RESEARCH ARTICLE

Polymorphism between Popular Rice Varieties of Uttar Pradesh, Uttarakhand, Madhya Pradesh, Bihar, West Bengal and Jharkhand through Highly Variable HvSSR Marker

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ABSTRACT

India possesses an immense wealth of Basmati and non Basmati aromatic rice varieties and land races exhibiting a wide variability in their grain quality and cooking characteristics. Among non basmati aromatic rices, an important and popular scented rice variety grown in Eastern Uttar Pradesh and other state. This variety is famous for its taste and aroma. In eastern India it is cooked in honour of guest or given as gift. Although all variety fast going out of cultivation, it is Pusa 1342 as non aromatic, Pusa Basmati 1 as slightly aromatic, Pusa Sugandha as aromatic and Pusa 1121 as highly aromatic indigenous aromatic rice variety if it is promoted properly, offers a great promise not only in domestic market but also as an export commodity. It can be boon for farmers of Eastern Uttar Pradesh and Tarai area of Bihar, collected from different parts of Eastern Uttar Pradesh were evaluated on the basis of grain quality for pure line selections and further improvement to attract more and more farmers for its cultivation again. 24 SSR molecular markers were selected covering as much as of the genome as possible. The selected primers and their physical location are summarized. Allele diversity was done using 24 distinct rice microsatellite markers dispersed throughout the rice genome. The average product range of resultant amplicons was 80-580bp. A total of 66 alleles were observed all additional rice variety Budhiluchai, Sorna, Sonam, Aamchur, MTU mention below.

Keywords: *Pusa Basmati 1, Pusa Sugandha, Pusa 1121 Grain quality, Non Basmati rice*

INTRODUCTION

Genetic diversity serves a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for survival in the environment. Such individuals are more likely to survive to produce offspring(s) bearing that allele. Assessment of genetic diversity is an integral part of plant breeding as exploitation of the genetic diversity helps the plant breeder to develop new varieties. Rice is the staple food for more than half of the World's population. Its rich genetic diversity in the form of thousands of landraces and progenitor species, besides its economic significance, has aroused unending interest among scientists for several decades. India is home to many varieties of rice, which have been obtained after centuries of breeding and selection by farmers based on various desirable characteristics such as yield, aroma, grain length, cooking quality, etc. Basmati rice also originates from India. The famous rice variety is valued for its slender grains, fragrance, and elongation upon cooking. In the present study, 42 different rice varieties were collected from other Indian states and then grouped according to their geographical location. Most of these rice varieties are local rice cultivars which are often preferred by the farmers for commercial cultivation based upon their choice of grain quality, yield, fragrance, market value, and also upon their cultivation practices. Most of these varieties have long been

known by their local names, and hence there are no or very few records of the genetic nature or background of these varieties. It is believed that some of these varieties may have originally belonged to some other state/region and then taken to other states due to their superior performance. Keeping these things in mind, the current study was planned to study the genetic diversity among these varieties of rice collected from different parts of India. For this, twenty-four genome-wide simple sequence repeat (SSR) molecular markers located one each on the two arms of each of the twelve rice chromosomes was used. SSR markers are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions. Genetic diversity is commonly measured by genetic distance or genetic similarity, implying that there are differences or similarities at the genetic level.

In rice, grain quality parameters are of utmost importance as most of the rice worldwide is consumed as whole grain or rice flakes. They are also the significant determinants of price both in national as well as international markets. Yield is the most noticeable characteristic to farmers while the crop is in the field. Still, when the crop product is harvested and milled rice reaches the market, quality becomes the key determinant of its value. In the present study, phenotypic analysis was also done to complement the genotypic data and establish a healthy relationship among the varieties. Grain quality evaluation comprised of grain length, cooking quality, alkali spreading value, and aroma. A dendrogram was finally obtained using cluster analysis depicting the genetic similarity among the rice varieties/groups.

MATERIALS AND METHODS

Rice Varieties

A total of 42 distinct rice varieties were collected from several Indian states. These varieties comprise lengthy grain basmati varieties, short-grain aromatic, short-grain non-aromatic, and some medium-grained rice varieties. The seeds were collected from several states, including Uttar Pradesh, Uttarakhand, Madhya Pradesh, Bihar, West Bengal, and Jharkhand. The seeds were freshly harvested from Kharif-2010. It may be noted that the seeds were collected from these locations from farmers, but initially these varieties may have belonged to some other place and may be genetically as well as phenotypically distinct from different types that may have originated from the exact place.

Genetic Diversity Analysis

Diversity analysis was done on selected 42 rice varieties using SSR markers. In all, 24 SSR markers covering the whole of the rice genome were selected and were amplified through PCR.

Selection of primers

SSR markers are short DNA fragments that are interspersed throughout the genome and can be amplified using primers that flank these regions. Such markers are co-dominant and are considered one of the most favored markers for several molecular biology applications, including genetic diversity studies, due to their multi-allelic nature, high reproducibility, co-dominant inheritance, abundance, and extensive genome coverage. For the current study, 24 SSR markers were selected (Table 1), from different locations in the genome. Attempts were made to choose one primer each from the short and long arms of each chromosome. The two chosen markers had a minimum physical distance of approximately 7 Mb. The annealing temperature of all the 24 primers was found to be 55°C using gradient PCR.

DNA Isolation and Quantification

Seeds of all the 42 rice varieties were germinated in a BOD incubator at 37°C for 10-15 days. DNA was then isolated from young leaf tissue using the CTAB method of Murray and Thompson (1980). All the reagents were freshly prepared in the lab. *Reagents for DNA isolation* 1M Tris-Cl (pH 8.0): 121g Tris-Cl base was dissolved in 800 ml double-distilled H_2O, and the pH was adjusted to 8.0 using concentrated HCl. The final volume of the solution was made up to 1000ml and then autoclaved. 0.5 M EDTA (pH 8.0):186g di-sodium salt of EDTA was dissolved in 800 ml double-distilled H_2O, and the pH was adjusted to 8.0 using NaOH pellets. The final volume was made up to 1000ml and then autoclaved. 4M NaCl: 234 g of NaCl was dissolved in 800 ml double distilled water. The final volume was adjusted to 1000ml and then autoclaved. 10% CTAB: 100 g of CTAB was dissolved in 1000ml of distilled water. DNA extraction buffer (100 ml working solution): 1M Tris-Cl (pH 8.0) - 10.0 ml (100mM) 0.5 M EDTA (pH 8.0) 4.0 ml (20mM). 4M NaCl - 35.0 ml, (1.4 M) 0% CTAB 20.0 ml (2%), β-Mercaptoethanol 200 µl (0.2%) Double distilled water 30.8 ml, 3M Sodium Acetate pH 5.2: RNase A (10mg/ml) 10 mg RNase A was dissolved in 1 ml Autoclaved double-distilled H_2O, vortexed well and then kept in water bath at 100°C for 10-15 minutes, cool down and store at -20 °C. Phenol Chloroform Isoamyl alcohol (PCI) mixture: Ready-to-use mixture of Phenol:Chloroform:Isoamyl alcohol (25:24:1) v/v was obtained from Amresco company. Chloroform: Isoamyl alcohol(24:1)v/v Freshly prepared by mixing

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96ml Chlorophorm and 4ml Isoamyl alcohol 10. Tris-EDTA buffer (TE): Tris (10mM): EDTA (1mM) buffer was prepared in 100 ml as follows,1mM Tris-Cl, pH 8.0 1 ml 0.5M EDTA pH 8.0, 0.2 ml Autoclaved double-distilled H_2O 98.8 ml.

DNA Isolation and Quantification-Genomic DNA were isolated from all the 42 rice varieties using the standard CTAB method. 2-4 grams of freshly harvested leaf tissue from 15-day old seedlings were ground to a fine powder in liquid nitrogen using sterile pre-cooled mortar and pestle. The ground powder was transferred to a 50 ml polypropylene centrifuge tube containing 20ml of pre-warmed DNA extraction buffer. The suspension was incubated at 65°C for one hour with intermittent mixing. The tubes were then cooled to room temperature, and an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by gentle inversion for about 5 minutes. The contents were then centrifuged at 12000 rpm for 10 minutes at room temperature. The aqueous phase was transferred to a fresh centrifuge tube with comprehensive bore tips to avoid DNA shearing, and 0.6 volumes of chilled isopropanol were added. Contents were mixed by gentle inversion and incubated at –20°C for 30 minutes for DNA precipitation. The precipitated DNA so obtained was pooled out by centrifugation at 10,000 rpm for 5 minutes. The DNA pellet was washed twice with 70% ethanol, dried at 37°C for one hour, and dissolved in 500 µl TE buffer (pH 8.0). DNA was quantified by gel quantification method, in which the samples along with the known concentration of λ genomic DNA as standard were loaded on 0.8% agarose gel prepared in 1X TBE buffer. The gel was then run at 80 volts for about 30 minutes. The intensity of individual samples was compared with a range of known amounts of λ DNA (50,100 and 150 ng/µl. Accordingly, the samples were diluted to a working concentration of 10 ng /µl for further use.

DNA Purification

The crude DNA extracted was purified by adding RNase (10mg/ml) to the sample at the rate of 5 µl/1g of leaf tissue used for extraction (approx.1µl/100µl of DNA solution in TE buffer) and was mixed gently by inversion. The mixture was incubated in a water bath for 1hour at 37°C with intermittent mixing. An equal volume of Phenol Chloroform Isoamyl alcohol (PCI) solution was added to the mixture, mixed gently by inverting for about 5 minutes. The mixture was centrifuged at 10000 rpm for 10 minutes, and the aqueous phase was taken out in a new eppendorf tube. Chloroform: isoamyl alcohol (24:1) extraction was performed twice to remove all impurities. To the aqueous phase, 1/10th volume of 3M sodium acetate (pH 5.2) was added, mixed gently, and then two volumes of chilled ethanol were added to the mixture. Contents were mixed gently and incubated at –20°Cfor 2 hr, centrifuged for 5 minutes at 10000rpm. The supernatant was discarded, and the pellet was washed twice with 70% ethanol. DNA Pellet was dried properly and dissolved in 100 µl TE buffer pH 8.0.

Amplification by Polymerase Chain Reaction (PCR)

PCR reactions were set up in 10µl volume containing 50 ŋg of template 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 sulfate), 1.5 mM $MgCl_2$ and 0.5 units of Taq DNA polymerase (all the reagents from Puregene). The volume was made up to 10µl by autoclaved Milli Q water. The cocktail was then subjected to PCR amplification in a thermal cycler (Biometra). The PCR cycling conditions were as follows: Initial denaturation at 94^{0}C for 5 min, denaturation at 94^{0}C for 1 min, annealing at 55^{0}C for 1 min, Primer extension at 72^{0}C for 30 seconds, repeat step 2, 34 times. Final extension at 72^{0}C for 10 min Step 7: Storage at 4^{0}C until electrophoresis. 2.4 Agarose Gel Electrophoresis of the PCR products 4% MetaPhor agarose (Lonza) was used to resolve the PCR amplified DNA. The gel was prepared by dissolving metaphor agarose in 1X TBE buffer. 10 ŋg ethidium bromide was added per 100 ml of gel, and the cooling gel was poured into the gel cassette with wide combs and allowed to polymerize at room temperature. The gel and tray and combs, was shifted to the electrophoresis tank, where the combs were removed carefully. PCR samples were mixed with 1X loading dye and loaded in the preformed wells. Electrophoresis was carried out in 1X TBE buffer at 120 volts for about 3-3.5 hours. Gel photographs were taken by gel documentation system (Flourochem/ AlphaImager™ image acquisition with CCD camera, San Leandro California).

Evaluation of SSR polymorphisms

Unambiguous and reproducible bands were scored according to their molecular weight and the concentration of the amplicon. The alleles were scored as absent (0) or present (1). The digit 9 was given for any missing alleles. A binary data input file was used for analysis through NTSYSpc software version 2.02. The binary data matrix was subjected to cluster analysis. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering was performed on squared Euclidean distance matrix and similarity matrix using Jacquard's coefficient utilizing the unweighted pair group method with arithmetic

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averages (UPGMA) method. Principle Component Analysis (PCA) was also done to determine the similarity between and among individual varieties/ groups.

Grain quality parameters

For the present study, phenotypic characterization of the 42 rice varieties was done to complement the genetic data. Some essential quality traits of rice were considered, such as grain length and width, cooking quality, alkali spreading value, and aroma.

Grain length and width

Five healthy grains were taken to measure length and width through 3X magnification on a graph sheet. The average height and breadth, along with the L/B ratio, were calculated.

Grain cooking quality

Dehulling of rough paddy was done using dehuller (model no NF 271, 0.5 HP motor at 1400 rpm). Dehulled grains were then milled mechanically and were then used for the estimation of various quality parameters.

Grain Elongation

To determine the cooking quality, the average length and breadth were calculated before and after cooking. The size and width of five milled rice grains were recorded and then cooked for 5 minutes in normal water. Readings were again taken after cooling them at room temperature. Kernel elongation ratio was calculated by dividing observed length after cooking by size before cooking.

Alkali spreading value

Gelatinization temperature is the temperature at which the starch granules in rice absorb water and lose their crystalline nature, an irreversible change. Rice starch usually gelatinizes between 65°C and 85°C. Rice with a gelatinization temperature at the lower end of the range often cooks to a softer texture and retrogrades less than rice with a gelatinization temperature at the upper end of the spectrum. Gelatinization is often measured by the alkali spreading method on a scale of 0-7.

The method of Little *et al.* (1958) was used for conducting the alkali spreading test. 10 milled rice grains from each variety were added to a glass perti plate containing 10 ml of freshly prepared 1.7% KOH solution (17 g KOH pellets dissolved in 500 ml double distilled water and the volume made up to 1000 ml) and incubated at 30°C for 23 hours. Grains were carefully separated using forceps, and the spreading value of the grains was scored by visual assessment using the method of Jennings *et al.* (1979).

Following three blind checks were used to improve the efficiency of recorded observations: Taraori Basmati (ASV= 4), IR64 (ASV= 5), and Pusa Basmati 1 (ASV= 7).

Aroma

Aroma is one of the most essential traits of rice, especially with respect to its commercial value. Determination 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, and samples were smelled and rated for aroma. Since smell is a subjective trait, a panel of five members independently checked for aroma. The samples were categorized into four classes: nonaromatic, slightly aromatic, moderately aromatic, and highly aromatic and given scores of 0, 1, 2, and 3, respectively. Following four blind checks were included to increase the accuracy of the scores: Pusa 1342 as non-aromatic (score 0), Pusa Basmati 1 as slightly aromatic (score 1), Pusa Sugandha as aromatic (score 2), and Pusa 1121 as highly aromatic (score 3).

RESULTS AND DISCUSSION

Genetic diversity analysis along with the phenotypic characterization of all the 42 rice varieties was done. Allele diversity was done using 24 distinct rice microsatellite markers dispersed throughout the rice genome. The average product range of resultant amplicons was 80-580bp. A total of 66 alleles were observed.

Selection of primers

24 SSR molecular markers were selected, covering as much of the genome as possible. The chosen primers and their physical location are summarized in table 1. 5 picomoles of each primer were used for amplification through PCR. Gradient PCR was done to identify the annealing temperature of the selected primers, which was found to be in a uniform range of 54-56°C (figure 1). Hence all the primers were annealed at 55°C.

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Table 1. Details of primers

Primer ID	Chr	Location (bp)	T_a	Forward sequence	Reverse Sequence
RM24	1	19,304,216	55	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG
RM578	1	8,407,910	55	GGCGTCGTGTTTTCTCTCTC	CAAAAAGGAGGAGCAGATCG
RM561	2	36,205,911	55	GAGCTGTTTTGGACTACGGC	GAGTAGCTTTCTCCCACCCC
RM1211	2	18,450,371	55	TACAGTGGCGAAAGGAATAC	CCATCACGCATGTTAGTTAG
RM15780	3	28,585,355	55	ACCTTCGACGCTATCAGATTTGG	ATAGCAAAGGAGTCGCAAAGACC
RM251	3	9,948,792	55	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC
RM273	4	23,859,081	55	GAAGCCGTCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC
RM307	4	11,114,917	55	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
RM1054	5	29,144,016	55	TGCATATGTACCGCAACCTC	TTTCTGCATGATCCCCTCTG
RM159	5	466,069	55	GGGGCACTGGCAAGGGTGAAGG	GCTTGTGCTTCTCTCTCTCTCTCTCTC
RM528	6	26,554,756	55	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
RM6818	6	16,581,413	55	GTCGCATTCGTCTCCACC	ACCATTTCCAGATGACTCGG
RM427	7	2,655,927	55	TCACTAGCTCTGCCCTGACC	TGATGAGAGTTGGTTGCGAG
RM432	7	18,957,896	55	TTCTGTCTCACGCTGGATTG	AGCTGCGTACGTGATGAATG
RM42	8	19,964,404	55	ATCCTACCGCTGACCATGAG	TTTGGTCTACGTGGCGTACA
RM25	8	4,377,460	55	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC
RM219	9	14,503,902	55	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG
RM24846	9	22,909,901	55	CGAACATTGTTTCGCCCTTTCC	CGAGGCACCCTACATACTACTCACG
RM6271	10	4,546,146	55	ACCTCAAGATTCCAGCTGTC	AAGCGGAAATGCTGCAGTAG
RM6100	10	18,558,474	55	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC
RM2110	11	19,423,211	55	ATGTGGACAATGATATATGT	CTCCGTTTCATATTATAAGA
RM7443	11	28,395,760	55	TGCTGCGTGTTACTTTGGTG	AACCCTTCATCAGGCTACGC
RM28346	12	20,919,548	55	GCCCAAAGTTAATATCGGT-GTCTCC	AGCCTGCCTAGCACTCATAGACC
RM17	12	26,954,668	55	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA

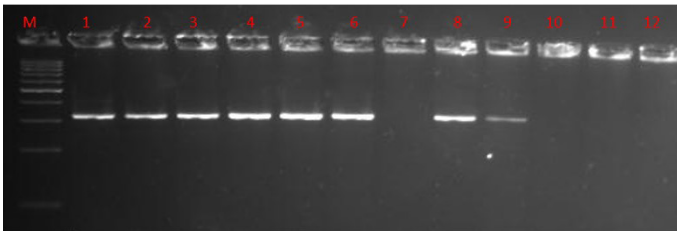

Fig.1. Gradient PCR profile of primer RM273 using a temperature range of 50.0 to 65.0°C [M:100 bp DNA ladder; 1:50°C; 2:50.4°C; 3:51.3°C; 4:52.5°C; 5:54.2°C; 6:56.4°C; 7:58.9°C; 8:61.7°C; 9:62.7°C; 10:63.9°C; 11:64.7°C; 12:65.0°C]

Allelic diversity among the rice varieties

A total of 66 different alleles were obtained with a total of 24 different rice microsatellite markers. Amplicons obtained by normal PCR were resolved on 4% agarose gel by horizontal gel electrophoresis (fig. 3). The results obtained based on analysis of 42 rice cultivars of rice using 24 random primers are tabulated in Table 2. Highly distinct and unambiguous bands were scored as present (1) or absent (0). Any missing allele was scored as 9. All of the SSR markers displayed polymorphism, with the number of alleles ranging between 2 and 4. The olymorphism Information Content (PIC) value was calculated for all the SSR markers using the formula PIC = 1-SP_i^2, where Pi is the allelic frequency of the i^{th} allele. The PIC values ranged from a minimum of 0.04 (RM6818) to a maximum of 0.66 (RM251). The product size range was 80-580bp, with RM6271 giving smallest product size of 80bp and RM24846 giving largest product size of 580bp.

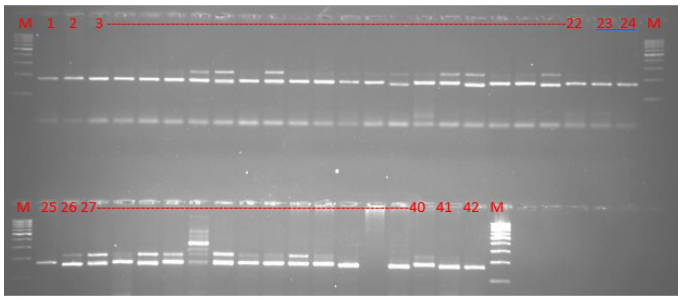

Fig. 2: Diversity analysis of 42 rice varieties using RM432 molecular marker. [M:100 bp DNA ladder; 1-42: Farmers' rice varieties. The PCR products were analyzed on 4% agarose gel.

Statistical analysis of the scored raw data was done through NTSysPC version 2.02h software. Accordingly, a binary data matrix input file was prepared. Using

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Table 2: Allelic distribution and PIC values of 24 rice microsatellite (SSR) markers across 42 farmers' rice varieties

Primer ID	Chr	Genotypes tested	Genotypes missing	Genotypes amplified	No. of alleles	Allelic distribution				Product range	PIC
						1	2	3	4		
RM24	1	42	1	41	4	34	4	5	1	150-220	0.29
RM578	1	42	0	42	3	25	7	9	0	280-310	0.57
RM1211	2	42	0	42	2	34	8	0	0	175-185	0.31
RM561	2	42	0	42	2	40	2	0	0	180-190	0.09
RM15780	3	42	0	42	2	27	15	0	0	130-150	0.46
RM251	3	42	0	42	4	15	18	6	3	110-145	0.66
RM307	4	42	1	41	3	34	7	5	0	135-200	0.27
RM273	4	42	1	41	2	24	19	0	0	190-200	0.44
RM159	5	42	1	41	2	25	16	0	0	250-260	0.48
RM1054	5	42	0	42	4	10	28	11	4	170-220	0.42
RM6818	6	42	0	42	2	41	4	0	0	110-150	0.04
RM528	6	42	0	42	4	30	13	11	2	290-360	0.32
RM427	7	42	0	42	2	40	0	2	0	190-200	0.09
RM432	7	42	1	41	3	32	7	12	0	180-230	0.28
RM25	8	42	2	40	3	10	27	4	0	130-150	0.47
RM42	8	42	5	37	2	16	21	0	0	130-140	0.49
RM219	9	42	1	41	4	13	21	6	2	200-300	0.61
RM24846	9	42	2	40	2	28	12	0	0	550-580	0.42
RM6271	10	42	4	38	2	31	7	0	0	80-90	0.3
RM6100	10	42	1	40	3	26	5	15	0	150-200	0.42
RM7443	11	42	0	42	3	31	11	20	0	160-200	0.16
RM2110	11	42	6	36	3	21	15	2	0	400-450	0.48
RM17	12	42	3	39	2	13	26	0	0	190-200	0.44
RM28346	12	42	1	41	3	29	11	4	0	180-210	0.42

this matrix, a similarity matrix was generated using Jacquard's coefficient. This similarity matrix was finally clustered using Sequential agglomerative hierarchical non-overlapping (SAHN) clustering utilizing the unweighted pair group method with arithmetic averages (UPGMA) method, generating a dendrogram wherein all the 42 rice varieties were clustered into groups.

MTU, a local landrace from Andhra Pradesh, and Sapaluchai, a famous local cultivar from Madhya Pradesh, displayed the highest level of genetic similarity (79%), followed by Jeera Shankar and Suranit (77%). A minimum similarity of 31 % was observed among the varieties and two major clusters (I and II) were formed, a small cluster containing Gonor-1 and BPT-52 and a large cluster containing the other 40 types. Cluster II was further subdivided into two unequal clusters at a similarity coefficient of around 0.34 (IIA and IIB). IIA contained three varieties (names) and IIB contained the remaining varieties. IIA formed the largest subgroup, which divides into two distinct subgroups (IIAi and IIBii) at a similarity coefficient of 0.37, one containing 9 varieties and the other containing 27 varieties (fig 4).

Grain Quality Evaluation

Grain quality holds a significant role for rice as they are the primary determinant of its price in the market. A general profile was generated for all the 42 rice varieties to characterize them into various groups according to their grain size (length and breadth), their aroma and alkali content, as well as their cooking qualities (like elongation ratio). Among all the varieties, the most extended grain length was recorded as 11.7 mm for SS20 variety, while the shortest distance was recorded for as 6.9 mm for Sulendas (fig. 5). Kakeria-2 showed the highest elongation ratio of 1.61 (fig. 6), while SS20 showed the lowest elongation ratio of 1.05. A few highly aromatic varieties were also identified, such as Komal, BPT-52, Tilakchandan, and Basmati-334, having a score of 3. A high level of phenotypic diversity was observed among the 42 rice varieties. The range of recorded length was 6.9-11.7mm. A diverse L/B ratio was also observed, ranging from 2.29 (Suranit) to 5.57 (SS20). Sensory analysis of aroma also yielded diverse results, covering the entire range of 0-3. The ASV score ranged from 1-7, with most of the varieties giving a score of 2 (fig. 7).

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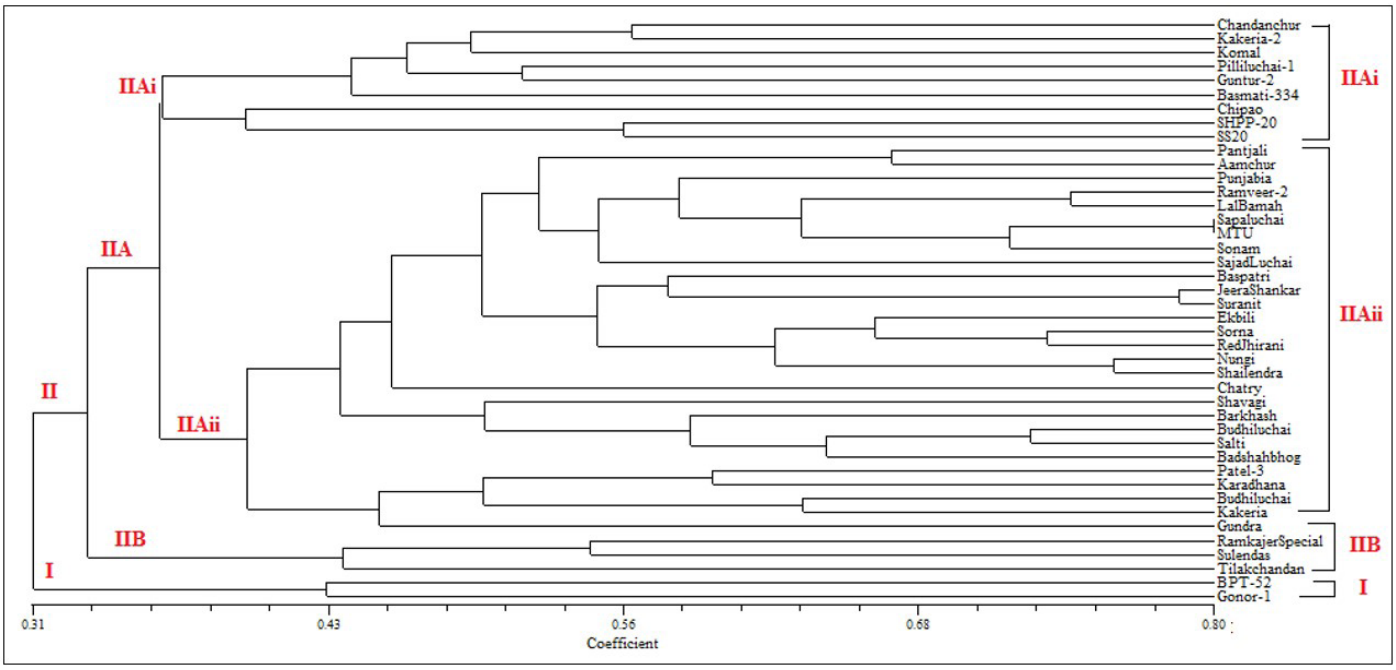

Fig. 4: Dendrogram depicting the genetic similarity among 42 rice varieties. The dendrogram was constructed using NTSysPC version 2,02 software.

Table 3. General grain characteristics of the 42 rice varieties used for diversity analysis

Sr. No	**Variety**	**State**	Length* (mm)	Breadth* (mm)	L/B ratio
1	Budhiluchai	Madhya Pradesh	7.9	2.4	3.29
2	Sorna	Madhya Pradesh	7.5	1.9	3.95
3	Sonam	Uttar Pradesh	8.5	2.7	3.15
4	Aamchur	Madhya Pradesh	7.7	2.9	2.66
5	Ramveer-2	Madhya Pradesh	8.7	2.7	3.22
6	Kakeria	Madhya Pradesh	7.5	2.6	2.88
7	MTU	Andhra Pradesh	8.3	3	2.77
8	Punjabia	Bihar	8.1	2.7	3
9	Sapaluchai	Madhya Pradesh	7.7	2.6	2.96
10	Pantjali	Bihar	8.4	2.7	3.11
11	Chipao	Madhya Pradesh	8.4	2.6	3.23
12	Suranit	Madhya Pradesh	7.8	3.4	2.29
13	Jeera Shankar	Madhya Pradesh	7.6	2.1	3.62
14	Gundra	Madhya Pradesh	7.9	2.8	2.82
15	BPT-52	Uttar Pradesh	8	2.6	3.08
16	Badshahbhog	Orissa	8.1	2.6	3.12
17	Shailendra	West Bengal	8.2	3.4	2.41
18	Sajad Luchai	Madhya Pradesh	10.3	2.1	4.9
19	Lal Bamah	Uttarakhand	8.2	2.7	3.04
20	Salti	Madhya Pradesh	8.1	2.7	3
21	Kakaria-2	Madhya Pradesh	10.6	2.1	5.05
22	Barkhash	Madhya Pradesh	8.3	2.7	3.07
23	Budhiluchai	Madhya Pradesh	8.1	2.9	2.79
24	Baspatri	Madhya Pradesh	8.1	2.9	2.79
25	Komal	Uttar Pradesh	9.4	2.3	4.09
26	Chandanchur	Bihar	9.7	2.1	4.62
27	Guntur-2	Orissa	7.9	2.4	3.29
28	Nungi	Madhya Pradesh	7.9	3.1	2.55
29	Pilliluchai-1	Madhya Pradesh	10.7	2	5.35

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30	Tilakchandan	Uttarakhand	8.3	2.2	3.77
31	Patel-3	Madhya Pradesh	7.7	2	3.85
32	Karadhana	Madhya Pradesh	8.5	2.1	4.05
33	Sulendas	Madhya Pradesh	6.9	2.5	2.76
34	Ekbili	Madhya Pradesh	8.5	2.7	3.15
35	Chatry	Madhya Pradesh	8.4	2.6	3.23
36	Red Jhirani	Orissa	8.6	3.1	2.77
37	Gonor-1	West Bengal	8.1	2.5	3.24
38	Ramkajer Special	Madhya Pradesh	8.5	3	2.83
39	Basmati-334	Jharkand	8.8	2.7	3.26
40	Shavagi	Jharkand	8.1	2	4.05
41	SS20	New Delhi	11.7	2.1	5.57
42	SHPP-20	New Delhi	9.5	1.9	5

*These are average of five observations

Table 4: Cooking quality of the 42 rice varieties used for diversity analysis

	Variety	Before Cooking*		After Cooking*				
		Length	Breadth	Length	Breadth	Elongation Ratio#	Aroma	ASV##
1	Budhiluchai	5.9	2.4	7.3	2.9	1.24	1	2
2	Sorna	5.3	1.6	7	1.6	1.32	1	1
3	Sonam	5.9	2.3	7.9	2.6	1.34	1	2
4	Aamchur	6.4	2.3	8.8	2.4	1.38	0	2
5	Ramveer-2	6.1	2.3	7.4	2.6	1.21	0	2
6	Kakeria	5.5	2.1	6.8	2.3	1.24	0	2
7	MTU	5.9	2.3	7.1	2.7	1.20	1	2
8	Punjabia	5.9	2.3	7.6	2.7	1.29	2	2
9	Sapaluchai**	-	-	-	-	-	-	-
10	Pantjali	6	2.3	7.8	2.7	1.30	0	2
11	Chipao	6.9	1.9	9.8	2.3	1.42	0	1
12	Suranit	6	2.6	6.9	2.6	1.15	0	2
13	Jeera Shankar	5.2	1.7	7.3	2.2	1.40	2	1
14	Gundra	6.1	2.1	9.3	2.8	1.52	1	3
15	BPT-52	6	2	7.9	2.8	1.32	3	2
16	Badshahbhog	5.9	2.1	7.3	2.5	1.24	2	2
17	Shailendra	5.7	2.5	6.9	2.8	1.21	2	2
18	Sajad Luchai	6.9	1.9	9.6	1.9	1.39	1	1
19	Lal Bamah	6	2.3	7.6	2.7	1.27	1	2
20	Salti	6.3	2.3	7.7	2.9	1.22	0	2
21	Kakaria-2	7.2	1.8	11.6	2.6	1.61	0	1
22	Barkhash	5.9	2.3	8.1	2.8	1.37	1	2
23	Budhiluchai	5.7	2.3	6.9	2.7	1.21	1	2
24	Baspatri	5.8	2.5	7.4	2.9	1.28	0	2
25	Komal	6	1.8	8.3	2.3	1.38	3	3
26	Chandanchur	7.1	1.9	9.2	2.3	1.30	0	1
27	Guntur-2	6	2.1	7.8	2.5	1.30	0	1
28	Nungi	6.5	2.3	8.5	3.1	1.31	0	2
29	Pilliluchai-1	7.5	1.7	10.3	2.3	1.37	2	2
30	Tilakchandan	6	1.7	8.3	2.3	1.38	3	1
31	Patel-3	5.5	1.7	7.3	2.1	1.33	0	1

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32	Karadhana	5.9	1.9	7.9	2.4	1.34	1	1
33	Sulendas	5.1	2	7.6	2.9	1.49	2	1
34	Ekbili	6	2.3	7.3	2.5	1.22	0	1
35	Chatry	6.3	2.3	7.5	2.5	1.19	0	2
36	Red Jhirani	6.1	2.1	8	2.6	1.31	0	1
37	Gonor-1	6.1	2.1	7.7	2.8	1.26	0	1
38	Ramkajer Special	6.2	2.4	7.9	2.8	1.27	1	2
39	Basmati-334	6.4	2.2	8.9	2.9	1.39	3	7
40	Shavagi	5.7	2.1	7.9	2.9	1.39	1	1
41	SS20	8.2	1.7	8.6	2.2	1.05	2	7
42	SHPP-20	7	1.6	7.5	3.1	1.07	1	7

*These are average of 5 observations; **Non-availability of seed; #Elongation ratio= length after cooking/ length before cooking ## Alkali Spreading Value

Fig. 5: Seeds of Kakeria-2 showed highest elongation ratio of 1.61

Fig.6: The shortest and the longest grain in the present study were found to be Sulendas (left) and SS20(right) respectively

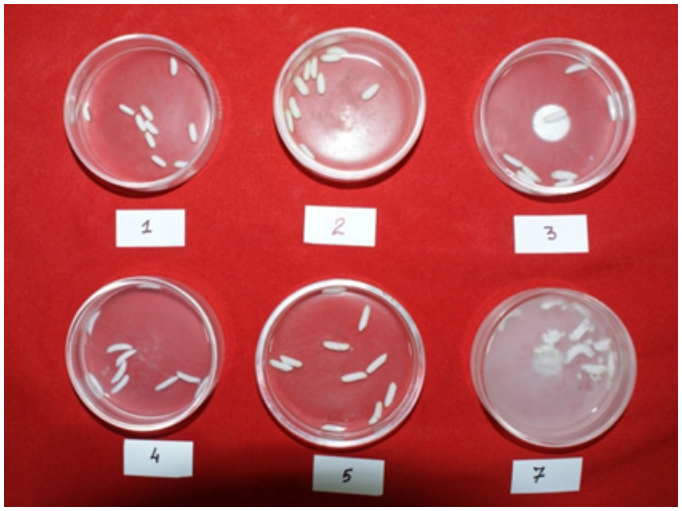

Fig.7: Analysis of Alkali Spreading Value (ASV) of all the 42 rice varieties using the method of Jennings et al (year). Scores were given as follows: 1-No change at all; 2- swelling of grain; 3- cloudy appearance; 4-disintegration of grains starts; 5-disintegration nearly complete; 6- grains start dissolving; 7-grains completely dissolve.

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