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*Full Length Research Paper*

# Comparison of traditional physico-chemical methods and molecular marker assays for characterization of Basmati rice (*Oryza sativa* L.)

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**This study was aim to review the efficiency of molecular markers and traditional physico-chemical methods for the identification of basmati rice. The study involved 44 promising varieties of *Indica* rices collected from geographically distant places and adapted to irrigated and aerobic agro-ecosystems. Quality data for physical characteristics and organoleptic evaluation observed in AICRP trials and fingerprints based on inter-SSR- PCR and SSR-PCR assays were used and all the varieties were grouped into two major distinct clusters based on distance matrices. The 22 varieties adapted to aerobic agro-ecosystem were classified into a single largest cluster. Both traditional and evolved basmati (EB) varieties were clustered with varieties adapted to irrigated agro-ecosystem. Estimates of genetic diversity between varieties adapted to irrigated and aerobic rice were more prominent than that observed between basmati varieties as was indicated by both the molecular assays. Estimates of physico-chemical quality parameters supplemented with organoleptic attributes were observed to be adequate to provide confirmatory unscrupulous identification of basmati rice.**

**Key words:** Basmati rice, physico-chemical characteristics, molecular markers, genetic diversity, organoleptic evaluation.

## INTRODUCTION

Traditional area of cultivation of basmati rice spread over 46 districts of six states of North-Western India under geographical indication (GI) for basmati rice which provides livelihood to more than 100 million people (Money, 2005). Genotype x environment interactions of important climatic components, soil and water of the region adjoining to Himalayas seems responsible for the quality of Basmati rice (Mani et al., 2005; Singh et al., 2000a). Cultivation of the traditional basmati varieties elsewhere in traditional rice-growing areas in the world will certainly lose its unique morphological and quality attributes (Anonymous, 1998). Therefore, Government of India, in view of demand of Basmati rice at premium price in the global market, has declared this area as agri export zone for basmati rice during March, 2001 (Money, 2005) to promote the production and productivity of Basmati rice in order to improve socio-economic status of farmers of the zone. Varieties of basmati rice grown only in GI are allowed to export under basmati category. The basmati varieties do not produce the same quality grains when grown outside their specific area of adaptation/GI. Recently, many varieties of inferior quality like sarbati, those visibly resemble with basmati are popular among farmers. Such varieties are used for adulteration in basmati, which lose credibility in the global market (Singh et al., 2008). Therefore, foolproof discriminative safe-guards are required to the protect quality of the export consignments of basmati. It was felt that traditionally used morphological and chemical parameters have not been found to be discriminative enough, warranting more precise techniques for maintenance of purity of the commodity (Nagaraju et al., 2002). A need has been felt to improve traditionally used physico-chemical methods by involving recently developed molecular marker based popular techniques used for characterization based on genetic differences within and among commercial

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varieties (Delacruz and Khush, 2000; Khush et al., 1979). Therefore, the present study was designed with the objective to compare the efficiency of traditional physico-chemical methods supplemented with organoleptic evaluation and the robust molecular markers SSR and their alternative form inter-SSR based methods (Zietkiewicz et al., 1994; Singh et al., 2000a) and to develop instant identification method that could detect (1) blending of "Sarbati" and non-basmati (NB) varieties which visibly resemble with basmati; (2) basmati rice grown outside the GI; (3) quality deterioration of basmati rice due to lodging, diseases and insects etc.

## MATERIALS AND METHODS

Forty-four (44) varieties of rice used in this study were collected from IRRI, the Philippines, Srilanka, Raipur (Chhattisgarh) and SVPUA&T, Meerut, India (Table 1). The varieties of irrigated agro-ecosystem (traditional method of rice cultivation that is puddled rice) included ten varieties of basmati rice (4 traditional and 8 evolved) and ten NB varieties. The varieties developed by crossing with traditional basmati varieties as one of the parents are defined as Evolved Basmati (EB) varieties. The rest 22 varieties adapted to aerobic agro-ecosystem that is, direct seeding rice (unpuddled) included 10 of IRRI, 5 of Central India, 5 of Sri Lankan origin and two variants of Sathi a farmers' variety being cultivated in aerobic conditions in Northern India for centuries. These 22 varieties are promising and well adapted in aerobic conditions of foothills of Himalayas, Raipur (Chhattisgarh), Srilanka and the Philippines (Table 1). The physico-chemical observations were also recorded on Vallabh Basmati-22, a basmati variety recently released in India. Genomic DNA was isolated using CTAB method (Moller et al., 1992) from 15 days old seedling of rice collected from field trial during Kharif (rainy season) 2007.

### Molecular markers assay

Forty-four (44) rice varieties were subjected to screen for the diversity with the help of 5', 3' anchored ISSR and SSR primers synthesized from Bangalore Genei, India. Each reaction mixture (20 ul), used ISSR and SSR primers consisted of 10x assay (10 mM Tris HCl, pH 8.0, 50 mM KCl with 15 mM $MgCl_2$), 1 unit/ul of red *Taq* DNA polymerase, 1.0 mM each of dATP, dTTP, dCTP and dGTP, 5.0 µm of primer (Bangalore Genei) and approximately, 25 ng of genomic DNA for inter-SSR and SSR, respectively. The PCR amplification conditions for ISSR analysis were as follows: initial extended step of denaturation at 94°C for 4 min followed by 45 cycles of denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min, elongation at 72°C for 2 min, followed by extension step at 72°C for 7 min and then, final hold at 4°C till electrophoresis. For SSR analysis, the number of cycles was 35 and annealing temperature was 55°C, respectively. PCR products was mixed with 5 µl of bromophenol gel loading dye (1 x buffer, Bromophenol blue, 0.1%; xylene cyanol 0.1% and glycerol in water, 50%). The amplification products were electrophoresed on 1.5 to 3% SFR (super fine resolution) agrose gel at 3 to 5 v/cm in 1 x TAE buffer. Genomic DNA was quantified by UV absorbance at 260 and 280 nm, using Bio-Rad Smart Tech$^{TM}$ spectrophotometer.

### Estimation of genetic diversity

Polymorphic products from ISSR-PCR and SSR-PCR assays were calculated qualitatively for the presence (1) or absence (0) from gel studies (Figure 1a and b). The proportion of bands that have been shared between any of the two varieties screened averaged over loci (ISSR) and SSR primers were used as the measure of similarity. Genetic diversity was calculated using the following formula (Botstein et al., 1980):

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where, $P_{ij}$ is the frequency of the $j$th allele for marker, $i$ and the summation extends over $n$ alleles. The calculation was based on the number of bands in ISSR and the number of bands per primer for SSR.

Cluster pattern was based on distance matrices by using the unweighted pair group method analysis (UPGMA) program in WINBOOT software (Yap and Nelson, 1996).

### Evaluation of grain quality

Seeds harvested from individual plants were analyzed for quality characters kernel length, breadth of milled, elongation of cooked rice, aroma and alkali spreading value (ASV). For the determination of the length breadth (L/B) ratio (Singh et al., 2000b), 20 fully developed wholesome milled rice kernels were measured for their length and breadth. The kernel elongation ratio was estimated as ratio of length of the cooked kernels to that of uncooked kernels. Determination of aroma was based on panel reports. The strength of the sample wise aroma was scored (Sood and Siddiq, 1978). ASV was estimated based on visual rating of starchy endosperm (Little et al., 1978). 20 rice kernels were incubated in 1.7% potassium hydroxide solution in a Petri dish for 23 h at 30°C and were rated for ASV.

### Organoleptic assessment for overall acceptability of the quality of basmati rice

Evaluation of quality of rice was also made by a penal of 10 experts in 3 replicates (Table 2) for overall acceptability ratings on account of appearance, cohesiveness, tenderness on touching and chewing, aroma, taste and flaky texture on cooking (Anonymous, 2008).

## RESULTS AND DISCUSSION

Molecular diversity, number of monomorphic and polymorphic bands for 44 rice varieties was estimated following ISSR-PCR and SSR-PCR assays and is given in Table 3. Scores for approved standards of physico-chemical characteristics acceptable in the global trade for Basmati varieties, VB-21, MAUB-57, Tarori Basmati and PB-1 and organoleptic (sensory) evaluation of Vallabh basamti-21, MAUB-57, Vallabh Basmati-22, Tarori Basmati and Pusa Basmati-1 by an expert panel are given in Tables 2 and 4, respectively. The diversity or similarities between varieties were estimated assay wise and jointly in the form of 3 dendrograms. For the sake of brevity, only one dendrogram (joint ISSR-SSR assay) is given in Figure 1.

**Table 1.** Rice varieties consisted of basmati, non basmati and coarse rice adapted to irrigated and aerobic agro-ecosystems, respectively across Asian countries.

| S/N | Variety name | Source/Origin | Agronomical characteristic feature |
|---|---|---|---|
| 1 | Pusa -2511 | IARI, New Delhi, India | Fine, semi dwarf |
| 2 | Type-3 | Nagina, U P, India | Basmati, tall, long duration, export quality |
| 3 | Ranbir Basmati | J.& K., India | Basmati, tall, short duration, export quality |
| 4 | Tarori Basmati | HAU, Kaul, India | Basmati, tall, long duration, export quality |
| 5 | Haryana Basmati-1 | HAU, Kaul,, India | Super fine, semi dwarf |
| 6 | Pusa Basmati-1 | IARI, New Delhi, India | Super fine, semi dwarf, export quality |
| 7 | CSR-30 | CSSRI,Karnal, India | Super fine, semi dwarf, export quality |
| 8 | Basmati-370 | Punjab, India | Basmati, tall, long duration, export quality |
| 9 | Pusa-1121 | IARI, New Delhi, India | Super fine, semi dwarf, export quality |
| 10 | Vallabh Basmati-21 | SVBPUA &T, India | Super fine, semi dwarf, short duration, export quality |
| 11 | MAUB-57 | SVBPUA&T, India | Dwarf, long duration, lodging resistant, export quality |
| 12 | Super Basmati | PAU, Punjab, India | Tall, long duration, lodging susceptible, export quality |
| 13 | N-22 | Nagina, U P India | Semi dwarf, non scented, drought resistant |
| 14 | Govind | GBPUA&T, India | Dwarf, non scented, short duration |
| 15 | NDR-118 | NADUA&T, India | Dwarf, non scented, medium duration |
| 16 | HKR-1 | HAU, Kaul, India | Semi dwarf, aromatic |
| 17 | Sarbati-B | Farmers variety, India | Fine, dwarf, short duration, lodging, disease susceptible |
| 18 | Sarbati-A | Farmers variety , India, | Fine, tall, short duration ,lodging, disease susceptible |
| 19 | Pusa Sungandha-2 | IARI, New Delhi, India | Fine, semi dwarf, medium duration, aromatic |
| 20 | Pant Dhan-10 | GBPUA&T, India | Semi dwarf, medium duration, coarse rice |
| 21 | Pant Dhan-12 | GBPUA&T, , India | Semi dwarf, medium duration, coarse rice |
| 22 | Vallabh Bangani | SVBPUA&T, India | Dwarf, medium duration, violet color foliage |
| 23 | Sathi-Safed | Chhatish Garh, India | Dwarf, short duration, aerobic, coarse, white grain |
| 24 | Sathi-Black | Farmers Variety India | Dwarf, short duration, aerobic, coarse, black grain |
| 25 | IR-74371-4-6-1-1 | IRRI,The Philippines | Semi dwarf, mid- duration, aerobic, LS |
| 26 | IR-71604-1-4-1 | IRRI,The Philippines | Semi dwarf, mid- duration,, aerobic, LS |
| 27 | IR-71527-44-1-1 | IRRI,The Philippines | Semi dwarf, mid- duration,, aerobic, LS |
| 28 | IR-74371-70-1-2 | IRRI, The Philippines | Semi dwarf, mid- duration,, aerobic, LS |
| 29 | IR-74371-70-1-1 | IRRI, The Philippines | Semi dwarf, mid- duration,, aerobic, LS |
| 30 | IR-80919 | IRRI, The Philippines | Semi dwarf, mid- duration,, aerobic, LS |
| 31 | IR-75298-59-3-1-3 | IRRI, The Philippines | Semi dwarf, mid- duration,, aerobic, non scented |
| 32 | IR-73888-1-2-7 | IRRI, The Philippines | Semi dwarf, mid- duration, aerobic, non scented |
| 33 | IR-75298-59-8-1-3 | IRRI, The Philippines | Semi dwarf, mid- duration,, aerobic, non scented |
| 34 | IR-71700-247-1-1-2 | IRRI, The Philippines | Semi dwarf, mid- duration,, aerobic, LS |
| 35 | BG-352 | Chhatish Garh, India | Semi dwarf, mid- duration,, aerobic, broad leaf |
| 36 | BG-358 | Chhatish Garh, India | Semi dwarf, mid- duration,, aerobic, broad leaf |
| 37 | BG-379-2 | Chhatish Garh, India | Semi dwarf, mid- duration, aerobic, parrot green |
| 38 | DGI-380 | Srilanka | Semi dwarf, mid- duration, aerobic, non scented |
| 39 | DGI-155 | Srilanka | Semi dwarf, mid- duration, aerobic, non scented |
| 40 | DGI-152 | Srilanka | Semi dwarf, mid- duration, aerobic, non scented |
| 41 | DGI-296 | Srilanka | Semi dwarf, mid- duration, aerobic, non scented |
| 42 | DGI-154 | Srilanka | Semi dwarf, mid- duration, aerobic, non scented |
| 43 | BG-94-1 | Chhatish Garh, India | Semi dwarf, mid- duration, aerobic, dark green |
| 44 | BG-359 | Chhatish Garh, India | Semi dwarf, mid- duration, aerobic, light green |

Ten ISSR and 28 SSR primers were used for PCR analysis to finger print the 44 rice varieties. Distance matrices based clustering categorized all the rice varieties into agro-ecosystem wise two major clusters. Twenty two (22) varieties of irrigated agro-ecosystem were further grouped into 5 clusters based on ISSR-PCR assay. The 11 varieties of quality rice adapted to irrigated agro-ecosystem were clustered into three 3 sub clusters

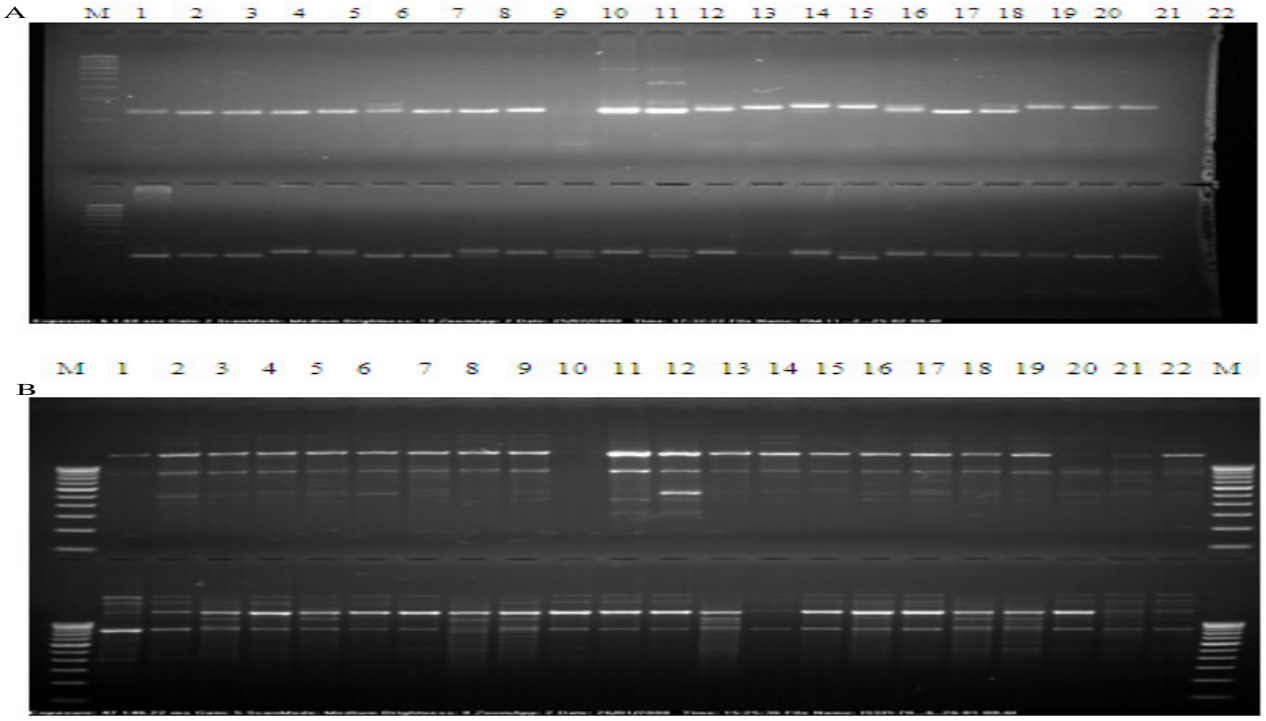


**Figure 1.** (A) SSR profiling pattern of 44 rice varieties with RM-11 primer. M indicates the molecular weight marker 100 bp; (B) ISSR profiling pattern of 44 rice varieties with ISSR-70 primer. M indicate the molecular weight marker 100 bp.

**Table 2.** Panel test, organoleptic scores (based only on expertise and skill of the expert; no physical measurement was taken) of entries in varietal trial on basmati rice recorded at DRR, Hyderabad during Kharif (rainy season) 2005 (Anonymous, 2005).

| Varieties | Appearance | Cohesiveness | Tenderness on | | Taste | Aroma | Elongation ratio | Overall acceptability |
|---|---|---|---|---|---|---|---|---|
| | | | Touching | Chewing | | | | |
| P B-1 (Yield check) | 4.40 | 3.2.0 | 3.93 | 4.03 | 3.20 | 3.40 | 2.93 | 3.48 **(Good)** |
| Tarori Basmati (Quality check) | 5.00 | 4.20 | 3.80 | 4.20 | 3.46 | 3.73 | 3.33 | 3.87 **(Good)** |
| MAUB 57 ( Vallabh Basmati 23 ) | 4.42 **(1)** | 3.92 **(1)** | 4.07**(2)** | 4.21 **(2)** | 3.21**(1)** | 3.71 **(1)** | 3.21 **(1)** | 3.70 **(1) (Good)** |
| Vallabh Basmati-22 | 4.57 **(1)** | 3.92 **(1)** | 4.42**(2)** | 4.57 **(2)** | 3.64 **(2)** | 3.92 **(2)** | 3.57 **(2)** | 3.96 **(2) (Good)** |
| Vallabh Basmati-21 | 4.42 (1) | 3.71 (1) | 4.07(2) | 4.7 0 (2) | 3.14 | 3.50 (2) | 2.57 | 3.47 (Good) |
| | 4.0-4.9 Creamish; 3.0-3.9 Red Streaks | 4.0-4.9 Partially separated: 3.0-3.9 Slightly sticky | 4.0-4.9 Moderately soft; 3.0-3.9 Moderately hard | 4.0-4.9 Moderately soft; 3.0-3.9 Moderately hard | 3.0-3.9 Desirable; 2.0-2.9 Tasteless | 4.0 Strong; 3.0-3.9 Optimum; 2.0-2.9 Mild; 1.0-1.9 No scent | 3.0-3.9 Good: 2.0-2.9 Moderate | 4.0-4.9 Excellent 3.0-3.9 Good; 2.0- 2.9 Acceptable |

MAUB-57 and Vallabh basmati-22 are significantly superior to PB-I for all seven parameters of quality. Vallabh Basmati-21 was superior to PB-1 for five parameters only as given earlier. In overall acceptability ratings, all the given varieties are almost equal to PB-I and Tarori Basmati and therefore, all were ranked 'good'. Value 1 in parenthesis indicated superiority over PB- I, while value 2 indicated superiority over both the check varieties PB-I and Tarori Basmati.

**Table 3.** Primer codes, annealing temperature, no. of polymorphic bands, no. of monomorphic bands and diversity value with forty-four varieties.

| S/N | Primer | Annealing temperature (°C) | Molecular weight range (bp) | Number of polymorphic band | Number of monomorphic band | Diversity (value of PIC) |
|---|---|---|---|---|---|---|
| **ISSR** | | | | | | |
| 1 | ISSR 61 | 50 | 100-2500 | 8 | 1 | 0.763 |
| 2 | ISSR 62 | 50 | 150-2500 | 12 | 1 | 0.647 |
| 3 | ISSR 63 | 50 | 100-2000 | 9 | 1 | 0.684 |
| 4 | ISSR 64 | 50 | 120-2500 | 12 | 1 | 0.543 |
| 5 | ISSR 65 | 50 | 120-2000 | 11 | 2 | 0.505 |
| 6 | ISSR 66 | 50 | 130-2000 | 14 | 2 | 0.457 |
| 7 | ISSR 68 | 50 | 190-2000 | 15 | 1 | 0.153 |
| 8 | ISSR 69 | 50 | 250-3000 | 17 | 1 | 0.391 |
| 9 | ISSR 70 | 50 | 300-2500 | 12 | 4 | 0.753 |
| 10 | ISSR 71 | 50 | 290-3000 | 9 | 1 | 0.656 |
| **SSR** | | | | | | |
| 1 | RM 04 | 55 | 70-600 | 3 | 2 | 0.713 |
| 2 | RM 05 | 55 | 90-800 | 4 | 3 | 0.876 |
| 3 | RM 104 | 55 | 80-1000 | 8 | 0 | 0.997 |
| 4 | RM 107 | 55 | 70-700 | 8 | 4 | 0.841 |
| 5 | RM 11 | 55 | 70-500 | 6 | 2 | 0.876 |
| 6 | RM 127 | 55 | 70-500 | 5 | 3 | 0.847 |
| 7 | RM 128 | 55 | 80-600 | 4 | 0 | 0.813 |
| 8 | RM 131 | 55 | 80-600 | 9 | 2 | 0.971 |
| 9 | RM 136 | 55 | 100-900 | 9 | 2 | 0.625 |
| 10 | RM 141 | 55 | 90-900 | 12 | 2 | 0.792 |
| 11 | RM 154 | 55 | 80-500 | 6 | 0 | 0.339 |
| 12 | RM 160 | 55 | 200-800 | 9 | 2 | 0.750 |
| 13 | RM 161 | 55 | 90-500 | 14 | 3 | 0.916 |
| 14 | RM 163 | 55 | 100-700 | 3 | 0 | 0.866 |
| 15 | RM 17 | 55 | 70-600 | 3 | 1 | 0.537 |
| 16 | RM 235 | 55 | 70-700 | 8 | 3 | 0.809 |
| 17 | RM 252 | 55 | 80-600 | 7 | 2 | 0. 682 |
| 18 | RM 273 | 55 | 80-800 | 7 | 4 | 0.834 |
| 19 | RM 30 | 55 | 70-700 | 3 | 2 | 0.695 |
| 20 | RM 302 | 55 | 70-500 | 7 | 2 | 0.914 |
| 21 | RM 330 | 55 | 70-600 | 7 | 2 | 0.501 |
| 22 | RM 38 | 55 | 80-800 | 5 | 2 | 0.819 |
| 23 | RM 42 | 55 | 80-500 | 3 | 1 | 0.822 |
| 24 | RM 43 | 55 | 70-800 | 4 | 1 | 0.845 |
| 25 | RM 51 | 55 | 60-700 | 3 | 2 | 0.997 |
| 26 | RM 55 | 55 | 70-800 | 11 | 2 | 0.949 |
| 27 | RM 84 | 55 | 60-700 | 3 | 2 | 0.937 |
| 28 | RM 85 | 55 | 70-900 | 5 | 0 | 0.992 |

**Table 4.** The quality standards (approved by Government of India and accepted in the global trade; Anonymous, 1998) of basmati varieties Vallabh Basmati-21, MAUB-57, Tarori Basmati and Pusa Basmati-1 were scored at National Quality Laboratories, DRR, Hyderabad (AP) and CRRI Cuttack (Orisa) (Anonymous, 2008). For the sake of brevity, results obtained at National Quality Laboratories at DRR, Hyderabad are given further.

| Component parameter of quality of Basmati | Required standard of quality of basmati (acceptable in the global trade) | Quality standards of PB -1 | Quality standard of Tarori Basmati | Quality standard of Vallabh Basmati-21 | Quality standard of MAUB-57 |
|---|---|---|---|---|---|
| Milling % | 65.00 (Minimum) | 67.0 | | | |
| Head rice recovery % | 45.00 (Minimum) | 52.1 | | | |
| Kernel length (mm) | 6.61 (Minimum) | 6.98 | | | |
| Kernel breadth(mm) | 2 .00(Maximum) | 1.78 | 1.79 | 1.79 | 1.74 |
| Length breadth ratio | 3.50 (Minimum) | 3.88 | 3.88 | 4.27 | 4.15 |
| Grain chalkiness % | 10 (Maximum) | rare | rare | rare | rare |
| Volume expansion ratio | 3.00 (Minimum) | 4.85 | 4.63 | 4.7 | 4.77 |
| Water uptake (ml) | 250 (Minimum) | 362 | 268 | 310 | 258 |
| Kernel length after cooking (mm) | 12.00 (Minimum) | 15.5 | 13.3 | 13.3 | 15.17 |
| Elongation ratio | 1.700 (Minimum) | 2.22 | 1.92 | 1.73 | 2.09 |
| ASV | 4-5 | 7.0 | 5.0 | 7.0 | 5.0 |
| Amylose content % | 20-25 | 24.81 | 24.44 | 24.48 | 23.75 |
| Aroma | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |

AICRP, All India Coordinated Research Project on rice; DRR, Directorate of Rice Research, Hyderabad, India; CRRI, Central Rice Research Institute, Cuttack, India.

(Figure 2). Four traditional basmati varieties Ranbir basmati, Type 3, Basmati 370 and Tarori Basmati along with 4 Evolved Basmati varieties, Pusa-1121, Haryana Basmati-1, CSR-30 and Pusa Basmati-1 were clustered together as earlier reported by Nagaraju et al. (2002). These varieties except Haryana Basmati-1 are still acceptable to consumers at national as well as international level at premium price. MAUB-57 and super Basmati both belonging to EB category were grouped together in a separate cluster in all three analyses (dendrograms). In addition, no Basmati variety was clustered with Vallabh Basmati-21. Such Evolved Basmati (EB) varieties possibly still retain a large genomic fraction of the non-basmati varieties which were used in the breeding programme designed to develop them. The rest 11 non-basmati (NB) varieties were grouped into two sub clusters. The lowest genetic diversity was observed among the traditional basmati varieties. High degree of genetic similarity among traditional basmati varieties indicated that possibly these were descendants of a single land race perhaps evolved in the valley of Dehradun during prehistoric time (Singh et al., 2009). Minor genetic variations preferred by farmers were traditionally conserved for over centuries. Such conserved variants were later on formally released for commercial cultivation and now are called traditional varieties of basmati rice. The first of such variety Basmati 370 was released for commercial cultivation in 1933 at the Rice Research Station, Kalashah Kaku (now in Pakistan) followed by Type-3 released from India. Most of the variants of traditional basmati maintained in India and Pakistan have been derived from Basmati-370 and Type-3. Traditional basmati and evolved basmati varieties expressed highest genetic diversity from other NB indica varieties due to high percentage of hybrid sterility. Both the marker assays indicated that the evolved variety CSR-30 expressed similarity with TB varieties. The adulteration in TB and EB grains with NB grains is very common due to significant price difference that hampers the basmati export. Hence, instant identification of genuine basmati varieties from the other Basmati-like varieties of inferior quality for example, Sarbati that visibly resembles with basmati is required. Small variations were noticed when SSR-PCR assay was applied. Pusa-2511 and Vallabh Basmati-21 adapted to irrigated agro-ecosystem were clustered with varieties of aerobic rice (that is, Sathi Black, BG-375-2, BG-358). Sathi safed expressed no closeness with any variety of aerobic agro ecosystems. It may therefore, be concluded that SSR markers were more robust and efficiently detected the miner differences in the genomic DNA which was the basis of molecular characterization of the genotypes (Nagaraju et al., 2002; Singh et al., 2008). But in joint analysis (Figure 2) Pusa-2511 and Vallabh Basmati-21 were clustered with Basmati group. Sathi Black, BG-375-2, BG-358 and Sathi safed were clustered with varieties of aerobic rice.

Morphological characters such as plant height, foliage color and disease resistance etc. (Table 1) could not have any significant bearing upon molecular markers ISSR and SSR based profiling of genotypes (Figure 2). Therefore, the varieties despite having such distinct morphological characters could not be identified by molecular markers used in this study. For example, tall

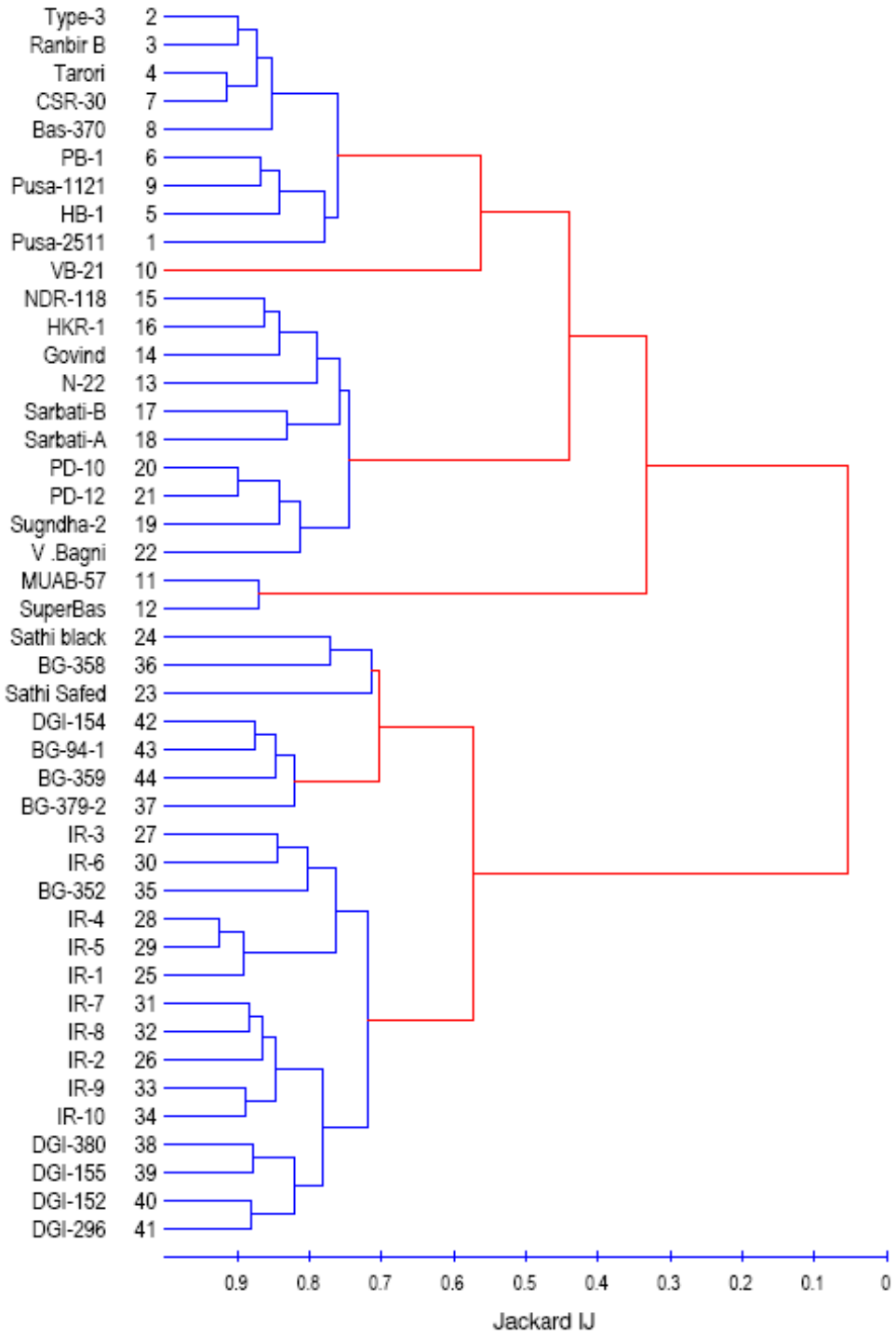


**Figure 2.** Dendrogram showing clustering of 44 rice varieties constructed using UPGMA based on Jacquard's similarity coefficient obtained from ISSR and SSR joint analysis.

and dwarf collections of farmers' variety "Sarbati", could not be identified by these markers and therefore, were consistently clustered together in both the assays and joint analysis. Likewise, MAUB-57 (dwarf) and Super Basmati (tall) were also clustered together. DNA sequences of minor genes responsible for such characters could not have significant bearing upon the clustering of these varieties. On the other hand, clustering of genotypes was mainly based on two complex characters adaptation reactions and quality (Table 1, Figure 2). Both quality and adaptation are governed by major QTLs spread over the genome represented by molecular markers used. Therefore,

respective genes responsible for some crucial components of quality and adaptation which greatly influenced clustering of varieties of both the agro-ecosystems seem to be tightly linked with ISSR and SSR markers. Adaptation to a particular agro-ecosystem requires a specific genetic mechanism which could have evolved in due course of time during evolutionary processes. The favorable genetic changes which have occurred spontaneously during course of evolution to acquire fitness in a particular agro-ecosystem seem to be the cause. Independence of clustering pattern of different genotypes of their geographical origin within agro-ecosystems indicated that all such genotypes of common gene pool were exchanged over the locations collected wherefrom, not long before. The time passed after shifting was short to attain adaptation as a consequence of a new genetic mechanism which could have required more time to evolve. The clustering of varieties VB-21 and Pusa-2511 of irrigated agro-ecosystem with varieties of aerobic agro-ecosystem might also have indicated their potential under aerobic conditions. Therefore, clustering of the genotypes obtained herein has depended upon adaptation to irrigated and aerobic agro-ecosystems within limits of regulations of the genetic mechanism evolved for adaptation, represented by ISSR and SSR sequences. Likewise, quality of Basmati rice is the resultant effect of a number of distinguished component traits (Table 4) governed by different genetic mechanisms. However, quality partially influenced the profiling of rice varieties within irrigated agro-ecosystem. Expression of similarity in quality characteristics of both evolved and traditional varieties could be accounted for within limits of regulations of these quality characteristics by the genes represented by ISSR and SSR sequences (Singh et al., 2008).

On the other hand, the newly identified elite genotype MAUB-57 (Tables 2 and 4) of basmati rice (Anonymous, 2008) and Super Basmati, a variety popular in Punjab were also not detected by molecular tools appropriately and therefore, were not clustered with the traditional and evolved varieties. Thus, misleading information was provided to the breeders, traders and consumers that reflected limited utility of molecular markers in basmati trade. Since the markers are working on DNA sequences and therefore, may identify varietal mixtures with limitations. Refinement of such techniques (Gupta et al., 2008) is also required in order to detect genetic differences more reliably at finer level among commercial varieties of Basmati rice traded in the global market. Such markers cannot detect variations observed in the quality of the commodity when produced beyond GI area (Mani et al., 2005) and deterioration due to disease and lodging, etc. that affected marketability significantly. In such cases the quality of basmati rice is influenced by environmental fluctuations that cannot presumably be detected by molecular marker unless the variation is caused at DNA level. Under such situations, only traditional methods seem capable to work. Export of basmati is linked with high premium price, credibility in the global market and international relations in the political circles too. Therefore, we can not afford to take any sort of risk to allow export of inferior quality. The matter assumed grave concern particularly in the recent era of global recession. As is evidenced from this study (Table 2), Physico-chemical analysis could provide foolproof information of basmati characteristics. Therefore, molecular markers can only be applied to supplement such traditional methods of basmati analysis and not as a substitute.

Quality of basmati is a resultant expression of a variety of component characters; therefore, application of potent markers for each component character may be studied *in toto* to have more reliable procedures to determine quality. A kit consisting of molecular markers potent enough to discriminate all the rice varieties based on specific traits may provide instant identification. It will perhaps need large number of markers. Still, there is no available procedure even to indicate to develop molecular markers for functional mechanism of taste buds of the tongue to make the consumer feel better when he eats rice of different varieties of basmati. It also seems difficult to develop molecular markers for some of the major quality traits such as appearance, cohesiveness, tenderness on touching and chewing, taste and flaky texture on cooking, etc. However, molecular markers for many other complex traits such as aroma can possibly be developed (Singh et al., 2000a). Most of them have already been developed. However, their efficient exploitation seems to be a remote goal. Such markers discriminative enough are used in the present study. The results indicated that their application is of value to assist traditional methods of basmati testing. However, as discussed, they cannot be used as their substitute. Therefore, determination of quality of basmati rice based on traditional methods of basmati testing is still the most reliable safeguard to protect Basmati export from adulteration till better alternative approaches are developed.

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