Molecular Characterization of Paddy Cultivars And Utility of SSR Markers

[1]Priyanka Verma, [2] Daksha Kaushik, [3]Prof. (Dr.) K.S. Chundawat,
[1, 2] Research Scholar, Department of Biotechnology, Mewar University, Chittorgarh (Raj.)
[3] Professor, Department of Life Sciences, Mewar University, Chittorgarh (Raj.)

Abstract : Morphological and molecular characterization and identification of varieties will be of great significance in varietal improvement, release and seed production programmes. From the perspective of a seed technologist, characterization of varieties will be helpful in maintenance of their genetic purity in the seed supply chain, in order to ensure the supply of good quality, genetically pure seeds to farmers.

Key Words : Molecular Characterization, SSR Markers

Introduction : One of the most challenging tasks in molecular diversity analysis is to identify a marker which can differentiate between closely related varieties. But, in the presented study, during the molecular characterization of basmati and non-basmati paddy cultivars, different groups of SSR marker have been generated, which can differentiate between/within (i) basmati and non-basmati (ii) aromatic and non-aromatic (iii) basmati cultivars (iv) non-basmati cultivars (v) traditional and evolved basmati paddy cultivars. The details about these markers have been discussed as below:

Utility of SSR Markers

Basmati and Non-Basmati Paddy Cultivars: A total of ten SSR markers (RM 44, RM 84, RM 121, RM 124, RM 128, RM 220, RM 302, RM 310, RM 447 and RM 528) have been found to be efficient in differentiating basmati and non-basmati paddy cultivars (Figure 4.22a). The polymorphism percentage of markers to differentiate basmati and non-basmati paddy cultivars was 20%. RM 44 marker present on chromosome 8 has been reported to be linked to cooked kernel elongation (Biswas *et al*., 2004). In non-basmati paddy cultivars, the cooked kernel length has been observed to be very low as compared to basmati ones. Therefore, this marker showed its potential in differentiating basmati and non-basmati paddy cultivars. Similarly, some SSR markers have been reported to amplify different alleles in basmati and non-basmatipaddy cultivars (Jain *et al*., 2004; Siwach *et al*., 2004). By comparing our experimental data with previous researchers, some SSR markers (RM 121,

RM 124, RM 128, RM 220, RM 302, RM 310, RM 447, RM 528) were found to be the additional SSR markers, which can be used to differentiate basmati and non-basmati paddy cultivars. SSR markers have been reported to produce a number of alleles that were shared among the basmati cultivars, whereas comparatively lower number of bands was common among basmati and non-basmati cultivars of paddy (Rabbani *et al*., 2010).

Aromatic and Non-Aromatic Paddy Cultivars: A total of ten SSR markers (RM 44, RM 166, RM 210, RM 216, RM 223, RM 253, RM 321, RM 495, RM 506 and RM 522) were found to be able in differentiating aromatic and non-aromatic paddy cultivars. Thus, 20% SSR markers showed their capability in differentiating P 44 and PR 118 from PB 1121, PB 1460, PB 1401, PS 5, PB 2 and Bas 370. SSR markers linked to aroma gene *(fgr)* were found to be highly polymorphic for different aromatic paddy cultivars (Jewel *et al*., 2011).

To the best of our knowledge, use of RM 321, RM 495, RM 506 and RM 522 to differentiate aromatic and non-aromatic paddy cultivars is not reported so far elsewhere. Thus, these markers may be further exploited to differentiate other aromatic and non-aromatic paddy varieties, which were not characterized in the presented study.

Basmati and Basmati Paddy Cultivars: A large number of SSR markers (RM 16, RM 18, RM 162, RM 174, RM 210, RM 282, RM 310, RM 431, RM 447, RM 465, RM 490, RM 502, RM 506, RM 220, RM 304, RM 516, RM 570, RM 549, RM 593) showed their potential contribution to estimate genetic divergence within basmati paddy cultivars. Thus, 38% of markers showed their potential in differentiating basmati paddy cultivars. SSR marker RM 162 was observed to be competent in differentiating PB 1121 from other basmati cultivars, whereas RM 310 differentiated PB 1460 from rest of the basmati varieties. Marker RM 16 gave two alleles in PB 1401 but a single allele in all other basmati paddy cultivars. RM 465 gave multiple alleles in all basmati cultivars but was able to differentiate PB 1460, PB 1401 from PB 1121, PB 2 and Bas 370. The maximum numbers of unique electromorphs were found with RM 304, RM 447 and RM 560 in differentiating basmati paddy cultivars. Similarly, unique electromorphs were recorded for basmati paddy cultivars by using SSR markers (Kaushik *et al.,* 2011). RM 252 was reported to be the most powerful marker for discriminating among closely related Basmati varieties (Jain *et al*., 2004).

Non-Aromatic and Non-Basmati Paddy Cultivars: A total of six SSR markers (RM 19, RM 121, RM 133, RM 282, RM 465 and RM 560) showed their potential in differentiating two medium grain non-basmati and non-aromatic paddy cultivars. Thus, only 12% of total tested markers were able to differentiate P 44 and PR 118. The low percentage of polymorphism by markers may be due to less number of cultivars to analyze. The other

reason is that both these cultivars have similar morphological and physico-chemical properties to a greater extent. Similar to our report, the lack of genetic difference between Pakistani paddy cultivars, i.e. 'Sugdasi-Sadagulab' and 'Sonehri-Sugdasi', and 'NIAB-IR9' and 'Shadab' has been observed, which could be due to either extremely low difference between the two varieties at the DNA level and having similar morphological and agronomic traits (Rabbani *et al*., 2010).

It has been also observed that the difference of allele size is very less for polymorphic markers in these two cultivars. Thus, these six polymorphic markers may be used in paddy breeding programs for the development and improvement of other closely related paddy cultivars, which are medium in shape and size with low amylose content.

Traditional and Evolved Basmati Paddy Cultivars: Some SSR markers (RM 81, RM 84, RM 133, RM 281, RM 441, RM 560) have been found to be efficient in discriminating traditional basmati paddycultivar (Bas 370) from evolved or cross-bed paddy cultivars (PB 1121, PB 1460, PB 1401, PB 2) as shown in Figure 4.22d. The results were in the agreement of previous researchers, in which the traditional basmati varieties were distinguished effectively from evolved basmati and non-basmati paddy varieties using SSR markers (Pal *et al*., 2004; Archak *et al*., 2007).

Conclusion :

Molecular markers have been found to be more effective in detecting adulteration among paddy cultivars as compared to physico-chemical approach. In case of molecular characterization, a single allele has been observed in pure sample, whereas two alleles at different positions were observed of adulterated sample.

DNA marker based identification of traditional basmati paddy may help in maintaining the integrity of this high quality genotype to the benefit of researchers, farmers and consumers (Rabbani *et al*., 2010).

References :

1. Brar, D. S. and Khush, G. S. 2003. Utilization of wild species of genus Oryza in rice improvement, p. 283- 309. In: Nanda, JS, Sharma SD (Eds.). Monograph on Genus Oryza.
2. Byerlee, D. 1996. Knowledge-Intensive Crop Management Technologies: Concepts, Impacts, and Prospects in Asian Agriculture. International Rice Research Conference, Bangkok, Thailand, 3rd -5th June, 1996.
3. Chakravarthi, B. K. and Naravaneni, R. 2006. SSR marker based DNA fingerprinting and diversity study in rice (Oryza sativa L.). African Journal of Biotechnology 5(9): 684-688.
4. Chang, T. T. 2003. Origin, domestication and diversification. In: Smith, C.W. (Ed.), Rice: Origin, history, technology and production. John Wiley & Sons, Inc. Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.
5. Q.J. Zhang, T. Zhu, E.H. Xia, et al. Rapid diversification of five Oryza AA genomes associated with rice adaptation Proc Natl Acad Sci U S A, 111 (46) (2014), pp. E4954-E4962 http://dx.doi.org/10.1073/pnas.1418307111
6. M. Sweeney, S. McCouch The complex history of the domestication of rice Ann Bot, 100 (5) (2007), pp. 951-957 http://dx.doi.org/10.1093/aob/mcm128
7. D.E. Johnson, M. Dingkuhn, M.P. Jones, et al. The influence of rice plant type on the effect of weed competition on O. sativa and O. glaberrima
8. Weed Res, 38 (3) (1998), pp. 207-216 http://dx.doi.org/10.1046/j.1365-3180.1998.00092.x
9. Linares OF African rice (Oryza glaberrima): History and future potential Proc Natl Acad Sci U S A, 99 (25) (2002), pp. 16360-16365 http://dx.doi.org/10.1073
10. C.J. Chen, W.C. He, T.Y. Nassirou, et al. Genetic diversity and phenotypic variation in an introgression line population derived from an interspecific cross between Oryza glaberrima and Oryza sativa PLoS One, 11 (9) (2016), Article e0161746
11. G.P. Cheng, J.H. Feng, G.H. Lang, et al. Identification of QTLs for agronomic traits associated with yield in a BC2F2 population between Oryza sativa and Oryza rufipogon Chin J Rice Sci, 20 (5) (2006), pp. 553-556 http://dx.doi.org/10.3321/j.issn:1001-7216.2006.05.017