ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

GENETIC DIVERSITY ANALYSIS OF ANTHER CULTURE DERIVED RICE PLANTS USING MOLECULAR MARKERS

***Hemaprabha K[1], Hemalatha T[2], UmaMaheswari T[3], Anbukkarasi K[3], Shanmugasundaram P[4]**

1. Department of Floriculture and Landscaping, College of Horticulture, TamilNadu Agricultural University, Coimbatore - 641003, India.

2. Department of Bioproducts, Central Leather Research Institute, Chennai - 600020, India.

3. Dairy Microbiology Division, National Dairy Research Institute, Karnal-132001, India.

4. Department of Plant Genetic Resources, TamilNadu Agricultural University, Coimbatore - 641003, India.

Manuscript Info

Manuscript History:

Received: 24 June 2013
Final Accepted: 07 July 2013
Published Online: July 2013

Key words:
RAPD, ISSR,
genetic diversity,
rice, anther culture

****Corresponding Author:***
Dr. K.Hemaprabha,
Assistant Professor (Biotechnology)
Department of Floriculture and Landscaping, College of Horticulture, TamilNadu Agricultural University, Coimbatore - 641003, India.
email:
hema.kswamy@gmail.com

Abstract

Random amplified polymorphic DNA (RAPD) analysis and inter-simple sequence repeats (ISSR) primers were used to determine the occurrence and extent of variation in rice (*Oryza sativa* L.) plants regenerated from anther culture. Genetic diversity among 27 regenerants of the cross CO43/Nootripathu and their parents were assessed using 25 RAPD primers and 19 ISSR primers. RAPD primers used in the study produced 285 polymorphic markers (81.65% polymorphism) and ISSR primers produced 201 polymorphic markers (79.37% polymorphism). The number of markers produced per primer ranged from 4-27 in case of RAPD with a mean of 14.04 and 7-23 in case of ISSR with a mean of 13.05. Dendrograms were constructed using similarity index values. RAPD markers grouped the 27 regenerants (A_0 generation) and their parents into four clusters and ISSR markers into three clusters. The Polymorphism information content (PIC) values ranged from 0.702 to 0.952 with a mean of 0.887 for RAPD markers. In ISSR analysis, the mean PIC value was 0.891 and the highest and lowest PIC values were 0.940 and 0.832 respectively. In the present study, RAPD markers were able to reveal greater genetic diversity among the regenerants screened than ISSR markers.

Introduction

Rice (*Oryza sativa* L.) is the most important staple crop and more than half of the World's population, depends upon it directly for food. It has also become a model organism for genome analysis, having a diploid chromosome number of 24 and the smallest genome size (430 Mb) of all major crop plants (Arumuganathan and Earle, 1991). Many environmental stress factors, such as drought, chilling temperature, acidified soil, saline soil etc., significantly reduce the productivity of this major crop and also limit the growing areas. Therefore, the development of new varieties with higher yield potential and tolerance to stress conditions will clearly contribute to increase rice production in many areas (Steponkus et al., 1980). Water limited condition (also referred to as drought), affecting 23 mha of rice regularly (Pandey et al., 2007) is a condition related to insufficient soil moisture available to support average crop production. Hence, crop improvement strategies focus on developing new rice varieties which could grow under water stress conditions with significant productivity. Suresh et al (2013) report that hybrids of CO43/Nootripathu, as superior ones for improving yield under water stress.

Haploids with their unique genomic constitution have potential for accelerating the production of homozygous new varieties. The production of rice haploids and subsequent homozygous diploid plants by *in vitro* anther culture has dramatically advanced in the last two decades (Premvaranon et al., 2011). The benefit of anther culture is distinct in accelerating the selection process, because of high degree of homozygous

doubled-haploid (DH) plants or pure lines which can be obtained at first generation in a single step. Selection process can be done earlier since anther culture technique allows early expression of recessive genes and increase selection efficiency as the number of plants required to obtain the desired recombinants are less than the conventional breeding (Dewi and Purwoko, 2001). In rice, DH lines have been produced in many genotypes using anther culture (Raina, 1989). Much of the gene mapping work has been carried out using DH lines derived from rice anther culture (Li et al., 2005; Zeng et al., 2006). Androclonal variation was also detected in anther derived rice lines using RAPD markers (Afza et al., 2001). Genetic markers can be combined with DH populations to develop genetic maps and to identify genetic markers linked to traits of interest (Forster and Thomas, 2005). DH system is fully compatible with other biotechnological approaches such as mutation techniques for gene character identification or genetic engineering etc.

DNA based molecular markers are highly heritable, available in high numbers and often exhibit enough polymorphism to discriminate closely related genotypes. PCR based markers such as random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and inter simple sequence repeats (ISSR) have been of great use in genetic diversity analysis, genome mapping and gene tagging because they are technically simple, time saving, highly informative and require small amounts of DNA (Thanh *et al.*, 1999).

In light of the above knowledge, the present study was taken up with an aim to analyze the genetic diversity among the androclones from hybrids of CO43/Nootripattu, (wherein CO43 is a high yielding rice variety released from TNAU, Coimbatore, India, while Noortipathu, is a rainfed landrace grown in southern Tamilnadu, India) using RAPD and ISSR markers.

MATERIALS AND METHODS

Field maintenance and collection of materials

Hybrids of CO43/Nootripattu were grown in fields of Tamilnadu Agricultural University, Coimbatore, India, for collection the anthers. The first two or three panicles were harvested at booting stage between 08.30am and 10.00 am for anther culture.

Anther culture

The induction of androgenesis is enhanced by giving cold pre treatment to the panicles. The cold treatment was given at 10°C for eight days in a BOD incubator. The ensheathing leaves were removed and the spikes from the middle of the panicles were removed and used for the experiment (Alejar et al., 1995). Embryogenic calli was observed from one week old culture tubes. The callus was maintained in MS medium with hormonal and additive combinations with constant subculturing. The calli after subculturing were transferred to the regeneration medium. The calli were incubated in the growth chamber at 25 ±2°C with 16 h of light. After rooting, the plantlets were subjected to primary and secondary hardening.

Evaluation of regenerants in field

The regenerants (A_0 generation) after primary and secondary hardening were transplanted to field in paddy breeding station (TNAU). The seedlings were transplanted with spacing of 30x20 cm. Recommended dose of fertilizers, pest management measures and irrigation schedules were given. Biometrical observations were recorded for each regenerant and statistically analysed.

Genetic diversity analysis

Source of plant material

The plant material from the regenerants (A_0 generation) along with the parental lines were collected and used for diversity analysis using morphological and DNA markers.

Primers

Twenty five RAPD primers (Operon Technologies, USA) and 19 ISSR primers (University of British Columbia) were used in this study.

Isolation and quantification of genomic DNA

DNA was isolated from fresh leaf samples collected from the seedlings of regenerants by the Cetyl Trimethyl Ammonium Bromide method (Gravel and Jarret, 1991). To assess the quality, all the genomic DNA samples were run on 0.8% agarose gel and gel was stained with ethidium bromide and bands were documented using Alpha Imager 1200 (Alpha Innotech Inc., USA). The quantity of DNA present in each sample was determined by reading the absorbance at 260nm in a Hoefer Dyna Quant 200 fluorometer (Hoefer, California, USA). Calf thymus DNA was used as the standard.

Molecular marker analysis

RAPD analysis

DNA from the A_0 regenerants was amplified using a set of 25 arbitrary oligonucleotide decamer primers. Polymerase chain reaction (PCR) amplification reaction was carried out in 15 µl reaction volume containing 20-25 ng of genomic DNA, 0.8 µM of primer, 0.66 mM each of dATP, dGTP, dCTP, dTTP,

1.5 mM assay buffer and 0.3 units of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). Amplification were performed in thermal cycler (PTC – 100^{TM} MJ Research Inc., USA) programmed for an initial denaturation of 94°C for 2 min, 35 cycles of 1 min. denaturation at 94°C, 1 min annealing at 37°C, and extension at 72°C for min, followed by final extension for 5 min at 72°C and then at 4°C for storage. PCR amplified products (10 µl) were subject to electrophoresis in a 1.5% agarose gel in 1x TBE buffer at 8V/cm for 4-5 hours using standard submarine gel electrophoresis unit. The bands were visualized and documented in gel documentation system (Alpha Imager, 1200, Alpha Innotech Corp., USA)

ISSR analysis

A total of 19 ISSR primers were used for amplifying the genomic DNA. Amplification was carried out in 15 µl reaction volume containing 20-25 ng of genomic DNA, 0.8 µM of primer, 0.66 mM each of dATP, dGTP, dCTP, dTTP, 1.5 mM assay buffer and 0.3 units of *Taq* DNA polymerase. Amplification was done using a PTC thermal cycler (MJ Research Inc.) programmed for initial denaturation at 94°C for 1 min, 45 cycles of 1 min denaturation at 94°C, 50 sec annealing at 46°C and 2 min extension at 72°C and final extension of 5 min at 72°C and then at 4°C for storage. PCR amplified products (10 µl) were subject to electrophoresis in a 1.5% agarose gel in 1xTBE buffer at 8V/cm for 4-5 hours using standard submarine gel electrophoresis unit. The bands were visualized and documented in gel documentation system (Alpha Imager, 1200, Alpha Innotech Corp., USA)

Data analysis

Data scoring

Clearly resolved, unambiguous polymorphic bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of a matrix with '1' and '0', which indicate the presence and absence of bands in each line respectively.

Cluster analysis

The binary data scored was used to construct a dendrogram. The genetic associations between varieties were evaluated, by calculating the Jaccards similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers (Jaccard, 1980), similarity matrix was generated using the NTSYS-PC software, version 2.02 (Rohlf, 2005). The similarity coefficients were used for cluster analysis and dendrogram was constructed by the Unweighted Pair– Group Method (UPGMA) (Sneath and Sokal, 1973).

Matrix comparison

The product-moment correlation (r) based on Mantel Z–value (Mantel, 1967) was computed to measure the degree of relationships between similarity index matrices produced by any two marker systems.

RESULTS

Genetic diversity analysis using molecular markers

In the present study, 27 regenerants along with their parents were evaluated for genetic diversity using RAPD markers and ISSR markers.

Isolation of genomic DNA

DNA was isolated from 15 days old seedlings following CTAB extraction method and the quantity and quality of DNA was tested by flourometry and agarose gel electrophoresis (0.8%), respectively to ensure the use of good quality DNA for the molecular marker studies.

Variability and polymorphism analysis

RAPD analysis

The details of markers amplified by 25 random primers among the 27 regenerants (A_0 generation) and their parents are given in Table 1. All the 25 random primers used in the study produced scorable, unambiguous markers (Fig. 1). The primers produced a total of 351 markers across 27 regenerants and their parents, out of which 285 were polymorphic. The number of markers produced by different primers ranged from 4 to 27 with an average 14.04 markers per primer. Among the primers used for RAPD analysis, the primer OPR02 gave the highest number of fragments (27), while the primer OPM 18 produced the lowest number of fragments (4). The total number of polymorphic markers was 285 and polymorphism percentage was 81.65.

ISSR analysis

The details of the amplified products by different ISSR primers are furnished in Table 2. All the 19 primers used in the study produced scorable, unambiguous markers (Fig. 2). The ISSR primers produced a total of 248 markers across 27 regenerants and their parents, out of which 201 were polymorphic. Among the primers used, the primer UBC 844 produced the highest number of fragments (23) followed by UBC 807, UBC 812 and UBC 864 producing 17 fragments, while the primer UBC 818 produced the lowest number of fragments (7).

Comparison of RAPD and ISSR analysis

A comparative account of RAPD and ISSR analysis is furnished in Table 3. Among the marker system used in the analysis, RAPD markers produced the highest number of markers (351). The average number of markers product per primer was 14.04 and 13.05 in RAPD and ISSR, respectively. The number of polymorphic markers produced was 285 and 201 for RAPD and ISSR which accounts 81.65 and 79.37 per cent, respectively.

Similarity index - RAPD

Similarity index values were obtained for each pair wise comparison among the 27 regenerants (A_0 generation) and their parents based as RAPD marker data. The similarity coefficients based on 351 RAPD markers ranged from 0.297 to 0.933. Among the 27 regenerants and their parents the highest similarity index (0.933) was observed between DHL 24 and DHL 23 and the lowest similarity index (0.297) was observed between DHL 8 and DHL 7.

Similarity index - ISSR

Similarity index values were obtained for each pair wise comparison among the 27 regenerants (A_0 generation) and their parents based as ISSR marker data. The similarity coefficients based on 248 ISSR markers ranged from 0.2818 to 0.9588. Among the 27 regenerants and their parents, the lowest similarity index (0.2818) was observed between the parent CO43 and DHL5 and the highest similarity index (0.9588) was observed between DHL 16 and DHL 10.

RAPD Cluster analysis

Cluster analysis was performed based on Jaccard's (Jaccard, 1980) similarity coefficient matrices, calculated from RAPD markers to generate a dendrogram of 27 regenerants along with their parents (Fig. 3). The similarity index values ranged from 0.48 to 0.93. The dendrogram separated 27 regenerants and their parents into four major clusters (Table 4). Hundred per cent similarity was found between DHL 23 and DHL 24 lines.

ISSR Cluster analysis

Cluster analysis was performed based on Jaccard's (Jaccard, 1908) similarity coefficients matrices calculated from ISSR markers to generate a dendrogram for 27 regenerants (A_0 generation) along with their parents (Fig. 4). The similarity coefficients ranged from 0.42 to 0.99. The dendrogram separated the 29 genotypes into three distinct clusters (Table 4).

Polymorphism information content (PIC)

The PIC values, a reflection of allele diversity and frequency among the regenerants were not uniform. The PIC values ranged from 0.702 to 0.952 with a mean PIC value of 0.887 for RAPD analysis (Table 5). In case of ISSR, analysis the mean PIC value was 0.891 and the highest and lowest PIC values were 0.940 and 0.832 respectively (Table 5). Comparison of different parameters among the two markers is given in Table 6.

Fig.1. RAPD marker profiles of 27 regenerants and their parents generated by the primer OPR2.

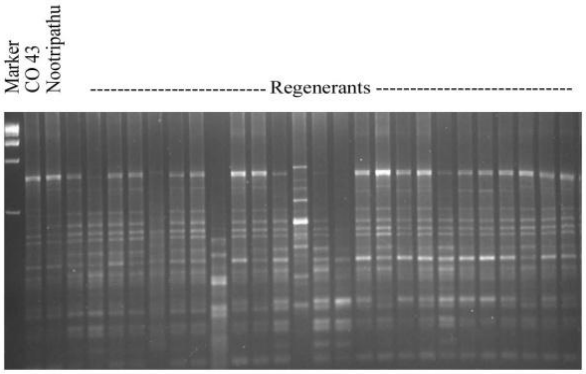


Fig.2. ISSR marker profiles of 27 regenerants and their parents generated by the primer UBC885.

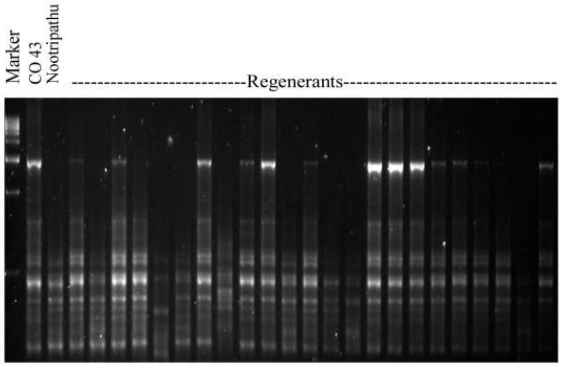
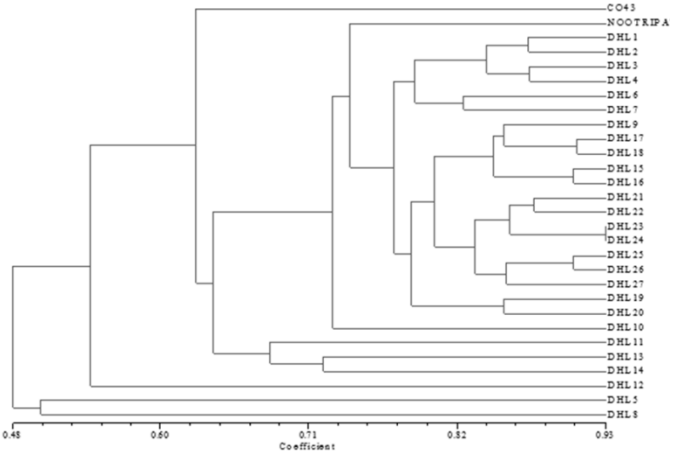


Fig. 3. Dendrogram based on 25 RAPD primers for the 27 regenerants.



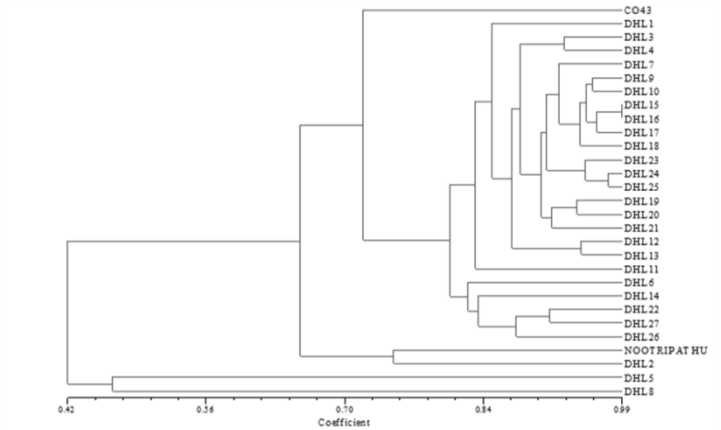


Fig. 4. Dendrogram based on 19 ISSR primers for the 27 regenerants.

Table 1. Details of RAPD markers produced across 27 regenerants (A_0 generation)

S.No.	Primer code	Sequence (5'-3')	Number of markers	Number of polymorphic markers	Number of monomorphic markers	Polymorphism (%)
1.	OPM11	GTCCACTGTG	11	07	04	63.63
2.	OPM12	GGGACGTTGG	9	7	2	77.77
3.	OPM14	AGGGTCGTTC	14	14	0	100
4	OPR02	CACAGCTGCC	27	22	5	81.48
5.	OPM18	CACCATCCGT	4	4	0	100
6.	OPM19	CCTTCAGGCA	13	9	4	69.23
7.	OPM20	AGGTCTTGGG	11	7	4	63.63
8.	OPR01	TGCGGGTCCT	13	13	0	100
9.	OPM16	GTAACCAGCC	14	12	2	85.71
10.	OPR05	GACCTAGTGG	12	8	4	66.66
11.	OPR07	ACTGGCCTGA	9	8	1	88.89
12.	OPR08	CCCGTTGCCT	8	8	0	100
13.	OPR09	TGAGCACGAG	11	5	6	45.45
14.	OPR10	CCATTCCCCA	13	11	2	84.61
15.	OPR11	GTAGCCGTCT	15	11	4	73.33
16.	OPR12	ACAGGTGCGT	17	12	5	70.59
17.	OPR13	GGACGACAAG	16	16	0	100
18.	OPR14	CAGGATTCCC	22	15	7	68.18
19.	OPR15	GGACAACGAG	15	13	2	86.67
20.	OPR16	CTCTGCGCGT	20	18	2	90
21.	OPAL11	GTCACGTCCT	16	13	3	81.25
22.	OPAL12	CCCAGGCTAC	17	17	0	100
23.	OPAL13	GAATGGCACC	22	20	2	90.90
24.	OPAL14	TCGCTCCCGTT	15	8	7	53.33
25.	OPAL17	CCGCAAGTGT	7	7	0	100
	Total		351	285	66	81.65

Table 2. Details of ISSR markers produced 27 regenerants (A_0 generation)

Primer code	Sequence (5'-3')	Number of markers	Number of polymorphic markers	Number of monomorphic markers	Polymorphism (%)
UBC 807	AGAGAGAGAGAGAGAGT	17	12	5	70.59
UBC 809	AGAGAGAGAGAGAGAGG	9	6	3	66.67
UBC 810	GAGAGAGAGAGAGAGAT	15	14	1	93.33
UBC 811	GAGAGAGAGAGAGAGAC	10	9	1	90.00
UBC 812	GAGAGAGAGAGAGAGAA	17	14	3	82.35
UBC 814	CTCTCTCTCTCTCTCTA	8	6	2	75.00
UBC 816	CACACACACACACACAT	10	8	2	80.00
UBC 818	CACACACA CACACACAG	7	3	4	42.86
UBC 820	GTGTGTGTGTGTGTGTC	15	13	2	86.67
UBC 826	ACACACACACACACACC	13	9	4	69.23
UBC 834	AGAGAGAGAGAGAGAGYT	16	15	1	93.75
UBC 840	GAGAGAGAGAGAGAGAYT	10	8	2	80.00
UBC 841	GAGAGAGAGAGAGAAYC	10	8	2	80.00
UBC 844	CTCTCTCTCTCTCTCTRC	23	20	3	86.96
UBC 856	ACACACACACACACACYA	11	9	2	81.81
UBC 857	ACACACACACACACACYG	14	12	2	85.71
UBC 864	ATGATGATGATGATGATG	17	14	3	82.35
UBC 880	GGAGAGGAGAGGAGA	14	12	2	85.71
UBC 885	HBHAGAGAGAGAGAGAG	12	9	3	75.00
Total		248	201	47	79.37

Single letter abbreviations for mixed base positions

R = (A, G)

Y = (C, T) W = (A,T) (Weak [2 H-bonds[)

N = (A,G,C,T) S = (G,C) (Strong [3 H-bonds])

R = (A,G) M = (A,C) (Amino in large groove)

Y = (C,T) K = (G,T) (Keto in large groove)

B = (C,G,T) (i.e. not A) V = (A,C,G) (i.e. not T) D = (A,G,T) (i.e. not C) H = (A,C,T) (i.e. not G)

Table 3. Level of polymorphism detected by RAPD and ISSR analysis across 29 genotypes

S.No	Parameters	Types of markers	
		RAPD	ISSR
1.	Genotypes screened	29	29
2.	Number of primers used	25	19
3.	Total number of markers	351	248
4.	Range of markers	4-27	7-23
5.	Average number of markers	14.04	13.05
6.	Number of monomorphic markers	66	47
7.	Number of polymorphic markers	285	201
8.	Percentage of polymorphism	81.65	79.37

Table 4. List of different clusters and the regenerants that fall under each cluster for RAPD and ISSR markers

Cluster numbers	No. of lines	List of lines included
RAPD		
I	1	CO43
II	25	NOOTRIPATHU, DHL 1, DHL 2, DHL 3, DHL 4, DHL 6, DHL 7, DHL 9, DHL 17, DHL 18, DHL 15, DHL 16, DHL 21, DHL 22, DHL 23, DHL 24, DHL 25, DHL 26, DHL 27, DHL 19, DHL 20, DHL 10, DHL 11, DHL 13, DHL 14
III	1	DHL 12
IV	2	DHL 5, DHL 8
ISSR		
I	25	CO43, DHL 1, DHL 3, DHL 4, DHL 7, DHL 9, DHL 10, DHL 15, DHL 16, DHL 17, DHL 18, DHL 23, DHL 24, DHL 25, DHL 19, DHL 20, DHL 21, DHL 12, DHL 13, DHL 11, DHL 6, DHL 14, DHL 22, DHL 27, DHL 26
II	2	NOOTRIPATHU, DHL 2
III	2	DHL 5, DHL 8

Table 5. PIC values of RAPD and ISSR primers

S.No	RAPD Primer	PIC value (RAPD)	ISSR Primer	PIC value (ISSR)
1.	OPM11	0.9026	UBC 807	0.911
2.	OPM12	0.813	UBC 809	0.834
3.	OPM14	0.895	UBC 810	0.898
4.	OPR02	0.952	UBC 811	0.878
5.	OPM18	0.702	UBC 812	0.905
6.	OPM19	0.922	UBC 814	0.832
7.	OPM20	0.875	UBC 816	0.876
8.	OPR01	0.911	UBC 818	0.840
9.	OPM16	0.903	UBC 820	0.913
10.	OPR05	0.897	UBC 826	0.916
11.	OPR07	0.857	UBC 834	0.931
12.	OPR08	0.839	UBC 840	0.889
13.	OPR09	0.890	UBC 841	0.888
14.	OPR10	0.917	UBC 844	0.940
15.	OPR11	0.906	UBC 856	0.893
16.	OPR12	0.919	UBC 857	0.905
17.	OPR13	0.915	UBC 864	0.918
18.	OPR14	0.933	UBC 880	0.882
19.	OPR15	0.907	UBC 885	0.888
20.	OPR16	0.936	**Mean**	**0.881**
21.	OPAL11	0.893		
22.	OPAL12	0.879		
23.	OPAL13	0.910		
24.	OPAL14	0.904		
25.	OPAL17	0.802		
	Mean	**0.887**		

Table 6. Comparison of different parameters among the two markers

Marker system	Mean number of markers	Level of polymorphism %	Mean PIC values	Number of clusters
RAPD (25)	14.04	285	0.887	4
ISSR (19)	13.05	201	0.891	3

DISCUSSION

Biotechnological tools complement breeding programmes in many ways, one of which is to be able to identify target genes (or mapped gene of agronomic importance) with the assistance of DNA markers, a process called marker assisted selection or MAS (Zheng et al., 1995). Anther culturability is a quantitative trait controlled by nuclear-encoded genes (Yamagishi et al., 1998). However, earlier genetic studies on haploidy merely determined whether there are differences in response among varieties, and whether the traits such as callus induction and plant regeneration are heritable. With the development of MAS system, these characteristics can now be detected at the molecular level.

In the present study, an attempt has been made to survey the 27 regenerants (A_0 generation) from the F_1 hybrid CO43/Nootripathu with 25 RAPD and 19 ISSR primers to generate marker profiles. The number of RAPD markers and ISSR markers ranged from 4-27 and 7-23 respectively. The number of RAPD markers (14.04 per primer) generated was more than the number of ISSR markers (13.05 per primer). The level of polymorphism was found to be more with RAPD (81.65%) than ISSR markers (79.37%).

Among the RAPD primers used, primer OPR02 produced a maximum of 27 markers each. Out of 19 primers used for generating ISSR markers a total of 7 primers produced more than 15 markers per primer. The percentage polymorphism obtained from RAPD primers was 81.65 % and from ISSR it was 79.37 %. Polymorphism information content (PIC) value of each marker is a measure of marker diversity. PIC also provides the estimate of discriminatory power of a locus by taking into account not only the number of alleles expressed, but also the relative frequency of those alleles. The average PIC value for RAPD markers was 0.887 and for ISSR it was 0.881.The high levels of polymorphism for RAPD markers were observed in rice by many workers involving DH lines (Russell et al., 1997). Vijay (2004, Tamilnadu Agricultural University, India, M.Sc., Thesis) used three different marker systems to survey a set 49 rice genotypes and found that the average polymorphism information content (PIC) value was low for ISSR markers (0.289) when compared to SSR (0.388) and AFLP (0.396). This contradictory situation of obtaining a higher PIC values in the present study may be attributed to the wide diversity among the regenerants (A_0 generation) and their parents.

Cluster analysis was carried out on two sets of marker profiling data based on RAPD and ISSR. The results based on the two DNA marker profiles revealed the grouping of 27 regenerants varied from each other. Using the RAPD marker profiles resulted in 4 clusters whereas ISSR markers grouped the DH lines into 3 clusters. In all the cases, parents of DH lines, CO43 and Nootripathu remained outside the purview of all the clusters indicating their divergence. The use of RAPD and ISSR markers resulted in the detection of 0.66 and 0.86 similarity coefficients between CO43 and Nootripathu respectively indicating the better efficiency of differentiating the materials because of their higher level of polymorphism percentage.

Molecular markers represent a sample of a plant genome, yet they are used to infer relationships of the entire genome among set of line or population. The distribution of the loci detected by individual methods will affect the precision of the resulting estimates of genetic distance (Nei, 1987). For estimation of genetic diversity it is ideal that the loci detected are randomly dispersed and sample the whole genome. The comparison of distributions of the different individual marker systems need to be based on the genetic map, but this is very time consuming (Lu et al., 1996). An alternative approach has been proposed to estimate the relationships among the genetic diversity derived from several marker techniques by using statistic such as Mantel's test.

In the present investigation, the correspondence between the similarity matrices generated by the two marker systems was evaluated by calculating product-moment correlation (Mantel's test). There was no close correspondence between the similarity matrices derived from RAPD and ISSR marker profiles. There was very low correlation indicating non-congruence between the clustering established by the two marker systems. The same trend was observed by Manifesto et al (2001) between two marker systems viz., AFLP and SSR (r=0.27) in wheat. Meenakshisundaram and Maheswaran (2008) found low correlation based on Mantel's test involving RAPD and ISSR marker systems in *Phyllanthus*.

The present study has been attempted to establish the differences among 27 regenerants generated from CO43/Nootripathu using molecular markers. The existence of variation in anther culture derived plants at molecular level was established in crops like wheat (Brown, 1991; Guzy-Wrobelska et al., 2007), maize (Belicuas et al., 2007) and rice (Sathish et al., 1995; Godwin et al., 1997; Afza et al., 2001). Associating variation at molecular level with phenotype level will facilitate easy discrimination of regenerants having the features of both the parents. Identifying and using suitable markers associated with positive attributes of parental combinations will help to sort out superior regenerants possessing fixed-heterosis for major agronomic traits. For this purpose, the DNA marker systems used in the present study could be easy tools since developing DNA markers using these systems do not need prior sequence information of the genome. Further, these marker systems are simple to use and cost effective with less technicalities making them amenable for use in any breeding programme.

In conclusion, it could be clearly stated that RAPD markers proved to be versatile in discriminating the 27 lines derived from anther culture, when compared to ISSR markers. Extreme types with wide variations were encountered which reaffirms the possibility of employing anther culture in rice improvement by restoring yield advantage under water stress conditions in advanced generations.

Acknowledgements

The award of Senior Research Associateship, from Council of Scientific and Industrial Research (CSIR), New Delhi, India, to T. Hemalatha is gratefully acknowledged.

References

Afza, R., Xie, J., Shen, M., Zapata-Arias, F.J., Fundi, H.K., Lee, K.S., Bobadilla-Mucino, E. and Kodym, A. (2001): Detection of androclonal variation in anther-cultured rice lines using rapds. In Vitro Cell. Develop. Biol. - Plant. 37: 644-647.

Aleijar, M.S., Zapata, F.J., Senadhira, D., Khush, G.S. and Datta, S.K. (1995): Utilization of anther culture as a breeding tool in rice improvement. In: Terzi M, Cella R, Falavigna A (eds) Current Issues in Plant Molecular and Cellular Biology. Kluwer Academic Pub. Netherlands: pp. 137-142.

Arumuganathan, K. and Earle, E.D. (1991): Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep., 9: 208-218.

Belicuas, P.R., Guimaraes, C.T. , Paiva, L.V. , Duarte, J.M. , Maluf, W.R. and Paiva, E. (2007): Androgenetic haploids and SSR markers as tools for the development of tropical maize hybrids. Euphytica 156: 95-102.

Brown, P.T.H. (1991): The spectrum of molecular changes associated with somaclonal variation. IAPTC News Lett., 66: 14-25.

Dewi, I.S. and Purwoko, B.S. (2001): Kultur antera untuk mendukung program pemuliaan tanaman padi. Bull. Agron., 29: 59-63.

Forster, B.P. and Thomas, W.T.B. (2010): Doubled Haploids in Genetics and Plant Breeding. In: Janick J, (ed) Plant Breeding Reviews, vol. 25. John Wiley & Sons, Inc., Oxford, UK.

Godwin, I.D., Sangduen, N., Kunanuvatchaidach, R., Piperidis, G. and Adkins S.W. (1997): RAPD polymorphisms among variant and phenotypically normal rice (*Oryza sativa* var.*indica*) somaclonal progenies. Plant Cell Rep., 16: 320-324.

Gravel, N.J. and Jarret, R.L. (1991): A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea* plant. Mol. Biol. Rep., 9: 262-266.

Guzy-Wrobelska, J., Labocha-Pawlowska, A., Kwasniewski, M. and Szarejko, I. (2007): Different recombination frequencies in wheat doubled haploid populations obtained through maize pollination and anther culture. Euphytica 156: 173-183.

Jaccard, P. (1980) Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat., 44: 223-270.

Li, Z.K., Yu, S.B., Lafitte, H.R., Huang, N., Courtois, B., Hittalmani, S., Vijayakumar, C.H.M., Liu, G.F., Wang, G.C., Shashidhar, H.E., Zhuang, J.Y., Zheng, K.L., Singh, V.P., Sidhu, J.S., Srivantaneeyakul S. and Khush. G.S. (2005): QTL environment interactions in rice. I. Heading date and plant height. Theor. Appl. Genet., 108: 141-153.

Lu, J., Knox, M.R., Ambrose, M.J., Brown, J.K.M. and Ellis, T.H.N. (1996): Comparative analysis of genetic diversity in pea assessed by RFLP- and PCR-based methods. Theor. Appl. Genet., 93: 1103-1111.

Manifesto, M.M., Schlatter, A.R., Hopp, H.E., Suarez, E.Y. and Dubcovsky, J. (2001): Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci., 41: 682-690.

Mantel, N.A. (1967): The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209-220.

Meenakshisundaram, P. and Maheswaran, M. (2008): RAPD and ISSR analyses reveal low levels of genetic diversity in *Phyllanthus amarus.* Acta Hort., 765: 179 – 188.

Nei, M. (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.

Pandey, S., Bhandari, H. and Hardy, B. (2007): Economic costs of drought and rice farmers' coping mechanisms: a cross-country comparative analysis. International Rice Research Institute, Manila, pp. 203.

Premvaranon, P., Vearasilp, S., Thanapornpoonpong, S., Karladee, D. and Gorinstein, S. (2011): *In vitro* studies to produce double haploid in Indica hybrid rice. Biologia. 66: 1074-1081.

Raina, S.K. (1989): Tissue culture in rice improvement: status and potential. Adv. Agron., 42: 339-397.

Rohlf, F.J. (2005) NTSYS-PC. Numerical taxonomy and multivariate analysis system version 2.0. Department of Ecology and Evolution. State University of New York.

Russell, J.R., Fuller, J.D., Macaulay, M., Hatz, B.G., Jahoor, A., Powell, W. and Waugh, R. (1997): Direct comparison of levels of genetic variation among barley accessions detected by of RFLPs, AFLPs, SSRs and RAPDs. Theor. Appl. Genet., 95: 714-722.

Sathish, P., Gamborg, O.L. and Nabors, M.W. (1995): Rice anther culture: callus initiation and androclonal variation in progenies of regenerated plants. Plant Cell Rep. 14, 432-436.

Sneath, P.H.A. and Sokal, R.R. (1973): Numerical taxonomy. Freeman, San Francisco.

Steponkus, P.L., Cutler, J.M. and O'Toole, J.C. (1980): Adaptation to water stress in rice. In: Turner, NC, Kramer PJ (eds) Adaptation of plants to water and high temperature stress. Wiley-Interscience: New York, pp. 231–254.

Suresh, R., Chandra, B.R., Michael, G.S. and Shanmugasundaram, P. (2013): Genetic analysis of yield traits in rice under irrigated and water stress environments. Indian J. Genet. Plant Breed., 73: 162-168.

Thanh, N.D., Thanh, N.D., Zheng, H.G., Dong, N.V., Trinh, L.N., Ali, M.L. and H.T. Nguyen. (1999): Genetic variation in root morphology and microsatellite DNA loci in upland rice (*Oryza sativa* L.) from Vietnam. Euphytica 105: 53-62.

Yamagishi, M., Otani, M., Higashi, M., Fukuta, Y., Fukui, K., Yano, M. and Shimada, T. (1998): Chromosomal regions controlling anther culturability in rice (*Oryza sativa* L.). Euphytica 103: 227-234.

Zeng, D.L., Guo, L.B., Xu, Y.B., Yasukumi, K., Zhu, L.H. and Qian, Q. (2006): QTL analysis of seed storability in rice. Plant Breed., 125: 57-60.

Zheng, K., Huang, N., Bennett, J. and Khush, G.S. (1995): PCR-based marker- assisted selection in rice breeding. IRRI Discussion Paper Series, No. 12. International Rice Research Institute, Philippines, pp. 24.

****