Evaluation on submergence tolerance characteristics on Nepalese rice

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Introduction

Flash flood is one of the major yield limiting factors in rice growing areas especially in the South Asia. In many places, the flash flooding can completely inundate plants for a few days to weeks (Vargera et al., 1976). Complete submergence due to frequent flooding can severely impair the plant growth and yield (Xu et al., 2006). Therefore, farmers are forced to plant with five times less yielding landraces that performs well in deepwater or in flash flooding conditions (Voesenek and Bailey-Serres, 2009). These landraces are considerably tolerant to flooding stress as a result of different adaptive mechanisms for surviving the hypoxic or anoxic conditions (Xu et al., 2006; Hattori et al., 2009).

Use of the Sub1A gene in rice breeding enhances the tolerance for this abiotic stress in rice production. However, the identification of the new flash flood tolerant rice genes and their use in regular breeding program is very important to enhance the efficiency of rice varietal development for incoming climate change hazards.

Materials and method

Ninety five rice lines including Nepalese rice landraces, modern varieties and breeding lines were used in this study. Samba Masuli Sub-1 was used as resistance check while IR64 was used as susceptible check. The 14 days old seedlings were submerged in the water tank for another 14 days. Shoot and seedling lengths, leaf area and chlorophyll content (SPAD reading) were recorded before submergence and just after de-submergence. The survival ability was scored 7 days after de-submergence and survival percent was calculated.

Genomic DNA of rice accessions was prepared following modified CTAB method as described by Sul and Korban (1996). After denaturing the DNA template at 94°C for 2 min, PCR was performed with 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 90 s, and final extension at 72°C for 10 min. PCR was performed using Sub1A specific primers for 44 selected accessions along with the check lines (Table 1).

Results

Highest survival was observed in Samba Masuli Sub-1 (93.73%), Radha-4 (89.87%) and Sugapankhi (88.29%). Some Nepalese rice landraces showed the presence of Sub1A alleles but having very low survival rate, however, rice landrace Sugapankhi showed good survival and regeneration ability but lacks Sub1A-1 allele (Table 3) while screening germplasm using molecular markers.

Increase on plant height during submergence showed the lowest survival and regeneration rate (r=-0.61171, p=0.000, N=97). Similarly, the SPAD value during submergence showed the positive correlation (r=0.429, p=0.000, N=97) with survival. However, average leaf area just before submergence showed negative relationship (r=-0.3929, p=0.005, N=97) for it. No any accessions showed tillering during submergence, however, accessions with high tillering habit on normal condition showed high survival percentage during submerged condition (r=0.327992, p=0.02, N=97) (Table 2).

Discussion

Rice accessions Seto Anadi, Gurdi-Hemja and Zyabdikhole showed the presence of the Sub1A-1 allele but have almost nil survivability after submergence treatment which may be due the extra costing of energy for rapid stem elongation response which is to be considered disadvantage for faster regeneration (Perata and Voesenek, 2007). Similalry, the significant level of submergence tolerance expressed by the rice accession Sugapankhi while lacking Sub1A-1 allele can be explained by hypothesizing the presence of another mechanism or the allele in this rice landrace which is equally effective for the submergence tolerance characteristics.

Table 1. List of primers used for Sub1A genes monitoring in selected rice accessions (Xu et al., 2006)

Primer name	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
Sub1A_1	GATGTGTGGAGGAGAAGTGA	GGTAGATGCCGAGAAGTGTA
Sub1A_3	CTCGGCACCTTCGACACC	AAGACGAACGGTGAACCATG
Sub1A_4	CTCGGCACCTTCGACACC	GGTAGATGCCGAGAAGTGTA
Sub1A_5	ATATTCACTGCTCACTAGTAAC	GTTTGTGGCCTTTGAGTAAG
Sub1A_6	GATGTGTGGAGGAGAAGTGA	TGTTTTGGTGGATCGATGGG
Sub1A_7	GATGTGTGGAGGAGAAGTGA	GTTTGTGGCCTTTGAGTAAG

Table 2. Relationship between different plant traits with the survial percentage after submergence conditon (N=97)

	Plant height just after submerge	SPAD just after submerge	Plant Survival % after submerge	Leaf area before submerge
SPAD before submerge	0.030 (0.775)			
SPAD just after submerge	-0.411 (0.000)			
Plant Survival % after submerge	-0.612 (0.000)	0.429 (0.000)		
Leaf area before submerge	0.641 (0.000)	-0.364 (0.010)	-0.393 (0.005)	
Tiller no. at 28 days on air	-0.024 (0.869)	0.232 (0.109)	0.328 (0.020)	-0.150 (0.299)

Note: number in parenthesis is probability value

Table 3. Sub1A-1 allele monitoring in some selected rice accessions collected from different parts of Nepal.

Accession	Survival %	Sub1A-1	Accession	Survival %	Sub1A-1	Accession	Survival %	Sub1A-1
Samba Masuli Sub-1	**93.72**	*Present*	Sabitri	**51.60**	*Absent*	Rango	**5.56**	*Absent*
Radha-4	**89.87**	*Present*	Amagura	**48.09**	*Present*	Rudhuwa-Ilam	**5.00**	*Absent*
Sugapankhi	**88.29**	*Absent*	Masuli	**46.25**	*Absent*	Dhanush Ban	**0.00**	*Absent*
Radha-12	**74.87**	*Present*	Kalanamak	**45.28**	*Present*	Ghandruk local	**0.00**	*Absent*
Silhat	**72.50**	*Present*	Jaya	**42.65**	*Absent*	Gurdi-Hemja	**0.00**	*Present*
Ramdhan	**71.59**	*Present*	IR-42	**40.35**	*Absent*	IR-64	**0.00**	*Absent*
Sona Masuli	**65.46**	*Absent*	Lalka Basmati	**36.92**	*Absent*	Jhinuwa-Lamjung	**0.00**	*Absent*
Anadi	**63.75**	*Absent*	Thapachini	**35.83**	*Absent*	Jumli Marshi Mehele	**0.00**	*Absent*
Jagarnathiya	**62.83**	*Absent*	Radha-11	**34.19**	*Absent*	Linde Basaha	**0.00**	*Absent*
Harinker	**62.53**	*Absent*	Kamod	**31.71**	*Absent*	Pahele	**0.00**	*Absent*
Rajala	**60.96**	*Absent*	Hardinath-2(BG-114)	**31.31**	*Absent*	Ramani-Bara	**0.00**	*Absent*
Makwanpur-1	**60.20**	*Absent*	Aanpjhutte	**25.00**	*Absent*	Seto Anadi	**0.00**	*Present*
Rambilash	**59.58**	*Absent*	Janaki	**12.50**	*Absent*	Thimaha-Chobo	**0.00**	*Absent*
Pakher	**58.32**	*Absent*	Sotwa	**10.29**	*Absent*	Zyabdikhole	**0.00**	*Present*
Radha-7	53.08	Absent	Sathiya	7.81	*Absent*			

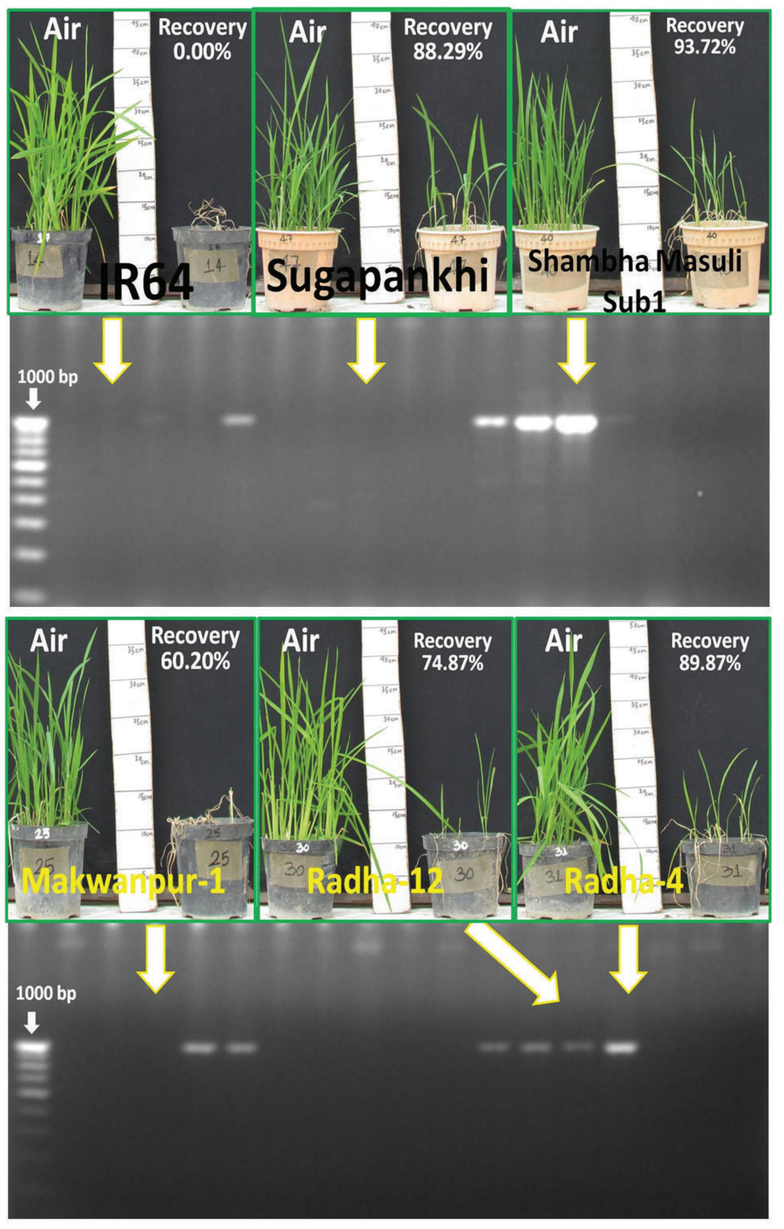

Photo 1. Response of Nepalese rice on submergence with respect to Sub1A-1 allele

Conclusion

Thus, we can conclude that the survival ability is not dependent on Sub1A-1 and this allelic form is not the only one to confer the tolerance. Therefore, widely used submergence tolerance allele Sub1A-1 is not the only determinant for the submergence tolerance characteristics in rice and this alone can't be used in the marker assisted selection program for the development of rice varieties with submergence tolerance. Similarly, many other mechanisms of the submergence tolerance are still need to explore and use for the development of the diverse rice varieties with submergence tolerance.

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