Indian Journal of Biotechnology
Vol. 19, October 2020, pp 292-298

Marker assisted selection and F_6 generation characterization of Katarni rice (*Oryza sativa* L.) derived lines

Kumari Suvidha, Kumari Neha, Sundaram Bharti, Satyendra, Tirthartha Chattopadhyay, Suresh Prasad Singh and Mankesh Kumar*

Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar-813210, India

Received 10 August 2020; revised & accepted 26 September 2020

Characterisation of promising progenies in the advanced generations of breeding schemes is essential to know about their distinctness from the parents as well as other existing cultivars. This also requires for developing them as a variety or pre-breeding line. In this study, an attempt was made for establishing distinctness of twenty five F_6 families of Katarni x Rajendra Sweta, Katarni x IR64 and Katarni X MTU7029 along with 14 rice genotypes. The genotypes were characterised with the help of thirty simple sequence repeat (SSR) markers. The markers generated 107 polymorphic alleles with an average of 3.56 alleles per locus. The values polymorphic information content (PIC), expected heterozygosity (H) and discriminating power (D) of markers varied from 0.41 to 0.48, 0.32 to 0.50 and 0.75 to 0.96, respectively. At 0.38 similarity coefficient four distinct clusters were formed and cluster I had highest number accessions of F_6 families. The study advocated use of (SSR) markers as a complementary tool to morphological descriptors to decipher the distinctiveness of the promising lines in advanced generations of a breeding programme.

Keywords: Katarni rice, *badh2*, *sd1*, SSR markers, similarity coefficient

Introduction

Aromatic rice is a small and special group of rice which is highly priced due to their specific pleasant aroma and other special qualities which comes after cooking[1]. Katarni is one of the famous landrace of rice of Bihar state which is renowned for its special aroma, unique medium fine grain and cooking qualities. Due to its special grain qualities and uniqueness, a tag for geographical indication (GI) has recently been granted to this rice in April, 2018 (Geographical Indication no. 553) (http://ipindiaservices.gov.in/GIRPublic/Application/Details/553) by the Office of Intellectual Properties Rights, New Delhi, India. However, like other traditional rice landraces, it also suffers from a problem of lodging due to its tall height, weak culm and uncontrolled use of nitrogenous fertilizers. Its culm is so weak that the plants often lodge due to the weight of fully matured grains resulting in very low yielding ability (25-30 q/ha)[2]. Hence, isolation and exploitation of dwarfness in Katarni will help not only in increasing production area but also safeguard the interest of the farmers.

Dwarf cultivars are more resistant to damage by lodging and it has more grain yield in response to fertilizer application. The semi-dwarf mutants (*sd1*) in rice are mainly deficient in gibberellic acid (GA) due to a deletion of 383 bp in a gene (at chromosome 1) coding for one of the enzymes encoded by GA20-oxidase gene (*GA20ox2*), catalysing the synthesis of bioactive GA[3]. Out of several compounds responsible for aroma in rice, a highly volatile 2-acetyl-1-pyrroline (2-AP) is the principal component[4] detected in all aerial plant parts of scented rice[5]. The semi-dwarf plant height and fragrance in rice is governed by recessive genes *sd1* and *badh2*, respectively and their presence can only be determined phenotypically by the final height of the plant after the flowering or late developmental stages. The DNA markers for these traits are widely used in rice improvement by marker-assisted selection (MAS) which has helped breeders to identify and eliminate the plants in early generation[6-7].

To reduce the height and maturity period, a marker assisted breeding (MAB) programme was initiated by crossing Katarni with three semi-dwarf rice varieties namely Rajendra Sweta, IR64 and MTU7029. Testing

*Author for correspondence:
mankesh2008@gmail.com,
mankesh_srivastava@rediffmail.com
Mobile phone: +91-9771602414

for distinctness in promising lines at genetically stable stage i.e. F_6 generation is needed to document them and to establish their distinctness from parental source lines. In the present study, assessment of the genetic variability of Katarni derived lines at F_6 generation alongwith their parental lines and aromatic landraces has been described using molecular markers.

Materials and Methods

In this forward breeding approach, tall and aromatic Katarni rice was crossed with three semi-dwarf varieties Rajendra Sweta, IR64 and MTU7029 with an objective to reduce the plant height retaining its aroma intact. The generation was advanced to F_6 and molecular characterisation of 25 entries in F_6 generation was done along with 9 aromatic local landraces namely Champaran Basmati, Shyamjira, Burma Bhushi, Kishanganj Basmati, Malbhog, Karibank, Hafsal, Jasua and Katarni and 5 cultivars namely Rajendra Kasturi, MTU7029, BPT5204, IR-64, Rajendra Sweta. Among 25 derived lines, 21 were F_6 generation of Katarni x Rajendra Sweta (denoted by $KRSF_6$), 3 were of Katarni x MTU7029 (denoted by $KMTUF_6$) and 1 was from Katarni x IR-64 cross (denoted by $KIRF_6$).

Genomic DNA was isolated by using rapid DNA isolation method of Kumar *et al.*[8]. The genotypes were characterised by using 30 SSR markers (Table 1). The sequence of SSR markers located throughout 12 chromosomes of rice is available in gramene markers database (https://www.gramene.org/archive). In addition, gene specific primers for semi dwarfism (*sd1*) and fragrance (*badh2*)[9] were used for selection of dwarf and fragrant progenies. The PCR amplification using SSR primer and gene specific primer was done in gradient thermal cycler (Applied Biosystems model VERITI). Twelve µl reaction volume of PCR consisted of 2 µl of genomic DNA, 0.5 µl of 10 µM forward and reverse primers, 3.5 µl of GeneDirex/SRL PCR mix and 5.5 µl of MilliQ water. For SSR markers, PCR was programmed as initial denaturation of template DNA at 94°C for 4 minutes followed by 35 cycles consisting of denaturation for 30 s at 94°C, annealing at 55°C for 40 s, extension for 40 s at 72°C and final extension for 10 minutes at 72°C. However, the annealing temperature for gene specific primers was set at 58°C keeping all remaining PCR conditions same. The amplified products were separated at 2% agarose gel in 1X sodium borate (SB) conductive media for electrophoresis[10] in SIGMA-SVI gel electrophoresis system. The gel was imaged under gel documentation system (UVTECH, Banglore Genei, Bengaluru, India).

Table 1 — Informativeness of molecular markers generated through PCR amplification in 39 rice accessions

Sl. No.	Primer Name	Chr. loc.	Allele	Range of allele sizes (bp)	H	PIC	D
1	RM413	5	4	65-110	0.38	0.46	0.94
2	RM481	7	5	147-220	0.33	0.48	0.96
3	RM206	11	5	141-200	0.33	0.48	0.96
4	RM236	2	3	168-191	0.44	0.43	0.89
5	RM164	5	5	239-326	0.32	0.48	0.96
6	RM257	9	3	137-169	0.45	0.43	0.89
7	RM21	11	5	100-170	0.32	0.48	0.96
8	RM566	9	3	213-239	0.44	0.43	0.89
9	RM431	1	3	249-275	0.44	0.43	0.89
10	RM514	3	3	246-269	0.44	0.43	0.89
11	RM212	1	5	96-262	0.41	0.45	0.92
12	RM204	6	5	105-143	0.33	0.48	0.96
13	RM519	12	3	124-146	0.45	0.43	0.89
14	RM314	6	3	114-133	0.44	0.43	0.89
15	RM131	4	2	196-202	0.50	0.41	0.75
16	RM3668	11	3	92-109	0.44	0.43	0.89
17	RM1024	5	2	135-142	0.50	0.41	0.75
18	RM528	6	3	235-258	0.44	0.43	0.89
19	RM404	8	3	213-240	0.45	0.43	0.89
20	RM515	8	2	218-245	0.50	0.41	0.75
21	RM3428	11	4	134-170	0.38	0.46	0.94
22	RM348	4	3	118-136	0.44	0.43	0.89
23	RM1345	8	3	105-135	0.46	0.43	0.87
24	RM3451	6	4	148-174	0.38	0.46	0.94
25	RM4924	6	5	121-170	0.32	0.48	0.96
26	RM214	7	4	100-129	0.38	0.46	0.94
27	RM7121	7	4	123-151	0.38	0.46	0.94
28	RM6370	10	2	51-90	0.50	0.41	0.75
29	RM457	11	3	228-256	0.44	0.43	0.89
30	RM241	4	5	111-152	0.32	0.48	0.96
	Average		3.56		0.41	0.44	0.90

The PCR amplification size (in bp) was scored for the presence and absence of the alleles throughout all 39 accessions as 1 and 0, respectively. Based on binary matrix the heterozygosity index (H), polymorphism information content (PIC) and discriminating power (D) of the markers were calculated by an online marker efficiency calculator (iMEC)[11]. The genetic association between 39 rice accessions was calculated by Jaccard's similarity coefficient followed by cluster analysis showing distance based inter-relationship among genotypes was generated using the unweighted pair group method with arithmetic averages (UPGMA) with the SAHN sub programusing NTSYS.pc version 2.1.

Results

Marker Assisted Selection Through *sd1* and *badh2* Gene Specific Markers

The validation of the F_1s (eighteen F_1s of Katarni x IR64, twenty five F_1s of Katarni x Rajendra Sweta) was done through the parental polymorphic SSR markers RM21 and RM242. Out of 18 F_1s of Katarni x IR64 and 25 F_1s of Katarni x Rajendra Sweta, 12 and 23 F_1s were found to be in heterozygous condition indicating the presence of both parental alleles and was used for further selfing. With *badh2* gene specific marker, Katarni had amplification of fragrant band (257 bp size) while Rajendra Sweta and MTU7029 had non-fragrant band (350 bp size) amplification. With *sd1* gene specific marker, Rajendra Sweta and MTU7029 had amplification of 280 bp while in tall Katarni with functional GA20 oxidase gene had shown amplification of nearly 731 bp. The 18 F_1s of Katarni x MTU7029 was screened for the presence of fragrant allele (~257 bp) in the PCR with *badh2* gene specific primer and 12 plants were selected for further selfing (Fig. 1).

In the previous generations i.e. from F_2 to F_5, promising lines were identified first on the basis of the presence of a 257 bp size band for aroma *badh2* gene and thereafter PCR amplification of the selected progenies with *sd1* gene specific primer. Presence of a band size of 280 bp for *sd1* genes in the selected semi-dwarf progenies and approximately 700 bp band in tall Katarni was obtained during PCR using the gene specific primers for *sd1* gene (Fig. 2). In a similar way, the aromatic and semi-dwarf progenies were selected and generation was advanced to F_5.

Molecular Characterisation of Katarni Derived Lines in F_6 Generation

A total of 107 alleles were detected at the loci of 30 microsatellite markers across 39 accessions of rice. The informativeness of the markers on different parameters has been shown in Table 1. Allele frequency of the markers ranged from 2 (RM131, RM1024, RM515 and RM6370) to 5 (RM481, RM206, RM164, RM21, RM212, RM204, RM4924 and RM241) with an average of 3.56 alleles per locus. Figure 3 shows a representative gel image of PCR amplification by primer RM1345 and RM204, respectively.

Expected heterozygosity (H) of a marker is the probability of heterozygosity of an individual for the locus in a population. It differed among the markers

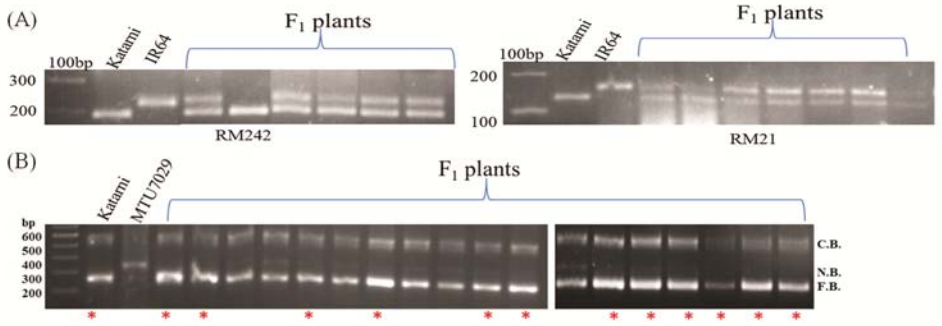

Fig. 1 — Validation of F1 plants of (A) Katarni x IR64 (through RM241) and (B) Katarni x MTU7029 cross through *badh2* marker (* indicated plants are fragrant gene positive plants)

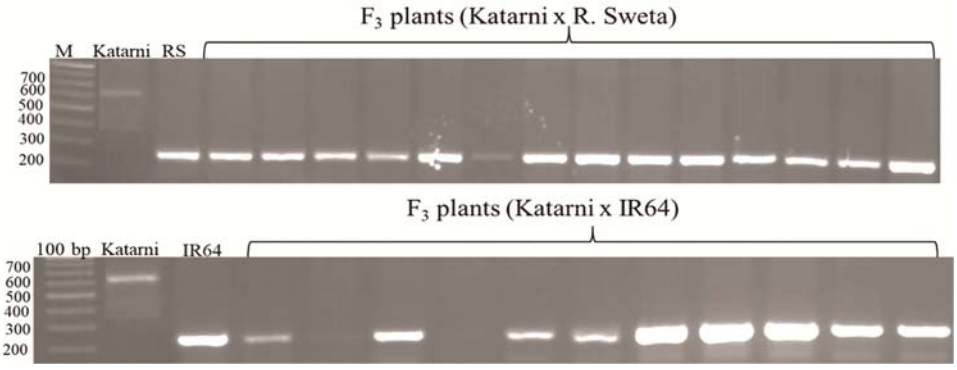

Fig. 2 — PCR screening of *badh2* gene positive plants through *sd1* gene specific primer in F_3 generation of Katarni x Rajendra Sweta and Katarni x IR64.

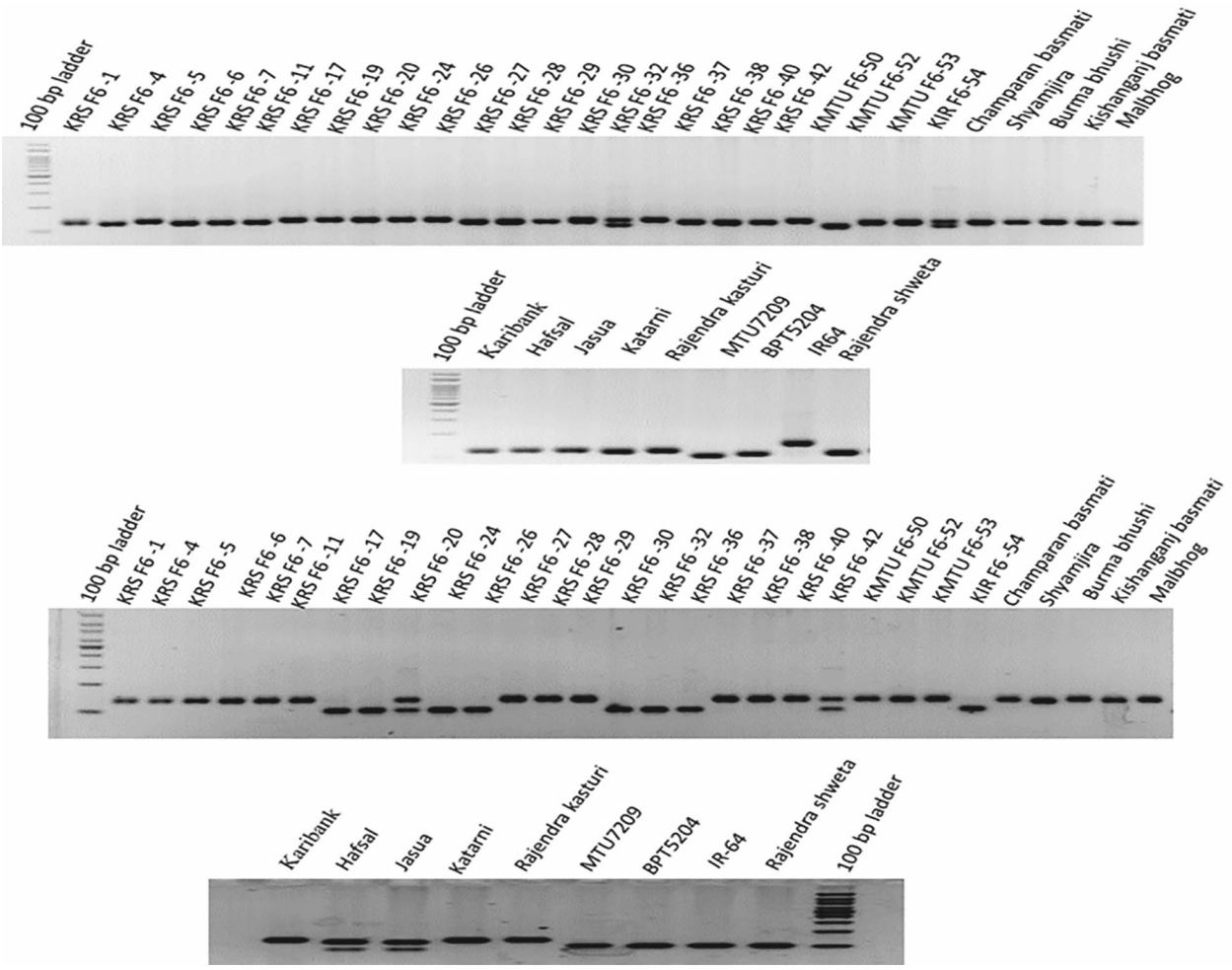

Fig. 3 — Gel image of PCR amplification of 39 genotypes of rice by using (A) RM204 and (B) RM1345

and ranged from 0.32 (in RM164, RM21, RM4924 and RM241) to 0.50 (in RM131, RM1024, RM515 and RM6370) with an average of 0.41. The polymorphism information content (PIC) value is a measure of polymorphism among varieties for a marker locus. In the present study, it ranged from 0.41 (in RM131, RM1024, RM515 and RM6370) to 0.48 (in RM481, RM204, RM206, RM64, RM21, RM4924 and RM241) with average of 0.44.The discriminatory power (D value) indicates the efficiency of a primer in varietal identification i.e. the probability that two randomly chosen entries differentiable from one another because of their different banding patterns. It ranged from 0.75 (in RM131, RM1024, RM515 and RM6370) to 0.96 (in RM481, RM204, RM206, RM164, RM21, RM4924 and RM241) with average of 0.90.

A similarity coefficient matrix based on alleles generated through 30 SSR markers was generated used to study the level of similarity/relatedness among Katarni derived F_6 lines, cultivars and landraces. The Jaccard's similarity coefficient varied from 0.08 to 0.89 indicating great variations among genotypes. Highest similarity coefficient value of 0.89 was found between the $KRSF_6$ entry no. 1 and 4 and between $KRSF_6$ entry no. 17 and entry no. 19 while least similarity coefficient value of 0.08 was found between the genotypes MTU7029 and $KRSF_6$ entry no. 11. The dendrogram showed four major clusters (Fig. 4) at 0.38 similarity coefficient distance as the threshold value for clustering. Major cluster (I) contained most of the rice genotypes (21 rice genotypes) followed by cluster II (8 genotypes) while clusters II and IV had 5 genotypes each. Interestingly, most of the F_6 Katarni x Rajendra Sweta derived lines were grouped in cluster I while cultivars and landraces were grouped both in cluster III and IV. Cluster-wise details of 39 rice accessions have been shown in Table 2.

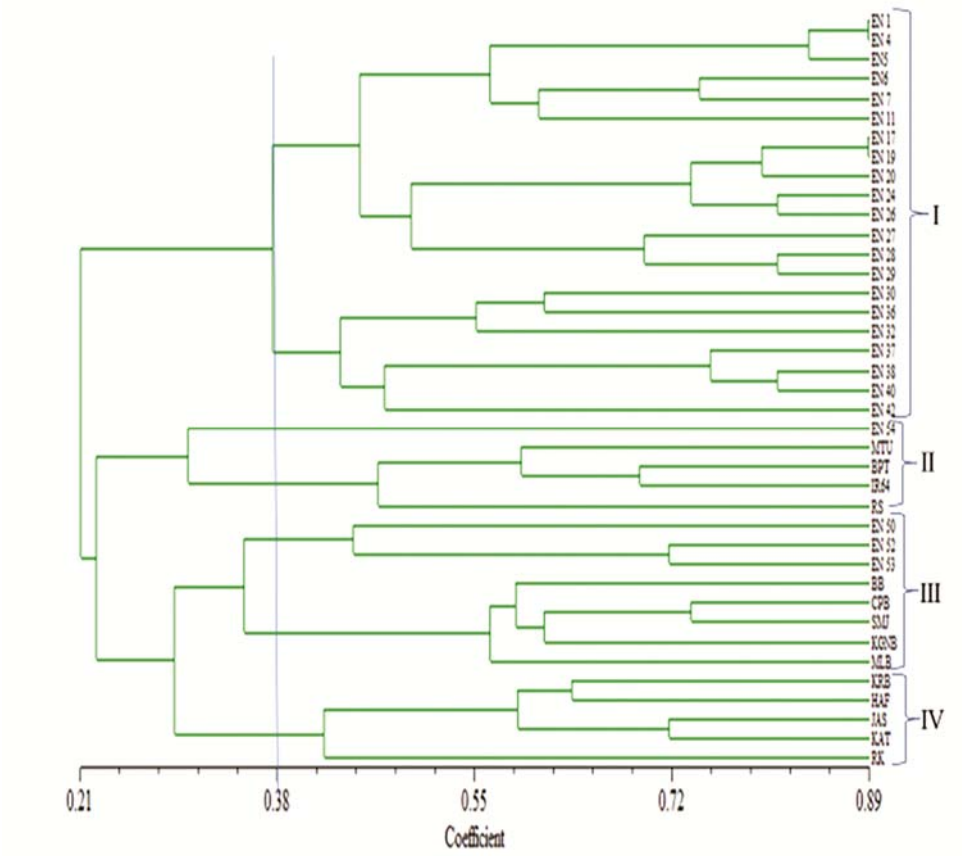

Fig. 4 — An UPGMA cluster dendrogram showing the genetic relationships among 39 accessions based on 30 SSR markers.

Table 2 — Clustering of 39 genotypes on the basis UPGMA analysis

Cluster No.	No. of genotypes	Genotypes in cluster
I	21	$KRSF_6$-1, 4, 5, 6, 7, 11, 17, 19, 20, 24, 26, 27, 28, 29, 30, 36, 32, 37, 38, 40, 41 and 42
II	5	$KIRF_6$-54, MTU7029, BPT5204, IR64 and Rajendra Sweta
III	8	$KMTUF_6$-50, 52, 53, Burma Bhusi, Champaran Basmati, Shyamjeera, Kishanganj Basmati, Malbhog
IV	5	Karibank, Hafsal, Jasua, Katarni and Rajendra Kasturi

Discussion

Assessment of diversity among of the derived lines in advanced generation of a cross can help plant breeders in establishing their distinctness with respect to parental lines as well as existing genotypes. As a result of several meiotic events generation after generation and objective oriented directional selection in a breeding program, the recombinants in advanced generation look very similar to each other phenotypically but have a variation at genetic level. The molecular marker gives reliable information about the distinctness and similarity among the genotypes[12] as they are less affected by environmental factors as compared to the morphological markers[13] and in recent years, microsatellite markers have a significant role to screen, characterize, and evaluate genetic diversity in many crop species[14]. In the present study, the molecular markers both for *sd1* and *badh2* genes were utilized which helped to select the semi-dwarf and fragrant plants not only in early generations, but also at early growth stage of the plant. Raina *et al*[15] also conducted a marker assisted introgression of *sd1* gene from semi-dwarf rice cultivar PAU148 into a tall and fragrant cultivar Ranbir Basmati by using its gene specific marker.

In this study, the F_6 generations derived lines of Katarni rice along with its parental lines and local landraces were characterised using 30 SSR. Being a co-dominant, SSR markers helped to isolate the true F_1s in the initial step of the breeding programme. The selection of molecular marker largely depends upon genomic coverage, its efficiency of polymorphism detection (PIC) and other informativeness in a population, because it can reduce the amount of

genotyping required for inference of ancestry. Wide range of H and D values among the 39 rice accessions using 30 SSR markers indicated the sufficient amount of genetic variation. In a similar study by Warusawithana *et al*[16] the H value varied from 0.198 (RM480) to 0.690 (RM418) in 20 rice accessions. The D value ranged from 0.9203 (RM493) to 0.4529 (RM18) in 24 rice genotypes[17]. It is clear from the molecular profiling of genotypes using different markers that, there was sufficient diversity among the parents and their derived lines in F_6 generation. In a similar study, the genetic diversity analysis of 24 genotypes with nineteen primers resulted in 6 clusters at 0.32 similarity coefficient. Additionally, the genetic diversity of 30 aman rice along with 4 check varieties was assessed with three primers by Shahriar *et al*[18] revealed 4 clusters at 0.36 similarity coefficient. Like *sd1* gene, *fgr* or *badh2* gene is recessive in nature hence can't be distinguished phenotypically unless in homozygous state. The availability of a molecular marker for *sd1* and *badh2* gene has helped in present breeding programme to test the presence of dwarf or tall genotypes with fragrance gene rapidly at early generations of the different crosses.

In a forward breeding approach, almost complete homozygosity can be reached after six generations of selfing and recombination can no longer change the genetic constitution of the line. Thus it has one major advantage that homozygous lines constitute a permanent resource that can be replicated indefinitely and a second advantage is that because they undergo several rounds of meiosis before reaching to the homozygosity (upto F_6 generation), the degree of recombination is higher compared to F_2 populations[19]. Present results confirmed the hypothesis that the recombination between the parents placed most of the F_6 lines of Katarni crosses into the different group in clustering. Previous attempts using conventional breeding to reduce the plant height of Katarni were failed owing to loss of its unique characters. Isolation of a semi-dwarf, early maturing and aromatic lines having exquisite quality parameters will help farmers in terms of productivity and income generation.

Acknowledgements

We sincerely acknowledge the grants received from the SERB, DST, New Delhi (Sanction no. SB/YS/LS-81/2013) and BAU, Sabour (State plan code: SNP/CI/2017-2). This paper bears BAU publication no. 826/2020.

References

1 Shao G N, Tang A, Tang S Q, Luo J, Jiao G A *et al*, A new deletion mutation of fragrant gene and the development of three molecular markers for fragrance in rice, *Plant Breed*, 130 (2011) 172-176.

2 Kumar M, Satyendra, Singh S P, Kumar A, Smriti *et al*, Characterisation of Katarni rice for its geographical indication in Bihar India, *J Agri Search*, 5 (2018) 223-229.

3 Spielmeyer W, Ellis M H & Chandler P M, Semidwarf (*sd-1*), ''green revolution'' rice, contains a defective gibberellin 20-oxidase gene, *Proc Natl Acad Sci USA*, 99 (2002) 9043-9048.

4 Buttery R G, Ling L C & Mon T R, Quantitative analysis of 2-acetyl-1-pyrroline in rice, *J Agric Food Chem*, 34 (1986) 112-114.

5 Hinge V R, Patil H B & Nadaf A B, Aroma volatile analysis and 2AP characterisation at various developmental stages in Basmati and non-Basmati scented rice (*Oryza sativa* L.) cultivars, *Rice (NY)*, 9 (2016) 38.

6 Bernardo R, Molecular markers and selection for complex traits in plants: Learning from the last 20 years, *Crop Sci*, 48 (2008) 1649-1664.

7 Collard B C Y & Mackill D J, Marker assisted selection: An approach for precision plant breeding in the twenty first century, *Philos Trans R Soc, B*363 (2008) 557-572.

8 Kumar V, Prasad A, Roy C & Chhattopadhyay T, Validation of a simple and rapid method for isolating genomic DNA from medicinal and aromatic plants for subsequent polymerase chain reaction, *Int J Curr Microbiol Appl Sci* , 7 (2018) 2562-2566.

9 Bradbury L M T, Fitzgerald T L, Henry R J, Jin Q & Waters D L E, The gene for fragrance in rice, *Plant Biotechnol J*, 3 (2005) 363-370.

10 Kern S E & Brody J R, Conductive media for electrophoresis- US7811437 (to Faster Better Media LLC, USA), Oct 12, 2010.

11 Amiryousefi A, Hyvönen J & Poczai P, iMEC: Online Marker Efficiency Calculator. *Appl Plant Sci*, 6 (2018) e1159.

12 Giancola S, Marcucci S, Poltri, Lacaze P & Hopp H E, Feasibility of integration of molecular markers and morphological descriptors in a real case study of a plant variety protection system for soybean, *Euphytica*, 127 (2002) 95-113.

13 Ovesna J, Polakova K & Lisova L, DNA analyses and their applications in plant breeding, *Czech J Genet Plant Breed,* 38 (2002) 29-40.

14 Miah G, Rafii M Y, Ismail M R, Puteh A B, Rahim HA *et al*, A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance, *Int J Mol Sci*, 14 (2013) 22499-22528.

15 Raina M, Salgotra R M, Pandotra P, Rathour R & Singh K, Genetic enhancement for semi-dwarf and bacterial blight resistance with enhanced grain quality characteristics in traditional Basmati rice through marker-assisted selection, *C R Biologies*, 342 (2019) 142-153.

16 Warusawithana T M, Samarasinghe W L G, Dassanayake P N, Ubeysekara N M & Jayarathna K G C N, Genetic diversity analysis of traditional rice variety 'Pachchaperumal' using SSR markers, *Int J Sci & Tech Res*, 6 (2017) 229-237.

17 Shakil S, Sultana S, Hasan Md, Hossain M, Ali Md *et al*, SSR marker based genetic diversity analysis of modern rice varieties and coastal landraces in Bangladesh, *Indian J Biotechnol,* 14 (2015) 33-41.

18 Shahriar H, Robin A, Begum S & Hoque A, Diversity analysis of some selected rice genotypes through SSR-based molecular markers, *J Bangladesh Agril Univ,* 12 (2014) 307-311.

19 Schneider K, Mapping populations and principles of genetic mapping. *In The handbook of plant genome mapping* (ed. K. Meksem and G. Kahl). Wiley-VCH Verlag, Weinheim 2005, 1-22.