Australian Journal of Crop Science AJCS
AJCS 7(7):923-932 (2013) ISSN:1835-2707

Molecular and morphological characterization of Indian farmers rice varieties (*Oryza sativa* L.)

Vinita Pachauri[1], Nilay Taneja[2], Prashant Vikram[3], Nagendra Kumar Singh[1] and Sanjay Singh[4]*

[1]National Research Centre on Plant Biotechnology, New Delhi, India
[2]Dr.DY Patil Institute of Biotechnology and Bioinformatics, Pune, Maharashtra, India
[3]Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute, Philippines
[4]Seed Production Centre, Indian Agricultural Research Institute, New Delhi, India

***Corresponding author: sanjay_singh777@yahoo.com**

Abstract

Genetic relatedness among Indian farmers' rice varieties were assessed using 24 distinct rice microsatellite markers. The 41 rice genotypes studied included 14 improved varieties and 27 landraces collected from different parts of India. The number of alleles per microsatellite locus ranged from 2 to 4, averaging 2.79 alleles per locus. Polymorphism information content (PIC) values ranged from 0 to 0.66, with an average of 0.38. The band size for a given microsatellite locus varied between 80 bp and 580 bp. Characterization of the varieties was also done for various morphological parameters including number of grains per panicle, thousand grain weight, length and breadth of whole and milled grains, cooking qualities like grain elongation ratio, grain aroma and alkali spreading value. Cluster analyses were used to group cultivars by constructing dendrograms based on SSR marker analysis and morphological characterization of grains. The dendrogram based on molecular marker analysis grouped the 41 rice cultivars into four diverse groups. Genetic relatedness was observed for most of the varieties in cluster II for grain quality traits- aroma and grain length/breadth. Clustering of these varieties according to their genotypes as well as phenotypes revealed the possible linkage or pleiotropic effects of the genomic regions associated with some grain quality traits. Information generated through cluster analysis based on phenotypic and genotypic data could be efficiently used in breeding rice varieties harboring grain quality traits. Pure-line selection can be made using farmer varieties that are characterized morphologically and phenotypically in the study.

Keywords: Cluster analysis, Genetic diversity, Grain and Cooking qualities, Microsatellite markers, Rice.
Abbreviations: MMGDA-Molecular Marker based Genetic Diversity Analysis, PCA-Principle Component Analysis, PIC-Polymorphism Information Content, UPGMA-Unweighted Pair Group Method with Arithmetic Average.

Introduction

Rice is undoubtedly the most important cereal of the world providing 21% of global human per capita energy and 15% of per capita protein (Maclean et al., 2002). South Asia, one of the major centers for rice domestication, has been described as the "food basket" and "food bowl" of Asia. Among all the Asian countries, India is the prominent rice growing country accounting for about 20% of all world rice production. Apart from the traditional varieties, India is home to wide varieties of rice cultivars, landraces and many lesser known varieties that have been under cultivation since ages by farmers as well as local entrepreneurs. These cultivars were developed through selections, based on desirable characters such as grain yield, aroma, grain length, cooking quality and adaptation to various abiotic stresses. Such process of selection resulted in a wide spectrum of rice varieties adapted to a wide range of agro-ecological conditions. That is why almost all the rice growing provinces of India have their own locally adapted cultivars suitable for particular agro-climatic conditions as well as local preferences (Singh et al., 2003). Many of these varieties are highly valued in the domestic market and were also patronized by many erstwhile royal families (Pachauri et al., 2010). Since ages, most of the locally grown cultivars have been known by their local/common dialect names and hence no or very few records of the genetic nature and background of such varieties are available. It is believed that some of these varieties may have originally belonged to some other state/region and have travelled a long route of domestication hundreds of years ago (Pratheepha, 2009). Therefore, there molecular and phenotypic characterization could reveal their phylogeny and this information would be quite useful in utilizing these germplasms in genetic improvement of the existing rice varieties. Molecular Marker based Genetic Diversity Analysis (MMGDA) has the potential for assessing changes in genetic diversity over time and space (Duwick, 1984). DNA markers are predominantly used in molecular characterization and diversity studies due to their abundance and repeatability (McCouch et al., 1997). Different molecular markers viz. Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphisms (SNPs) have been used to assess genetic diversity of various rice cultivars throughout the world (Joshi et al., 2000). Among different PCR based markers, the microsatellite markers based on simple sequence repeats (SSRs) are preferred over other molecular markers due to their ease of application, high reproducibility, rapid analysis, low cost, easy scoring patterns and greater allelic diversity (Chen et al., 1997). These markers are distributed relatively

uniformly throughout genome and detect a high level of allelic diversity in cultivated varieties and distantly related species (McCouch et al., 1997). Studies in various organisms provide evidence that the number of microsatellite sequences in a genome, their length, composition, mutation rate and chromosomal distribution can vary drastically among taxa (Temnykh et al. 2000). Although rice germplasm characterization and diversity analysis has been done by several workers, variability studies of the common landraces and cultivars grown by Indian farmers is limited. Considering the wide cultivation and use of such varieties in the Indian sub-continent, the present study was undertaken with 14 improved varieties and 27 landraces of rice to assess their genetic and morphological diversity. Molecular characterization using microsatellite markers covering the entire twelve chromosomes of the rice genome and phenotypic characterization for various grain quality parameters was carried out. Three major objectives were defined for present investigation: (i) genetic differentiation of 41 rice cultivars by generating a DNA fingerprint for each, (ii) morphological characterization of the genotypes for grain and cooking quality parameters, and (iii) grouping the cultivars according to their genotypes and then establishing their genetic relationship for various grain quality traits.

Results

Estimation of morphological and grain quality features

Observations on basic characteristic features including days to fifty percent flowering, panicle length, and number of grains per panicle, 1000 grain weight and color/appearance of grain husk were recorded (Table 1). Days to fifty percent flowering (DFF) also known as heading date, a crucial trait for adaptation to different cultivation areas and cropping seasons is defined as when 50% of the plants in a single line completely exerted at least one panicle. DFF of the phenotypes characterized in Kharif 2011 ranged 95 days (Ekbili) to 155 days (Jeera Shankar and Tilakcandan). Phenotyping of the genotypes was done for grain quality characters viz., grain length, grain breadth, length/ breadth ratio, elongation ratio, alkali spreading value and aroma (Supplementary Table 1). The range and mean estimates of various physiological, grain and cooking quality traits of the 41 rice varieties are presented in Table 2. Head rice of rice genotypes under investigation was recorded. The longest grain length (unmilled and milled) was recorded as 11.67±0.41mm and 8.2±0.38mm respectively for SS20, while Sulendas had shortest grain length of 6.93±0.37mm and 5.07±0.15mm respectively. The shape of rice grain is also determined by its length/ breadth ratio and rice varieties can be broadly classified in to three broad categories viz., slender (L/B ratio >3.0), medium (L/B ratio 2.1-3.0) and bold (L/B ratio <2.1). Diverse L/B ratio (unmilled grain) was observed, ranging from 2.29±0.24mm (Suranit) to 5.66±0.22mm (SS20). Linear kernel elongation without increase in girth is another important quality characteristic (Khush et al., 1979) and is a desirable property of good quality rice like Basmati. Highest kernel elongation ratio was observed in Kakeria-2 (1.608±0.19), while SHPP-20 showed the lowest elongation ratio of 1.078±0.06. Alkali spreading value (ASV) of starch endosperm gives information about gelatinization temperature, the cooking temperature at which water is absorbed and the starch granules swell irreversibly in hot water with a simultaneous loss of crystallinity and birefringence. A low ASV corresponds to the high gelatinization temperature. Most of the rice varieties under present investigation had an ASV of 2 and 1. Rice aroma is a qualitative trait, sensory analysis is usually considered as the first step to identify/discriminate fragrant varieties from the non-fragrant ones (Tragoonrung et al., 1996). Sensory analysis of grain aroma revealed the range of sensory scores between 0 and 3. Highly aromatic varieties such as DPT-52, Tilakchandan and Basmati-334 having a sensory aroma score of 3 as well as moderately aromatic varieties with a sensory score of 2 have been identified along with some non-aromatic and less aromatic varieties (Table 2).

Detection of polymorphism through SSRs

A total of 24 SSR loci were evaluated for their efficiency of polymorphism across 14 improved varieties and 27 landraces of rice. All the markers were found to be polymorphic among the rice cultivars and generated reproducible and informative allelic profiles. The distribution of alleles for SSR locus *viz.* RM432 across a section of 24 rice cultivars is presented in Fig 1. A total of 1036 scorable bands were obtained for 67 different alleles. The allelic frequency ranged from 2-4, with an average of 2.79 alleles per locus. The product size ranged from 80bp (RM6271) to 580bp (RM24846). A variety was assigned null allele for a microsatellite locus whenever an amplification product could not be detected for a particular genotype-marker combination. In the set of 41 genotypes, 14 loci showed null alleles. The frequency of rice cultivars showing null allele ranged from a minimum of one (for RM24, RM307, RM273, RM159, RM432, RM219, RM6100 and RM28346) to a maximum of 6 (for RM2110).
Polymorphism Information Content (PIC) values, a reflection of allele diversity and frequency among the varieties, were calculated for all the markers. The average PIC value was 0.38 and ranged from a low of 0 (RM427) to a high of 0.66 (RM251). The frequencies of null alleles were not included in the calculation of PIC values for each SSR locus. The details of markers used along with the number of alleles for each locus and PIC values are given in Table 3.

Clustering of rice cultivars

Cluster analyses was done to group the improved varieties and landraces by constructing dendrograms based on the grain and cooking quality data (Fig 2,3,4 and 5) and allelic information gathered from the genotype-marker interaction (Fig. 6). The Genetic Similarity (GS) index ranged from a minimum of 58% to a maximum of 91% (Jeera Shankar and Suranit). The UPGMA-dendrogram clustered the 41 genotypes into four major clusters (I, II, III and IV) (Fig 6). Cluster II comprising of both improved varieties (Aamchur, Sorna, MTU and Sonam) and landraces (Pantjali, Punjabia, Nungi, Shailendra, Ramveer-2, Lal Bamah, Red Jhirani, Sajad Luchai and Chatry) that showed more genetic relatedness among the varieties as compared to any other cluster. It was observed that the clustering based on the allelic and morphological data was conserved to some extent for some genotypes like Pantjali, Ramveer-2, Lal Bamah, Sonam, Red Jhirani, Aamchur, etc (most of these belong to group II).

Comparative analysis of genotypic and phenotypic diversity analysis results

Interestingly, one of the group constructed based on aroma, included 15 lines (Patanjali, Aamchur, Shailendra, Gonor-1, Chatry, Ekbili, Lal Bamah, Sonam, Red Jhirni, Suranit, Sorna, Salti, Ramveer-2, Nungi and Kakeria) out of which 12

Table 1. Basic morphological features and state of collection of the 41 rice genotypes used in the study.

S. No.	Cultivar	State from where collected	No. of grains/panicle* (Mean±SD)		Panicle length (cm)* (Mean±SD)		1000 grain weight (g)* (Mean±SD)		DFF#	Color of husk
IMPROVED VARIETIES										
1	Aamchur	Madhya Pradesh	244.8	± 12.21	23.6	± 0.47	26.3	± 0.78	142	Black
2	DPT-52	Uttar Pradesh	281.8	± 10.28	26.32	± 0.37	24.56	± 0.47	125	Yellow
3	Gonor-1	West Bengal	324.6	± 5.86	30.84	± 0.15	30.14	± 0.75	122	Reddish
4	Gundra	Madhya Pradesh	331.2	± 10.87	30.3	± 0.41	28.08	± 0.68	115	Yellow
5	Guntur-2	West Bengal	310.4	± 14.89	29.66	± 0.19	29.76	± 0.83	125	Reddish
6	Jeera Shankar	Madhya Pradesh	223.4	± 15.45	28.12	± 0.33	29.38	± 0.6	155	Reddish
7	Komal	Uttar Pradesh	320.4	± 8.71	28.36	± 0.27	24.62	± 1.27	115	Yellow
8	MTU	Andhra Pradesh	294.8	± 7.76	27.32	± 0.47	30.3	± 0.6	120	Yellow
9	Patel-3	Madhya Pradesh	338.8	± 11.03	28.58	± 0.26	27.88	± 0.79	119	Yellow
10	Shavagi	Jharkhand	290.2	± 7.05	25.76	± 0.15	29.38	± 0.56	110	Yellow
11	SHPP-20	New Delhi	356	± 10.3	32.38	± 0.28	27.62	± 0.77	126	Yellow
12	Sonam	Uttar Pradesh	313.4	± 11.35	27.46	± 0.52	27.06	± 1.27	115	Yellow
13	Sorna	Madhya Pradesh	304.6	± 16.07	29.34	± 0.82	25.38	± 0.51	120	Yellow
14	SS20	New Delhi	340.2	± 9.83	30.76	± 0.23	30.54	± 0.65	145	Yellow
LANDRACES										
15	Badshahbhog	Orissa	245.4	± 9.66	27.3	± 0.45	21.9	± 0.47	150	Yellow
16	Barkhash	Madhya Pradesh	290.2	± 8.76	27.46	± 0.34	32.3	± 0.83	112	Yellow
17	Basmati-334	Jharkhand	234.8	± 7.5	26.72	± 0.24	26.44	± 0.53	105	Yellow
18	Baspatri	Madhya Pradesh	256	± 8.75	25.42	± 0.5	30.24	± 0.79	146	Yellow
19	Budhiluchai-1	Madhya Pradesh	250.4	± 5.46	24.62	± 0.51	29.32	± 1.16	148	Black & white striped
20	Budhiluchai-2	Madhya Pradesh	304.8	± 10.69	26.3	± 0.19	33.5	± 0.94	144	Whitish Yellow with black stripes
21	Chandanchur	Bihar	202.2	± 10.18	24.34	± 0.36	34.56	± 1.15	151	Yellow
22	Chatry	Madhya Pradesh	215.6	± 6.91	24.3	± 0.23	29.36	± 1.08	100	Yellow
23	Chipao	Madhya Pradesh	302.8	± 13.27	27.42	± 0.38	27.44	± 0.47	130	Light yellow
24	Ekbili	Madhya Pradesh	194.6	± 9.69	29.38	± 0.26	25.62	± 0.66	95	Yellow
25	Kakeria	Madhya Pradesh	343.8	± 7.66	29.52	± 0.43	24.3	± 0.85	100	Black
26	Kakeria-2	Madhya Pradesh	302.6	± 10.6	28.1	± 0.22	24.32	± 0.74	105	Black
27	Karadhana	Madhya Pradesh	297.6	± 13.39	27.22	± 0.16	31.18	± 0.99	127	Yellow
28	Lal Bamah	Uttarakhand	210	± 12.75	24.34	± 0.39	27.16	± 0.43	140	Yellow
29	Nungi	Madhya Pradesh	210.2	± 8.58	23.32	± 0.2	23.24	± 0.5	115	Yellow
30	Pantjali	Bihar	245.4	± 11.59	26.34	± 0.23	32.3	± 0.89	130	Yellow
31	Pilliluchai-1	Madhya Pradesh	298	± 11.9	26.42	± 0.41	34.92	± 0.84	148	Whitish Yellow with black stripes
32	Punjabia	Bihar	300.8	± 12.4	27.76	± 0.15	29.36	± 0.74	145	Reddish
33	Ramkajer Special	Madhya Pradesh	300.8	± 8.9	29.42	± 0.18	35.44	± 0.42	150	Black & white striped
34	Ramveer-2	Madhya Pradesh	275.6	± 6.84	25.76	± 0.11	25.14	± 0.92	150	Yellow
35	Red Jhirani	Orissa	300	± 9.8	29.34	± 0.18	22.6	± 0.7	134	Red
36	Sajad Luchai	Madhya Pradesh	322.2	± 14.86	29.78	± 0.24	24.58	± 0.4	140	Yellow
37	Salti	Madhya Pradesh	217.4	± 8.62	25.82	± 0.39	27.32	± 0.76	100	Yellow
38	Shailendra	West Bengal	321.2	± 8.53	29.36	± 0.32	29.48	± 1.03	145	Yellow
39	Sulendas	Madhya Pradesh	234.8	± 8.73	27.18	± 0.08	32.54	± 0.61	135	Yellow
40	Suranit	Madhya Pradesh	225.6	± 10.5	25.34	± 0.18	23.32	± 0.46	145	Yellow
41	Tilakchandan	Uttarakhand	211.2	± 6.46	23.5	± 0.34	22.62	± 0.73	155	Yellow

#Days to fifty percent flowering; *Average of five individual observations.

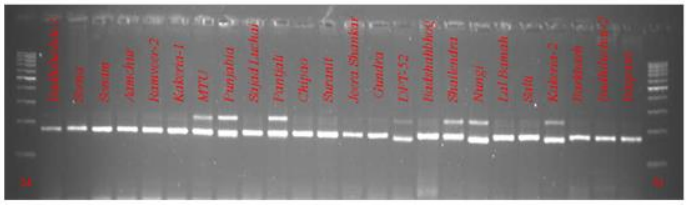

Fig 1. Allele profiles of a subset of 24 rice genotypes for SSR locus RM 432. M: 100bp DNA ladder

lines were from genotypic cluster II. Therefore 80 % (12/15 × 100 = 80%) of the lines belonged to common genotypic group (Fig 5). Similarly, one of the group based on milled grain length included 13 lines (Patanjali, Punjabia, Aamchur, Barkhash, MTU, Budhiluchai-1, Lal Bamah, Chatry, Ekbili, Sonam, Salti, Ramveer-2, Nungi) out of which 10 were from cluster II (except Barkhash, Budhiluchai-1 and Salti). Therefore, 77 % (10/13 × 100 = 77%) varieties belonged to common phylogenetic group i.e. cluster II (Fig 4). Therefore varieties of phylogenetic cluster II showed a promise compared to other groups and indicated the presence of conserved genomic regions governing grain quality traits.

Discussion

Success of a crop improvement program depends on the magnitude of genetic variability and the extent to which the desirable characters are heritable (Ravi et al., 2003). Hence assessment of genetic diversity becomes important in establishing relationships among different cultivars (Sivaranjani et al., 2010, Kibria et al., 2009, Nagaraju et al., 2002). According to an FAO report (2002), genetic diversity has been utilized and preserved partially during the process of domestication and cultivation; not even 15 percent of potential genetic diversity has been utilized in crop plants. A number of valuable allelic variations of traits of great economic importance are still unutilized. Significant genetic improvement of rice can be done through introgression of valuable genes/genomic regions from wild progenitors/ landraces/traditional varieties which are presently grown in some parts of India, one of the major domestication centers of rice. Some landraces and traditional varieties had been under cultivation by farmers since time immemorial, usually on the basis of practices inherited from their forefathers and easy availability of seeds. These cultivars are preferred by the farmers due to their good taste, impressive cooking qualities and suitability in particular agro-climatic regions. For example, varieties like Kalanamak and Adamchini in Eastern Uttar Pradesh are well known for their taste, Dhagaddeshi in Chhattisgarh and Nootripathu in Tamil Nadu provinces of India are still the preferred choice of farmers even today because of their tolerance to drought prone environments (Singh et al., 2003). Large numbers of such cultivars are also present in other Indian states- Madhya Pradesh, Orrisa, Bihar, etc. Assam Rice Collection is another example of a set of locally adapted cultivars collected and preserved in India from the northeastern provinces (Hore, 2005). In the present study we collected 41 different rice cultivars from farmers that are grown by them for both own consumption as well as for commercial use, since a very long time in their region usually situated in remote rural parts of the country. During the course of the study, a traditional basmati variety Basmati-334 and a short grain aromatic rice variety and Tilakchandan were also collected from the farmers of remote villages of Jharkhand and Uttarakhand respectively. Both are aromatic, but fall in two different diverse clusters/groups. On morpho-physiological analyses, it was observed that the variety 'Basmati-334' collected from the farmers did not comply with the parameters of standard basmati rice as notified by the Government of India (GOI office memorandum, 2008). The length and breadth of milled grains (6.4±0.15mm and 2.2±0.18 respectively) and kernel elongation ratio (1.40±0.09) were less than the minimum value/range for any variety to be qualified as Basmati. However characteristic fragrance was present. There is a possibility of true Basmati-334 variety being out-crossed or intermixed with some other variety and continuous propagation of this had been there since a long time. Interestingly, it has been reported as a drought tolerant cultivar (Vikram et al. 2012). In another case, there were two different rice genotypes of the name Budhiluchai (Budhiluchai 1 and 2) which were collected from different villages/regions of Madhya Pradesh. For convenience, they were given a suffix of numerals 1 and 2. The study revealed that although these two variants are present in the same cluster yet they share only about 75% similarity at the genetic level. There were few differences in the grain dimensions, cooking quality parameters as well as physical appearance of the two variants. Similarly, the two varieties by the name Kakeria (Kakeria and Kakeria-2) shared only 64% genetic similarity and had considerable morphological as well as grain quality differences. By convention it is assumed that the two variants should have been the same and must be genetically similar, but our genetic diversity analysis contradicts this fact. Such kind of cultivar identity crisis is often witnessed throughout the world. Such discrepancies emphasize the importance of molecular characterization of rice germplasm prior to their deployment in varietal improvement. It is highly likely that, there is more number of such cultivars whose genetic background is not yet unveiled and are used by innocent farmers under unknown/false identities. In the current investigation, the rice genotypes were clustered into four distinct groups. Most of the members of cluster II were found to be similar in terms of phenotypic traits like grain aroma and milled grain length (Fig 4 and 5). Also they shared more genetic similarity. This phenotypic and genotypic co-relation for quality traits -grain aroma and grain length/breadth indicates towards either linkage or pleiotropic effect of the genomic regions associated with phenotypic expression of these traits. It is likely that in the due course of evolution, genomic regions responsible for different grain quality traits have moved somehow together in some locally adapted farmer's varieties. Morphological and molecular characterization of such cultivars followed by their use in rice varietal improvement could lead to substantial gain. Basmati rice is considered to be the best rice in India and fetches a handsome price at both national as well as international market. Department of Agriculture and Co-operation, Ministry of Agriculture, Government of India has laid down certain quality standards for milled basmati rice varieties. Based upon these parameters, we identified a few lines from among the 41 rice varieties in the present study having good grain quality and cooking quality parameters. These parameters although are not at par with Basmati standards, but could be used as the criteria for selection and standardization of non-basmati rice varieties with potential market value, good taste and cooking qualities (Table 4). In this study, at least six varieties viz. Chipao, Gundra, Sajad luchai, Kakeria-2, Chandanchur and Pilliluchai-1 have exceptionally good grain and cooking qualities. These varieties have high grain number (more than 290 grains per panicle, except for Chandanchur with 202.2±10.18 grains per panicle) and pleasant aroma with kernel elongation ratio greater than 1.3. They are also good in appearance to eat with

Table 2. Range and Mean estimates of various physiological, grain and cooking quality traits of 41 rice varieties under study.

Characters	Range		Mean ± SD
	Min	Max	
DFF	95	155	129.24 ± 17.68
No. of grains/panicle	195	356	277.8 ± 45.96
Panicle length (cm)	23.5	32.4	27.33 ± 2.23
1000 grain weight (g)	21.9	35.4	28.08 ± 3.57
Unmilled grain length(L)(mm)	6.93	11.67	8.47 ± 0.34
Unmilled grain breadth(B)(mm)	1.93	3.4	2.54 ± 0.20
L/B Ratio of unmilled grain	2.31	5.66	3.54 ± 0.30
Pre-cooked Milled grain length(Lp)(mm)	5.07	8.2	6.14 ± 0.34
Pre-cooked Milled grain breadth(Bp)(mm)	1.6	2.6	2.10 ± 0.17
L/B Ratio of uncooked grain	2.27	4.76	3.01 ± 0.32
Cooked grain length(Lc)(mm)	6.8	11.73	8.1 ± 0.50
Cooked grain breadth(Bc)(mm)	1.6	3.07	2.57 ± 0.24
Kernel Elongation ratio (Lc/Lp)	1.08	1.6	1.32 ± 0.11
Grain aroma	0	3	-
Alkali Spreading Value (ASV)	1	7	-

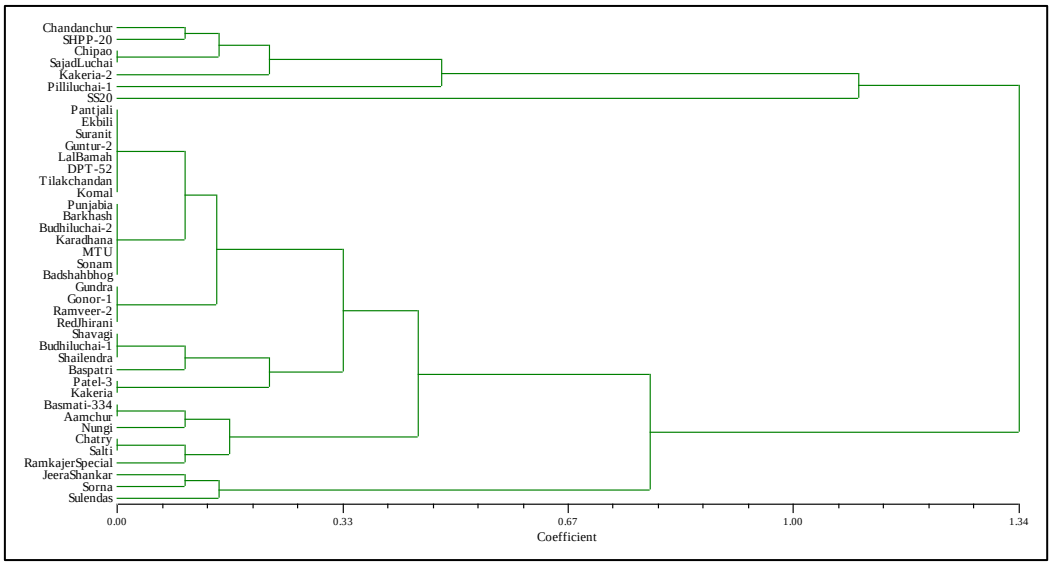

Fig 2. Dendrogram derived from UPGMA cluster analysis using Nei's similarity coefficient based on length of milled grain depicting the associations among the 14 improved varieties and 27 landraces of rice.

an average cooked grain length greater than 9 mm. On the basis of grain and cooking qualities, 12 varieties were selected and two different standard markers for grain number (nksrssr 04-11) and grain length (SF28) (Fig 7) were used to observe their amplification. Both the markers gave expected amplification pattern suggesting their potential use in rice improvement through Marker-Assisted Selection (MAS).

There are records about several local rice cultivars that perform well in adverse environmental conditions and are at par in quality with various traditionally and commercially grown varieties. For example Kalanamak, a traditional variety grown in eastern Uttar Pradesh and Nagina-22, a selection from Rajbhog, a landrace grown in foot hills of Himalayan region in Nepal (Vikram et al., 2011), performed well under salt (Kalanamak) and drought (Nagina-22) stress conditions respectively. Many of such genotypes are known, grown and consumed locally, but have less call at the international platform. Some genotypes included in the present investigation are used/have been used briefly for various programs, but other varieties do have great potential to be used in similar context. Efforts are required to identify, improve and promote cultivation of such rice cultivars for the domestic and international market and for their potential use in various breeding and crop improvement programs.

Materials and methods

Collection of rice varieties

14 improved varieties and 27 landraces of rice were collected from different regions/zones of seven Indian states- Madhya Pradesh, Uttar Pradesh, Uttarakhand, West Bengal, Orissa, Andhra Pradesh and Bihar during 2010. Farmers in the remote areas of these regions were surveyed to get information about their preferences of rice varieties. A diverse collection of long and short grain aromatic as well as non-aromatic and local landraces was made (Table 1). Some varieties were known to the farmers of different locations/area of same state by similar names, but were different in physical appearances. Such varieties were given a suffix of 1 and 2.

Germination of rice varieties

Healthy mature seeds collected from the farmers' field were stored in moisture free environment and germinated during Kharif-2011 in square nursery plot design in panicle to row pattern in rice fields at Division of Genetics, Indian Agricultural Research Institute, New Delhi, India. After about 22 days, each cultivar was transplanted in three rows

Table 3. Allelic distribution and PIC values of 24 microsatellite markers across 41 genotypes.

S. No.	Marker ID	Repeat Motif	Chr	Product Size range (bp)	Null alleles/Genotypes missing*	Total no. of alleles	Allelic distribution 1	2	3	4	PIC Value
1	RM24	(GA)29	1	150-220	1	4	34	3	5	1	0.25
2	RM578	(GA)19	1	280-310	0	3	24	7	9	-	0.58
3	RM1211	(AG)14	2	175-185	0	2	33	8	-	-	0.31
4	RM561	(GA)11	2	180-190	0	2	39	2	-	-	0.1
5	RM15780	(CGC)7	3	130-150	0	2	27	14	-	-	0.45
6	RM251	(CT)29	3	110-145	0	4	15	18	5	3	0.66
7	RM307	(AT)14(GT)21	4	135-200	1	3	33	7	5	-	0.27
8	RM273	(GA)11	4	190-200	1	2	23	17	-	-	0.49
9	RM159	(GA)19	5	250-260	1	2	24	16	-	-	0.48
10	RM1054	(AC)17	5	170-220	0	4	10	27	10	4	0.44
11	RM6818	(TCT)9	6	110-150	0	2	40	4	-	-	0.04
12	RM528	(AGAT)9	6	290-360	0	4	30	12	10	1	0.31
13	RM427	(TG)11	7	190-200	0	3	39	13	2	-	0
14	RM432	(CATC)9	7	180-230	1	3	32	7	11	-	0.25
15	RM25	(GA)18	8	130-150	2	3	10	22	4	-	0.6
16	RM42	(AG)6(AG)2T(GA)5	8	130-140	5	2	16	20	-	-	0.49
17	RM219	(CT)17	9	200-300	1	4	13	20	6	2	0.62
18	RM24846	(TTG)8	9	550-580	2	2	27	12	-	-	0.43
19	RM6271	(CTC)10	10	80-90	4	2	30	7	-	-	0.3
20	RM6100	(CGA)8	10	150-200	1	3	25	5	15	-	0.45
21	RM7443	(GTTT)7	11	160-200	0	3	30	11	19	-	0.18
22	RM2110	(AT)21	11	400-450	6	3	20	15	2	-	0.49
23	RM17	(GA)21	12	190-200	3	2	12	26	-	-	0.43
24	RM28346	(AT)38	12	180-210	1	3	28	11	4	-	0.42

*Out of total 41 genotypes.

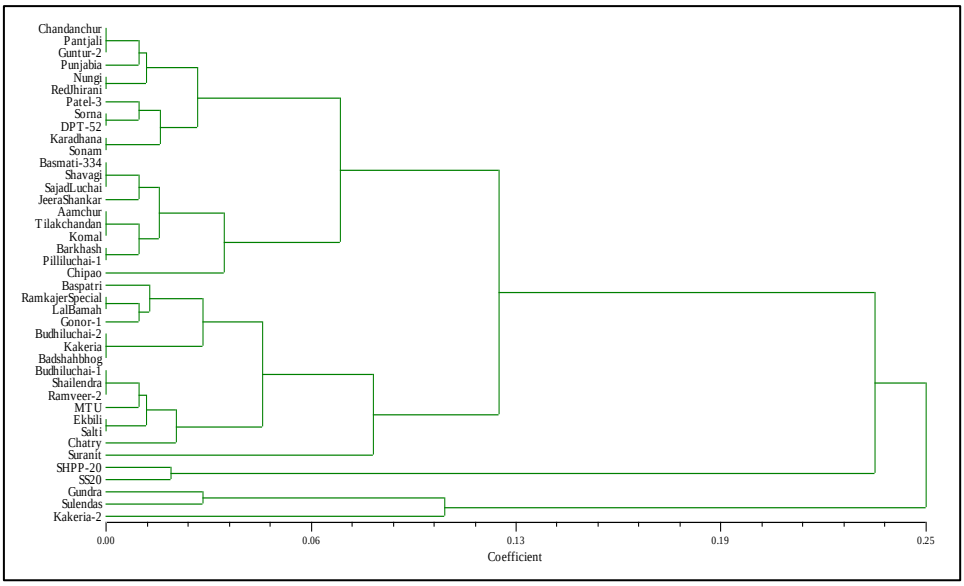

Fig 3. Dendrogram derived from UPGMA cluster analysis using Nei's similarity coefficient based on Kernel elongation ratio depicting the associations among the 14 improved varieties and 27 landraces of rice.

with 15 single plants per row at spacing of 30cm between rows and 20cm between plants in augmented randomized complete block design. Five single plants were selected in the middle of the row avoiding border plants for data collection.

Estimation of morpho-physiological parameters

Days to 50% flowering

Days to 50% flowering (DFF) or maturity was recorded as the number of days from sowing to initiation of flowering of 50% of plants based on visual observation of the each variety.

Panicle length

Panicle length was measured in cm from the panicle neck to the tip (excluding awn) at ripening stage and averaged over five plants.

Number of grains per panicles

It was measured by counting number of grains in a healthy panicle per plant at harvesting stage and averaged over five plants.

Table 4. Comparison of grain and cooking qualities of 12 selected rice varieties from the present investigation with Basmati standards. Six varieties highlighted in red are presented as potentially good candidates for use in breeding programs.

	Average pre-cooked milled rice length (mm)	Average pre-cooked milled rice breadth (mm)	Average length/breadth ratio of pre-cooked milled rice	Average cooked rice length (mm)	Kernel elongation ratio	Aroma
Value as per Basmati Standards	>6.61	<2	>3.5	>12	>1.7	Present
Value for Non-Basmati Standards	>5.8	<2.1	>2.9	>9	>1.25	Present
Chandanchur	7.07	1.87	3.78	9.2	1.3	Present
Chipao	6.87	1.93	3.56	10	1.46	Present
DPT-52	6	2	3	8.07	1.35	Present
Gonor-1	6.07	2.07	2.93	7.73	1.27	Absent
Gundra	6.07	2.13	2.85	9.4	1.55	Present
Kakeria-2	7.2	1.8	4	11.53	1.6	Present
Karadhana	5.87	1.87	3.14	7.93	1.35	Present
Patel-3	5.47	1.67	3.28	7.33	1.34	Present
Pilliluchai-1	7.53	1.67	4.51	10.27	1.36	Present
Red Jhirani	6.13	2.13	2.88	7.93	1.29	Present
Sajad luchai	6.87	1.93	3.56	9.6	1.4	Present
Tilakchandan	6	1.67	3.59	8.27	1.38	Present

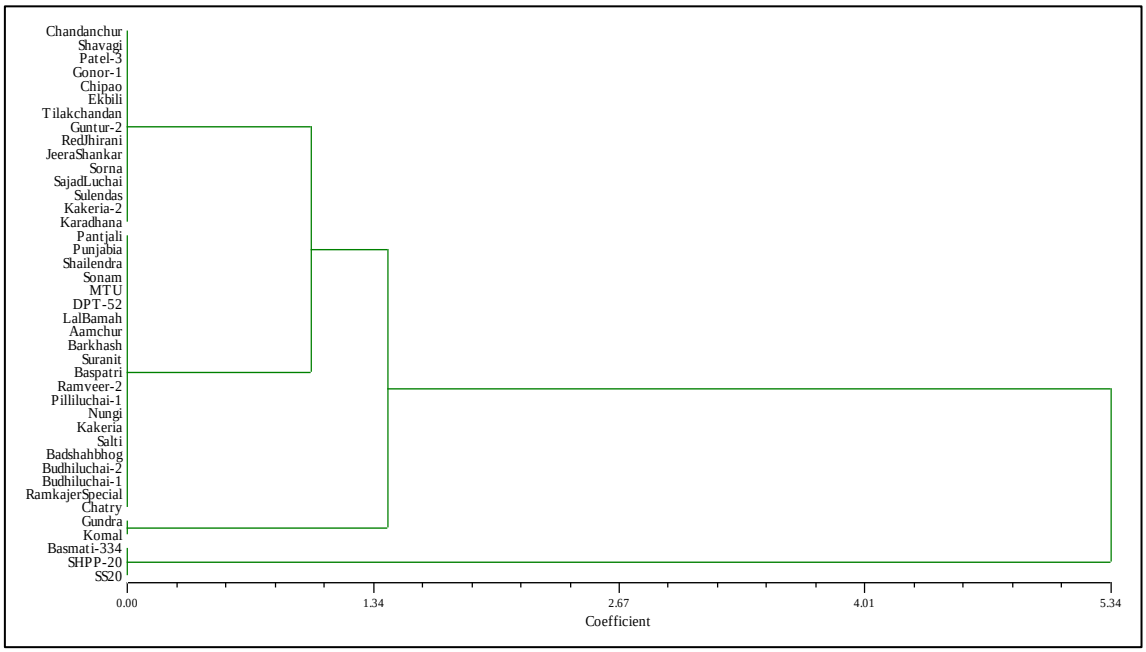

Fig 4. Dendrogram derived from UPGMA cluster analysis using Nei's similarity coefficient based on alkali spreading scores depicting the associations among the 14 improved varieties and 27 landraces of rice.

1000-grain weight

1000-grain weight was measured in grams by weighing 1000 filled grains and averaged over five samples for each variety.

Estimation of unmilled grain and cooking quality parameters

For full grain length and width, five unmilled healthy grains from a single plant were measured in mm through 3X magnification on a graph sheet. Average length, breadth and length/breadth ratio was calculated and averaged over five plants. For grain and cooking quality analysis, 10 g of full grains from each sample were dehusked in a Satake Testing Rice Husker (Satake Co. Ltd. Tokyo, Japan). The brown rice obtained was polished in a single pass rice miller (Satake Co. Ltd. Tokyo, Japan). Only normal shaped rice kernels (unbroken) were used for estimation of kernel elongation ratio, aroma evaluation and alkali spreading value (ASV). Length and breadth was recorded for five grains before and after cooking the grains in normal water for 10 min and averaged over five plants. Kernel elongation ratio was calculated by dividing observed grain length after cooking by grain length before cooking.

Grain Aroma

Sensory evaluation for the presence of aroma was made according to the method suggested by Sood and Siddique (1978). Ten milled grains were placed in petri dishes and 10 ml of freshly prepared 1.7% KOH solution was added. The plates were then incubated for ten minutes at room temperature. They were then opened one by one, smelled and rated for aroma. Since smell is a subjective trait, a panel of five members who had prior experience independently

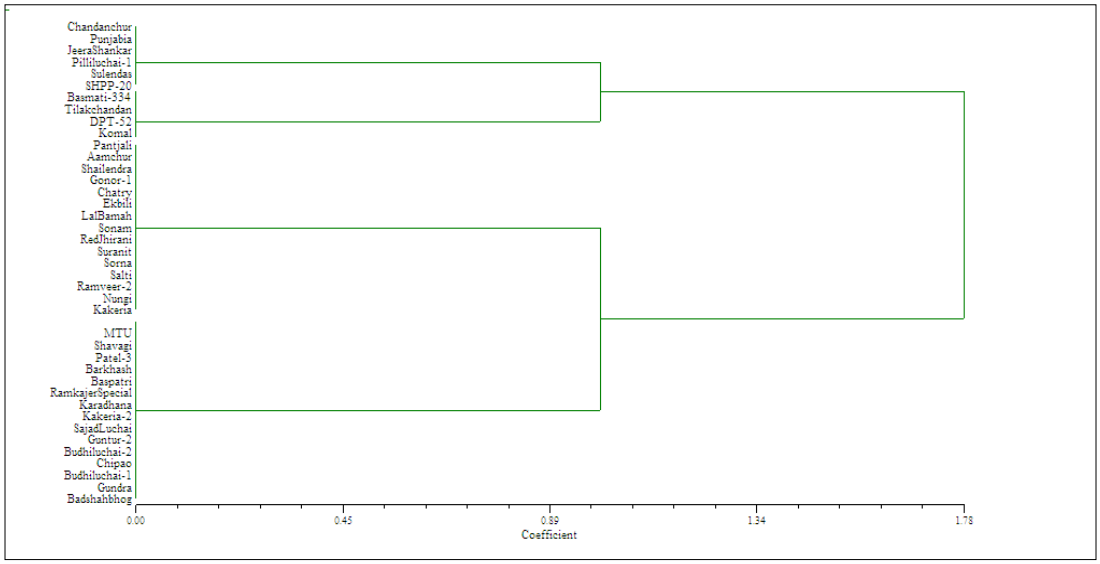

Fig 5. Dendrogram derived from UPGMA cluster analysis using Nei's similarity coefficient based on the sensory aroma scores depicting the associations among the 14 improved varieties and 27 landraces of rice.

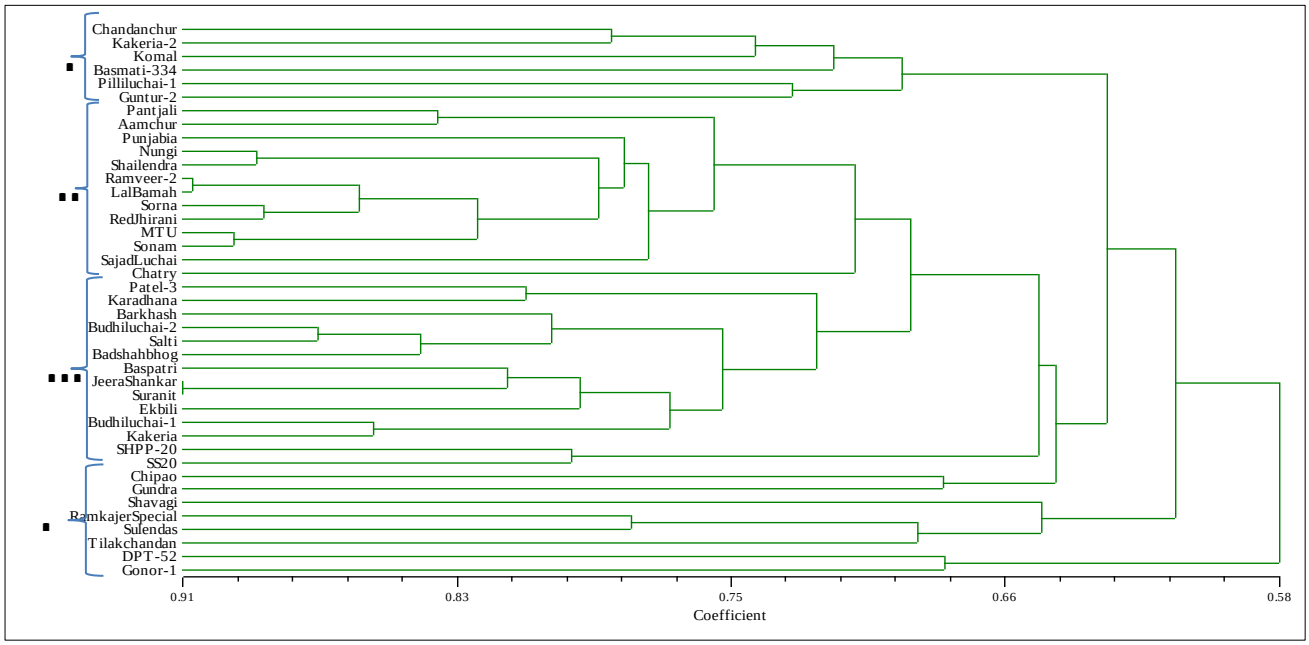

Fig 6. Dendrogram derived from UPGMA cluster analysis using Nei's similarity coefficient based on 24 SSR markers depicting associations among the 14 improved varieties and 27 landraces of rice.

Fig 7. Amplification pattern of 12 selected rice varieties with SF28, a CAPS marker associated with grain length (GS3 gene) on 4% agarose gel. Two bands 110bp (marked with arrow) and 26bp (not visible above) are obtained after restriction digestion of the 136bp fragment by *PstI* enzyme at 37^0C for 1 hour. M: 100bp DNA ladder.

checked for grain aroma. The samples were rated on an arbitrary scale of 0-3 (0 for non-aromatic, 1 for less aromatic, 2 for moderately aromatic and 3 for highly aromatic samples). Mode of the scores of the five panelists was taken as the final score. Blind checks were included to increase the accuracy of the scores.

Alkali Spreading Value

Alkali spreading value is known to be inversely related to the temperature at which gelatinization of rice occurs (Lanceras et al., 2000).The method of Little et al. (1958) was used for conducting the alkali spreading test. Five milled rice grains were immersed in freshly prepared 1.7% potassium hydroxide solution and incubated at 30^oC for 23 hours. Grains were carefully separated using forceps, and the spreading value of the grains was scored on a scale of 1-7 by visual assessment using the method of Jennings et al. (1979). Mode of five grains was taken as the final score.

Molecular analysis through SSR

A total of 24 SSR/microsatellite markers having repeats ranging from dimmers to tetramers were chosen with one marker from each long and short arm of the 12 rice chromosomes. To increase the efficiency of genotyping, the two markers selected from the same chromosome had a minimum physical distance of approximately 7 Mb. DNA was isolated from fresh leaves of about 12-15 day old seedlings by CTAB method as proposed by Murray and Thompson (1980). The annealing temperature of all the markers was analyzed using gradient PCR with a temperature range of $50\text{-}65^0C$. PCR amplification was performed in a 10µl volume containing 45 ŋg of template genomic DNA, 5 pmol (13 ng) each of forward and reverse primers, 0.2 mM dNTPs, 1X PCR buffer (10mM Tris, pH 8.0, 50 mM KCl and 50 mM ammonium sulphate), 1.5 mM $MgCl_2$ and 0.5 units of Taq DNA polymerase (all the reagents from Puregene Tauras Scientific). Template DNA was initially denatured at 94°C for 5 min followed by 35 cycles of repeated PCR amplification with the following parameters in a sequential order: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, primer extension at 72°C for 30 sec with a final extension at 72°C for 10 min. The amplified products were electrophoretically resolved on 4% Meta-Phor Agarose gels (Lonza, USA) prestained with ethidium bromide (0.1mg/ml). The gels were photographed and visualized by transillumination under short-wave UV light by gel documentation system AlphaImager (Flourochem 5500).

Validation through standard molecular/gene-based markers

Some potential candidates identified on the basis of their grain characteristics were also genotyped with two standard markers; nksrssr 04-11 and SF28 which are genetically linked to grain number and grain length respectively. nksrssr04-11 (forward primer sequence 5'-CCATCAGTTGAAGGGCTCTC-3' and reverse primer sequence 5'-CTTTTATGGCATGGGCAACT-3') is an SSR marker associated with the QTL for grain number ($qPB_{4\text{-}2}$) located on the long arm of chromosome 4 (Deshmukh et al., 2010). SF28 (forward primer sequence: 5'-TGCCCATCTCCCTCGTTTAC-3; and reverse primer sequence (5'-GAAACAGCAGGCTGGCTTAC-3') is a CAPS (Cleavage Amplified Polymorphic Sequence) marker developed on the C/A polymorphism in the *GS3* gene responsible for grain length in rice. SF28 amplifies a 136bp segment of the second exon of the *GS3* gene (Fan et al., 2006). The CTGCAG/CTGAAG polymorphism results in a restriction site change of *PstI* restriction enzyme. After restriction digestion of the 136 bp product with *PstI* enzyme at 37^oC for 1 hour, two fragments of 110 bp and 26 bp were produced for the genotypes having CTGCAG sequence. In genotypes with CTGAAG sequence, the 136bp amplicon was left undigested due to the lack of *PstI* site. The conditions used for amplification through PCR and electrophoresis were same as used for SSR markers.

Data analysis and clustering

Grain quality and cooking data was analyzed by calculating mean and standard deviation of five different observations. For genetic diversity analysis, unambiguous and reproducible bands were scored for all the 24 SSR markers qualitatively according to their product size. The alleles were scored in the form of a binary data matrix as absent (0) or present (1) and the numeral 9 was given for null alleles. All of the SSR markers displayed polymorphism, with the number of alleles ranging between 2 and 4. Polymorphism Information Content (PIC) values were calculated for all the markers using the following formula given by Nei et al. (2002):

$PIC_i = 1\text{-}\Sigma P_{ij}^2$,
Where; P*ij* is the frequency of the j^{th} allele for marker *i*.

The two sets of data obtained (phenotypic grain characters/cooking quality parameters and genotypic profile) were subjected to cluster analysis. Clustering was done for four grain characteristics *viz*. milled (precooked) grain length, kernel elongation ratio, alkali spreading value and sensory aroma score. Clustering of the matrix data obtained through allelic profiles was done on the basis of distance (similarity) matrices through NTSYSpc software version 2.1. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering was performed on squared Euclidean distance matrix and similarity matrix using Jacquard's coefficient using the unweighted pair group method with arithmetic averages (UPGMA) method. Principle Component Analysis (PCA) determined the level of similarity between and among individual varieties/groups.

Conclusion

India harbors a huge resource of rice cultivars that are lesser known at the market front but hold great significance not only for farmers but also for the local consumers. An effort was made to collect a set of 41 cultivars including 14 improved varieties and 27 landraces of rice and to assess their genetic and morphological diversity. Their genetic diversity with molecular markers (SSR markers) and phenotypic characterization with grain quality traits indicated toward linkage or pleiotropic effect of genomic regions involved in their phenotypic expression. Some genotypes (Patanjali, Punjabia, Aamchur, MTU, Lal Bamah, Chatry, Ekbili, Sonam, Ramveer-2, Nungi) were also characterized and offered promise in their use in the genetic improvement of rice cultivars for grain quality.

Acknowledgements

The authors are highly grateful to the various farmers who provided knowledge and information as well as seeds of the various cultivars preferred by them. We are also thankful to ICAR for providing financial assistance to conduct the research work.

References

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95: 553–567.

Deshmukh R, Singh A, Jain N, Anand S, Gacche R, Singh AK, Gaikwad KS, Sharma TR, Mohapatra T, Singh NK (2010) Identification of candidate genes for grain number in rice (*Oryza sativa* L.). Funct Integr Genomics 10: 339–347.

Duwick DN (1984) Genetic diversity in major farm crops on the farm and reserve. Econ Bot 32: 161-178.

Fan C, Yu S, Wang C, Xing Y (2008) A causal C–A mutation in the second exon of GS3 highly associated with rice grain length and validated as a functional Marker. Theor Appl Genet 118(3): 465-472

FAO (2002) A report on: Crops and drops – making the best use of water for agriculture. Food and Agriculture Organization of the United Nations. Rome.

Government of India Office memorandum No. 1514, New Delhi, October 29, 2008

Hore DK (2005) Rice diversity collection, conservation and management in northeastern India. Genetic Resources and Crop Evolution 52: 1129–1140.

Jennings PR, Coffman WR, Kauffman HE (1979) Grain Quality. In: Rice improvement. Los Banos (Philippines) Intl Rice Res Inst 101-120

Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS (2000) Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus Oryza. Theor Appl Genet 100; 1311-1320.

Khush GS, Paule CM, Cruz NMDL (1979). Rice grain quality evaluation and improvement at IRRI. Proceedings workshop on chemical aspects of rice grain quality. IRRI, Los Banos, Laguna, Philippines, 21-31.

Kibria K, Nur F, Begum SN, Islam MM, Paul SK, Rahman KS, Azam SMM (2009) Molecular marker based genetic diversity analysis in aromatic rice genotypes using SSR and RAPD markers. Int J Sustain Crop Prod 4(1): 23-34.

Lanceras JC, Huang ZL, Naivikul O, Vanavichit A, Ruanjaichon V Tragoonrung S (2000) Mapping of genes for cooking and eating qualities in Thai Jasmine rice (KDML105). DNA Res. 7: 93-101.

Little RR, Hilder GB, Dawson EH (1958) Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chem 35: 111-126.

Maclean JL, Dawe DC, Hardy B, and Hettel GP (ed.) (2002). Rice Almanac, Los Baños (Philippines): International Rice Research Institute, Bouaké (Côte d'Ivoire): West Africa Rice Development Association, Cali (Colombia): International Center for Tropical Agriculture, Rome (Italy): Food and Agriculture Organization.

McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Chao YG, Huang N, Ishii T, Blair M (1997) Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Mol Biol 35(1-2): 89-99.

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res 8: 4321-4325.

Nagaraju J, Kathirvel M, Kumar R, Siddiq EA, Hasnain SE (2002) Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc Natl Acad Sci. 99(9): 5836-5841.

Nei J, Pewter M, Mackill BJ (2002) Evaluation of genetic diversity in rice sub species using microsatellite markers. Crop Sci. 42: 601-607.

Pachauri V, Singh MK, Singh AK, Singh S, Shakeel NA, Singh VP, Singh NK (2010) Origin and genetic diversity of aromatic rice varieties, molecular breeding and chemical and genetic basis of rice aroma. J Plant Biochem Biotechnol. 19(2): 127-143.

Prathepha P (2009) The fragrance (*fgr*) gene in natural populations of wild rice (*Oryza* rufipogon Griff.) Genet Resour Crop Evol 56: 13-18.

Ravi M, Geethanjali S, Sameeyafarheen F, Maheswaran M (2003) Molecular marler based genetic diversity analysis in rice (Oryza sativa L.) using RAPD and SSR markers. Euphytica 133: 243-252.

Singh RK, Singh US (2003) A Treatise on Scented Rices of India, Kalyani Publishers, New Delhi.

Sivaranjani AKP, Pandey MK, Sudharshan I, Kumar GR, Madhav MS, Sundaram RM, Varaprasad GS, Rani NS (2010) Assessment of genetic diversity among Basmati and non-Basmati aromatic rices of India using SSR markers. Current Sci. 99(2): 221-226.

Sood BC, Siddiq EA (1978) A rapid technique for scent determination in rice. Ind J Genet Plant Breed 38: 268-271.

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100: 697-712.

Tragoonrung S, Sheng JQ, Vanavichit A (1996) Tagging an aromatic gene in lowland rice using bulk segregant analysis. International Rice Research Institute, Rice genetics III.

Vikram P, Swamy BPM, Dixit S, Sta Cruz, Ahmed HU, Singh AK, Kumar A (2011) qDTY1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. BMC Genet. 12, 89.

Vikram P, Swamy BPM, Dixit S, Ahmed HU, Sta Cruz, Singh AK, Kumar A (2012). Bulk Segregant Analysis: "An effective approach for mapping consistent-effect drought grain yield QTLs in rice" Field Crops Res. 134:185-192.