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Short Communication

Molecular mapping of a fertility restorer gene in basmati rice using microsatellite markers

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At the Indian Agricultural Research Institute, New Delhi work is underway on the development of rice hybrids having basmati quality. One of the major limitations faced so far while developing basmati quality rice hybrids was the non-availability of perfect restorers in basmati germplasm. In order to overcome this, selective inter-mating was followed in F_2 and subsequent generations of partially restored basmati quality F_1 hybrids with intensive selection for enhanced restoration. This approach of restorer breeding led to the development of several promising iso-cytoplasmic restorers combining basmati quality [1]. These restorers are presently being used for the development of basmati quality rice hybrids. But the lack of morphological markers that help early detection of the potential plants possessing the fertility restorer gene, makes the breeding process inefficient and equally intense only at anthesis stage. Molecular tagging of fertility restorer gene in these restorer lines would help in the transfer of the restorer gene(s) to desirable agronomic background without involving a sterile cytoplasm or extensive test crossing, development of restorers with normal cytoplasm, pyramiding of restorer genes, selection for restorer genes independent of environmental influence on restoration, identification of plants with restorer genes at early seedling stage by employing the marker assisted selection to augment backcross breeding. Ultimately the identification of closely linked markers would help in map based cloning of fertility restorer gene to understand the molecular and biochemical nature of fertility restorer gene.

In the present paper, we report the identification of a microsatellite marker linked to the fertility restorer gene in a basmati restorer line Pusa Rice Restorer 78 (PRR-78) developed at Division of Genetics, IARI, New Delhi. The line PRR-78 is an effective restorer of WA (wild abortive) cytoplasm. An F_2 mapping population was generated by crossing IR 58025A, a CMS (cytoplasmic male sterile) line carrying WA cytoplasm with PRR- 78. A total of 235 plants were characterized for pollen fertility following the procedure described earlier [2].

DNA isolated from 10 fully fertile (pollen fertility > 90%) and 10 fully sterile (pollen fertility 0%) plants was used for constituting the fertile and sterile bulks for bulked segregant analysis [3]. Forty-two rice microsatellite (RM) markers selected from different chromosomes (at least two from each chromosome) were used for parental polymorphism survey. All microsatellite markers located on the chromosome 10 as reported earlier [4], were included in the analysis since this chromosome is reported to harbour restorer genes in non-basmati sources [5, 6]. Out of forty-two markers studied, ten were found polymorphic between the parents. Four of the markers namely RM 263 on chromosome 2 and RM 216, RM 258 and RM 228 on chromosome 10, differentiated the bulks in the bulked segregant analysis (BSA). Genotyping of 100 F_2 individual plants was carried out using these markers. The data obtained from individual plants using the marker RM 263 did not confirm the BSA result. While high degree of segregation distortion was observed with respect to RM 228 locus, however, the markers RM 216 and RM 258 showed independent segregation with respect to RM 228 indicating that this segregation distortion at RM 228 locus did not have any influence on segregation at RM 216 and RM 258 loci. The marker RM 216 ,mapped at a distance of 30.4 cM from the marker locus RM 258 with a LOD score of 4.41. RM 258, which clearly differentiated the bulks (Fig. 1) mapped at a distance of 9.5 cM from the restorer locus with a LOD score of 11.23. Based on

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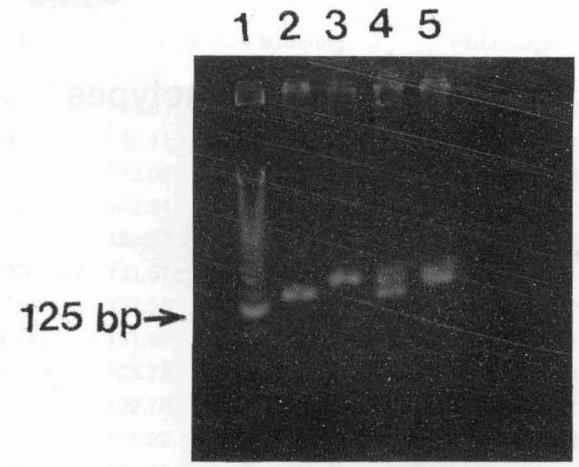

Fig. 1. A rice microsatellite marker RM 258 identified to be linked with fertility restorer gene in PRR 78 using bulked segregant analysis. DNA marker (lane 1), restorer line PRR 78 (lane 2), CMS line IR 58025A (lane 3), fertile bulk showing heterozygous pattern (lane 4) and sterile bulk showing homozygous pattern for IR 58025A allele (lane 5).

the results obtained with RM 258, two additional microsatellite markers RM 294A and RM 171 [7] expected to be closer to the fertility restorer locus on chromosome 10 were used for survey of parental polymorphism but were found monomorphic. No cleaved amplified polymorphic sequence (CAPS) could be generated even on the restriction of amplified fragments from fertile and sterile parents with three tetra cutter enzymes i.e. *Hae* III, *Sau*3AI and *Taq*I and one rare cutter enzyme *Hind* III

In addition, 5 RAPD primers i.e. OPB07, 0PB18, OPK05, OPU10 and OPW01 reported to be linked to fertility restorer gene(s) is non- basmati sources were also screened to study the parental polymorphism, Monomorphic amplification was observed with 3 primers OPB07, OPU10 and OPW01 while the remaining two OPB18, OPK05 did not show any amplification.

Further, the end sequence information of two RFLP markers G 2155 [5] and C1361 [8] reported to be linked to fertility restorer gene *Rf*-1 in other sources were used to develop the PCR based markers by designing forward and reverse primers of 19 and 25 bases respectively for RFLP clone G 2155 and of 20 and 22 bases respectively for RFLP clone C 1361. G 2155 based primer pairs amplified a monomorphic fragment of 200 bp, while no amplification was obtained with C1361 based primer pairs. Restriction digestion of the fragments amplified in fertile and sterile parents by the G 2155 based primer pair with enzymes *Hae*III, *Sau*3A, Taq1 and *Hind*III did not yield any CAPS. It is proposed that while the microsatellite marker RM 258 can be employed for the detection of the fertility restorer gene in the sister lines or related pedigree lines in basmati rice hybrid development programme, single nucleotide polymorphism (SNPs) which can be generated between the fragments amplified in fertile and sterile parents by RM 294A, RM 171 and G 2155 based PCR primers might be useful in designing a new set of primers, to identify more molecular markers. Currently, the efficacy of the marker RM 258 is being validated across a set of known restorers and non-restorer lines of both basmati and non basmati origin, to test its utility in identifying the restorer lines.

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