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Phenotypic and molecular characterization of advanced breeding lines of aromatic Katarni rice

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Abstract

Katarni is a non-basmati aromatic rice of Bihar, India, whose tall stature, weak culm and long maturity limit its productivity. Katarni was crossed with the semi-dwarf varieties Rajendra Sweta and BPT5204, and advanced breeding lines were developed through marker-assisted backcrossing and forward breeding. Twenty-two lines and three parental checks were evaluated during *kharif* 2023 for yield, quality and molecular traits using 13 trait-linked SSR markers and four gene-specific markers. High heritability with high genetic advance was observed for plant height, flag leaf length and grain yield, among other traits. Plant height and flag leaf length correlated positively with grain yield. SSR markers grouped the 25 genotypes into four clusters. The *badh2* (aroma) and *sd1* (semi-dwarfism) alleles were present in 19 and 21 lines, respectively. Lines EN10 and EN15 combined semi-dwarf stature, earlier flowering, the aroma allele and grain yield at par with the checks, and are proposed for multi-location evaluation.

Keywords: *badh2*, functional markers, Katarni rice, *sd1*, SSR markers

INTRODUCTION

Rice is the most widely cultivated staple cereal crop feeding more than 60 % of the world population after wheat and supplements 25–44 % of dietary protein and 32–59 % of the dietary energy (Prabhu *et al.,* 2017). Rice is endowed with vast genetic diversity which makes its adaptation to diverse agroclimatic conditions and provides a very rich genetic resource for crop improvement (Hour *et al.,* 2020). Basmati rice is prevalently grown in foothills of Himalayas comprising North Indian and Western Pakistan states. The unique qualities and popcorn-like aroma after cooking of these rice varieties come only when they are grown in certain geographical areas and hence, tagged with geographical indication. The extra slender grain of Basmati elongates excessively after cooking and becomes fluffy textured. Besides slender grain Basmati rice, some traditional non-Basmati, medium slender to short grained aromatic rice are also being grown in some patches of India. Katarni rice is one of the finest short grained non-basmati aromatic traditional rice which is native of eastern Indian state Bihar and also got the GI (geographical indication) tag due to its location specific uniqueness in 2018 (Kumar *et al.,* 2018). But this genotype is very poor yielder (25–30 q/ha), prone to lodging at the time of maturity due to its weak culm and has very late maturity period of about 155–160 days which makes it unfit for traditional rice-wheat cropping system.

Out of 100 compounds responsible for pleasant aroma in Basmati and other scented rice varieties, 2-acetyl-1-pyrroline is the prevalent compound which is synthesized in plant mainly due to loss of function of *betaine aldehyde dehydrogenase* (*BADH2*) gene located on chromosome 8 (Chen *et al.,* 2008). Gibberellic acid (GA), a key hormone for controlling height in rice, is deficient in GA-sensitive semi-dwarf (*sd1*) mutants and exploited extensively during the “green revolution” in the 1960s and 1970s by breeders for the development of semi-dwarf varieties (Ashikari and Matsuoka, 2002). Apart from aroma, quality in rice is also determined by grain extrinsic factors like length, breadth, L/B ratio and intrinsic factors like amylose content, gelatinization temperature, gel consistency value etc. Several high-yielding, semi-dwarf aromatic varieties of basmati and non-basmati rice have been developed through conventional breeding. However, retaining their exquisite quality parameters without the help of molecular markers remains a challenge, since associated quality traits are often lost in successive generations. The linked molecular marker in general and perfect markers in particular for quality and other agronomical traits in rice have been utilized widely by the breeders in the selection of desirable plants in a very precise way.

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Development of a dwarf, early maturing and fragrant derivative of Katarni rice can enhance the profitability of Katarni growing farmers as well as the stakeholders associated with its business. With the objective to reduce the height and maturity period, a marker assisted breeding programme was initiated by crossing Katarni with two semi-dwarf rice varieties *viz*., Rajendra Sweta and BPT5204. Marker assisted foreground, recombinant and background selection were done and the segregants with 82 to 94 % background recovery were further advanced through selfing (Suvidha *et al.,* 2020). These backcross-derived lines have also been morphologically characterized for yield and its contributing traits in earlier generations (Kumar *et al*., 2019). Replicated trials of the promising entries can be done after attaining approx. 98 % homozygosity after 6–7 generations of repeated selfing or backcrossing in marker assisted breeding programme in rice (Collard and Mackill, 2008). Assessment of genetic variability, association of characters, and direct and indirect effects contributed by each character towards yield assists the selection process and most importantly in choosing the best genotype for varietal release. This study deals with the morphological and molecular characterization of Katarni rice derived lines to identify semi-dwarf and early maturing lines with similar quality characteristics of Katarni.

MATERIALS AND METHODS

Morphological observation and statistical analysis

The twenty-two derived lines of Katarni rice (denoted EN1 to EN22), comprising backcross-derived lines (BC_1F_5 to BC_3F_4 generation) from the crosses Katarni x R. Sweta (KRS) and Katarni x BPT5204 (KBPT), and forward-bred, selfed lines (F_7 generation) from the KRS cross (**Table 1**), were evaluated along with three parental checks i.e., Katarni (EN23), BPT5204 (EN24) and R. Sweta (EN25) (**Table 1**) during *kharif* season, 2023 in a randomized block design (RBD) with three replications. The data were recorded for plant height, days to 50% flowering, number of tillers per plant, panicle length, flag leaf length and number of grains per panicle. Post-harvest data were taken on 1000 grain weight, kernel length, kernel breadth, L/B ratio, grain yield per plant in kg per hectare, hulling percentage, milling percentage and amylose content. Amylose content of genotypes was estimated by using 1 ml acetic acid, 2 ml of KI (Potassium Iodide) and I_2 (Iodine) reagent and calculated according to the formula given by Juliano (1971). Statistical analysis was done through INDOSTAT to calculate coefficient of variance, heritability and genetic advance as percentage of mean (GAM).

Molecular analysis and PCR conditions

Thirteen SSR markers which have been previously reported to be linked with various quality traits in rice were used for genotyping of 25 accessions under study. The PCR amplification products were scored as a binary (0, 1) presence/absence matrix for each amplified fragment, as required for input into iMEC software (Amiryousefi *et al.,* 2018) and used in marker efficiency calculation to calculate expected heterozygosity, polymorphism information content, mean heterozygosity and discriminating power of SSR markers. The Jaccard's similarity coefficient and dendrogram were drawn using NTSYS software. The gene specific or perfect markers for semi-dwarfism (*sd1*), heading date (*Hd3A*), grain size (*GS3*) and fragrance (*badh2*) were used to confirm the introgression of target genes in the advanced generations of Katarni derived lines. List of the molecular markers, chromosomal location and linked traits are shown in **Table 2**.

DNA amplification was carried out in thermal cycler (Applied Biosystems model VERITI) using SSR and gene specific primers. PCR reaction was carried out in 12 µl reaction volume containing 2 µl (100 ng) of extracted genomic DNA, 3.5 µl of SRL/Xcelris PCR

Table 1. List of Katarni rice derived lines under study

Genotype	Description	Generation	Genotype	Description	Generation
EN1	KRS BC -63	BC_3F_4	EN 12	KRS BC-298	BC_1F_6
EN 2	KRS BC-97	BC_3F_4	EN 13	KRS BC-311	BC_2F_5
EN 3	KRS BC-104	BC_3F_4	EN 14	KBPT BC-319	BC_2F_5
EN 4	KRS BC-119	BC_3F_4	EN 15	KRS F_7-3	F_7
EN 5	KRS BC-127	BC_3F_4	EN 16	KRS F_7-6	F_7
EN 6	KRS BC-221	BC_3F_4	EN 17	KRS F_7-7	F_7
EN 7	KRS BC-223	BC_3F_4	EN 18	KRS F_7-8	F_7
EN 8	KRS BC-224	BC_3F_4	EN 19	KRS F_7-10	F_7
EN 9	KRS BC-166	BC_1F_5	EN 20	KRS F_7-11	F_7
EN 10	KRS BC-275	BC_1F_5	EN 21	KRS F_7-17	F_7
EN 11	KRS BC-288	BC_1F_6	EN 22	KRS F_7-18	F_7

KRS = Katarni × Rajendra Sweta; KBPT = Katarni × BPT5204. Except EN14 (KBPT), all listed lines (EN1–EN13 and EN15–EN22) were derived from the KRS cross.

Table 2. List of SSR and gene specific or perfect markers used for molecular characterization of Katarni derived lines

S. No.	Primers	Chr. No.	Linked traits	Reference
SSR Markers				
1	*RM222*	10	Number of grains/panicle	Gaikwad *et al*. (2014)
2	*RM289*	5	Spikelet fertility	Gaikwad *et al*. (2014)
3	*RM220*	1	Number of spikelets/panicle	Gaikwad *et al*. (2014)
4	*RM282*	3	Fragrance in rice	Singh *et al*. (2007)
5	*RM5474*	3	Fragrance in rice	Singh *et al*. (2007)
6	*RM218*	3	Grain Length/Breadth (L/B) Ratio	Pandey *et al*. (2013)
7	*RM480*	5	Kernel Breadth, L/B, Kernel Length	Pandey *et al*. (2013)
8	*RM21260*	7	Alkali Spreading Value	Pandey *et al*. (2013)
9	*RM324*	2	Grain Length, 1000 GW	Swamy *et al*. (2012)
10	*RM262*	2	1000 GW	Swamy *et al*. (2014)
11	*RM340*	6	1000 GW	Feifei *et al*. (2016)
12	*RM564*	3	Post-harvest seed dormancy	Bharti *et al*. (2019)
13	*RM190*	6	Amylose content	Biselli *et al*. (2014)
Perfect Markers				
	Hd3A	6	Heading date	Nayyeripasand *et al*. (2013)
	sd1	1	Semi-dwarfness	Spielmeyer *et al*. (2002)
	badh2	8	Fragrance	Bradbury *et al*. (2005)
	GS3	3	Grain size	Ramkumar *et a*l. (2010)

mix, 0.75 µl of 10 µM forward and reverse primer each and 5 µl of milliQ water. Template DNA was initially denatured at 94°C for 4 minutes followed by 35 cycles (30 sec denaturation at 94°C, 40 sec annealing at 55–67°C and 40 sec of extension at 72°C) of PCR amplification and final extension of 72°C for 10 minutes followed by hold at 4°C. The annealing temperature in the PCR using gene specific primers was kept at 58°C.

RESULTS AND DISCUSSION

Morphological observations

Mean value of eleven pre- and post-harvest morphological traits in 25 genotypes was calculated (**Table 3**). The plant height ranged from 66 cm (EN17) to 161 cm (EN9) with a general mean of 89 cm, while days to 50% flowering ranged from 93 (EN16) to 121 (EN5) days with an average value of 108 days indicating the reduced height and early maturity of the segregants with respect to Katarni rice. Similar variability was observed for the rest of the yield and quality contributing traits as shown in **Table 3**. Grain yield is the most important and complex trait, resulting from the associated expression of different yield-related component traits as mentioned above and is directly related to the profitability of the farmers. In the present study, it varied from 1554 kg/ha (EN13) to 4142 kg/ha (EN2) with a general mean of 2760 kg/ha. None of the genotypes were found to be significantly superior to the parental checks i.e., BPT5204 (3812 kg/ha) and R. Sweta (3930 kg/ha). However, genotypes *viz*., EN1, EN2, EN3, EN10 and EN15 were found to be on par in performance with respect to the above checks.

The milling percentage ranged from 59.3 (EN5) to 68.1 % (EN3) with mean of 64.9 %, amylose content ranged from low amylose of 15.5 (EN16) to high amylose of 26.5% (EN2) with mean of 20.4 % and hulling percentage varied from 69.8 (EN11) to 79.9 (EN16) with mean of 76.3% (**Table 3a**).

The broad sense heritability varied from 44 % (for TGW) to 97 % (for plant height) whereas, genetic advance as percent of mean varied from 7.69 % (kernel breadth) to 51.65 % (grain yield). High heritability (>60 %) coupled with high genetic advance (>20 %) was observed in characters like plant height, number of tillers per plant, flag leaf length, kernel length, L/B ratio and grain yield indicating the presence of less environmental effect with additive gene action and selection for these traits may be useful in next generations. Number of grains per panicle and 1000 grain weight showed moderate-to-low heritability (56 % and 44 %, respectively) despite high genetic advance (21.7 % and 21.0 %), suggesting comparatively greater environmental or non-additive influence on these two traits. Similar findings were reported by Dhurai *et al.* (2014) in rice for characters like plant height, number of grains per panicle and grain yield per plot. Roy and Shil (2020) also studied heritability and genetic advance in F_3 and F_5 generation in the cross of aromatic and modern cultivars for various morphological traits. Similar observations of high heritability coupled with high genetic advance for plant height, days to 50 % flowering and seeds per panicle in aromatic rice genotypes were also reported by Sravan *et al.* (2016).

Table 3. Mean performance of 11 morphological traits in 25 genotypes

Genotypes	DFF	PH	NTPP	PL	FLL	NGPP	TGW	GYKPH	KL	KB	L/B
EN 1	117	122	12	24	20	250	13	3574	7.99	2.12	3.76
EN 2	100	80	11	20	31	251	13	4142	7.65	1.79	4.28
EN 3	100	82	8	20	34	234	13	4009	7.48	1.74	4.30
EN 4	118	96	8	22	25	197	10	2626	7.40	1.73	4.29
EN 5	121	92	11	25	29	186	9	1973	6.67	1.61	4.15
EN 6	113	91	9	23	27	194	11	2762	7.62	1.73	4.41
EN 7	118	85	13	21	26	220	12	1578	7.06	1.91	3.70
EN 8	115	88	8	20	25	152	12	2922	7.19	1.82	4.01
EN 9	115	161	10	26	32	241	13	3209	7.60	1.88	4.04
EN 10	107	92	8	24	33	188	9	3384	5.82	1.80	3.23
EN 11	103	89	10	22	25	182	13	2637	7.48	1.78	4.21
EN 12	118	79	8	24	24	168	12	1998	7.38	1.87	3.95
EN 13	106	88	9	23	33	241	14	1554	7.60	1.80	4.22
EN 14	104	83	9	22	26	229	13	3041	7.40	1.87	3.98
EN 15	104	82	10	22	22	219	17	4018	7.09	1.92	3.70
EN 16	93	72	9	19	26	249	11	1658	7.62	1.67	4.57
EN 17	105	66	15	18	21	257	19	2038	8.96	1.89	4.77
EN 18	103	67	8	19	20	289	17	1732	8.64	1.92	4.51
EN 19	106	76	9	19	25	179	16	2312	8.47	1.78	4.76
EN 20	103	85	8	22	26	255	16	2252	8.58	1.84	4.67
EN 21	103	74	11	22	34	270	15	2295	8.89	1.84	4.85
EN 22	105	88	8	23	26	256	13	2560	5.68	1.98	2.88
Katarni	114	137	8	25	32	223	14	2972	7.58	1.87	4.06
BPT5204	103	78	8	22	31	245	12	3812	7.44	1.75	4.26
R. Sweta	100	80	10	21	36	252	13	3930	7.67	1.97	3.94
Mean	108	89	9	22	28	225	13	2760	7.56	1.84	4.14
Range (L)	93	66	8	18	20	152	9	1554	5.68	1.61	2.88
Range (H)	121	161	15	26	36	289	19	4142	8.96	2.12	4.85
S.E. (±m)	1.1	1.9	0.6	0.5	1.2	15.8	1.2	252.0	0.11	0.05	0.12
C.D. (5%)	3.2	5.4	1.8	1.5	3.5	45.0	3.7	716.8	0.33	0.14	0.34
h^2 (Broad Sense) %	93.0	97.0	68.0	82.0	81.0	56.0	44.0	76.0	93.0	52.0	82.0
GAM (at 5%)	13.5	48.2	29.1	16.9	30.4	21.7	21.0	51.6	20.6	7.7	20.1

DFF- days to 50% flowering ; PH- plant height (cm); NTPP- number of tillers per plant; PL- panicle length (cm); FLL- flag leaf length (cm); NGPP- number of grains per panicle; TGW- 1000-grain weight (g); GYKPH- grain yield (kg/ha); KL- kernel length (mm); KB- kernel breadth (mm); L/B- kernel length/breadth ratio.

Correlation in combination of path coefficient analysis is important for identifying the degree of association and direct and indirect influence of one character on another and also on grain yield. Days to 50% flowering and plant height had positive and significant association with panicle length (r = 0.53 and r = 0.69, respectively); number of tillers per plant had positive and significant association with kernel length (r = 0.27) and kernel breadth (r = 0.24); while number of grains per panicle had positive and significant association with thousand grain weight (r = 0.36) and kernel length (r = 0.32) (**Table 4**). These findings were similar to the previous reports of Koli and Punia (2012) who reported positive correlation of panicle length and test weight with grain yield in aromatic rice. Kernel length had maximum direct phenotypic effect (1.27) while 1000 grain weight had minimum direct positive phenotypic effect (0.07) on grain yield (**Table 5**). Archana *et al.* (2018) reported direct positive effect of plant height on grain yield. Priyanga *et al.* (2022) also reported positive direct effect of plant height, panicle

Table 3(a). Hulling percentage (HP), milling percentage (MP) and amylose content (AP, %) of 25 genotypes

Genotype	HP (%)	MP (%)	AP (%)
EN1	78.9	63.9	22.0
EN2	78.4	63.1	26.5
EN3	78.1	68.1	26.0
EN4	74.9	66.9	21.2
EN5	75.2	59.3	20.2
EN6	78.0	66.1	20.3
EN7	72.8	68.0	20.5
EN8	73.8	63.6	18.5
EN9	79.5	65.6	21.0
EN10	73.6	60.0	21.8
EN11	69.8	67.0	16.6
EN12	78.9	67.1	17.3
EN13	76.6	66.2	17.7
EN14	76.9	67.7	17.0
EN15	77.9	60.5	18.4
EN16	79.9	67.2	15.5
EN17	76.3	61.9	18.8
EN18	74.8	60.4	19.0
EN19	75.6	65.9	18.4
EN20	74.9	67.1	17.5
EN21	77.0	64.8	21.3
EN22	72.9	61.7	21.9
Katarni	78.2	65.8	23.3
BPT5204	75.9	68.0	24.0
R. Sweta	78.4	66.3	24.5
Mean	**76.3**	**64.9**	**20.4**

Table 4. Phenotypic correlation coefficient matrix

Traits	PH	NTPP	PL	FLL	NGPP	TGW	KL	KB	L/B	GYKPH
Days to 50% flowering	0.49**	0.12	0.53**	-0.14	-0.39**	-0.31**	-0.22	0.06	-0.24*	-0.16
Plant height		0.01	0.69**	0.19	0.00	-0.21	-0.18	0.18	-0.26*	0.22*
Number of tillers per plant			-0.11	-0.15	0.15	0.19	0.27*	0.24*	0.10	-0.13
Panicle length				0.27*	-0.15	-0.32**	-0.44**	0.02	-0.41**	0.12
Flag leaf length					0.05	-0.25*	-0.21	-0.19	-0.07	0.27*
Number of grains per panicle						0.36**	0.32**	0.23*	0.14	0.03
1000-grain weight							0.49**	0.32**	0.25*	-0.00
Kernel length								0.07	0.82**	-0.17
Kernel breadth									-0.49**	0.15
Kernel length/breadth										-0.24*

*, **: Correlation is significant at p = 0.05 and 0.01, respectively

PH- plant height; NTPP- number of tillers per plant; PL- panicle length ; FLL- flag leaf length; NGPP- number of grains per panicle; TGW- 1000-grain weight ; KL- kernel length; KB- kernel breadth L/B- kernel length/breadth ratio; GYKPH- grain yield

Table 5. Direct (diagonal) and indirect effects of component traits attributing to grain yield at phenotypic level

Traits	DFF	PH	NTPP	PL	FLL	NGPP	TGW	KL	KB	L/B	GYKPH
Days to 50% flowering	-0.37	-0.18	-0.05	-0.20	0.05	0.15	0.12	0.08	-0.03	0.09	-0.16
Plant height	0.19	0.38	0.01	0.26	0.08	0.00	-0.08	-0.07	0.07	-0.10	0.22*
Number of tillers per plant	-0.01	0.00	-0.06	0.01	0.01	-0.01	-0.01	-0.02	-0.02	-0.01	-0.13
Panicle length	-0.06	-0.07	0.01	-0.11	-0.03	0.02	0.03	0.05	0.00	0.04	0.12
Flag leaf length	-0.03	0.04	-0.03	0.05	0.19	0.01	-0.05	-0.04	-0.04	-0.02	0.27*
Number of grains per panicle	0.06	0.00	-0.02	0.02	-0.01	-0.15	-0.06	-0.05	-0.04	-0.02	0.03
1000-grain weight	-0.02	-0.01	0.01	-0.02	-0.02	0.02	0.07	0.03	0.02	0.02	-0.00
Kernel length	-0.28	-0.23	0.35	-0.56	-0.27	0.41	0.63	1.27	0.09	1.05	-0.17
Kernel breadth	-0.05	-0.13	-0.18	-0.02	0.14	-0.17	-0.24	-0.05	-0.73	0.36	0.15
Kernel length/breadth	0.40	0.44	-0.18	0.69	0.13	-0.23	-0.42	-1.37	0.82	-1.66	-0.24*

DFF- days to 50% flowering ; PH- plant height ; NTPP- number of tillers per plant; PL- panicle length; FLL- flag leaf length; NGPP- number of grains per panicle; TGW- 1000-grain weight ; KL- kernel length; KB- kernel breadth; L/B- kernel length/breadth ratio; GYKPH- grain yield

length, grain length and grain width on single plant yield in rice landraces, which are partly in conformity with the present findings (plant height and grain length showed positive direct effects in both studies, whereas panicle length and kernel breadth showed negative direct effects in the present study). The residual effect, representing the influence of factors other than the ten component traits included in the model, was 0.86, indicating that a large proportion of the variation in grain yield is governed by additional yield-contributing traits and environmental factors not measured in this study.

PCR profiling of derived lines

The molecular diversity assessed using thirteen trait-linked SSR markers and four gene-specific markers (**Table 2**) also revealed a significant difference between the derived lines in advanced generation of Katarni rice. Uses of functional markers or gene specific markers are more preferred over random molecular markers as they can be used in any population regardless of the genetic background. These markers are developed from the sequence of the gene itself; hence have complete linkage to the gene of interest. Further the problem of linkage drag associated with random markers is overcome through functional markers due to very lesser recombination within the flanking regions (Lau *et al.,* 2015).

The *i*MEC analysis of PCR amplified products through a set of 13 trait-linked SSR markers (**Table 6**) indicated the variation of expected heterozygosity (H value) from 0.269 (RM220 and RM564) to 0.435 (RM282 and RM218) with an average value of 0.372. H value measures the probability that an individual is heterozygous for the locus in the population. The PIC value is a measure of polymorphism among varieties for a marker locus used in linkage analysis. It ranged from 0.280 (RM282 and RM218) to 0.339 (RM220 and RM564) with an average value of 0.304. Mean heterozygosity (H. av.) value of a marker is the heterozygosity of the polymorphic fraction and its value was 0.004 for all markers except RM289 (0.006) and RM564 (0.002). The discriminatory power value (D value), which is the efficiency of a molecular marker in varietal identification, ranged from 0.900 (RM282 and RM218) to 0.976 (RM220) with an average value of 0.936. Suvidha *et al.* (2020) also studied the PIC, H value and D value of 30 SSR markers in 25 families of Katarni rice in F_6 generation.

The Jaccard's similarity coefficient based on the NTSYS analysis of amplification profile ranged from 4 % to 73 %. Maximum similarity of 73 % was found between sister line genotypes viz., EN2 and EN3, EN4 and EN5, EN7 and EN8 and between genotypes EN17 and EN18 of the same cross combination. In cluster analysis using UPGMA method (**Fig. 1**), twenty-five genotypes were grouped into four clusters out of which, cluster I had the maximum number of genotypes (10) followed by cluster III (8), cluster II (5) and cluster IV (2). The dendrogram indicated that all the genotypes differed from each other.

Hd3A gene is located in rice on chromosome number 6 and reported to control flowering time in rice. Nayyeripasand *et al.* (2013) used the gene specific marker for *Hd3A* gene and found PCR products of size 98 and 144 bp for late and early flowering genotypes, respectively. As expected, *Hd3A* marker amplified allele B of 98 bp size in Katarni only and allele A type in 11 genotypes including BPT5204 and R. Sweta, while the rest of the genotypes were in heterozygous state (**Fig. 2a**). *GS3* gene is located in rice on chromosome number 3 which controls grain size and C to A mutation in the 2nd exon of the gene has been reported to be functionally associated with long and short grain size. According to Fan *et al.* (2009), allele C is associated with grain length from 6.4 to 8.8 mm, while allele A

Table 6. Informativeness of 13 microsatellite markers among 25 rice genotypes

Name of Marker	PIC	H. Value	H av.	D Value
RM222	0.305	0.375	0.004	0.939
RM289	0.285	0.425	0.006	0.909
RM220	0.339	0.269	0.004	0.976
RM282	0.280	0.435	0.004	0.900
RM218	0.280	0.435	0.004	0.900
RM480	0.308	0.365	0.004	0.944
RM324	0.312	0.354	0.004	0.949
RM262	0.301	0.385	0.004	0.934
RM5474	0.308	0.365	0.004	0.944
RM340	0.301	0.385	0.004	0.934
RM564	0.339	0.269	0.002	0.975
RM21260	0.294	0.403	0.004	0.924
RM190	0.305	0.375	0.004	0.939
Average	0.304	0.372	0.004	0.936

PIC, polymorphism information content; H. Value, expected heterozygosity; H av., mean heterozygosity of the polymorphic fraction; D Value, discriminating power (marker index).

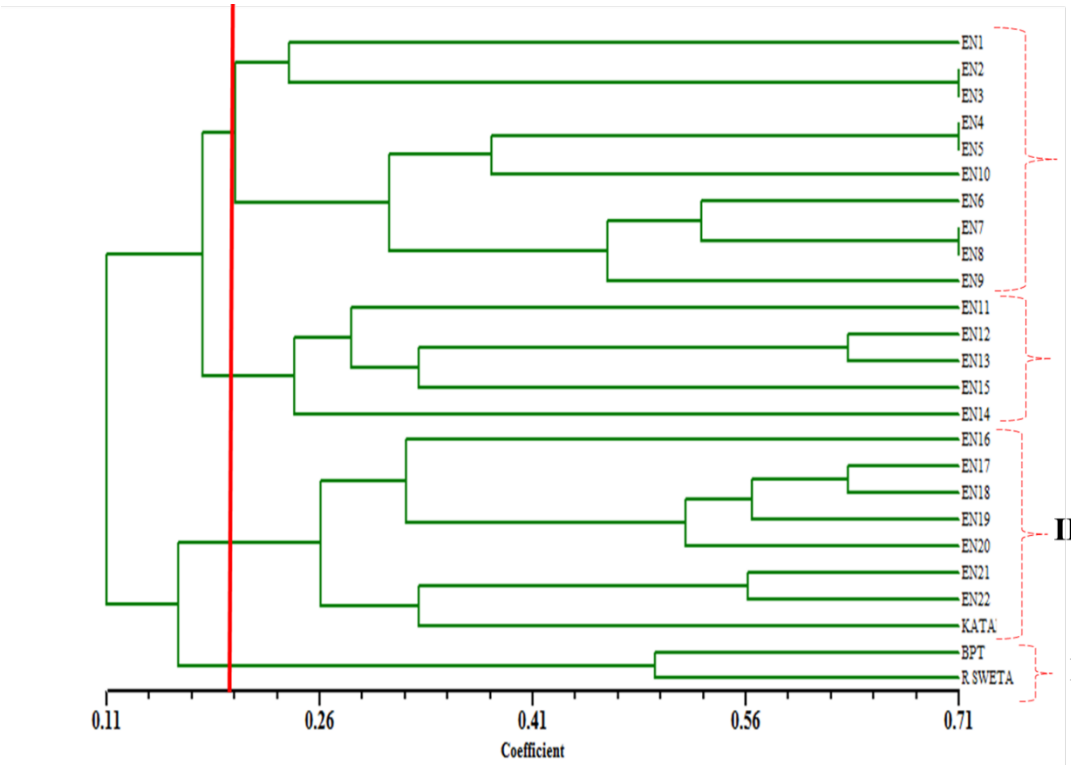


Fig. 1. Clustering of 25 rice genotypes on the basis of molecular data by UPGMA method

is associated with grain length from 8.8–10.7 mm. The gene specific marker for *GS3* as suggested by Ramkumar *et al.* (2010) was used to screen the 25 genotypes in the present study and all the genotypes were found to be amplified with the C-type allele (**Fig. 2b**) which was also in congruity with the grain length data (**Table 3**).

The main objective of the present study was to develop semi-dwarf, early maturing genotypes retaining the

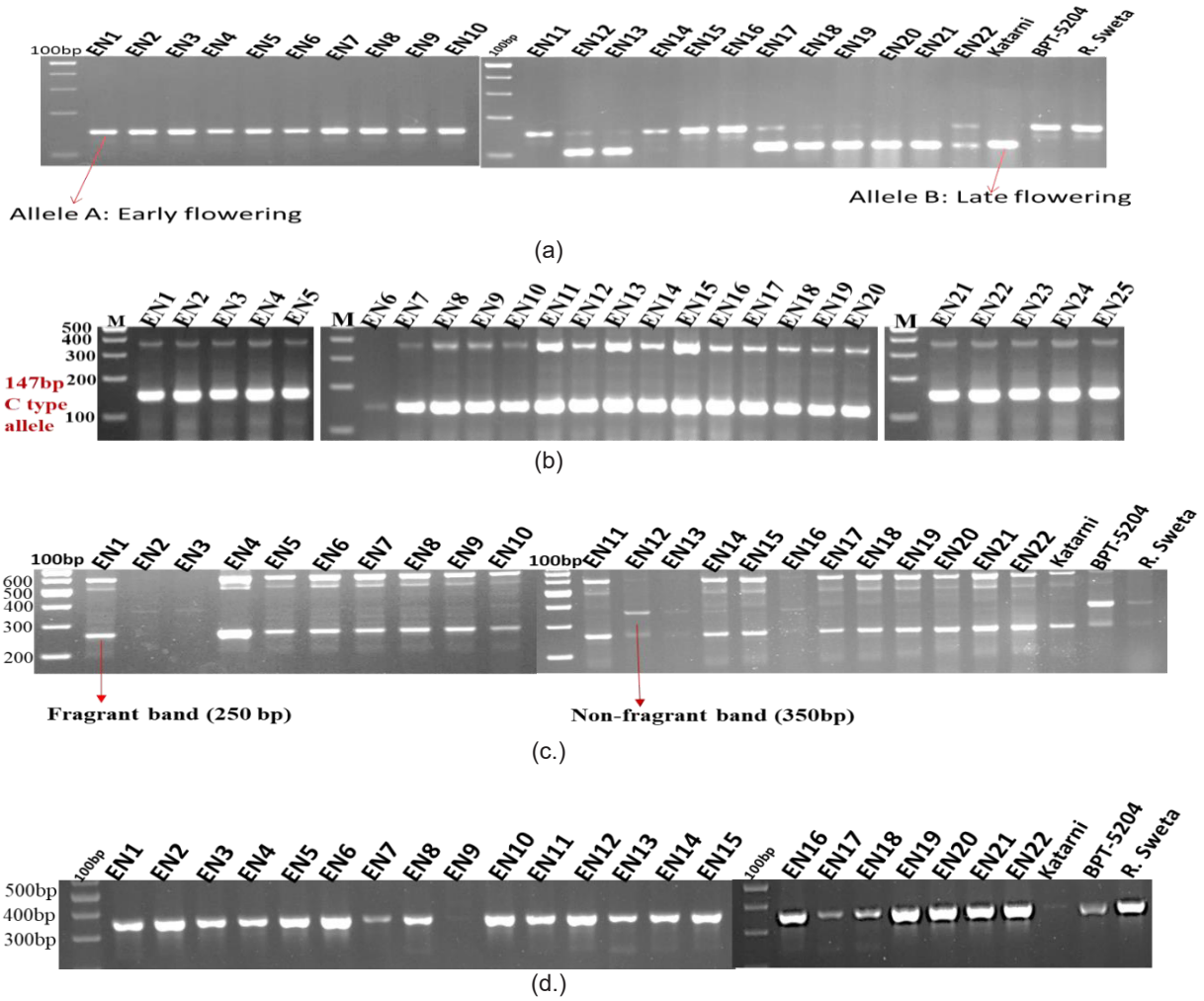


Fig. 2. (a.) Amplification with Hd3A gene-specific marker showing presence of two alleles (A and B); (b.) GS3 gene-specific marker amplification of the C-type allele (147 bp); (c.) Screening of 25 genotypes using the badh2 gene-specific marker for the fragrant and non-fragrant alleles; (d.) Amplification of the 350 bp sd1 allele in 25 genotypes

aroma of Katarni. For confirming the presence of aroma gene in the derived lines of Katarni, a *badh2* gene specific marker, located on chromosome 8 (Bradbury *et al.,* 2005) was used to screen the 25 genotypes. PCR results indicated amplification of the fragrant allele (250 bp) in all genotypes except EN2, EN3, EN13 and the non-aromatic parents BPT5204 and R. Sweta, which carried the non-fragrant allele (350 bp) (**Fig. 2c**). The semi-dwarfing gene *sd1* is located in rice on chromosome number 1 with three exons and two introns. Gene specific marker has been designed on the basis of 383 bp deletion from exon 1 to exon 2 in such a way that it gives an amplicon of 350 bp size in semi-dwarf genotypes (Spielmeyer *et al.,* 2002). All the genotypes except EN9 (161 cm) and Katarni (137 cm) had shown 350 bp amplification showing the presence of *sd1* gene and the field data on plant height which ranged from 66 to 122 cm was also in congruity with the *sd1* gene amplification pattern (**Fig. 2d**). The gene specific primer for *sd1* for semi-dwarfism, *GS3* for grain length and *badh2* for grain aroma were also used in this study and the PCR amplification pattern was in congruity with the morphological data of the genotypes. However, the *Hd3A* gene specific primer PCR profiling data was not matching with the flowering date data of the segregants, which may be due to the involvement of other important genes controlling this trait or to recombination between the Hd3A locus and the causal flowering-time gene(s) during the backcross and selfing generations. Taken together, the morphological and molecular data identified EN10 and EN15 as the most promising lines, combining semi-dwarf stature, earlier flowering, retention of the aroma allele and grain yield at par with the parental checks.

Substantial variability for agronomic and quality traits was observed among the backcross- and forward-bred

families of Katarni rice evaluated in this study. Trait-linked SSR and gene-specific (perfect) markers for *sd1*, *GS3* and *badh2* were effective in confirming introgression of semi-dwarfism, grain length and aroma into the advanced lines, and their use is recommended for accelerating selection in similar marker-assisted breeding programmes. Lines EN10 and EN15, which combined reduced plant height, early flowering, retention of the aroma allele and grain yield at par with the parental checks, are the most promising among the lines evaluated and are proposed for multi-location trials leading to varietal release as improved, semi-dwarf, early-maturing aromatic cultivars.

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