EVALUATION OF MICROSATELLITE MARKERS FOR SELECTION OF SALT TOLERANT RICE VARIETIES IN SRI LANKA

B.L.V. Rasangi[1], H.M.Y.G.S.B.K. Herath[1], D.N. Sirisena[2], S.W. Abeysekera[2] and P. Samaraweera [1]*

[1]Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya
[2]Rice Research and Development Institute, Bathalagoda

Introduction

Soil salinity is currently a major problem faced by rice farmers in Sri Lanka. Thousands of hectares that are technically suited for rice production are left uncultivated or are grown with very low yields because of saline soils, especially after the recent tsunami incidence. Therefore it is highly desirable to develop salt-tolerant rice varieties that can grow in salt affected lands. Nevertheless, screening for salt tolerance by observing phenotypic characters during rice breeding is very tedious and time consuming. The DNA markers allow simple method for monitoring incorporation of specific genes into plants at very early stage of development. Thereby they reduce the amount of work that needs to be done in order to select plants with desirable traits and accelerate the breeding programmes. The present study was conducted to evaluate the applicability of two microsatellite markers for identification of local salinity tolerant rice varieties.

Materials and Methods

Plant material

A collection of nine salt sensitive and tolerant parental rice varieties and ten F7 hybrids with varying degree of salt tolerance (Table 1) obtained from the Rice Research and Development Institute (RRDI), Bathalegoda were used in the study.

Isolation of DNA

Rice seeds were presoaked for 24 h in water and allowed to germinate for 48 h on wetted filter papers. The germinated seeds were grown in trays. DNA was extracted from two-week old rice leaves using rapid DNA extraction protocol of Kangle *et al.* (1995) which uses sodium dodecyl sulfate in extraction buffer. The quality of DNA was assessed by standard UV absorption and electrophoretic methods.

Testing of DNA for microsatellite markers

The microsatellites RM223 and RM315 (Lang *et al.*, 2001) of genomic DNA were amplified by polymerase chain reaction (PCR). The volume of the reaction mixture was 10 µl and contained 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 pmol/µl of each primer, 0.04 Units / µl of *Taq*-DNA polymerase and 150 ng of template DNA. The forward and reverse primers were, GAGGTA CTTCCTCCGTTTCAC and AGTCA GCTCACTGTG CAGTG for RM315 and GAGTGAGCTTGGGCTGAA AC and GAAGGCAAGTCTTGGC ACTG for RM223 respectively. The PCR programme consisted of initial

denaturation at 94 °C for 5 min, 35 amplification cycles with template DNA denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min and strand extension at 72 °C for 2 min, and a final extension step at 72 °C for 5 min. PCR products were then separated by electrophoresis on 1 % agarose gels and visualized by ethidium bromide staining.

Effect of salinity on seed germination

The seeds were first soaked in a 45 dS m^{-1} salt solution or distilled water (control) (Abeysiriwardena, 2004) for nine days and then allowed to germinate on filter papers moistened with distilled water for six days. Germination was considered normal if the seeds produced both radical and coleoptiles at the end of six days (Subasinghe *et a.*, 2007). The salinity tolerance levels were grouped according to percentage of seeds that showed normal germination.

Results and Discussion

The salt tolerance characteristics of ten hybrids and nine parental rice lines used in the study are shown in column 3 of Table I. In the field the hybrids displayed either moderate or high tolerance to salinity. The presence of two salt tolerant microsatellite markers RM315 and RM223 (Lang *et al.*, 2001) in the rice varieties was tested by amplifying the two sequences in rice genomic DNA samples using PCR. Figure 1 shows the PCR product separated on 1 % agarose gels and stained with ethidium bromide. The results indicated amplification of a DNA fragment of ~130 bp for RM315 with all salt tolerant rice lines, hybrids and parents. The sensitive varieties did not yield any product. Similarly, PCR of all the salt tolerant lines, but sensitive lines gave amplification for RM223 (data not shown). These results indicate that the characteristic amplifications with the primers used in the study occur only in salinity tolerant varieties. Therefore these primers can be used to monitor the transfer of salt tolerant genes into salt sensitive plants. All RM315 and RM223 positive lines displayed salinity tolerance during seed

Table 1. Rice varieties and their characteristics.

(1) Rice line	(2) Line number	(3) Phenotype (salinity tolerance)	(4) RM315	(5) RM223	(6) Tolerance to salinity at germination
Bg 450		sensitive	-	-	sensitive
Pokkali		high	+	+	high
Nonabokra		moderate	+	+	high
At 401		moderate	+	+	moderate
At 354		moderate	+	+	high
Bw 400		moderate	+	+	moderate
Bg 94-1		sensitive	-	-	highly sensitive
Bg 300		sensitive	-	-	highly sensitive
Bg 304		highly sensitive	-	-	highly sensitive
Bg 300 x At 401	16-119-1	high	+	+	high
Bg 94-1 x At 354	23-5	high	+	+	high
Bg 304 x At 401	15-49	high	+	+	high
Bg 300 x Pokkali	4-91	high	+	+	moderate
Bg 750 x Pokkali	5-110	moderate	+	+	moderate
Bg 750 x Pokkali	5-127	moderate	+	+	high
Bg 94-1 x Nonabokra	11-139	high	+	+	moderate
Bg 304 x At 354	21-8	high	+	+	high
Bg 450 x Bw 400	13-249	moderate	+	+	high
Bw 400 x At 401	14-368	moderate	+	+	moderate

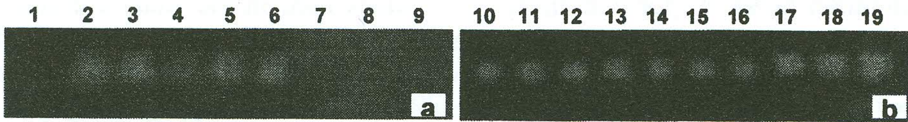

Figure 1. PCR products produced by RM315 primers. The products were separated on 1% agarose gels and stained with ethidium bromide. Panel a, parental lines; Panel b hybrids. Lanes 1-19: Bg 450, Pokkali, Nonabokra, At 401, At 354, Bw 400, Bg 94-1, Bg 300, Bg 304 and lines 16-119-1, 23-5, 15-49, 4-91, 5-110, 5-127, 11-139, 21-8, 13-249, 14-368 respectively.

germination (Table 1, column 6). However, their level of tolerance to salinity varied; some lines were highly tolerant to salinity where as the others displayed moderate tolerance. The differences may be due to the presence or absence of additional tolerant genes of this quantitative trait. For many lines the tolerance level observed during germination was close to that observed under field conditions (Table 1, column 6).

Conclusion

Rice microsatellite markers RM315 and RM223 show characteristic PCR amplification only in salt tolerant rice varieties. Therefore, they can be used to monitor transfer of salt tolerant genes to salt sensitive Sri Lankan rice varieties during breeding.

Acknowledgements

This study was supported by the University of Peradeniya research grants RG/2006/49/S and RG/2007/C-1/39/S.

References

Abeysiriwardena, D.S.Z. (2004) A simple screening technique for salinity tolerance in rice: germination rate under stress. International Rice Research Notes, 29(2): 78.

Kangle, Z, Prasanta, KS, Domingo, J, Gerard, M, and Huang, N (1995). Rapid DNA isolation for marker assisted selection in rice breeding. *Rice Genetics News Letter* (IRRI), 12: 255.

Lang, NT, Li, Z, and Buu, BC (2001) Microsatellite markers linked to salt tolerance in rice. *Omonrice,* 9: 9.

Subasinghe, A, Nissanka, NAASP and Weerakoon, WMW (2007) Identification of salt tolerant rice varieties at the seed germination stage and its relationship to seed husk thickness and iron absorption. Tropical Agricultural Research, 19: 219.