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CONTENTS

Marker assisted selection for aromatic and semi-dwarf segregants in cross of aromatic Katarni rice

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ABSTRACT: 'Katarni Rice' is the most prevalent, ceremonial and finest quality scented rice landrace of Bihar, India. Like Basmati, this aromatic rice is most preferred due to its flavour, palatability and popcorn like essence before and after cooking. However, it is low yielder (25-30 Q/ha) due to its tall and week stature and lodging tendency at the time of maturity. To overcome existing problems of Katarni rice, introgression of semi-dwarfing (*sd1*) gene from rice variety Rajendra Sweta was attempted with the help of marker assisted back crossing and present study is emphasizing on selection of dwarf and aromatic progenies in BC_1F_2 and F_3 generation of Katarni x R. Sweta.51 plants in BC_1F_2 and 31 in F_3 population were selected on the basis of 1.7% KOH sensory test for aroma. The segregation ratio of non-aromatic *vs.* aromatic plants in 325 BC_1F_2 plants was 3:1 confirming the monogenic inheritance of aroma. The selection of aromatic and semi-dwarf plants in both the population was further done through the trait specific markers for these traits in rice. The semi-dwarf plant height and early maturity of selected 49 aromatic plants in BC_1F_2 and 25 F_3 plants of Katarni x R. Sweta advocated the utilization of markers in selection of desirable segregants.

Key words: *Badh2*, Katarni, marker assisted breeding, rice, *sd1*

Rice (*Oryza sativa* L.) is a major food grain crop for the two third of the population in the world. Rice including basmati and non- basmati occupy major share in India's total cereal export. Aroma in rice is considered as one of the most important quality traits, for consumer acceptance. The popcorn like smell, soft texture and palatability of aromatic rice makes it a premium quality product in the market (Bradburry, 2009). Consumers nowadays have become more conscious of the quality of the rice that they consume and often prefer fragrant rice due to their characteristics and pleasant odour. The accumulation of 2-acetyl-1-pyrroline in aromatic rice genotype may be explained by the presence of mutation resulting in a loss of function of the *fgr* gene product (Hashemi *et al.,* 2013). The sensory test of leaf tissue and grain after reacting them with solution of 1.7% (w/v) KOH are used for aroma test. However, this method is not reliable as the test may be biased due to saturation of nostril sense at different level. Semi- dwarf stature of rice is valuable because these cultivars are not only more resistant to lodging but they also respond to fertilizer application. Gibberellic acid is a key hormone for height in plants. Some dwarf mutants like the GA- insensitive mutant *Rht* (Reduced height) and the GA-Sensitive *sd1* (semi-dwarf), were exploited by breeders during the "green revolution" in the 1960s and 1970s for the production of semi-dwarf varieties (Ashikari *et al.*, 2002).

Bihar state of India is very rich in genetic resources of rice including aromatic rice varieties. Katarni is one of the famous fine aromatic landrace of rice in this region which is renowned for its special aroma, unique medium fine grain and excellent cooking qualities. It is in high demand throughout the year but, is poor yielder (25-30 t/ha), and prone to lodging due to its tall and weak stature (140 to 160 cm in height) (Jha and Sinha, 2014). Lodging causes considerable yield losses and a reduction in grain quality. Earlier attempts to reduce its plant height using conventional breeding methods failed owing to loss of its unique quality characters. So, isolation and exploitation of dwarfness in Katarni without deterioration of its exquisite quality parameters will help in increasing area, production and return under its cultivation. Further, the improved, early maturing photo insensitive Katarni rice cultivar may fit into the multiple cropping systems. Therefore, in the present study, an attempt has been made to identify the photo insensitive, dwarf and aromatic progenies in the segregating population by taking the advantage of marker assisted selection in F_3 and BC_1F_2 populations.

MATERIALS AND METHODS

In the present marker assisted breeding programme, a rice variety, Rajendra Sweta was used as the donor for semi-dwarfness and early maturity. The recipient parent Katarni is an aromatic land race of rice which is tall, late maturing and lodging prone with fine grain and excellent cooking qualities. In year 2016, sowing of total 8000 BC_1F_2 plants and 4500 F_3 plants was done in the raised nursery beds. Morphological observations were taken on plant height (cm), days to 50% flowering, panicle length (cm), flag leaf length (cm) and grain length/breadth ratio. The strength of aroma was scored in 0-3 scale where, 0 denotes no aroma, 1 denotes slight aroma, 2 denotes moderate aroma and 3 denotes strong aroma. Sensory test for aroma was performed in leaf by 1.7% (w/v) KOH solution as a sensory test at room temperature (Sood and Siddiq, 1978). For knowing the inheritance of aroma gene, about 325 BC_1F_2 seedlings obtained from single seed descent method was transplanted separately with a spacing of 20 x 25 cm for morphological observations. For statistical analysis, the data were classified based on the class interval and chi-square test was done for goodness of fit in the segregating population. The segregating populations were distributed according to their frequency and class interval. Marker assisted selection for aroma and semi-dwarfness in BC_1F_2 and F_3 plants was done with a set of four gene specific primers as suggested by Bradbury *et al.* (2005) for badh2 and sd1 gene specific primer as suggested by Spielmeyer *et al.* (2002), respectively.These primers for aroma gene have been designed on the basis of 8 bp deletion of the 7th exon of wild type *Badh2* loci in such a way that it amplifies a 257 bp and 355 bp products in aromatic and non-aromatic genotype, respectively. A common band of 580 bp size was amplified in both genotypes as control. The sequence of the primer for *sd1* gene was based on a 383 bp deletion in *GA20ox* gene which results in the truncation of the penultimate enzyme GA20 oxidase which has role in bioactive gibberellic acid synthesis (Ashikari *et al.*, 2002).

Genomic DNA from individual plants was extracted using rapid DNA isolation method (Kumar *et al.,* 2017). DNA amplification was carried out in automated thermal cycler (Applied Biosystems model Veriti) using 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 PCR pre-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 58°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.

RESULTS AND DISCUSSION

40% plants had 0 score i.e. no aroma for leaf aroma in BC_1F_2 population, 37% plants had 1 score i.e. slight aroma, 20% plants had 2 score i.e. moderate aroma and only 3% plants had 3 score with strong aroma in 1.7% w/v potassium hydroxide (KOH) solution. The positive control Katarni and negative control Rajendra Sweta scored 3 and 0, respectively. Chi-square test in BC_1F_2 population for sensory score in leaf was conducted to test the goodness of fit by comparing observed and expected frequency of aroma in leaves in each plant. The goodness of fit with

Table1: Screening of BC_1F_2 and F_3 plants for selection of semi-dwarf and aromatic segregants

Sl. No.	Particulars	BC_1F_2 population	F_3 population
1.	Plants in population	Approx. 8000	Approx. 4500
2.	Selection on the basis of plant height and flowering time in the field	90	74
3.	Selection on the basis of 1.7% KOH sensory test for aroma	51	31
4.	Selection on the basis of PCR amplification of *badh2* gene specific primer	50	25
5.	Total number of Plants selected on the basis of foreground selection of *badh2* and *sd1* gene	49	25

Table 2: Morphological features and *sd1* and *badh2* gene response of selected BC_1F_2 and F_3 plants of Katarni x R. Sweta

Generation/Parents	BC_1F_2		F_3		Katarni	R. Sweta
Number of plants	49		25			
badh2	FB		FB		FB	NF
sd1	+		+		-	+
Traits	Mean	Range	Mean	Range		
DOF	113.5±7.1	102-144	116.5±4.8	97-123	124	105
PH	99.0±6.6	85.7-124	83.31±7.5	64.8-96.0	155	95
PL	32.8±8.6	20.5-42.5	20.3±2.05	16.25-26.50	21	18.5
FLL	25.2±6.8	13-35.5	25.72±6.3	16.5-44.0	32.5	23.5
L/B ratio	3.3±0.37	2.6-4.14	3.28±0.4	2.42-4.14	3.5	3.05

Note: DOF: Days to 50% flowering, PH: Plant Height (cm), PL: Panicle length (cm), FLL: Flag leaf length (cm), L/B ratio: Length/Breadth ratio, FB: Fragrant band, NF: Non-fragrant band

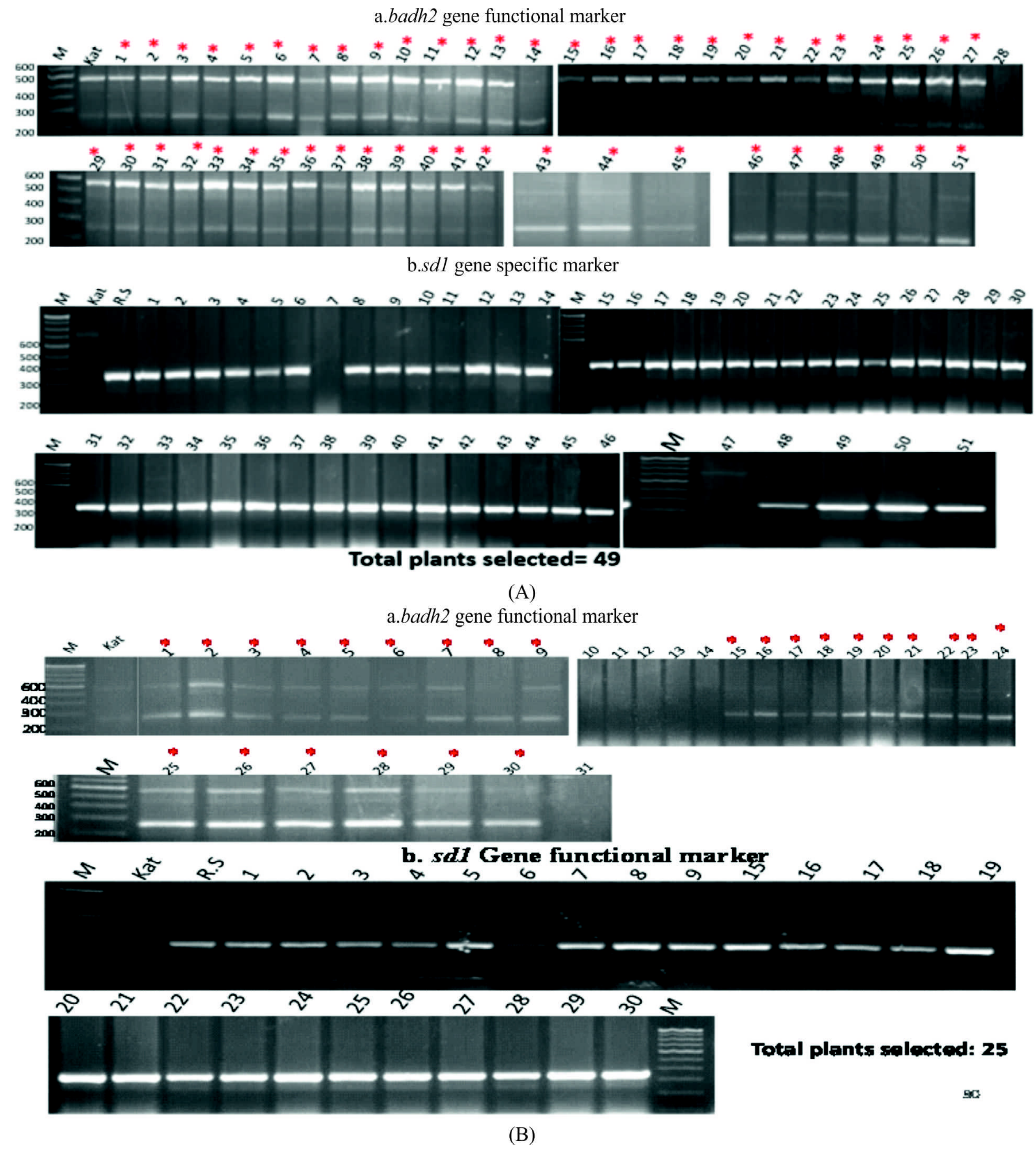

Figure 1: Markers assisted selection in (A) BC_1F_2 and (B) F_3 plants through PCR amplification of gene specific primers for *badh2* and *sd1* genes

chi-square (χ^2) test for two gene model showed the expected segregation ratio of 3:1 (at p< 0.05 and p<0.01; where 'p' is the probability that deviation of the observed frequency from the expected frequency is due to the chance alone).

Functional markers were used for the foreground selection of dwarf and aromatic plants in BC_1F_2 population because of the reduced risk of false selection due to recombination between the molecular markers and the gene of interest. Recurrent parent Katarni showed the amplification of 257 bp product of fragrant allele while R. Sweta had amplification of non-fragrant allele of 355bp (Figure1).The summary of selection based on morphological and molecular data in BC_1F_2 and F_3 population has been given in Table 1. Out of approximately 8000 plants in BC_1F_2 andof approximately 4500 plants in F_3, 90 and 74 plants respectively were selected on the basis of semi-dwarf early maturing phenotype and 51 BC_1F_2 and 31 plants, respectively were further identified on the basis of 2 to 3 aroma score in 1.7% KOH sensory test. Among the selected BC_1F_2 and F_3 plants, 50 and 31 plants, respectively showed the presence of fragrant band (257 bp) while rest showed the presence of non-fragrant band (355 bp).The fragrance gene (badh2) positive plants were further screened for the presence of semi-dwarfing gene sd1. 49 plants in BC_1F_2 and 25 plants in F_3 population had presence of approximately 350bp for *sd1* gene (Figure 2).Tall parent had no amplification of sd1 gene and hence the segregants showing the same amplification pattern of Katarni were rejected from the population. Morphological data was taken on the selected *badh2* and *sd1*gene positive plants in BC_1F_2 and F_3 population. Table 2 shows morphological features along with *sd1* and *badh2* gene response of selected BC_1F_2 and F_3 plants of Katarni x R. Sweta.

Panicle length and flag leaf length in BC_1F_2 population had average value of 32.8±8.6cm and 25.2±6.8cm, respectively while it was 20.3±2.05cm and 25.72±6.3cm, respectively in F_3 population. The average L/B ratio in BC_1F_2 and F_3 was 3.3±0.37 and 3.28±0.4, respectively. The morphological data clearly indicated a reduction in plant height and days to 50% flowering of selected aromatic plants in BC_1F_2 and F_3 population with respect to Katarni. The average plant height of BC_1F_2 and F_3 population was 99±6.6cm and 83.31±7.5cm, respectively which was comparable to the plant height of donor plant R. Sweta i.e. 95cm.

Katarni is being grown since ancient times in few blocks of Bhagalpur, Banka and Munger districts of Bihar and due to its territorial specific uniqueness, it has been granted geographical indication tag (Geographical Indication no. 553; Certificate number: 312) by the office of Intellectual Properties Rights, New Delhi, Govt. of India (Kumar *et al*., 2018).However, traditional Katarni is poor yielder (25-30 t/ha), weak strawed, tall and very late maturing. In present study, sd1 gene from rice variety R. Sweta was introgressed in Katarni with the help of marker assisted back crossing (MABC) to isolate the semi-dwarf, early and aromatic lines on the basis of morphological and molecular observations.In a similar study, Srivastava *et al.* (2019)attempted the introgression of semi-dwarfing gene (*sd1*) from rice variety CSR-10 with the help of marker assisted breeding for improving the aromatic, traditional, tall Kalanamak rice variety and assessment was conducted for *sd1* gene and for the presence of aroma gene with the help of *badh2* derived primer.

The performance of selected plants of BC_1F_2 and F_3 in Katarni x R. Sweta cross with respect to their parents indicated effectiveness of selection for aroma and plant height in desirable range. Average plant height was 80 to 90cm which was nearer to the plant height of donor parent R. Sweta. Earliness of a genotype in rice is a desirable trait; however aroma development requires a particular day length and climatic conditions. Mean value of days to 50% flowering was 113-116 days in both the population hence were desirable for development of dwarf and early aromatic Katarni rice.Presence of considerable variation of entries for each trait may be due to mortality of the backcross and F_3 generation and the remaining homozygosity can be achieved in successive selfing of the backcross progenies. The segregation ratio of non-aromatic to aromatic plants was 3:1 which confirmed the monogenic inheritance of aroma and controlled by a recessive gene. The inheritance pattern of aroma in rice has been reported in several crosses of non-aromatic and aromatic varieties and aroma in rice is to found to be genetically controlled as monogenic recessive (Sood and Siddiq, 1978) trait. The segregation ratio of non-aromatic to aromatic plants was 3:1 in F_2 plants which confirmed the monogenic inheritance of aroma. The recent inheritance studies on aroma in rice by Patil *et al.*(2012) also reported the monogenic inheritance of aroma in rice.

CONCLUSION

Since aroma and semi-dwarfism in rice is mainly controlled by recessive genes, screening of the individuals in offspring population at the seedling stage for their presence was difficult. The most common KOH sensory method to detect the aroma in rice relies mainly on human sense which is poor in accuracy (Peng *et al.*, 2018). In the present study, the functional molecular markers of aroma and semi-dwarfism gene had provided a convenient way to screen the progenies in the backcross generation, increase the accuracy and reduced the duration of the breeding process to improve the traditional Katarni rice. The identified lines will be evaluated for yield and various agronomic traits in multilocation trials.

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REFERENCES

Ashikari, M., Sasaki, A., Ueguchi-Tanaka,M., Itoh, H., Nishimura, A., Datta, S., Ishiyama, K., Saito,T., Kobayashi, M., Khush, G.S., Kitano, H. and Matsuoka, M. (2002). Loss-of-function of a Rice Gibberellin Biosynthetic Gene, *GA20 oxidase* (*GA20ox-2*), Led to the Rice 'Green Revolution'. *Breeding Sci.,* 52: 143–150. 10.1270/jsbbs.52.143.

Bradbury, L.M.T., Fitzgerald T.L., Henry R.J., Jin Q. and Waters D.L.E. (2005). The gene for fragrance in rice. *Plant Biotech. J.,* 3: 363–370.

Bradbury, L.M.T. (2009). Identification of the gene responsible for fragrance in rice and characterization of the enzyme transcribed from this gene and its homologs. Ph.D. thesis, Southern Cross University, Lismore, Australia,Pp-11.

Hashemi, F.S.G., Rafii, M.Y., Ismail, M.R., Mahmud, T. M. M., Rahim, H. A., Asfaliza, R., Malek, M. A. and Latif, M. A. (2013). Biochemical, Genetic and Molecular Advances of Fragrance Characteristics in Rice.*CRC Crit Rev Plant Sci.,* 32: 445–457.

Jha, B.K. and Sinha, R.K. (2014). A study of diversification of Katarni to HYV Paddy in Eastern India.Book Chapter India Studies in Business and Economics,Pp. 89-95. Sprigerlink.

Kumar, M., Satyendra, Singh, S.P., Kumar, A., Shashikant, Singh, P.K. and Sohane, R.K. (2018).Characterization of Katarni rice for its Geographical Indication in Bihar, *Indian J. AgriSearch,* 5(4):223–229.

Kumar, V., Prasad, A., Roy, C. and Chattopadhyay, T. (2017).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. App. Sci.,* 7(8): 2562–2566.

Patil, K.G., Nagesh, Patil, V.G., Kulkarni, R.S. and Shashidhar, H.E. (2012).Inheritance of aroma in aromatic rice (*Oryza sativa* L.) genotypes. *Ann. Biol. Res.,* 3(12): 5472–5474.

Peng,B., Zuo, Y.H., Hao, Y.L., Peng, J., Kong,D.Y.,Peng,Y., Nassiron,T.Y., He,L.L., Sun,Y.F., Liu, L., Pang,R.H.,Chen,Y.X.,Li,J.T.,Zhou,Q.Y., Duan,B., Song,X.H., Song,S.Z. and Yuan, H.Y.(2018).Studies on aroma gene and its application in Rice Genetics and Breeding.*J. Plant. Stud.,* 7(2):29–40.

Sood, B.G. and Siddiq, E.A. (1978). A rapid technique for scent determination in rice. *Indian J. Genet. PlantBreed.,*38: 268–271.

Spielmeyer, W., Ellis, M.H. and Chandler, P.M.(2002). Semidwarf (*sd-1*), ''green revolution'' rice, contains a defective gibberellin 20-oxidase gene. *Proc. Natl. Acad. Sci.* USA., 99(13):9043–8.

Srivastava, D., Shamim, M., Mishra, A., Yadav, P., Kumar, D., Pandey, P., Khan, N.A. and Singh, K.N.(2019).Introgression of semi-dwarf gene in Kalanamak rice using marker-assisted selection breeding. *Curr. Sci.,* 116:4–10.

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