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Marker assisted selection for aromatic and submergence tolerant rice genotypes for Tripura

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ABSTRACT

Screening for submergence tolerance and aroma in selected rice varieties of Tripura and their segregants were done both phenotypically and using linked molecular markers. Allelic specific amplification using primers derived from putative BAD2 gene for aroma could identify parents and two heterozygous lines derived from Basmati 370 x Pyzum and three homozygous aromatic lines from Pyzum x Binni cross. SSR marker, RM219 linked to Sub-1, could identify one submergence tolerant line derived from Basmati 370 x Pyzum. Although no aromatic line was found submergence tolerant in the current investigation, but the consistency of this screening process, would help in selecting aromatic lines with submergence tolerance from hybridization program involving traditional varieties of Tripura.

Key words:*: rice, submergence tolerance, aroma, molecular marker*

As a North-eastern state of India, Tripura possesses many diversified valuable rice germplasm, kept unused in the breeding programme. There are many indigenous aromatic land races in Tripura (Sardana, 1997). Some parts of Tripura suffer flashflood for a sapm of 10-15 days. Substantial level of submergence tolerance would be beneficial for sustainable production of quality rice in these regions. Pyzum and Binni are among the many indigenous popular varieties grown by tribal farmers of Tripura. Pyzum has moderate yield potentiality, moderate level of submergence tolerance and high bran content. Binni has high aroma and medium long sticky grain. This is mainly used for making *petha* (sweet) by the tribal farmers. Fragrance of rice is controlled by a recessive gene associated with increased level of 2-acetyl-1-pyrroline. This is due to a blockage in a biochemical pathway caused by the presence of a non-functional betaine aldehyde dehydrogenase 2 (BAD2) enzyme, resulted from a 8bp deletion in a gene (*fgr*) present on chromosome 8 (Bradbury *et al*., 2005). Submergence tolerance was also observed to be controlled mainly by a single locus, designated by *Sub1* present on chromosome 9 (Xu *et al*., 2004). In the present investigation, both phenotypic and established marker based selection for aroma and submergence tolerance was done in high yielding segregants from crosses involving indigenous varieties from North-eastern India with view for process could be put into forward for concurrent selection for both the traits.

56 F_3 segregants from crosses of Basmati 370 x Pyzum and 41 F_3 segregants from crosses of Pyzum x Binni along with their parents were evaluated for seed yield, aroma and submergence tolerance in wet season of 2006. Twelve segregants of Basmati 370 x Pyzum and 3 segregants from Pyzum x Binni cross were selected finally on the basis of high seed yield as compared to other lines. The average plant yield of selected Basmati 370 x Pyzum cross was 5.5 g while average yield of selected lines of Pyzum x Binni cross was 4.3 g. Aroma was scored (0-2 scale) from 10 F_4 seeds individually from each line following the method described by Berner and Hoff (1986). Screening for submergence was done under controlled condition at the Rice Reserach Station, Chinsurah, West Bengal, followed by the standard procedure (Xu and Mackill,

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1996) with minor modifications. Ten days after transplanting, seedlings were submerged in more than 30 cm water depth for 10 days. After 7 days of removal of water plants were identified as those, survived (1) or not survived (0). In the present study for detection of aromatic lines, a single tube Allele Specific Amplification (ASA) was done by using four primers designed (Bradbury *et al*., 2005) from a putative *BAD2* gene. Another SSR primer, RM219 was employed which was found linked to *Sub1* by 3.4cM (Xu *et al*., 2004) for screening submergence tolerance lines. DNA was extracted from 1g tender leaves of single plant of parents and their F_3 crosses by using the standard method (Dellaporta *et al.*, 1983). Extracted DNA samples were stored at -20°C. DNA samples extracted from the lines selected on the basis of high yield have only been used for PCR amplification. The 25 ìl PCR reaction mixture contained: 4 ìl 2.5 mM dNTPs, 1 ìl of 20 ng DNA in 2.5 ìl 10x *Taq* polymerase assay buffer, 1.5 ìl MgCl2 and 1.5 U *Taq* polymerase enzyme (Bangalore Genei Pvt Ltd., India). For SSR reaction, 1 ìl each of forward and reverse primers while for ASA, 0.5 ìl each of four primers of 10 pmol were added with each reaction mixture. PCR products were analyzed on a 2.5% low melting high resolution agarose gel (Sigma) in 1X TBE buffer and bands were visualized by ethidium bromide staining (0.5 ìg/ml).

A single tube allele specific amplification showed that Basmati 370 and Binni were having a 257bp fragment while non-aromatic Pyzum showed a 355bp fragment. A 580bp fragment was present in all the lines, considered as indicator of successful PCR reaction. Two segregants (line 2 and line 3) of Basmati 370 x Pyzum were carrying both the bands (257bp and 355bp) and detected as heterozygote for that trait. Some seeds of line 2 and line 3 of Basmati 370 x Pyzum cross were found aromatic (score 1) and some of them were non-aromatic (score 0). Non-aromatic genotypes were found homozygous with only one allele (355bp). Three segregants belonging to Pyzum x Binni cross were carrying 257bp amplicon. The scale value of aroma in F_4 grains showed the linearity with marker as expected. RM219 amplified a 230bp fragment from two susceptible aromatic parents (with high mortality) but amplified a 204bp fragment from the tolerant parent, Pyzum (Fig 1). Except one (line 6) none of the segregating line from Basmati 370 x Pyzum contained 204 bp fragment as tolerant parent. This line was

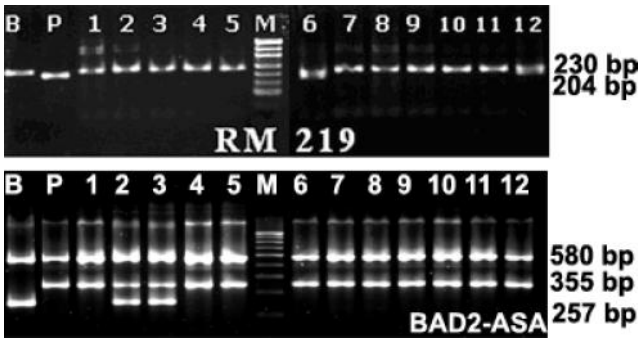

Fig. 1. SSR profile obtained by RM219 linked with submergence tolerance (*sub1*) and a single tube Allele Specific Amplification with primers derived from BAD2 gene for detection of aroma (*fgr*) in rice varieties and their crosses, viz., B: Basmati-370; P: Pyzum, 1-12: Segregants of Basmati370/Pyzum and M: 50 bp DNA ladder for RM219 and 100 bp DNA ladder for BAD2-ASA.

tolerant to submergence stress. On the other hand all the aromatic lines of Pyzum x Binni cross were found susceptible to submergence stress. All these lines carried 230bp fragment.

In the present investigation BAD2-ASA could differential aromatic and non-aromatic rice genotypes. On the other hand, although RM 219 was found polymorphic, the specific allele (204bp) was found co-segregating with tolerant genotypes in earlier studies (Xu *et al.*, 2004). In the present study also the low mortality (<20%) of Pyzum under submergence was well substantiated by the presence of the 204bp allele indicating submergence tolerance. No genotype was identified carrying both aroma and submergence tolerance alleles. But these markers would provide the necessary tools for introgression of important genomic regions for the development of submergence tolerance cultivar with Basmati type long grain and strong aroma of local landraces.

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