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Molecular diversity in rice (*Oryza Sativa* L.) accessions of Chhattisgarh based on grain length using ISSR markers

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Abstract
A total of ten ISSR primers were taken to assess the genetic diversity. The 10 primers yielded a total of 46 amplified fragments from 48 rice genotypes and out of these, 29 alleles were polymorphic. The number of scorable bands produced per primer ranged from 2 to 6 with an average of 4.6, and the average number of polymorphic fragments per primer was 2.9. The highest number of alleles (6) was detected on each of locus UBC 809, UBC 834, UBC 841, UBC 842, UBC 873 and the lowest number of alleles (2) was detected on locus UBC 824. The polymorphism percentage ranged from 33.33% (primer UBC 834) to 100% (primer UBC 824, UBC 841, UBC 856) with an average polymorphism of 60% across all the genotypes. The UPGMA based clustering analysis using similarity coefficient grouped these genotypes into two major and three sub-clusters. Cluster I and II consists of 24 genotypes. The grouping resembled the ancestry of the genotypes under study.

Keywords: Rice, Molecular diversity, Grain length, ISSR

Introduction
Rice is the staple food for two thirds of the Indian population. It contributes 43 per cent of caloric requirement and 20-25% of agricultural income. In India, rice is grown in an area of 43.5 million ha (23% of gross cropped area) with an annual production of 90 million tons (Viraktamath and Sundaram, 2010) [19]. Rice and agriculture are still fundamental to the economic development of most of the Asian countries. In much of Asia, rice plays a central role in politics, society and culture, directly or indirectly employs more people than any other sector. A healthy rice industry, especially in Asia's poorer countries, is crucial to the livelihoods of rice producers and consumers alike.

Landraces are the local or traditional varieties of a domesticated plant species which have developed over time through adaptation to their natural environment (Jones *et al*., 2008) [5]. The demand for productive and homogenous crops has led to development of a small number of standard, high yielding varieties. This has consequently resulted to tremendous loss of heterogeneous traditional cultivars through genetic erosion. Landraces preserve much of this lost diversity and are known to harbor great genetic potential for breeding new crop varieties that can cope with environmental and demographic changes. There are more than 400,000 rice varieties worldwide but the major categories include; indica, japonica, basmati and glutinous. These varieties differ in their grain qualities which include: milling quality, grain shape, cooking quality, nutritional quality and aroma.

Genetic improvement mainly depends on the amount of genetic diversity present in different genotypes in the population. The estimation of genetic diversity between different genotypes is the first and foremost process in plant breeding (Rajesh *et al*., 2012) [11]. Among numerous techniques available for assessing the genetic variability and relatedness among crop germplasm, DNA based markers provide very effective and reliable tools for measuring genetic diversity in crop germplasm and studying evolutionary relationships. Compared to morphological analysis, molecular markers can reveal differences among the genotypes at molecular level. They provide the information that helps in deciding the distinctiveness of species and their ranking according to the number of close relatives and phylo-genetic position (Rahman *et al*., 2007) [9]. Molecular markers also serve as a valuable tool to assess the genetic variation, varietal classification and germplasm identification of rice (Singh *et al*., 2006) [16].

ISSR markers are widely used for assessment of genetic variability in crop species. ISSR involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. ISSR uses long primers (15-30 bp), which permit the subsequent use of high anneling temperature leading to high stringency and reproducibility (Zietkiewicz *et al.*, 1994) [21]. This technique always allows the examination of genomic variation without prior knowledge of DNA sequences. These markers are considered unbiased and neutral marker for genetic mapping application. Moreover, these markers reveal high degree of polymorphism, generating reliable information for DNA analysis and with the necessary sensibility to distinguish genetically related individuals in rice (Joshi *et al*., 2000) [6].

Materias and Methods

The current research study was conducted at Research cum Instructional farm, College of Agriculture, IGKV, Raipur (C.G.), Department of Genetics and Plant Breeding and R. H. Richhariya research laboratory, College of Agriculture, IGKV, Raipur (C.G.). Rice genotypes used in this study are listed in the Table 1. Seeding was done in the well prepared seed beds. Fresh leaf samples from 15 days old seedlings were used for the extraction of DNA.

Table1: List of rice genotypes used in the current study

S.No.	Name	Source (Village/Block/Distt.)	S.No.	Name	Source (Village/Block/Distt.)
1	Lokti Machhi	Bade Rajpur/Bade Rajpur/Bastar	25	Farsa phool	Koyalibeda/Koyalibeda/Bastar
2	Atma Sital	Antagarh/Antagarh/Bastar	26	Jay Bajrang	Fingeshwar/Fingeshwar/Raipur
3	Lokti Machhi	Narayanpur/Narayanpur/Bastar	27	Gilas	Enhoor/Durgkondal/Bastar
4	ADT:27	Rajim/Fingeshwar/Raipur	28	Khatia pati	Odan/Palari/Raipur
5	Anjania	Pandarbhattha/Bemetara/Durg	29	Mani	Rajim/Rajim/Raipur
6	Kanak Jira	Dadesara/Durg/Durg	30	Khatriya pati	Odan/Palari/Raipur
7	Jhumera	Martara/Bemetara/Durg	31	Girmit	Kokodi/Kirnapur/Balaghat
8	Kakeda (I)	Kuamalji/Pandariya/Bilaspur	32	Lanji	Deverda/Baldevgarh/Tikamgarh
9	Dubraj II	Chandkhuri/Arang/Raipur	33	Banreg	Khutgaon/Deobhog/Raipur
10	Bhulau	Gidhpuri/Palari/Raipur	34	Ruchi	Kusumi/Kusumi/Sarguja
11	Rani kajar	Garra/Palari/Raipur	35	Safed luchai	Nagajhare/Barghat/Seoni
12	Sundar mani	Kodohatha/Deobhog/Raipur	36	Kanthi deshi	Vijaipali/Barghat/Seoni
13	Bhado kanker	Turanga/Pusaur/Raigarh	37	Piso III	Barghat/Barghat/Seoni
14	Jhumarwa	Charbhatha/Fingeshwar/Raipur	38	Kakdi	Kukanar/Darma/Bastar
15	Bishnu	Bishnupur/Baikundpur/Sarguja	39	Gajpati	Kosamghat/Ghar Ghoda/Bastar
16	Basa Bhog	Pratappur/Pratappur/Sarguja	40	Gadur sela	NA/Mohala/Rajnandgaon
17	Krishna Bhog	Mohgaon/Mandla/Mandla	41	Aadan chilpa	Kesherpal/Bastar/Bastar
18	Hira Nakhi	Khekha/Bichhiya/Mandla	42	Unknown	NA/NA/NA(CG)
19	Lokti Maudi	Abujhmad/Abujhmad/Bastar	43	Saja chhilau	Kanker/Kanker/Bastar
20	Kariya bodela bija	Kodo/Abujhmad/Bastar	44	Parmal Safri	Tilda/Tilda/Raipur
21	Gganja Kali	Kudum Kala/Ghar Ghoda/Raigarh	45	Safri	Varasioni/Waraseoni/Balaghat
22	Banas KupiII	Jhilwada/Waraseoni/Balaghat	46	Narved	Muraina/NA/Muraina
23	Dhangari Khusha	Darrabhatha/Saraipali/Raipur	47	Nagbel	Dev Bhog/Dev Bhog/Raipur
24	Bhaniya	Fashakar/Durgkondal/Bastar	48	Mudariya	Abhanpur/Abhanpur/Raipur

Genomic DNA Isolation

DNA was extracted from the leaves of rice genotypes following CTAB (Cetyl Tri-methyl Ammonium Bromide) method as described by Doyle and Doyle (1990) [2] with some modifications. Quantification of DNA was performed using UV visible spectrophotometer (Nanodrop, ND-1000 USA) and visualized on gel documentation unit (Flour Chem. ™ Alpha innotech, USA. ISSR profiling was performed using a set of 10 ISSR primers obtained from Biotechnology Laboratory, University of British Columbia, Canada.

PCR reaction

2µl of diluted template DNA of each genotype was dispensed at the bottom of PCR plate (AXYGEN). Separately cocktail was prepared in an Eppendorf tube as described in table. About 8µl of cocktail was added to each sample and the PCR were set up as the profile depicted in Table-2 and temperature profile used is depicted in Table-3.

Table 2: PCR mix for one reaction (Volume 20 µl)

Reagent Stock	concentration	Volume (□l)
Nanopure H2O	-	13.5
PCR buffer A	10 X	2.0
dNTPs (Mix)	1.0 mM	1.0
Primer (forward)	5 ρmol	0.5
Primer (reverse)	5 ρmol	0.5
Taq polymerase	1 U/ µl	0.5
DNA template	50 ηg/ µl	2.0
	Total	20

Table 3: Temperature profile used for PCR amplification using Inter-simple sequence repeats Markers

Steps	Temperature (°C)	Duration (min.)	Cycles	Activity
1	94	2	1	Denaturation
2	93	0.45		Denaturation
3	48-54	1	35	Annealing
4	72	1		Extension
5	72	8	1	Final Extension
6	4	∞		Storage

PCR products were separated in five percent polyacrylamide gels (vertical) by electrophoresis in 1X tris-boric acid- EDTA buffer. The DNA profiles were visualized on a UV trans illuminator in Gel Documentation.

Scoring and analysis of data

The banding pattern of population developed by each set of primer was scored separately. The size of amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 50 base pairs (bp) DNA ladder. Particular base pair position was scored as "1" and absence of band for that particular base pair position was scored as "0" (zero). For analysis NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) software was used to construct a UPGMA (Unweighted Pair Group Method with Arithmetic averages) dendrogram showing the distance-based interrelationship among the genotypes.

Result and Discussion

Assessment of genetic diversity and diversity based grouping of rice genotypes using molecular markers is the prime objectives of the study. Molecular markers, which assess genome sequence composition, enable detection of differences in the genetic information of the different genotypes; which is useful to asses and to utilize the genetic variability for breeding programme. The use of molecular markers to study the genetic diversity and relationships among the different cultivars has been previously reported by Davierwala *et al.*, 2000 [1], Neeraja *et al.*, 2002 [8], Saker *et al.*, 2005 [13].

The 10 primers yielded a total of 46 amplified fragments from 48 rice genotypes and out of these, 29 alleles were polymorphic. The number of scorable bands produced per primer ranged from 2 to 6 with an average of 4.6, and the average number of polymorphic fragments per primer was 2.9. The highest number of alleles (6) was detected on each of locus UBC 809, UBC 834, UBC 841, UBC 842, UBC 873 and the lowest number of alleles (2) was detected on locus UBC 824. Out of 10 ISSR markers, two makers UBC 818, UBC 885 exhibited monomorphic reaction for all the accessions whereas rest 8 showed polymorphic reaction. The polymorphism percentage ranged from 33.33% (primer UBC 834) to 100% (primer UBC 824, UBC 841, UBC 856) with an average polymorphism of 60% across all the 48 long and short grain length rice genotypes. Similar to our study Fatehi *et al.* (2011) [3] have found moderate level of average polymorphism (45%) in their studies. High level of polymorphism has been reported by Sofalian *et al.* (2008) [17], Zhu *et al.* (2011) [20] and Sadigova *et al.* (2014) [12]. The suitability of the ISSR technique for genetic diversity studies and germplasm evaluations has been shown in many studies (Shukla *et al*., 2011, Tiwari *et al*., 2013, Kumbhar *et al*., 2013 and Samal *et al*., 2014) [15, 18, 7, 14].

Table 4: List of 10 ISSR markers with their PIC value, No. of alleles percentage polymorphism found among 48 rice accessions

Marker	No. Of Alleles	PIC VALUE	Total No. of bands	No. of polymorphic bands	Percentage Polymorphism
UBC 808	3	0.25	3	2	66.67
UBC 809	6	0.29	6	5	83.33
UBC 818	3	0.00	3	0	0.00
UBC 824	2	0.50	2	2	100.00
UBC 834	6	0.07	6	2	33.33
UBC 841	6	0.50	6	6	100.00
UBC 842	6	0.07	6	3	50.00
UBC 856	5	0.46	5	5	100.00
UBC 873	6	0.15	6	4	66.67
UBC 885	3	0.08	3	0	0.00

Similarity coefficient analysis and Clustering

The relationships among rice genotypes were estimated by a UPGMA cluster analysis of genetic similarity matrices. ISSR similarity coefficient between different genotypes ranged from 0.52 to 1.00. Two major clusters were formed and are sub-divided into three sub-clusters. 1st cluster consists of 24 genotypes whereas 2nd cluster also consisted of 24 rice genotypes (Fig 1).

The accessions that are derivatives of genetically similar dropped in one group. Group I exhibited 76.5% similarity coefficient among all the accessions of the group which include 24 genotypes. It is further subdivided into three sub-clusters, sub-cluster I consists of 12 long grain genotypes which are Farasaphool, Jay Bajarang, Khatia Pati, Khatriya Pati, Ruchi, Kanthi deshi, Mani, Banreg, Gilas, Lanji, Girmit, Safed luchai. Sub- cluster II consists of 10 short grain genotypes which are Atma Sital, Dubraj II, Anjania, Kanak Jira, Jhumera, Kakeda (I), Bhulau, Rani Kajar, Sundar Mani and ADT: 27. Sub-cluster III comprised of two short grain genotypes that are Lokti Machhi and Lokti Machhi (II).

Group II consists of 24 genotypes at 84% similarity and sub-divided into three sub-clusters. Sub-cluster I consists of six short grain genotypes which are Kakdi, Basa Bhog, Krishna Bhog, Lokti Maudi, Kariya Bodela Bija and Bhaniya. Sub-cluster II consists of 16 genotypes which are Piso III, Gajan Kali, Banas Kupi III, Dhangri Khusha, Gajpati, Gadursela, Aadan Chilpa, Unknown, Saja Chhilau, Nagbel, Mudariya, Bhado Kanker, Jhumarwa, Bishnu, Narved and Hira Nakhi. Sub-cluster III comprised of two long grain genotypes that are Parmal Safri and Safri.

Rice similarity index reveals that high degree of similarity to the extent of 100% exists in many genotypes, in sub-group I

under group I Farsa Phool, Jay Bajrang, Khatia Pati, Khatriya Pati, and Ruchi shows 100% similarity. Again in same sub-group Mani and Banreg also exhibited 100% similarity. Again under group I, in sub-group II Anjania, Kanak Jira, Jhumera and Kakeda (I) shows 100% similarity whereas in same sub-group 100% similarity exists between Bhuau, Rani Kajar and Sundar Mani. Under group II in sub-group II 100% similarity exists between Gajpati, Gadursela, Adanchilpa, Unknown, Sajachhilau, Nagbel and Mudariya in the same sub-group Bhado Kanker, Jhumarwa and Bishnu shows 100% similarity, again in the same sub-group Ganja Kali, Banas Kupi III, and Dhangri Khusha shows 100% similarity.

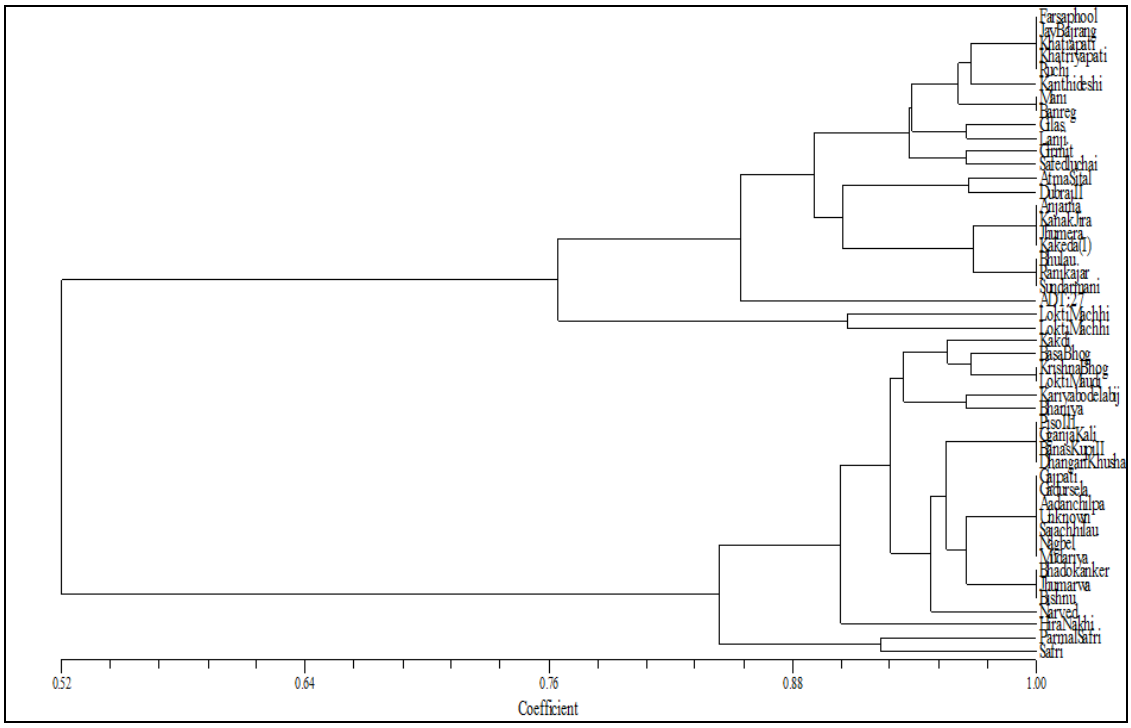

Fig 1: UPGMA-based molecular dendogram of ISSR marker showing 48 rice germplasm

Polymorphism Information Content

Polymorphism Information Content provides an estimate of determining power of a marker based on the number of alleles at a locus and relative frequencies of these alleles. PIC value represents the relative informativeness of each marker and in the present study, PIC values ranged between 0 for UBC 818 to 0.5 for UBC 824 and UBC 841 followed by 0.46 for UBC 856 with an average PIC value of 0.24 (Table 4). ISSR markers are frequently used for varietal diagnostic purposes in many crop species (Raina *et al*., 2001 and Gorji *et al*., 2011) [4]. Phenotypic analysis and genotypic analysis did not conceded with each other because the grain length is a quantitative trait and is affected by number of genes/ QTLs.

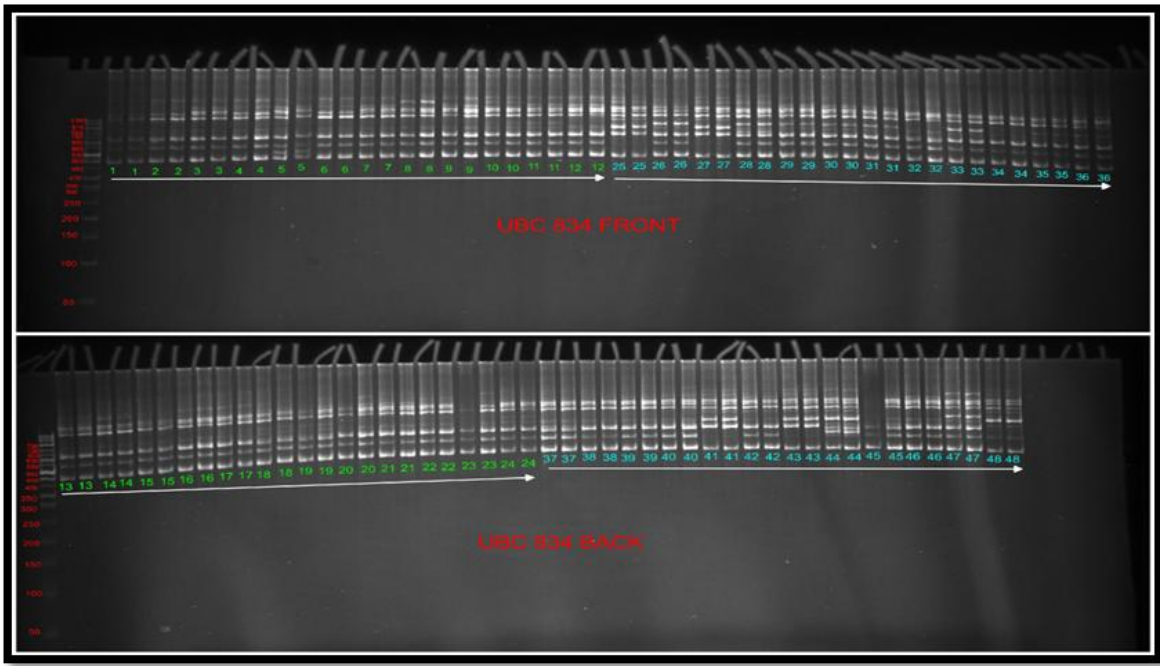

Fig 2: PCR amplification of 48 short and long grain accessions of rice with ISSR primer UBC 834.

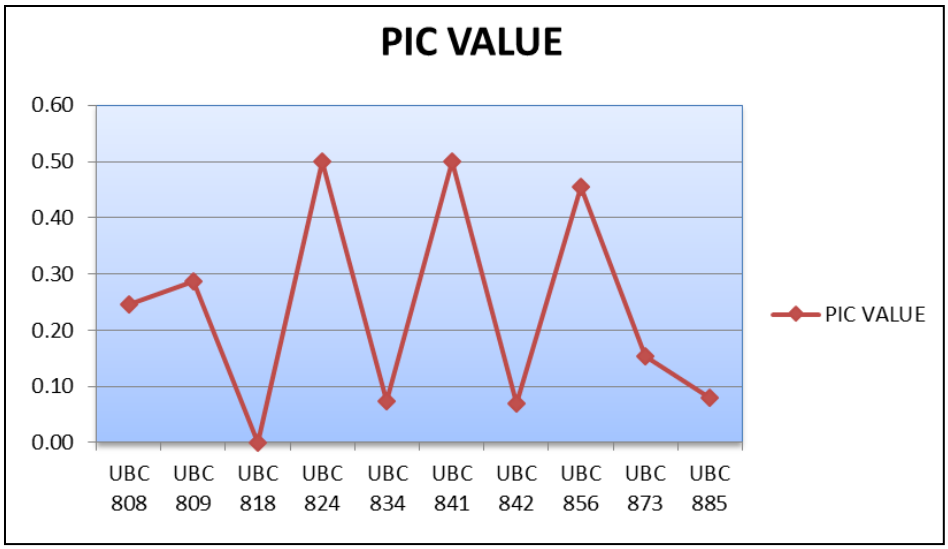

Fig 3: PIC value of ISSR marker

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