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Genetic diversity analysis in rice (*Oryza sativa* L.) germplasm of Jammu region of Jammu and Kashmir

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Abstract

The rice landraces/farmers' varieties maintained by farming communities over centuries under diverse agro-climatic situations of Jammu region remained unexploited for utilizing useful allelic diversity in breeding programs. A set of 25 rice genotypes including landraces, introductions, farmers' collections and released varieties were subjected to morphological characterization considering panicle traits, spikelet descriptors and 13-grain yield attributing traits. Molecular characterization of the germplasm was also done using a set of 30 microsatellites (SSR) markers. The genotypes showed highly significant variations for panicle length, panicles per plant, no. of tillers per plant, grain length, 1000 grain weight and grain yield per plant. Principal component analysis revealed that GIZA14 exhibited maximum distance from other genotypes. Based on multivariate analyses, the traditional and local genotypes, including landraces, showed maximum genetic distance from released check genotypes. The analyses based on genotyping with SSR markers indicated closer genetic similarity within Farmers' collection from Kishtwar (KRC) and Paddar (PRC) groups, suggesting their common origin. Basmati genotypes were grouped together in one sub-cluster, and many of the farmers' collections/landraces with check varieties in another indicated that many of the farmers' collections were released (used as check varieties), saved and recycled by farmers over the years. PRC 2, PRC 3, and Ratta Ranjha were grouped closer. Thus, molecular marker (SSR) based genomic characterization of genotypes supported by matching phenotypic characterization patterns proved helpful in characterizing genetic diversity, identifying diverse and similar genotypes, and identifying specific genotypes for specific traits of agronomic/economic importance.

Keywords: Rice, landraces, SSRs, farmer's collections, molecular characterization, morphological characterization

Introduction

The Jammu and Kashmir lying between latitude 32°17'N and 37°05'N and longitude 72°31'E and 80°20'E is known for very diverse agro-climatic conditions. The region has variable climates such as subtropical in plains, intermediate in foothills, and semi-temperate to temperate in high hills and mountains. The geographic and climatic variability is reflected in genetic variability among crop plants and other flora and fauna. The genetic purity of rice genotypes is maintained due to the self-pollinating behavior of the crop. The rice landraces/farmers' varieties maintained over centuries under diverse agro-climatic situations of Jammu region have retained peculiar genetic traits but remained unexploited for utilizing useful allelic diversity in breeding programs. The landraces, local germplasm complexes, and farmers' varieties with distinct genetic structure hold prospects for future rice crop improvement. The local varieties/germplasm also serve as a repository of genes for breeding varieties against biotic and biotic stresses and specific agronomic traits of economic importance, particularly under changing climatic conditions. The broad genetic and phenotypic diversity of rice genetic resources, adapted to a range of environmental conditions, has been significantly reduced during the decades of cultivation. The loss of genetic diversity due to intensive artificial selection

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and breeding caused bottlenecks favoring alleles that control important agronomic traits (Buckler et al. 2008). For achieving production targets on a sustainable basis, it is essential to conserve, characterize and utilize rice genetic diversity of different geographical regions and exploit unutilized valuable allelic variations for traits of economic significance.

Morphological descriptors and molecular markers are frequently used to study genetic diversity to develop conservation strategies and facilitate their management (Saeed et al. 2011). Several studies have described the aromatic rice cultivars of North Himalayas and proposed strategies for their sustainable improvement. The nutritional quality of the rice grain produced by certain traditional landraces due to their more effective accumulation of bioactive compounds is greater than that of the grain produced by conventional and modern varieties (Bhat and Riar 2015; Berni et al. 2018). Nutritional profiling of pigmented and scented rice genotypes of Kashmir region has also been done in the past. Husaini and Sofi (2018) described rice biodiversity in Cold Hill Zones of Kashmir Himalayas and suggested measures for their conservation and utilization in breeding programs.

Microsatellite/simple sequence repeat (SSR) markers have been abundantly applied for characterizing genetic diversity in many crop species, including rice, for obtaining unique DNA profiles of genotypes because of having a high level of polymorphism and greater information (Miah et al. 2013; Rathi et al. 2014; Singh et al. 2016 Donde et al. 2019; Madhubabu et al. 2020). Das et al. (2013) characterized genetic diversity in 83 rice landraces from Eastern and North Eastern States of India using simple sequence repeat (SSR) profiles. Aljumaili et al. (2018) used SSR markers to the genetic diversity of aromatic rice germplasm and concluded that SSR markers could be used to identify potential parents to achieve heterosis in future aromatic rice breeding

Table 1. A list of rice genotypes used in the study

S. No.	Genotype	Source	Brief description
1	Giza 14	SKUAST-J	An Egyptian rice introduction was well adopted and recommended in the hill zone of the Jammu region.
2	K 343 (Chenab)	SKUAST-K	Released from SKUAST-K for hill zone of Jammu region
3	K 448 (Jhelum)	SKUAST-K	Released from SKUAST-K for hill zone of Jammu region
4	Duggu Rice	Bhaderwah (Doda)	Farmers' collection with red pericarp from Bhaderwah. A Japanese rice introduction well adapted in Bhaderwah and adjoining areas of Doda district.
5	Chandki Basmati	Poonch	Aromatic rice landrace from Chandak area of Poonch district
6	Basmati 370	SKUAST-J	Released as basmati cultivar for sub-tropical areas of Jammu
7	Ranbir Basmati	SKUAST-J	Released as basmati cultivar for sub-tropical areas of Jammu
8	Nagina	SKUAST-J	Aromatic non-basmati rice variety from UP
9	KRC 1	Chingam, Kishtwar	Farmers' collection with red pericarp
10	KRC 2	Muller, Kishtwar	Farmers' collection with red pericarp
11	KRC 3	Passar Koot, Kishtwar	Farmers' collection
12	KRC 4	Passar Koot, Kishtwar	Farmers' collection
13	KRC 5	Rahal, Chatroo, Kishtwar	Farmers' collection
14	KRC 6	Parna, Kishtwar	Farmers' collection
15	KRC 7	Inderwal, Kishtwar	Farmers' collection
16	KRC 8	Singpora, Kishtwar	Farmers' collection
17	K 39	SKUAST-K	Released forJammu region
18	SJR 5	SKUAST-J	Released from SKUAST-Jammu
19	PRC 1	Atholi, Paddar, Kishtwar	Farmers' collection with red pericarp
20	PRC 2	Atholi, Paddar, Kishtwar	Farmers' collection
21	PRC 3	Massu, Paddar, Kishtwar	Farmers' collection
22	DHMAS	CSK, HPKV, Palampur	A doubled haploid rice variety pyramided with three blast resistance genes,
23	Ratta Ranjha	Sungri Budhal, Rajouri	Landrace
24	RML 22	CSK, HPKV, Palampur	A rice variety with blast resistance gene Pi9
25	Bansali	Topa Darhal, Rajouri	Landrace with red pericarp

programs. Park et al. (2019) assessed the genetic diversity of rice germplasms characterized by black-purple and red pericarp color using simple sequence repeat markers and showed the importance of protecting germplasm resources and the molecular markers that can be derived from them. Molecular and morphological characterization of germplasm from tropical and temperate regions, including Indian origin rice germplasm, has been well accomplished by several researchers (Pachauri et al. 2013; Kumbhar et al. 2015; Donde et al. 2019). The present investigation aimed to assess and quantify the genetic variation in the rice landraces and varieties grown by the farmers in the Jammu region of India using morphological traits and microsatellite/SSR markers.

Materials and methods

Experimental design and material used in the study

The study was carried out at Genomics Laboratory and Experimental Farm, School of Biotechnology, SKUAST-Jammu, 2016-2018. A set of 25 rice genotypes, including landraces, introductions, farmers' collections and released varieties collected/obtained from different areas/sources from Jammu region of Jammu and Kashmir, India were used in the present study (Table 1). Of these, 15 genotypes were collected from different rice growing ecologies of Jammu region, while others were established and released varieties used as checks to carry out the study. During Kharif 2016 and 2017, all genotypes were sown and transplanted in a randomized complete block design (RCBD) with three replications. The nursery for each genotype was transplanted in a 12.5 m^2 plot of 5.0 m length at a planting density of 15 × 20 cm. Recommended agronomic practices for raising a healthy crop were followed throughout the experiment.

Genetic diversity characterization at morphological level

To characterize genetic diversity at morphological level, five randomly tagged plants were used to record data from each plot. However, observations on days to 50 percent flowering, maturity, and duration of grain filling were recorded on plot basis. Data on yield contributing traits, namely plant height, panicle length, number of panicles per plant, number of tiller per plant, spikelet per panicle, grain length, grain breadth, grain length breadth ratio, yield per plant and 1000 grain weight were recorded. The data were recorded for two successive years and then pooled and subjected to statistical analysis using software such as SPSS 20.0 (Anonymous 2011) and PAST3 (Hammer et al. 2001). The analysis of variance (ANOVA) for all characters was carried to establish the significance and extent of genetic variability among the experimental genotypes. Principal Component Analysis (PCA) was determined from coefficients output from Euclidian coefficients matrix using software PAST3. The cluster analysis of the distance matrix was done to form an agglomerative hierarchical tree through UPGMA (Unweighted Pair Group Method using Arithmetic Averages) available in PAST3.

Genetic diversity characterization at molecular level

To assess genetic diversity at the molecular level, total genomic DNA of each genotype was extracted from leaf samples harvested from 4-5 leaf seedlings using a modified CTAB method (Clarke 2009). Amplification of primer specific region of DNA using a panel of 30 SSR primers was carried out in polymerase chain reaction (PCR) tubes containing 10 µL reaction mixture {1 µl of template DNA (50ng/µl), 0.5 µl 0.1mM of each forward and reverse primers, 1 U of Taq polymerase (Sigma Aldrich, USA), 2.2 µL of 10X PCR buffer with $MgCl_2$, 2.5 mM of each dNTP (dTTPs, dGTPs, dCTPs, dATPs)}. An initial denaturation step of 4 minutes was programmed in the thermocycler, followed by a loop of 30 cycles, each consisting of denaturation (94°C for 30 seconds), annealing (55°C–67°C for 30 seconds depending on the marker used), and extension (72 °C for 30 seconds). The final extension was performed at 72°C for 7 minutes. The PCR products were then stored at 4°C.

Parameters like Polymorphism Information Content (PIC), number of alleles per locus, heterozygosity, and total number of alleles were calculated using Power Marker software version 3.25 (Liu and Muse 2005). Diversity analysis and population structure analysis were done with allelic frequency level data, i.e., binary data with the help of NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) software version 2.02e (Rohlf 1997). A pairwise, individual-by-individual (N x N) genetic distance matrix was generated from binary data by Computer matrices of genetic distance coefficients from gene frequency. Symmetric Genetic Dissimilarity matrix was produced as Euclidean metric coefficient (GD_{NL}) (Nei and Li's 1979) following these measures

$$GD_{NL} = 1 - [2N_{11}/ (2N_{11} + N_{10} + N_{01})]$$

Where N_{11} is the number of bands-alleles present in both individuals; N_{10} is the number of bands–alleles present only in the individual i; N01 is the number of bands alleles present only in the individual j N represents the total number of bands-alleles.

Modified location model such as Principal Component Analysis (PCA) was determined from Correlation matrix.

Results and discussion

Genetic diversity characterization at morphological level

The genotypes used in the study exhibited significant variation in panicle laxity/compactness and panicle branching pattern (Fig. 1). Panicle traits are the most important features of rice germplasm resources that have been utilized to determine its identity, genetic diversity,

Fig. 1. (a) Panicle compactness and (b) branching pattern in

Fig. 2. Variation in spikelet type and aleurone layer color

Table 2. Mean performance (pooled over two years) of genotypes for yield and yield contributing traits

Genotype	Days to 50% Flowring	Days to maturity	Duration of grain filling	Plant height (cm)	Panicle length (cm)	Panicle/ plant	Tiller/ plant	Spikelets/ panicle	Grain length (mm)	Grain breadth (mm)	Grain L/B Ratio	Grain yield/ Plant (g)	1000 Grain weight (g)
GIZA14	97	129	32	108.43	21.91	10.13	10.13	207.06	5.11	2.95	1.74	29.51	24.36
K343	85	115	30	124.09	25.59	12.93	12.93	118.66	5.95	2.52	2.37	30.75	27.28
K448	74	105	31	118.43	24.89	14.40	14.40	134.80	5.67	2.31	2.45	26.58	23.86
Duggu	85	117	32	120.46	25.07	11.87	11.87	143.13	6.06	2.57	2.39	23.63	27.17
Chandki Basmati	97	127	30	133.09	24.75	13.00	13.20	148.13	6.18	2.43	2.55	26.64	23.19
Basmati 370	103	142	39	182.99	32.21	16.67	16.67	146.73	7.03	1.85	3.80	26.69	23.14
Ranbir Basmati	97	129	32	162.11	29.85	19.00	20.87	95.26	7.09	1.76	4.03	20.00	19.84
Nagina	80	110	30	130.27	22.93	17.93	19.07	128.60	5.54	2.33	2.38	33.20	20.73
KRC1	74	106	32	129.81	25.15	12.67	13.33	115.53	5.73	2.53	2.27	28.39	25.21
KRC2	74	104	30	130.11	24.83	14.33	14.53	118.00	6.29	2.65	2.40	31.58	26.54
KRC3	70	100	30	124.01	24.37	17.33	17.40	115.00	6.07	2.49	2.45	30.88	25.21
KRC4	67	99	32	125.98	25.62	15.27	15.27	132.93	6.105	2.41	2.53	30.71	25.01
KRC5	67	97	30	122.24	23.11	13.67	14.00	134.93	6.13	2.54	2.42	22.88	26.69
KRC6	70	102	32	127.61	24.83	15.80	14.67	118.20	6.18	2.531	2.44	21.09	25.44
KRC7	70	100	30	126.59	25.21	16.07	16.07	125.13	5.97	2.61	2.29	31.26	25.39
KRC8	96	126	30	140.09	25.67	13.53	14.20	120.73	5.83	2.88	2.03	25.99	30.37
K39	78	108	30	127.45	26.37	19.47	20.67	107.66	5.95	2.44	2.44	35.07	26.12
SJR5	85	117	32	129.07	26.63	15.20	15.20	179.00	7.16	2.28	3.14	38.73	25.90
PRC1	72	102	30	158.28	26.04	11.40	11.40	105.93	6.22	3.02	2.08	31.03	33.72
PRC2	72	104	32	110.99	22.83	16.53	16.53	115.93	5.65	2.60	2.20	28.83	26.20
PRC3	82	112	30	134.34	25.18	21.00	23.00	122.00	6.11	3.02	2.02	38.65	31.93
DHMAS	97	129	32	132.58	27.18	15.00	15.00	153.80	6.65	2.10	3.17	33.38	24.22
Rata Ranjha	97	130	33	122.36	25.89	15.73	16.20	125.80	5.09	2.83	1.81	32.50	24.90
RML22	85	114	29	118.53	21.69	18.40	19.33	113.93	5.28	2.59	2.04	32.99	24.19
Bansali	100	133	33	120.85	24.31	16.40	17.47	141.33	5.70	2.72	2.11	27.89	23.66
SD	11.89	12.91	1.97	16.16	2.24	2.65	3.10	23.84	0.5	0.31	0.55	4.78	3.02
CV (%)	3.67	5.59	6.15	4.98	9.13	10.10	9.26	8.05	8.19	6.11	11.36	8.33	11.51
CD @ 1%	3.00	4.28	5.62	4.06	6.86	6.75	6.92	6.28	6.30	4.48	9.02	6.38	8.72

yield, and quality. Lei et al. (2018) found that each cultivar included in the study manifested a unique panicle trait. The study on the morphological diversity of Kam fragrant glutinous rice landraces provided novel information that may be utilized sustainably in rice improvement. The assessment of rice genetic diversity is critical for a trait-specific varietal development program. Madhubabu et al. (2020) studied genetic variability for micronutrient content and agro-morphological traits in rice (*Oryza sativa* L.) and found six principal components analysis indicated 76% of total variation ranging from 7 to 19%. Genotypes such as Nagina, KRC 8, PRC 2, PRC 3, and Bansali had spikelets with awns, although they varied in color (Fig. 2). Analysis of variance for grain yield-related traits indicated highly significant variations among genotypes. Genotypes KRC 4 and KRC 5 matured in a minimum number of days thus falling in early maturing group. On the other hand, Basmati 370 and Ranbir Basmati took a maximum number of days to complete the life cycle, thus grouped into the late maturity group. RML 22 showed the lowest duration for grain filling, whereas panicle length, panicles per plant, no. of tillers per plant, grain length, grain yield per plant, and 1000 grain weight showed highly significant variation (Table 2).

The Euclidean distance matrix indicated GIZA 14 exhibiting maximum distance from other genotypes, whereas Basmati 370 and Ranbir Basmati showed similar distance pattern from other genotypes combined. Maximum genetic distances were observed between GIZA 14 and Ranbir Basmati; GIZA 14 and K39; Giza 14 and PRC 1 and Ranbir Basmati and SJR 5. GIZA 14 has been particularly known for its shattering resistance against hail storms hitting the cold and temperate hill zone of Jammu and Kashmir, Thus it can serve as a potential parent for this and other useful traits in breeding programs with other genotypes found genetically distant from this genotype. Similar studies have been attempted by Chakravarthi and Naravaneni (2006), Parimala and Devi (2016) and Dahl et al. (2016). Principal Component Analysis (PCA) based on all 13 traits has been presented in a 2D plot (Fig. 3). GIZA 14, SJR 5, PRC 1, Ranbir Basmati, and Basmati 370 formed the outermost layers showing maximum diversity from other genotypes. DHMAS, Bansali, Chandki Basmati, Ratta Ranjha and KRC 8 appeared genetically distant from others on GIZA 14 extension. Duggu Rice and Nagina separated themselves on separate node. Then, a major cluster was formed in the centre with the rest of the genotypes.

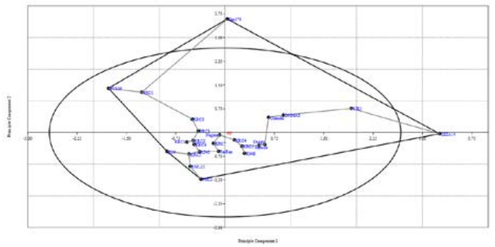

Fig. 3. Principle Component analysis based on yield related

Genetic diversity characterization at molecular level

Diversity analysis using 30 SSR polymorphic markers generated a total of 108 bands. Differential and comparable band patterns for genotypes indicated genetic variation in the genotypes used (Figs. 4a, b, and c). The number of alleles per locus produced by different primers ranged from 2-6 with an average of 3.6 alleles per primer/locus, and level of polymorphism was found to be significantly high, which indicated that most of the primers used in the study were efficient to characterize variability in the germplasm. Primers RM219 and RM413 produced a maximum of 6 alleles per locus, indicating these markers could decipher maximum allelic diversity at the target loci compared to other markers used (Table 3). Tarang et al. 2020) used 60 microsatellite markers in analyzing 63 rice genotypes of Central and West Asia and reported 252 polymorphic

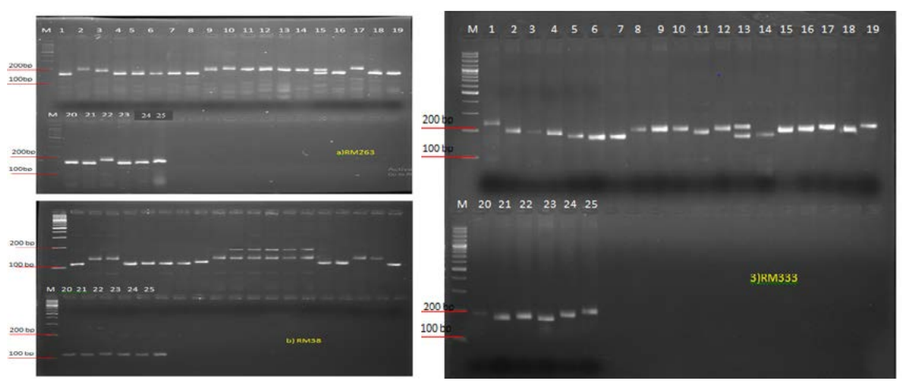

Fig. 4. SSR banding profile of the genotypes with the markers, a) RM 263, b) RM38, and c) RM333

Table 3. Major allelic frequency and PIC content

Marker	Major Allele Frequency	Genotype No	Allele No.	Hetero-zygosity	PIC
RM21	0.54	5	4	0.04	0.58
RM38	0.30	5	5	0.20	0.69
RM80	0.48	3	3	0.00	0.56
RM85	0.57	5	4	0.04	0.55
RM110	0.44	4	3	0.08	0.51
RM125	0.54	4	4	0.92	0.56
RM133	0.48	6	5	0.08	0.61
RM168	0.74	4	3	0.12	0.38
RM216	0.40	6	5	0.08	0.66
RM219	0.30	7	6	0.08	0.76
RM220	0.40	5	5	0.00	0.66
RM225	0.54	4	3	0.04	0.52
RM240	0.60	3	3	0.00	0.42
RM261	0.80	2	2	0.00	0.27
RM263	0.58	4	3	0.04	0.46
RM270	0.48	3	3	0.00	0.56
RM317	0.40	3	3	0.00	0.56
RM330	0.77	3	2	0.37	0.29
RM333	0.40	6	5	0.04	0.66
RM334	0.60	3	3	0.00	0.48
RM413	0.36	7	6	0.04	0.69
RM431	0.36	5	5	0.00	0.70
RM440	0.56	2	2	0.00	0.37
RM441	0.52	3	3	0.00	0.54
RM444	0.48	4	3	0.08	0.51
RM445	0.79	2	2	0.00	0.27
RM491	0.47	4	4	0.00	0.62
RM541	0.48	4	4	0.00	0.61
RM555	0.64	2	2	0.00	0.35
RM590	0.44	3	3	0.00	0.56
Mean	0.52	4.03	3.6	0.07	0.53

alleles amplifying an average of 4.2 alleles per primer. In this study, the mean number of effective alleles was 3.78, which RM490 and RM5423 markers had the lowest and the RM225 and RM246 markers had the highest value for this index.

Most of the primers showed high PIC values and therefore found to be more efficient in characterizing genetic diversity. High PIC value of more than 0.6 were observed for 7 primer pairs i.e. RM431 (0.70), RM413 (0.69), RM220 (0.66), RM216 (0.66), RM38 (0.69) and RM333 (0.66). Das et al. (2013) analyzed genetic diversity in 83 landraces of aromatic and non-aromatic rice selected from North Eastern states. They found that allelic frequency varied in these states, and the germplasm collected from West Bengal showed higher values indicating higher genetic diversity. Some of the SSR markers amplified unique alleles specific to a particular genotype and could distinguish them from the rest. In present study, highly informative SSRs also revealed high genetic diversity among the genotypes studied suggesting their potentiality in future genetic improvement. However, primer RM219 was a more effective and useful tool to elucidate the genetic differences among the rice genotypes and to study the phylogenetic relationships. The major allele frequency varied from 0.30 (RM38, RM219) to 0.80 (RM261). Out of all the 30 SSRs used, RM219 and RM413 identified maximum of 7 sets of genotypes. The value of heterozygosity ranged from 0.00 to 0.92. As many as 15 markers reflected heterozygosity of

genotypes for corresponding loci, the highest being with marker RM125 (0.92) followed by RM330 (0.37). Several studies characterizing genetic diversity in rice germplasm distributed across the world have published their reports on allelic diversity with unique alleles and classified the genotypes in different clusters and genetic similarity and dissimilarity (Allhgholipour et al. (2014), Salgotra et al. (2015), Anupam et al. (2017), Aljumaili et al. (2018) and Park et al. (2019).

A symmetrical genetic dissimilarity matrix depicting pairwise comparisons between the tested genotypes indicated closer genetic similarity within KRC and PRC groups, suggesting their common origin and geographical occurrence (Supplementary Table S1). On the other hand it indicated the maximum distance between K 448 and Chandki Basmati, Duggu Rice and KRC 3, K 448 and PRC 1, RML 22 and KRC 4, PRC 2 and KRC 4, PRC 1 and K 343. These genetically highly dissimilar genotypes belonged to different climatic situations and could act as prospective parents for transgressive breeding and exploitation of heterosis in hybrid breeding programs. Tartang et al. (2020) also used SSR markers to determine the genetic diversity in 63 rice germplasm lines and reported that Nei gene diversity and amount of PIC showed that RM23 and RM212 markers had the highest value and the RM3 marker had the lowest value for these two indices. Genetic distance between populations revealed that the higher range of genetic distance supported the present findings. Principle Component Analysis (Supplementary Fig. 1) depicted that K 343 and K 448 showed a greater distance from the vector on one side. Duggu Rice and RML 22 showed closeness and were placed near GIZA 14. PRC 2 and PRC 3 were placed on the outer rim but close to each other. KRC 1 and KRC 2 were grouped and Basmati 370 and Ranbir Basmati. Bansali was grouped with Ratta Ranjha. Basmati 370 and Ranbir Basmati were the closest to the vector. KRC 8, PRC 1, and Chandki Basmati positioned themselves near each other. The remaining genotypes were arranged at an almost equal distance from the vector and fanning out around vector.

The results obtained concerning genetic distances of the genotypes from the vector and concerning each other are in broad agreement with the geographical distribution of the genotypes. The genetic diversity analysis of different rice species using various marker systems were successfully conducted earlier by many researchers (Singh 1 et al. 2016; Thomas and Dominic 2016; Park et al. 2018). Results of multivariate analysis such as UPGMA and Principle Component Analysis (PCA) based on phenotypic distance matrix supported the findings based on the molecular analysis. Thus, molecular marker (SSR) based genetic characterization of genotypes supported by matching phenotypic characterization patterns proved helpful in characterizing genetic diversity, identifying diverse and similar genotypes, and identifying specific genotypes for specific traits agronomic/economic importance. The study indicated that many of the farmers' collections were released varieties such as K 448 saved and recycled by the farmers over the years. Farmers' varieties like Bansali, Chandaki Basmati, Ratta Ranjha, KRC 1, KRC 2, PRC 1 and Duggu are unique to this region and can be involved in breeding programs with other improved rice varieties for exploitation of untapped allelic diversity.

Supplementary materials

One supplementary table and one Supplementary Figure are supplied

Authors' Contribution

Conceptualization of research (MS, GMA, RKS); Designing of the experiments (MS, GMA); Contribution of experimental materials (MS, RKS, VS, AKS); Execution of field/lab experiments and data collection (GMA, SH, AS, P); Analysis of data and interpretation (MS, GMA, RKS); Preparation of the manuscript (MS, P, AKS).

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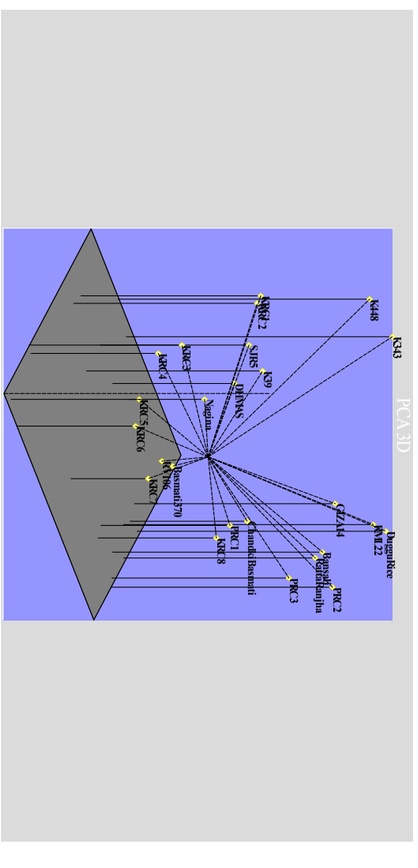
Supplementary Table S1. Nei and Li's genetic distance (GD_{NL}) matrix

Genotype	GIZA14	K 343	K 448	Duggu	Chandki	Bas 370	R. Bas.	Nagina	KRC 1	KRC 2	KRC 3	KRC 4	KRC 5	KRC 6	KRC 7	KRC 8	K 39	SJR 5	PRC 1	PRC 2	PRC 3	DHMAS	Ratta Ranjha	RML 22	Bansali
GIZA14	0.00																								
K 343	0.88	0.00																							
K 448	1.10	0.37	0.00																						
Duggu	0.30	0.66	1.00	0.00																					
Chandki	0.51	1.03	1.98	0.46	0.00																				
Bas 370	0.57	0.93	1.29	0.60	0.46	0.00																			
R.Bas.	0.66	0.97	1.32	0.72	0.51	0.07	0.00																		
Nagina	1.36	1.07	1.10	1.23	0.76	0.69	0.73	0.00																	
KRC 1	1.16	0.55	0.54	1.16	1.04	1.13	1.07	0.76	0.00																
KRC 2	1.05	0.46	0.56	1.05	0.93	1.02	0.96	0.88	0.15	0.00															
KRC 3	1.24	0.75	0.73	1.35	1.20	1.46	1.35	1.13	0.33	0.26	0.00														
KRC 4	1.49	0.82	0.79	1.82	1.32	1.46	1.49	1.04	0.47	0.39	0.25	0.00													
KRC 5	1.31	0.90	0.87	1.43	1.16	1.52	1.43	1.43	0.49	0.42	0.24	0.24	0.00												
KRC 6	1.35	0.99	0.95	1.49	1.20	1.32	1.24	1.24	0.63	0.54	0.25	0.42	0.17	0.00											
KRC 7	1.05	0.97	1.11	1.05	0.89	0.76	0.76	0.82	0.73	0.78	0.83	0.70	0.60	0.59	0.00										
KRC 8	0.81	1.08	1.47	0.74	0.85	0.78	0.68	0.97	1.18	1.07	1.50	1.25	1.22	1.05	0.41	0.00									
K 39	1.04	0.68	0.95	1.13	1.10	1.20	1.24	1.35	0.58	0.49	0.69	0.69	0.71	0.83	0.97	0.81	0.00								
SJR 5	1.00	0.88	0.69	1.32	1.29	1.07	0.92	0.92	0.49	0.56	0.66	0.60	0.80	0.87	0.94	1.12	0.54	0.00							
PRC 1	0.56	1.78	1.81	0.68	0.65	0.85	0.81	1.25	1.40	1.27	1.15	1.25	1.04	1.25	0.91	0.76	0.89	0.85	0.00						
PRC 2	0.63	1.14	1.29	0.46	0.60	0.80	0.84	1.20	1.49	1.34	1.61	1.79	1.38	1.20	0.85	0.53	1.20	1.17	0.65	0.00					
PRC3	0.85	1.05	1.44	0.58	0.61	0.61	0.65	1.22	1.25	1.13	1.63	1.63	1.29	1.11	0.74	0.49	1.11	1.19	0.87	0.39	0.00				
DHMAS	1.34	0.97	0.71	1.34	0.99	0.99	0.85	0.93	0.97	0.90	0.89	0.97	0.96	0.97	1.04	1.24	0.89	0.71	0.95	1.08	1.00	0.00			
Ratta Ranjha	0.73	0.88	1.46	0.44	0.51	0.57	0.54	1.13	1.27	1.25	1.24	1.64	1.20	1.04	1.14	0.89	1.24	1.00	0.81	0.41	0.33	0.93	0.00		
RML 22	0.58	0.71	1.19	0.53	0.75	0.82	0.78	0.93	1.05	1.03	1.22	1.81	1.41	1.22	0.95	0.87	1.47	1.19	0.95	0.44	0.63	1.32	0.53	0.00	
Bansali	0.73	0.88	1.20	0.54	0.57	0.76	0.80	0.80	1.16	1.05	1.35	1.13	1.31	1.13	0.76	0.68	1.13	1.20	0.81	0.57	0.37	1.34	0.44	0.42	0.00



Supplementary Fig. 1: Principle component analysis of genotypes with vector in three dimensions