.53	2.92	47.58	24.10	5.00	53.00
14	IET 1444	(I)	5.65	2.42	45.97	24.56	7.00	61.33
15	GZ 1368-S-5-4	(I)	5.82	2.39	27.11	24.50	7.00	47.67
16	GZ 6903-1-2-2-1	(J)	5.15	1.83	40.56	18.99	6.00	88.67
17	CIASEM	(I)	5.41	1.98	34.59	23.08	4.00	62.33
18	Korea 27	(I/J)	4.92	1.77	39.94	19.97	7.00	86.33
19	AC 2882	(J)	4.99	2.15	32.37	19.74	6.00	88.00
20	GZ 10595-9-1	(J)	5.91	1.98	44.64	17.27	5.00	94.67
21	GZ7769-2-1-1-2	(J)	5.24	1.78	37.72	18.53	7.00	89.33
22	GZ 8714-7-1-1-2	(J)	4.73	1.74	52.56	18.53	7.00	90.67
23	AC 1605-M20-14	(J)	5.01	1.74	41.78	20.72	7.00	85.00
24	IR 12 L 355	(I)	6.65	2.99	44.15	25.85	7.00	42.33
25	IR 12 L 339	(I)	6.68	3.07	45.04	28.11	5.00	37.67
26	IR 11 L 465	(I)	6.67	2.76	39.37	23.03	6.00	47.67
27	IR 88628-B	(I)	6.91	3.31	41.79	21.82	5.00	57.67
28	WAB 56-104	(S/G)	7.47	3.93	28.53	23.51	5.00	52.00
29	IRAT 170	(I)	5.72	2.15	31.98	28.04	5.00	35.67
30	NERICA -4	(S/G)	6.58	2.92	66.69	27.29	5.00	37.00
31	A 22	(I)	5.74	2.74	39.10	27.55	5.00	46.00
32	IR 65600-127-6-2	(TJ)	5.95	2.15	33.01	19.97	7.00	84.33
33	IR 69853-70-3-1-1	(TJ)	5.34	2.04	34.52	17.44	3.00	90.67
34	IR 68011-15-1-1	(TJ)	6.60	2.45	35.27	21.74	5.00	62.00
35	TCCP266-1-3B-10-2-1	(I)	5.85	2.76	28.43	27.50	5.00	42.67
36	IR 66160-121-4-5-3	(TJ)	5.51	2.02	27.47	18.19	7.00	94.33
37	IR 68552-55-3-2	(TJ)	4.92	2.03	40.94	16.34	4.00	98.00
38	KATY	(TJ)	6.58	2.35	37.42	22.49	5.00	72.33
39	IRGA 318-11-6-2-6	(I)	6.51	2.31	58.75	26.10	5.00	52.67
40	IRGA440-22-3-6	(I)	5.84	2.87	56.38	21.74	7.00	70.00

Japonica: (J), Indica (I); Indica / Japonica (I/J); Tropical Japonica (TJ) and Hybrid between Oryza sativa with Oryza galliberema (S/G).

Table 4. Distribution of 40 rice genotypes within each cluster.

Cluster	Number of genotypes	Name of genotypes included
I	5	GZ 6296-12-1-2-1, IET 1444, IR 12 L 355, IR 12 L 339, A 22
II	4	Sakha 105, Sakha 107, GZ 8714-7-1-1-2, IRGA440-22-3-6
III	8	GZ 1368-S-5-4, CIASEM, AC 2882, WAB 56-104, IRAT 170, IR 65600-127-6-2, IR 68011-15-1-1, TCCP266-1-3B-10-2-1
IV	16	Giza 178, Sakha 101, Sakha 102, Sakha 106, GZ 9730-1-1-1-1, GZ 9626-2-1-3-2, GZ 6903-1-2-2-1, Korea 27, GZ 10595-9-1, GZ7769-2-1-1-2, , AC 1605-M20-14, IR 11 L 465, IR 88628-B, IR 69853-70-3-1-1, IR 68552-55-3-2(NPT), KATY
V	2	NERICA -4, IRGA 318-11-6-2-6
VI	5	Giza 177, Giza 179, Sakha 104, SKC 2015-1, IR 66160-121-4-5-3(NPT)

Table 5. Intra cluster distances (Bold) and inter cluster distances (D2) for 40 rice genotypes.

Cluster	I	II	III	IV	V	VI
I	3.126	9.961	13.209	8.740	18.386	17.898
II		3.495	19.925	11.231	14.827	22.602
III			4.280	9.286	31.479	5.897
IV				3.173	24.604	11.433
V					4.026	35.627
VI						2.885

They utilize available genetic resources to develop novel combinations thanks to useful breeding procedures that rely on modern and adaptive understanding of genetic diversity among genotypes. The genotype-environment interaction as well as the genotype's genetic contribution are indicated through morphological markers. (Rajasekaran and Thenmozhi, 2013).

Polymorphism of microsatellite markers

All of the SSR motifs utilized in the current study were polymorphic, resulting in a varying of alleles with distinct size ranges (Table 6). The overall size of amplified products ranged from 114bp in locus RM111 to 272bp in locus RM528. The effective number of alleles varied from 2.253 in locus RM276 to 5.019 in locus RM204 with an average of 3.894 alleles which was much higher than the average of 2.1903 alleles reported by Kibria *et al.* (2009). The 40 rice genotypes had a total of 39 alleles, with an average of 4.875 alleles per locus. The number of alleles per locus ranged from 3 in RM276 to 6 in both of RM204 and RM340 (Table 6). This value was lower to the average of 5.86 per microsatellite locus reported by (Narjes Tabkhkar *et al.*,2012), while it was higher than the average of 4.23 alleles per locus reported by Ghneim *et al.,* (2008) for Venezuelan rice cultivars and the average of 3 alleles per locus found by Kibria *et al.,* (2009) using microsatellite markers linked to genes controlling rice grains aroma. Moreover, the average number of alleles per locus found in this study was lower than that found in earlier studies.

Kuroda et al., (2007), for example, detailed an average of 9.28 alleles per locus more than 7 SSR loci. This could be anticipated and well accepted due to the differences in the rice genotypes studied.

Polymorphism information content (PIC) is a useful measure of the polymorphism level of the genotypes under investigation; values approaching 1 suggest a high degree of genetic variety and are related with a large number of alleles, whilst values less than 0.5 indicate a low polymorphism level. PIC for microsatellite loci ranged from 0.626 in RM276 to 0.804 in RM204 with an average of 0.735 that was similar to the average of 0.72 per microsatellite locus reported by Narjes Tabkhkar *et al.*, (2012) using seven SSR markers in 40 rice varieties. The result was greater than the average of 0.524 revealed by Ghneim *et al.*, (2008) in 11 Venezuelan rice varieties using 48 SSR markers, and the average of 0.50 reported by Bounphanousay *et al.*, (2008) utilizing 24 SSR markers. Furthermore, utilizing three SSR markers, Kibria *et al.*, (2009) got an average of 0.119 in aromatic rice genotypes, which was less than that found in the current study.

Table 6. Attributes of SSR markers linked to three characteristics of rice cooking and eating quality in all studied genotypes.

No.	Marker Name	Repeat Motif	expected allel size bp	Allele size bp	No. of total alleles	No. of effective alleles	Major allel frequency	Heterozigosity	PIC
1	RM204	(CT)44	169	119-191	6	5.019	0.25	0.810	0.804
2	RM584	(CT)14	169	124-162	5	3.785	0.4	0.711	0.696
3	RM111	(GA)9	124	114-132	4	3.081	0.45	0.728	0.694
4	RM527	(GA)17	233	228-256	5	4.070	0.325	0.784	0.768
5	RM276	(AG)8A3(GA)33	149	124-146	3	2.253	0.45	0.666	0.626
6	RM528	(AGAT)9	232	244-272	5	4.050	0.3	0.774	0.763
7	RM340	(CTT)8T3(CTT)14	163	134-192	6	4.831	0.375	0.776	0.766
8	RM412	(GA)22	198	194-231	5	4.065	0.3	0.774	0.766
	Total				39	31.154	2.85	7.525	5.883
	Mean				4.875	3.894	0.35625	0.752	0.735

Cluster analysis and genetic relationships

Cluster analysis was performed using the UPGMA method to classify the studied varieties based on the simple matching coefficient of similarity. The dendrogram separated the 40 genotypes into two main groups with genetic similarity of 0.65 (Figure 1). The cophenetic correlation coefficient between the dendrogram output matrix of the cluster analysis and the similarity matrix of the simple matching coefficient was 0.93 (the highest value among the similarity coefficients), indicating that the similarity coefficient and the cluster analysis method used was reasonable to group rice varieties depends on the data obtained from the SSR markers. The first main group comprised 24 rice genotypes and was divided into two subgroups. The first subgroup belonged to the japonica type (it contained 16 varieties of Egyptian rice and improved lines) with a similarity coefficient of around 76%. All genotypes in this subgroup were classified as having good cooking and eating properties with low amylose content, low gelatinization temperature and soft gel consistency.

The second sub-cluster was further divided into two groups. The first group comprised six genotypes belonged to Tropical-japonica type with genetic similarity of 78%. Four genotypes were classified with low amylose content and two are intermediate (IR68011 and Katy). All the tropical-japonica genotypes had either low GT (two genotypes) or intermediate (three genotypes), while the remaining one (IR69853) had a high GT. All the six tropical-japonica genotypes had soft gel consistency. The second group had two genotypes belonging to the same origin (west Africa) are namely NERICA-4 and WAB56-104. In spite of they have same origin but have different cooking and eating properties, NERICA-4 had high AC and hard GC and WAB56-104 had intermediate in both AC and GC. On the other hand, both of them had intermediate gelatinization temperature. In general, these results are in agreement with those reported by Fitzgerald *et al.*, (2009). The second major cluster consisted of 16 rice genotypes which belonged to either indica (12 genotype) or indica/japonica type (4 genotypes) with genetic similarity of 0.68 (Figure 1), and was further divided into two sub-clusters. The two indica/japonica Egyptian commercial varieties came separately in the first sub-cluster at about 90% genetic similarity. Both of the two varieties were classified with low amylose content, low gelatinization temperature and soft gel consistency. The second sub-cluster was divided into two groups with 70% similarity. The first group consisted of four genotypes, all of them belonged to indica type except one belonged to indica/japonica type (GZ6296).

These genotypes showed different values in both of AC and GC ranged from intermediate to high, in case of GT showed intermediate values. The second group was further divided into two sub-groups with 77% similarity. The first sub-group was comprised of 8 genotypes with different origin. These genotypes showed different values in both of AC and GC ranged from intermediate to high, except Korea 27 (indica/japonica type) showed low AC and soft GC, in case of GT, the genotypes showed different range of low to intermediate values. The second sub-group contained only two indica genotypes namely; IRGA 318 and IRGA 440 with about 79% similarity coefficient. In spite of they have same origin but they showed different cooking and eating properties, and ranged from intermediate to high in case of AC. On the other hand, IRGA440 had low GT and soft GC, while, IRGA318 had intermediate in both GT and GC.

The findings revealed that genotypes with the same origins were grouped into the same classes. Furthermore, genotypes with the same amylose classes were grouped. Consequently, the SSR markers used were relevant for the evaluation of genetic variability and cooking and eating quality characteristics of the rice genotypes. Using SSR markers in different Egyptian rice cultivars, El-Refaee *et al.*, (2011) investigated genetic diversity related to agronomic traits and biotic stresses. They reported that the within-group similarities for the Egyptian japonica, indica, and indica / japonica rice genotypes were greater than 74%, 82%, and 84%, respectively. Lapitan *et al.*, (2007) reported that SSR markers can identify quality rice subspecies and classify cultivars with the same quality of cooking and

consumption. Kibria *et al.,* (2009) used SSR markers to classify 14 aromatic rice genotypes into two main groups, and the dendrogram showed that the genotypes are genetically identical type derivatives clustered together.

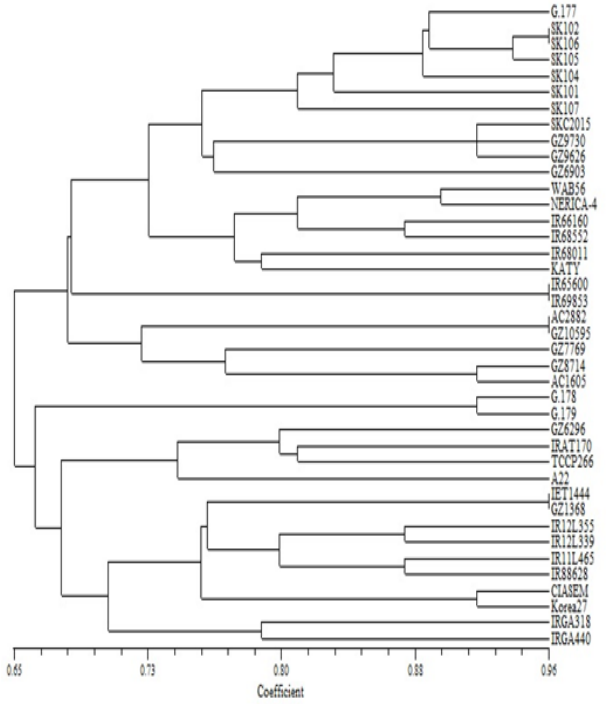

Fig. 1. Dendrogram of cluster analysis by UPGMA method based on simple matching coefficient of eight microsatellite markers linked to grain quality characteristics in 40 studied rice genotypes.

CONCLUSION

This study revealed that there might be a great possibility to get the best new combinations by crossing the genotypes that have highest genetic distance. In addition, the findings showed that the utilization of microsatellite markers linked to grain quality attributes, particularly those closely linked to the wx and alk genes, can discriminate rice types for quality characteristics. SSRs are also thought to be a good tool for marker assisted selection (MAS) to select high-quality rice genotypes.

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تحليل التنوع الوراثى باستخدام المعلمات الجزيئية المرتبطة بمواقع الصفات الكمية الرئيسية لصفات جودة الطهى والاكل فى الارز

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يعد احد الاهداف الريئسية لبرامج التربية فى الارز هو تحسين صفات جودة الطهى والاكل. يعد استخدام المعلمات الجزيئية لدراسة التنوع الوراثى فى مناطق معينة فى جينوم الارزمؤشرا هاما والذى يمكن من خلاله تطبيق الانتخاب بمساعدة المعلمات الجزيئية. تم استخدام ثمانية من المعلمات الجزيئية والمرتبطة ارتباطا وثيقا بمواقع الصفات الكمية الرئيسية والتى تتحكم فى ثلاثة من صفات جودة الطهى والاكل فى الارز(محتوى الاميلوز، درجة حرارة الجلتنة و درجة تماسك الجلى) ل 40 تركيب وراثى من الارز فى هذة الدراسة. اعتمادا على المسافة الوراثية تم تقسيم ال 40 تركيب وراثى تحت الدراسة الى 6 مجموعات. كانت المجموعة الرابعة هى الاكبر واحتوت على 16 تركيب وراثى تلاها المجموعة الثالثة بثمانية تراكيب وراثية بينما كان هناك تركيبان وراثيا فقط فى المجموعة الخامسة. كانت اعلى مسافة وراثية بين المجاميع الداخلية (35,627) بين المجموعتين الخامسة والسادسة بينما كانت اقل مسافة وراثية بين المجموعتين الثالثة والسادسة (5,897) . وكان العدد الاجمالى للاليلات متعددة الاشكال المظهرية 39 اليلا بمتوسط 4,875 اليلا لكل معلم جزيئي وتراوحت عدد الاليلات لكل معلم جزيئي 3 اليلات للمعلم RM 276 الى 6 اليلا للمعلم RM 204 , RM 340. تراوح العدد الفعال للاليلات من 2,253 الى 5,019 اليلا للمعلم RM 204, RM 276 على التوالى بمتوسط 3,894 اليلا لكل موقع. كان متوسط عدم التماثل الوراثى اعتمادا على اساس التنوعى الوراثى الجينى بمقدار 0,752 مما يشير الى وجود تباين عالى بين التراكيب الوراثية تحت الدراسة. قسم التحليل العنقودى باستخدام طريقة UPGMA الاربعين تركيب وراثى تحت الدراسة الى ستة مجموعات وتم فصل التراكيب الوراثية المصرية ذات جودة الطهى والاكل جيدة بعيدا عن التراكيب الوراثية الاخرى. لقد صنفت التراكيب الوراثية المصرية من الطراز اليابانى من الارز بخصائص طهى واكل جيدة مع محتوى منخفض من محتوى الاميلوز ودرجة الجلتنة المنخفضة ودرجة تماسك الجلى الناعمة. اشارت نتائج هذة الدراسة الى ان المعلمات الجزيئية SSR والمرتبطة بالجينات او مواقع الصفات الكمية والتى تتحكم فى صفات جودة الطهى والاكل تعتبر اداة فعالة للانتخاب بمساعدة المعلمات الجزيئية والتى تحدد جودة الحبوب فى الارز.