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Characterization of Rice Genotypes for *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) Gene

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ABSTRACT

The present study was performed in *kharif* (June-December) 2019 at Bihar Agricultural College, Sabour, Bhagalpur, Bihar, India to screen gene specific markers developed from *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) gene and identify the rice genotypes for the presence of *SNORKEL* genes. A total of 13 rice genotypes were used out of which 8 genotypes Vaidehi, RYC743, FR13A, Desaria, Dudhi, Birar, Kalaladora and Kajargod indicated the presence of both *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) gene using SK1-1, SK1-2, SK2-1 and SK2-2 markers. Therefore, these genotypes have tolerance ability of deep water flooding due to higher rates of elongation where water stagnates for longer than two weeks. However, results from the present study also revealed that the genotype Desaria and RYC743 had higher rates of elongation and Satyam, Sudha, Purnendu, FR13A, Pansoradhan and Swarna Sub1 had lower shoot elongation rate compared to other genotypes. This study provides gene specific markers for the identification of varieties/donors tolerant to deep water flooding as well as donors to be used in deep water rice breeding programs. Thus, the marker developed and validated in this study will aid rice breeders in quickly and efficiently identifying and introgressing the *SNORKEL* genes. The results would be a helpful in the development of rice varieties with both submergence tolerance and elongation ability in order to improve the yield stability in flood-prone areas.

KEYWORDS: Rice, elongation, flooding, genotypes, internode, marker, *SNORKEL* gene

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Rice is a staple food for billions of people worldwide. Although, rice productivity has grown significantly since the 1960s, yields still need to be double to fulfil estimated demands by 2050 (Samal et al., 2018, Wang et al., 2016). More than 30% of the rice in Asia and 40% of the rice cultivated in Africa is in lowland or deep water areas. High water level in flood prone areas is one of the environmental stresses that limits rice plant growth (Singh et al., 2011). Bihar has about 33 lha area under rice. About 10% of this is under deep water rice cultivation. Deep water rice is cultivated in flooded conditions with water deeper than 50 cm for one month or longer during the growing season (Voesenek and Bailey-Serres, 2009, Luo et al., 2011, Sarkar and Bhattacharjee, 2012, Singh et al., 2014). Many districts of Bihar are flooded during the rice growing season every year and deep water rice varieties are grown in this area. These rice varieties have special unique feature for the survival under deep water. The major characteristic feature is rapid internode elongation in response to rising water level which helps the uppermost leaves come protruding the water level and ensures photosynthesis (Vergara et al., 1976, Catling, 1992, Kende et al., 1998, Septiningsih et al., 2013). Deep water rice has the ability to tolerate flooding via escape strategy as well as quiescence strategy (Bailey-Serres et al., 2010). Under flooding, it has been observed that ethylene enhances the biosynthesis of gibberellic acid causing elongation of internode in deep water rice (Fukao and Bailey-Serres, 2008). Few landraces can elongate by more than 20 cm per day, but have poor grain quality and are low yielding (Septiningsih et al., 2013). The non-deep water rice varieties show little elongation under deep water condition. Many studies have identified QTLs for deep water traits, such as internode elongation and number of elongated internodes (Hattori et al., 2007, Hattori et al., 2008, Nagai et al., 2012). It was reported that the QTL on chromosome 12 contributed the most rapid internode elongation in deep water stress conditions during the vegetative stage growth (Septiningsih et al., 2013, Vergara et al., 2014, Tamang and Fukao, 2015). By positional cloning, two genes within QTL were identified namely, *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) as key regulators for the survival under deep water. *SNORKEL* genes have an AP2/ERF domain involved in ethylene signal transduction and its expression is induced by ethylene treatment or response to submergence in deep water rice (Ashikari and Matsuoka, 2006, Fukao et al., 2006, Nakano et al., 2006). These genes are also reported in *Oryza rufipogon*, *Oryza nivara*, and *Oryza glumaepatula* (Hattori et al., 2009, Sasayama et al., 2018). However, the modern rice does not have internodal elongation ability due to lack of *SNORKEL* genes. Introgressing the *SNORKEL* genes to high-yielding modern rice varieties could potentially result in rice crops that can survive in flooding and produce more yield even in flooded conditions. Thus, the aim of this study is to develop gene-based marker from *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) gene for differentiation of deep water from non-deep water rice varieties and also to identify the genotypes with higher elongation rate in response to submergence which may provide better protection against flooding stress.

2. MATERIALS AND METHODS

The field experiment was conducted at Rice Research Farm, Bihar Agricultural College, Sabour, Bhagalpur which is geographically located between 25°15'40" N latitude to 87°2'42" E longitude at 46 m above mean sea level during *kharif* (June-December), 2019 . A total of thirteen rice genotypes known for submergence tolerance (FR13A, Swarna Sub1) and suitability to deep water (Satyam, Sudha, Vaidehi, RYC743, Purnendu, Desaria, Pansoradhan, Dudhi, Birar, Kalaladora, Kajargod) were obtained from the Rice Section, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India. The seeds of 13 rice genotypes were seeded in nursery bed and twenty-five days old seedlings were transplanted in randomized complete block design (RCBD) with two replications. Each genotype was raised in a two row plot of 4 m length by adopting a spacing of 15×15 cm^2. The forty-five day old plants of rice genotypes were completely submerged with turbid water of 1.5 m height for 14 days. Water depth was checked daily to ensure complete submergence of the material as per the requirement. The field was de-submerged after 14 days of submergence. After 14 days of de-submergence, the survival percentage of the seedlings was recorded. For elongation rate of shoot, shoot length before submergence and after de-submergence was recorded. All the recommended agronomic packages of practices were adopted during the entire crop period. Statistical analysis of data was done using R software.

DNA isolation from the 13 rice genotypes was done using a protocol described by Sinha et al. (2017). The gene specific primer pairs were designed based on already submitted sequences to NCBI of the *SNORKEL1* (Gene bank accession number AB510478.1) and *SNORKEL2* (Gene bank accession number AB510481.1) gene of rice (Table 1). BatchPrimer3 (You et al., 2008) software was used for primers designing. The PCR amplification was carried out in 15 µl reaction volume containing 1×PCR buffer, 0.25 mM dNTPs, 0.25 µM each forward and reverse primer, 0.5 U Taq DNA Polymerase (Xcelris, India) and 40 ng of template DNA using a thermal cycler (Agilent Mastercycler, USA). Touchdown-PCR reactions were performed as follows: 4 min initial denaturation at 94°C, followed by 94°C for 30 s, 60°C for 60 s, and 72°C for 60 s in the first

Table 1: Details of the designed gene specific primers

Sl. No.	Gene	Primer Name	Primer Sequence	Expected product size
1.	*SNORKEL1*	SK1-1	F-CACCGCCTTCAGCATCTT	709
			R- CTCGTCCTTGTCGTTCTCCT	
2.	*SNORKEL1*	SK1-2	F- GTTCCACGGCATCCACAT	693
			R- CGCGATTATCGATCTCCTCT	
3.	*SNORKEL2*	SK2-1	F- TGCGGAGAGAACGATAACAA	691
			R- TCTCGTTTTCCACGCATACA	
4.	*SNORKEL2*	SK2-2	F- CCTTCTTTTGAGGGAGTTGG	973

cycle, then decreasing the annealing temperature by 1°C cycle^{-1} for 10 cycles, followed by 94°C for 30 s, 55°C for 60 s, and 72°C for 60 s for 25 cycles and ending with 5 m of elongation at 72°C. The amplified PCR products along with 100bp DNA ladder as molecular marker were resolved in 1.2% agarose gel stained with ethidium bromide (0.5 µg ml^{-1}). Gel was visualized under UV light and documented in gel documentation system (Uvitec gel doc system, UK).

3. RESULTS AND DISCUSSION

Marker-assisted selection is a useful tool in plant breeding programs to maximize selection efficiency. Molecular markers make it possible to analyse a large number of samples and identify desired alleles in early stage of plant and significantly shorten the breeding cycle. Molecular markers are widely used to characterize the genotypes due to their simplicity and reproducibility. The four pairs of primers designed based on the sequence of *SNORKEL1* and *SNORKEL2* genes were used to amplify the target sequence on the 13 rice genotypes. In present study, all of the gene specific markers developed from *SNORKEL* genes were amplified and produced a clear, sharp band. A DNA fragment of 709, 693, 691 and 973 bp was amplified in all the analyzed deep water rice genotypes except in Satyam, Sudha, Purnendu and Pansoradhan using primer pairs SK1-1, SK1-2, SK2-1 and SK2-2, respectively (Figure 1 (A–D)). The reason for not detecting the *SNORKEL* genes in Satyam, Sudha, Purnendu and Pansoradhan could be because of existence of different source of genes that could be responsible for deep water traits.

Further, the forty-five day old plants of deep water rice genotypes were completely submerged in submergence pond with turbid water of 1.5 m height for 14 days to check the survival percentage and elongation rate. After 14 days of de-submergence, the survival percentage of plants was scored according to the IRRI Standard Evaluation System (Anonymous, 1988). The genotypes RYC743, Purnendu, FR13A, Desaria, Dudhi, Birar, Kalaladora, Kajargod and Swarna Sub1 showed rapid recovery after de-submergence compared to Satyam, Sudha, Vaidehi and Pansoradhan

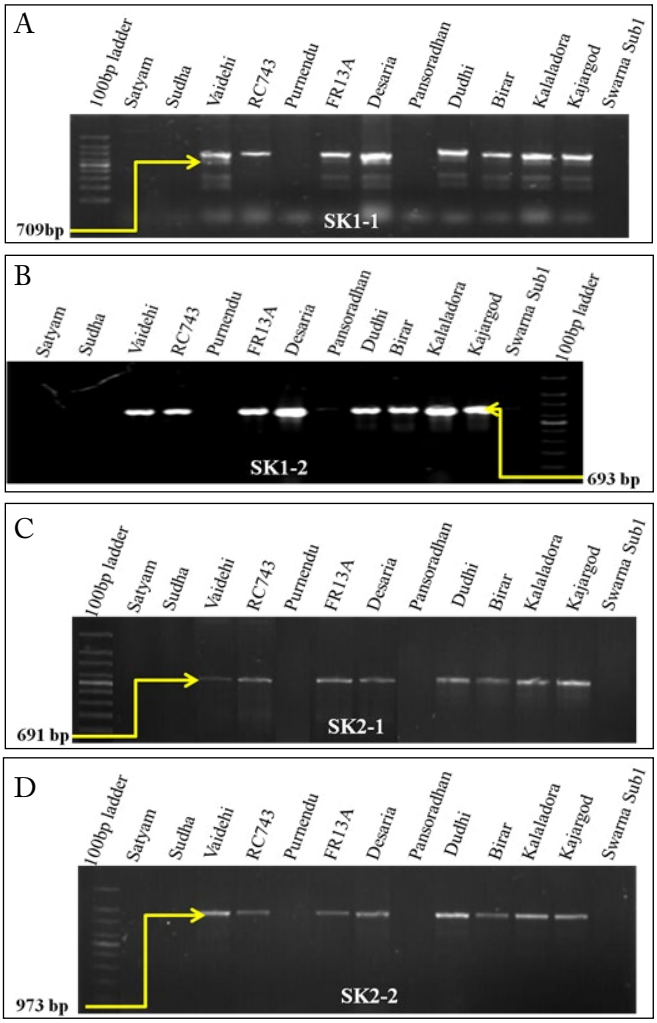

Figure 1: PCR amplification of gene-specific primers on 13 rice genotypes A) SK1-1 B) SK1-2 C) SK2-1 and D) SK2-2

(Table 2). The genotype Desaria showed fastest regained growth soon after de-submergence while Satyam showed the slowest growth. The shoot length was measured before submergence and after 14 days of submergence to calculate the shoot elongation percentage. The shoot elongation percentage ranged from 6.19% to 23.24%. Swarna Sub1 showed the least shoot elongation percentage of 6.19,

Table 2: Survival percentage, shoot length before and after submergence and shoot elongation percentage of 13 genotypes

Sl. No.	Genotypes	Survival percentage after 14 days of de-submergence	Shoot length (cm) before submergence	Shoot length (cm) after submergence	Shoot elongation percentage (%)
1.	Satyam	24.00^{c}	82.50^{e}	89.50^{f}	8.49^{c}
2.	Sudha	31.50^{bc}	114.00^{ab}	125.00^{bcd}	9.65^{bc}
3.	Vaidehi	42.50^{b}	113.50^{ab}	126.00^{bcd}	11.01^{abc}
4.	RYC743	96.50^{a}	76.50^{e}	93.50^{f}	22.22^{ab}
5.	Purnendu	92.00^{a}	118.00^{a}	128.50^{bc}	8.90^{c}
6.	FR13A	97.00^{a}	116.00^{a}	126.50^{bc}	9.05^{bc}
7.	Desaria	98.00^{a}	120.50^{a}	148.50^{a}	23.24^{a}
8.	Pansoradhan	37.50^{b}	101.00^{d}	110.50^{e}	9.41^{bc}
9.	Dudhi	92.50^{a}	104.00^{bcd}	117.50^{cde}	12.98^{abc}
10.	Birar	90.50^{a}	101.50^{cd}	114.50^{de}	12.81^{abc}
11.	Kalaladora	91.00^{a}	120.00^{a}	133.00^{b}	10.83^{abc}
12.	Kajargod	92.00^{a}	113.00^{abc}	128.00^{bc}	13.27^{abc}
13.	Swarna Sub1	95.50^{a}	48.50^{f}	51.50^{g}	6.19^{c}
	CD (p=0.001)	5.24	2.63	2.89	
	CV	7.17	5.29	4.71	

which is on par with genotype Satyam and Purnendu. The genotype Desaria and RYC743 had higher rates of elongation (of what) 23.24% and 22.22%, respectively. The study has revealed that Satyam, Sudha, Purnendu, FR13A, Pansoradhan and Swarna Sub1 has lower shoot elongation rate compared to other genotypes. Moreover, Satyam, Sudha, Purnendu, Pansoradhan and Swarna Sub1 were lacking *SNORKEL* genes. While FR13A possessed *SNORKEL* genes as well as *Sub1* but had lower elongation rate which might be due to interaction between *SNORKEL* and *Sub1* genes. However, it is not the case with RYC743. In deep water rice genotypes, when plants are covered in water, stimulating cell division in their stems due to the presence of *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) genes help them to elongate. The internode elongation in deep water rice genotypes is induced by ethylene and GA production, which is contrasting to that of the *Sub 1* mode of action. The *Sub1* alone is not sufficient in conditions where flooding lasts for more than 15 days. The correlation analysis between shoot elongation percentage and survival percentage showed a non-significant correlation (0.38). The genotypes Purnendu, FR13A and Swarna Sub1 showed slower rate of shoot elongation but had better survival under submergence. Generally, the deep water rice genotypes have fast shoot elongation ability which in turn consumes high amount of carbohydrate. Singh et al. (2017) reported that the deep water genotypes elongate by 25 cm per day as the flood water level increases. The rapid elongation allows the leaf tips to extend above the water surface and thus, enables the rice plants to efficiently photosynthesize and exchange gases for respiration (Vergara et al., 1976). A work of Fukao et al. (2006) and Xu et al. (2006) suggests that the molecular mechanism underlying reduced shoot elongation as an adaptive strategy for submergence tolerance. It was reported that plants conserve carbohydrates and energy under prolonged flooding conditions and recover normal growth and development after the flood subsides (Kende et al., 1998, Bui et al., 2019). Thus, rice genotypes having slower shoot elongation ability under submergence are preferred for cultivation in the areas affected with flash flooding, while the genotypes having faster shoot elongation ability are considered as suitable for deep water areas (Sarkar et al., 2012, Vergara et al., 2014).

Our study suggests that the rice genotypes RYC743 and FR13A have both *Sub1* locus (data not shown) and *SNORKEL* genes. Therefore, these genotypes have tolerance ability of flash flood as well as deep water flooding. These two genotypes can be used as donors in breeding programme to develop deep water rice variety. Most of deep water rice genotypes survive by elongation of stems, whereas non-deep water rice genotypes lack this characteristic and are destroyed by deep water. Deep water flooding tolerance linked markers can be precisely used for differentiation of deep water from non-deep water rice genotypes.

4. CONCLUSION

The present study provided the new gene specific markers namely, SK1-1, SK1-2, SK2-1 and SK2-2

for the identification of the deep water rice varieties having *SNORKEL* genes and differentiation of the genotypes can be done even at the seedling stage which will save time and money. The results clearly revealed the utility of this gene specific markers in breeding applications like germplasm screening and marker assisted selection. The study has also revealed that the rice genotypes Desaria and RYC743 had higher rates of shoot elongation in response to submergence.

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