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Short Communication

Marker Assisted Selection for Semi-Dwarf Trait in F_2 population of *Basmati* and Red Rice

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Rice is a staple food crop for more than half of the world's population. India is the largest producer of rice with an area of 47.88 million hectares and total production of 137.83/million tons (https://www.agriwelfare.gov.in/). In Himachal Pradesh, it was cultivated in 68.46 thousand hectares with production of 138.46 thousand tonnes (https://agriculture.hp.gov.in/en/production-2/). HPR 2795 is a high yielding red rice variety but it is tall and highly prone to lodging, however, semi-dwarf varieties can with stand lodging due to their short height. The present study was undertaken to develop plant types combining the desirable traits of PB 3 (Basmati type i.e. long aromatic grains and dwarfness) and HPR 2795 (red coloured grain). The semi-dwarfing rice gene (*sd-1*) has been deployed in modern rice breeding. It is a recessive trait which results in shortened culm and leads to improved lodging resistance and higher harvest index, and allows increased use of nitrogen fertilizers (Walcott and Laing, 1976). The *sd-1* gene was first identified in the Chinese variety Dee-geo-woo-gen (DGWG), and was crossed in the early 1960s with variety Peta (tall) to develop the semi-dwarf cultivar IR8, which produced record yields throughout Asia and became the basis for the development of new high-yielding, semi-dwarf plant types (IRRI, Annual report 1966). Spielmeyer *et al.* (2002) reported that the semi-dwarf (*sd-1*) "green revolution" rice phenotype is a result of a deficiency of active GAs in the elongating stem arising from the defective 20-oxidase GA biosynthetic enzyme. The combined phenotypic selection for semi-dwarf plant type and red cotyledon colour is difficult in F_2 population through pericarp breeding approach. In contrast, the use of molecular markers provide precise and robust selection. Keeping this in view, the present study was undertaken to select plants with red seed colour and semi-dwarf plant types based on morphological and marker assisted selection.

The F_1 seed was produced by crossing varieties PB 3 and HPR 2795 during *Kharif* 2019 followed by selfing to produce F_2 seed. The F_2 seeds were grown in nursery, followed by their transplanting with the row to row and plant to plant spacing of 20x15 cm, respectively following recommended package and practices. Genomic DNA was

isolated from young leaf tissue of each F_2 plant following CTAB method as described by Murray and Thompson (1980). PCR was performed by deploying selected primers using gene specific marker for *sd1* gene with the sequence of 'F: *5'CACGCACGGGTTCTTCCAGGTG 3'* and R: *5'AGGAGAATAGGAGATGGTTTACC*3'. Scoring of alleles for homozygous dwarf, heterozygous and homozygous plants was done. Association between marker, genotype and plant height was established.

A population of 165 F_2 plants were grown in the field which showed typical one gene segregation for plant height. Out of these 55 promising F_2 plants along with parents were used for molecular analysis. Information on the presence or absence of the molecular band amplified with the *sd1* primer is given in Table 1 and Fig 1. HPR 2795 showed no amplification with the *sd1* gene specific primer whereas, PB 3 exhibited clear amplification. The plant height of HPR 2795 ranged from 115-125 cm with red grain pericarp colour, while in PB 3, it ranged from 99-105 cm with white grains. The molecular analysis showed 300 bp band size in HPR 2795 and 280 bp in PB 3 on 2% agarose gel. Out of 55 F_2 plants, 47 plants were *sd1* positive, while the remaining plants were *sd1* negative. The presence of the positive *sd1* allele in the segregating progenies confirmed the introgression of the gene *sd1* from PB 3.

Among the 47 $F_{2,}$ plants with white seed colour and positive for *sd1*, 6 plants numbered as P4, P6, P11, P24, P36 and P41 exhibited plant height >100cm whereas, in 21 plants numbered P8, P 13, P16, P18, P21, P23, P25, P29, P32, P33, P35, P40, P48, P49 and P51 plant height varied from 101- 110cm. (Table 1). Among the 47 F_2 plants, 26 plants exhibited red pericarp and were positive for the semi-dwarfing gene *sd1*. Out of these 26 plants, 5 plants numbered as P15, P19, P26, P 39 and P55 were having red coloured grains and their height was >100 cm. The plants numbered as P2, P9, P10, P12, P14, P17, P20, P22, P28, P34, P37, P38, P42, P43, P44, P45, P46, P50, P52, P53 and P 54 were red seeded and their plant height ranged from 101-110 cm. Reduced height of these plants indicated that these plants may carry *sd1* allele.

These findings are consistent with earlier studies of Kumari *et al.* (2020) who successfully used marker-assisted selection (MAS) to screen aromatic, semi-dwarf, and photo sensitive genes in an F_6 population of the cross Katrani × Rajendra Sweta. Similarly, Srivastava *et al.* (2019) reported introgressing the *sd1* gene from donor CSR10 to develop semi-dwarf backcross derivatives using MAS. Raina *et al.* (2019) also employed marker-assisted backcross breeding to introgress *sd1* gene along with bacterial blight resistance genes (*Xa21* and *Xa13*) into a tall, lodging-prone *Basmati* variety, highlighting the utility of MAS in rice improvement.

Table 1: Information on F_2 plants showing presence and absence of *sd1* gene band and plants with red seed coat and white seed coat colour in cross HPR-2795 x PB-3

***sd1* gene specific bands**					
Present (less height) with				**Absent (more height)**	
White pericarp		**Red pericarp**		**White pericarp**	**Red pericarp**
Plant height .>100 cm	Plant height 101-110 cm	Plant height >100 cm	Plant height 101-110 cm	Plant height 111-130 cm	Plant height 111-130 cm
P4, P6, P11, P24, P36 and P41	P8, P13, P16, P18, P21, P23, P25, P29, P32, P33, P35, P40, P48, P49 and P51	P15, P19, P26, P39 and P55	P2, P9, P10, P12, P14, P17, P20, P22, P28, P-34, P37, P38, P42, P43, P44, P45, P46, P50, P52, P53 and P 54	P1, P3, P7, P27, P31 and P47	P5 and P30

Based on desirable plant height, seed colour and molecular analysis, 5 F_2 homozygous plants, numbered P15, P19, P26, P39 and P55 possessing *sd1* allele along with red pericarp were advanced to develop dwarf lines with red pericarp and basmati characteristics.

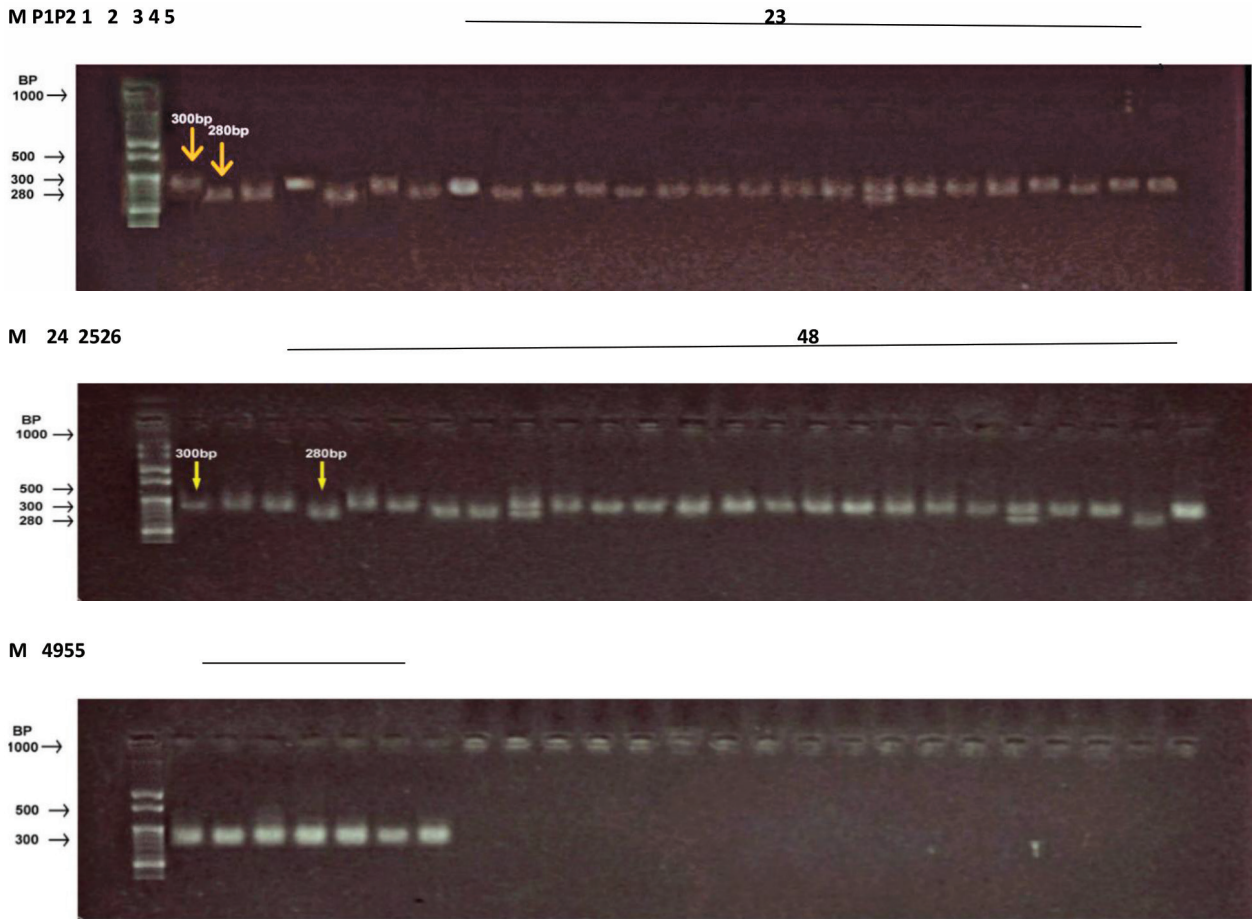

Fig 1: A representative gel depicting the F_2 plants of cross PB 3 X HPR 2795 with *SD1* gene-derived SSR marker *sd1*. P1=Punjab Basmati 3 (*sd1*); P2 = HPR- 2795.

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Authors' Contributions

AV, DB, AKB, KT conceptualized and executed the research work, compiled and edited the manuscript; SV, AM, OP helped in the statistical analysis of the findings and in editing of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

The article doesn't contain any study involving ethical approval.

Use of Generative AI or AI assisted technologies

Authors declare that no Generative AI or AI assisted technologies have been used inpreparation of this manuscript.

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