SCREENING OF BC_1F_1 POPULATION (BG 379-2/ IR 07F102 // BG 379-2) OF RICE (*Oryza sativa* L.) FOR SUBMERGENCE TOLERANCE USING MOLECULAR MARKERS

R. N. Nawarathna[1], A. L. T. Perera[1] and W. L. G. Samarasinghe[2]

ABSTRACT

Rice is susceptible to submergence from germination to early vegetative stage and affects wetland paddy cultivation areas of Sri Lanka is affected by flash floods. There are no submergence tolerant varieties available locally. Therefore, this research was designed to evaluate the BC_1F_1 population (Bg 379-2/ IR 07F102 // Bg 379-2) morphologically for submergence tolerance and to select polymorphic molecular markers between the donor parents IR07F102 and recurrent parent, Bg 379-2, and to screen and select plants of the BC_1F_1 population for submergence tolerance using identified molecular markers. The 108 individuals of BC_1F_1 of rice were morphologically screened for plant lodging, leaf senescence and plant elongation and molecular screening was done by using 5 Rice microsatellites and 1 In Del marker. Out of those, molecular marker, RM464A for SUB1 gene conferring submergence tolerance, was used to screen the BC_1F_1 population. Five plants were selected as submergence tolerant individuals as they were positive in both morphological and molecular screening.

Keywords: *Molecular markers, Rice (Oryza sativa L.), Submergence tolerance*

INTRODUCTION

Rice (*Oryza sativa L.)*, is one of the major cereals grown worldwide and also the staple food of the people of Asia and Sri Lanka. Rice is mainly grown as a wetland crop due to its semi-aquatic nature and ability to survive in water logged soils, however the vegetative stage of the plant is highly susceptible to submergence caused by flash floods of 50cm or more during heavy monsoon rains (Mackill *et al.,* 1996). Most rain-fed submergence-prone rice lands located in wet zone of Sri Lanka receive a mean annual rainfall of more than 2500 mm. Flash floods, a rapid surge of flooding that subsides after several d and lasts no longer than 10 d, are common phenomena in the Kalutara, Gampaha, Ratnapura and Matara districts and around 75,000 ha of low lying lands of coastal flood plains located from Wennappuwa to Dondra remain unproductive due to poor drainage and long term submergence as a result of accumulation of runoff and flowing of rivers (Jayawardena, 1984).

Sensitive rice cultivars die within a week under complete submergence conditions. Even if the plant is only partially submerged, it reduces tillering, induces rapid elongation of shoots and increases lodging (Pereta, 2007). Most of the currently recommended high yielding rice varieties are not tolerant to submergence. This affects most of the farmers in Low Country Wet Zone (LCWZ) , therefore, many farmers cultivate submergence tolerant, photosensitive traditional varieties such as *"Ma wee", "Madael", "Thavalu"*, *'Molligoda',* and *'Soola'* in order to cope with submergence. However, these local traditional varieties generate poor yields of 0.75 to 1.0 t/ha (Jayawardena, 1984).

[1] *Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.*
[2] *Rice Research and Development Institute (RRDI), Batalagoda, Sri Lanka.*

In 1996, a major QTL *SUBMERGENCE 1 (SUB1)* was mapped on chromosome 9 of *indica* rice line, IR 4093 l-26 (Xu and Mackill, 1996). This QTL contributes to 70% of phenotypic variation together with minor QTLs located on chromosomes 1, 2, 6, 7, 8, 10, 11 and 12 in tolerant low land rice cultivars (Nandi *et al.,* 1997; Toojinda *et al.,*2003). Mapping of *SUB1* facilitates the improvement of submergence tolerant cultivars through Marker Assisted Backcrossing (MAB) (Neereja *et al*., 2007).

The requirement for developing submergence tolerant, high yielding rice varieties has been identified as an urgent need of national importance. The objectives of this research was to meet this challenge by using molecular markers to screen a BC_1F_1 population of the cross between exotic line *IR07F102* having flood tolerance QTL, *SUB1* and the local susceptible variety, *Bg 379-2* in order to obtain a flood tolerant line.

MATERIALS AND METHODS

The examined population comprised of 108 BC_1F_1 plants and 25 plants of each of the tolerant parent, *IR 07F102* and recurrent parent, *Bg 379-2* a local variety susceptible to submergence. The seeds were provided by Rice Research and Development Institute (RRDI), Batalagoda, Sri Lanka.

Morphological screening

A total of 108, two-week old seedlings of BC_1F_1 population were morphologically screened together with the parents by following a slightly modified version of Vergera and Mazaredo method as followed by Septiningsih *et al.,* (2009) at RRDI, Batalagoda. They were submerged in 58 cm of water for 14 d under water temperature of 33.52^{o}C. The de-submerged plants were scored from 3 d after de-submergence up to 14 d of recovery for Plant Elongation (PE), Leaf Senescence (LS) and Lodging.

Plant height and height of sheath were measured from soil level up to the tip of longest leaf before submergence, 6 days after submergence and 3 days after de-submergence using a meter ruler. The colour of leaves and thickness of stem were recorded by visual observation after 3 days from de-submergence up to 14 days of recovery. The responses of plants were scored for lodging using a scale developed by GSR project of IRRI with slight modifications as shown in Table 01.

Molecular screening

DNA was extracted from 10-day old leaf tissues by using a rapid DNA extraction protocol (Anushka *et al*., 2008). The PCR analysis was carried out by using 6 rice microsatellite primers (RM219, RM464A, RM23869, RM316, RM285, RM105) and one In Del primer (ART5) (Integrated DNA Technologies, Coralville, LA, USA).

The PCR was performed in a mixture of 15µL containing 3µL of template DNA, 3µL of 5X PCR buffer (100 mM Tris–HCl (pH 8.3), 250 mM KCl), 0.9 µL of 25 mM $MgCl_2$, 0.15µL of 10mM dNTPs, 1µL each of 5µM Forward primer and Reverse primer and 0.2µL *Taq* DNA polymerase (5U/µL) (Promega BioSciences, CA, USA). After initial denaturation for 5 min at 94 °C, PCR was carried out with a profile of 35 cycles at 94 °C for 1 min, 59.1 °C for 1 min, 72 °C for 2 min with a final extension at 72 °C for 5 min and a hold at 4 °C using My cycler (BIORAD laboratories, CA, USA). The PCR products were analyzed in 8% polyacrylamide gels for 6.15 hr at 100 V using vertical gel electrophoresis (Life Technologies, Paisley, Scotland).

RESULTS AND DISCUSSION

Submergence tolerance was assessed using the investigated morphological parameters independently. Morphological screening was carried out in order to test the relationship of morphological traits such as LS, PE

and lodging with submergence tolerance. Accordingly a total of 32 plants which elongated less than 12cm under submergence were considered submergence tolerant (Figure 01) and 25 plants that retained dark green leaves under submergence were also selected as tolerant (Figure 02).

In addition, 28 plants of lodging index 1 and 2 upon desubmergence were considered as tolerant (Figure 03). The plant numbers 52, 72, 97, 116, 64, 15, 98 and 49 were positive for all three traits in the morphological screening.

Table 01. Plants selected in screening

Plant no	Morphological Screening			Molecular Screening	Plant no	Morphological Screening			Molecular Screening
	E	L S	L			E	L S	L	
33	-	+	-	-	28	-	+	+	-
50	+	+	-	-	96	-	-	+	-
63	+	+	-	-	47	+	-	+	-
***52**	**+**	**+**	**+**	**+**	95	-	-	+	+
118	+	-	-	-	25	-	+	-	-
82	-	-	-	+	11	-	-	+	+
2	+	-	-	-	71	-	-	+	-
78	-	-	+	-	127	-	-	+	-
130	-	+	-	-	4	-	-	+	+
44	+	-	-	-	98	+	+	+	-
74	+	-	-	-	***49**	**+**	**+**	**+**	**+**
54	+	-	-	-	134	+	-	-	-
51	-	+	-	-	55	+	-	+	-
69	-	+	+	-	79	+	-	+	-
***72**	**+**	**+**	**+**	**+**	67	+	-	+	-
***97**	**+**	**+**	**+**	**+**	61	+	-	-	-
5	+	-	-	-	77	-	-	-	+
16	+	-	-	-	64	+	+	+	-
57	+	+	-	-	34	-	+	+	-
113	+	+	-	+	40	+	-	+	-
119	-	+	+	-	43	-	-	+	-
30	+	-	-	-	66	+	+	-	-
15	+	+	+	-	10	+	-	+	-
110	-	-	-	+	68	-	+	-	-
84	+	-	-	-	88	-	+	-	-
87	+	-	-	-	126	-	+	+	-
125	+	-	-	-	133	-	-	+	-
104	-	-	-	+	38	-	-	-	+
***116**	**+**	**+**	**+**	**+**	13	-	+	-	-

(+) = Selected, (-) = Not Selected, E= Elongation, LS= Leaf Senescence, L =Lodging, *= Selected in both molecular and morphological screening

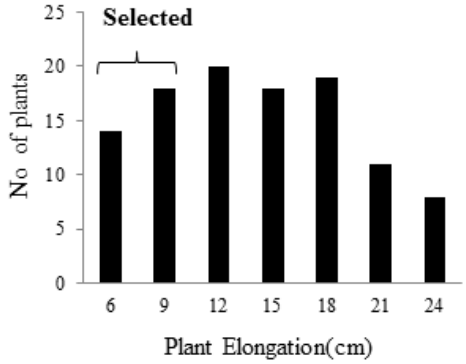

Figure 01: Distribution pattern for plant elongation of BC_1F_1 population of Bg 379-2/ IR 07F102 Bg 379-2.

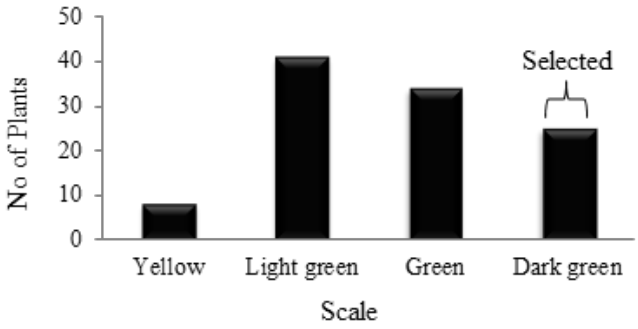

Figure 02: Distribution pattern of leaf senescence of BC_1F_1 population of Bg 379-2/ IR 07F102 // Bg 379-2.

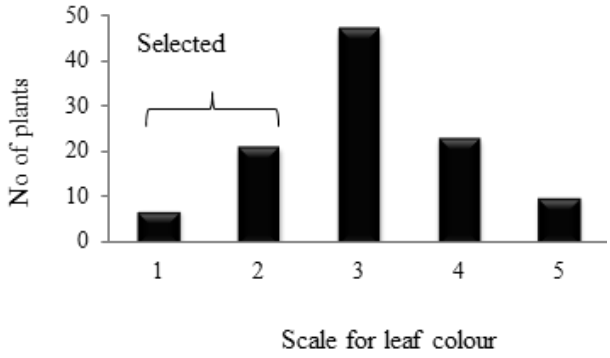

Figure 03: Distribution pattern for lodging scores of BC_1F_1 population of Bg 379-2/ IR 07F102 // Bg 379-2 ; 1-5: 1= No lodging, 2= Slightly lodged, 3= Moderately lodged, 4= Nearly lodged, 5= Completely lodged.

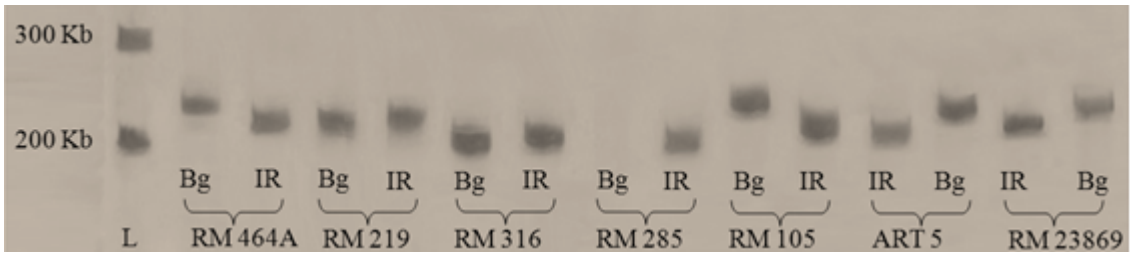

Figure 04: PCR products of parents to check for polymorphic markers ; L= 100bp ladder, Bg= Bg 379-2, IR= IR 07F102.

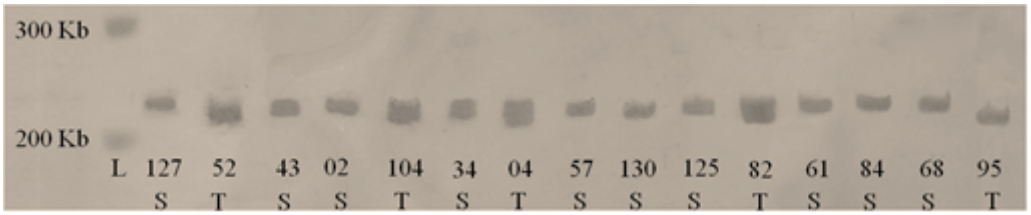

Figure 05: Amplified products of randomly selected individuals of BC_1F_1 population of Bg 379-2/ IR 07F102 // Bg 379-2 by RM 464A; S= Susceptible, T= Tolerant, L= 100bp ladder ; 127, 52, 43, 02, 104, 34, 04, 57, 130, 125, 82, 61, 84, 68 , 95 = Plant numbers of BC_1F_1 individuals .

Individuals of BC_1F_1 population showed significant variation in terms of PE (Figure 01) and ranged from 6 cm to 24 cm. Some individuals of BC_1F_1 showed rapid elongation of first two leaves compared to tolerant parent after 2 d of submergence (data not shown) and attempted to escape stress by reaching the surface of water column with their leaf tips, in order to utilize atmospheric oxygen. However, plants which showed higher initial PE accompanied by LS collapsed upon de-submergence. This showed that a combination of moderate submergence tolerance and moderate elongation ability may be desirable under stagnant long-term partial flooding. Therefore, the plants which showed lower PE together with plants categorized under lodging scales of 1 and 2 which remained erect were considered as tolerant individuals. Because of vigorous plants having erected stature with lower elongation of sheaths and leaves can escape from submergence stress during vegetative stages can withstand submergence and bear heavy panicles at the reproductive stage (Mackill *et al*., 1996).

Plants were selected against LS, since photosynthesis is affected by LS and survival of plant is greatly determined by degree of resistance to LS. Therefore, high chlorophyll content in plants which remained green may add to survival over other susceptible individuals.

Molecular screening was carried out to identify plants carrying *SUB1* gene in order to precise identification of BC_1F_1 plants to carry out breeding programmes. Out of tested markers, RM464A, RM 105, RM 23869 and ART 5 showed polymorphism between the parents, IR07F102 and Bg 379-2 in 8% polyacrylamide gel (Figure 04).

From these, the tightly linked marker RM464A was used to screen the BC_1F_1 population (Figure 05) and intogression of *SUB 1* was assured by tightly linked marker RM 464A.

A total of 14 BC_1F_1 plants of number 4, 52, 82, 72, 97, 113, 49, 110, 11, 77, 104, 116, 95 and 38 were selected by molecular screening based on their heterozygous nature for presence of *SUB1*.

The 5 plants 72, 52, 97, 116 and 49 were selected as submergence tolerant in both morphological and molecular screening (Table 02).

Amplicons for *SUB 1* is identified with sizes between 200 kb and 300 kb for all checked markers. Xu *et al.,* (2004) found that rice microsatellite markers, RM464A linked to *SUB1* by 0.7 cM can be used for foreground selection of the *Sub1* over wide range of recipients due to lower recombination events between marker and QTL to enhance precision. Therefore, intogression of *SUB 1* locus was assured by tightly linked marker, RM 464A

as suggested by Xu *et al.,* (2004). Identified polymorphic In Del marker, ART 5 which targets 15bp insertion in promoter region of *SUB1C* is a potential marker to use together with *SUB1A* markers in final screening, to ensure the introgression of both *SUB1A* and *SUB1C* into newly developed submerges tolerant lines.

Though breeding for submergence has been initiated since 1980s, this is the first attempt on intogression of *SUB1* into local new improved variety *via* MAS in Sri Lanka. Improvement for both abiotic stresses such as drought, salinity, iron toxicity and biotic stresses together with submergence tolerance is essential in rice breeding programmes to address the rising food demand in Sri Lanka.

CONCLUSION

In summary, five plants from the BC_1F_1 population derived from Bg 379-2/ IR 07F102 // Bg 379-2 were selected for submergence tolerance considering both morphological and molecular traits. The polymorphic marker, RM 464A can be used in MAS to cope with flash floods to mitigate the impact of climate change in rice production in Sri Lanka.

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