UTILIZATION OF SSR PRIMERS TO DISCRIMINATE BETWEEN DIFFERENT SUBSPECIES OF RICE (*Oryza sativa*, L.)

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Rice (*Oryza sativa* L.) is one of the most important cereal crops. It is a major source of food for more than 2.7 billion people most of them in developing countries and is planted on about one-tenth of the earth's arable land. Besides its economic significance, rice is characterized with large genetic diversity in the form of thousands of land races and related wild species (Nagaraju *et al*., 2002).

The majority of cultivated rice belongs to subspecies indica and japonica (Oka, 1988). Indicia cultivars are widely grown in lowland areas of the tropics and subtropics, whereas japonica ones are cultivated in both temperate and tropical regions. Based on morphological and physiological characters, the japonica cultivars can be classified into temperate and tropical sub-classes, the first predominantly cultivated across Northeast Asia, Europe, USA and Australia, the second is common in Southeast Asia, Southern China and the uplands of Latin America and Africa by using isozyme markers (Glaszmann, 1987).

Both types of japonica rice have a very close genetic relationship and have in general a lower genetic diversity than the indica subspecies (Glaszmann, 1987; Mackill, 1995; Ni *et al.*, 2002; Vitte *et al*., 2004; Gao *et al.*, 2005). However, comparing allele richness in temperate versus tropical japonica, Garris *et al.* (2005) found a statistically significant larger richness in the first group.

Since salt tolerance has low heritability, phenotypic selection is difficult and progress through conventional breeding has been slow. Conventional breeding will be enhanced greatly by biotechnological tools, allowing breeders to formulate more proficient breeding strategies (Collard and Mackill, 2007). Although the possibilities are indefinite, there is still a gap between genomics and its application to breeding (Xu *et al*., 2005). Usefulness of a given molecular marker is dependent on its capability in revealing polymorphisms allowing discrimination between alleles. Microsatellites or simple sequence repeats (SSRs) increased the hope to find out salt tolerant rice genotypes (Bhowmik *et al.*, 2009). Molecular marker technology has wide and diverse applications. The availability of an array of molecular markers and genetic maps, Marker assisted selection (MAS) has become possible both for traits governed by major genes as well as

for quantitative trait loci (QTL) (Francia *et al.*, 2005).

In the present study, we assist the potentiality of 274 selected SSR primers to detect the genetic variation between temperate, tropical japonica and indica rice types.

MATERIALS AND METHODS

Plant materials

Nine rice (*Oryza sativa* L.) genotypes were used in the present study. The three varieties; Gaori, Sakha 104 and Giza 177, were temperate japonica types and among the other six (obtained from recomptenant inbreed liens (RILs); three, TCCP266-2-49-B-B-3, Giza 178 and IR 29, were indica types; and three, IR 65598-112-1-2, IR65564-44-5-1 and IR66160-5-2-3-2 were tropical japonica types; Seeds were obtained from the Rice Research and Training Center (RRTC), Sakha, Kafer El-Sheikh, Egypt. All lines used were either salt tolerant, sensitive, or moderate (Table 1).

DNA isolation and verification

DNA extraction and purification from the 45 individual plant samples (five plants for each line) was carried out using CTAB (Cetyl-tetramethyl ammonium bromide) method according to Murray and Thompson (1980). Analysis was performed on bulked DNA from 5 highly resistant and 5 highly susceptible individuals among the present cultivars. A total of 274 SSR simple sequence repeats (SSR) primers were used to amplify the DNA template (Table 2). Primers were designed according to three different sources: http://gramene. org/marker, Chen *et al.*, (1997) and http://ricelab.plbr.cornell.edu. PCR amplification conditions were as follow: each 25 µl PCR volume contained 1.0 µl (50 ng template DNA), 1.0 µl dNTPs (10 mM), 2.5 µl $MgCl_2$ (25 mM), 2.0 µl 10 X buffer (10 mM tris, pH 8.0, 50 mM KCl and 50 mM ammonium sulphate), 2.0 µl of 10 mM forward and reverse primers, 0.25 µl Taq polymerase (5 u/ µl). The amplification protocol of 5 min at 94°C, 35 cycles, was performed with 45 sec at 94°C; 45 sec at lower annealing temperature of the primer about 50 up to 68°C; 1 min at 72°C, and a final extension step of 10 min at 72°C (Plaschke *et al.*, 1995; Roder *et al.*, 1998).

Agarose (3%) was used for resolving the PCR products and 1 kb DNA ladder marker as a standard DNA were used in the present study. The run was performed for 3.5 hour at 80 volt in SDE-PLAS submarine (10 cm x 10 cm). Bands were detected on UV-trans-illuminator, photographed by Gel documentation system and according to analysis by Phoretix program 1D gel analysis software version 4.01.

Diversity analysis

The amplified SSR DNA bands representing different alleles were scored as different genotypes. For each marker, allelic bands were compared against a 100

bp DNA ladder. Then, fragment data was converted into the binary encoded allelic data to apply the multivariate analyses.

Genetic distance, the ratios of shared DNA bands and genetic similarities were estimated from the allele binary formatted data set using Nei and Li's coefficient (Nei and Li, 1979). Genetic distance was calculated as follows:

GDn = 1-[2N11/ (2N11 + N10 + N01)]

Where: N (1,1) is the number of loci having bands present in both accession, N (1,-) is the number of loci having a band present in the first accession, N (-,1) is the number of loci having a band present in the second accession

The accessions were clustered based on the matrix of genetic similarities using the un-weighted pair group method with arithmetic averages (UPGMA).

Polymorphic information content (PIC) values were calculated for each microsatellite based on the allelic frequency detected in the accessions studied using this formula.

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where: P_{ij} is the frequency of the j^{th} allele for i^{th} marker and summation extends over n alleles. Polymorphic loci were defined as those whose most frequent allele had a frequency of less than 0.95.

Genetic diversity of the entries/ populations (based on set of measured molecular data) was estimated using diversity parameters other than PIC (Sun *et al.*, 2001). These are calculated as follows: Percentage of polymorphic loci (PPL):

$$P = (k/n) \times 100\%$$

Where: k is the number of polymorphic loci, n is the total number of loci investigated.

Average number of alleles per locus (A):

$$A = \Sigma Ai / n$$

Where: Ai is the number of alleles at the i^{th} locus and n is the total number of loci investigated.

Average number of alleles per polymorphic locus (Ap): $Ap = \Sigma Api/np$

Where: Api is the number of alleles at a certain polymorphic locus and np is the total number of polymorphic loci investigated.

Percentage of polymorphic alleles (PPA)

$$PPA = (\Sigma Api/ \Sigma Ai) \times 100\ \%$$

The similarity matrix using Nei and Li (1979) genetic distance for SSR characterization was also used for principal coordinate analysis (PCoA) with the Dcenter, Eigen, Output, and Mxplot subprograms in NTSYS-PC.

Quantity one software (Gel Doc, Bio-Rad Laboratory, Inc.) was used to estimate the length of amplification product and for capturing gel images. NTSYS Pc 2.1 was used in cluster analysis and principal coordinate analysis. Power Marker version 3.25 was used to construct the tree by 3130XI Genetic Analyzer Program.

RESULTS AND DISCUSSION

SSR markers diversity analysis

A total of 274 SSR markers were used for screening of rice cultivars (Table 3), among them 219 SSR loci were polymorphic. The maximum number of alleles per locus were five alleles and they are located at nine loci; RM260, RM218, RM310, RM160, RM206, RM259, RM570, RM276 and RM21in all accessions (Fig. 1A). Four alleles per locus were located at 27 loci, three alleles at 86 loci and two alleles at 97 loci (Fig. 1B).

In case of temperate japonica accessions, the maximum number of alleles per locus were 3 in RM335, RM159, RM276, RM162, and RM160 loci (Fig. 1C) whereas in indica accessions, the maximum number of alleles were 3, found in 21 loci (Fig. 1D), and in tropical japonica accessions, the maximum number was 3, found in RM70, RM259, RM454, RM206, RM578, RM224 and RM254 (Fig. 1E).

Twelve SSR markers were able to discriminate between the temperate japonica, indica, and tropical japonica rice accessions under this study (Table 3 and Fig. 1F). Although these SSR markers are useful for distinguishing between rice cultivars; they need to be tested for construction of a Bar Code distinguishing subspecies.

SSR polymorphism profiles

Total numbers of 645 alleles were generated at 219 SSR loci in the nine accessions under analysis, and produced 605 alleles. The temperate japonica accessions have 105 alleles at 50 polymorphic loci. Within three indica accessions, 266 alleles were detected at 122 polymorphic loci. The three tropical japonica accessions have 129 alleles detected at 61 polymorphic loci (Table 4). These results are consistent with the RFLPs analysis of chloroplast, mitochondrial and nuclear DNA of eight cultivars of *O. sativa*, where the average genetic distance within indica subspecies was much larger than those within japonica subspecies (including tropical japonica) (Ishii *et al.*, 1993). The finding of Junjian *et al.* (2002) demonstrates that indica rice had a higher level of genetic variation than japonica. Our results are in good accordance with those of Mackill (1995); Yang *et al.* (1994) and Zhang *et al.* (1992).

In the present study the average number of alleles per locus (A) 2.49, 1.2, 1.55 and 1.25 were obtained in temperate japonica, indica, and tropical japonica accessions, respectively. The average number of alleles per polymorphic locus (Ap) 2.76, 2.1, 2.18 and 2.11 were estimated in temperate japonica, indica, and tropical japonica accessions, respectively (Table 4).

The Polymorphic loci data were used to estimate polymorphic information content (PIC). Percentages of polymorphic loci for total, temperate japonica, indica, and tropical japonica were 84.55%, 19.30%, 47.10% and 23.55%, respectively. The percentages of polymorphic alleles

for total, temperate japonica, indica, and tropical japonica were 93.79%, 16.27%, 41.24% and 20.00%, respectively. The average number of gene diversity (PIC) was 0.51 which indicating moderately high variation among the nine accessions.

The RM260 loci with (CT=34) showed the highest PIC value (0.79) and also the highest five number of polymorphic alleles. The level of polymorphism at microsatellite loci can be expressed as a result of (chick the meaning) gene diversity. These results are in agreement with (Innan *et al.,* 1997) however, the measure of polymorphism depends on the length of DNA fragment or repeats (Jack and Mayes, 1993).

RM294, RM434, RM409, RM560, RM598, RM532, RM564, RM565, RM168, RM60 and RM561 showed the lowest PIC value of 0.19. The RM488, RM527, RM55 and RM269 with (GA= 17) repeat motif amplified 1-4 alleles and PIC values were 0.71, 0.57, 0 and 0, respectively. The SSR markers RM488, RM159, RM286, and RM21 with (GA) repeat motif of 17, 19, 16, and 18 showed the same PIC value of 0.71, the RM21 marker showed five alleles while the other markers showed four alleles. The RM145 with (GA= 31) repeat motif showed 3 alleles and PIC value 0.56. RM430, RM287, RM578, RM527 and RM26 markers with (GA) repeat motif of 25, 21, 19, 17 and 15 also showed three alleles and PIC value of 0.56. The PIC values did not show any direct correlation in the present study. The number of amplified alleles per a primer and its PIC Values are depend upon the repeated number and the repeated sequence of the microsatellite (Temnykh *et al*., 2000; Temnykh *et al*., 2001; Yu *et al*., 2003; Juneji *et al.,* 2006). The higher number of alleles and the higher PIC values are associated with GA repeats (Ni *et al*., 2002). Profiles of the nine rice accessions are summarized in the Table (4) according to the 12 chromosomes of rice. For chromosome 1, a total of 35 SSR loci were detected and 31 loci were polymorphic. The alleles were varied from 2 to 5. The polymorphic information contents were varied from 0.35 to 0.75 and the average of PIC value was 0.52. This represents moderately high gene diversity of chromosome 1. For chromosome 2, a total of 31 SSR loci were detected and 27 loci were polymorphic. The alleles were varied from 2 to 4. The PICs were varied from 0.20 to 0.67 and the average of PIC was 0.47. This represents moderately low gene diversity of chromosome 2. For chromosome 3, a total of 34 SSR loci were observed and 25 loci were polymorphic. The alleles were varied from 2 to 5. The PICs were varied from 0.20 to 0.77 and the average of PIC was 0.49. This shows a moderately low gene diversity of chromosome 3. For chromosome 4, a total of 23 SSR loci were mentored and 17 loci were polymorphic. The alleles were varied from 2 to 4. The PIC values were varied from 0.20 to 0.64 and the average PIC was 0.47 representing moderately low gene diversity for chromosome 4. In chromosome 5, a total of 28 SSR loci were studied and 23 loci were polymorphic. The alleles were varied from 2 to 4.

The PIC values were varied from 0.20 to 0.72 and the average PIC was 0.50 which representing moderately high gene diversity for chromosome 5. For chromosome 6, a total of 23 SSR loci were studied and 17 loci were polymorphic. The alleles were varied from 2 to 5. The PIC values were varied from 0.35 to 0.72 and the average PIC was 0.58 which showed moderately high gene diversity in chromosome 6. In chromosome 7, a total of 15 SSR loci were studied and 12 loci were polymorphic. The alleles were varied from 2 to 4. The PIC values were varied from 0.20 to 0.67 and the average PIC was 0.52 which showed moderately high gene diversity for chromosome 7. In chromosome 8, a total of 21 SSR loci were studied and 17 loci were polymorphic. The alleles were varied from 2 to 5. The PIC values were varied from 0.35 to 0.77, and the average PIC was 0.52 which showed moderately high gene diversity for chromosome 8. In chromosome 9, a total of 15 SSR loci were studied and 13 loci were polymorphic. The alleles were varied from 2 to 5. The PIC values were varied from 0.20 to 0.77, and the average PIC was 0.51 which showed moderately high gene diversity in chromosome 9. In chromosome 10, 20 SSR loci were studied and 15 loci were polymorphic. The alleles were varied from 0.20 to 0.72 and the average PIC was 0.50 which showed moderately high gene diversity in chromosome 10. In chromosome 11, 14 SSR loci were studied and 13 loci were polymorphic. The alleles varied from 2 to 5. The PIC values varied from 0.44 to 0.77 and the average PIC was 0.61 which showed moderately high gene diversity in chromosome 11. In chromosome 12, 15 SSR loci were studied and nine loci were polymorphic. The alleles varied from 2 to 5. The PIC values were varied from 0.35 to 0.79, the average PIC was 0.53 which showed moderately high gene diversity exited in chromosome 12.

Among all chromosomes, the chromosome 11 and 2 showed the highest and the lowest average of PIC values (0.61 and 0.47, respectively) and also for average alleles per polymorphic locus AP (3.23 and 2.52, respectively). For the temperate japonica, indica, and tropical japonica subspecies, the maximum AP value of 2.5 were found were for chromosome 9, 2.33 for Chromosome 9 and 3.0 for chromosome 7.

The average alleles per locus Alas showed in Table (3) were ranged from 2.14 (chromosome 12) to 3.07 (chromosome 11). Among the three subspecies, temperate japonica showed maximum value of 1.33 (chromosome 2), indica showed 1.82 (chromosome 1), and tropical japonica showed 1.71. Percentage of polymorphic loci ranged from 64.29% (chromosome 12) to 92.86% (chromosome 11). The percentages of polymorphic loci among the three subspecies were 33.33% (chromosome 2) in temperate japonica, 64.71% (chromosome 1) in indica, and 50% (chromosome 11) in tropical japonica. The percentage of polymorphic alleles ranged from 83.33% (chromosome 12) to 97.67% (chromosome 11). The maximum percentage of polymorphic alleles among the three subspecies was

50% (chromosome 2) in temperate japonica, 80.65% (chromosome 1) in indica, and 70.83% (chromosome 11) in tropical japonica.

According to our genotyping variation of the nine accessions, the highest value was found in chromosome 11 which showed the highest values in average alleles per polymorphic locus AP, average alleles per locus AL, percentage of polymorphic loci, percentage of polymorphic alleles, and average PIC.

Within temperate japonica accessions, the highest variation was observed in chromosome 2 with highest value of average alleles per locus AL, percentage of polymorphic loci and percentage of polymorphic alleles. The indica accessions has the highest variation was occurred in chromosome 1 which conveyed the highest value of average alleles per locus AL, percentage of polymorphic loci and percentage of polymorphic alleles. The tropical japonica accessions has the highest variation detected in chromosome 11 which explicit the highest value of average alleles per locus (A), percentage of polymorphic loci and percentage of polymorphic alleles.

Genetic similarity

The genetic similarity matrix was calculated based on the 605 alleles at 219 SSR loci in nine accessions using Nei and Li genetic distance (Dice similarity coefficient). Indica accessions showed a lower level of similarity than the japonica ones (Table 5). Temperate japonica and tropical japonica accessions showed high similarity with each other. This result is in consistent with the findings of Garris *et al*. (2005) who studied the genetic structure of 234 accessions of rice using 169 nuclear SSR and two chloroplast loci. Based on nuclear SSR diversity, the alleles at all 15 monomorphic loci in the temperate japonica group were identical in size to the most frequent allele present in the tropical japonica.

Clustering of SSR variations

The genetic similarity matrix was used for cluster analysis based on UPGMA. The dendrogram showed two major clusters. Cluster I consisted of temperate and tropical japonica accessions. Each group was represented by tolerant, intermediate, and sensitive accessions. Group Ib contained of tropical japonica, while cluster II comprised indica accessions (Fig. 2).

Japonica and indica groups joined to genetic similarities of 0.33. Temperate japonica and tropical japonica accessions showed 0.61 similarities. Temperate japonica accessions were grouped together at 0.855 similarities. Sakha 104 and Giza 177 (moderate tolerant and sensitive to salinity) were grouped together at 0.89 similarities. The similarity between tolerant, moderate and sensitive to salinity of temperate and tropical japonica accessions was 0.855 and 0.820, respectively.

In cluster II, indica accessions, TCCP 266 (tolerant) was explicit from moderate and sensitive accessions at 0.657

similarities. Giza 178 and IR 29 (moderate tolerant and sensitive to salinity) were grouped together at 0.6716 similarities. The tolerant, temperate japonica (Gaori), tropical japonica (IR 65598- 112-1-2), and indica (TCCP266) were separated from a cluster of moderate and sensitive salt tolerance accessions.

Principal coordinate analysis

The two-dimensional principal coordinate analysis showed similar topology with the cluster analysis (Fig. 3). Eigen values were extracted using NTSYS Pc. Version 2.1. First three principal coordinates 61.03 % of total variations. PC1 possessed 33.24%, PC2 and PC3 had 15.75% and 12.04% of total variations (Table 6).

SUMMARY

Nine rice (*Oryza sativa* L.) genotypes of indica and japonica types were used in the present study. Total number of 274 SSR simple sequence repeats primers were used to detect the genetic variation and genetic markers specific for each rice genotype. The results showed that the 219 SSR primers were generate a total 645 amplified alleles, among them 605 alleles were polymorphic. The temperate japonica accessions showed 105 alleles at 50 polymorphic loci. Within three indica accessions, 266 alleles were detected at 122 polymorphic loci. Within the three tropical japonica accessions, 129 alleles were detected at 61 polymorphic loci, respectively. The average number of alleles per locus (A) 2.49, 1.2, 1.55 and 1.25 were observed in total, temperate japonica, indica, and tropical japonica accessions, respectively. Average number of alleles per polymorphic locus (Ap) 2.76, 2.1, 2.18 and 2.11 were observed in total, temperate japonica, indica, and tropical japonica accessions, respectively. The average number of gene diversity (PIC) was 0.51, indicating moderately high variation among the nine accessions. Twelve SSR markers were able to discriminate among the temperate japonica, indica, and tropical japonica rice accessions under the present study.

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Table (1): Parental lines and their basic characteristics.

No.	Name	Lines	Type	Salt tolerant
1	Gaori	IR5591-1-1-1-1/Taikeng7(IRRI)	Temperate Japonica	Tolerant
2	Sakha 104	GZ 4046-8-1/GZ 4100-9-1(Egypt)	Temperate Japonica	Moderate
3	Giza 177	Giza171/Yomji No.1//Giza175/Milyange 49(Egypt)	Temperate Japonica	Sensitive
4	TCCP 266-2-49-B-B-3	Pokkali somaclonal variant(IRRI)	Indica	Tolerant
5	Giza 178	Giza 175/Milyang 49(Egypt)	Indica	Moderate
6	IR29	IR833-6-2-1-1///IR1561-149-1//IR661-140-3*4/*O.nivara*(IRRI)	Indica	Sensitive
7	IR65598-112-1-2	Shen-Nung89-366/Genijah WangKal (B 4667) (IRRI)	Tropical Japonica	Tolerant
8	IR65564-44-5-1	Shen-Nung89-366/Gundil Kuning(IRRI)	Tropical Japonica	Moderate
9	IR66160-5-2-3-2	IRRI line	Tropical Japonica	Sensitive

Table (2): List of microsatellites markers used in the study.

Chromosomes No.	Total primers	Microsatellites markers primers
1	35	RM495, RM428, RM84, RM323, RM220, RM151, RM272, RM259, RM243, RM578, RM579, RM572, RM23, RM24, RM493, RM9, RM5, RM488, RM237, RM246, RM443, RM403, RM128, RM543, RM302, RM486, RM265, RM315, RM472, RM431, RM104, RM529, RM414, RM14, RM568.
2	31	RM154, RM211, RM279, RM53, RM555, RM145, RM71, RM452, RM438, RM27, RM324, RM561, RM341, RM475, RM106, RM263, RM526, RM221, RM525, RM497, RM450, RM530, RM240, RM112, RM250, RM213, RM207, RM535, RM138, RM569, RM3732.
3	34	RM60, RM132, RM22, RM231, RM489, RM545, RM517, RM546, RM218, RM251, RM563, RM282, RM554, RM338, RM156, RM411, RM16, RM135, RM426, RM49, RM532, RM55, RM186, RM168, RM448, RM416, RM293, RM571, RM143, RM565, RM514, RM570, RM442, RM85.
4	23	RM307, RM401, RM537, RM551, RM335, RM518, RM261, RM456B, RM185, RM471, RM417, RM142, RM564, RM119, RM252, RM241, RM451, RM317, RM131, RM127, RM280, RM559, RM5320.
5	28	RM153, RM159, RM267, RM592, RM593, RM574, RM437, RM289, RM169, RM516, RM509, RM598, RM430, RM164, RM39, RM440, RM161, RM173, RM534, RM188, RM233B, RM26, RM274, RM87, RM480, RM538, RM334, RM249.
6	23	RM435, RM597, RM190, RM586, RM587, RM204, RM253, RM276, RM121, RM136, RM527, RM465B, RM541, RM454, RM275, RM162, RM343, RM528, RM30, RM340, RM412, RM141, RM217.
7	15	RM427, RM125, RM214, RM432, RM560, RM10, RM70, RM505, RM234, RM429, RM118, RM172, RM420, RM82, RM47.
8	21	RM337, RM407, RM152, RM38, RM25, RM310, RM44, RM339, RM42, RM223, RM284, RM210, RM556, RM149, RM80, RM230, RM433, RM502, RM447, RM281, RM3395.
9	15	RM316, RM464, RM219, RM321, RM105, RM460, RM409, RM434, RM257, RM242, RM201, RM160, RM215, RM245, RM205.
10	20	RM474, RM330A, RM222, RM216, RM239, RM311, RM467, RM184, RM271, RM269, RM258, RM304, RM228, RM294A, RM484, RM147, RM333, RM496, RM590, RM591.
11	14	RM286, RM332, RM167, RM552, RM441, RM202, RM287, RM229, RM21, RM473E, RM206, RM254, RM224, RM144.
12	15	RM20A, RM19, RM453, RM247, RM491, RM512, RM83, RM277, RM511, RM260, RM309, RM463, RM270, RM235, RM17.

Table (3): Alleles specific SSR primers among temperate japonica, indica and tropical japonica.

Chromosomes No.	Total polymorphic	Microsatellites markers
1	31	RM246
2	27	RM438
3	25	RM282
4	17	-
5	23	-
6	17	RM528, RM340, RM141
7	12	RM214, RM505, RM82
8	17	-
9	13	RM205
10	15	RM496
11	13	RM144
12	9	-
Total	219	12

Table (4): Summary of DNA profiles according to chromosome in the nine accessions studied.

Chromosomes No.	DNA profile	Total	Temperate japonica	Indica	Tropical japonica	PIC
1 to 12	Total number of alleles	645.00	312.00	403.00	325.00	
	Total number of accessions	9.00	3.00	3.00	3.00	
	Average alleles per locus(A)	2.49	1.20	1.55	1.25	
	Number of polymorphic loci	219.00	50.00	122.00	61.00	
	Number of polymorphic alleles	605.00	105.00	266.00	129.00	
	Av. Alleles per poly. Locus (Ap)	2.76	2.10	2.18	2.11	
	Percentage of polymorphic loci	84.55	19.30	47.10	23.55	
	Percentage of polymorphic alleles	93.79	16.27	41.24	20.00	
	Average gene diversity (PIC)					0.51

Table (5): Genetic similarity matrix based on dice coefficient of the nine rice accessions studied (Nei and Li, 1979).

	A	B	C	D	E	F	G	H
B	0.8716							
C	0.8288	0.8872						
D	0.2763	0.2957	0.3074					
E	0.2879	0.3113	0.3035	0.6602				
F	0.2763	0.3113	0.2957	0.6525	0.6680			
G	0.5837	0.5798	0.6109	0.3541	0.3463	0.3541		
H	0.6304	0.6304	0.6498	0.3385	0.3463	0.3774	0.8405	
I	0.5992	0.6031	0.6187	0.3580	0.3580	0.4086	0.7860	0.8716

A-Gaori B-Sakha 104 C- Giza 177 D- TCCP 266-2-49-B-B-3 E- Giza 178
F- IR29 G- IR65598-112-1-2 H-IR65564-44-5-1 I-IR66160-5-2-3-2

Table (6): Principal coordinates (PCs) of 259 loci of present the nine accessions.

	PIC1	PIC2	PIC3
Eigenvalue	142.64	67.59	51.68
% of Variance	33.24	15.75	12.04
Cumulative %	33.24	48.99	61.03

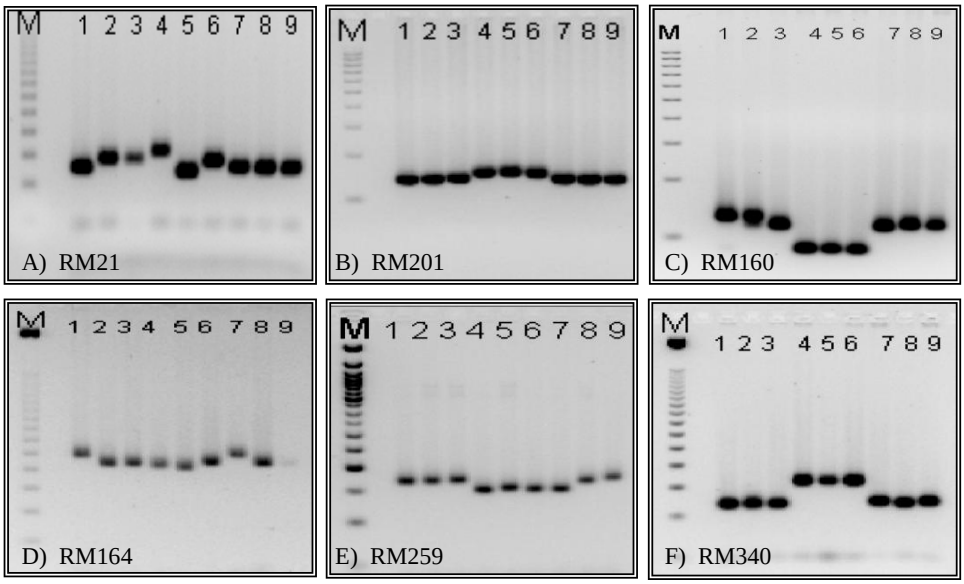

Fig. (1): Genomic amplification using primers specific in rice lines from left to right are: 1-Gaori 2-Sakha 104 3- Giza 177 4- TCCP 266-2-49-B-B-3 5- Giza 178 6- IR29 7- IR65598-112-1-2 8-IR65564-44-5-1 9-IR66160-5-2-3-2. Lane 1, 2 and 3 are temperate japonica, 4, 5 and 6 are indica and 7, 8 and 9 are tropical japonica. M is a 50 bp ladder.

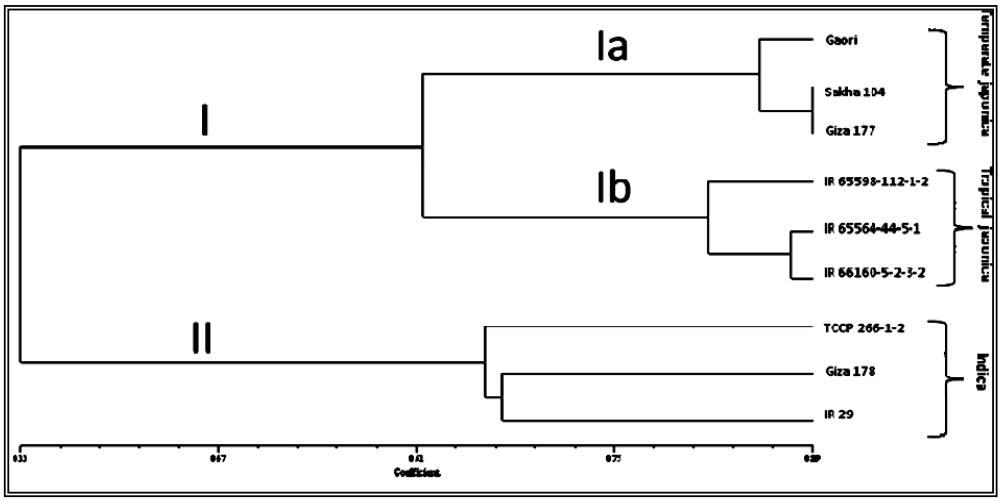

Fig. (2): Dendrogram of rice accessions based on Nei and Li's genetic distance between 259 SSR loci.