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RESEARCH ARTICLE

Fingerprinting of some Egyptian rice genotypes using Intron-exon Splice Junctions (ISJ) markers

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ABSTRACT: DNA fingerprinting has become an important tool for diversity assessment and varietal identification in plant breeding programs. Semi- random PCR primers targeting intron-exon splice junctions (ISJ) were used to evaluate the potential of these markers in identification and classification of rice genotypes. A total of 12 ISJ primers were used for screening fourteen Egyptian rice genotypes, including six Japonica, four Indica and four Indica/Japonica rice genotypes. A total of 117 amplified fragments were generated among which 76 fragments were polymorphic revealing average polymorphic ratio of 58.9%. Number of amplified fragments per genotype across the primers ranged from 65 in Japonica rice variety Sakha101 to 85 in Indica/Japonica rice variety Giza179. Number of polymorphic amplified fragments ranged from 3 for primer ISJ-1 to 24 for primer ISJ-2. The average numbers of amplified bands per primer per genotype were 16.71 and 10.24, respectively. Polymorphic information content (PIC) values ranged from 0.289 for ISJ-9 to 0.480 for ISJ-1 with an average of 0.375. The coefficient of similarities based on semi-random data among the studied genotypes ranged from 0.53 to 0.9 with an average of 0.66. All genotypes clearly grouped into two major clusters in the dendrogram at 58% similarity based on Jaccard's similarity index. The first cluster represents the Indica and Indica/Japonica rice genotypes, while the second cluster represents the Japonica genotypes. These results indicate that fingerprinting using semi-specific DNA markers may be an efficient tool for varietal identification and assessing genetic diversity in rice. The results highlight the existing diversity among the studied genotypes and hence their potential use in breeding programs. The simplicity and reproducibility of ISJ markers indicates the potential utilization for molecular characterization, identification and purity assessment of rice genotypes.

KEYWORDS: Rice, DNA fingerprinting, ISJ markers, Similarity coefficient

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INTRODUCTION

Rice (*Oryza sativa*, L.) is one of the world's most important crops, providing a staple food for nearly half of the global population (FAO, 2004). About 20% of the total calorie supply worldwide comes from rice, and particularly in Asia where more than 2 billion people derive 60 - 70% of their daily energy requirement exclusively from rice (Matsumoto et al, 2006). In Egypt, rice is considered the second important cereal crop, following wheat, as a main food for Egyptian population (Bastawisi et al, 2003). During 2015 season, the total production of rice was 5.9 million tons (4.1 million tons, milled basis) (FAO, 2016). The identification of rice cultivars and lines and assessment of genetic diversity are essential and a prerequisite for genetic improvement program, variety registration system, distinctness, uniformity and stability (DUS) testing and the protection

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of plant variety and breeders' rights (Ichii et al, 2003; Kwon et al, 2005). So, a clear-cut identification of elite crop varieties and hybrids is vital for protection and prevention of unauthorized commercial use (Nandakumr et al, 2004). Recent advances in molecular genetics are routinely used for many conservation aspects such as characterizing plant genetic diversity for purposes of improved acquisition, maintenance and use (Karp et al, 1997). DNA markers use in applied breeding programs can range from facilitating appropriate parental selection for hybrids, to mapping/tagging of gene blocks associated with economically important traits (often termed QTLs). DNA markers may be hybridization based (e.g., RFLP), or polymerase chain reaction (PCR) based (e.g., RAPD and SSR); they may detect single locus, oligo loci, or multiple loci differences. The markers may be inherited in either presence/absence (dominant), or co-dominant manner (Hash and Bramel-Cox, 2000). DNA-based markers are highly heritable, available in high numbers, and exhibit enough polymorphism; hence they can be used to discriminate closely related genotypes of a plant species (Yashitola et al, 2002; Wang et al, 2005). Thus, DNA marker systems has become essential tool for fingerprinting and germplasm management (McGregor et al, 2000). The use of semi-random marker system targeting intron-exon splice junctions (ISJ), which was first proposed by Weining and Langridge (1991) and developed by Rafalski et al. (1997), has proven to be very useful for analyzing cultivars and DNA fingerprinting (Przetakiewicz et al, 2002). This approach is universally applicable in plants because it relies on primers based on consensus plant ISJ sequences (7- 9 bases in length), which are necessary for effective intron splicing (Rafalski et al, 1997). The primers' lengths are then increased by adding additional selective bases at random to 3' or 5' ends; this raises their annealing temperatures, yielding more reproducible PCR amplifications. A key advantage of semi-specific primers is that they do not target the heterochromatic regions of the plant genome (Przetakiewicz et al, 2002). The technique has been employed in several monocotyledonous and dicotyledonous plants, all of which have confirmed the markers versatility (Gawel et al, 2002). ISJ markers have been successfully used for genetic analysis in a number of plant species including rye (Rafalski et al, 2002), maize (Rafalski et al, 2001), wheat (Gawel et al, 2002), rice (El-Moghazy, 2007; Ramadan, 2009).

MATERIALS AND METHODS

Plant materials and genomic DNA isolation

Fourteen rice genotypes, including six Japonica, four Indica and four Indica/Japonica rice genotypes were selected for the current study. Seeds were obtained from Rice Research and Training Center (RRTC). The studied genotypes names, type and pedigree are presented in Table 1.

Field evaluation

This investigation was conducted in the Experimental Farm of RRTC during rice growing seasons 2015-2016. Seeds of the studied genotypes were sown in the nursery and after 30 days from sowing, seedlings of each genotype were individually transplanted in the permanent field in 15 rows. Each row was five meters long with 20 cm between rows comprised 25 hills each of a single plant. The experiment was laid out in Randomized Complete Block Design (RCBD) according to Snedecor and Cochran (1967). All agricultural practices were carried out as recommended. Data were collected on yield (t/ha), plant height (cm), duration (days), panicles plant^{-1}, panicle length (cm), 1000-grain weight (g) and spikelet fertility (%).

Molecular analysis

Genomic DNA was isolated from young leaves of ten plants of each genotype using CTAB method described by (Murray and Thompson, 1988) with some minor modifications. The quantity and quality of DNA was assessed with 0.8% agarose gel electrophoresis using diluted uncut lambda phage DNA as size standard. The concentration of DNA was adjusted to approximately 15 ng / µl for PCR reaction. Twelve Semi-random primers were initially screened out of which seven reproducible ones were selected for DNA fingerprinting of the studied genotypes. Primers names and sequences are listed in Table 2. PCR amplification reactions were performed in 15 µl reaction mixtures, containing 1.5 µl of template DNA, 1.5 µl of ISJ primer, 7.5 µl of PCR master mix (Promega) and 4.5 µl ddH_2O. Thermal cycler was carried out with initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at T_m - 2°C for 30 seconds and elongation at 72°C for 1 min, and a final extension at 72°C for 10 min. Five µl of

Table 1. Names, type, parentage and parentage of studied rice genotypes

No.	Genotypes	Type	Parentage
1	Giza177	Japonica	Giza171 / Yomjo No.1 // PiNo.4
2	Sakha101	Japonica	Giza176 / Milyang79
3	Sakha102	Japonica	GZ4098-7-1 / Giza177
4	Sakha104	Japonica	GZ4096-8-1 / GZ4100-9-1
5	Saka105	Japonica	GZ5581-46-3 / GZ4316-7-1-1
6	Sakha107	Japonica	Giza177 / BL1
7	Giza178	Indica \ Japonica	Giza175 / Milyang49
8	Giza179	Indica\ Japonica	GZ1368-S-5-4 / GZ6296-12-1-2-1-1
9	GZ9399-4-1-1-3-2-2	Indica\ Japonica	Giza178 / IR65844-29-1-3-1-2
10	GZ6296-12-1-2-1-1	Indica\ Japonica	AC1225 / Hualien Yu202
11	Giza182	Indica	Giza181 / IR 39422-161-1-3 // Giza181
12	Egyptian Yasmine	Indica	IR262-43-8-11 / KDML105
13	GZ1368-S-5-4	Indica	IR1615-31 / BG94-2-1
14	Egyptian Hybrid1	Indica	IR69625A / Giza178

Table 2. List of sequences and summary of ISJ used primers

Primer	Sequences (5'- 3')	TM*	NN	Size of bands (bp)	TNB	NPB	P%	PIC
ISJ-1	CAG ACC TGC A	32	10	160 - 867	10	3	30	0.480
ISJ-2	ACT TAC CTG AGG CGC CAC	58	18	199 - 1682	28	24	85.71	0.350
ISJ-3	TGC AGG TCA G	32	10	280 - 1245	14	6	42.86	0.461
ISJ-5	CAG GGT CCC ACC TGC A	56	16	338 -1593	10	4	40	0.344
ISJ-7	TGC AGG TCA GGA CCC T	53	16	192 - 2230	25	18	72	0.341
ISJ-9	AGG TGA CCG ACC TGC A	53	16	373 -2098	16	9	56.25	0.289
ISJ-10	ACT TAC CTG CAT CCC CCT	56	18	350 - 1513	14	12	85.71	0.361

PCR products were loaded into 3% agarose gel supplemented with ethidium bromide in 0.5 X TAE buffer and 50 bp DNA ladder (0.5 µg/µl, Fermentas). Electrophoresis was performed at 60 Volts for 2.5 hours and gels were then visualized and photographed using Biometra gel documentation unit (BioDoc, Biometra, Germany). Primer results such as total number of bands (TNB), number of polymorphic bands (NPB), and polymorphism ratio (P %) were recorded. Polymorphism information content (PIC_i) of a band was calculated according to Anderson et al. (1993) as follow:

$$PIC_i = 1 - \Sigma f_{ij}^2$$

Where f_{ij} is the frequency of the j^{th} pattern of the i^{th} band. Then, the PIC of each primer was calculated as:

$$PIC = 1/n \Sigma PIC_i$$

Where n is the NPB for that primer.

The amplified fragments were scored as present or absent (1, 0, respectively). Pair-wise similarity matrices based on DNA profile data were estimated using Jaccard's similarity coefficient (Sneath and Sokal, 1973). Genetic relationships among the genotypes studied were calculated using UPGMA cluster analysis and Principal Component Analysis (PCA) of the similarity matrix. All analyses were computed with the program PAST, ver. 1.90 (Hammer et al, 2001).

RESULTS

Field performance

Analysis of variance showed highly significant differences among the tested genotypes for all studied traits (Table 3). The results proved the existence of considerable amount of genetic variability among the tested genotypes. The mean performances of studied characters are presented in Table 4. Grain yield is the ultimate objective of any crop improvement program. The mean performance of grain yield for the tested genotypes ranged from 9.3 to 13.0 t/ha (Table 4). GZ1368-S-5-4

Table 3. Mean square estimates of ordinary analysis and combining ability analysis for studied traits during seasons 2015-2016 and their combined

S.O.V.		df	Yield (t/ha)	Plant height (cm)	Duration (days)	Panicles Plant^{-1}	Panicle length (cm)	1000 Grain weight (g)	Spikelet fertility %
	Y1	-	-	-	-	-	-	-	-
Years	Y2	-	-	-	-	-	-	-	-
	Comb.	1	0.46*	0.11	13.76*	31.82**	27.43**	3.05**	5.55
	Y1	2	0.15	0.50	0.74	0.18	0.56	0.03	0.30
Reps	Y2	2	0.01	0.29	5.17	0.02	3.03	0.38	1.22
	Comb	4	0.08	0.39	2.95	0.10	1.79	0.20	0.76
	Y1	13	2.86**	87.22**	197.22**	32.61**	19.79**	11.76**	19.39**
Gen.	Y2	13	2.60**	121.03**	254.69**	22.84**	25.29**	12.34**	15.27**
	Comb.	13	5.36**	200.30**	447.10**	54.18**	41.87**	23.61**	31.14**
	Y1	-	-	-	-	-	-	-	-
Gen. × Years	Y2	-	-	-	-	-	-	-	-
	Comb.	13	0.10	7.95	4.81**	1.27	3.21**	0.49	3.52**
	Y1	26	0.08	3.94	2.25	0.35	0.39	0.27	1.56
Error	Y2	26	0.06	6.18	0.83	1.33	1.13	0.13	0.68
	Comb.	52	0.07	5.06	1.54	0.84	0.76	0.20	1.12

Table 4. Mean performance of some traits in the studied rice genotypes during seasons 2015-2016 (Combined)

Genotypes	Yield (t/ha)	Plant height (cm)	Duration (day)	Panicles Plant^{-1}	Panicle length (cm)	1000 Grain weight (g)	Spikelet fertility %
Giza 177	10.0	101	125	19	21.3	27.4	95.5
Sakha 101	11.5	90	140	23	23.7	28.2	94.3
Sakha 102	10.0	106	125	20	20.4	27.9	97.1
Sakha 104	11.0	105	135	21	20.1	28.5	94.1
Sakha 105	10.8	100	124	20	19.8	28.0	96.2
Sakha107	9.7	97	123	18	18.9	27.3	95.7
Giza 178	11.0	106	135	26	23.6	21.2	91.3
Giza 179	11.4	98	122	25	22.9	27.5	92.7
GZ9399-4-1-1-3-2-2	11.1	103	118	26	21.4	26.6	93.8
GZ6296-12-1-2-1-1	10.4	96	127	24	20.3	25.8	90.6
Giza 182	11.3	100	134	21	24.3	26.0	92.3
E. Yasmine	9.6	112	148	22	27.4	27.3	91.1
GZ1368-S-5-4	9.3	104	137	14	19.2	23.3	90.7
Hybrid1	13.0	107	135	27	26.5	25.2	88.5
LSD 5%	0.426	3.689	2.037	2.013	1.429	0.612	4.752
LSD 1%	0.568	4.918	2.715	2.684	1.906	0.816	6.335

possessed minimum yield potential (9.3 tons/ha), while Hybrid1 had maximum grain yield with 13 tons/ha. Short stature rice plant is a desirable trait and rice variety with appropriate plant height will have a non-lodging behavior which is important for high yield potential. The trait mean values significantly varied and ranged from 90 to 112 cm in rice cultivars Sakha101 and Egyptian Yasmine, respectively. Among studied rice genotypes; GZ6296-12-1-2-1-1, Sakha107 and Giza179 are short statured with plant height of 96.0, 97.0 and 98.0, respectively. Early

maturing varieties are important to overcome irrigation water shortage. Minimum days to maturity were recorded for GZ9399-4-1-1-3-2-2 (118.0 day) while maximum value was found with Egyptian Yasmine (148.0 day). Concerning panicles plant^{-1} trait, the rice genotypes Hybrid1, Giza178, GZ9399-4-1-1-3-2-2 and Giza179 showed the highest mean values of 27, 26, 26 and 25 panicles, respectively. While, the genotypes; GZ1368-S-5-4, Sakha107 and Giza177 revealed the lowest mean values of 14, 18 and 19 panicles plant^{-1}, respectively. Wide range of panicle length (cm) was observed. The results indicated that Sakha107 had shortest panicles of 18.9 cm while Egyptian Yasmine had maximum value of 27.4 cm. Thousand-grain weight (g) significantly varied (Table 4). Among studied genotypes, lowest value for 1000-grain weight was exhibited by Giza178 (21.2 g), while maximum value was recorded for Sakha 104 (28.5 g). Also, Sakha101, Sakha104 and Sakha105 among rice genotypes performed well for 1000-grain where they had more than 28g. Spikelet's fertility (%) was also varied among the genotypes. Hybrid1, GZ6296-12-1-2-1-1 and GZ1368-S-5-4 indicated minimum values with of 88.5, 90.6 and 90.7% while Sakha102, Sakha105 and Sakha107 had maximum fertility among rice genotypes with 97.1, 96.2 and 95.7%, respectively

Molecular analysis

A total of 117 amplified bands were generated and 76 of them were polymorphic bands using 7 selected ISJ primers. The size of amplified fragments ranged from 160 to 2230 bp.. Index of polymorphism, which was estimated as the proportion of polymorphic loci in the total number of loci studied, was 58.9% (average over all loci), varying from 30% for primer ISJ-1 to 85.71% for primers ISJ-2 and ISJ-10. Number of amplified fragments per primer ranged from 10 for primers ISJ-1 and ISJ-5 to 28 for primer ISJ-2 (Table 5). Total number of amplified fragments per genotype ranged from 65 in Japonica rice variety Sakha101 to 85 in Indica/Japonica rice variety Giza179. Number of polymorphic amplified bands ranged from 3 for primer ISJ-1 to 24 for primer ISJ-2. The average number of bands per primer and genotypes were 16.71 and 10.24, respectively. In addition, Indica rice genotypes revealed the highest average number of amplified fragments per genotype compared with Japonica rice genotypes. Also, the PIC values ranged from 0.289 for ISJ-9 to 0.480 for ISJ-1 with an average of 0.375.

The PCR amplified fragments produced by the highest polymorphic ISJ markers in the current study ISJ-2, ISJ-7 and ISJ-10 are shown in Figure 1. The amplification pattern of highly informative loci is found in Figure 2.

From figure 2 it can be found that about 28 polymorphic amplified bands were generated. Among them 15 bands were related to the subspecies. The bands ISJ-1-599, ISJ-2-1087, ISJ-3-10-444, ISJ-10-2-1179 and ISJ-10-4-910 were present in Japonica rice genotypes, while they were completely absent from all Indica genotypes. Bands ISJ-2-852, ISJ-2-530, ISJ-2-593 and ISJ-7-7-986 were found in some Japonica and Indica / Japonica genotypes but they were absent from pure Indica genotypes. The amplified fragments ISJ-2-917, ISJ-3-8-505, ISJ-3-8-505, ISJ-3-12-334, ISJ-5-4-1089, ISJ-7-6-1056 and ISJ-9-9-873 were generated in Indica and Indica / Japonica genotypes meanwhile they were absent from Japonica genotypes. These obtained results indicate that these amplified bands may be considered as specific DNA markers for distinguishing among rice genotypes related to different rice subspecies.

The similarity coefficient values for the studied genotypes

Table 5. Number of amplified fragments for each marker and rice genotype

Genotype / Marker	Giza177	Sakha101	Sakha102	Sakha104	Saka105	Sakha107	Giza178	Giza179	Giza182	E. Yasmine	GZ1368- S	GZ9399-4	GZ6296-12	E. Hybrid1
ISJ-1	8	9	9	9	8	8	9	9	9	9	7	9	9	9
ISJ-2	12	14	15	14	15	14	20	18	15	11	13	14	13	9
ISJ-3	11	8	8	10	10	10	11	12	10	11	11	10	11	11
ISJ-5	7	6	7	7	8	8	9	8	9	9	9	8	9	7
ISJ-7	13	10	12	11	13	14	14	15	11	13	12	10	9	17
ISJ-9	12	13	11	12	12	11	15	13	14	14	12	12	10	10
ISJ-10	5	5	6	6	7	5	4	10	5	9	9	8	3	4
Total	**68**	**65**	**68**	**69**	**73**	**70**	**82**	**85**	**73**	**76**	**73**	**71**	**64**	**67**

Table 6: similarity coefficient among studied genotypes based on ISJ markers

Genotypes	Giza177	Sakha101	Sakha102	Sakha104	Saka105	Sakha107	Giza178	Giza179	Giza182	E. Yasmine	GZ1368- S	GZ9399-4	GZ6296-12	E. Hybrid1
Giza177	1.00													
Sakha101	0.82	1.00												
Sakha102	0.84	0.87	1.00											
Sakha104	0.85	0.89	0.90	1.00										
Saka105	0.74	0.75	0.81	0.80	1.00									
Sakha107	0.82	0.73	0.82	0.78	0.79	1.00								
Giza178	0.63	0.63	0.63	0.62	0.60	0.58	1.00							
Giza179	0.56	0.56	0.61	0.59	0.60	0.58	0.67	1.00						
Giza182	0.53	0.57	0.57	0.56	0.54	0.54	0.80	0.68	1.00					
E. Yasmine	0.57	0.57	0.60	0.59	0.57	0.59	0.74	0.71	0.80	1.00				
GZ1368- S-5-4	0.58	0.55	0.58	0.58	0.60	0.61	0.65	0.68	0.72	0.77	1.00			
GZ9399-4-1-1-3-2-2	0.60	0.62	0.64	0.65	0.58	0.58	0.68	0.71	0.76	0.79	0.78	1.00		
GZ6296-12-1-2-1-1	0.59	0.57	0.61	0.60	0.57	0.65	0.66	0.64	0.69	0.73	0.69	0.80	1.00	
E. Hybrid1	0.61	0.59	0.63	0.60	0.59	0.63	0.62	0.67	0.61	0.70	0.59	0.64	0.72	1.00

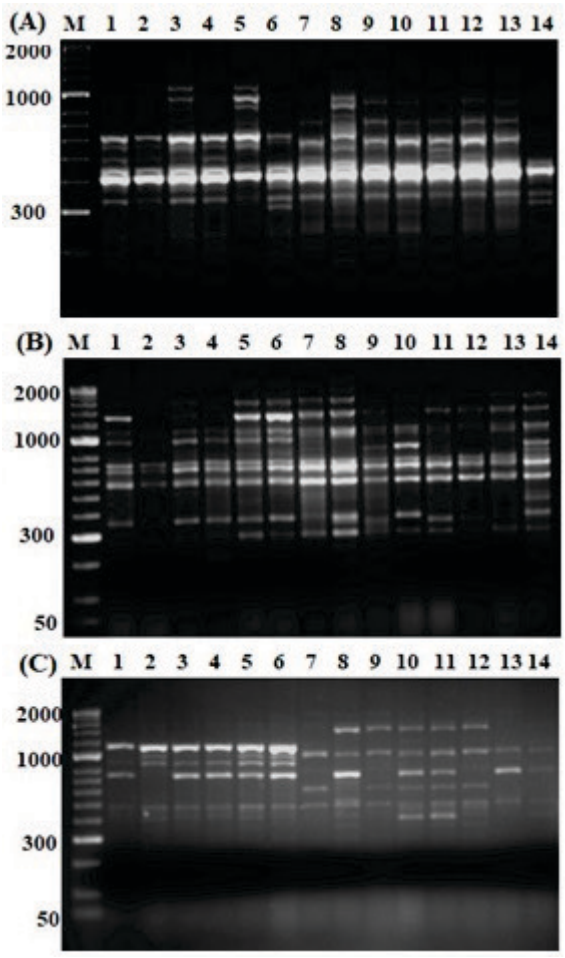

Figure 1. Agarose gel electrophoresis of PCR amplified fragments for the highest polymorphic ISJ markers. ISJ-2 (**A**), ISJ-7 (**B**) and ISJ-10 (**C**). **M:** 50 bp DNA ladder; **1:** Giza177; **2:** Sakha101; **3:** Sakha102; **4:** Sakha104; **5:** Sakha105; **6:** Sakha107; **7:** Giza178; **8:** Giza179; **9:** GZ9399-4-1-1-3-2-2**; 10:** GZ6296-12-1-2-1-1**; 11:** Giza182; **12:** E. Yasmine; **13:** GZ1368- S-5-4 and **14:** E. Hybrid1

are presented in Table 6. In general, the coefficient of similarities based on semi-random data among the studied genotypes ranged from 0.53 to 0.9 with an average similarity index of 0.66. The lowest genetic similarity was observed between the pure Indica variety Giza182 and the pure Japonica variety Giza177, while the highest similarity was observed between both Japonica varieties Sakha102 and Sakha104. Among Japonica genotypes, genetic similarity coefficients ranged from 0.73 and 0.9 with an average of 0.81. The lowest similarity coefficient was observed between Sakha107 and the high yielding rice variety Sakha101. On the other hand, a mong Japonica and Indica and Indica/Japonica genotypes the coefficients of similarity varied from 0.53 and 0.65 with an average of 0.59. The highest similarity coefficient was observed between the Indica/Japonica line GZ6296-12-1-2-1-1 and the Japonica variety Sakha107. Among Indica/-Japonica genotypes the coefficients of similarity ranged from 0.59 and 0.80 with an average of 0.70. The lowest similarity coefficient was found between Egyptian Hybrid1 and GZ1368-S-5-4, while the highest similarity coefficients were observed between Giza182 and both varieties Giza178 and Egyptian Yasmine and between both lines GZ6296-12-1-2-1-1 and GZ9399-4-1-1-3-2-2 The genetic relationships among studied rice genotypes are presented in a dendrogram based on Jaccard's similarity index and UPGMA method (Figure 3). All Genotypes clearly grouped into two major clusters in the dendrogram at 58% similarity. This result indicates the presence of high amount of genetic variation between the two rice subspecies indicating their potential in successful

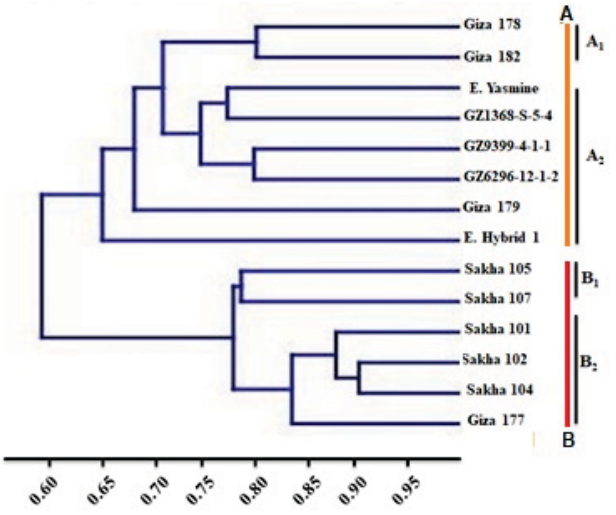

Figure 3. Dendrogram derived from UPGMA cluster analysis of 14 rice genotypes based on Jaccard's similarity coefficient using ISJ markers

crossing and breeding program. The first cluster "A" represents the Indica and Indica/Japonica rice genotypes, while the second cluster "B" represents the Japonica genotypes. The main group "A" was further divided in two sub clusters A1and A2 at about 71% similarity. Similarly, the main group "B" was further separated into two sub clusters, B1 and B2 at about 78% similarity (Figure 3). The sub clusters A1 included the Indica rice variety Giza182 and the wide spread Indica/Japonica rice variety Giza178, while the sub cluster A2 included the both Indica rice genotypes Egyptian Yasmine and GZ1368-S-5-4 and both Indica Japonica rice genotypes GZ9399-4-1-1-3-2-2 and GZ6296-12-1-2-1-1. The genotypes Giza179 and E. hybrid1 didn't group into any sub cluster. On the other hand, the sub cluster B1 included both rice genotypes Sakha105 and Sakha107. The other Japonica genotypes were found in sub cluster B2.

DISCUSSION

Cultivated rice varieties are developed as a result of many years of selection from the available genetic diversity that found in different environments and human cultures. Morphological markers depending on simply and complexly traits have been used for varietal identification and characterization for a long time. The low number of morphological markers, poor or unknown in genetic control, environmental effects on the phenotypic expression, procedural difficulties and stage specific identification are determining factors for using these markers as genetic markers in varietal identification (Ravi et al., 2003). In the present investigation, both morphological and DNA markers have been used for fingerprinting of fourteen elite Egyptian rice genotypes. Analysis of variance indicated the presence of considerable amount of variation among the studied rice genotypes. A considerable amount of variation was observed for grain yield ha^{-1} (from 9.3 to 13 ton ha^{-1}). However, all studied rice genotypes are identified as high yielding. Egyptian Hybrid1 scored the highest values for the traits grain yield $hectare^{-1}$, $panicles\ plant^{-1}$ and panicle length. The yield increase was 13.04% over the high yielding Egyptian rice variety Sakha101 and 18.18% over Giza178 (the restorer line of Hybrid1). This result reflects the fact that heterosis increases growth and yield of rice plant by about 20%. Each of Giza178, Giza179 and Giza182 are high yielding varieties (11.0 to 11.4 t/ha) and rice genotypes under the current study lie in the first group (118 – 127 days). The aromatic rice variety Egyptian Yasmine lies in the third group with 148 days. All studied rice genotypes are characterized by semi-dwarfing so all of them are resistant for lodging except for Sakha102.

Molecular markers introduce an important tool for varietal identification. There are a large numbers of DNA markers varied from dominant to co-dominant, specific to random and PCR-based to non PCR-based markers. In the current report, Intron-exon Splice Junctions (ISJ) primers have been used for fingerprinting of some Egyptian rice varieties and lines. The most important advantage of ISJ markers is that they avoid targeting the heterochromatic sites of the plant genome (Przetakiewicz et al., 2002). In addition, these makers can be used to identify the polymorphism in a specific gene family or conserved DNA region and the primers can be designed from genomic or EST databases of various regions of the genome (Poczai et al. 2013).

The highest number of polymorphic bands was observed for both markers ISJ-2 and ISJ-7 indicating that these markers can be used for varietal fingerprinting. The highest number of amplified fragments was observed for both Indica / Japonica rice varieties Giza178 and Giza179 indicating the rich of their genetic backgrounds with polymorphism explaining their ability to adaptation under different environments. Five markers i.e. ISJ-1-599, ISJ-2-1087, ISJ-3-10-444, ISJ-10-2-1179 and ISJ-10-4-910 were specific for Japonica rice genotypes and can be used for Japonica rice distinguishing. The lowest coefficient of similarity was observed between the indica variety Giza182 and Japonica variety Giza177.

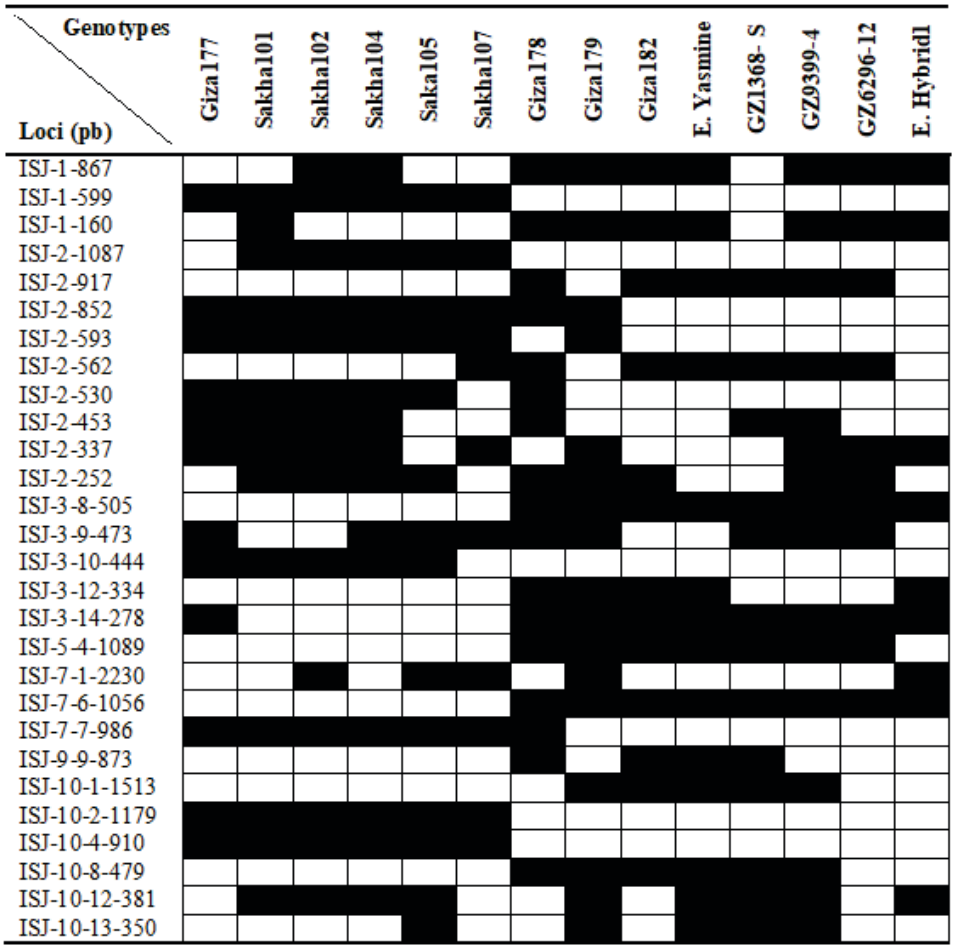

Figure 2. Schematic representation of semi-random profiling of studied rice genotypes. Shaded block indicate the presence of allelic bands by the unique respective semi-random loci

The cluster analysis divided the studied genotypes into two major branches at 58% similarity. The ISJ results obtained here are coherent with that of Petroudi et al. (2010) who obtained high number of polymorphic amplified bands ranging from 1 to 21 and high number of amplified bands per primer and genotypes (15.41 and 11.56, respectively) using semi-random primers. Also the large number of amplified bands in Indica genotypes found in this study was interesting. This finding may be due to the large amount of genetic variability presented in Indica rice. This result agreed with Gao et al. (2005) who reported that average allele number in the indica rice was 1·40 times higher than that in the japonica rice. PIC value refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency; thus, it provides an estimate of the discriminating power of the marker (Nagy et al, 2012). In the current study the PIC values ranged from 0.289 for ISJ-9 to 0.480 for ISJ-1 with an average of 0.375. Similarity coefficient results showed significant level of diversity among genotypes. The similarity among genotypes ranged from 0.53 to 0.9 with an average similarity index of 0.66. These results were similar to Petroudi et al. (2010) where wide similarity values ranged from 0.47 to 0.95 with an average similarity index of 0.75. The results of similarity index among and within rice subspecies were in agreement with Chakravarthi and Naravaneni (2006) and Ramadan et al. (2015) who reported low similarity coefficient between japonica type and indica type genotypes, and Kanawapee et al. (2011) who reported relatively high level of similarity between closely related genotypes. Clustering genotypes in the constructed dendrogram was largely dependent on their genetic background. The ability of cluster analysis to divide rice genotypes according to their genetic background and/or origin has been reported in different papers. El-Malky et al. (2007) reported that cluster analysis grouped the studied genotypes into two groups, one included the indica genotypes and the other included

the japonica genotypes. Also, Zeng et al. (2004) found that all genotypes clearly grouped into two major branches in the dendrogram with less than 10% similarity based on Jaccard's similarity index, one branch represents the subspecies, japonica rice and another branch represents the subspecies, indica, or the hybrids between japonica rice and indica rice. The obtained results indicated that ISJ marker is an efficient marker for varietal identification and classification in rice and other species. Introns are considered as a source of polymorphism because of their moderate sequence evolution (Kimura, 1983). In addition, Introns can be used for the construction of genetic maps, because they reflect variation that found within genes (Han et al, 2006). The results indicated the high resolution power of ISJ as a semi-specific marker. High number of amplified fragments has been generated in the studied genotypes. All Japonica genotypes were grouped in one cluster B while all Indica and Indica rice genotypes were grouped in another cluster A. The current study encourages using larger number of ISJ markers for identifying and fingerprinting larger number of genotypes in rice and different plant species.

CONCLUSION

The obtained results indicated that ISJ marker is an efficient marker for Varietal identification and classification in rice and other species. Introns are considered as a source of polymorphism because of their moderate sequence evolution (Kimura, 1983). In addition, Introns can be used for the construction of genetic maps, because they reflect variation that found within genes (Han et al, 2006). The results in current paper indicated the powerful of ISJ as a semi-specific marker. High number of amplified fragments has been generated in the studied genotypes. The highest number of polymorphic bands was observed for both markers ISJ-2 and ISJ-7 indicating that these markers can be used for varietal fingerprinting. The highest number of amplified fragments was observed for both Indica / Japonica rice varieties Giza178 and Giza179 indicating the rich of their genetic backgrounds with polymorphism explaining their ability to adaptation under different environments. Five markers i.e. ISJ-1-599, ISJ-2-1087, ISJ-3-10-444, ISJ-10-2-1179 and ISJ-10-4-910 were specific for Japonica rice genotypes and can be used for Japonica rice distinguishing. The lowest coefficient of similarity was observed between the indica variety Giza182 and Japonica variety Giza177. The cluster analysis divided the studied genotypes into two major branches at 58% similarity. All Japonica genotypes were grouped in one cluster B while all Indica and Indica rice genotypes were grouped in another cluster A. The current study encourages using larger number of ISJ markers for identifying and fingerprinting larger number of genotypes in rice and different plant species.

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انگشت‌نگاری برخی از ژنوتیپ‌های مصری برنج با استفاده از نشانگرهای محل اتصال اینترون-اگزون (ISJ)

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چکیده

انگشت‌نگاری DNA یک ابزار مهم برای بررسی تنوع و شناسایی ارقام در برنامه‌های اصلاح گیاه می‌باشد. آغازگرهای نیمه تصادفی PCR که مکان‌های اتصال اینترون-اگزون (ISJ) را هدف قرار می‌دهند برای ارزیابی پتانسیل این نشانگرها در شناسایی و دسته‌بندی ژنوتیپ‌های برنج مورد استفاده قرار گرفتند. برای غربالگری ۱۴ ژنوتیپ برنج مصری، شامل ۶ ژنوتیپ ژاپونیکا، ۴ ژنوتیپ ایندیکا و ۴ ژنوتیپ ایندیکا/ژاپونیکا از ۱۲ آغازگر ISJ استفاده شد. در مجموع ۱۱۷ قطعه تکثیر شده تولید شد که ۷۶ قطعه چند شکل بوده و نسبت متوسط چندشکلی برابر با ۵۸/۹٪ را نشان داد. تعداد قطعات تکثیر شده به ازای ژنوتیپ در آغازگرها از ۶۵ در رقم برنج ژاپونیکای Sakha101 تا ۸۵ در رقم برنج ایندیکا/ ژاپونیکا Giza179 متغیر بود. تعداد قطعات چندشکل تکثیرشده از ۳ برای آغازگر ISJ-1 تا ۲۴ برای آغازگر ISJ-2 تفاوت داشت. متوسط تعداد باندهای تکثیر شده به ازای آغازگر و به ازای ژنوتیپ به ترتیب ۱۶/۷۱ و ۱۰/۲۴ بود. مقادیر PIC از ۰/۲۸۹ برای ISJ-9 تا ۰/۴۸۰ برای ISJ-1 با میانگین ۰/۳۷۵ متغیر بود. ضریب تشابه بر اساس اطلاعات نیمه تصادفی میان ژنوتیپ‌های مطالعه شده بین ۰/۵۳ تا ۰/۹ با میانگین ۰/۶۶ بود. همه ژنوتیپ‌ها بوضوح در دو گروه اصلی در تشابه ۵۸٪ براساس شاخص مشابهت جاکارد دسته‌بندی شدند. در گروه اول ژنوتیپ‌های ایندیکا و ایندیکا/ژاپونیکا و در گروه دوم ژنوتیپ‌های ژاپونیکا قرار دارند. این نتایج نشان می‌دهد که انگشت‌نگاری با استفاده از نشانگرهای DNA نیمه اختصاصی می‌تواند یک ابزار سودمند برای شناسایی و ارزیابی تنوع ژنتیکی در برنج باشد. نتایج وجود تنوع میان ژنوتیپ‌های مطالعه شده و پتانسیل استفاده از آنها در برنامه‌های اصلاحی را نشان می‌دهد. سهولت استفاده و تکرارپذیری نشانگرهای ISJ حاکی از توانایی بالقوه آنها برای توصیف مولکولی، شناسایی و ارزیابی خلوص ژنوتیپ‌های برنج می‌باشد.

کلمات کلیدی: برنج، انگشت‌نگاری DNA، نشانگرهای ISJ، ضریب تشابه