



Agro-morphological Characterization and Molecular Analysis of Rice Landraces Collected from Tribal Belts of Maharashtra

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ABSTRACT

Background: India has a large number of locally adapted landraces with specific traits like aroma, taste, medicinal properties, high nutritional value, disease resistance, etc. Genetic diversity in crop germplasm is estimated using morphological and molecular markers, which is an important criterion for plant improvement.

Methods: A total of 144 landraces and 16 farmers' varieties of rice collected from nine districts of Maharashtra were evaluated in a field trial at Jawhar block in the *kharif* season, which was conducted in a randomized block design (RBD) in triplicate during 2016 to 2019. The morphological characters were recorded as per DUS guidelines and among them, 45 landraces were evaluated by using molecular markers at the BAIF Development Research Foundation, Pune.

Result: Morphological characters revealed a wide range of variation within the landraces and farmers' varieties. The landraces *Singa*, *Masura*, *Padharwanji*, *Masuri*, *Sudi*, *Gadagutawanji*, *Pandari Luchi* and *Kasbai* gave significantly higher grain and straw yields. In addition, these landraces were tall and non-lodging. PCA analysis of the morphological characters showed character-specific grouping, also indicating higher variability for quantitative traits. The five Inter Simple Sequence Repeats (ISSRs) markers revealed 90.02% polymorphism. Dendrogram and PCoA revealed an absence of geographical structuring in the landraces, possibly due to the introduction of gene pools from different locations.

Key words: Agro-morphological characterization, DUS, Genetic diversity, ISSRs, Landraces.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal crop and primary staple food for more than half of the global population (<http://ricepedia.org/rice-as-food>). Rice is grown on 160.6 million hectares across the world with a total production of 738.20 million tonnes and a productivity of 3424.41 kg/ha (Kujur *et al.*, 2017). As one of the centres of origin for rice, India harbours a large number of landraces, which are adaptable to different agro-climatic zones with unique characteristics like aroma, taste and disease resistance. National Bureau of Plant Genetic Resources New Delhi (NBPGR), have been recorded and maintained more than 87000 rice landraces of the Indian subcontinent ([http://genebank.nbpgr.ernet.in/CropSpecieswith ICEC Wise.aspx?CropCode=2047](http://genebank.nbpgr.ernet.in/CropSpecieswith%20ICEC%20Wise.aspx?CropCode=2047)). Apart from dietary importance, many rice landraces are integral parts of traditional rituals and are used as medicine (Deb *et al.*, 2015).

The drive for high-yielding varieties of rice has unfortunately led to the extinction of thousands of landrace varieties and the remainder are rapidly approaching the same fate (Sangeetha *et al.*, 2020). In this scenario, the collection, restoration and conservation of this enormously valuable germplasm is a need of the hour. For the efficient utilization of germplasm, characterization, evaluation and development of a workable retrieval system is a prerequisite (Reddy *et al.*, 2006; Prajapati *et al.*, 2018) and it may serve as a valuable genetic resource for crop improvement programs (Sujata *et al.*, 2021). Characterization and evaluation of germplasm provide basic information on

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desirable traits for breeding programmes to produce improved varieties (Ferguson, 2007). Nowadays, many modern molecular tools are available for germplasm characterization; however, morphological markers have their own significance and field utility and also serve as an aid for germplasm identification. There are a series of reports on germplasm characterization and diversity analyses on rice landraces from the northeast (Hore, 2005; Parikh *et al.*, 2012; Sinha and Mishra, 2013; Roy *et al.*, 2015; Anupam *et al.*, 2017; Umakanth *et al.*, 2017).

Understanding the intricate interactions between rice diversity, cultivation practices and traditions can also be aided by studying the genetic diversity and relationships among landraces (Roy *et al.*, 2015). The western and eastern districts of Maharashtra have enormous diversity,

but systematic studies are scanty. To bridge this gap, here, rice landraces collected from various districts of Maharashtra and their genetic diversity was studied using morphological markers. In addition, genetic diversity of a small set of landraces were also analysed using molecular markers such as Inter simple sequence repeats (ISSRs). ISSRs are inter-tandem repeats of short DNA sequences, which provide a great potential to determine inter and intra-genomic diversity since they reveal variation within unique regions of the genome at several loci simultaneously (Al-Turki and Basahi, 2015). The ISSR makers were characterised by high polymorphism, informative and cost-effective in determining the genetic diversity of rice germplasm (Al-Turki and Basahi, 2015; Alhasnawi *et al.*, 2015; Singh and Sengar, 2015).

MATERIALS AND METHODS

Plant material collection and characterization

The 144 landraces and 16 farmers varieties of rice were collected from different farmers in Thane, Palghar, Raigarh, Nasik, Pune, Ahmednagar, Sindhudurg, Ratnagiri and Gadchiroli districts of Maharashtra during 2008-2016 (Supplementary Table 1).

The field study was conducted in a randomized block design (RBD) at Jawhar block with three replications in *kharif* seasons during 2016 to 2019. Around 8-21 days old individual seedlings were transplanted at 25 cm × 25 cm spacing. The recommended package of practices (POP) was followed to optimize plant growth. Observations on different morphological and agronomic characters of collected accessions were recorded at different stages of growth as per DUS guidelines (<http://plantaauthority.gov.in/crop-guidelines.htm>). The visual observations were recorded for 34 qualitative and 16 quantitative characters (Table 1).

DNA extraction

Out of 144 landraces, 45 landraces were randomly selected for the genetic diversity study. Liquid nitrogen was used to grind fresh leaves into a fine powder and the modified CTAB method was used to extract DNA (Murray and Thompson, 1980). The genomic DNA was examined for purity and quantity using 1% agarose gel electrophoresis and then diluted using Nanodrop (Thermo, India) to 20 ng/μl for PCR amplification. A set of five ISSR primers (Table 2), which were chosen based on unique and repeatable banding patterns and were used for genetic fingerprinting. PCR reactions were performed in 20 μl final volumes by adding 10 mM ISSR primer, 2X Go Taq green master mix (Promega, India) and 20 ng genomic DNA. PCR cycling was as follows; initial denaturation at 94°C for 5 minutes, 45 cycles at 94°C for 30 seconds, annealing at 50°C for 45 seconds, extension at 72°C for 60 seconds and finally a final extension step at 72°C for 5 minutes (MJ research PTC-200) (Nimbalkar *et al.*, 2018; Takawale *et al.*, 2019; Jade *et al.*, 2021). The amplified products were separated

Supplementary Table 1: List of landraces and farmers' varieties of rice used in the study.

Name of landrace/ Farmers variety	Accession no.	Biological status
Saag Bhat*	BAIF/Jawhar/Rice/2	Landrace
Masala Bhat*	BAIF/Jawhar/Rice/3	Landrace
Malghudya	BAIF/Jawhar/Rice/4	Landrace
Kolpi (Halvi)*	BAIF/Mokhada/Rice/5	Landrace
Dhundune	BAIF/Vikramgad/Rice/7	Landrace
Dangi(safed)*	BAIF/Jawhar/Rice/8	Landrace
Zini(Halvi))	BAIF/Shahapur/Rice/9	Landrace
Dhaval Bhat*	BAIF/Mokhada/Rice/10	Landrace
Dula-1	BAIF/Jawhar/Rice/11	Landrace
Kavla Bhat	BAIF/Jawhar/Rice/12	Landrace
Dangi Red*	BAIF/Jawhar/Rice/15	Landrace
Tamkudai*	BAIF/Jawhar/Rice/18	Landrace
Lalya	BAIF/shahapur/Rice/19	Landrace
Kali Khadsi-1	BAIF/Jawhar/Rice/20	Landrace
Vanai	BAIF/Jawhar/Rice/22	Landrace
Kolpi (Nimgarvi)*	BAIF/Mokhada/Rice/27	Landrace
Mahadi (Nimgarvi)*	BAIF/karjat/Rice/28	Landrace
Wada Zini*	BAIF/Wada/Rice/29	Landrace
Pacheki	BAIF/Jawhar/Rice/31	Landrace
Zini(Nimgarvi)	BAIF/Jawhar/Rice/34	Landrace
Varangal (Sukewali)	BAIF/Mokhada/Rice/35	Landrace
Chimansal*	BAIF/Trimbekshwar/Rice/40	Landrace
Surti Kolam*	BAIF/Wada/Rice/41	Landrace
Juna Kolam*	BAIF/Jawhar/Rice/47	Landrace
Rajghudya*	BAIF/Jawhar/Rice/49	Landrace
Gujrat zini*	BAIF/shahapur/Rice/51	Landrace
Kolpigarvi (Iahan)*	BAIF/Mokhada/Rice/53	Landrace
Sal bhat*	BAIF/Trimbakeshwar/Rice/56	Landrace
Najarbhat	BAIF/Jawhar/Rice/64	Landrace
Kasbai (Jawhar)	BAIF/Jawhar/Rice/67	Landrace
Mahadi(Garvi)*	BAIF/karjat/Rice/72	Landrace
Masuri (Shahapur)*	BAIF/shahapur/Rice/73	Landrace
Tornya*	BAIF/Vikramgad/Rice/76	Landrace
Vakvel	BAIF/Mokhada/Rice/77	Landrace
Banglya	BAIF/Jawhar/Rice/79	Landrace
Masura (Jawhar)*	BAIF/Jawhar/Rice/80	Landrace
Kasvel*	BAIF/Jawhar/Rice/81	Landrace
Dodgi	BAIF/Jawhar/Rice/83	Landrace
Javyachi gundi	BAIF/Mokhada/Rice/84	Landrace
Dula-2	BAIF/Jawhar/Rice/85	Landrace
Suklya	BAIF/shahapur/Rice/117	Landrace
Tulshya*	BAIF/Jawhar/Rice/125	Landrace
Raat Bhat*	BAIF/Dahisar/Rice/131	Landrace
MurbadMahadi*	BAIF/Murbad/Rice/134	Landrace
Masuri (Jawhar)	BAIF/Jawhar/Rice/136	Landrace
Sonphal*	BAIF/panvel/Rice/140	Landrace
Kala dhavul-New*	BAIF/Jawhar/Rice/146	Selection
Kala girvel-new*	BAIF/Jawhar/Rice/147	Selection

Supplementary Table 1: Continue....

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Kala bagad-New	BAIF/Jawhar/Rice/148	Selection
Gavatizini*	BAIF/murbad/Rice/150	Landrace
Masura (Murbad)*	BAIF/murbad/Rice/152	Landrace
Jira zini*	BAIF/murbad/Rice/153	Landrace
Kasbai-1	BAIF/Talasari/Rice/286	Landrace
Kasbai-1	BAIF/Talasari/Rice/291	Landrace
Kali Khadsi-2	BAIF/Jawhar/Rice/319	Landrace
Ashwini*	BAIF/Jawhar/Rice/366	Farmer
		variety
Kirti*	BAIF/Jawhar/Rice/367	Farmer
		variety
Sadhana*	BAIF/Jawhar/Rice/368	Farmer
		variety
Kamal bhat*	BAIF/Jawhar/Rice/369	Farmer
		variety
Hali Kolapi*	BAIF/Akole/Rice/3	Landrace
Tamkudai*	BAIF/Akole/Rice/2	Landrace
Kalbhat*	BAIF/Akole/Rice/1	Landrace
Jirvel*	BAIF/Akole/Rice/8	Landrace
Khadkya*	BAIF/Junner/Rice/21	Landrace
GariKolapi*	BAIF/Akole/Rice/11	Landrace
Raibhog Kusalya*	BAIF/Akole/Rice/24	Landrace
Raibhog Tambada*	BAIF/Akole/Rice/6	Landrace
Juna 57*	BAIF/Junner/Rice/10	Landrace
Ambemohor*	BAIF/Junner/Rice/9	Landrace
Varangali	BAIF/Akole/Rice/13	Landrace
Dhavul	BAIF/Akole/Rice/15	Landrace
Manor	BAIF/Akole/Rice/20	Landrace
Noon	BAIF/Etapalli/Rice/1	Landrace
Lal Luchi	BAIF/Etapalli/Rice/2	Landrace
Kokde	BAIF/Etapalli/Rice/3	Landrace
Gandha	BAIF/Etapalli/Rice/4	Landrace
Khodakuri	BAIF/Etapalli/Rice/6	Landrace
Brahma Luchi	BAIF/Etapalli/Rice/7	Landrace
Yerekusma	BAIF/Etapalli/Rice/8	Landrace
Sudi	BAIF/Etapalli/Rice/9	Landrace
DeepaKode	BAIF/Bhamragad/Rice/10	Landrace
Pitris	BAIF/Etapalli/Rice/11	Landrace
PandariLuchi	BAIF/Etapalli/Rice/12	Landrace
Katewanji	BAIF/Etapalli/Rice/13	Landrace
Lal kabra	BAIF/Etapalli/Rice/14	Landrace
Yermaluchi	BAIF/Etapalli/Rice/15	Landrace
Godal	BAIF/Etapalli/Rice/16	Landrace
Koteng	BAIF/Bhamragad/Rice/17	Landrace
Selkawanji	BAIF/Bhamragad/Rice/18	Landrace
Ehwanji	BAIF/Etapalli/Rice/19	Landrace
Badge	BAIF/Etapalli/Rice/20	Landrace
Singa	BAIF/Etapalli/Rice/21	Landrace
Sapri	BAIF /Etapalli/Rice/22	Landrace
Karja	BAIF /Bhamragad/Rice/23	Landrace
Gadagutawanji	BAIF /Bhamragad/Rice/24	Landrace

Supplementary Table 1: Continue....

Supplementary Table 1: Continue....

Batasi	BAIF /Etapalli/Rice/25	Landrace
Dhopachuding	BAIF /Bhamragad/Rice/26	Landrace
RengalLuchi	BAIF /Bhamragad/Rice/27	Landrace
Chivkus	BAIF /Etapalli/Rice/28	Landrace
Padharwanji	BAIF /Etapalli/Rice/29	Landrace
MothiLuchi	BAIF /Etapalli/Rice/30	Landrace
Goti	BAIF /Etapalli/Rice/32	Landrace
Nomoku	BAIF /Etapalli/Rice/33	Landrace
Sambalpuri	BAIF /Etapalli/Rice/34	Landrace
DRK-1	BAIF /Nagbhid/Rice/35	Farmer
		variety
DRK-2	BAIF /Nagbhid/Rice/36	Farmer
		variety
DRK-3	BAIF /Nagbhid/Rice/37	Farmer
		variety
HMT	BAIF /Nagbhid/Rice/38	Farmer
		variety
Vijay Nanded	BAIF /Nagbhid/Rice/39	Farmer
		variety
Nanded 92	BAIF /Nagbhid/Rice/40	Farmer
		variety
GRK	BAIF /Nagbhid/Rice/41	Farmer
		variety
Nanded Hira	BAIF /Nagbhid/Rice/42	Farmer
		variety
Kate HMT	BAIF /Nagbhid/Rice/43	Farmer
		variety
NandedChennur	BAIF /Nagbhid/Rice/45	Farmer
		variety
Kate Chennur	BAIF /Nagbhid/Rice/47	Farmer
		variety
Jay Shriram	BAIF /Nagbhid/Rice/48	Farmer
		variety
Kular	BAIF /Bhamragad/Rice/53	Landrace
Karishma	BAIF /Etapalli/Rice/57	Landrace
Parvatichennur	BAIF /Lakhni/Rice/58	Landrace
JunaKesar	BAIF /Lakhni/Rice/59	Landrace
Shriram	BAIF /Lakhni/Rice/60	Landrace
Kudyarak	BAIF /Etapalli/Rice/61	Landrace
Ludkus	BAIF /Etapalli/Rice/62	Landrace
Pikosang	BAIF /Etapalli/Rice/63	Landrace
Gadulsinga	BAIF /Etapalli/Rice/64	Landrace
SutWanji	BAIF /Etapalli/Rice/65	Landrace
Gujrating	BAIF /Etapalli/Rice/66	Landrace
Bodhalavanji	BAIF/Etapalli/Rice/67	Landrace
Ludaki	BAIF/Bhamragad/Rice/73	Landrace
Koshawar	BAIF/Bhamragad/Rice/74	Landrace
Walaylal	BAIF/Kudal/Rice/28	Landrace
JaadMunga	BAIF/Ratnagiri/Rice/38	Landrace
PandhariWalay	BAIF/Kankavali/Rice/29	Landrace
Sorti	BAIF/Kudal/Rice/30	Landrace

Supplementary Table 1: Continue....

Supplementary Table 1: Continue....

Lal Patni(digas)	BAIF/Kudal/Rice/49	Landrace
Lal Patni	BAIF/Ratnagiri/Rice/50	Landrace
BarikPatni	BAIF/Ratnagiri/Rice/39	Landrace
Safed Bela	BAIF/Kudal/Rice/36	Landrace
Masura (Kudal)	BAIF/Kudal/Rice/66	Landrace
Ghati Pankaj –Lal	BAIF/Kudal/Rice/42	Landrace
YelkatPatni	BAIF/Kudal/Rice/55	Landrace
Kothimbiri	BAIF/Kudal/Rice/52	Landrace
Yalkat(Hirlok)	BAIF/Kudal/Rice/51	Landrace
KharaMunga	BAIF/Kudal/Rice/37	Landrace
Khochri	BAIF/Kankavali/Rice/46	Landrace
Pathere Bhat	BAIF/Ratnagiri/Rice/56	Landrace
Karmali	BAIF/Kudal/Rice/64	Landrace
Dodat	BAIF/Kudal/Rice/40	Landrace
Sanana	BAIF/Kudal/Rice/59	Landrace
Bela	BAIF/Kudal/Rice/33	Landrace
Rajvel	BAIF/Kankavali/Rice/57	Landrace
Saravat	BAIF/Ratnagiri/Rice/69	Landrace
Mahadi	BAIF/Ratnagiri/Rice/70	Landrace
Damaga	BAIF/Kudal/Rice/72	Landrace
Sonfal	BAIF/Vaibhawnadi/Rice/73	Landrace
Munga	BAIF/Vengurle/Rice/74	Landrace
Walay-Lal(Pendur)	BAIF/Malavan/Rice/75	Landrace
GhatiVarangal	BAIF/Kudal/Rice/76	Landrace
Yalkat(Bav)	BAIF/Kudal/Rice/77	Landrace
Ghati Pankaj-safed	BAIF/Kudal/Rice/67	Landrace

*Samples used for marker analysis.

using 2.0% Ethidium Bromide staining and a gel documentation system (BIO-RAD, India) was used to document the results.

Data analysis

Morphological analysis

Analyses of variance were performed on each morphological data to test the significance of variation between landraces by using Microsoft Excel 2016. Principal Component Analysis (PCA) was performed by using PAST software version 4.4 (Hammer *et al.*, 2001).

Molecular marker

Every identifiable and well-resolved fragment was manually rated for band presence (1) or absence (0). Polymorphism information content (PIC), which detects polymorphism within a population by considering the number of alleles that are expressed and the relative frequencies of those alleles that are expressed and the relative frequencies of those alleles, was calculated (Nagy *et al.*, 2012). The ISSR primer index (SPI) was calculated by adding the PIC values of loci amplified by a given primer. The binary data were used for the Unweighted Pair Group Method with Arithmetic Means (UPGMA) cluster analysis. PAST software was used for the construction of the Dendrogram, PCOA (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

Qualitative characters

Polymorphism was found in 31 of the 34 qualitative traits studied; the non-polymorphic traits were the presence of leaf auricle, collar and ligule. Coleoptile colour, varied as colourless (39%), purple (14%) and green (47%). Basal leaf sheath colour is green (84.4%), light purple (8.1%), purple line (6.9%) and uniform purple (0.6%). Leaf intensity of green colour, light (11.9%), medium (41.3%), dark (46.9%). Only eight landraces had anthocyanin coloration on leaf margins and two landraces with anthocyanin coloration on leaf tips. The anthocyanin coloration was observed on the leaf sheath of 34 landraces and the intensity of purple colour varied as very weak (2 landraces), weak (12 landraces), medium (7 landraces), strong (11 landraces), very strong (2 landraces). In auricles colour, colourlessness was the dominant character (95%) over light purple (3%) and purple (2%). The anthocyanin colouration of the collar was observed only in 11% of landraces. Ligule colour varied as white (84%), light purple (15%) and purple (1%). The anthocyanin-coloured stem was observed only in 16% of landraces.

Leaf pubescence varied as weak (33%), medium (54%), strong (6%) and very strong (7%). The ligule shape varied as split (79%), truncate (11%) and acute (10%); Flag leaf blade attitude was observed as erect (78%), semi-erect (19%), horizontal (3%) and deflexed (5%). Erect flag leaf is one of the important trait related to high-yielding ability (Yan *et al.*, 2012) in present study, 44% of landrace had erect flag leaf and these could be potential donors of the trait for high-yielding.

Culm attitude was observed as erect (69%), semi-erect (11%), open (20%) while culm strength was observed as strong (47%), moderately strong (33%), intermediate (18%) and weak (3%). The culm strength correlated with lodging trait so strong culm strength could be used as a donor in the development of non-lodging varieties.

The curvature of the panicle main axis varied as straight (6%), semi-straight (31%), deflexed (38%) and dropping (25%). Panicle secondary branching was observed as strong (54%), weak (21%) and clustered (25%). Secondary branching is an important yield-contributing trait and landraces with strong and clustered landraces could be utilized in combination with other traits to increase the potential yield of rice landraces. Ogunbayo *et al.* (2014) observed 37% of the genotypes with strong panicle secondary branching. The attitude of panicle branches varied as erect (5%), erect to semi-erect (24%), semi-erect (19%), semi-erect to spreading (39%) and spreading (13%). Panicle exertion was observed as partly exerted (3%), mostly exerted (28%) and well-exerted (70%); panicle threshability easy (33%), intermediate (57%) and difficult (9%). The sustenance of panicle exertion through maintaining growth during moisture stress was found as a significant trait associated with the grain yield through minimizing spikelet sterility (Subashri *et al.*, 2009) and most

(70%) studied landraces that well-exserted panicles are potential trait donors in a breeding program.

Lemma and palea colour was one most diverse character among that straw colour (51%) was most dominant trait over brown furrows on straw (31%), brown (9%), gold furrows on straw background (4%), reddish to light purple (2%), black (1%) and brown spots on straw background (1%). (Sinha *et al.*, 2015) also recorded similar observations like straw coloured lemma palea as the dominant trait while black coloured lemma palea least dominant trait in the West Bengal rice collection. The hairiness of lemma and palea was observed as short hairs (59%), hairs on upper portion (35%), hairs on lemma keel (4%) and glabrous and long hairs 1% for each. Apicus colour was also a diverse character, varied as straw (64%), brown (22%), white (8%), black (3%), red and red apex 2% for each. Only 12% of landraces were observed with awns; awn colour varied as yellowish white (4%), yellowish brown (3%), red (2%), 1% landraces each for reddish brown, light red and black; awn length could be classified as very short (1%), short (4%), medium (3%) and long (4%). The presence of awn, length and colour of awn were unique characters and can be utilized as morphological markers for specific landraces. Parikh *et al.* (2012) reported 72.3% of genotypes in their collection were awnless.

The grain shape observed as short slender (10%), short bold (33%), medium slender (12%), long bold (34%), long slender (8%), long slender basmati type (3%) and extra-long slender (1%). The grain colour varied as white (69%), light red (13%), red (14%), dark red (2%), light brown (1%) and dark brown (1%), while only 15% of landraces were scented. Traits like decorticated grain index, colour and aroma have a direct effect on the marketability or commercial success of rice cultivars (Sinha *et al.*, 2015). Among the studied accessions *DRK-2* and *Parvati chennur* had long slender, white and scented grains, while *Dula-2*,

Pitris, *Suklya*, *Tulshya* and *Vakvel* had medium slender, white and scented grains.

Quantitative characteristics

The mean values of quantitative characters of rice landraces are illustrated in Table 1. According to the results of the analysis of variance, every character among the accessions was highly significant. The studied landraces showed leaf length and width ranged between 10.7 to 71.8 cm and 0.5 to 2.3 cm, respectively. Sinha and Mishra (2013) reported such a wide range in leaf length in the West Bengal rice collection. Productive tillers per plant varied from 3.33 (*Saag bhat*) to 33.8 (*Hali Kolapi*); average grains per panicle were 181.2, minimum grains were observed in *Kalikhads-2* (50) and maximum grains in *Kasbai* (401); panicle length ranged between 9.8 cm (*Lal-Patani*) to 35.0 cm (*Chimansaal*). The 1000-grain weight ranged between 9.8 g (*Zini*) to 42.3 g (*Kala Dhavul*). Traits such as thousand grain weight, number of productive tillers per plant, grains per panicle and flag leaf length may be effectively utilized as selection indices to enhance yield in rice breeding programs (Hariharan *et al.*, 2025). Grain length ranged between 5.4 mm and 10.6 mm, while decorticated grain length ranged from 3.9 mm to 8.0 mm. Grain width ranged between 1.6 mm and 4.3 mm, while decorticated grain width ranged from 1.2 mm to 3.7 mm.

Days required for crop maturity varied from 75 to 155 days in studied accessions. *Namoku* required a minimum span (75 to 80 days) for maturity, while landrace *Kasbai* required a maximum time (150 to 155 days) for maturity. In general, depending on days to maturity, rice germplasm is classified as very early (<100 days), early (101-120 days), medium (121-140 days), late (141-160) and very late (>160 days). In the present study, 38 accessions required less than 100 days for maturity *i.e.* very early maturing and 62 accessions required 101 to 120 days (early maturing) for crop maturity. The plant height varied

Table 1: Quantitative characters of rice landraces.

Characters	Min	Max	Mean	SD	SEM ($\pm$)	CV%
Leaf blade length (cm)	10.7	71.8	42.5	11.78	0.93	27.7
Leaf blade width (cm)	0.5	2.3	1.2	0.39	0.03	33.34
Panicle length (cm)	9.8	35	23.8	4.03	0.32	16.91
Productive tillers/plant (Nos)	3.3	33.8	14.5	5.56	0.44	38.23
Days to maturity	75	154	113.4	17.06	1.69	15.05
1000-grain weight (g)	9.8	42.3	21.5	6.26	0.5	29.17
Grain length (mm)	5.4	10.6	8.1	0.93	0.07	11.52
Grain width (mm)	1.6	4.3	2.7	0.47	0.04	17.65
Decorticated grain length (mm)	0.6	8.0	5.9	0.8	0.06	13.43
Decorticated grain width (mm)	1.2	3.7	2.2	0.44	0.03	19.71
Plant height (cm)	55.7	174.3	116.9	25.06	1.98	21.44
No. of grains per panicle	50	401	181.2	74.45	5.89	41.08
Kernel shape index	1.6	5.3	3.1	0.66	0.05	21.19
Grain yield (q/ha)	16.1	59.9	38.6	10.54	0.83	27.3
Straw yield (q/ha)	29.5	98.5	60.2	17.29	1.37	28.74

from 55.7 cm (*Ghativarangal*) to 174.3 cm (*Kasbai-Jawhar*). The plant height of very early maturing accessions varied between 70.9 cm (*Lal Patni-Pirdavane*) and 156 cm (*Katewanji*); grain yield varied from 16.12 q/ha (*Namoku*) to 57.74 q/ha (*Gandha*), while straw yield varied from 29.64 q/ha (*Murbad-Mahadi*) to 90.35 q/ha (*Pandharwanji*). Plant height of early maturing accessions ranged between 56.8 cm (*Sanana*) and 162.17 cm (*Sadhana*); grain yield varied from 20.56 q/ha (*Kala Bagad-New*) to 55.05 q/ha (*Kular*), while straw yield varied from 29.48 q/ha (*Zini-Nimgarvi*) to 98.50 q/ha (*Pandhariluchhi*). The 52 accessions required 121 to 140 days (medium maturing) for crop maturity. Plant height of medium maturing accessions ranged between 55.7 cm (*Ghati-Varangal*) to 174.30 cm (*Kasbai-Jawhar*); grain yield varied from 22.50 q/ha (*Munga*) to 55.89 q/ha (*Jayshriram*), while straw yield varied from 35.70 q/ha (*Mahadi-Garvi*) to 98.50

q/ha (*Masura*). Only 5 accessions were recorded as late (140-160 days). Plant height ranged between 91.8 cm (*Yelkat-Patani*) and 148.17 cm (*Kasbai-1*); grain yield varied from 28.20 q/ha (*Yelkat-Patani*) to 59.85 q/ha (*Kasbai-2*), while straw yield varied from 70.69 q/ha (*Masura*) to 87.50 q/ha (*Karmali*). The traits grain yield per plant, number of productive tillers per plant, days to 50% flowering, days to maturity, number of grains per panicle and plant height contributes most to genetic diversity (Ashok *et al.*, 2017). Landrace *Namoku* produced the lowest grain yield (16.12 q/ha), while *Kasbai-2* produced the highest grain yield (59.85 q/ha). Landrace *Zini-nimgarvi* produced the lowest straw yield (29.48 q/ha) and landrace *Pandhariluchi* and *Masura* produced the highest (98.50 q/ha) straw yield.

Accessions *Singa*, *Masura*, *Padharwanji*, *Masuri*, *Sudi*, *Gadagutawanji*, *Pandari Luchi* and *Kasbai* found superior

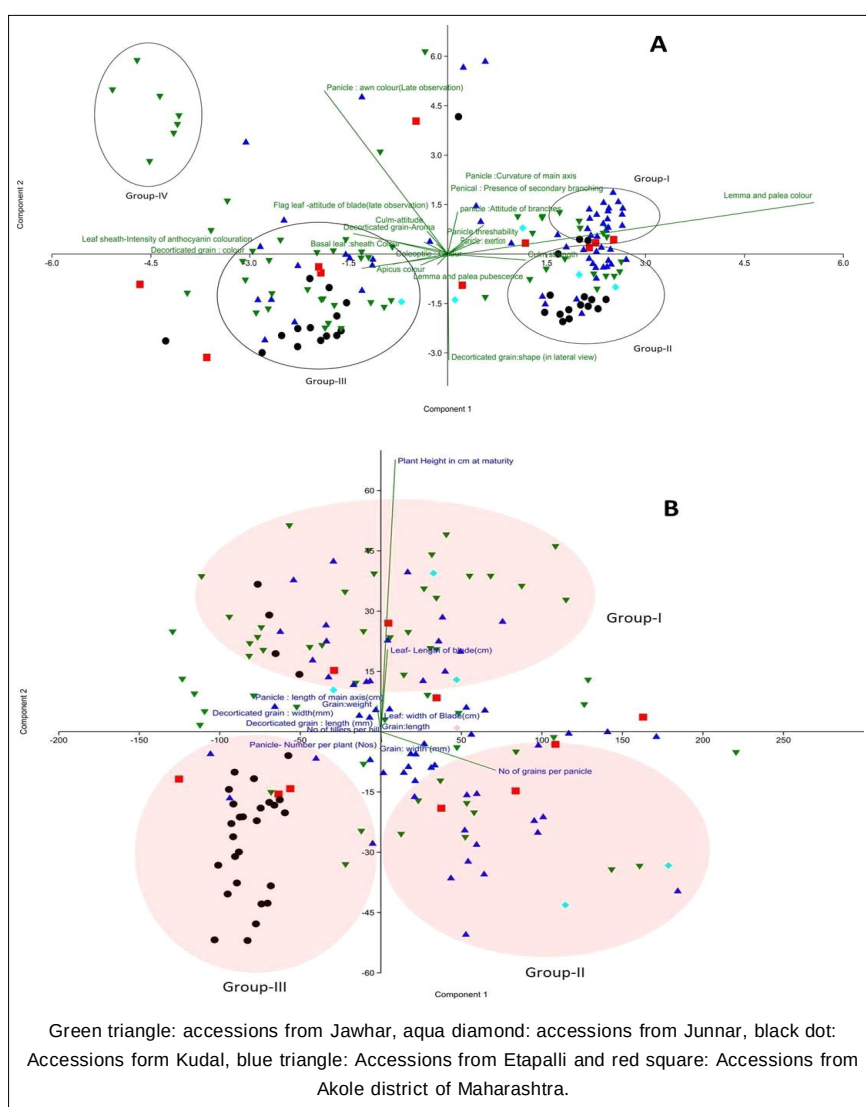


Fig 1: Principal component analysis (PCA) showing grouping of rice landraces with reference base on A) Qualitative traits. B) Quantitative traits.

with respect to high grain yield, non-lodging, straw yield and plant height. These landraces are being contributed to fodder security as they have better straw yield.

Principal component analysis (PCA)

PCA has proven to be a very useful multivariate analysis method that allows for the simultaneous analysis of many measurements on each individual (Ashok *et al.*, 2017); therefore, widely used for genotype selection and genetic diversity analysis. In the present study, PCA was carried out to determine the variability within the accessions for qualitative and quantitative traits. PCA (Fig 1A and 1B) revealed 42.89% variation by the first two PCs for the qualitative traits, suggesting low variability for the qualitative traits. Whereas, 97.23% for quantitative traits among the landraces contributed by the first two PCs, indicating higher variability for quantitative traits. Ashok *et al.* (2017) studied 64 rice genotypes for heritable diversity and reported that the first eight PCs contributed to 83.97% variability for yield and morphological characters. The 72.48% variability was recorded by the first four PCs for morphological characters for 124 accessions of rice (Pachauri *et al.*, 2017).

PCA for qualitative traits (Fig 1A) demonstrated that Group I contain 18 landraces from the Etapalli, 5 landraces from Jawhar and 2 landraces each from Akola and Kudal were associated with the vector of traits lemma and pale colour. Group II contains 18, 9, 12 and 2 landraces from Etapalli, Jawhar, Kudal and Junnar areas, respectively, associated with the vector decorticated grain shape (in lateral view) and culm strength. Group III was the larger group than the others and was composed of 50 landraces. Moreover, this group is composed of various vector traits such as lemma and palea pubescence, apicus colour, coleoptile colour and basal leaf: sheath colour, leaf sheath intensity of anthocyanin coloration and decorticated grain: colour. Group IV is mainly associated with the vector trait panicle awn colour and contains the 7 landraces from the Jawhar area. PCA for quantitative traits (Fig 1B) resulted in three main groups. Group I associated with vector traits plant height, Leaf length of blade and contains 55 landraces. Group-II contains 28 landraces associated with vector number of grains per panicle and grain width. Whereas, Group III was composed of 36 landraces associated with the vector trait panicle number per plant.

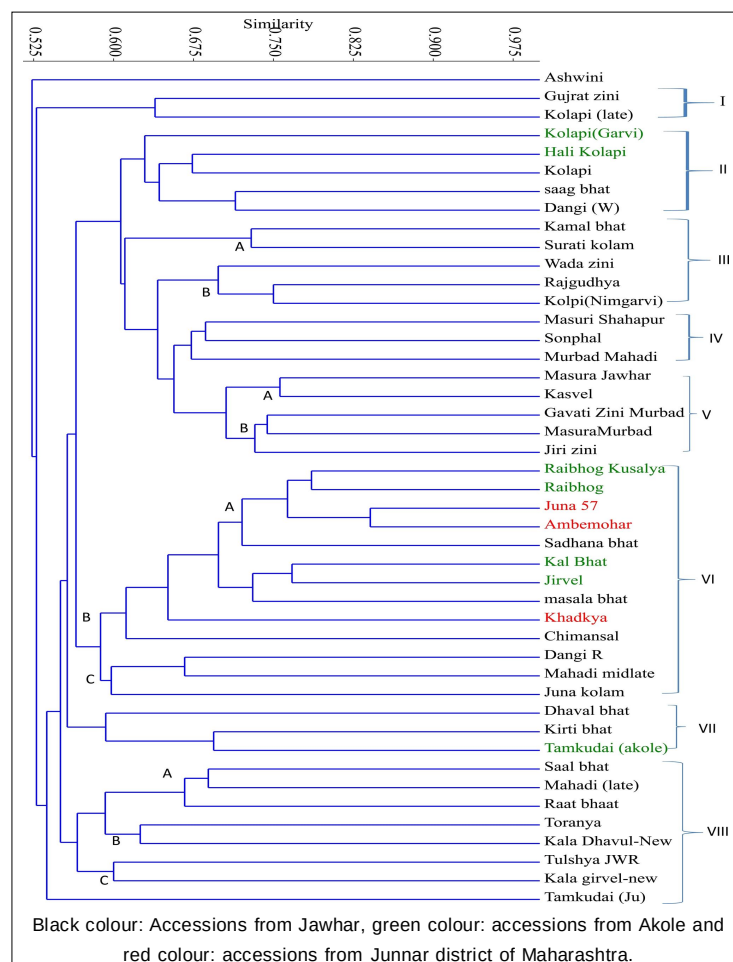
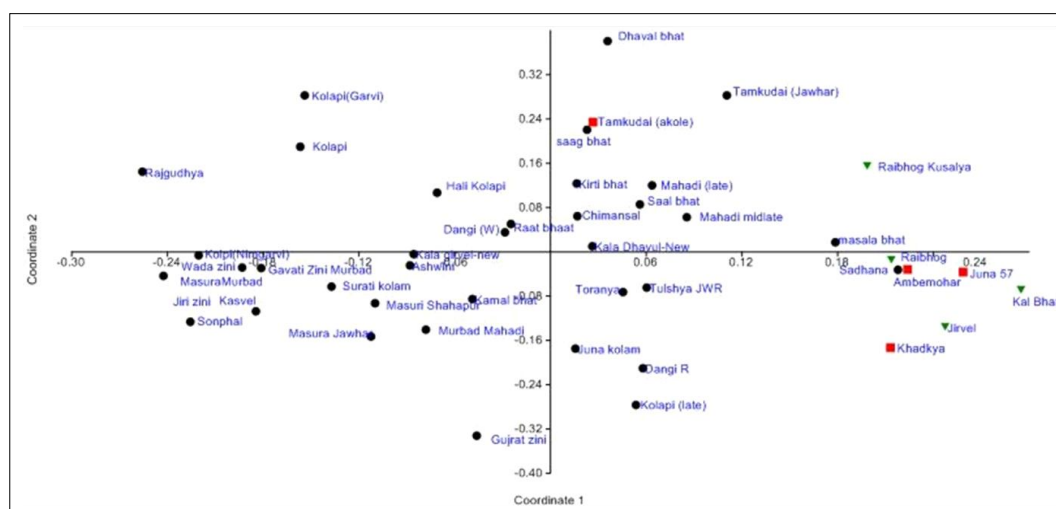


Fig 2: UPGMA cluster analysis showing the genetic relationship among 45 rice landraces generated by using 5 ISSR primers.

Table 2: Details of five ISSR primers use for generating fingerprinting profiles in 45 rice landraces, total number of loci, polymorphic loci, percentage polymorphism, polymorphic information content, primer index.

Primer sequences (5'-3')	Number of amplified loci	Total number of polymorphic loci	Percentage polymorphism	Polymorphic information content (PIC)	ISSR primer Index (SPI)
(CT) 8G	23	23	100	0.37	15.9
(AG) 8YC*	27	26	96	0.41	18.2
(AC) 8YT*	24	23	96	0.33	14.5
(CA) 8RC*	17	11	65	0.47	19.3
(GA) 8T	15	12	80	0.5	21.7
Total	106	95	90		

* Y= C or T (Pyrimidine), R = A or G (purine).

**Fig 3:** Scatter plot Principal coordinate analysis of 45 rice landraces using binary data of ISSR markers, indicating no geographical structuring, Black dots: Jawhar; red square: Junnar; Green inverted triangle: Akole district of Maharashtra.

Molecular marker analysis

Out of 12 primers tested, five dinucleotide repeat ISSR primers were found informative. Polymorphic bands ranged from 17 (ISSR-9) to 27 (AG8YC) (Table 2). A total of 106 bands were recorded for five primers, out of which 95 bands were found polymorphic. Overall, 89.62% polymorphism was observed. Primer CT8G scored 100% polymorphism, whereas primer ISSR09 scored 65% polymorphism and the other three primers scored 80 to 96% polymorphism (Table 2). Al-Turki and Basahi (2015) reported 90.02% polymorphism from 11 different ISSR primers for 27 Hassawi rice genotypes, whereas 75 to 100% polymorphism from 10 ISSR primers for rice was reported by Singh and Sengar (2015). The dinucleotide repeats ISSR primers were found to be very informative and yielded higher polymorphism results are analogous to the previous reports (Al-Turki and Basahi, 2015; Nimbalkar *et al.*, 2018; Takawale *et al.*, 2019). Polymorphic Information Content (PIC) refers to the value of a marker for detecting polymorphism within a population, based on the number of present alleles and the frequency of their distribution (Singh and Sengar, 2015). PIC values obtained ranged from 0.33 to 0.5, whereas, The PIC values of ISSR markers used to assess the genetic diversity of rice genotypes, as reported by Jegadeeswaran *et al.* (2024) ranged from

0.359 to 0.846 and SPI values ranged from 14.5 to 21.7. The dendrogram revealed all 45 landraces grouped into 8 major clusters with 10 sub-clusters and landrace *Ashwini* was out-grouped. Cluster VI represented the highest number of landraces, followed by cluster VIII. The dendrogram indicated that the genetic variation of most of the landraces did not resemble geographical structuring (Fig 2).

Principal coordinate analysis (PCoA) (Fig 3) demonstrated all landraces intermix across the coordinates, supporting the dendrogram that confirms the absence of geographical structuring in the landraces. It might be due to the introduction of a gene pool from different locations in the course of the development of rice varieties. Similar results were reported by (Singh *et al.*, 2013) for Indian rice varieties.

CONCLUSION

Higher genetic diversity in rice was observed for morphological parameters. The landraces *Singa*, *Masura*, *Padharwanji*, *Masuri*, *Sudi*, *Gadagutawanji*, *Pandari Luchi* and *Kasbai* gave significantly higher grain and straw yields. In addition, these landraces were tall and non-lodging. These landraces can be utilized for further rice breeding programmes. The overall molecular analysis revealed the absence of geographical structuring.

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Conflict of interest

All authors declared that there is no conflict of interest.

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