MOLECULAR CHARACTERIZATION OF DROUGHT TOLERANCE IN NINE EGYPTIAN RICE GENOTYPES USING RAPD, SCoT AND SSR MARKERS

AZIZA A. ABOULILA[1], OLA A. GALAL[1] AND M. I. ABO-YOUSSEF[2]

1. Genetics Department, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafr El-Sheikh, Egypt

2. Rice Research and Training Center, Agricultural Research Center, Sakha, Kafr El-Sheikh, Egypt

Rice is one of the most important food crops. It has high nutritional benefits while it is rich in calories, fiber, vitamins and minerals. Rice has also low amounts of cholesterol and sodium therefore it is a healthy source of energy (Sellamuthu *et al*., 2011).

Rice is very sensitive to drought stress which can result in a high reduction in grain yield (reached to 50%) during reproductive development (Pandey and Bhandari, 2009; Srividhya *et al*., 2011). Drought is a major abiotic problem that limits rice productivity and affects grain quality (Yang *et al*., 2008; Bimpong *et al*., 2011). Increasing of climate changes causes serious problems due to drought stress effects. Therefore, the development of highly-yielding and drought-tolerant rice varieties was a major goal for rice breeding.

Response to drought in plants is a complex manner involving physiological, biochemical and molecular changes (Atkinson and Urwin, 2012). Therefore, drought tolerance is a result of expression of some traits during drought stress period. So, no single trait is responsible for crop productivity improvement in response to water deficits (Kamoshita *et al*., 2008; Farooq *et al*., 2009).

Using molecular markers to detect quantitative trait loci (QTLs) controlling drought tolerance related traits has the potential to accelerate breeding for drought tolerance. RAPD (Random Amplified Polymorphic DNA) analysis was extensively used to detect and generate molecular markers for drought response in different species including rice (Boopathi *et al*., 2001; Youssef *et al*., 2010).

Start Codon Targeted (SCoT) polymorphism is a simple DNA marker technique developed by Collard and Mackill (2009). It uses a single primer targeting the short consensus conserved region flanking the translation initiation codon in most genes ATG. This marker was extensively used in the study of genetic diversity in rice varieties (Collard and Mackill, 2009).

The microsatellite (SSR) markers are the most suitable for rice because of their reproducibility, multiallelic nature, hyper variability, codominant inheritance, relative abundance and genome-wide cov-

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erage (Tautz, 1989; Morgante and Olivieri, 1993; Powell *et al*., 1996).

Genetic variability at the molecular level could help identifying and developing unique rice germplasm. It is important to study the genetic diversity among the Egyptian rice varieties for food security. The aims of this study are to evaluate the genetic diversity of nine Egyptian rice genotypes and characterize these genotypes for drought tolerance in addition to interpretation of the relationships among these nine genotypes for breeding purposes.

MATERIALS AND METHODS

This study was conducted at Genetics Department Laboratory, Faculty of Agriculture, Kafrelsheikh University, Egypt to study the molecular genetic diversity of nine Egyptian rice genotypes.

Plant material

Nine rice genotypes (commercial varieties) belonging to Japonica and Indica/Japonica types were used in this study as shown in Table (1).

Isolation of genomic DNA

Total genomic DNA was extracted from 100-150 mg fresh young leaves at tillering stage by using Cetyl trimethyl ammonium bromide (CTAB)-based procedure for plants as described by Murray and Thompson (1980). DNA samples were diluted to a final concentration of 40 ng/µl before PCR amplification.

Genotyping using RAPD and SCoT markers

A set of eighteen oligonucleotide decamer RAPD primers (Bio Basic Inc, Canada) and three SCoT primers (iNtRON Biotechnology, Inc, Korea) with GC content of 60% were used. The sequences of RAPD and SCoT primers are listed in Table (2).

Genotyping using SSR markers

A total of 16 SSR primers (iNtRON Biotechnology, Inc, Korea) as shown in Table (3) were tested to find polymorphisms among the nine rice genotypes.

PCR amplification

The PCR amplification was performed according to Williams *et al*. (1990) using 2X PCR Master mix solution [(i-Taq^{TM}) iNtRON Biotechnology] in 10 µl reaction volume on a thermal cycler (Perkin Elmer Cetus) programmed.

Electrophoresis

The amplification products were separated by a horizontal gel electrophoresis unit using 1.5% agarose gel for RAPD and SCoT markers and 3% agarose gel for SSR markers. Gels were then run for 1.5 to 2.0 h. at current 70 V. Bands were documented using Benchtop UV-transilliminator and photographed using photo Doc-ItTM imaging system. A known DNA Ladder ready-to-use (Thermo Scientific O'GeneRuler DNA Ladder

Mix, Cat-no: SM1173) was run against the RAPD-PCR products, while the molecular size of the SCoT and SSR amplified products was determined against 1 Kb plus DNA ladder (TIANGEN).

Data analysis

For RAPD, SCoT and SSR analyses, data were introduced as binary values of (1) and (0) for the presence or absence of an amplification product. The presence or absence of specific amplification bands was scored and used to determine the number of alleles per SSR primers and polymorphic information content (PIC) value of all the three types of markers. The dendrograms of the nine rice genotypes; based on RAPD, SCoT and SSR markers, were generated using the unweighted pair grouped method arithmetic average (UPGMA) to determine the genetic diversity and relationships among the tested genotypes. PIC values were calculated with the formula as reported by Roldan-Ruiz *et al*. (2000). The similarity matrix was also used for principal coordinate analysis (PCoA).

RESULTS AND DISCUSSION

Genetic diversity and molecular characterization of the Egyptian rice varieties

- ***RAPD and SCoT polymorphism***

The size and number of total amplified bands, number of polymorphic bands, percentage of polymorphism and polymorphic information content obtained per each RAPD and SCoT primer are shown in Table (4). All the tested eighteen RAPD and the three SCoT primers produced polymorphic bands among the rice genotypes.

The results of RAPD markers (Fig. 1 and Table 4) showed that the size of the amplified bands ranged from 100 bp (OPB-10 primer) to 2983 bp (OPH-02 primer). A total number of 377 bands; ranged from 13 to 29 bands with an average of 20.94 bands per primer, were amplified and 68.70% out of them were polymorphic with an average of 14.39 bands per primer. The OPB-08 primer showed the highest polymorphism percentage (92.59%), while the OPH-04 primer showed the lowest percentage of polymorphism (41.18%), indicating a considerable amount of polymorphism. The PIC values ranged from 0.16 to 0.35 with an average value of 0.24.

Several studies confirmed the importance of RAPD marker for DNA fingerprinting, establish genetic relationships and also to detect molecular markers for drought tolerance. Tuinstra *et al*. (1992) used RAPD markers to identify quantitative trait loci (QTLs) linked to drought tolerance within some sorghum recombinant inbred lines. Schneider *et al*. (1997) detected four RAPD markers in one population and five in another associated with drought tolerance in common bean (*Phaseolus vulgaris* L.). Boopathi *et al*. (2001) found a RAPD-positive marker that could be used to identify drought-tolerant genotypes in segregating rice populations. Abdel-Tawab *et al*. (2003)

used RAPD analysis to detect some markers for drought tolerance in wheat. Pakniyat and Tavakol (2007) found some markers associated with drought tolerance in bread wheat genotypes using RAPD-PCR. Also, Nazari and Pakniyat (2008) introduced some markers related to drought tolerance in cultivated and wild barley using RAPD markers.

For the tested SCoT primers (Fig. 1), the results summarized in Table (4) showed that the three SCoT primers amplified 45 polymorphic bands out of 52 total bands with average of 17.33 bands per primer and the percentage of polymorphism was 86.54%. Number of bands per primer ranged from 17 to 18 while the PIC values spanned from 0.23 to 0.35 with an average value of 0.28.

The SCoT analysis proved to be a good method in studying genetic diversity. Due to reproducibility and simplicity of this method, it was used in the assessment of genetic diversity and taxonomy in barley (Aboulila and Mansour, 2017; Dora *et al*., 2017).

- ***SSR polymorphism and fingerprinting***

To access genetic diversity level in the studied nine rice genotypes, 16 SSR primers were used to study DNA polymorphism among the rice genotypes. Table (5) showed the total number of alleles, positive unique marker, polymorphism percentage, allele size, highest frequency allele, annealing temperature and PIC values of each microsatellite locus in the studied rice genotypes. All of the 16 SSR primers used in this study generated polymorphic and scorable amplification bands among rice genotypes and no band was found to be monomorphic (Fig. 2 and Table 5).

A total of 104 alleles were detected for the 16 SSR markers among the nine rice genotypes. The number of alleles per locus ranged from one (RM301 and RM302) to 14 (RM3825), with an average of 6.5 alleles across the 16 loci. The overall size of the amplified products ranged from 85 bp (RM545) to 1172 bp (RM228). The differences in molecular size between the smallest and the largest allele for a given SSR locus varied from 57 bp (RM201) to 1069 bp (RM228). The PIC values for the SSR loci ranged from 0.20 (RM302 and RM518) to 0.49 (RM301), with an average of 0.31. The highest PIC value (0.49) was obtained for RM301 followed by RM228 (0.44) and RM553 (0.41) (Table 5).

Among the 16 studied SSR markers, 14 markers spread across five chromosomes (chr. 1, 3, 4, 9 and 12) were found to be useful in fingerprinting for the nine genotypes of the present study. It was found that fingerprinting of Sakha 102, Sakha 103 and Giza 178 varieties could be done by six different markers for each, followed by Sakha 101 and Giza 171 varieties with five markers and Sakha 106 and E. Hybrid varieties with three markers. Fingerprinting of the remaining genotypes (Giza 104 and Giza 179) could be done using at least one marker.

A total of 57 unique alleles were detected at 14 SSR loci (Table 5). All rice genotypes had positive unique alleles for at least one SSR locus. The RM301 SSR marker amplified one specific allele only in the four rice varieties sensitive to drought (Sakha 101, Sakha 103, Sakha 106 and Giza 171).

The different SSR primers gave different degrees of polymorphism. An average of 6.5 alleles per locus was recorded in this study. Cho *et al*. (2000) and Wong *et al*. (2009) used different classes of SSRs and reported an average of 2.0-5.5 alleles per locus. Also, polymorphism level as assessed by PIC values was high and varied among the SSR loci with an average of 0.31. Akkaya and Buyukunal-Bal (2004) reported that high values of PIC can be attributed to the use of informative markers. In this study PIC values showed that the best marker for genetic diversity analysis was RM301 followed by RM228 and RM553. These DNA sequences can help in the future for studying rice germplasm.

In this study, RM301 marker was able to produce specific alleles in four drought-sensitive varieties only that could be used for identifying the sensitive genotypes from the others. Moreover, the RM212, RM518 and RM553 SSR primers generated positive unique markers only in the sensitive drought genotypes. While, RM20A and RM302 primers produced positive unique markers in a moderate (Sakha 102) and a tolerant drought genotype (Giza 178), respectively. These results can help in identification and characterization of rice genotypes. Thus, SSR fingerprinting proved to be a valuable tool in the determination of rice drought tolerant genotypes.

Generally, molecular characterization revealed polymorphism percentages of 68.70% for RAPD markers, 86.54% for SCoT markers and 100% for SSR markers, indicating high levels of polymorphism among the studied rice genotypes. Similar result was found by Rahman *et al*. (2012) who reported that all SSR primers generated polymorphic bands and no monomorphic bands were found. Results of the present study were consistent with Racchi *et al*. (2014), who reported that SSR markers are more polymorphic than SCoT markers. On contrast, our results were not in agreement with Bahraminejad and Mohammadi-Nejad (2015), who reported that the polymorphism detected by SCoT markers were low as compared to RAPD markers. This difference was perhaps explained by the difference in the DNA segments which targeted by the three markers. RAPD marker was previously used by Malik *et al*. (2000) who revealed that RAPD marker had a great potential to detect polymorphism between two wheat genotypes namely; PK81 (drought resistant) and ABA18 (drought susceptible).

Genetic distance-based analysis

- ***RAPD markers***

The dendrogram based on RAPD markers was constructed using the

UPGMA method (Fig. 3a). The dendrogram was classified the nine rice genotypes into two main groups. The three drought tolerant varieties (Giza 178, Giza179 and E. Hybrid) that belonged to the type of Indica/Japonica were isolated in a single group (cluster I) with a genetic similarity percentage of 81.0%, while the second group collected all the studied Japonica types which divided into two subgroups with a similarity percentage of 84.4%. The two moderate tolerant varieties (Sakha 102 and Sakha 104) as well as the sensitive variety (Sakha 106) were isolated in a single subgroup (cluster II), while the second subgroup contained the other three drought sensitive varieties (Sakha 101, Sakha 103 and Giza 171) in cluster III.

The principal coordinate analysis (PCoA) results represented 46.24% and 19.79% of total variation based on RAPD data with 93.9% level of variation as shown in Fig. (3b). In this analysis three main groups were appeared. The first group of PCoA contained the three drought tolerant rice genotypes used in this study. This was in the same line of the direction of that obtained from the UPGMA clustering analysis which located the drought tolerant varieties (Giza 178, Giza179 and E. Hybrid) in group I with a genetic similarity percentage of 81.0% (Fig. 3a). The second group contained all the drought sensitive genotypes in addition to Sakha 102 which was moderate tolerant variety. The third group contained only Sakha 104 variety.

- ***SCoT markers***

The UPGMA-based dendrogram (Fig. 4a) showed the genetic relationships among the rice genotypes for SCoT analysis. The dendrogram of the nine rice genotypes using UPGMA procedure clustered these genotypes into three major groups in accordance with their reaction to drought tolerance response and the type of these genotypes. Interestingly, the three drought tolerant rice varieties (Giza 178, Giza 179 and E. Hybrid) which belong to Indica/Japonica type were far from the other genotypes and were clustered together in one group (cluster I). This result supported the previous result obtained from the dendrogram based on RAPD data. A moderate drought-tolerant variety, Sakha 104 (Japonica type) was closer to the drought tolerant genotypes (cluster II). The drought-sensitive rice genotypes were very close to each other and grouped in the same cluster (cluster III) which they are Japonica type. Sakha 102 (a moderate drought-tolerant variety) was very similar to the drought sensitive genotypes and might be considered in the same group (cluster III).

PCoA was performed based on the data of the three SCoT primers which investigated in this study to discriminate the nine genotypes according to their drought response. The two axes explained 46.68% and 14.85% of the total variation in response to drought tolerance for axis 1 and axis 2, respectively. The nine genotypes were grouped into three main clusters (I, II and III) as shown in Fig. (4b). The clus-

ter I gathered together the three drought tolerant rice varieties (Giza 178, Giza 179 and E. Hybrid). These genotypes were separated together in one group (cluster I) with a genetic similarity percentage of 67.9% in UPGMA clustering (Fig. 4a). On other hand, cluster II encompassed the other rice genotypes used in this study, except Sakha 104 variety which was separated in one group alone. These results completely agreed with our UPGMA clustering analysis.

- ***SSR markers***

The UPGMA-based dendrogram; which was obtained from the binary data deduced from the DNA profiles using the 16 SSR markers of the analyzed samples, added a new dimension to the genetic similarity perspective. Four distinct groups were created from the analysis of the pooled SSR marker data at a similarity percentage of 20.8% (Fig. 5a). However, the cluster analysis showed high genetic variations among the rice genotypes.

The dendrogram of the nine rice genotypes clustered them into four major groups almost in accordance with their drought tolerance response and their Indica/Japonica type or Japonica type (Fig. 5a). The drought tolerant varieties; E. Hybrid and Giza 179 (Indica/Japonica types), formed a distinct group (cluster I) suggested that they were distantly related from the other genotypes. Group II included the moderate drought-tolerant genotypes (Japonica type) which clustered together with only Giza 178 variety which should be outside this group. Groups III and IV comprised the four drought-sensitive genotypes which were Japonica type.

PCoA based on SSR data are presented in (Fig. 5b). The three axes explained 57.72% of the total variation in response of the genotypes to drought tolerance (24.45%, 20.94% and 12.33% for axis 1, axis 2 and axis 3, respectively). This analysis is a useful tool for studying the genetic relations among these rice genotypes and reveals that most of these genotypes are grouped according to their drought tolerance level and type. The nine genotypes were grouped into four main clusters (I, II, III and IV). The presence of rice genotypes in all the four groups indicated a high genetic diversity in these genotypes. For drought tolerance, PCoA results represented that the highly drought tolerant varieties (E. Hybrid and Giza 179) in addition to the moderate drought tolerant variety (Sakha 104) were separated together in one group. On the other hand, the four drought sensitive genotypes were separated into two groups, the first group contained Sakha 101 and Sakha 103 varieties and the second group contained Sakha 106 and Giza 171 varieties. These results were in the same line of UPGMA clustering results, since the sensitive rice genotypes were separated together in groups III and IV.

Genetic relationships among the rice genotypes obtained from using the UPGMA method in this study revealed

that three clusters with 71.6 and 63.2% of similarity were obtained for RAPD and SCoT markers, respectively, while four clusters with 20.8% of similarity were obtained for SSR markers. In general, this genetic diversity is likely to be associated with the substantial variation in drought tolerance, ecological and climatic conditions and especially breeding and ancestral history. In this study, the grouping of these genotypes based on RAPD, SCoT and SSR markers was well corresponded to their known pedigree data. Interestingly, each of the drought-tolerant, moderate drought-tolerant and drought-sensitive rice genotypes were clustered in the same group showing the maximum divergence for each cluster from the other clusters. Indeed, drought-tolerant rice genotypes constitute a special group of rice strains that are well known for the presence of superfine genes for drought tolerance and grain quality (Jain *et al*., 2004).

In conclusion, results of the present study can be useful in identification of markers which are associated to drought tolerance response in rice which facilitate future breeding efforts aimed at improving this trait through marker-assisted selection (MAS). Although, SSR markers showed a higher percentage of polymorphism than RAPD and SCoT markers. These results suggested that one characteristic alone is not a good predictor for genetic marker and the three markers together could be useful for DNA and genomic fingerprinting.

SUMMARY

Nine Egyptian rice (*Oryza sativa* L.) genotypes were assessed for DNA polymorphism using three different types of molecular markers (RAPD, SCoT and SSR). RAPD primers produced 68.70% of polymorphism and an average PIC value of 0.24. SCoT primers generated 86.54% of polymorphism and 0.28 average value of PIC. All tested SSR markers yielded amplified products and generated 104 alleles (average 6.5 alleles/marker) with PIC values ranged from 0.20 to 0.49 per marker. RM301 SSR marker produced specific alleles only in the four drought sensitive rice varieties that could readily distinguished the sensitive genotypes from the others. While, RM20A and RM302 SSR markers produced one positive unique marker in the moderate (Sakha 102) and drought tolerant (Giza 178) varieties, respectively. These markers may be usefully exploited for molecular breeding of rice for drought tolerance. On the other hand, clustering analysis using UPGMA method classified the nine rice genotypes into three groups using RAPD and SCoT markers and four groups using SSR marker. The results of principal coordinate analysis (PCoA) were closely related with those of the clustering analysis. These results could be used by breeders to develop drought tolerant rice genotypes and new breeding protocols for rice improvement.

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Table (1): Rice genotypes which used in this study and their drought tolerance status.

Genotype No.	Genotype name	Type	Response to drought
1	Sakha 101	Japonica	Sensitive
2	Sakha 103	Japonica	Sensitive
3	Sakha 106	Japonica	Sensitive
4	Giza 171	Japonica	Sensitive
5	Sakha 102	Japonica	Moderate
6	Sakha 104	Japonica	Moderate
7	Giza 178	Indica/Japonica	Tolerant
8	Giza 179	Indica/Japonica	Tolerant
9	Egyptian hybrid	Indica/Japonica	Tolerant

Table (2): Names and nucleotide sequences of RAPD and SCoT primers which used to amplify DNA in this study.

No.	Primer name	Sequence (5'→3')
RAPD primers		
1	OPA-09	GGGTAACGCC
2	OPA-14	TCTGTGCTGG
3	OPA-20	GTTGCGATCC
4	OPB-01	GTTTCGCTCC
5	OPB-05	TGCGCCCTTC
6	OPB-06	TGCTCTGCCC
7	OPB-07	GGTGACGCAG
8	OPB-08	GTCCACACGG
9	OPB-10	CTGCTGGGAC
10	OPB-11	GTAGACCCGT
11	OPB-12	CCTTGACGCA
12	OPB-14	TCCGCTCTGG
13	OPB-17	AGGGAACGAG
14	OPH-01	GGTCGGAGAA
15	OPH-02	TCGGACGTGA
16	OPH-03	AGACGTCCAC
17	OPH-04	GGAAGTCGCC
18	OPH-05	AGTCGTCCCC
SCoT primers		
1	SCoT-7	ACAATGGCTACCACTGAC
2	SCoT-8	ACAATGGCTACCACTGAG
3	SCoT-9	ACAATGGCTACCACTGCC

Table (3): Names and nucleotide sequences of SSR primers which used in this study.

No.	SSR Loci	Position on LG	Sequence (5'→3')		Expected PCR product size (bp)
			Forward	Reverse	
1	RM9	Chr. 1	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC	136
2	RM20A	Chr. 12	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	140
3	RM55	Chr. 3	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG	226
4	RM201	Chr. 9	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	158
5	RM212	Chr. 1	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	136
6	RM215	Chr. 9	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	148
7	RM228	Chr. 10	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC	154
8	RM246	Chr. 1	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT	116
9	RM261	Chr. 4	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC	125
10	RM301	Chr. 2	TTACTCTTTGTGTGTGTGTGAG	CTACGACACGTCATAGATGACC	153
11	RM302	Chr. 1	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC	156
12	RM451	Chr. 4	GATCCCCTCCGTCAAACAC	CCCTTCTCCTTTCCTCAACC	207
13	RM518	Chr. 4	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC	171
14	RM545	Chr. 3	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG	226
15	RM553	Chr. 9	AACTCCACATGATTCCACCC	GAGAAGGTGGTTGCAGAAGC	162
16	RM3825	Chr. 1	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG	147

LG: linkage group.

Table (4): Size and number of total amplified bands, polymorphic bands, percentage of polymorphism and polymorphic information content (PIC) values of the studied rice genotypes for RAPD and SCoT markers.

Primer name		size (bp)	No. of bands		Polymorphism (%)	PIC Value
			Total	Polymorphic		
RAPD	OPA-09	113-2368	18	10	55.56	0.21
	OPA-14	185-1345	14	10	71.43	0.27
	OPA-20	159-1268	19	9	47.37	0.18
	OPB-01	263-2410	15	10	66.67	0.21
	OPB-05	162-2526	22	11	50.00	0.18
	OPB-06	232-2355	23	14	60.87	0.21
	OPB-07	105-1471	22	14	63.64	0.24
	OPB-08	115-2781	27	25	92.59	0.35
	OPB-10	100-2040	24	19	79.17	0.33
	OPB-11	166-2919	29	24	82.76	0.30
	OPB-12	137-2414	28	24	85.71	0.32
	OPB-14	225-2243	13	11	84.61	0.29
	OPB-17	112-1651	22	10	45.45	0.16
	OPH-01	181-2947	23	21	91.30	0.30
	OPH-02	193-2983	22	19	86.36	0.31
	OPH-03	157-2403	23	13	56.52	0.21
	OPH-04	264-2530	17	7	41.18	0.16
	OPH-05	122-1902	16	8	50.00	0.18
Total		-	377	259	68.70	4.41
Average		-	20.94	14.39	-	0.24
SCoT	SCoT-7	92-1202	18	17	94.44	0.27
	SCoT-8	289-1350	17	16	94.12	0.35
	SCoT-9	176-1143	17	12	70.59	0.23
Total		-	52	45	86.54	0.85
Average		-	17.33	15	-	0.28

Table (5): Total number of alleles, allele size, highest frequency allele and polymorphic information content (PIC) values of the different rice genotypes for 16 SSR markers.

No.	SSR loci	No. of alleles			Polymorphism (%)	Allele size (bp)		Highest frequency allele		Annealing temperature (°C)	PIC value
		Total	Positive unique marker (bp)			Range (bp)	Difference	Size (bp)	Frequency		
			No.	Genotype							
1	RM9	7	3	189 (Sakha 102) 200 (Sakha 103) 395 (Giza 179)	100	145-540	395	212	0.667	65	0.34
2	RM20A	2	1	374 (Sakha 102)	100	226-374	148	226	0.444	68	0.35
3	RM55	11	7	373 & 481 & 607 (Sakha 103) 309 & 399 (Giza 171) 418 & 729 (Giza 178)	100	194-729	535	280 & 194	0.556	58	0.28
4	RM201	4	2	143 (Sakha 101) 174 (Sakha 102)	100	143-200	57	162 & 200	0.222	62	0.27
5	RM212	5	3	222 (Sakha 101) 448 & 724 (Sakha 103)	100	114-724	610	114	0.778	65	0.26
6	RM215	12	8	97 & 241 & 560 (Sakha 101) 286 (Sakha 103) 668 (Giza 171) 743 (Sakha 102) 320 & 598 (Giza 178)	100	96-743	647	160	0.556	65	0.27
7	RM228	3	0	-	100	103-1172	1069	1172	0.556	65	0.44
8	RM246	11	6	110 & 155 & 359 (Giza 171) 146 (Sakha 102) 345 (Sakha 104) 264 (Giza 178)	100	99-359	260	99	0.778	62	0.30

Table (5): Cont'

9	RM261	4	2	129 (Sakha 104) 192 (Giza 178)	100	121-192	71	121	0.556	62	0.33
10	RM301	1	0	-	100	152	0	152	0.444	68	0.49
11	RM302	1	1	148 (Giza 178)	100	148	0	148	0.111	68	0.20
12	RM451	10	5	110 & 139 (Sakha 101) 127 (Sakha 106) 310 & 343 (E. Hybrid)	100	92- 343	251	235	0.556	62	0.33
13	RM518	3	2	150 (Sakha 101) 227 (Sakha 106)	100	150-227	77	175	0.889	62	0.20
14	RM545	12	7	187 (Sakha 103) 85 & 495 & 615 & 881 (Sakha 106) 98 & 365 (E. Hybrid)	100	85- 881	796	284	0.778	65	0.27
15	RM553	4	1	267 (Giza 171)	100	165-305	140	165 & 170	0.444	68	0.41
16	RM382 5	14	9	288 & 349 & 510 (Sakha 103) 131 & 311 (Giza 171) 186 (Sakha 102) 482 (Giza 178) 229 & 541 (E. Hybrid)	100	131-541	410	174	0.444	65	0.26
Total		104	57		100						5
Average		6.5	4.07								0.31

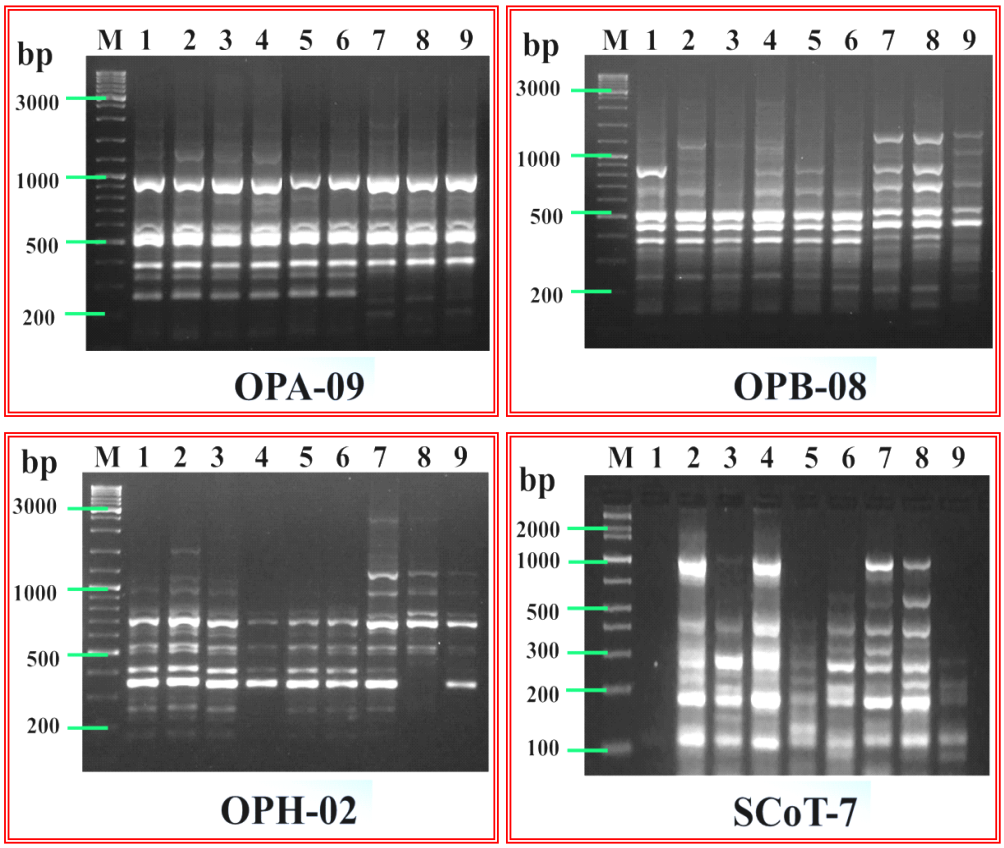

Fig. (1): Results of amplification based on the use of RAPD primers (OPA-09, OPB-08 and OPH-02) and SCoT-7 primer in the nine rice genotypes. M: 100 bp DNA ladder and lanes 1-9: the genotypes of Sakha 101, Sakha 103, Sakha 106, Giza 171, Sakha 102, Sakha 104, Giza 178, Giza 179 and E. hybrid, respectively.

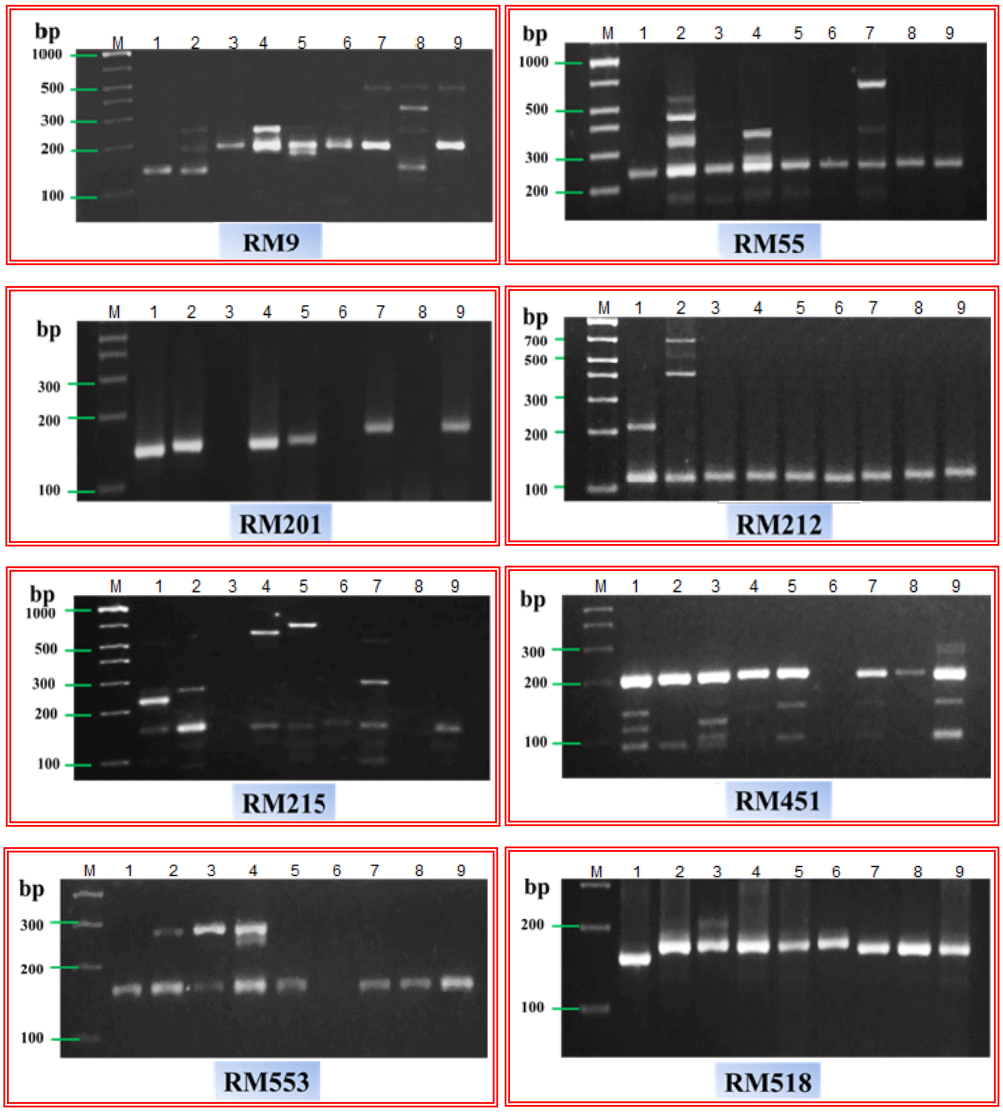

Fig. (2): Results of SSR amplification based on the use of RM9, RM55, RM201, RM212, RM215, RM451, RM518 and RM553 primers in the nine rice genotypes. M: 100 bp DNA ladder and lanes 1-9: the genotypes of Sakha 101, Sakha 103, Sakha 106, Giza 171, Sakha 102, Sakha 104, Giza 178, Giza 179 and E. hybrid, respectively.

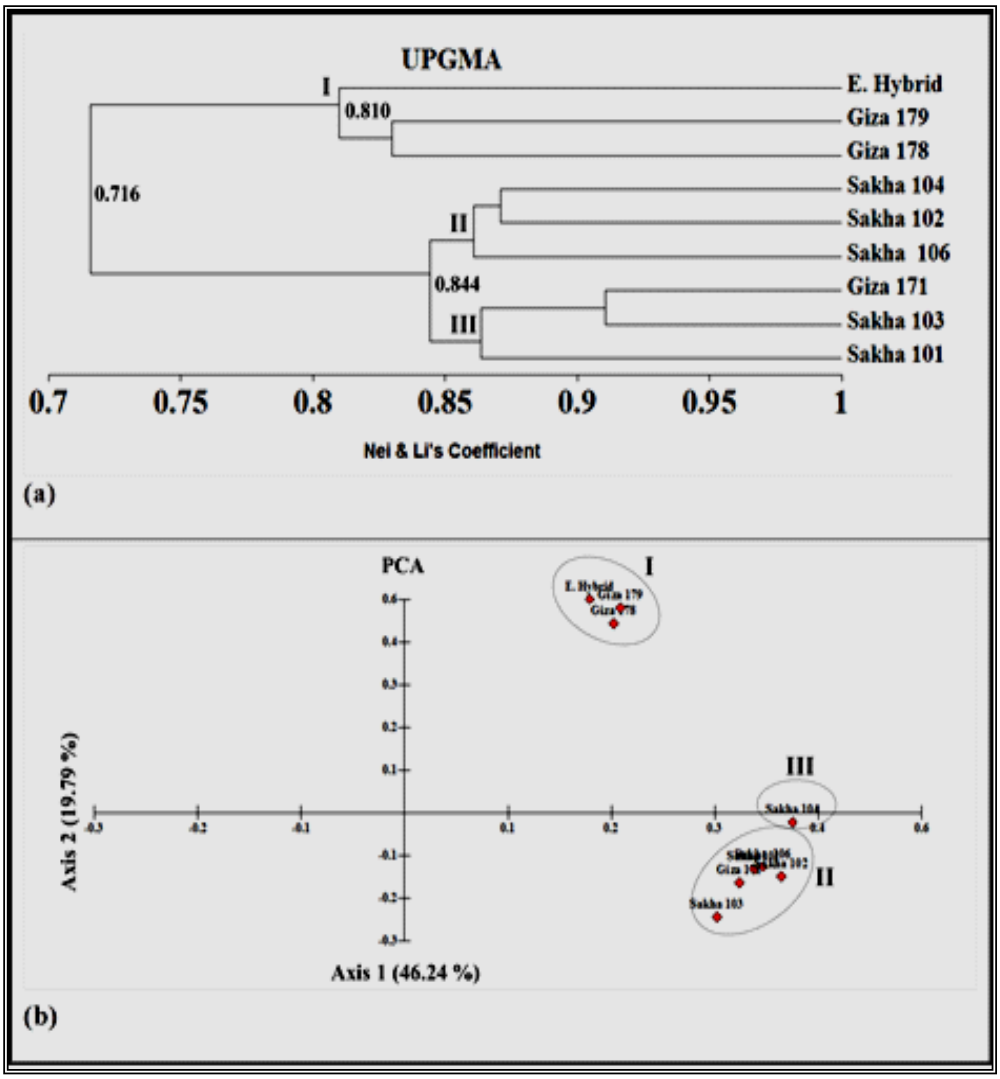

Fig. (3): (a) A dendrogram and (b) principal coordinate analysis (PCoA) of the tested nine rice genotypes based on the eighteen used RAPD primers. I, II and III represented separate clusters.

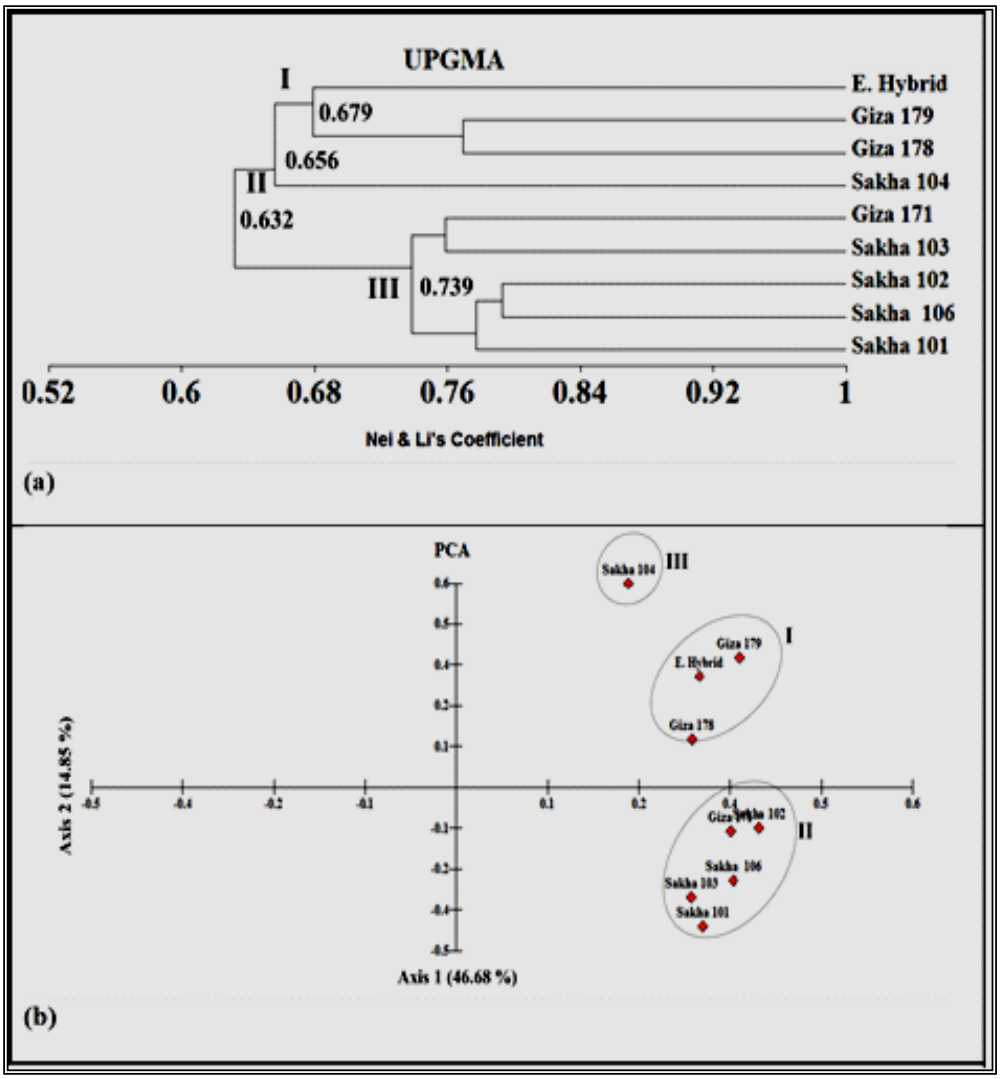

Fig. (4): (a) A dendrogram and (b) principal coordinate analysis (PCoA) of the tested nine rice genotypes based on the three used SCoT markers. I, II and III represented separate clusters.

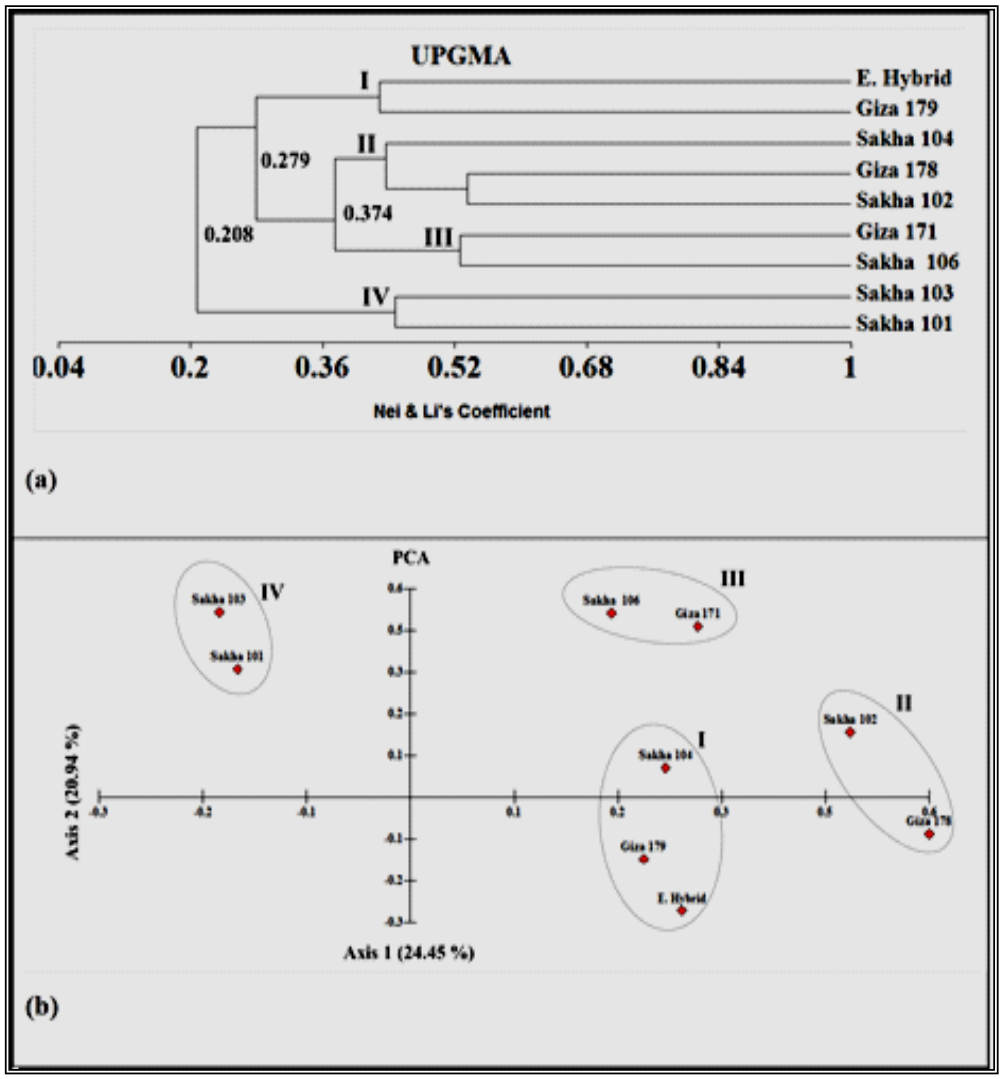

Fig. (5): (a) A dendrogram and (b) principal coordinate analysis (PCoA) of the tested nine rice genotypes based on the alleles detected by the 16 used SSR markers. I – IV represented separate clusters.