International Journal of Plant & Soil Science

Volume 35, Issue 20, Page 284-301, 2023; Article no.IJPSS.104839
ISSN: 2320-7035

Molecular Characterization of Rice Genotypes Using Molecular Markers

Thete A. M. [a], Dahat D. V. [a], Jambhale V. M. [b] and Chaudhari S. R. [a*]

[a] *Department of Genetics and Plant Breeding, College of Agriculture, Pune, MS, India.*
[b] *Department of Agricultural Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri, MS, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i203809

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/104839

Original Research Article

Received: 22/06/2023
Accepted: 29/08/2023
Published: 20/09/2023

ABSTRACT

Rice, (*Oryza sativa* L. 2n=24) belongs to the family Gramineae and subfamily Oryzoideae. *Oryza* has two cultivated species: *Oryza sativa* and *Oryza glaberrima*. Breeding for good-quality traits requires the selection of parents with a wider genetic diversity. Molecular markers are used in molecular biology and biotechnology to identify a sequence of DNA. Molecular markers have played an increasing role in rice breeding for cultivar improvement, screening, selection, and germplasm collections. The present investigation is undertaken to study the genetic diversity among thirty rice genotypes using the ISSR marker.

Out of 40 ISSR primers, 9 amplified and showed polymorphism, viz., ISSR 807, ISSR 808, ISSR 809, ISSR 811, ISSR 816, ISSR 823, ISSR 826, ISSR 827 and ISSR 829. A total of 76 loci were generated by amplification with 9 polymorphic primers, out of which 66 loci were polymorphic with an average of 86.84 percent polymorphism. Among ISSR primers, ISSR 807 produced the maximum number of 11 loci. A dendrogram constructed using NTSYSpc 2.02i software grouped all 30 genotypes into two major clusters (clusters A and B). Cluster A Kalbhat alone is present in one sub-cluster at 69.2% similarity with Karjat 2, Jaya, Sairam, Saubhagya Dhan, RPBIO 226, Karjat 6, Ratnagiri 1, DRR Dhan 44, DRR Dhan 45, DRR Dhan 46, MTU 10010, Karjat 7, Swarna Shreya,

---

**Corresponding author: E-mail: snehalchaudhari737@gmail.com;*

Sugandha, and Madhumati. In Cluster B Karjat 3 alone forms one sub- cluster and has 70 % similarity with MTU 1001, PKVHMT, JGL 1798, Indrayani, Phule Samruddhi, Phule Maval, Sonsal, Pavana, DRR Dhan 41, kundalika, Bhogawati, Ambemohar, and Phule Radha.

Keywords: Rice; molecular markers; PCR; ISSR; genetic diversity.

1. INTRODUCTION

Rice, (*Oryza sativa* L. 2n=24) belongs to the family Gramineae and subfamily Oryzoideae. *Oryza* has two cultivated species, *Oryza sativa* and *Oryza glaberrima*. Rice (*Oryza sativa* L.) is a true diploid (2n=24) with twelve chromosome pairs and contains 5.8 x 10^5 kb/haploid genome (Bonnet and Smith, 1976). There is ample polymorphism in rice DNA and it is highly recombinogenic compared to other plants. Moreover, rice is also an ideal model plant for the study of grass genetics and genome organization due to its diploid genetics. It is the staple food for more than half of the world's population and occupies almost one-fifth of the total land area covered under cereals It is an important food crop in the world both in terms of area (163.43 million ha) and production (498.95 million metric tons). In India, rice contributes to about 45 percent of cereal production and is the main food source for more than 60 percent of the population. We will have to produce 40 percent more rice by the year 2030 to satisfy the growing demand without adversely affecting the resource base [1-3].

The recent development of DNA markers has provided new opportunities for the genetic improvement of rice grain quality. Genetic diversity shows Genetic divergence among the genotypes plays an important role in the selection of parents with wider variability for different characters. Genetic divergence analysis quantifies the genetic distance among the selected genotypes. For the assessment of genetic diversity, molecular markers have been generally superior to morphological and biochemical data [4-7].

Molecular markers provide reliable estimates of genetic diversity, may improve screening efficiency for many traits through their linkage, having alleles with small (quantitative traits) and large (qualitative traits) effects and will provide the first understanding of the biology and architecture of quantitative traits at the DNA level. Molecular markers have played an increasing role in rice breeding for cultivar improvement, screening, selection. Molecular marker based genetic diversity analysis also has the potential to assess changes in genetic diversity over time and space. Among various PCR- based markers, SSR and ISSR markers are more popular in rice because they are highly informative, mostly monolocus, SSR marker is codominant, easily analyzed and cost-effective. Inter simple sequence repeats (ISSR), which involve PCR amplification of DNA using a single primer composed of a microsatellite sequence anchored at the 3" or 5" end by 2-4 arbitrary nucleotides, is one of the DNA based molecular markers that could be used to assess genetic diversity [8]. It has been successfully employed to assess genetic diversity within and between populations in several plant species (Liu and Wendell, 2001). The molecular characterization and fingerprinting of these released varieties using ISSR markers will provide sufficient knowledge on diversity among them at the molecular level, which will help the breeders to develop strategies for the future, and the variety-specific fingerprints will enable them to identify and characterize each variety released. The present-day gene pool accommodates high yielding rice varieties every year which are evolved through breeding programme continuously which result in difficulty in identification and cataloguing. Molecular markers are able to reveal variation present between them at DNA level (Ni et al. 2002, Chakravarthi and Naravaneni, 2006). These DNA-based markers are highly precise, dependable and not prone to environmental conditions and fluctuations [9-13].

Thus, the present investigation is undertaken to analyze genetic diversity among different 30 rice genotypes using ISSR markers.

2. MATERIALS AND METHODS

The current study on “Molecular characterization of rice genotypes using molecular markers” was carried out at the Division of Botany, Biotechnology laboratory, College of Agriculture, Pune.

2.1 Plant Materials

Thirty high-yielding rice varieties obtained from the Agricultural Research Station, Maval, Pune, were used in this study (Table 1).

Table 1. List of selected rice genotypes and their features

Sr. No.	Genotype Name	Characteristic Features
1.	Karjat – 2	Dwarf stature, long slender grains, moderately resistant to blast and neck blast, recommended for rainfed region.
2.	MTU-1010	Short slender, semi- dwarf (108 cm), resistant to blast & tolerant to Brown Plant Hopper.
3.	Karjat – 3	Dwarf stature, short bold grain, resistant to blast suitable for rainfed uplands as well as irrigated areas for *kharif* and *rabi* season, Grains: short bold and scented.
4.	Sairam	Short slender grain, early maturity.
5.	Swarna Shreya	Long slender, suitable for rainfed low land and direct seeded aerobic condition with maturity period of 120-125 days, can withstand drought and also tolerance to many diseases and insects.
6.	Sugandha	Tall (130-140 cm), grains: Medium Slender with aroma, white, moderately resistant to BLB and Pest complex.
7.	Madhumati	Scented, long slender, late maturity.
8.	Karjat – 7	Dwarf stature, long slender grain, early maturity, moderately resistant to brown plant hopper and bacterial leaf blast.
9.	Karjat – 6	Short slender grain, resistant to bacterial leaf blast & brown plant hopper, suitable for midland under rainfed & irrigated conditions.
10.	Ratnagiri-1	Long bold grain, Semi dwarf (100-105 cm), early maturity, moderately resistant to blast and neck blast, grains: long bold, moderately susceptible to BLB.
11.	DRR Dhan-44	Short duration, medium slender, varieties with moderate tolerance to biotic (BPH and blast) and abiotic stress (drought).
12.	DRR Dhan-45	Mid early duration culture (~130 days) with long slender grains, high zinc content (18.18 ppm) with high yield potential.
13.	DRR Dhan-46	Long slender, mid late, high zinc with high yield potential and moderate tolerance to Brown Plant Hopper and blast and drought.
14.	Saubhagya Dhan	Photo insensitive and resistance to lodging long slender
15.	Jaya	Dwarf (82 cm), grains: long bold, white, moderately susceptible to bacterial blight, Brown Plant Hopper, and resistant to blast
16.	Kalbhat	Slender, scented, high Fe Content, mid late crop, grow well on all kinds of soil and can withstand vagaries of rainfall.
17.	MTU-1001	Grain type: Medium slender, special features: semi dwarf (115 cm), tolerant to BPH & blast
18.	RP-BIO-226	Needs assured irrigation, 135-140 days, resistance to bacterial blight, excellent grain and cooking quality features, bacterial blight resistant, high yielding and fine grain variety of rice.
19.	PKV-HMT	Short slender grain, early maturity, moderately resistant to leaf blight and blast
20.	JGL-1798	Grain Type – Medium, slender, Straw glume, days to maturity - 125-130, special features - resistant to gall midge biotype 2 and 3, tolerant to blast and BLB, susceptible to cold
21.	Indrayani	Semi dwarf, mid late, grains: Long slender, scented, moderately susceptible to BLB & leaf scald, resistant to blast and major pests.
22.	Kundalika	Early maturity, Grains: short slender, resistant to blast & major pest.
23.	Phule Samruddhi	Long slender grain, high yield, mid late, moderately resistant to blight and blast.
24.	Ambemohar	Short, bold, scented and late maturity.
25.	Phule Radha	Early maturity, grains: short slender; resistant to Bacterial Leaf Blight, blast and scald.
26.	Sonsal	Semi dwarf, grains: superfine, slender, mid late.

Sr. No.	Genotype Name	Characteristic Features
27.	Phule Maval	Long bold quality, mid late.
28.	Pavana	Early maturity, semi dwarf, grains: long slender, scented, resistant to blast and moderately resistant to leaf scald, tolerant to major pests, awned.
29.	DRR Dhan-41	High yield, mid late, long slender.
30.	Bhogawati	Mid late, long slender, scented, height 85-90 cm, resistant to blast, moderately resistant to leaf scald.

2.2 Growing of Seedlings

The seeds of thirty rice genotypes were grown in the tray at College of Agricultural, Pune. The leaves were cut and taken when the seedlings were of 20 days old for DNA extraction.

2.3 Methods

2.3.1 Isolation and purification of genomic DNA

The genomic DNA was isolated from different rice genotypes using the CTAB extraction method suggested by Doyle and Doyle [14] with slight modifications. A 2-gm leaves sample of rice varieties was crushed in a mortar and pestle using liquid nitrogen. After crushing, 1ml of CTAB extraction buffer is added to the crushed sample and mixed properly in the centrifuge tube. Then the tubes were incubated at 65ºC for 45 minutes in a water bath with occasional mixing. Chloroform and Isoamyl alcohol (24:1) were added in equal volume and mixed by gentle inversion for 5-6 times and these tubes were centrifuged at 12000 rpm for 10 minutes in a centrifuge machine. The contents were separated into two distinct phases. The aqueous phase containing DNA was pipetted out, an equal volume of chilled isopropanol and 100 µl 3M sodium acetate was added to it, and it was incubated at -20 0C for overnight. On the next day, the solution was centrifuged at 12000 rpm for 10 minutes. The pellet obtained was washed with 100 µl of 70 percent ethanol and centrifuged at 10000 rpm for 10 minutes. Pellets were air-dried and dissolved in 50 µl double distilled water. This DNA was stored at -20°C for further use.

The purification of DNA samples was done to remove RNA, proteins, and Polysaccharides, which were the major contaminants. RNA and proteins were removed with RNase A and Proteinase K treatment, respectively. 1µl of RNase A and Proteinase K were added to the DNA sample and incubated at 37°C for 20 min. after each addition. The PVP used during extraction helps to eliminate polysaccharides.

2.4 DNA Quality Checking

The quality of the DNA in the sample was determined by agarose gel electrophoresis with isolated DNA on a 0.8 percent agarose gel. The agarose gel electrophoresis unit was cleaned properly with 70% ethanol before use. Agarose gel was prepared by dissolving 0.8 g of agarose in 100 ml of 1X TBE buffer and heated it in a microwave oven. 7 µl of ethidium bromide was added to it after cooling down to 45 to 50ºC. The gel was poured into a casting tray in which a comb had been inserted previously and kept for 30 minutes. After the gel solidified, the comb was removed. 2 µl of DNA was mixed with 1µl of 6X gel loading dye and loaded on the gel. The electrophoresis was carried out at 3 to 5 volts per cm of run using 1X TBE buffer. The gel was then visualised under the gel documentation unit to check the results.

Table 2. Details components CTAB buffer

Sr. No.	Components	Stock concentration	Working concentration	Volume for 100 ml
1	Tris HCl buffer	1M	100 mM	12.11 ml
2	NaCl	4M	1.4 M	23.37 ml
3	EDTA	0.5M	20 Mm	14.61 ml
4	CTAB (percent)	10	2	10 ml
5	Bmercaptoethanol	4%	0.2	4 gm
6	PVP	10%	1	1 gm
7	Sterile double distilled water			
	Total			**100 ml**

2.5 DNA Quantification and Purity Analysis

The quantification of isolated DNA was done by measuring absorbance at 260nm wavelength and purity was checked by taking the ratio of absorbance (A260/A280). Detailed absorbance and purity are given in (Table 3). The A260/A280 ratio was around 1.85 which indicated the purity of the genomic DNA obtained using modified CTAB method.

2.6 Molecular Marker Assay

Diversity analysis of rice was carried out using the Inter simple Sequence Repeats (ISSR) marker. This was carried out by amplifying the DNA using one primer design to be complementary to the SSR regions, the amplification could occur in the region between two SSR's, detecting polymorphisms in the amplification pattern. The good-quality genomic DNA isolated from leaf samples of the selected genotypes was subjected to an ISSR assay as per the procedure reported by Zietkiewicz et al. [15]. A total of nine ISSR primers with good resolving power were selected after an initial forty primers screening listed in (Table 4).

2.7 DNA Amplification by PCR Technique

The PCR reaction was set for the 20 µl reaction volume given in (Table 5). A master mix was prepared for each primer with the above reagents and divided into different PCR tubes.2 µl of different genomic DNA samples was added to master mix which led to a final quantity of 20 µl. PCR tubes were placed in a thermal cycler for amplification of the genomic DNA.

Table 3. DNA quality assay of taken genotypes

Sr. No.	Sample name	OD at 260 (nm)	OD at 280 (nm)	Ratio of 260/280	Concentration (µg/ul)
1.	Karjat – 2	0.495	0.275	1.80	24.75
2.	MTU-1010	0.393	0.256	1.54	19.65
3.	Karjat – 3	0.386	0.196	1.97	19.3
4.	Sairam	0.405	0.223	1.82	20.25
5.	Swarna Shreya	0.401	0.221	1.81	20.05
6.	Sugandha	0.393	0.215	1.83	19.65
7.	Madhumati	0.395	0.210	1.88	19.75
8.	Karjat – 7	0.414	0.233	1.78	20.7
9.	Karjat – 6	0.405	0.216	1.88	20.25
10.	Ratnagiri-1	0.413	0.226	1.83	20.65
11.	DRR Dhan-44	0.415	0.238	1.74	20.75
12.	DRR Dhan-45	0.414	0.239	1.73	20.7
13.	DRR Dhan-46	0.413	0.219	1.89	20.65
14.	Saubhagya Dhan	0.414	0.214	1.93	20.7
15.	Jaya	0.416	0.224	1.86	20.8
16.	Kalbhat	0.396	0.229	1.73	19.8
17.	MTU-1001	0.413	0.219	1.89	20.65
18.	RP-BIO-226	0.402	0.225	1.79	20.1
19.	PKV-HMT	0.42	0.236	1.78	21
20.	JGL-1798	0.417	0.249	1.67	20.85
21.	Indrayani	0.398	0.219	1.82	19.9
22.	Kundalika	0.406	0.217	1.87	20.3
23.	Phule Samruddhi	0.432	0.236	1.83	21.6
24.	Ambemohar	0.412	0.218	1.89	20.6
25.	Phule Radha	0.401	0.209	1.92	20.05
26.	Sonsal	0.435	0.239	1.82	21.75
27.	Phule Maval	0.437	0.246	1.78	21.85
28.	Pavana	0.419	0.220	1.90	20.95
29.	DRR Dhan-41	0.417	0.234	1.78	20.85
30.	Bhogawati	0.418	0.231	1.81	20.9

Table 4. Details of primers selected for ISSR assay

Sr. No	Primer	Nucleotide sequence (5'–3')	Sr. No	Primer	Nucleotide sequence (5'–3')
1	UBC801	ATATATATATATATATT	21	UBC821	GTGTGTGTGTGTGTGTT
2	UBC802	ATATATATATATATATG	22	UBC822	TCTCTCTCTCTCTCTCA
3	UBC803	ATATATATATATATATC	23	UBC823	TCTCTCTCTCTCTCTCC
4	UBC804	TATATATATATATATAA	24	UBC824	TCTCTCTCTCTCTCTCG
5	UBC805	TATATATATATATATAC	25	UBC825	ACACACACACACACACT
6	UBC806	TATATATATATATATAG	26	UBC826	ACACACACACACACACC
7	UBC807	AGAGAGAGAGAGAGAGT	27	UBC827	ACACACACACACACACG
8	UBC808	AGAGAGAGAGAGAGAGC	28	UBC828	TGTGTGTGTGTGTGTGA
9	UBC809	AGAGAGAGAGAGAGAGG	29	UBC829	TGTGTGTGTGTGTGTGC
10	UBC810	GAGAGAGAGAGAGAGAT	30	UBC830	TGTGTGTGTGTGTGTGG
11	UBC811	GAGAGAGAGAGAGAGAC	31	UBC831	ATATATATATATATATYA
12	UBC812	GAGAGAGAGAGAGAGAA	32	UBC832	ATATATATATATATATYC
13	UBC813	CTCTCTCTCTCTCTCTT	33	UBC833	ATATATATATATATATYG
14	UBC814	CTCTCTCTCTCTCTCTA	34	UBC834	AGAGAGAGAGAGAGAGYT
15	UBC815	CTCTCTCTCTCTCTCTG	35	UBC835	AGAGAGAGAGAGAGAGYC
16	UBC816	CACACACACACACACAT	36	UBC836	AGAGAGAGAGAGAGAGYA
17	UBC817	CACACACACACACACAA	37	UBC837	TATATATATATATATART
18	UBC818	CACACACACACACACAG	38	UBC838	TATATATATATATATARC
19	UBC819	GTGTGTGTGTGTGTGTA	39	UBC839	TATATATATATATATARG
20	UBC820	GTGTGTGTGTGTGTGTC	40	UBC840	GAGAGAGAGAGAGAGAYT

Note-Single letter abbreviations for mixed base positions R=(A,G);Y=(C,T).

Table 5. Components of PCR reaction mixture

Sr. No.	Components	Stock concentration	Working concentration	Volume for one tube
1	10X PCR buffer	10.0 X	1.00 X	2.0 µl
2	MgCl2	25.0 mM	1.50 mM	1.2 µl
3	dNTPs	10.0 mM	200 µl	0.4 µl
4	Taq DNA polymerase	3.0 U/µl	1.00 U	1.0 µl
5	Primer	100 pmol/µl	10.0 pmol/µl	1.0 µl
6	Genomic DNA	30.0 ng/µl	60.0 ng	2.0 µl
7	Sterile double distilled water			12.4 µl
	Total			**20.0 µl**

2.8 PCR Reaction for ISSR Primers

The PCR amplification was done by using the 20µl PCR reaction. Annealing temperature for each of the primer pair was optimized by using different temperatures in gradient PCR. The annealing temperatures optimized for each of five primer set is given (Table 6). Based on consistency of bands, the optimum concentration of PCR reaction mixture which gave reproducible results was used for nine ISSR primers analyzed in 30 rice varieties.

Table 6. Annealing temperature optimized for chosen 9 ISSR primers

Sr. No.	Primer name	Annealing temp (^0C)	Sr. No.	Primer name	Annealing temp (^0C)
1	ISSR807	50	6	ISSR823	52
2	ISSR808	52	7	ISSR826	52
3	ISSR809	52	8	ISSR827	52
4	ISSR811	52	9	ISSR829	52
5	ISSR816	50			

Table 7. PCR program for ISSR assay

Step No.	Temp. (°C)	Duration	Cycles	Function
1	95	5 min		Initial denaturation
2	95	1 min	30	Denaturation
3	50 or 52	1 min		Annealing
4	72	1 min	Cycles	Extension
5	72	10 min		Final extension
6	4	Pause		Final hold

2.9 Data Scoring and Analysis

Amplification profiles of all thirty rice genotypes with different primers were compared with each other, and bands of DNA fragments were manually scored as (1) or (0) depending on the presence or absence of a particular band, respectively. The dendrogram was constructed using the Unweighted Pair Group Method of Arithmetic Averages (UPGMA), (Mathew et al., 2000). The data was analyzed using the Numerical Taxonomy System of Multivariate Statistical Program NTSYpc 2.02i (Rohlf, 1998) software package.

3. RESULTS AND DISCUSSION

The PCR amplification of template DNA produced a total of 172 bands among the 30 genotypes. The number of polymorphic markers obtained on analysis of 30 genotypes was 9. These were used to characterize and estimate genetic diversity among 30 genotypes of rice.

3.1 Molecular Analysis Using ISSR Marker

The molecular characterization of 30 genotypes of rice with 9 ISSR markers was done and presented in terms of PIC value for each primer & diversity analysis was done through similarity matrix and dendrogram obtained by using NTSYS-PC. All the selected ISSR primers were found to be polymorphic in the present study. These results are presented here under two subheads. Among the ISSR primers, 807 produced maximum number of 11 bands followed by 811 and 827 (10 bands). However, the least number of bands was amplified by ISSR 823 primer (5 bands). The highest (100%) polymorphism was shown by ISSR 807, ISSR 808 primer while ISSR 827 primer showed minimum that is 60% polymorphism given in (Table 8).

All the selected ISSR primers were found to be polymorphic in the present study. These results are presented here under two sub heads. Among the ISSR primers, 807 produced maximum number of 11 bands followed by 811 and 827 (10 bands). However, least number of bands was amplified by ISSR 823 primer (5 bands). The highest (100%) polymorphism was shown by ISSR 807, ISSR 808 primer while ISSR 827 primer showed minimum that is 60% polymorphism given in (Table 8).

The PIC values were calculated to find out the efficiency of primers in distinguishing individual genotypes. The polymorphism information Content (PIC) values of ISSR primers ranged from 0.40 to 0.80 in ISSR primer. Further it was observed that there was no correlation between per cent polymorphism and PIC values as ISSR primers ISSR 807, ISSR 808 showed maximum

Table 8. PIC value, allele information and product size of ISSR primer

ISSR Primer	No. of allele	Polymorphic bands	Monomorp hic bands	Percent Polymorphism	PIC Value	Product size (bp)
ISSR807	11	11	0	100	0.80	300 to 1500
ISSR808	9	9	0	100	0.61	400 to 3000
ISSR809	9	8	1	88	0.56	300 to 1000
ISSR811	10	9	1	90	0.51	300 to 3000
ISSR816	8	7	1	87.5	0.45	400 to 3000
ISSR823	5	4	1	80	0.53	400 to 1500
ISSR826	6	5	1	83.33	0.40	500 to 1500
ISSR827	10	6	4	60	0.46	400 to 3000
ISSR829	8	7	1	87.5	0.42	400 to 1500

per cent polymorphism and ISSR primer 807 showed maximum PIC value followed by 808 and 823 Further it was revealed that minimum per cent polymorphism showed by ISSR 829 while ISSR 826 showed minimum PIC indicating ISSR 807 was more informative and ISSR primer 808 and 823 were moderately informative [16-17].

The ISSR primer, 823 amplified 5 fragment out of which 4 were polymorphic, 1 was monomorphic and 1 was unique (Table 8, Fig. 6). The unique fragment having size 400 bp was present in Kalbhat genotype. The ISSR 809 amplified 9 fragment out of which 8 were polymorphic, 1 was monomorphic and 1 was unique (Table 8, Fig. 3).

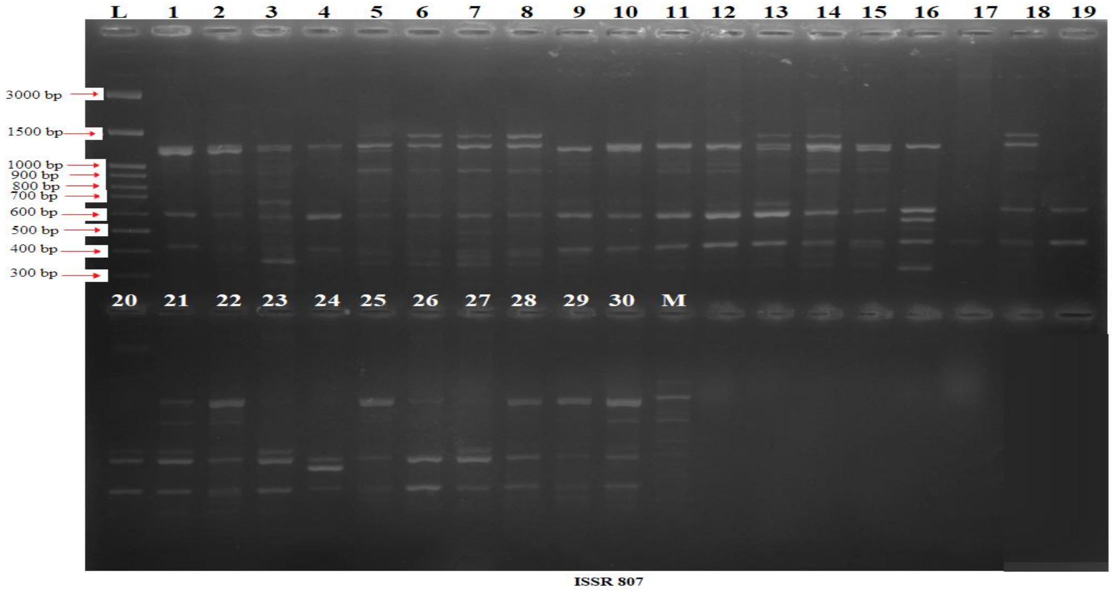


Fig. 1. ISSR banding profile of thirty rice genotypes with ISSR 807 primer

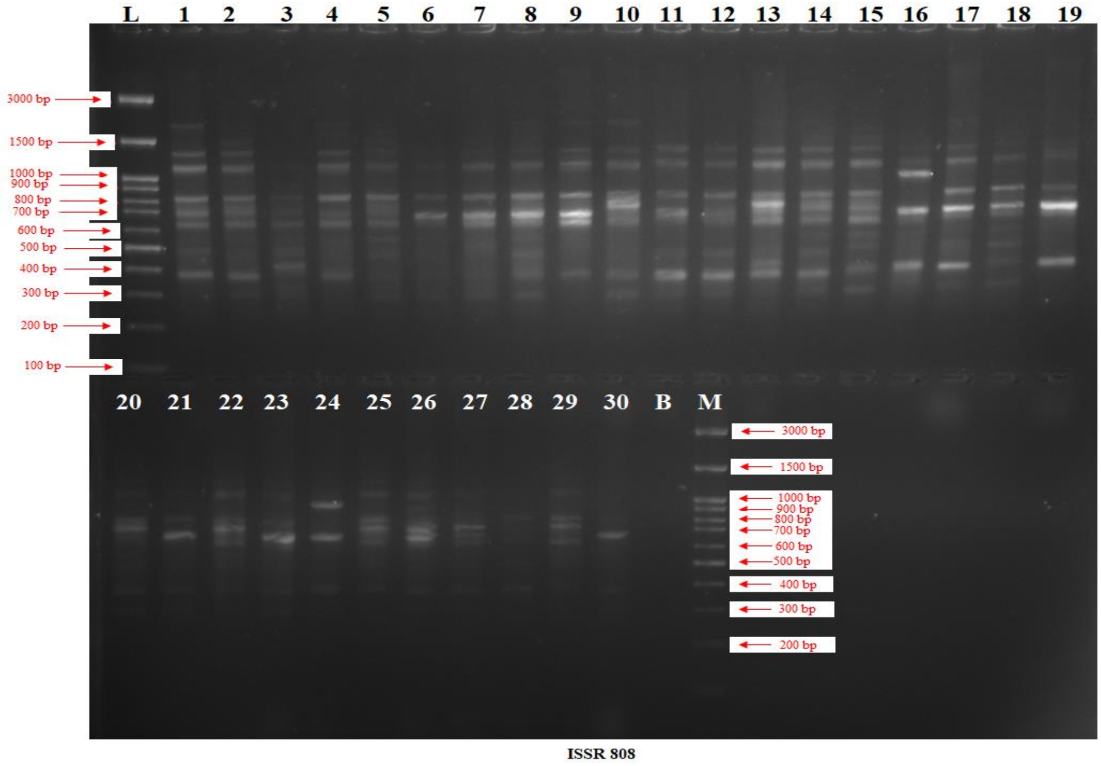


Fig. 2. ISSR banding profile of thirty rice genotypes with ISSR 808 primer

The unique fragment having size 500 to 600 bp and was present in Kalbhat genotype only. The ISSR primer, 808 amplified 9 fragment and all 9 were polymorphic, and 1 were unique (Table 8, Fig. 2). The unique fragment having size 1 kb was present in Kalbhat and Ambemohar genotype only.

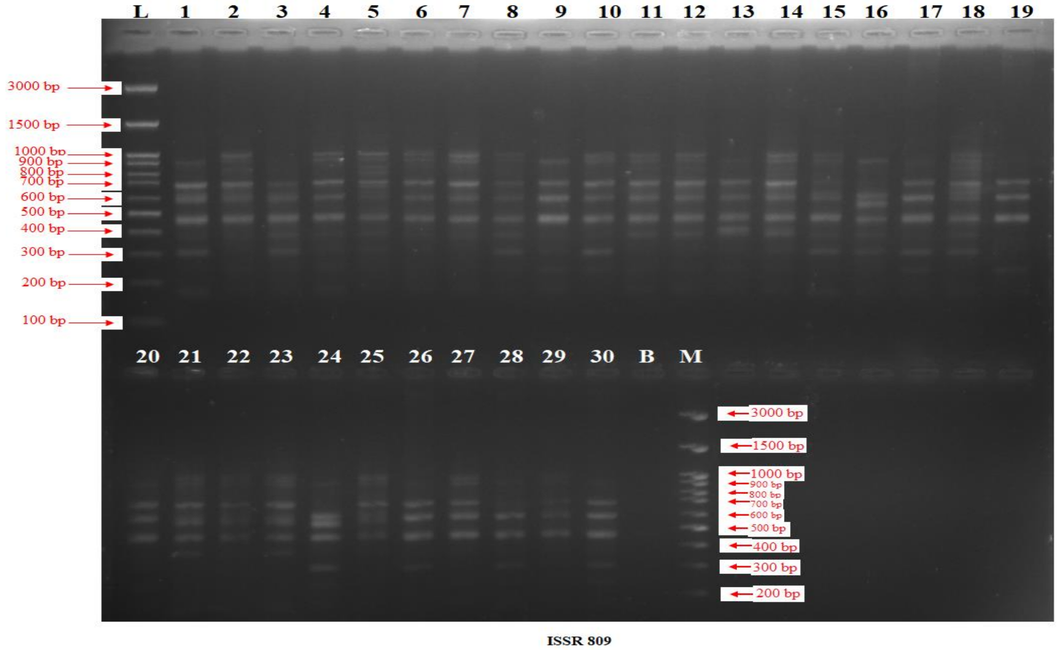


Fig. 3. ISSR banding profile of thirty rice genotypes with ISSR 809 primer

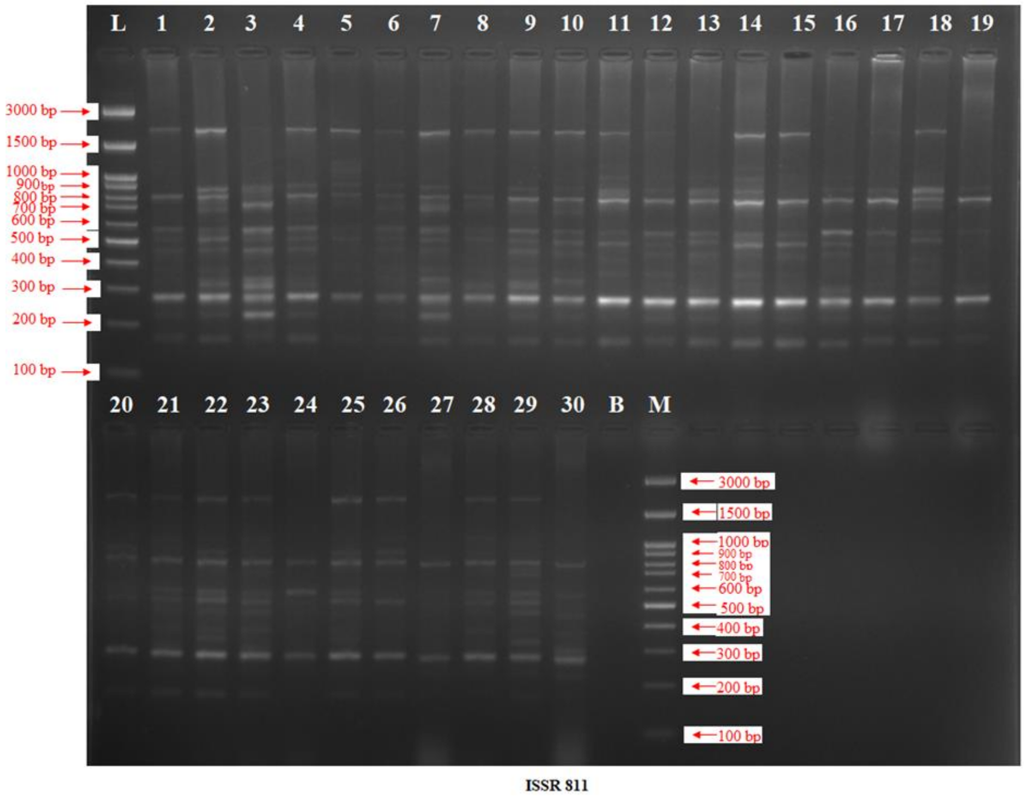


Fig. 4. ISSR banding profile of thirty rice genotypes with ISSR 811 primer

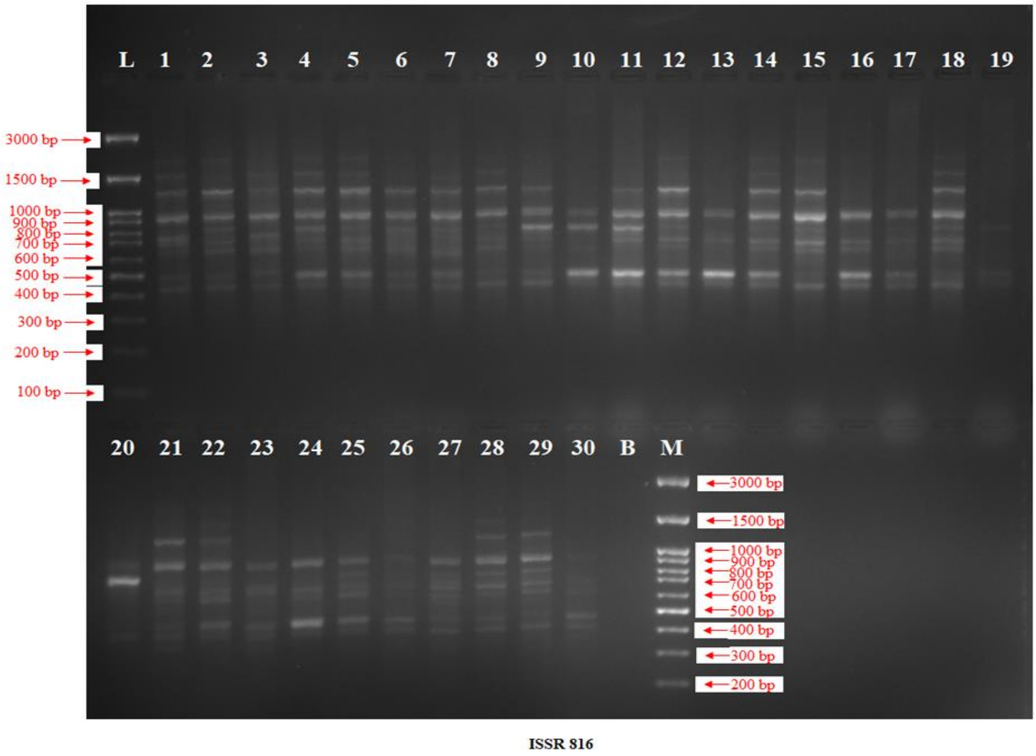


Fig. 5. ISSR banding profile of thirty rice genotypes with ISSR 816 primer

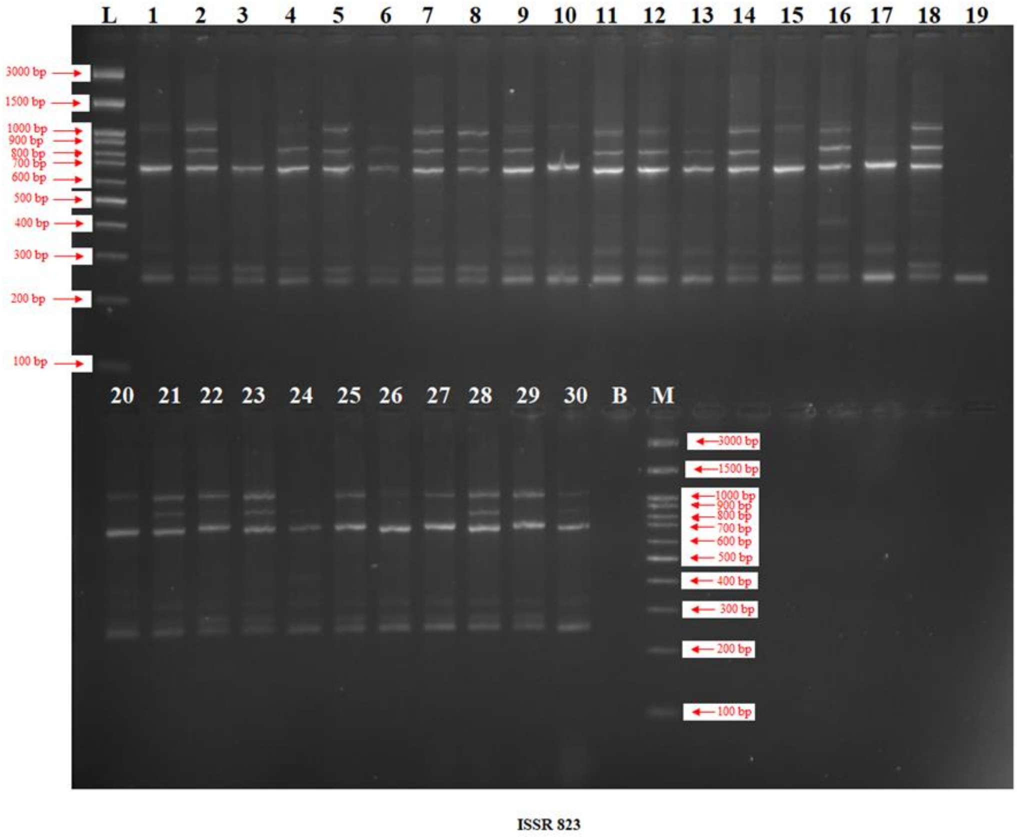
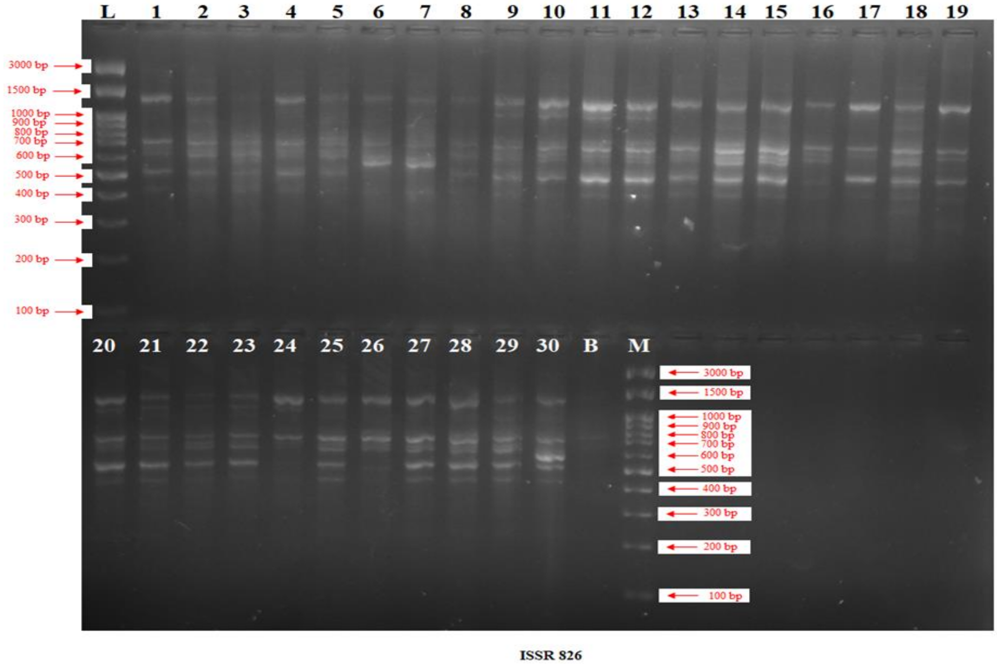


Fig. 6. ISSR banding profile of thirty rice genotypes with ISSR 823 primer



.Fig. 7. ISSR banding profile of thirty rice genotypes with ISSR 826 primer

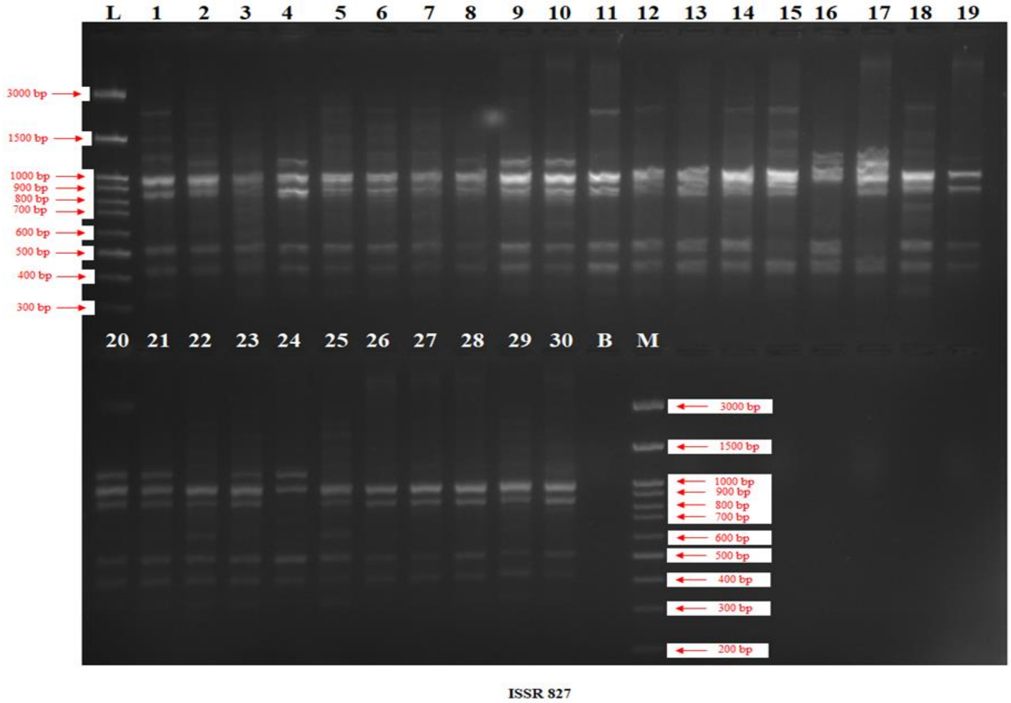


Fig. 8. ISSR banding profile of thirty rice genotypes with ISSR 827 primer

3.2 Genetic Diversity Analysis by ISSR Primers

Genetic similarity matrix was obtained by using NTSYS-PC for ISSR data (Table 10). The pair wise similarity values ranged from 0.50 to 0.92. Maximum similarity value of 0.92 was noticed between DRR Dhan 44 and DRR Dhan 45. Minimum similarity value of 0.50 was observed between rice genotype Ambemohar and Swarna Shreya. From this study, it is revealed that rice landraces are less divergent indicating that a large part of the genome may be similar among themselves [18-20].

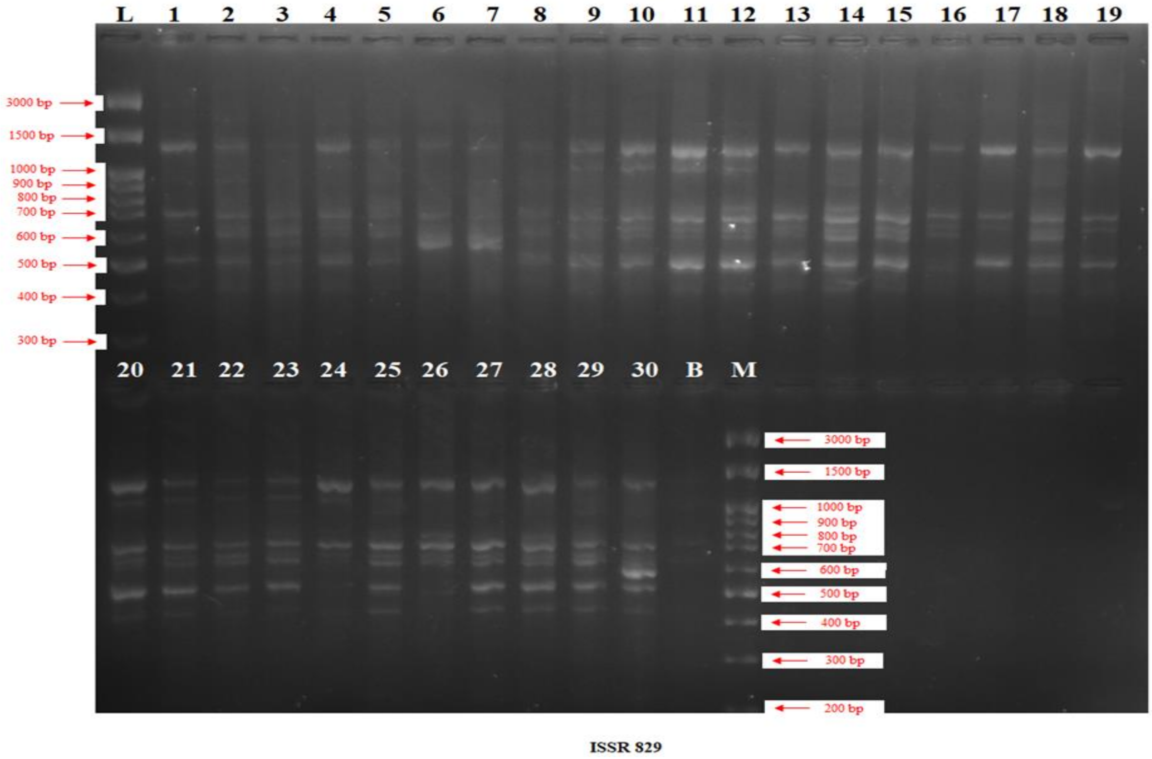


Fig. 9. ISSR banding profile of thirty rice genotypes with ISSR 829 primer
L-DNA Ladder, 1)Karjat – 2, 2)MTU-1010, 3)Karjat – 3, 4)Sairam, 5)Swarna Shreya, 6)Sugandha, 7)Madhumati, 8)Karjat – 7, 9)Karjat – 6, 10)Ratnagiri-1, 11)DRR Dhan-44, 12)DRR Dhan-45, 13)DRR Dhan-46, 14)Saubhagya Dhan, 15)Jaya, 16)Kalbhat, 17)MTU-1001, 18)RP-BIO-226, 19)PKV-HMT, 20)JGL-1798, 21)Indrayani, 22)Kundalika, 23)Phule Samruddhi, 24)Ambemohar, 25)Phule Radha, 26)Sonsal, 27)Phule Maval, 28)Pavana, 29)DRR Dhan-41, 30)Bhogawati

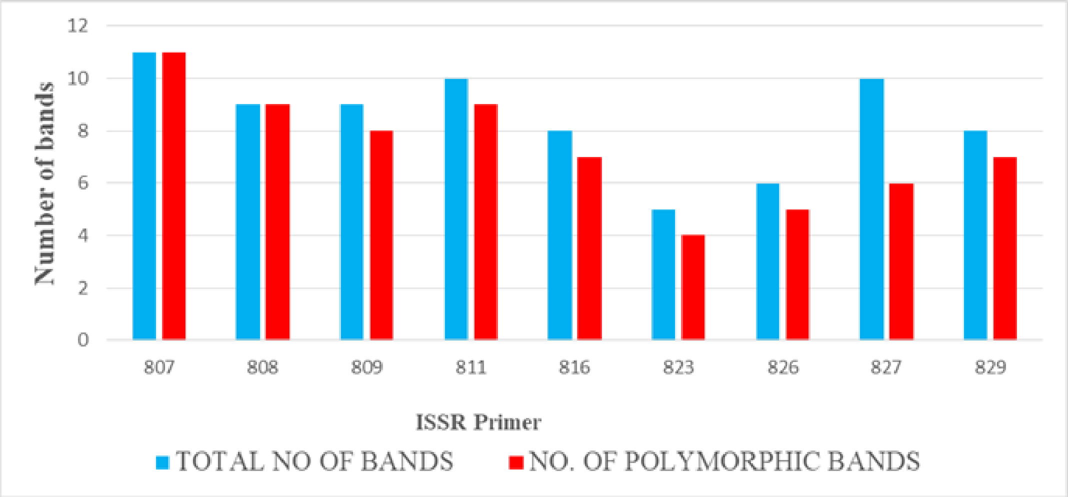


Fig. 10. Total bands and polymorphic bands amplified by ISSR primer

The UPGMA based dendrogram of thirty rice genotypes generated with NTSYSpc 2.02i program was presented in (Fig. 12). It was observed that two major clusters A and B were generated with dendrogram. Cluster A consists of genotypes Karjat 2, Jaya, Sairam, Saubhagya Dhan, RPBIO 226, Karjat 6, Ratnagiri 1, DRR Dhan 44, DRR Dhan 45, DRR Dhan 46, MTU 10010, Karjat 7, Swarna Shreya, Sugandha, Madhumati, Kalbhat. Among cluster A, DRR

Table 10. Similarity matrix among thirty rice genotypes based on ISSR marker

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.0000	0.7632	0.6711	0.7895	0.7500	0.6974	0.6842	0.7500	0.7237	0.7632	0.6842	0.7105	0.7237	0.8026	0.8026
2		1.0000	0.7237	0.8421	0.8026	0.7500	0.8158	0.8289	0.8289	0.7895	0.7632	0.7895	0.7500	0.7763	0.7763
3			1.0000	0.6711	0.6842	0.7105	0.6184	0.7105	0.7105	0.7500	0.6711	0.6974	0.7632	0.6842	0.6579
4				1.0000	0.7500	0.7237	0.7632	0.8026	0.8289	0.8158	0.7895	0.8158	0.7500	0.8553	0.7763
5					1.0000	0.8158	0.7763	0.7632	0.6842	0.6711	0.6974	0.7237	0.6842	0.8158	0.7105
6						1.0000	0.8553	0.7368	0.7105	0.6711	0.7237	0.7237	0.6579	0.7632	0.6842
7							1.0000	0.8026	0.7500	0.7105	0.7368	0.7368	0.6447	0.7237	0.7237
8								1.0000	0.7632	0.7237	0.7237	0.7500	0.6842	0.7368	0.7105
9									1.0000	0.8553	0.8553	0.8553	0.7632	0.7895	0.7368
10										1.0000	0.8158	0.7895	0.7763	0.7763	0.7763
11											1.0000	0.9211	0.8026	0.8553	0.7237
12												1.0000	0.7500	0.8289	0.7763
13													1.0000	0.8158	0.6579
14														1.0000	0.7895
15															1.0000

1)Karjat–2, 2)MTU-1010, 3)Karjat –3, 4)Sairam, 5)Swarna Shreya, 6)Sugandha, 7)Madhumati, 8)Karjat – 7, 9)Karjat – 6, 10)Ratnagiri-1, 11)DRR Dhan-44, 12)DRR Dhan-45, 13)DRR Dhan-46, 14)Saubhagya Dhan, 15)Jaya, 16)Kalbhat, 17)MTU-1001, 18)RP-BIO-226, 19)PKV-HMT, 20)JGL-1798, 21)Indrayani, 22)Kundalika, 23)Phule Samruddhi, 24)Ambemohar, 25)Phule Radha, 26) Sonsal, 27)Phule Maval, 28)Pavana, 29)DRR Dhan-41,30)Bhogawati

Genotypes	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
1	0.6974	0.7237	0.7763	0.6842	0.6842	0.6974	0.6974	0.7105	0.6447	0.7237	0.7368	0.7105	0.6842	0.7632	0.6447
2	0.6974	0.7500	0.7763	0.7368	0.7105	0.6974	0.6974	0.7632	0.5921	0.7500	0.7105	0.7105	0.7368	0.7895	0.6974
3	0.6316	0.7105	0.6316	0.6711	0.6974	0.7105	0.7105	0.6711	0.6842	0.6579	0.6974	0.7237	0.7237	0.7237	0.7105
4	0.7500	0.7500	0.8026	0.7632	0.7105	0.7500	0.6447	0.7632	0.5921	0.6974	0.7105	0.7368	0.7105	0.7368	0.6974
5	0.6053	0.6316	0.7368	0.5921	0.5658	0.6316	0.6316	0.6184	0.5000	0.6579	0.6184	0.5921	0.5921	0.6974	0.5789
6	0.6842	0.6579	0.7105	0.6711	0.6447	0.6842	0.5789	0.6711	0.6579	0.6842	0.6711	0.6711	0.6447	0.6974	0.5789
7	0.6974	0.7237	0.7500	0.7105	0.6579	0.6711	0.5921	0.7368	0.5921	0.6711	0.6579	0.6579	0.6316	0.7105	0.6184
8	0.6842	0.7368	0.7632	0.6974	0.6974	0.7368	0.6579	0.8026	0.6053	0.6579	0.6711	0.7237	0.7237	0.7763	0.7368
9	0.7105	0.7895	0.7632	0.7763	0.7500	0.7632	0.7105	0.8026	0.6053	0.7105	0.6974	0.7237	0.6974	0.7237	0.6316
10	0.6711	0.7763	0.7500	0.7368	0.7895	0.7500	0.6974	0.7632	0.6184	0.7500	0.6842	0.7368	0.6842	0.7105	0.6184
11	0.6711	0.6974	0.7500	0.7368	0.6579	0.7237	0.6974	0.7632	0.5921	0.6711	0.6579	0.7105	0.6842	0.6842	0.6184
12	0.7237	0.6711	0.7500	0.7632	0.6579	0.7500	0.7237	0.7632	0.6184	0.6711	0.6316	0.7368	0.6579	0.6842	0.6974
13	0.6579	0.6842	0.7105	0.6974	0.6447	0.6579	0.6316	0.6711	0.5789	0.6579	0.6974	0.6974	0.7237	0.6711	0.6316
14	0.6842	0.6842	0.8421	0.6974	0.6447	0.6842	0.6579	0.6711	0.5789	0.6842	0.6711	0.6974	0.6711	0.6974	0.6053
15	0.7368	0.7105	0.7632	0.6974	0.6974	0.6842	0.6316	0.6974	0.6579	0.6579	0.6447	0.6974	0.6447	0.6974	0.6053
16	1.0000	0.6579	0.6842	0.7237	0.6711	0.7632	0.6316	0.7500	0.7368	0.6053	0.7237	0.6974	0.6711	0.6447	0.7368
17		1.0000	0.7105	0.7500	0.7763	0.6842	0.6316	0.7500	0.6316	0.6842	0.7500	0.7237	0.6974	0.7500	0.6316
18			1.0000	0.6974	0.6447	0.6842	0.6842	0.7237	0.6053	0.7105	0.6447	0.6711	0.6711	0.6711	0.6316
19				1.0000	0.8158	0.7237	0.6711	0.7895	0.6974	0.6711	0.7632	0.7632	0.6842	0.6842	0.7500
20					1.0000	0.8026	0.7763	0.8421	0.7237	0.7500	0.8158	0.8158	0.8158	0.7895	0.7237
21						1.0000	0.7632	0.9079	0.7632	0.7105	0.7500	0.8026	0.8026	0.7500	0.8158
22							1.0000	0.7763	0.6579	0.7632	0.7500	0.7237	0.7763	0.8026	0.7632
23								1.0000	0.7500	0.7237	0.7895	0.8684	0.8158	0.7895	0.8026
24									1.0000	0.6579	0.7763	0.8026	0.7500	0.7500	0.7632
25										1.0000	0.7763	0.7500	0.7763	0.7763	0.6053
26											1.0000	0.8158	0.7895	0.8158	0.7237
27												1.0000	0.8158	0.8158	0.7763
28													1.0000	0.8684	0.7763
29														1.0000	0.7500
30															1.0000

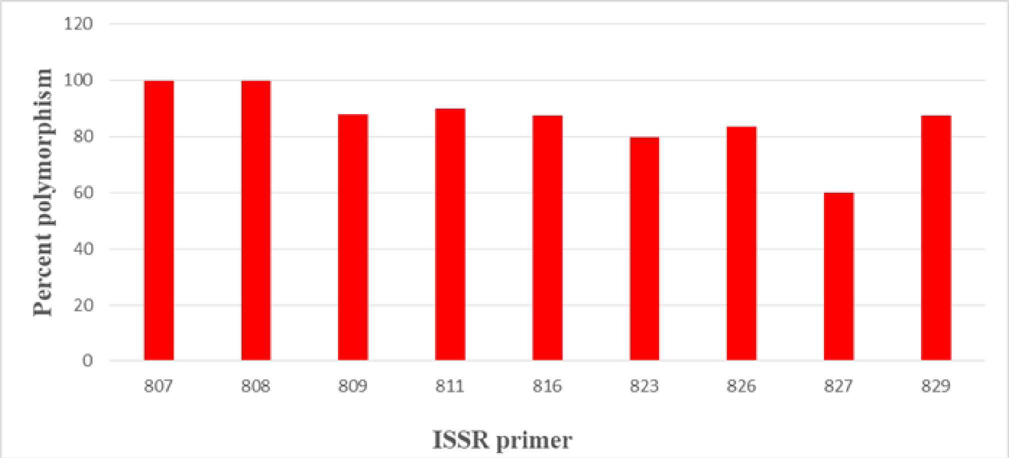


Fig. 11. Total percent polymorphism detected by ISSR primer used in this research

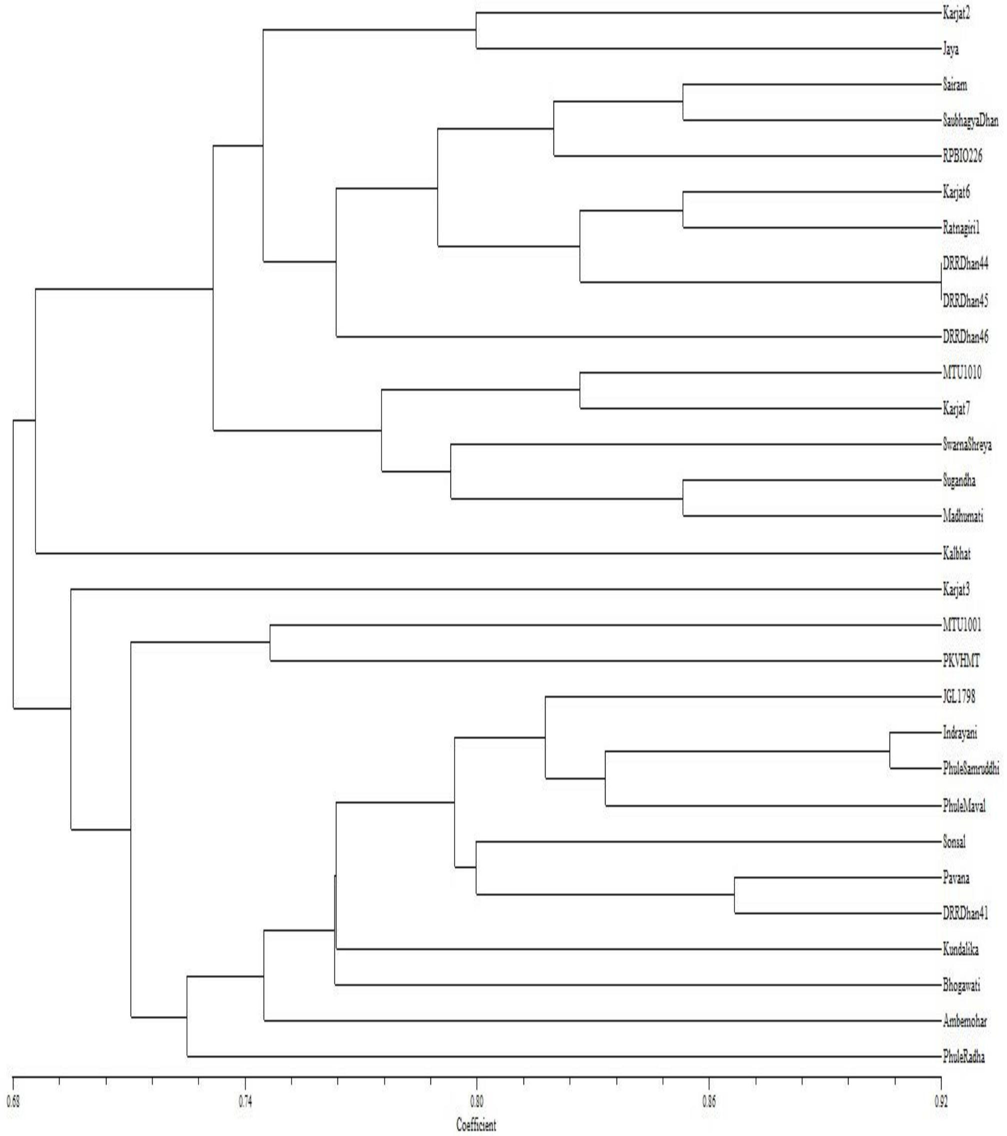


Fig. 12. Dendrogram showing phylogenetic relationship among thirty rice genotypes generated from nine ISSR primer

Dhan 44 and DRR Dhan 45 has highest similarity that is 92 percent. Further cluster A is diversified in to two sub clusters. Kalbhat alone is present in one sub cluster with 69.2% similarity with second sub cluster of A. The second sub cluster of A had varieties viz., Karjat 2, Jaya, Sairam, Saubhagya Dhan, RPBIO 226, Karjat 6, Ratnagiri 1, DRR Dhan 44, DRR Dhan 45, DRR Dhan 46, MTU 10010, Karjat 7, Swarna Shreya, Sugandha, Madhumati. The second sub cluster consists of two groups. The first of the two groups consist of genotypes Karjat 2, Jaya, Sairam, Saubhagya Dhan, RPBIO 226, Karjat 6, Ratnagiri 1, DRR Dhan 44, DRR Dhan 45, DRR Dhan 46. In the first group maximum similarity value of 0.92 was noted between DRR Dhan 44 and DRR Dhan 45. The second group is comprised of MTU 10010, Karjat 7, Swarna Shreya, Sugandha, Madhumati. In the second group Sugandha and Madhumati have 85% similarity [21-23].

Cluster B consists of Karjat 3, MTU 1001, PKVHMT, JGL 1798, Indrayani, Phule Samruddhi,Phule Maval, Sonsal, Pavana, DRR Dhan 41, kundalika, Bhogawati, Ambemohar, Phule Radha. Karjat 3 alone forms one sub cluster and has 70% similarity with second sub cluster of B. The second sub cluster of B have varieties MTU 1001, PKVHMT, JGL 1798, Indrayani, Phule Samruddhi,Phule Maval, Sonsal, Pavana, DRR Dhan 41, kundalika, Bhogawati, Ambemohar, Phule Radha. This second cluster comprised of two groups at 71% similarity. The first group had MTU 1001, PKVHMT with 75% similarity [24-26].

The second group has JGL 1798, Indrayani, Phule Samruddhi,Phule Maval, Sonsal, Pavana, DRR Dhan 41, kundalika, Bhogawati, Ambemohar, Phule Radha. In this group Indrayani and Phule Samruddhi has 91% similarity. From the dendrogram constructed on the basis of results obtained by ISSR 807, ISSR 808, ISSR 809, ISSR 811, ISSR 816, ISSR 823, ISSR 826, ISSR 827 and ISSR 829 primer, it is concluded that crosses should be made between cluster A and Cluster B, also crosses should be made between Kalbhat and other genotypes in cluster A and between Karjat 3 and other genotypes in cluster B [27,28].

4. CONCLUSION

In this study, Swarna Shreya and Ambemohar were found to be most diverse than other genotypes and Kalbhat, Ambemohor and karjat 3 had shown some unique bands so they could be utilized for developing superior hybrids through various breeding strategies. All screened primers are found to be polymorphic with high polymorphic information content which efficiently discriminating the varieties at molecular level. By looking at the PIC values and polymorphic percentage, it can be concluded that the ISSR marker can be suitably used for assessing the genetic diversity among different rice genotypes.

CONFERENCE DISCLAIMER

Some part of this manuscript was previously presented in the conference: 6th International Conference on Strategies and Challenges in Agricultural and Life Science for Food Security and Sustainable Environment (SCALFE-2023) on April 28-30, 2023 in Himachal Pradesh University, Summer Hill, Shimla, HP, India. Web Link of the proceeding: https://www.shobhituniversity.ac.in/pdf/Souvenir-Abstract%20Book-Shimla-HPU-SCALFE-2023.pdf

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mengistu MA. ISSR fingerprinting, Barbas CF, Burton DR, Scott JK, Silverman GJ. Quantification of DNA and RNA. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA. Phage Display. 2001;4-6.
2. Bostein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphism. American Journal of Human Genetics. 1980;32:314-331.
3. Blair MW, Panaud O, Mc Couch SR. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theor. Appl. Genet. 1999;98:780-792.
4. Davierwala AP, Chowdari KV, Kumar S, Reddy APK, Ranjekar PK, Gupta VS. Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. Genetica. 2000;10(7):69-84.
5. Dharmaraj K. Studies on varietal identification of rice genotype using ISSR marker. J. Pharma. Phytochemistry. 2018; 1(3):2808-2812.

6. Giasuddin ASM. Polymerase chain reaction technique: Fundamental aspects and applications in clinical diagnostics. J. Islamic Aca. Sci. 1995;8:29-32.
7. Girma TK, Bekele E. ISSR analysis of wild and cultivated rice species from Ethiopia Gezahegn. Afr. J. Biotechnol. 2010; 9(32):5048-5059.
8. Qian W, Ge S, Hong DY. Genetic variation within and among population of a wild rice Oryza granulate from China detected by RAPD and ISSR markers. Theo.Appl. Gene. 2001;102:440-449.
9. Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS. Genetic diversity and phylogenetic relationship as revealed by Inter Simple Sequence Repeat (ISSR) polymorphism in the genus Oryza Theor. Appl. Genet. 2000;100:1311-1320.
10. Kaizman M, Jakse J, Baricevic D, Javornik B, Prosek M, Robust. CTAB- activated charcoal protocol for plant DNA extraction. Acta Agriculture Slovenica. 2006;87-2:427-433.
11. Kladmook M, Kumchoo T, Hongtrakul V. Genetic diversity analysis and sub species classification of Thailand rice landraces using DNA markers. Afr. J. Biotechnol. 2012;11(76):14044- 14053.
12. Lal S, Mistry NK, Thaker R, Shah SD, Vaidya PB. Genetic diversity assessment in six medicinally important species of ocimumfrom central Gujarat (India) utilizing RAPD, ISSR and SSR markers. I. J. A. B. R. 2012;2(2):279-288.
13. Lodhi, Muhammad A, Guang-Ning Ye, Norman F Weeden, Bruce I Reisch. A simple and efficient method for DNA extraction from grapevine cultivars, Vitis species and Ampelopsis. Plant, molecular Biology Reporter. 1994;12(1):6-13.
14. Doyle JJ, Doyle JL. A rapid total DNA preparation procedure for fresh plant tissue. Focus. 1990;12:13-15.
15. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics. 1994; 20:176–183.
16. phenotypic variability and trait associations in released and elite rice (*Oryza sativa* L.) genotypes of Ethiopia. A thesis submitted to the School of Graduate Studies, Addis AbabaUniversity; 2011.
17. Muthusamy S, Kanagarajan S, Ponnusamy S. Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. Ele. J. Biotechnol. 2008;11: 3.
18. Nagaraju J, Kathirvel M, Kumar R, Siddiq EA, Hasnain SE. Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence- based ISSR-PCR and SSR markers. Proc. Nat. Acad. Sci. USA. 2002;99(9):5836- 5841.
19. Nagaraju J, Kathirvel M, Kumar R, Siddiqi EA, Hasnain SE. Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence- based ISSR-PCR and SSR markers. Centre for Research on Sustainable Agricultural and Rural Development, Chennai, India; 2002.
20. Racharak R, Eiadthong RS. Genetic relationship among subspecies of Musa acuminate and A-genome consisting edible cultivated bananas assayed with ISSR markers. J. Sci. Technol. 2007; 29(6):1479-1489.
21. Rani B, Sharma VK. A modified CTAB method for quick extraction of genomic DNA from rice seed/grain/leaves for PCR analysis. Human J. Res. Art. 2016;4:254-260.
22. Rychlik W, Spencer WJ, Rhoads RE. Optimization of the annealing temperature for DNA amplification in vitro. Nucleic Acid Res. 1990;18:6409-6412.
23. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Nature. 1988;239:487–97.
24. Saini N, Jain N, Jain S, Jain RK. Assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. Euphytica. 2004;140:133-146.
25. Sarla N, Bobba S, Siddiq EA. ISSR and SSR markers based on AG and GA repeats delineate geographically diverse *Oryza nivara* accessions and reveal rare alleles. Curr. Sci. 2003;84:683-690.
26. Sheethal Y, Poonam R, Navinder S, Sunita J, Rajinder JK. Assessment of genetic diversity among rice genotypes with differential adaptations to salinity using physio-morphological and molecular

markers. J. Plant Biochem. And Biotechnol. 2008;17(1).

27. Turkie TA. Assessment of ISSR based molecular genetic diversity of Hassawirice in Saudi Arabia. Saudi J. Bio. Sci. 2015;18:7-11.
28. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingy SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990;18:6531–6535.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/104839