Phenotyping of Breeding Populations in Complement with Molecular Markers to Select Submergence Tolerant Rice (*Oryza sativa)*

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Introduction

Over 22 million hectares of lowland rain fed rice lands which occupy 18 % of global supply of rice are vulnerable to flash flooding worldwide and severe in Asian countries such as India, Bangladesh and Thailand. Most of these fields are cultivated with submergence tolerance landraces FR13A and FR43B with poor yield of 2 Mt/ha (Neeraja *et al.*, 2007). As reported by the respective data sources, up to the end of January 2013, approximately 75,000 ha of paddy lands have been affected due to flood condition prevailed throughout the season. Therefore rice breeders should select the appropriate varieties for those areas with the higher yield. The study was undertaken to improve submergence tolerance in popular Sri Lankan rice variety Bg360 through identifying submergence tolerant individuals in BC_2F_1 population of Bg360 / Swarna Sub1 // Bg360 by phenotypic and molecular screening.

Methodology

This experiment was carried out in the field and laboratory at the Bio technology Division of Rice Research and Development Institute, Batalagoda which is in the Low country Intermediate Zone of Sri Lanka from May to October 2013. Two rice varieties namely Bg360 which is three and half month, submergence susceptible rice variety popularly grown in Sri Lanka, and Swarna sub1 which is a developed submergence tolerant rice variety grown in India and 526 seeds from BC_2F_1 population of cross of Bg360 and Swarna sub1 were grown in nursery trays for 10 days and submerged under 1 m height of water for 10 days. Number of survived plants were taken at de-submergence and number of recovered plants were numerated 14 days after de-submergence. Height difference was scored before and after submergence.

A rapid DNA extraction protocol modified by Rice Research and Development Institute (RRDI), Bathalagoda was used for DNA extraction. Peatan a df DNA in she samples were confirmed by using agarose gel (1%) electrophoresis with 50 mV for 45 minutes. RM 219 microsatellite (Table 1) was used to dbtaeoa polymorphism between Bg360 and Swarna sub1. In PCR amplification, single preheat at 94 ^{0}C, 35 cycles of denaturation at 94 ^{0}C for 1 min, annealing at 59.1 ℃ for 1 min and elongation for 72 C for 2 min and final extension at 72 ^{0}C for 5 min were used for 15 µl of reaction volume which consist of 0.06 U Taq DNA Polymerase, 1X Buffer, 1.5 mM $MgCl_2$, 0.1 nM dNTPs, 0.07 þmol forward and reverse Primers and 20 ng/µl template DNA. Agarose gel (2%) was used in 0.5X TBE buffer for electrophoresis for 2 hours under 50mV voltages to analyze the amplified DNA.

Table 1. Details of RM 219 marker.

Primer type	Primer name	Primer sequence	Melting temperature
SSR	RM 219 F	5'-TCGGATGATGTAAAGCCT-3'	54.8 ℃
	RM 219 R	5'-CATATCGGVATTCGCCTG-3'	53.8 ℃

Results and Discussion

The results in table 2 showed that Bg360 was not able to withstand submergence and its leaves and stems showed rotting symptoms and died. Swarna sub1 plants were not affected because it showed 91.84 % survival and 88.88% recovery after 10 days of submergence and leaves remained greenish colour than the Bg360. After de-submergence, Swarna sub1 plants and some plants in BC_2F_1 population were able to recover rapidly and regained the greenness of leaves whereas Bg360 plants were not able to recover after de-submergence.

Table 2. Survival and recovery percentages of the parents and BC_2F_1 population after de-submergence

Plant identity	Survival percentage	Recovery percentage
Swarna sub 1	91.84	88.88
Bg 360	0	0
62	84.61	18.18
68	75.00	22.22
72	85.96	25.51
73	95.94	32.39
75	95.12	17.95
76	91.42	28.13
77	90.71	30.72
80	82.05	4.5

When considering the phenotypic characters, purple coloured stem is a dominant character in Bg360 and it can be used as a phenotypical marker et Swarna sub1 does not show purple coloured stem. The total number of survival plants in the population is 129 out of 526 seedlings and 7 plants did not show purple coloured stem. The height difference of survived plant is ranging from 0 to 10.5 cm. However, some plants showed lower height difference than the parents. Therefore, phenotypically this population is selected for further screening and for further experiments.

Initially the Sub1 locus was monitored by markers shown to be closely linked with the gene (Xu et al. 2000, 2004). Use of tightly linked (RM 464 A) and flanking (RM 219, RM 316) markers, as suggested by Hospital and Charcosset (1997) ensured efficient foreground and recombinant selection. RM 219 was applied as downstream marker which showaw the polymorphism with Bg360 and Swarna Sub1 (Plate 1).

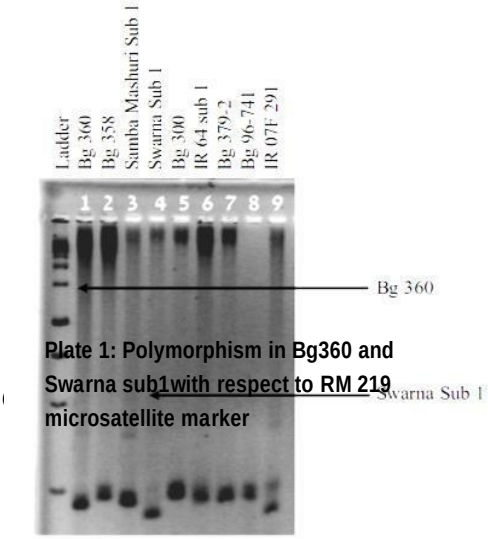

Plate 1: Polymorphism in Bg360 and Swarna sub1with respect to RM 219 microsatellite marker

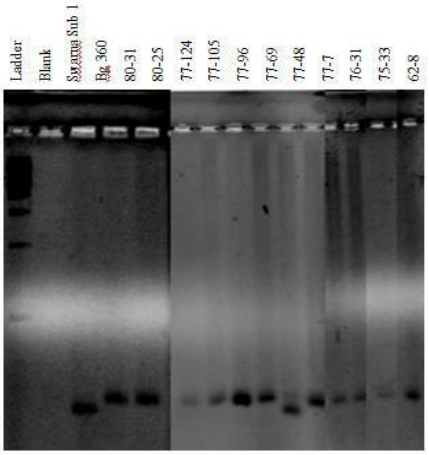

Plate 1. Polymorphism in Bg360 and Swarna sub1with respect to RM 219 microsatellite marker.

There is 11.6 % chance for recombination when using the marker RM219 (Neeraja et al., 2007). Though population comes to the second backcross, donor gene should be in the heterozygous stage, it may not show the heterozygotasy only for the RM 219 due to high recombination percentage. The plants which showed heterozygous for the Sub1 gene with double bands are 72-20, 72-18, 72-11, 72-3 and 77-69 (Plate 1). Some plants showed homozygous by presenting single band as the recurrent parent and those plants cannot be rejected at this level. For acquiring of higher accuracy, those plants should be again checked by using an upstream marker.

Conclusions

In morphological screening 129 plants were recovered after submergence. Plants which are having green colour stems were rejected and plants with lower height difference and purple colour stem were selected for molecular screening. Microsatellite marker RM 219 which is tightly linked to Sub1 gene is a reliable polymorphic marker for molecular screening of cross of Bg360 and Swarna Sub1. Plant numbered 72-20, 72-18, 72-11, 72-3 and 77-69 plants selected from molecular screening are submergence tolerance individuals in the BC_2F_1 population which is having heterozygous loci for RM 219. Plants which are shown homozygous and they should be subjected to further analysis by using an upstream marker to check whether they are recombinants or not.

References

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