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Molecular genetic diversity of landraces, cultivars and wild relatives of rice of Goa

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We studied 51 rice varieties to understand their genetic diversity. Out of 19 ISSR primers, 15 primers produced reproducible bands. Out of 110 ISSR bands, 104 were polymorphic bands with an average of 6.93 bands per primer. The amount of polymorphism varied from 50% to 100%, with an average of 92%. Genetic identity value ranged from 0.5091 to 0.9727, with an average of 0.740. Dendrogram revealed the formation of four major clusters. Wild rice *Oryza rufipogon* formed a separate clade, indicating its uniqueness. Our study opens up avenues for use of traditional rice varieties for rice breeding, genome-wide association mapping and conservation of rice germplasm.

Keywords: Genetic diversity, ISSR markers, landraces, *Oryza sativa*, *Oryza rufipogon*.

MOLECULAR genetic diversity of rice germplasm has been evaluated intensively on a large scale using molecular markers[1–3]. Consequently, the global studies present an outstanding overview of the cultivated rice population structure. However, an in-depth knowledge on local germplasm of rice could not be provided. Hence, various local rice germplasm studies have been taken up at the national or state level to understand the genetic diversity of rice in a particular area[4–8]. Molecular markers have been used as an important tool for assessing the genetic relations, identification and for the desirable genotype selection in breeding programmes and germplasm conservation[9]. In this communication, we present the molecular genetic diversity among landraces, cultivars and wild rice in Goa.

During the field survey, we collected a total of 50 varieties of rice from different talukas of Goa (28 landraces, 22 high yielding rice varieties), India. We also included wild rice *Oryza rufipogon* from Goa, and a salt-tolerant rice variety Pokkali from Kerala (Tables 1 and 2). The seeds were germinated in laboratory conditions and allowed to grow for 20 days. Genomic DNA was extracted from the fresh/frozen rice leaf material using standard protocol[9]. The universal random oligonulceotide primers, specifically inter-simple sequence repeat (ISSR), were obtained from Metabion International AG (Martinsried, Germany). The primers used during this analysis of molecular genetic diversity of rice are listed in Table 3.

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Amplification via polymerase chain reaction (PCR) was performed using 25 µl as final volume for each sample. All chemicals required for PCR analysis were obtained from Merck Specialities Private Limited, Bengaluru, India.

PCR amplification was carried out using a Mastercycler gradient (Eppendorf AG, Hamburg, Germany)[10]. Initial denaturation of template DNA was done at 94°C for 5 min and then 30 cycles of amplification using PCR with 1 min of denaturation at 94°C was done, followed by 1 min of annealing at different temperatures (Table 3). The primer extension was carried out for 2 min at 72°C. Final extension (10 min) was provided at 72°C for DNA amplification. The amplified PCR product was mixed with 1 µl gel loading dye containing bromophenol blue, and then loaded in the wells of 2% agarose gel with ethidium bromide. Electrophoresis was carried out at room temperature using TBE buffer (1×) with pH 8.0. The gel was observed, photographed and analysed using gel documentation system under ultraviolet light (Alpha-DigiDoc™, Alpha Innotech Corporation, Canada).

Each ISSR amplified product was named by primer code. The banding pattern varied from primer to primer. The experiment was repeated (three replications) to obtain reproducible results. ISSR bands were accurately scored by using binary code 0 (if no band) and 1 (for presence of band). Each informative ISSR band was scored independently. The polymorphism percentages were calculated taking into account the proportion of polymorphic bands over the total number of bands. Dendrogram and genetic distance were generated by clustering according to the unweighted paired group method with arithmetic mean (UPGMA) using the computer software NTSYS-pc Version-2 (ref. 11).

Out of 19 primers screened, 15 showed consistent and reproducible bands. Four primers namely ISSR-809, IB-3, IB-4 and ISSR-6 did not amplify. Amplification profiles of the primers ISSR-834 and ISSR-7 are provided in Figures 1 and 2 respectively. The 110 ISSR bands were obtained from 15 different primers (average 7.33 bands per primer) (Table 4). Out of 110 bands, 104 were polymorphic for all the rice varieties with an average of 6.93 bands per primer. The number of amplified bands ranged from 2 (ISSRA1) to 10 (1SSR-7). The polymorphism percentage among all samples varied from 50% to 100% (average 92%). Polymorphic banding pattern of 100% was obtained using primers ISSR-808, ISSR-834, UBC-828, UBC-811, 1SSR-7, ISSR-2, ISSR-807, ISSR-812 and ISSRA3, while the lowest polymorphism (50%) was observed in ISSRA1 primer (Table 4).

Pair-wise genetic similarities were computed from ISSR data, the genetic identity values varied from 0.5091 to 0.9727 (average 0.740). Among 51 rice varieties, the salt-tolerant AVT-1908 and salt-tolerant AVT-1918 shared maximum genetic identity (0.9727), whereas the traditionally cultivated rice varieties (landraces) Ek Kadi and Dhave showed a similar range of genetic identities (0.9273). The traditional salt-tolerant rice varieties Kalo

Table 1. Traditionally cultivated rice varieties (landraces) collected from Goa

Variety	Place of collection	Taluka
Assgo	Neura-O-Grande	Tiswadi
Barik Kudi	Siolim	Bardez
Bello	Sigonem	Sanguem
Damgo	Corjuem	Bardez
Dhave	Bati	Sanguem
Ek Kadi	Ozorim	Pernem
Ghansal	Torxem	Pernem
Girga	Amberem	Pernem
Jiresal	Savoi-Verem	Ponda
Kalo Damgo	Mandrem	Pernem
Kalo Korgut	Assolna	Salcete
Kalo Novan	Naroa	Bicholim
Karo Mungo	Parcem	Pernem
Karz	Barcem	Quepem
Kendal	Ponchavadi	Ponda
Khochro	Naneli	Satari
Kolyo	Gaodongrem	Canacona
Korgut	Navelim	Tiswadi
Kotimirsal	Gaodongrem	Canacona
Muno	Cumbarjua	Tiswadi
Kusago	Davanvado	Pernem
Novan	Paroda	Salcete
Patni	Sancordem	Dharbandora
Sal	Poinguinim	Canacona
Shiedi	Amone	Bicholim
Taysu	Usgao	Dharbandora
Tamdi	Cotorem	Satari
Valay	Pirla	Quepem

Table 2. High yielding, scented and hybrid rice cultivars collected from ICAR, Goa and other regions

Variety	Place of collection
Annapurna	Mandrem (Pernem)
CSR-27	ICAR
IR-8	Tivrem (Ponda)
Jaya	Saligao (Bardez)
Jyoti	Siolim (Bardez)
Karjat-3	Paroda (Salcete)
Karjat-5	Amona (Quepem)
Kasturi	ICAR
KRH-2	ICAR
MO-7	ICAR
MO-9	ICAR
MO-17	ICAR
Mugadh Sugandh	ICAR
Pusa Basmati-1	ICAR
Pusa Sugandh-2	ICAR
Pusa Sugandh-3	ICAR
Pusa Sugandh-5	ICAR
R-6857	ICAR
Sahyadri-1	ICAR
Salt Tolerant AVT-1901	ICAR
Salt Tolerant AVT-1918	ICAR
Vasmati	ICAR
Pokkali	Kerala
Oryza rufipogon (wild rice)	Taleigao (Tiswadi)

Table 3. ISSR primers screened, annealing temperature and number of amplified bands

Primer name	5′–3′ sequence	AT	Amplified primer	Amplified bands
ISSR-810	GAGAGAGAGAGAGAGAT	49.4	+	8
1SSR-808	AGAGAGAGAGAGAGAGC	51.8	+	7
ISSR-809	AGAGAGAGAGAGAGAGG	51.8	–	–
1SSR-834	AGAGAGAGAGAGAGAGYT	51.4	+	9
UBC-828	TGTGTGTGTGTGTGTGA	49.4	+	8
UBC-811	GAGAGAGAGAGAGAGAC	51.8	+	9
IB-3	TCTCTCTCTCTCTCTCC	51.8	–	–
IB-4	ACACACACACACACACC	51.8	–	–
1SSR-7	GGCGGCGGCGGCGGCTA	66.2	+	10
ISSR-2	AAGAAGAAGAAGAAGGC	47.0	+	9
ISSR-3	AAGAAGAAGAAGAAGTG	44.5	+	9
ISSR-6	AGCAGCAGCAGCAGCCG	59.0	–	–
ISSR-807	AGAGAGAGAGAGAGAGT	49.4	+	7
ISSR-812	GAGAGAGAGAGAGAGAA	49.4	+	6
RM-ST1	CACGTGAGACAAAGACGGAG	58.4	+	8
RM-ST2	GAGAGAGAGAGAGAGAYG	53.8	+	8
ISSRA1	GAAGGCAAGTCTTGGCACTG	58.4	+	2
ISSRA2	ACTATGCAGTGGTGTCACCC	58.4	+	3
ISSRA3	TGGCCTGCTCTCTCTCTCTC	58.45	+	7

+, Amplified; –, Not amplified; AT, Annealing temperature.

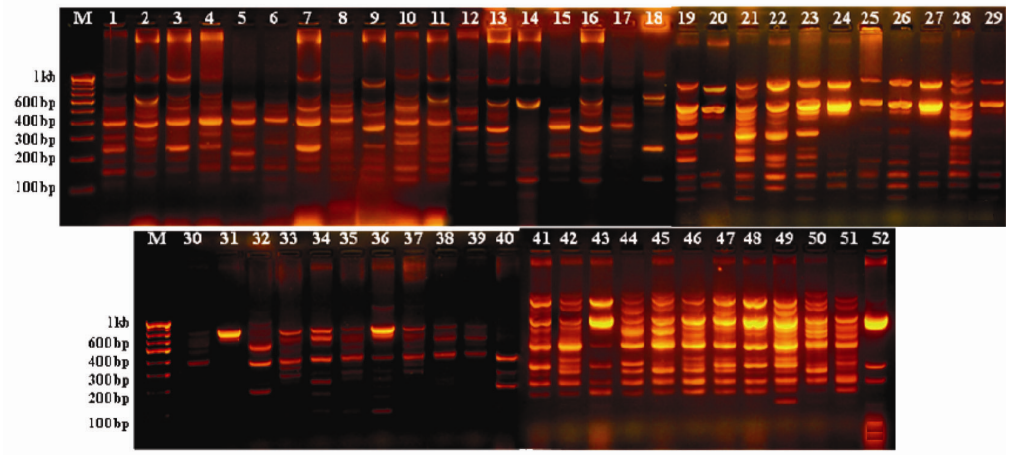

Figure 1. ISSR amplification profile of 51 rice varieties and a wild rice *Oryza rufipogon* with primer ISSR-834. Lane 1. Gene ruler^{TM} 1 kb DNA ladder marker; 1, Barik Kudi; 2, Bello; 3, Dhave; 4, Ek Kadi; 5, Kalo Novan; 6, Kalo Damgo; 7, Karz; 8, Kendal; 9, Khochro; 10, Kolyo; 11, Kusago; 12, Novan; 13, Patni; 14, Sal; 15, Taysu; 16, Tamdi; 17, Valay; 18, *Oryza rufipogon*; 19, Ghansal; 20, Girga; 21, Jiresal; 22, Kotimirsal; 23, Pusa Basmati-1; 24, Pusa Sugandh-2; 25, Pusa Sugandh-3; 26, Pusa Sugandh-5; 27, Kasturi; 28, Vasmati; 29, Mugadh Sugandh; 30, Assgo; 31, Damgo; 32, Kalo Korgut; 33, Karo Mungo; 34, Korgut; 35, Muno; 36, Shiedi; 37, CSR-27; 38, Salt tolerant-1901; 39, Salt tolerant-1918; 40, Pokkali; 41, Annapurna; 42, IR-8; 43, Jaya; 44, Jyoti; 45, Karjat-3; 46, Karjat-5; 47, MO-7; 48, MO-9; 49, MO-17; 50, R-6857; 51 and KRH-2; 52, Sahyadri-1.

Korgut and Kalo Damgo showed genetic identity value of 0.9000. The lowest genetic identity value (0.5091) was observed in *O. rufipogon* (wild rice) and local scented rice variety Girga.

ISSR data of 51 rice varieties and *O. rufipogon* (wild rice) were used for generating the dendrogram. Dendrogram generated from ISSR data revealed clustering of rice varieties (Figure 3). It revealed four major clusters which include (i) high yielding rice varieties; (ii) scented rice varieties (iii) salt-tolerant rice varieties, and (iv) traditional rice varieties of Goa. The first cluster comprised 12 varieties belonging to high yielding rice varieties (Annapurna, IR-8, MO-17, R6857, Jaya, MO-9, Jyoti, Karjat-3, Karjat-5, MO-7, KRH-2 and Sahaydri-1). The second cluster consisted of 10 rice varieties which included scented landraces of rice (Ghansal, Jiresal and Kotimirsal) and high yielding scented rice (Pusa Sugandh-2, Kasturi, Vasmati, Mugadh Sugandh, Pusa sugandh-5, Pusa Basmati-1 and Pusa Sugandh-3). The third group consisted of 11 rice varieties belonging to salt-tolerant landraces of rice (Assgo, Kalo Korgut, Muno, Shiedi, Karo Mungo, Korgut, Damgo, Pokkali) and high yielding salt-tolerant rice varieties (salt-tolerant AVT-1901, salt tolerant AVT-1918, CSR-27). The fourth cluster comprised 17 landraces of rice which were traditionally cultivated by farmers (Barik Kudi, Dhave, Ek Kadi, Bello, Karz, Kendal, Kolyo, Khochro, Patni, Tamdi, Valay, Kusago, Novan, Kalo Novan, Kalo Damgo, Sal and Taysu).

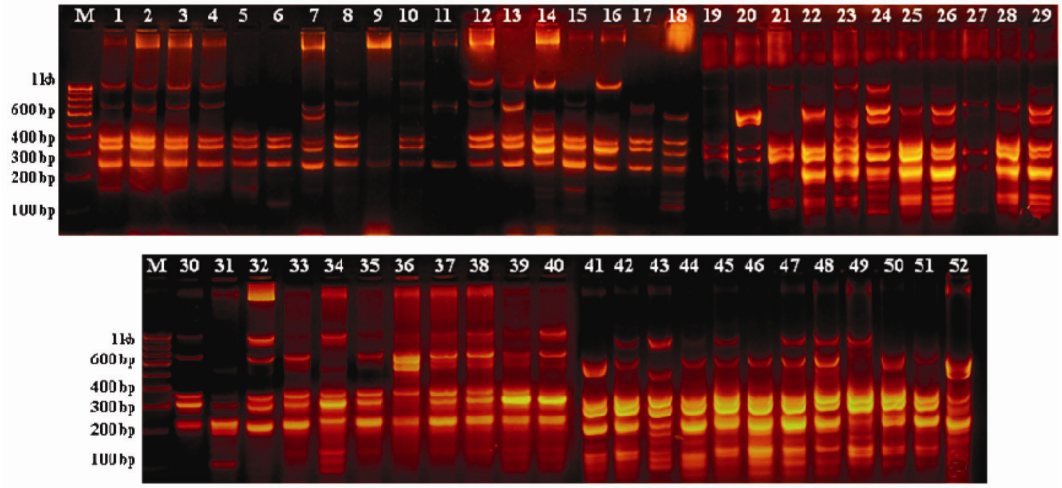

Figure 2. ISSR amplification profile of 51 rice varieties and a wild rice *Oryza rufipogon* with primer ISSR-7. Lane 1. Gene ruler™ 1 kb DNA ladder marker; 1, Barik Kudi; 2, Bello; 3, Dhave; 4, Ek Kadi; 5, Kalo Novan; 6, Kalo Damgo; 7, Karz; 8, Kendal; 9, Khochro; 10, Kolyo; 11, Kusago; 12, Novan; 13, Patni; 14, Sal; 15, Taysu; 16, Tamdi; 17, Valay; 18, *Oryza rufipogon*; 19, Ghansal; 20, Girga; 21, Jiresal; 22, Kotimirsal; 23, Pusa Basmati-1; 24, Pusa Sugandh-2; 25, Pusa Sugandh-3; 26, Pusa Sugandh-5; 27, Kasturi; 28, Vasmati; 29, Mugadh Sugandh; 30, Assgo; 31, Damgo; 32, Kalo Korgut; 33, Karo Mungo; 34, Korgut; 35, Muno; 36, Shiedi; 37, CSR-27; 38, Salt tolerant-1901; 39, Salt tolerant-1918; 40, Pokkali; 41, Annapurna; 42, IR-8; 43, Jaya; 44, Jyoti; 45, Karjat-3; 46, Karjat-5; 47, MO-7; 48, MO-9; 49, MO-17; 50, R-6857; 51, KRH-2 and 52, Sahyadri-1.

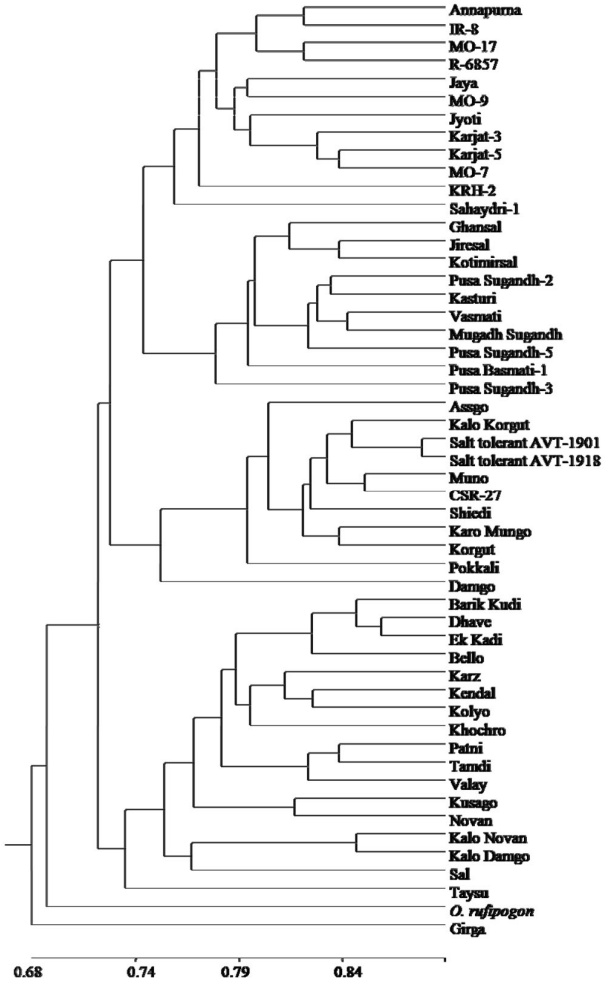

Figure 3. Dendrogram of Nei's genetic distance of landraces, high yielding rice varieties and a wild rice *Oryza rufipogon* based on ISSR data.

Table 4. Number of amplified bands, polymorphic bands and percentage of polymorphism

Primer name	No. of amplified bands	No. of polymorphic bands	Polymorphism (%)
ISSR-810	8	7	87.5
1SSR-808	7	7	100
1SSR-834	9	9	100
UBC-828	8	8	100
UBC-811	9	9	100
1SSR-7	10	10	100
ISSR-2	9	9	100
ISSR-3	9	8	88.8
ISSR-807	7	7	100
ISSR-812	6	6	100
RM-ST1	8	7	87.5
RM-ST2	8	7	87.5
ISSRA1	2	1	50.0
ISSRA2	3	2	66.6
ISSRA3	7	7	100
Total	110	104	–
Mean	7.33	6.93	92.0

Surprisingly, a scented rice variety Girga remained separate and not clustered with any group of rice varieties studied. Wild rice *O. rufipogon* formed a separate clade indicating its uniqueness and distance.

ISSR primers have been found helpful in identifying the genetic diversity and population structure of coffee[12], barley[13] and orchids[10,14]. Molecular markers, like random amplification polymorphic DNA (RAPD), have been employed successfully to ascertain the genetic diversity in various species including rice[15], however, RAPD has several limitations together with dominance, uncertain locus homology, sensitivity and low reproducibility. To

solve these problems, inter-simple sequence repeat (ISSR) amplification was used to assess genetic diversity and distance[16]. In our study, a total of 110 bands were amplified, of which 104 were polymorphic for all rice varieties. It was observed that AG and GA based primers were given 100% polymorphism. Similar findings of AG and GA based primers have been revealed to amplify clear bands in rice[17,18]. Indigenous knowledge of landraces gathered from local farmers has provided a strong background to understand the genetic relationships of native rice varieties of Goa, India.

The joint evaluation of landraces with known cultivars has permitted genome-wide association mapping and suggests scope to revise more rice landraces collected from different geographical regions[19]. Among the rice varieties studied, a local scented variety Girga was not clustered with scented group as expected, and it formed a separate clade showing some uniqueness, however, it needs further study. Wild rice *O. rufipogon* formed a separate clade representing its distinctiveness. It has been reported that the genus *Oryza* consists of two cultivated species and about 20 wild species[20]. The Asian cultivated rice *O. sativa* has evolved in the following sequence as *O. rufipogon* (wild perennial) to *O. nivara* (wild annual) and then the cultivated annual *O. sativa*[21]. Our study may help in comprehending genetic closeness and diversity between the landraces (traditionally cultivated rice varieties) and cultivars (high yielding rice varieties). It opens up an avenue for the use of traditional rice varieties for breeding programmes, genome-wide association mapping and conservation of rice germplasm.

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