academicJournals

Vol. 14(1), pp. 42-51, 7 January, 2015
DOI: 10.5897/AJB2013.13101
Article Number: 27F891C49435
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Application of molecular markers to find out classificatory determinants of rice (*Oryza sativa* L.)

D. Singh[1]*, Anil Sirohi[2], Pooran Chand[1], Preeti Sirohi[1], Parul Sirohi[1], Suresh Chand[1], Ravinder Kumar[1], S.K. Mishra[1], Navneet Kumar[1], Anuj Kumar[1], Rajender Kumar[3], Atar Singh[1], L.K. Gangwar[1] and Shalini Gupta[2]

[1]Molecular Biology Laboratory, Department of Genetics and Plant Breeding, SVP University of Agriculture and Technology, Meerut, India-250 110.
[2]College of Biotechnology, SVP University of Agriculture and Technology, Meerut, India-250 110.
[3]Department of Agriculture Biotechnology, SVP University of Agriculture and Technology, Meerut, India-250 110.

Received 31 July, 2013; Accepted 8 December, 2014

The present communication is aimed to find out determinants of molecular marker based classification of rice (*Oryza sativa* L) germplasm using the available data from an experiment conducted for development of molecular fingerprints of diverse varieties of Basmati and non Basmati rice adapted to irrigated and aerobic agro-eco systems. The distance-matrix based dendrogram was developed following UPGMA method that grouped all the 44 varieties agro-eco systems wise. We examined the clustering of Basmati and non- Basmati varieties in detail. The results suggest that the easily distinguishable simple characters viz. plant height, photosensitivity, leaf color, awn's color, awns' number etc., governed by major genes did not contribute significantly towards classification of rice genotypes used. The complex characters such as adaptation to agro-eco systems and rice quality may play vital role in determination of molecular marker based classification of rice genotypes.

Key word: Basmati rice, molecular markers, genetic diversity, agro-eco systems, rice quality.

INTRODUCTION

The traditional varieties of Basmati rice are tall, long duration, photosensitive and contained minimum genetic diversity. Therefore, this crop could not be genetically benefitted even during green revolution era. However, low yielding, disease susceptible traditional varieties of Basmati rice still remained in cultivation due to their high quality that fetch high premium price in the local as well as international market.

Lodging and disease appearance in such basmati rice varieties results into poor productivity and inferior quality (DelaCruz and Khush, 2002) which in turn results into less remuneration to the farmers in high input agriculture (Singh and Gupta, 1985). Furthermore, short duration and input efficient rice varieties of inferior quality like Sarbati resembling with basmati in appearance became a challenge for Basmati trade.

Due to high price difference such varieties are used for blending in export consignments that loses national

*Corresponding author. E-mail: devisingh11@gmail.com. Tel: +919358918146. Fax: +91 121 2888505.

credibility in the global market. Triggering efforts, to promote production and maintaining purity of Basmati rice, Government of India has declared this traditional basmati growing area as Agri Export Zone for Basmati rice (Singh et al., 2011; Singh and Sirohi, 2005). Traditionally used morphological and physico-chemical parameter does not provide more precise techniques for maintenance of purity of the commodity (Nagaraju et al., 2002). Therefore, foolproof instant safeguards are required to be developed to detect adulteration of any type of rice blended in the export consignments

In parallel, reliability and precision of such discriminatory functions will apparently depend upon adequacy of molecular markers used and characters contributing towards estimates of genetic diversity. Once adequate molecular markers and efficient determinants are identified, further studies will be intensified to get information at finer level which could be potent to precisely detect admixture in commercial varieties (Botstein et al., 1980; Vos et al., 1995). Alternatively, it is needed to find out characteristics/determinants contributing to genetic diversity (Singh, 2010) which is the basis of classification/clustering of genotypes.

So, the present study was undertaken with the objective to fingerprint/characterize different varieties of Basmati and non-Basmati rice adapted to different agro-ecological systems by using SSR and its alternative approach inter-SSR assays (Zietkiewicz et al., 1994, Singh et al., 2000a, 2000b) and to examine the results in order to work out efficient determinants for molecular marker based classification.

MATERIALS AND METHODS

Plant materials and molecular markers assay

Forty four varieties of Basmati and non-Basmati rice along with their variety wise place of development/ origin and visibly distinguishable characteristics are listed in Table 1. The genomic DNA of 44 rice varieties was isolated using CTAB method (Moller et al., 1992). 10 ISSR and 28 SSR molecular markers (Table 4) were used for genetic analysis. The ISSR-PCR technique (Zietkiewicz et al., 1994) was used to enhance the speed of sensitivity of detection of molecular markers. The 5', 3' anchored ISSR, and SSR primers synthesized by Bangalore Genei were used. Each reaction mixture (20 ul) that used ISSR and SSR primers for amplification consisted of 10x assay (10 mM Tris HCl, pH 8.0, 50 mM KCl with 3.0 mM $MgCl_2$): 1 U of *Red Taq* DNA polymerase, 1.0 mM each of dATP, dTTP, dCTP and dGTP, 10 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. For SSR analysis, the number of cycles was 35 and annealing temperature was 55°C, respectively. PCR products were mixed with 5 µl of gel loading dye (1x 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 agrose gel at 3 to 5 V/cm in 1x TAE buffer. Genomic DNA was quantified by UV absorbance at 260 nm and 280 nm, using UV Bio-Rad spectrophotometer.

Data analysis and detection of genetic diversity

Forty-four rice varieties were used to estimate genetic diversity. Polymorphic products from ISSR-PCR and SSR-PCR assays were calculated qualitatively for presence (1) or absence (0). The proportion of bands that have been shared between any of the two varieties 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):

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Pij 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). The diversity or similarities between varieties were given in the form of dendrogram (Figure 1). For the sake of brevity, dendrogram for combined ISSR-SSR assays is given in Figure 1.

Evaluation of grain and quality

For determination of the length breadth (L/B) ratio, kernel length, breadth of milled and cooked rice, aroma and alkali spreading value (ASV) (Dela Cruz and Khush, 2000), 20 fully developed wholesome milled rice kernels were measured. The kernel elongation ratio (ER) was estimated as ratio of length of the cooked kernels to that of uncooked kernel.

Determination of aroma was based on panel reports. Traditional Basmati varieties Taroari Basmati and evolved basmati variety Pusa Basmati-1 were used as standards. The strength of the aroma was scored sample wise (Sood and Siddiq, 1978) as Strongly Scented (SS) and Mildly Scented (MS). ASV was estimated based on visual rating of starchy endosperm (Little et al., 1978). Twenty rice kernels were incubated in 1.7% potassium hydroxide solution in a Petri dish for 23 h at 30°C.

In addition, evaluation of quality was also made by a panel of experts for overall acceptability ratings on account of its acceptability for appearance, cohesiveness, tenderness on touching and chewing and, aroma, taste, elongation and flaky texture on cooking (Anonymous, 2007).

RESULTS AND DISCUSSION

The molecular marker based available techniques are used to estimate genetic diversity that may correspond to contribution of different characteristics in the categoryzation of germplasm. Development of molecular markers was cost effective by using RFLPs, AFLP, microsatellites, inter-SSR and RAPDs. Primary applications of molecular markers are: molecular characterization, classification of germplasm and mapping of the genes. Therefore, these markers are available in public domain. A variety of SSR markers, the most robust molecular markers is potent enough to tag the genes with small differences of 2 to 3 nucleotides for development of genetic maps and

Table 1. Rice varieties (*Oryza sativa*) consisted of Basmati, scented non-Basmati, non-scented fine and coarse rice groups adapted to Irrigated and aerobic agro-eco systems across Asian countries.

Variety Name	Origin	Characteristic features
Pusa -2511	IARI, New Delhi, India	Irri, dwarf, medium duration, super fine, disease, LS, scented
Type-3	Nagina, UP, India	Irri, tall, long duration, photosensitive, disease, TB
Ranbir Basmati	J & K, India	Irri, tall, long duration, photosensitive, disease , TB
Taroari Basmati	CCS HAU, Kaul, India	Irri, tall, long duration, photosensitive, disease, TB
Haryana Basmati-1	CCS HAU, Kabul, India	Irri, semi dwarf, super fine, disease, L S, Basmati, EB
Pusa Basmati-1	IARI, New Delhi, India	Irri, semi dwarf, long duration, large awns, disease, EB
CSR-30	CSSRI, Karnal, India	Irri, tall, long duration, photosensitive, disease, EB
Basmati-370	PAU, Ludhiana, India	Irri, tall, long duration, photosensitive, disease, TB
Pusa-1121	IARI, New Delhi, India	Irri, semi dwarf, medium duration, disease, EB
Vallabh Basmati-21	SVPUAT, Meerut, India	Irri and aerobic, semi dwarf, medium duration, EB
MAUB-57	SVPUAT, Meerut, India	Irri, semi dwarf, long duration, photosensitive, EB
Super Basmati	PAU, Ludhiana, India	Irri, tall, long duration, photo sensitive, disease, EB
N-22	Nagina, UP, India	Irri, semi dwarf, drought resistant, disease, non -scented,
Govind	GBPUAT, UK, India	Irri, dwarf, non-scented, short duration, coarse, L S, non-scented
NDR-118	NDAUT, UP, India	Irri, dwarf, medium duration, coarse, L S, non- scented
HKR-1	CCS HAU, Kaul, India	Irri, semi dwarf, medium duration, disease, LS, scented
Sarbati-B	Farmers' variety UP, India	Irri, semi dwarf, fine, short duration, lodging, disease, non-scented
Sarbati-A	Farmers' variety UP	Irri, tall, fine, short duration, lodging, disease, non- scented
Pusa Sungandha-2	IARI, New Delhi, India	Irri, fine, semi dwarf, medium duration, L S, scented
Pant Dhan-10	GBPUAT, UK, India	Irri, semi dwarf, medium duration, L S, non- scented
Pant Dhan-12	GBPUAT, UK, India	Irri, semi dwarf, medium duration, L S, non- scented
Vallabh Bangani	SVPUAT, Meerut, India	Irri, dwarf, medium duration, violet color foliage, non-scented
Sathi-Safed	Farmers' Variety UP	Aerobic, semi dwarf, short duration, disease, coarse, non -scented
Sathi-Black	Farmers' Variety UP	Aerobic, semi dwarf, short duration, disease, coarse, non- scented
IR-74371-4-6-1-1	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, L S, non -scented
IR-71604-1-4-1	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, LS, non- scented
IR-71527-44-1-1	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, LS, non- scented
IR-74371-70-1-2	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, LS, non -scented
IR-74371-70-1-1	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, LS, non -scented
IR-80919	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, LS, non- scented
IR-75298-59-3-1-3	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, non -scented
IR-73888-1-2-7	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, non- scented
IR-75298-59-8-1-3	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, non- scented
IR-71700-247-1-1-2	IRRI, the Philippines	Aerobic, semi dwarf, mid- duration, LS, non- scented
BG-352	Chhattisgarh, India	Aerobic, semi dwarf, mid- duration, broader leaves, non- scented
BG-358	Chhattisgarh, India	Aerobic, semi dwarf, mid- duration, broader leaves, non- scented
BG-379-2	Chhattisgarh, India	Aerobic, semi dwarf, mid- duration, parrot green leaves, non-scented
DGI-380	Srilanka	Aerobic, semi dwarf, mid- duration, non- scented
DGI-155	Srilanka	Aerobic, semi dwarf, mid- duration, non -scented
DGI-152	Srilanka	Aerobic, semi dwarf, mid- duration, non -scented
DGI-296	Srilanka	Aerobic, semi dwarf, mid- duration, non- scented
DGI-154	Srilanka	Aerobic, semi dwarf, mid- duration, non -scented
BG-94-1	Chhattisgarh, India	Aerobic, semi dwarf, mid- duration, dark green, non -scented
BG-359	Chhattisgarh, India	Aerobic, semi dwarf, mid- duration, light green, non -scented

Irri: Adapted to Irrigated agro-eco system, Aerobic: Adapted to aerobic agro-eco system, LS: Long slender grain, Disease: Variety susceptible to diseases, TB: Traditional Basmati variety, EB: Evolved Basmati variety.

molecular characterization of rice germplasm (Wu and Tanksley, 1993; Yang et al., 1994). SSR markers are simple, PCR based, locus specific, more reliable, reproducible and typically co-dominant markers.

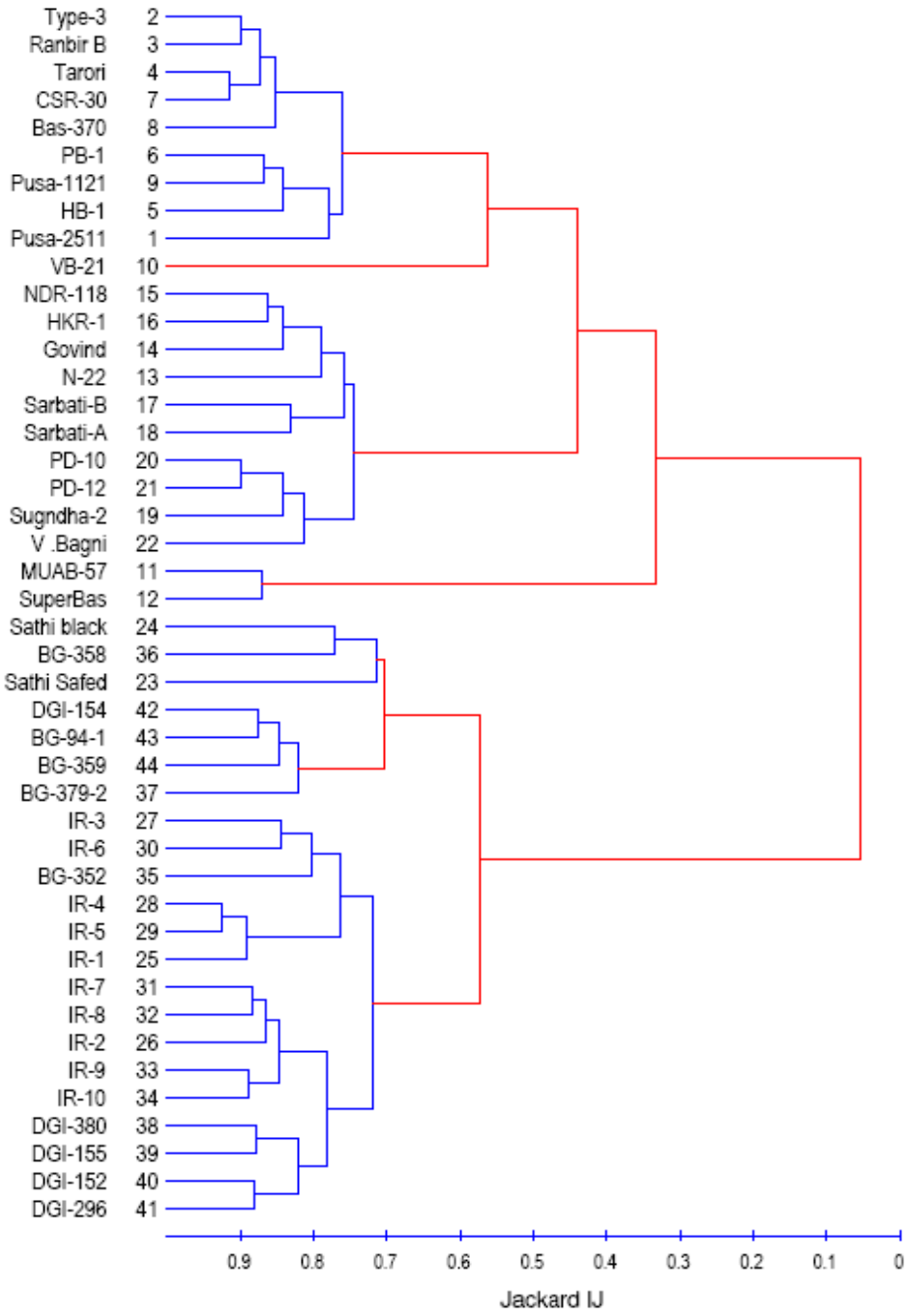

Figure 1. Dendrogram showing clustering of 44 rice varieties constructed using UPGMA based on Jacquard's similarity coefficient obtained from ISSR and SSR joint analysis. Ranbir B, Ranbir Basmati; Bas. 370, Basmati 370; PB-1, Pusa Basmati 1; HB-1, Haryana Basmati-1; VB-21, Vallabh Basmati-21; CSR-30, Basmati CSR-30; N-22, Nagina-22; PD-10, Pant Dhan-10; PD-12, Pant Dhan-12; V. Bagni, Vallabh Bangani; MAUB-57, Vallabh Basmati - 23; Super Bas, Super Basmati.

The present data used for characterization of rice genotypes was generated involving ten ISSR, 20 eight SSR molecular markers and 44 Basmati and non-Basmati rice varieties. The 22 varieties of Basmati and non-Basmati rice adapted to Irrigated agro ecosystem considered presently were developed in Indo-Gangetic plains of Northern India (Singh et al., 2000a) where rice is cultivated mainly in Irrigated puddled conditions (puddle

that is, anaerobic conditions, the traditional method of rice cultivation in Northern India). The rest 22 rice genotypes collected from four different sources (1) IRRI, the Philippines (2) Sri Lanka (3) Central India (4) Indo-Gangetic plains of Northern India, were adapted to aerobic agro-ecosystem that is, direct seeding rice (unpuddled). Out of 22 varieties adapted to aerobic conditions, two varieties Sathi white and Sathi black were Farmers' varieties collected from Indo-Gangetic plains of Northern India. Both the Sathi strains/ farmers' varieties mature in 75 days (seed to seed) with minimal water requirement and remained in cultivation in aerobic conditions over decades in the area of their adaptation before deployment of irrigation facilities. Also, Vallabh Basmati-21, the variety recently released for Irrigated agro-eco system (Singh et al., 2011) has expressed excellent harvests in aerobic conditions (Singh, 2008).

In ISSR-PCR assay, all the 44 rice varieties were classified agro-ecosystem wise into two major distinct clusters. Twenty two (22) varieties of irrigated agro ecosystem were further grouped into 5 clusters based on variations in quality. Out of these, 11 varieties of super fine quality were distributed over 3 clusters depending upon minor variations in quality. Four traditional Basmati varieties Ranbir Basmati, Type 3, Basmati 370 and Taroari Basmati along with four evolved basmati varieties Pusa-1121, HB-1, CSR-30 and PB-1 were clustered together as also reported earlier by Nagaraju et al. (2002).

None of the variety was clustered with Vallabh Basmati 21 and therefore, it was put in a separate cluster. The improved dwarf genotype MAUB 57 recently identified as an elite genotype of Basmati rice for UP and Haryana by AICRP (Anonymous, 2007) and Super Basmati popular in Punjab were clustered together in a separate cluster consistently in all the three assays. The rest 11 varieties of non-basmati rice adapted to irrigated agro-eco system were grouped into two different clusters.

Furthermore, all the 22 varieties adapted to aerobic conditions were further grouped into two sub clusters. Three varieties BG 352, BG 358 and Sathi Black were grouped into one sub cluster. The rest 19 genotypes were distributed over five sub clusters. Within agro-eco systems, the distribution of varieties in the main as well as in sub clusters reflected independence of their geographical origin. The varieties of aerobic rice despite having their origin in the Philippines, Sri Lanka, Northern India (Sathi strains) and Central India were grouped in only one major cluster indicating presence of common base sequence repeats in the genomic DNA corresponding to ISSR markers used. Such DNA sequences could perhaps have evolved, due to different kinds of selection pressures required to acquire fitness well for survival, in due course of time. The explanation could also be forwarded that all the genotypes of common gene pool would have exchanged over the locations collected there from not long before. In other words, the time passed after shifting of such germplasm was too short to attain adaptation as a consequence of a new genetic mechanism which could have evolved in due course of time. Such sequences were detected by the molecular markers used herein. Therefore, it might be concluded that the base sequences which determined ISSR markers were corresponding to the gene complexes responsible for quality and adaptation reactions.

Examination of SSR-PCR assay reflected, minor variations in clustering pattern. However, clustering of the 8 varieties of basmati rice involving traditional and evolved groups remained the same. Likewise, both the evolved varieties of basmati rice MAUB-57 and Super Basmati were consistently grouped in a separate cluster. PS-2511 and Vallabh Basmati-21 adapted to irrigated agro ecosystem were clustered with varieties of aerobic rice, that is, Sathi Black, BG-375-2, and BG-358. Vallabh Basmati-21 being adapted to aerobic conditions might have genes common to those adapted to aerobic conditions.

However, it could not be detected by ISSR markers. It suggested that PS-2511 requires further evaluation in aerobic agro ecosystem for adaptation reactions. Additionally, it might be concluded that SSR markers were, in general, more robust and therefore, could detect even the minor variations more efficiently in the genomic DNA. Such variations are taken as a basis of molecular characterization of the genotypes (Wu and Tanksley, 1993; Yang et al., 1994).

Furthermore, the clustering pattern of joint assay was more similar to that of ISSR clustering pattern. However, it varied slightly when joint assay was taken into consideration for DNA profiling of these varieties (Figure 1). PS-2511, a super fine non Basmati variety was also included into the cluster containing traditional as well as evolved varieties of basmati rice. PS-2511 expressed quality characteristics of Basmati of inferior quality (Table 2) reflecting its limited closeness to Basmati group. This is why; despite continues efforts, PS- 2511 could not be popularized as Basmati among consumers. It also holds true in the present context as reflected by its inconsistent clustering with Basmati group. However, its molecular lineage with Basmati was detected only by ISSR+SSR joint assay (Figure 1). However, impact of adaptation and quality on classification of varieties remained distinct.

In parallel, all the genotypes suitable for aerobic conditions were clustered in a distinct major cluster almost consistently in all the three assays. More variation was noticed in clustering pattern, in general, among the varieties adapted to irrigated agro-eco system over all the three analyses. The varied minor genetic changes which have occurred spontaneously during course of evolution to acquire fitness in a particular agro-eco system seem to be the only cause. It could be an evolutionary adaptation that serves to discriminate between genotypes on the basis of their quality. Examination of dendrogram (Figure 1) and the results discussed above suggested that

Table 2. The component characteristics of rice quality (approved by Government of India and acceptable in the global trade) were scored at quality laboratories, DRR, Hyderabad (AP) and CRRI Cuttack (Orissa). For the sake of brevity, results only of some of basmati varieties Vallabh Basmati-21, MAUB-57, Taroari Basmati and Pusa Basmati-1 obtained at DRR, Hyderabad are given below.

Quality parameter	Quality standards approved by DAC, GOI and expressed by Basmati varieties						
	Approved by DAC, GOI and also acceptable in global trade	Quality parameters expressed by commercially popular and recently released/identified varieties of Basmati rice					
		PB-1	P S-2511	Haryana Basmati	Taroari Basmati	Vallabh Basmati-21	Vallabh Basmati-22
Milling %	65.0 (minimum)	67.0	71.0	70.4	69.2	66.2	69.37
Head rice recovery %	45.0 (minimum)	52.1	58.9	44.5	52.2	51.0	52.01
Kernel length (mm)	6.61 (minimum)	6.98	7.95	6.62	6.94	7.64	7.45
Kernel breadth (mm)	2.00 (maximum)	1.78	1.89	1.72	1.79	1.79	1.72
Length breadth ratio	3.5 (minimum)	3.88	4.21	3.85	3.88	4.27	4.30
Volume expansion ratio	3.0 (minimum)	4.85	3.75	5.50	4.63	4.7	4.74
Water uptake (ml)	250 (minimum)	362	315	285	268	310	205.50
Kernel length after cooking (KLAC) mm	12.0 (minimum)	15.5	14.4	11.0	13.3	13.3	14.80
Elongation ratio (ER)	1.70 (minimum)	2.22	1.8	1.51	1.92	1.73	1.98
Alkali Spreading Value (ASV)	4-7	7.0	7.0	7.0	5.0	7.0	5.08
Amylose content %	20-25	24.81	24.60	24.12	24.44	24.48	23.47
Gel Consistency (GC)	>61	466.6	47.0	47.0	49.66	47.00	54.66
Aroma score (1-3)	3.0	3.0	3.0	3.0	3.0	3.0	3.0

AICRP- All India Coordinated Research Project, DRR- Directorate of Rice Research, Hyderabad, India. CRRI- Central Rice Research Institute, Cuttack (Orissa), India. DAC- Department of Agriculture and Co-operation, GOI- Government of India.

clustering of 44 rice genotypes was mainly based on two factors that is, adaptation and quality (Table 1 and 2). Both adaptation and quality of rice are complex traits.

Adaptation is governed by several gene complexes. Adaptation is a function of the genes possessed by the variety for the regulation of biochemical and physiological processes during growth and development and how well these are matched with the available environmental resources and possible hazards. The adaptation results from a genetic mechanism which influences the matching of their growth and development processes within the environment (Fisher and Scott, 1993). Thus, process of adaptation is viewed as changes in the genetic constitution of genotypes as they accumulate genes or a change in gene frequencies within population which better match growth and development within the environment (Byth, 1981; Clements et al., 1983; Cooper and Hammer, 1996). Adaptation is more complicated but its elegant genetic studies are lacking. Quality of rice is the resultant effect of a number of component traits.

Contrarily, quality of Basmati rice is a complex character consisting of 13 physicochemical component characteristics (Table 2). In addition, finally quality is judged/ examined by a panel of experts. Each component character is governed by different genetic mechanisms. Comprehensive information about genetic mechanism of quality components is available in the literature and some of such studies are listed in the Table 3. Though, aroma is an essential component of Basmati. However, among non-Basmati rice genotypes such as PS- 2511 also carried aroma.

The study reveals that visibly distinguishable qualitative characters like plant height, photosensitivity, disease resistance, colour, size and number of awns, and sensory characters such as aroma etc. could not significantly influence clustering of genotypes. Therefore, the varieties differ for such characters could not be identified by using such molecular tools. For example, tall and dwarf collections of farmers' variety Sarbati could not be identified by the markers presently used and, therefore, were clustered together in a single cluster in all the three cases. Likewise, morphologically distinct genotypes MAUB-57 and Super Basmati were also clustered together. Such results indicated limited utility of molecular markers to the breeders and traders. It appears self-explanatory that small size of DNA sequences of genes responsible for such characters does not have significant bearing on the clustering of these varieties. DNA sequences of small size representing genes responsible for such characters could not be detected by the markers used. However, trait specific markers could be used to detect such characters. In such cases it seems easier to classify the varieties on the basis of morpho-physico-chemical characteristics and application of molecular markers seems of limited value. In the study, impact of any individual component character of quality on classification could not be noticed. None of the variety was classified on the basis of aroma alone. DNA

Table3. Studies describing genetic mechanisms of quality components of rice.

Characters	QTLs/Chrom. arm	References
Aroma	Aro3-1/3S, aro4-/4L, aro8-1/8L	Ahn et al.1992; Petrov et al., 1996; Lorieux et al., 1996; Wanchana et al., 2005; Chen et al., 2006; Amarawathi et al., 2008
Alkali spreading value	Asv6-1/6S	McKenzie and Rutger, 1983; He et al., 1999; Amarawathi et al., 2008
Amylose content	Amy6-1/6S	McKenzie and Rutger, 1983; He et al., 1999; Kumar and Khush, 1996; Amarawathi et al., 2008
Kernal elongation ratio	Elr11-1/11L	Ahn et al., 1993; Amarawathi et al., 2008
Length/breadth ratio	Lbr7-1/7L, Lbr7-2/7L	Redona and Mackill, 1998; Rabiei et al., 2004; Amarawathi et al., 2008
Grain breadth	Grd7-1/7L, gbr7-2/7L	Lin et al., 1995; Redona and Mackill, 1998; Sarkar et al., 1994; Amarawathi et al., 2008
Grain length	Grl11-1/1L, grl7-1/7Lgrl7-2/7L	Lin et al., 1995; Redona and Mackill, 1998; Aluko et al., 2004; Wan et al., 2006; Amarawathi et al., 2008
Gel consistency	qGC-4,qGC-6,qGC-4a,qGC-4b,qGC-5, qGC-8a, qGC-8b, qGC-12a, qGC-12b	Tan et al., 1999; He et al., 1999; Lanceras et al., 2000; Umemoto et al., 2002; Tian et al., 2005; Sabouri et al., 2009

Table 4. Primers sequence (SSR and ISSR).

Primer code		Sequence	Maker
RM 4	Forward	TTGACGAGGTCAGCACTGAC	IDT
	Reverse	AGGGTGTATCCGACTCATCG	
RM 5	Forward	TGCAACTTCTAGCTGCTCGA	IDT
	Reverse	GCATCCGATCTTGATGGG	
RM11	Forward	TCTCCTCTTCCCCCGATC	IDT
	Reverse	ATAGCGGGCGAGGCTTAG	
RM17	Forward	TGCCCTGTTATTTTCTTCTCTC	IDT
	Reverse	GGTGATCCTTTCCCATTTCA	
RM30	Forward	GGTTAGGCATCGTCACGG	IDT
	Reverse	TCACCTACCACACGACACG	
RM38	Forward	ACGAGCTCTCGATCAGCCTA	IDT
	Reverse	TCGGTCTCCATGTCCCAC	
RM42	Forward	ATCCTACCGCTGACCATGAG	IDT
	Reverse	TTTGCTCTAAGTGGCGTACA	
RM44	Forward	TCGGGAAAACCTACCCTACC	IDT
	Reverse	ACGGGCAATCCGCCCAACC	
RM51	Forward	TCTCGATTCAATGTCCTCGG	IDT
	Reverse	CTACGTCATCATCGTCTTCCC	
RM55	Forward	CCGTCGCCGTAGTAGAGAAG	IDT
	Reverse	TCCCGGTTATTTTAAGGCG	
RM84	Forward	TAAGGGTCCATCCACAAGATG	IDT
	Reverse	TTGCAAATGCAGCTAGAGTAC	
RM85	Forward	CCAAAGATGAAACCTGGATTG	IDT
	Reverse	GCACAAGGTGAGCAGTCC	
RM104	Forward	GGAAGAGGAGAGAAAGATGTGTGTCG	IDT
	Reverse	TCAACAGACACACCGCCACCGC	
RM107	Forward	AGATCGAAGCATCGCGCCCGAG	IDT
	Reverse	ACTGCGTCCTCTGGGTTCCCGG	
RM127	Forward	GTGGGATAGCTGCGTCGCGTCG	IDT
	Reverse	AGGCCAGGGTGTTGGCATGCTG	

Table 4. Contd.

RM128	Forward	AGCTTGGGTGATTTCTTGGAAGCG	IDT
	Reverse	ACGACGAGGAGTCGCCGTGCAG	
RM131	Forward	TCCTCCCTCCCTTCGCCCACTG	IDT
	Reverse	CGATGTTCGCCATGGCTGCTCC	
RM136	Forward	GAGAGCTCAGCTGCTGCCTCTAGC	IDT
	Reverse	GAGGAGCGCCACGGTGTACGCC	
RM141	Forward	CACCACCACCACCACGCCTCTC	IDT
	Reverse	TCTTGGAGAGGAGGAGGCGCGG	
RM154	Forward	ACCCTCTCCGCCTCGCCTCCTC	IDT
	Reverse	CTCCTCCTCCTGCGACCGCTCC	
RM160	Forward	AGCTAGCAGCTATAGCTTAGCTGGAGATCG	IDT
	Reverse	TCTCATCGCCATGCGAGGCCTC	
RM161	Forward	TGCAGATGAGAAGCGGCGCCTC	IDT
	Reverse	TGTGTCATCAGACGGCGCTCCG	
RM163	Forward	ATCCATGTGCGCCTTTATGAGGA	IDT
	Reverse	CGCTACCTCCTTCACTTACTAGT	
RM235	Forward	AGAAGCTAGGGCTAACGAAC	IDT
	Reverse	TCACCTGGTCAGCCTCTTTC	
RM252	Forward	TTCGCTGACGTGATAGGTTG	IDT
	Reverse	ATGACTTGATCCCGAGAACG	
RM273	Forward	GAAGCCGTCGTGAAGTTACC	IDT
	Reverse	GTTTCCTACCTGATCGCGAC	
RM302	Forward	TCATGTCATCTACCATCACAC	IDT
	Reverse	ATGGAGAAGATGGAATACTTGC	
RM330	Forward	CAATGAAGTGGATCTCGGAG	IDT
	Reverse	CATCAATCAGCGAAGGTCC	

Table 4. Contd.

Primer code	Sequence	Maker
ISSR-1F	5’GGCGGCGGCGGCGGCAT3’	IDT
ISSR-2F	5’AAGAAGAAGAAGAAGGC3’	IDT
ISSR-3F	5’AAGAAGAAGAAGAAGTG3’	IDT
ISSR-4F	5’AAGAAGAAGAAGAAGCC3’	IDT
ISSR-5C	5’AGCAGCAGCAGCAGCCA3’	IDT
ISSR-6F	5’AGCAGCAGCAGCAGCCG3’	IDT
ISSR-7F	5’GGCGGCGGCGGCGGCTA3’	IDT
ISSR-8F	5’AGCAGCAGCAGCAGCGA3’	IDT
ISSR-9F	5’AAGAAGAAGAAGAAGCG3’	IDT
ISSR-10F	5’CCAGTGGTGGTGGTG3’	IDT

sequences accounted for by QTLs responsible for several component characters of adaption as well as quality were spread over the whole genome and were detected by the corresponding 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-eco systems seem to be largely represented by ISSR and SSR markers’ DNA sequences. Clustering of the genotypes used herein greatly depended upon adaptation to IRRI gated and aerobic agro-eco systems within limits of regulations of the genetic mechanism evolved for adaptation, represented by ISSR and SSR sequences. Likewise, within irrigated agro-eco system quality determined classification of varieties.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The senior author is greatly thankful to Prof. H.S. Gaur, Hon'ble Vice Chancellor, for providing funds generously for strengthening Molecular Biology Laboratory where present study was undertaken. We are also thankful to scientists of DRR, Hyderabad and AICRP centers on rice involved in conduct of trials and management of data used herein.

REFERENCES

Ahn SN, Bollich CN, McClung AM, Tanksley SD (1992) RFLP tagging of a gene for aroma in rice. Theor. Appl. Genet. 84:825-828.

Ahn SN, Bollich CN, McClung AM, Tanksley SD (1993) RFLP analysis of a genomic region associated with cook kernel elongation in rice, Theor. Appl. Genet. 87:27-32.

Aluko G, Martinez C, Tohme J, Castano C, Bergman CJ, Oard JH (2004) QTL mapping of grain quality traits form the interspecific cross *Oryza sativa* x *O. glaberrima.* Theor. Appl. Genet. 109:630-639.

Amarawathi Yellari, Singh Rakesh, Singh Ashok K, Singh Vijai P, Mohapatra Trilochan, Sharma TR, Singh NK (2008). Mapping of quantitative trait loci for basmati quality traits in rice (*Oryza sativa* L.). Mol. Breed. 21:49-65.

Anonymous (2007) Varietal Improvement, All India Coordinated Rice Improvement programme. Progress Report, 43rd Annual Rice Group Meeting, Raipur, April 15-18, 2007. pp.1.310.

Botstein DR, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32(3):314-331.

Byth DE (1998). Genotype x environment interaction and environment adaptation in plant improvement - an overview. In: Byth, D.E. and Mungomery, V.E. (eds.), Interpretation of Plant Response and Adapataion to Agricultural Environments, Australian Institute of Agricultural Science, Queensland Branch. pp. 2-10.

Chen S, Wu J, Yang Y, Shi W, Xu M (2006). The *fgr* gene responsible for rice fragrance was restricted with in 69 kb. Plant Sci. 171:505-514.

Clements RJ, Hayward MD, Byth DE (1983). Genetic adaptation in pasture plants. In: McIvor, J.G. and Bray, R.A. (eds.) Genetic Resources of Forage Plants, CSIRO, East Melbourne. pp. 101-115.

Cooper M, Hammer GL (1996). Plant adaptation and crop improvement (Ed.), Published by CAB International in association with the International Rice Research Institute, Manila, Philippines and International Crops Research Institute for Semi-Arid Tropics, Patancheru, Andhra Pradesh. p. 623.

DelaCruz NM, Khush GS. (2000) Rice grain quality evaluation procedures, In: Aromatic rices (Singh RK, Singh US, and Khush GS, Eds), Oxford & IBH publishing Co, New Delhi.15-28

Fisher JA, Scott BJ (1993). Are we justified in breeding for tolerance to acid soils in southern New South Wales? In: Randall, P.J. Delhaize, E., Richards, R.A. and Munns, R. (eds) Genetic Aspects of Plant Mineral Nutrition, Kluwer Acadmic Publishers, The Netherlands. pp. 1-8.

He P, Li SG, Qian Q, Ma YQ, Li JZ, Wang WM (1999). Genetic analysis of rice grain quality. Theor. Appl. Genet. 98:502-508.

Khush GS and Dela Cruz NM (2002). Developing basmati rices with high yield potential, In: Duffy R(ed.) Specialty rices of the world: breeding, production and marketing, Science pub, Inc, Enfield, USA.15-18.

Kumar I, Khush GS (1996). Inheritance of amylase content in rice (*Oryza sativa* L.). Euphytica 38: 261-269.

Lanceras J, 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.

Lin HX, Min-Shao K, Xiong ZM, Qian HR, Zhuang JY, Lu J, Huang N, Zheng K, Lin HX, Min SK, Xiong ZM, Qian HR, JY Zhuang, Lu J, Huang N, Zheng KL (1995). RFLP mapping of QTLs for grain shape traits in indica rice (*Oryza sativa* L. subsp. *indica).* Sci. Agric. Sin. 28:1-7.

Little RR, Hilder GB, Dawson EH (1978) Differentiation effect of dilute alkali on 25 varieties of milled whole rice. Cereal Chem. 35: 111-126.

Lorieux M, Pertrov M, Huang N, Guiderdoni E and Ghesquiere A (1996). Aroma in rice: genetic analysis of quantitative of trait. Theor. Appl. Genet. 93:1145-1151.

McKenzie KS, Rutger JN (1983). Genetic analysis of amylose content, alkali spreading score, and grain dismensions in rice. Crop Sci. 23:1145-1151.

Moller EM, Bahnweg G, Sandermann H, Geiger HH (1992). A simple and efficient protocol for isolation of high molecular weight DNA from filament fungi, fruit bodies and infected plant tissue. Nucleic Acids Res. 20:6115-6116.

Nagaraju J, Kathirvel M, Kumar RR, 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. USA. 99: 5836-5841.

Petrov M, Dnazart M, Giampaoli P, Faure J, Richard H (1996). Rice aroma analysis: discrimination between a scented and a non-scented rice. Sci. Aliments 16:347-360.

Rabiei B, Valizadeh M, Ghareyazie B, Moghaddam M, Ali AJ (2004). Identification of QTLs for rice grain size and shape of Iranian cultivars using SSR markers. Euphytica 137:325-332.

Redona ED, Mackill DJ (1998). Quantitative trait locus analysis for rice panicle and grain characteristics. Theor. Appl. Genet. 96:957-963.

Sabouri H (2009). QTL detection of rice grain quality traits by microsatellite markers using an *indica* rice (*Oryza Sativa* L.) combination. J. Genet. 88(1):81-85.

Sarkar RK, Nanda BB, Dash AB, Lodh SB (1994). Grain characteristics and cooking quality of aromatic and non-aromatic, long and slender varieties of rice (*Oryza sativa* L.). Indian J. Agric. Sci. 64: 305-309.

Singh D (2010) On the use of Mahalanobis' Generalized Distance D^2: proposed measures to avoid its misleading applications, *Vegetos* 23 (2):197-203.

Singh D and Gupta PK (1985). Selection of diverse genotypes for heterosis in yield and reponse in toria (*Brassica campestris L*.), Theor. Appl. Genet. 69:515-517.

Singh D, Sirohi A (2005). Basmati rice Research and Status of Agri. Export Zone for Basmati rice at Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut, In: National Symposium on "Basmati Rice Research: Current Trends and Future Prospects", held on 6-8 Sept. 2005 at SVBP University 0f Ag. &Technology, Meerut, pp. 101-118.

Singh D, Kumar A, Sirohi A, Kumar P, Singh J, Kumar V, Jindal A, Kumar S, Kumar N, Kumar V, Sharma V, Gupta S, Chand S (2011). Improvement of Basmati rice (*Oryza sativa* L.) using traditional breeding technology supplemented with molecular markers. Afr. J. Biotechnol. 10 (4): 499-506.

Singh D (2008). Development of Vallabh Basmati-21 variety of quality rice for export through traditional and molecular breeding, In: souvenir and abstracts national symposium on "Advances in biotechnology Research for Crop Improvement and Food Security, 6-7 March 2008, SVBPUA &T Meerut. pp. 33-45.

Singh RK, Singh US, Khush GS (2000a). Breeding aromatic rice for high yield and improved grain quality, In: Aromatic Rices. Oxford & IBH Publishing Co. Pvt Ltd, New Delhi. pp. 71-105

Singh RK, Singh US and Khush GS (2000b). Genetics and biotechnology of quality traits, In: Aromatic Rices, Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi.

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

Tan YF, Li JX, Yu SB, Xing YZ, Xu CG, Zhang QF (1999) The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. Theor. Appl. Genet. 99: 642-648.

Tian R, Jiang G H, Shen LH, Wang LQ, He YQ (2005). Mapping quantitative trait loci underlying the cooking and eating quality of rice using a DH population. Mol. Breed. 15:117-124.

Umemoto T, Yano M, Satoh H, Shomura A, Nakamura Y (2002). Mapping of a gene responsible for the difference in amylopectinstructure between japonica-type and indica-type rice varieties. Theor. Appl. Genet. 104: 1-8.

Vos P, Hogers R, Bleekr M, Reijans M, Zabeau M (1995). AFLP: a new

technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.
Wan XY, Wan JM, Jiang L,Wang JK, Zhai HQ, Weng JF, Wang HL, Lei CL, Wang JL, Zhang X, Cheng ZJ, Guo P (2006) QTL analysis for rice grain length and fine mapping of an identified QTL with stable and major effects. Theor. Appl. Genet. 112:1258-1270.
Wanchana S, Kanmolsukyunyong W, Ruengphayak S, Toojinda T, Tragoonrung S, Vanavichiy A (2005). A rapid construction of a physical contig across a 4.5cM region for rice grain aroma facilitate marker enrichment for positional cloning. Sci. Asia 31: 299-306.
Wu KS, Tanksley SD (1993) Abundance, polymorphism and genetic mapping of micro satellites in rice. Mol. Gen. Genet. 241:225-235.
Yang GP, Saghai Maroof MA, Xu CG, Zhang Q, Biyashev RM (1994). Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. Mol. Gen. Genet. 245:187-194.
Yap I, Nelson RJ (1996). WINBOOT: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms, IRRI Discussion Paper Series No. 14, International Rice Research Institute, Manila, Philippines.
Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176-183.