15. Identification and mapping of tightly linked SSR marker for aroma trait for use in marker assisted selection in rice

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To meet the needs of ever growing population, there is an immense need to increase the rice production with the concomitant improvement in grain quality and nutrition. Among all the grain quality characteristics, aroma (fragrance) represents a high value added trait. The traditional Basmati genotypes (long grain aromatic rice) valued for their impressive cooking qualities are generally low yielders. To increase the productivity, there is an emergent need to develop high yielding rices which possess the typical quality attributes of Basmati. In order to achieve this, rice breeders need a simple and inexpensive assay to identify aroma trait in breeding populations, as even today aroma is quantified through sensory evaluation, which is subjective and prone to errors in judgment. Marker assisted selection (MAS) is being explored as an important supplement to the phenotypic selection in rice breeding, since marker assays have the advantage of being inexpensive, simple, rapid and only requiring small amount of tissue (Cordeiro et al., 2002). The level of aroma is generally associated with the increased levels of the compound 2-acetyl-1-pyrroline (2-AP) (Buttery et al., 1983). It has now been established that a single recessive gene (*fgr*) is responsible for aroma as determined by the level of 2-AP (Lorieux et al., 1996). This *fgr* gene was mapped on chromosome 8 with the RFLP markers, the nearest marker RG28 showed the genetic distance of 4.5 cM(Ahn et al., 1992 Lorieux et al., 1996). Based on this locus, several PCR-based co-dominant markers were developed, which can differentiate between fragrant and non-fragrant rice cultivars (Garland et al., 2000 Cordeiro et al., 2002 Jin et al., 2003). But these markers were also located physically away from the gene; hence they may not be efficient markers to use in marker assisted selection of aroma trait. Later, Bradbury et al. (2005) reported that the *fgr* gene encodes betaine aldehyde dehydrogenase 2 (*BAD2*), which has significant polymorphisms in the coding region (8bp deletion in exon 7). Based on this polymorphism, a four marker-multiplex PCR assay was developed (Bradbury et al., 2005). But use of this marker system in MAS is relatively cumbersome. Hence, the best marker for routine MAS programme would be an SSR marker very close to the aroma gene as it will have the advantages of both SSR and functional markers.

With this background, we have selected the genomic region of about 20 Mb upstream as well as downstream of the 8 bp deletion in the *BAD2* gene and this genomic region (which includes the total nucleotide sequence of *BAD2*) was used for the identification of SSRs. The SSRs were identified and localized using the software Simple Sequence Repeat Identification Tool (SSRIT) and the motifs which were repeated ≥ 5 times were only identified (Temnykh et al., 2001). To test the utility of these SSRs as potential genetic markers to distinguish aromatic from non-aromatic rice, 6 SSRs with a maximum repeat length of ≥ 20 nt were selected and primers were designed using the software FastPCR with standard parameters as described by Chen et al., 1997. These primer pairs were used for amplification of DNA isolated from 40 genotypes including basmati and non-basmati varieties. Of the 6 primers, one marker named ARSSR-3 (forward: GACACGCACCTCTGTCTAGC; reverse GTTTAATTGGTGAGGAAGTGG)targeting a $(TA)_{29}$ motif showed clear polymorphism between aromatic and non aromatic rices with an allele size of 290 bp for the former and 320 bp for the latter (Fig. 1). It was observed that ARSSR-3 is physically located 97 kb away from the *BAD2* gene. In order to study the utility of this marker, genetic linkage analysis was performed in three backcross (BC_1F_1) mapping populations derived from the crosses Taroari Basmati/B 95-1, Basmati 386/BPT 5204 and Vasumati/B 95-1 and one F_2 population derived from the cross Basmati 386/BPT 5204; each population consisted of 200 individuals. The analysis revealed that ARSSR-3 tightly linked (0.3 cM) to the aroma trait (Fig. 2). The earlier reported SSR markers (SCU-SSR1, Garland et al., 2000 RM42, RM22 Chen et al., 1997) which were developed based on RG28 locus showed many recombinants in the linkage analysis. This may be attributed to the greater physical distance (1.3 Mb) of these markers from the *BAD2* gene. Hence, we propose ARSSR-3 as an alternative to the earlier reported allele specific multiplex markers for regular use in breeding programmes aimed at marker assisted introgression of aroma trait into the genetic background of elite lines, since it is being very close to the gene and have the advantage of functional marker also.

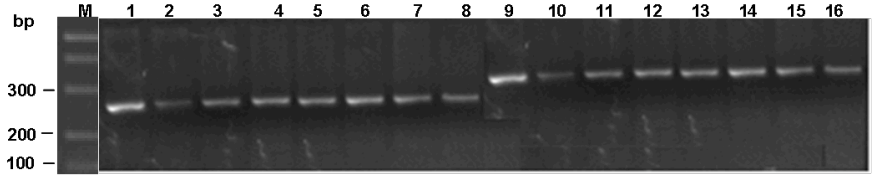

Fig. 1 Agarose gel showing marker distinguishing between aromatic(1-8) and non aromatic(9-16) rice varieties.1-TaroriBasmati,2-Basmati-386,3-Basmati370, 4-Type-3,5-Pusa Basmati, 6-Haryana Basmati,7-Dehradun Basmati8-Kasturi,9-Jaya,10-SambaMahsuri,11-IR-64,12-Swarna,13-Vijeta,14- TN-1,15- Kavya 16-Rasi

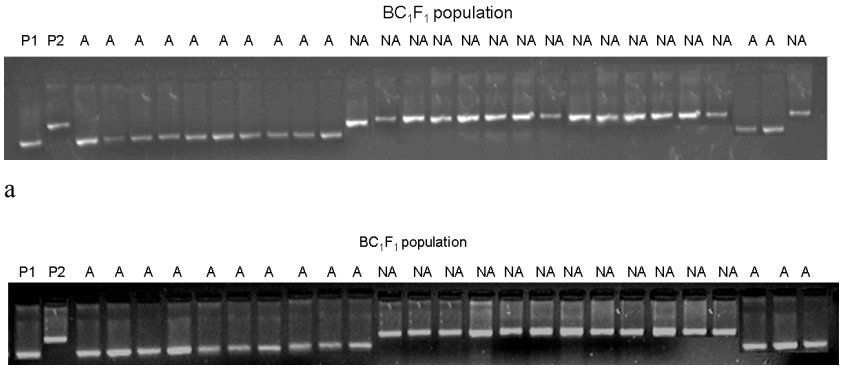

Fig. 2 Validation of ARSSR-3 in two different BC_1F_1populations. a, P1=Taroari Basmati, P2 =B95-1:b P1= Basmati 386, P2 =B95-1. The PCR fragments were loaded in to 3% agarose gel. A= Aromatic, NA= Non aromatic; phenotyping of each plant was carried out with gas chromatography in three replications.

References

Ahn S. M., C. M. Bollich and S. D. Tanksley, 1992. RFLP tagging of a gene for aroma in rice. Theor. Appl. Genet. 84: 825-828.

Buttery R. G., L. C. Ling, B. O. Juliano and J. G. Turnbaugh, 1983. Cooked rice aroma and 2-acetyl-1-pyroline. J. Agric. Food Chem. 31: 823-826.

Bradbury L. M. T., R. J. Henry, Q. Jin, R. F. Reinke and D. L. E. Waters, 2005. A perfect marker for fragrance genotyping in rice. Mol. Breeding. 16: 279-283.

Chen S., J. Y. Wu, W. Yang and M. Xu, 2006. The *fgr* gene responsible for rice restricted within 69 kb. Plant Science. 17: 505-514.

Cordeiro G. M., M. J. Christopher, R. J Henry and R. F. Reinke, 2002. Identification of microsatellite markers for fragrance in rice by analysis of the rice genome sequence. Mol. Breeding. 9: 245-250.

Garland S., L. Lewin, A. Blakeney and R. Reinke, 2000. PCR based molecular markers for fragrance gene in rice (*O. sativa* L.). Theor. Appl. Genet.101: 364-371.

Jin Q., D. Waters, G. M. Cardeiro, R. J. Henry and R. F. Reinke, 2003. A single nucleotide polymorphism marker linked to the fragrance gene in rice (*O. sativa* L). Plant Science.165: 359-364.

Lorieux M., M. Petrov, N. Huang, E. Guiderdoni and A. Chesquier, 1996. Aroma in rice: genetic analysis of quantitative trait. Theor.Appl.Genet. 93: 1145-1151.

Temnykh S., G. Declercke, A. Lukashova, L. Lipovich, S. Cartinhour and S. R. McCouch, 2001. Computational and experimental analysis of microsatellite in rice (*O. sativa* L) frequency, length variation, transposon associations and genetic marker potential. Genome Res. 11: 1441-1452.