Using Morphological Charachters and ISJ Markers for Assessment of Genetic Diversity among Some Rice Genotypes

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ABSTRACT

Twenty-four rice genotypes were used to study the morphological and ISJ molecular characterization data combined over the two rice growing seasons of 2014 and 2015 at the experimental farm of Rice Research and Training Center. There were significant differences among the genotypes for all traits, indicating high genetic divergence among rice germplasm. The 24 studied genotypes were divided to 11 clusters. Cluster III included the highest number of genotypes, followed by cluster X which included four genotypes. According to the rank for desirable traits among all clusters mean values, hybridization of IR 68011-15-1-1, KATY of cluster VI with promising genotypes of cluster VII (moroberekan), cluster IX (IR 67954-46-1-3-2) and cluster XI (IR 68167-28-3-2-2, IR 69432-54-1-1-2-2 (NPT)) may be recommended as started materials for breeding program, in order to isolating transgressive segregants lines. Twelve primers of intron-exon splice junction (ISJ) were utilized in this study, out of 89 bands were detected and 44 bands showed polymorphic. The genetic similarity between genotypes ranged from 0.794 to 0.955 with an average similarity index of 0.873. Cluster analysis based on Dice's similarity coefficient using UPGMA procedure divided the studied rice genotypes into six major clusters. Furthermore, some genotypes located in one cluster alone based on either morphological or molecular dendrograms, such as genotypes number 6 (Moroberekan) and 7 (Katy).

Key words: Rice, genetic diversity, morphological traits, ISJ primers.

INTRODUCTION

Rice is an important food crop for the entire world population. Genetic improvement essentially relies on the extent of genetic divergence present in the accessions, which is an appearance feature of all species in nature. Several researchers, such as Dutta *et al.*, (2013) have stressed the importance of genetic diversity in the selection of suitable genotypes for hybridization in different crops.

Genetic divergence in plants has been conventionally evaluated using morphological traits. Therefore, genetic divergence for morphological traits is the key factor of breeding programs to increase the genetic background of rice. The assessment of phenotype may not be a reliable measure of genetic differences as gene expressions influenced by environment. On the other hand, assessment of genetic variations based on DNA polymorphism are abundant and independent of environmental factor. DNA markers that differentiate genotypes are more effective and proper than morphological traits in identification of genetic variability (Zeng *et al.,* 2004).

The plenty of information on DNA sequences help in design sequence-associated primers for PCR amplification (Rafalski *et al.,* 2002). Intron- exons splice junction (ISJ) primers, used and developed by Rafalski *et al.,* (1997). Przetakiewicz *et al.,* (2002) reported that ISJ primers are very effective for genotypes fingerprinting. The genetic variations, as identified by morphological traits and molecular markers, could be an effective and useful tool in the breeding program using suitable parental genotypes. Therefore, the present investigation was undertaken with the objective of estimating genetic diversity in a set of genotypes using ISJ markers and morphological traits.

MATERIALS AND METHODS

1. Plant Materials and Field Experiments

Twenty-four rice genotypes were used in this study. These genotypes were selected from the Genetic Stock of Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt. The origin of these genotypes is presented in Table 1. Genotypes were transplanting at a Randomized Complete Block Design with three replications during two successive rice growing seasons (2014 and 2015). All agricultural practices were done according as recommend. Each plot contained four rows; the seedlings were planted at spacing of 20 x 20 cm row to row and plant to plant. Ten competitive random plants from the middle rows of the experimental plots were selected for recording the data on the following traits: days to 50% heading, plant height (cm), panicle exsertion (%), panicle length (cm), number of panicles plant^{-1}, number of filled grains panicle^{-1}, 1000-grain weight (g), grain yield plant^{-1} (g), spikelets fertility (%), hulling (%), milling (%), head rice (%), grain length (mm), grain shape (L/W ratio), amylose content (%) and gelatinization temperature.

2. DNA Isolation and ISJ Method

Total DNA of rice plants was extracted form 100-mg of three weeks old fresh leaves tissue using DNA extraction Mini Kit (DNA secure Plant Kit

Cat. no. Dp320). Twelve Semi-random primers (ISJ) according to Sawicki and SzczeciÒska (2007) were tested. Primers names and sequences of primers are shown in (Table 2). PCR amplification reactions were done in 20µl reaction mixtures containing, 3µl of template DNA, 1µl of each primer, 10µl of PCR master mix (My Tag^{TM} Red Mix) and 6µl ddH2O. PCR amplification profile: an pre denaturation step was done at 95°C for 2min, followed by 35 cycles for denaturation step at 95°C for 15s, annealing step at Tm+ for 20s and primer elongation at 72°C for 1min and then a final extension at 72°C for 5min. Amplified products were stored at -20°C until further use. PCR amplified products were separated by using electrophoresis on 1.2% agarose gels at 50 V in 0.5 × TBE buffer. Gels were stained by using ethidium bromide and were imaged by Biometra (UV-solo model) gel documentation system. Each PCR reaction was repeated tow times and reproducible bands were considered for analysis only.

Table 1: List of 24 rice genotypes and their origin with their salient features of grain shape.

No.	Entry	Origin	Grain shape
1	IR 65600- 77 -4-2-1	IRRI	medium
2	IR 65600-127-6-2	IRRI	medium
3	IR 65600-129-1-1-2	IRRI	bold
4	IR 69093-41-2-3-2	IRRI	bold
5	IR 69853-70-3-1-1	IRRI	bold
6	IR 68011-15-1-1	IRRI	medium
7	IR 65603-57-4-2	IRRI	bold
8	IR 65598-27-3-1	IRRI	bold
9	IR 65564-44-2-3	IRRI	bold
10	IR 65597-29-3-2-3	IRRI	bold
11	IR 68167-28-3-2-2	IRRI	bold
12	IR 66158-38-3-2	IRRI	bold
13	IR 66159-189-5-5-3 (NPT)	IRRI	bold
14	IR 66160-121-4-5-3 (NPT)	IRRI	bold
15	IR 67962-40-6-3-3 (NPT)	IRRI	bold
16	IR 67966-188-2-2-1 (NPT)	IRRI	bold
17	IR 68544-29-2-1-3-1-2 (NPT)	IRRI	bold
18	IR 68552-55-3-2 (NPT)	IRRI	bold
19	IR 69116-67-3-2-3 (NPT)	IRRI	bold
20	IR 69432-54-1-1-2-2 (NPT)	IRRI	bold
21	IR 67954-46-1-3-2	IRRI	bold
22	IR 67964-46-1-3-2	IRRI	medium
23	moroberekan	West Africa	medium
24	KATY	IRRI	medium

IRRI: International Rice Research Institute.

Table 2: List of 12 ISJ primers used in this study.

Primer Name	Sequence 5′ ⟶ 3′	Tm
ISJ 1	CAG ACC TGC T	32 ˚C
ISJ 2	ACT TAC CTG AGG CGC CAC	58 ˚C
ISJ 3	TGC AGG TCA G	32 ˚C
ISJ 4	GTC GGC GGA CAG GTA AGT	58 ˚C
ISJ 5	CAG GGT CCC ACC TGC A	56 ˚C
ISJ 6	ACT TAC CTG AGC CAG CGA	56 ˚C
ISJ 7	TGC AGG TCA GGA CCC T	53 ˚C
ISJ 8	GAC CGC TTG CAG GTA AGT	56 ˚C
ISJ 9	AGG TGA CCG ACC TGC A	53 ˚C
ISJ 10	ACT TAC CTG CAT CCC CCT	56 ˚C
ISJ 11	TGC AGG TCA AAC GTC G	51˚ C
ISJ 12	GGA CTG GAG CAG GTA AGT	56˚ C

3. Statistical Analysis

Morphological data were measured by using SPSS (version 13.0) software. Genetic variability was used following the Mohalanobis's generalized distance (D^2) method extended by Rao (1952). Amplified fragments were scored as present or absent (1, 0). The ISJ matrices were then studied by using (NTSYS) version 2.1 (Rohlf, 1998). Similarity for semi-random data was computed using the Dice's similarity index and similarity estimates were analyzed by the un-weighted pair group method with arithmetic average (UPGMA) algorithm. The resulting clusters were expressed as dendrogram.

RESULTS AND DISSCUTION

1. Morphological traits

Significant differences were observed among rice genotypes for all studied morphological traits as shown in Table 3. These results illustrate the presence of high genetic divergence among studied rice genotypes.

1.1. Distribution of genotypes

The results shown in Table 4 indicated that the studied 24 rice genotypes distributed in 11 clusters with different number of genotypes. Cluster III contained the highest number of genotypes (five genotypes) while, cluster X, cluster II, cluster IV, cluster VI and cluster XI contained 4, 3, 3, 2 and 2 genotypes, respectively. The lowest clusters were I, V, VII, VIII and IX that included the same number of genotype only one for each one.

1.2. Cluster analysis for mean values

Eleven cluster mean values of 17 different morphological traits for 24 genotypes of rice were presented in Table 5. Among 17 traits, it is evident that clusters I, III and X were marked for two traits viz. amylose content% and gelatinization temperature. However, cluster I was distinguished for panicle exsertion% (16.38), cluster III for milling% (71.50) and cluster X for plant height (87.21). While, cluster IV recorded high mean values for hulling% (81.06), milling% (71.79) and gelatinization temperature (6.0). Cluster II and V were featured of one character only amylose content. While, it was characterized by short plant height (87.21cm) and cluster V number of filled grains panicle^{-1} (233.38).

Among 17 traits, cluster VI stood first for six traits viz. days to 50% heading (97.75days), panicle exsertion% (16.21), milling% (71.67), grain length (6.60), grain shape (2.40) and gelatinization temperature (5.0). Cluster VII had one genotype viz. moroberekan which was outstanding types by its performance in most of the agronomic traits, this cluster was characterized by the following essential features viz. high in panicle exsertion% (15.08), 1000-grain weight (35.05), spiklets fertility% (97.30), grain elongation% (68.75) and gelatinization temperature (5.0).

Table 3: Analysis of variance and mean squares of combined data for the studied morphological traits in rice genotypes.

S.O. V. / Traits	Years	Reps. / years	Genotypes	Genotypes / years	Error
d.f.	1	4	23	23	92
Days to 50% heading	36.50**	0.38	252.95**	0.70	0.52
Plant height (cm)	26.84	5.76	1671.09**	0.51	0.88
Panicle exsertion%	6.52*	0.46	361.14**	0.27	0.63
Panicle length (cm)	46.46**	0.95	25.04**	0.90	0.59
No. of panicles plant^{-1}	3.89	4.37	14.89**	0.18	0.51
No. of filled grains panicle^{-1}	904.11*	95.51	3886.61**	20.56	37.15
1000 grain weight (g)	0.19	0..04	80.17**	0.71	0.53
Grain yield plant^{-1} (g)	41.93**	1.39	146.77**	0.23	0.92
Spiklets fertility%	23.93	1.12	137.67**	0.39	0.95
Hulling%	5.90	1.67	45.48**	0.41	0.77
Milling%	1.77	2.08	48.10**	0.12	0.63
Head rice%	2.33	1.14	192.64**	0.12	1.81
Grain length (mm)	0.01	0.01	1.58**	0.01	0.01
Grain shape	0.02	0.01	0.28**	0.01	0.01
Elongation%	0.01	1.22	533.72**	0.01	1.60
Amylose content%	0.09	0.01	77.08**	0.01	0.05
Gelatinization temperature	0.02	0.04	15.53**	0.00	0.03

**: Significant at 0.01 probability level.

Table 4: Distribution of 24 rice genotypes within each cluster

Cluster	Number of genotypes	Name of genotypes included
I	1	I 67962-40-6-3-3 (NPT)
II	3	IR 68544-29-2-1-3-1-2 (NPT), IR 68552-55-3-2(NPT), IR 69116-67-3-2-3 (NPT)
III	5	IR 65600-77-4-2-1, IR 65600-129-1-1-2, IR 65603-57-4-2, IR 65598-27-3-1, IR 65564-44-2-3
IV	3	IR 69093-41-2-3-2, IR 67966-188-2-2-1 (NPT), IR 67964-46-1-3-2
V	1	IR 69853-70-3-1-1
VI	2	IR 68011-15-1-1, KATY
VII	1	MOROBEREKAN
VIII	1	IR 66158-38-3-2
IX	1	IR 67954-46-1-3-2
X	4	IR 65597-29-3-2-3, IR 66159-189-5-5-3 (NPT), IR 66160-121-4-5-3 (NPT), IR 65600-127-6-2
XI	2	IR 68167-28-3-2-2, IR 69432-54-1-1-2-2 (NPT)

Genotype IR 66158-38-3-2 originated from IRRI which was included in cluster VIII, was high cluster mean value for four traits viz., hulling% (81.01), milling% (71.05), head rice% (65.44) and amylose content% (16.27). Also, it was high in number of filled grains panicle^{-1} next to cluster V. On the other hand, cluster IX was marked for five traits viz., panicle exsertion% (16.79), panicle length (29.15), number of panicles plant^{-1} (17.42), grain yield plant^{-1} (47.94) and gelatinization temperature (7.0). Cluster XI consisted of two genotypes (IR 68167-28-3-2-2 and IR 69432-54-1-1-2-2) with a characteristic feature of relatively high panicle exsertion% (15.51), number of panicles plant^{-1} (16.81), hulling% (81.10), milling% (72.06), amylose content% (18.54) and gelatinization temperature (6.0).

Obviously, there were none cluster comprised genotypes with all the desirable traits which could be selected and involved in breeding programme. Moreover, most of the minimum and maximum clusters mean values were distributed in relatively distant clusters. These findings in agreement with conclusion drown by Eswaran (2012). In addition, the results revealed that the parental lines, which selected from the clusters viz. VI, VII, IX and XI, could be used in a hybridization rice programme. The crossing between superior genotypes of above diverse cluster pairs may provide desirable transgressive segregants for improvement new high yielding varieties of rice. Based on the rank for desirable traits among all clusters mean values, hybridization of IR 68011-15-1-1, KATY of cluster VI with promising genotypes of cluster VII (MOROBEREKAN), cluster IX (IR 67954-46-1-3-2) and cluster XI (IR 68167-28-3-2-2 and IR 69432-54-1-1-2-2 (NPT)) may be recommended for isolating transgressive segregants. These results are in good agreement with those reported by Nayak *et al.,* (2004), Chaturvedi and Maurya (2005), Yadav *et al.,* (2011) and Chakravorty and Ghosh (2012).

1.3. Clusters based on morphological dendrogram

The results of cluster analysis of mean values of 17 morphological traits for rice genotypes are demonstrated in Fig.1. The genotypes were comprised of six major clusters at a cut-off similarity coefficient 70%. The major six clusters were comprised into eleven sub-clusters by using UPGMA cluster trees method. The 11 sub-clusters used to reveal the relationships among these genotypes as basic request step for the comparative with the molecular results.

Out of the different clusters, the results obtained also revealed that cluster size varied from one to four sub-clusters. The cluster I was consisted of two sub-clusters namely, 1 and 4 that had the same plant height, panicle length, number of panicles plant^{-1}, number of filled grains panicle^{-1}, spikelts fertility%. Cluster II comprised four sub-clusters (2, 3, 10 and 11), which were possessed the same traits viz., days to 50% heading, panicle length, No. of panicles plant^{-1}, grain elongation% and amylose content%. Cluster III, IV and VI each of them were included only one sub-cluster 6, 7 and 9, respectively. As for cluster III was superior for panicle exsertion%, grain length, grain shape and early maturity. While, cluster IV had the better traits of panicle exsertion%, 1000-grain weight, spiklets fertility% and grain elongation%. Cluster VI had good performance for panicle exsertion%, panicle length, number of panicles plant^{-1}, grain yield plant^{-1} and it had the lowest amylose content% (waxy rice) among all the studied sub-clusters. Two sub-clusters namely, 5 and 8 were included in cluster V, which were possessed the highest number of filled grains panicle^{-1} among the sub-clusters. In contrast, the two previous sub-clusters had the lowest content of gelatinization temperature among all the sub-clusters and the same characteristics of spiklets fertility% and grain shape.

Table 5: Cluster mean values of the 11 clusters for 17 studied traits in 24 rice genotypes and their contribution to total divergence

Character \ Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand mean	LSD 0.05	LSD 0.01
vegetative traits														
Days to 50% heading	124.83	109.61	107.60	109.22	99.33	97.75	107.17	106.00	109.83	105.75	98.92	106.91	0.82	1.11
Plant height (cm)	97.31	87.81	101.24	95.98	103.20	135.42	147.25	92.96	103.24	87.21	92.78	104.04	2.67	3.65
Panicle exsertion%	16.38	0.00	10.00	3.82	4.73	16.21	15.08	0.00	16.79	6.38	15.51	9.54	2.01	2.74
Panicle length (cm)	23.77	21.28	23.50	24.37	27.48	25.25	26.78	24.98	29.15	22.61	22.80	24.72	1.08	1.47
Yield and its components														
No. of panicle plant^{-1}	14.63	15.82	15.44	14.89	15.65	14.81	12.43	13.70	17.42	16.18	16.81	15.25	1.01	1.37
No. of filled grains panicle^{-1}	184.05	160.99	159.35	187.11	233.38	159.34	179.67	217.48	205.13	143.61	132.36	187.41	11.03	15.05
1000- grain weight (g)	27.93	30.19	34.25	33.09	28.63	33.89	35.05	33.03	29.90	30.02	28.13	31.28	0.80	1.10
Grain yield plant^{-1}(g)	39.98	32.84	43.03	45.21	42.81	40.63	44.06	34.30	47.94	36.93	39.59	40.67	1.88	2.56
Spiklets fertility%	90.78	94.57	89.94	91.07	92.67	93.14	97.30	92.63	78.95	88.78	88.71	90.78	1.74	2.37
Grain quality														
Hulling%	75.84	78.30	80.26	81.06	78.47	79.57	78.32	81.01	75.89	74.98	81.10	78.62	1.30	1.78
Milling%	69.45	66.67	71.50	71.79	68.42	71.67	70.11	71.05	66.12	66.65	72.06	69.59	1.07	1.46
Head rice%	62.78	50.33	59.43	62.97	53.64	50.57	59.72	65.44	51.02	55.38	57.12	57.13	2.13	2.91
Grain length (mm)	5.28	5.08	5.80	5.69	5.34	6.60	6.51	5.58	4.88	5.54	5.32	5.60	0.05	0.07
Grain shape	1.80	1.69	1.97	1.92	1.94	2.40	2.31	1.91	1.67	1.99	1.85	1.95	0.05	0.07
Elongation%	20.24	38.95	33.56	37.93	34.52	36.35	68.75	32.89	52.49	34.37	32.38	38.40	1.43	1.95
Amylose content%	19.20	15.87	18.10	21.95	17.44	22.12	26.10	16.27	7.76	17.64	18.54	18.27	0.19	0.26
Gelatinization temperature	7.00	3.33	6.00	6.00	3.00	5.00	5.00	3.00	7.00	7.00	6.00	5.30	0.11	0.15
Rank of desirable traits	3	2	3	3	2	6	5	4	5	3	6			

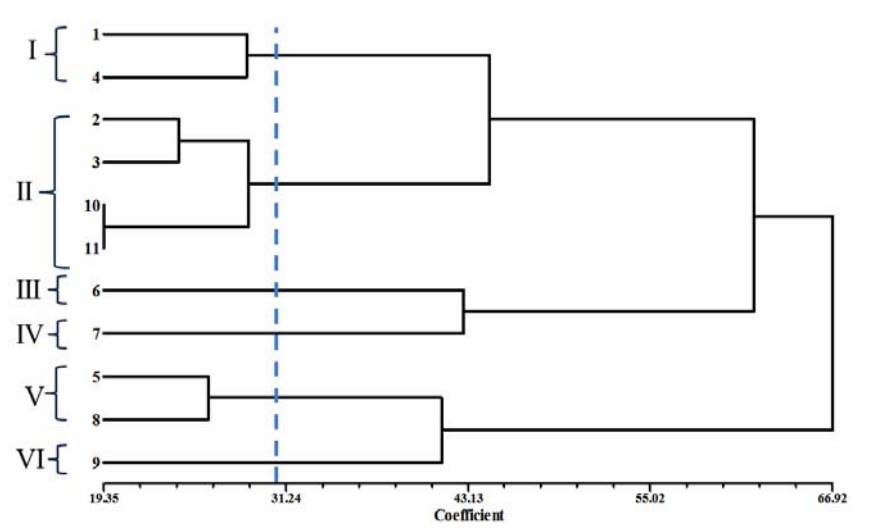

Figure 1: Dendrogram of some rice genotypes using ward method based on Euclidian distance for morphological traits.

Due to the useful breeding programs are depending on modern and adapted knowledge of genetic diversity among varieties to utilize the available genetic resources to create new genotypes. The morphological markers reflect the interaction of genotype with environment effects as well as genetic contribution of the genotype (Thenmozhi and Rajasekaran, 2013).

2. Molecular Markers

Out of 24 rice genotypes, only 11 were selected to represent the eleven sub-clusters based on morphological traits (according to the morphological distribution).

2. 1. ISJ data analysis

Intron-exon splice junctions (ISJ) primers were used to analyze the rice genome in order to assess the potential of these markers for identification and classification of rice genotypes. Out of 12 ISJ markers used in this study, only eight primers amplified and generated polymorphic alleles (ISJ 1, ISJ 3, ISJ 5, ISJ 6, ISJ 7, ISJ 9, ISJ 10 and ISJ 11) in all rice genotypes.

The sizes of amplified fragments were ranged from 300 to 2000 bp. A total of amplifications products were observed 89 reproducible and were scored 44 polymorphic bands. The numbers of polymorphic amplified products were ranged from 2 for both primers ISJ 1 and ISJ 7 to 11 for primer ISJ 11 (Table 6). The average number of bands per primer and genotypes were 11.13 and 8.09, respectively. The three markers ISJ 10, ISJ 3 and ISJ 11 were the most-informative locus for DNA profiling and differentiation because they were recorded the highest percent of polymorphic bands 75, 61.54 and 61.11, respectively. While, the lowest percent of polymorphic bands was obtained with primer ISJ 7. An example of an ISJ pattern produced using ISJ 10 and ISJ 11 primers are demonstrated in Figure 2.

2.2. Genetic Similarity Matrix

The coefficient of similarities based on semi-random data among genotypes ranged from 0.794 to 0.955 with an average similarity index of 0.873, indicating a moderate level of genetic variation among the studied genotypes (Table 7). The lowest genetic similarity was observed between the genotypes namely, IR 66158-38-3-2-1 and KATY while the highest ones belonged to IR 65600- 77-4-2-1 and IR 69116-67-3-2-3 (NPT) genotypes.

Table 6: Data of selected primers were used in semi-random amplification

Primer	Total number of bands	Number of polymorphic bands	Percent of polymorphic bands
ISJ 1	7	2	28.57
ISJ 3	13	8	61.54
ISJ 5	10	6	60
ISJ 6	12	6	50
ISJ 7	9	2	22.22
ISJ 9	12	3	25
ISJ 10	8	6	75
ISJ 11	18	11	61.11
Total	89	44	

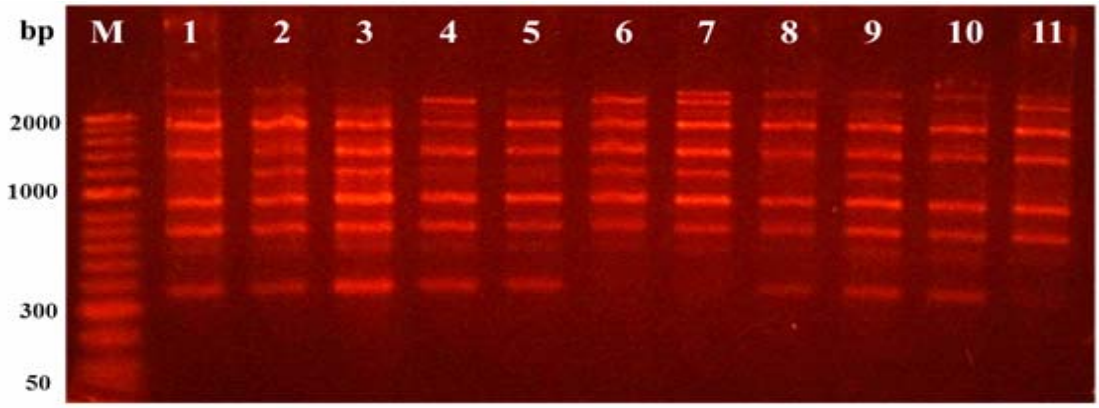

Figure 2: DNA profile of 11 rice genotypes using primer ISJ (6).

Table 7: Dice's similarity coefficient matrix for 11 rice genotypes based on semi-random amplification data.

Serial number of genotype	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	1.000										
II	0.929	1.000									
III	0.937	0.955	1.000								
IV	0.896	0.889	0.936	1.000							
V	0.880	0.886	0.908	0.905	1.000						
VI	0.824	0.816	0.865	0.901	0.857	1.000					
VII	0.861	0.840	0.863	0.859	0.855	0.878	1.000				
VIII	0.847	0.825	0.863	0.873	0.841	0.794	0.806	1.000			
IX	0.880	0.886	0.921	0.919	0.875	0.857	0.869	0.899	1.000		
X	0.895	0.861	0.909	0.893	0.877	0.872	0.830	0.871	0.918	1.000	
XI	0.901	0.867	0.876	0.859	0.828	0.811	0.836	0.849	0.869	0.898	1.000

I- IR 67962-40-6-3-3 (NPT) II- IR 69116-67-3-2-3 (NPT) III- IR 65600- 77 -4-2-1
IV- IR 67966-188-2-2-1 (NPT) V- IR 69853-70-3-1-1 VI- KATY
VII- MOROBEREKAN VIII-IR 66158-38-3-2-1 IX- IR 67954-46-1-3-2
X- IR 65597-29-3-2-3 XI- IR 68167-28-3-2-2

2.3. Clusters based on molecular dendrogram

The similarity values obtained for each pair wise comparison of ISJ markers for the studied genotypes were used to construct dendrogram based on Dice's coefficient. The genotypes were divided into six major clusters at a cut-off similarity coefficient 89% (Fig.3).

Out of the different clusters, the results obtained also revealed that cluster size varied from one to six genotypes. Only one genotype grouped in each of the clusters II, III, IV, V and VI that were namely, IR 69853-70-3-1-1, IR 68167-28-3-2-2, IR 66158-38-3-2-1, KATY and MOROBEREKAN, respectively. While, cluster I contained maximum number (6) of genotypes that were IR 67962-40-6-3-3 (NPT), IR 69116-67-3-2-3 (NPT), IR 65600- 77 -4-2-1, IR 67966-188-2-2-1 (NPT), IR 67954-46-1-3-2 and IR 65597-29-3-2-3 rice genotypes.

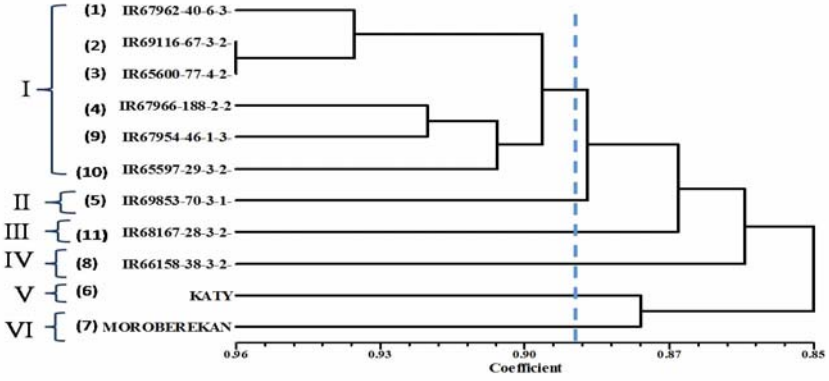

Figure 3: Dendrogram of some rice genotypes using UPGMA method based on Dice's coefficient for ISJ data.

Molecular markers have the ability to detect genetic variability and to help the manipulating of plant genetic resources. In contrary of morphological traits, molecular markers can demonstrate the differences among genotypes at the DNA level, providing a more direct, accurate and efficient tool for characterization and management of rice germplasm. The second generation DNA markers include ISJ markers which were used to determine the variation within rice accessions. So ISJ markers almost succeed in differentiation of rice genotypes. These results are good agreement with that reported by Drikvand *et al.* (2012) and Thenmozhi and Rajasekaran (2013).

2.4. Relationship between Morphological Traits and ISJ Polymorphisms

The morphological and ISJ markers data illustrated two dendograms with six major clusters that were not completely corresponding. However, there were some concordances between them.

Both dendrograms morphological and molecular were more or less similar as presented earlier in Fig. 1 and 3 for the previous results that obtained. In addition, it could be observed from the cluster analysis based on morphological traits that may have molecular basis, Drikvand *et al.* 2012 observed the same trend. In this respect, sub-clusters 2, 3 and 10 were closely related based on morphological traits (cluster-II) and the genotypes that belong to these sub-clusters are also closely related based on ISJ markers (Cluster-I). Furthermore, some genotypes located in one cluster alone based on either morphological or molecular dendrograms, such as genotypes number 6 and 7. This finding indicating that it could be using this molecular marker as a tool for selection in the early generations of selection program. In this aspect, Semagn (2002) suggested two reasons for low correlation between DNA markers and morphological data: (1) DNA markers cover a larger proportion of the genome, including coding and non-coding regions, than the morphological markers. (2) DNA markers are less subjected to artificial selection compared with morphological markers. However, Martinez *et al.* (2003) and Salem *et al.* (2008) believed that the correspondence between different methods might be improve by analyzing more morphological characters, and DNA markers.

In conclusion, the present study showed a large amount of genetic variation that exists among rice genotypes could be utilized efficiently to select parent for improved genotypes. The genotypes that have the greatest genetic distance can be exploited as parental genotypes in breeding programs.

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الملخص العربي

استخدام المعلمات الجزيئية (ISJ) والصفات المورفولوجية لتحديد التنوع الوراثي في بعض التراكيب الوراثية للأرز

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تهدف هذه الدراسة إلى تحديد التنوع الوراثي لأربعة وعشرين تركيبا وراثياً للأرز باستخدام التوصيف المورفولوجي والجزيئي (ISJ) والمقارنة بينهما، وذلك في موسمين زراعيين ٢٠١٤ ، ٢٠١٥ في المزرعة البحثية لمركز البحوث والتدريب في الأرز بسخا كفر الشيخ.

وقد وجدت اختلافات معنوية بين التراكيب الوراثية المستخدمة في هذه الدراسة لجميع الصفات مما يدل على وجود تباينات وراثية بين جيرمبلازم التراكيب الوراثية للأرز المستخدمة في هذه الدراسة. وقد قسمت التراكيب الوراثية الأربعة والعشرون التي تمت دراستها على أساس التباعد الوراثي إلى ١١ مجموعة (عنقود). وكانت المجموعة الثالثة أكبر المجاميع حيث احتوت على خمسة تراكيب وراثية وتلاها المجموعة الخامسة والتي احتوت علي أربعة تراكيب وراثية.

توصي هذه الدراسة طبقا لأهمية وترتيب الصفات المرغوبة بناء على قيم متوسطات المجاميع أن التهجين بين التراكيب الوراثية IR 68011-15-1-1 وKATY في المجموعة السادسة مع التراكيب الوراثية المبشرة في المجموعة السابعة (MOROBEREKAN) والمجموعة التاسعة (IR 67954-46-1-3-2) والمجموعة الحادية عشر (IR 68167-28-3-2-2, IR 69432-54-1-1-2-2 (NPT) ينتج عنها تويفات جديدة قد تحتوى على إنعزالات وراثية فائقة الحدود.

اعتماداً على النتائج الجزيئية باستخدام ١٢ معلم جزيئي من المعلمات الخاصة بالمناطق البينية الغير مشفرة من الجينوم (ISJ) تم تحديد ٨٩ حزمة نتجت عنها ٤٤ حزمة ذات تعدد للأشكال المظهرية. وتراوحت درجة التشابه الوراثي بين التراكيب الوراثية المستخدمة من ٠،٧٩٤ إلى ٠،٩٥٥ بمتوسط درجة تشابهه ٠،٨٧٣. وقد قسمت التراكيب الوراثية المستخدمة إلى ستة مجاميع كبرى(عناقيد) اعتمادا على التحليل العنقودي باستخدام UPGMA المرتبط بدرجات التشابه الوراثي. وعلاوة على ذلك فان بعض التراكيب الوراثية تقع في مجموعة واحدة منفردة وذلك على أساس كلا من المخططين المورفولوجيى والجزيئي، مثل التراكيب عدد ٦ (MOROBEREKAN) و ٧ (KATY).