International Journal of Genetics and Genomics
2018; 6(4): 44-51
http://www.sciencepublishinggroup.com/j/ijgg
doi: 10.11648/j.ijgg.20180604.12
ISSN: 2376-7340 (Print); ISSN: 2376-7359 (Online)

Identification of Novel Submergence Tolerant Local Rice Cultivars of Bangladesh

Al Amin[1, *], Khandakar Md Iftekharuddaula[1], Animesh Sarker[4], Ashraf Hossain Talukder[4], Sharmishtha Ghoshal[1], A. K. M. Shalahuddin[1], Tamal Lata Aditya[2], Md Ansar Ali[3], Bertrand Collard[5]

[1]Plant Breeding Division, Bangladesh Rice Research Institute, Gazipur, Bangladesh

[2]Director (Research) Office, Bangladesh Rice Research Institute, Gazipur, Bangladesh

[3]Director (Administration and Common Services) Office, Bangladesh Rice Research Institute, Gazipur, Bangladesh

[4]Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Tangail, Bangladesh

[5]Department of Breeding and Varieties, Sugar Research Australia (SRA), Gordonvale, Australia

Email address:

alamingenetics@gmail.com (A. Amin), kiftekhar03@yahoo.com (K. M. Iftekharuddaula), animesh_du_geb@yahoo.com (A. Sarker), ashraf82_bmb@yahoo.com (A. H. Talukder), sharmistha.ghosal@yahoo.com (S. Ghoshal), jojsau@gmail.com (A. K. M. Shalahuddin), tamaladitya@yahoo.com (T. L. Aditya), maalibrri@yahoo.com (M. A. Ali), bcycollard@gmail.com (B. Collard)

[*]Corresponding Author

To cite this article:

Al Amin, Khandakar Md Iftekharuddaula, Animesh Sarker, Ashraf Hossain Talukder, Sharmishtha Ghoshal, A. K. M. Shalahuddin, Tamal Lata Aditya, Md Ansar Ali, Bertrand Collard. Identification of Novel Submergence Tolerant Local Rice Cultivars of Bangladesh. *International Journal of Genetics and Genomics*. Vol. 6, No. 4, 2018, pp. 44-51. doi: 10.11648/j.ijgg.20180604.12

Received: December 16, 2018; **Accepted**: December 28, 2018; **Published**: January 28, 2019

Abstract: Bangladesh had harbored numerous submergence tolerant rice local cultivars or landraces (popularly known as Joli Aman). As the local cultivars endure intervallic flash-flooding year after year, it is presumed that genes/Quantitative Trait Loci (QTLs) that give resilience are probably going to be available in these genotypes. In submergence screening, the performance of three tested accessions (Lakhi, Atshotti and Damsi) demonstrated them to be as tolerant as the check FR13A. Other germplasm accessions (DSL-78-8, Laldepa, Putidepa, Laxmi digha) performed great recuperation capacity after de-submergence. Damsi, Rajasail, Haloi, Lal Digha, Manik Digha, Bhawalia, DSL-78-8, Putidepa, Atshotti and Jalda IRRI don't have the *SUB1* QTL and furthermore not like the BR5, a submergence susceptible check variety. Bajal, Horkoach, Pathornuti, Laxmi Digha, Horinga Digha, Bhawalia Digha and BR5 was not amplified by Gns2 conferring *SUB1* QTL (submergence tolerance specific QTL). Significantly, the local cultivars i.e. DSL-78-8, Putidepa and Sadadanga boro were identified as having better submergence tolerance however having no resistant alleles of GnS2 and Sub1C173, indel markers specific to *SUB1* QTL. The result of molecular screening revealed new submergence tolerant rice cultivars which do not possess *SUB*1 QTL. To discover new submergence tolerant QTLs other than *SUB1* through QTL mapping, newly identified submergence tolerant germplasm can be utilized.

Keywords: Submergence, Molecular Screening, Accessions, Landraces, *SUB1* QTL, Indel Markers

1. Introduction

The rice cultivation in the lowland of Bangladesh is affected by flash floods many times in a year. The local germplasm are the best adapted to their rainfed lowland environment by thriving to withstand different abiotic and biotic stresses [1-3]. The local germplasm, having the intermediate gene pool between wild progenitors and modern cultivars, harbor many novel genes and has the prospective to tolerate abiotic and biotic stresses [4-6]. As the local germplasm survive intervallic flooding for many years, it is implicit that genes/QTLs that confer submergence tolerance are likely to be present in these local genotypes. Therefore it is high time to select potential tolerant rice cultivars for breeding program to develop more submergence tolerant rice

as well as flash flood tolerant rice variety [7]. Earlier findings had revealed that submergence tolerance is controlled by a particular major quantitative trait loci or QTL resided on the chromosome 9, in participation with a few number of minor QTLs. The Sub1 QTL was fine-mapped using 2950 F2 segregating population and its genomic region of approximately 0.06 cM [8]. The sequencing of *Sub1*QTL containing genomic region in FR13A-derived breeding lines unveiled the presence of three genes namely *Sub1A*, *Sub1B* and *Sub1C* those encode putative ethylene responsive factors (ERF). *Sub1A*, *Sub1B* and *Sub1C* genes were successively recognized as the major factor of submergence tolerance with the coefficient of determination or R^2 value of 69% and a Logarithm of the Odds (LOD) score of 36. *Sub1* QTL causes withstand for complete submergence up to two weeks [9]. It was also found that, the *Sub1A* gene is key contributor for tolerance. The landrace FR13A has been used in all these studies, which is one of the remarkable submergence-tolerant local donor varieties. An important survey of 13 rice genotypes having Sub1A found that the capacity of submergence tolerance positively associates with the expression level of Sub1A during submergence [10].

Out of three genes, only *Sub1A* reduces ethylene production as well as boosts mRNA and protein production of two negative regulators of gibberellic acid (GA) signaling, resulting in the suppression of the energy-consuming escape response [11-13]. Reliably, transcriptome analysis demonstrated that Sub1A regulates the plenty of mRNAs linked with ethylene and gibberellic acid (GA) production and also signaling during submergence [14]. One of the utmost promising solutions is to introgress *SUB1* QTL into high yielding varieties and that may be promptly adopted by the farmers in the submergence prone area. It is also revealed that submergence tolerance is controlled by a single major quantitative trait locus (QTL) which is found on chromosome 9, along with a number of minor QTLs [15].

This QTL has been successfully introgressed using marker-assisted backcrossing (MAB) into a popular high-yielding Indian rice variety namely Swarna, within two years' time frame [16]. Swarna-Sub1, the best example of a submergence-tolerant well adopted variety, is currently being cultivated in submergence-prone and rainfed lowland areas of India and Bangladesh. The agronomic performance, grain yield and grain quality between Swarna and Swarna-Sub1 were observed similar under non-submerged control conditions indicating complete restoration of the Swarna background in Swarna-Sub1, but Swarna-Sub1 produces a double or higher yield advantages over Swarna after submergence for two weeks or more during the vegetative stage [17-19]. These results indicated the good opportunity to develop further high-yielding varieties that are well adapted to other environments. Moreover, hybrid rice has been assumed as a hopeful approach that can be used as a vector to increase rice production. Recently, there has been growing interest to cultivate hybrid rice in a number of countries, and the prospective use of Sub1 in hybrid rice has great potential for flood-prone regions. Here, current advances in converting additional mega varieties to submergence-tolerant varieties are described and the role of Sub1 in different genetic backgrounds established. The significance of the Sub1A and Sub1C genes for tolerance is further investigated, and the potential use of Sub1 QTL in hybrid rice is evaluated. Inferences for the improvement of additional submergence-tolerant varieties with Sub1, and the development of varieties with a higher level of tolerance under longer durations of submergence are needed.

This Sub1 QTL also has been successfully introgressed into some popular high yielding rice varieties at the International Rice Research Institute (IRRI) and Bangladesh Rice Research Institute (BRRI) [20-23]. Bangladesh Rice Research Institute (BRRI) and the Bangladesh Institute of Nuclear Agriculture (BINA) so far released BRRI dhan51 (Swarna-Sub1) and BRRI dhan52 (BR11-Sub1), BRRI dhan79 (BRRI dhan49-Sub1) and BINA dhan11 (Ciherang-Sub1) and BINA dhan12 (Sambha Mahshuri-Sub1) respectively.

Submergence stress not only depends on the duration of submergence but also depends on various environmental factors such as water temperature, depth of floodwater, water turbidity, dissolved O_2 and CO_2, number of sunny days and soil fertility. Submergence is a polygenic trait and only the *SUB1* QTL does not completely represent the trait alone [24]. Novel submergence-tolerant QTLs that supplement *SUB1* need to be discovered and then pyramided with SUB1 for increased level of tolerance. Thus far, however, very few sources of submergence tolerant accessions have been detected that are not derived from the original FR13A donor [19]. The first step in identifying new QTLs for submergence tolerance is to identify new germplasm accessions having a level of submergence tolerance similar to that of Sub1 lines but that do not have the same FR13A-derived SUB1 QTL allele. In Bangladesh, limited information is available on the allelic diversity among submergence-tolerant germplasm at the molecular level. Molecular markers can reveal differences among accessions at the DNA level and provide a more direct, reliable and efficient tool for crop improvement in plant breeding. This study was performed to identify new sources of submergence tolerant donors through characterization of the allelic diversity within the SUB1 region using 20 selected DNA markers genotyped across a subset of newly characterized submergence-tolerant accessions from Bangladesh. The output of this study is expected to pave the way towards unveiling new submergence tolerance QTLs that are additive to SUB1 that can be used to breed for higher levels of submergence tolerance.

Genetic diversity of crop plants is the key resource for maintaining agricultural productivity [25]. For these reasons, characterization and quantification of genetic diversity has been a major objective in evolutionary biology. The genetic diversity information within and among nearly related varieties is important for rational practice of genetic resources. The study of genetic unevenness within and among breeding materials is of critical and very important to

plant breeders [26]. Currently the progresses of molecular biology and biotechnology allow easier analysis of large number of loci distributed throughout the plant genome. As it has been proven that, molecular markers are powerful tools in the calculation of genetic variation and in the interpretation of genetic relationships within and among species. This experiment aims to study the presence of Sub1 QTL region in the native landraces, collected from submergence prone environment by Genetic Resource and Seed Division of Bangladesh Rice Research Institute (BRRI), so as to generate important information towards our understanding of the molecular mechanisms linked to submergence tolerance in rice. The output of molecular screening is expected to unveil the way of discovering new submergence tolerant QTLs other than *SUB1* through QTL mapping using newly identified submergence tolerant germplasm.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted at the Submergence Tank and Molecular Biology Laboratory of Plant Breeding Division, Bangladesh Rice Research Institute (BRRI), Gazipur -1701, Bangladesh.

2.2. Morphological Screening of Germplasm

A total of 20 landraces or local cultivars, 1 susceptible check BR5 and 1 tolerant check FR13A were tested in this experiment. Fourteen days old seedlings were transplanted in the Plant Breeding Division's submergence tank. The field layout was RCB design with three replications. The spacing was 20 cm × 20 cm and 1 row (30 hills/row) was maintained for each genotype. The genotypes were submerged for around 15 days at a depth of 98-105 cm at 14 days after transplanting. During submergence period, the water of the tank was made turbid twice daily and the light intensity in upper level (normal), mid-level (30 cm below the water surface) and lower level (75 cm below the water surface) of the tank water were measured through light meter (LI-250) (Table 1). The water pH and temperature were also measured and recorded (Table 1). At 15 days after submergence, the water was drained out from the submergence tank. Sanitation was done at 7 days after subsiding of water. Date of seeding, date of transplanting, date of submergence and date of de-submergence were 17/04/15, 01/05/15, 20/5/15 and 4/6/15, respectively. Fertilizers were applied @ 90 kg urea, 50 kg TSP, 70 kg MP, 56 kg gypsum and 8 kg zinc sulphate/ha. Total amount of P, S and Zn and two-third MP were applied at the time of final land preparation. Weeding and other cultural operations were done in time. Data for different parameters were taken following the methodologies as described below:

Seedling height (cm): Seedling heights of 10 plants were measured just after transplanting.

Final seedling height (cm): Seedling heights of 10 plants were measured at 5 days after de-submergence.

Seedling weight (grams): Seedling weight was measured from 10 plants taken just before transplanting and after drying in an oven at 50°C for 72 hours.

Seedling strength (g/cm): This parameter was measured by the following formula:

$$\text{Seedling strength} = \frac{\text{Seedling weight (g)}}{\text{Seedling height (cm)}}$$

$$\text{Survival}\% = \frac{\text{Number of surviving plants 5 days after de-submergence}}{\text{Plant numbers just before submergence}} \text{ X } 100$$

$$\text{Elongation}\% = \frac{\text{Seedling height 5 days after de-submergence - Seedling height just before submergence}}{\text{Seedling height just before submergence}} \text{ X } 100$$

Recovery ability and tolerance score: These were recorded at 5 and 30 days after de-submergence on the basis of tillering ability,% survival and vegetative growth, etc., following the IRRI (2002) standard evaluation system (SES).

The correlation coefficient of survival percentage with seedling height (cm), seedling weight (g), seedling strength (g/cm), final seedling height (cm) and elongation percentage was calculated using correlation analysis.

Table 1. *Range of light intensity, pH and temperature of water during submergence period.*

Water level	Light intensity (w/m^2)		Water pH	Water temperature (˚C)
	Before turbidity	After turbidity		
Upper level	100-135	-	7.1-7.4	30-31.5
Mid-level	22-27	1-1.9		
Lower level	2.5-6.4	0-0.021		

2.3. Molecular Screening of New Submergence Tolerant Germplasm of Bangladesh

2.3.1. Plant materials and DNA markers

A total of 20 landraces, 1 susceptible check BR5 and 1 tolerant check FR13A were tested in this experiment. (Table 2). Two Sub1-region primers in the Chromosome 9 with clear amplifications were selected for molecular characterization of those 20 genotypes (Table 3). GnS2, a gene-based CAPS marker specific to putative Sub1A gene within *SUB1* QTL and other gene-based STS/indel marker Sub1C173 were used to confirm presence of *SUB1* QTL in the 20 landraces [16-19].

Table 2. *List of genotypes used for molecular characterization.*

SN.	Designation	SN.	Designation
1.	Lakhi	12.	Songatapi
2.	Saita	13.	Laxmi Digha
3.	Haloi	14.	Damsi
4.	Bajal	15.	Bhaualia Digha
5.	Horcoach	16.	Lal Digha
6.	Manik Digha	17	Puti depa
7.	DSL 78-8	18	Atshotti
8.	Rajasail	19	Sadadanga Boro
9.	Pathor Nuti	20	Jalda IRRI
10.	Hoinga digha	21	FR13A (Resistant Check)
11.	Bhaualia	22	BR5 (Susceptible Check)

Table 3. *List of primers used for molecular characterization of 20 landraces.*

SN	Primers	Position	Allele Size	Forward	Reverse	Repeat motif
1.	Gns2	Exon of Sub1A	251 and 97, 130	CTTCTTGCTCAACGACAACG	TCGATGGGGTCTTGATCTCT	N/A
2.	Sub 1C173	Exon of Sub1C	158–176	CTACTTCAATGTCACCAACG	TAGAAGATGGAAGACCTGAT	$(AGC)_{10}$

2.3.2. Genotyping Protocol

DNA was extracted from young leaves following CTAB method [27]. The concentration of extracted DNA was estimated by Spectrophotometer method using Nano Drop spectrophotometer (ND 2000). PCR for 1 indel Sub1-region primer Sub1C173 and CAPS marker GnS2 were carried out in a 20 µl reaction volume containing 2 µl of 10X PCR buffer (Mg^{++}), 0.4 µl of 10 mM dNTPs, 2 µl of 5 U/µL Taq DNA polymerase, 1 µl of 10 µM forward and reverse primers, 1 µl DMSO (Dimethyl Sulphoxide), 8.6 µl Nano pure water and 4 µl (25ng/µg) of DNA using a 96 well thermal cycler. The mixture was overlaid with 10 µl of mineral oil to prevent evaporation.

The temperature profile used for PCR amplification of marker GnS2 comprised 94°C for 5 min (initial denaturation) followed by 35 cycles of 94°C for 30 second (denaturation), 55°C for 30 second (annealing), 72°C for 1 min 30 second (extension) with a final extension for 7 min at 72°C at the end of 35 cycles. The PCR product was preserved at 4°C temperature in the thermal cycler. After determining the amplification success, the PCR product was used for restriction digestion. The amplification of the PCR product was checked initially by adding 2 µl of 10X loading dye with 4 µl of PCR product and by loading 2 µl of the mixer solution in PAGE. The gel running time was 2 hours with 100 volt. After that the PCR product (without adding loading dye) was digested with *Alu1* restriction enzyme in the incubator keeping the temperature constant at 37°C for 12 hours. The protocol for digestion reaction was *Alu1* = 0.35 µl, NE Buffer 2 = 2.0 µl, H_2O = 10.65 µl, PCR product = 7.0 µl. The digested product was separated in 6% PAGE and the gel running time was just an hour.

The temperature profile used for PCR amplification of marker Sub1C173 comprised 94°C for 5 min (initial denaturation) followed by 35 cycles of 94°C for 45 second (denaturation), 58°C for 45 second (annealing), 72°C for 1 min 30 second (extension) with a final extension for 7 min at 72°C at the end of 35 cycles. The PCR products were mixed with 10X gel loading dye (Bromophenol blue, Xylene cyanol and Glycerol) and electrophoresed in 6% polyacrylamide gel using vertical polyacrylamide gels for high throughput manual genotyping. 2-3 µl of amplification products were resolved by running gel in 0.5X TBE buffer for 1.5hrs to 2.5hrs depending upon the allele size at around 100 volts and 200 mA electricity. As a standard marker 1 Kb^{+} DNA ladder was used to determine the amplicon size. The gels were stained in 1 µg/ml ethidium bromide and were documented using Molecular Imager gel documentation unit (XR System, BIO-RAD, Korea).

3. Results and Discussion

3.1. Screening of Local Germplasm Under Complete Submergence

During the period of submergence, the average light intensity was 1050–1400 µmol/m^2/s in the upper level before turbidity, but was 410–60 in the mid-level and 55–2 µmol/m^2/s in the lower level of the tank. On average, water temperature was 28–32°C, pH was 7.9–8.1 and dissolved O_2 was 2.8–4.0 mg/L. Under this condition and a longer period of submergence (18 days), screening results showed a range of 0.001–0.004 gm/cm for seedling strength, 20.1–273.75% for elongation and 5.26–93.75% for survival across all the tested accessions. The performance of three tested accessions (Lakhi, Atshotti and Damsi) showed them to be as tolerant as the check FR13A. The survival of Lakhi and Saita was 88.89% and 83.3% and 84.62% while the survival of tolerant check FR13A was 93.75%. Other germplasm accessions (DSL-78-8, Laldepa, Putidepa, Laxmi digha) performed reasonably well according to their good recovery ability after de-submergence, even though their tolerance was not high as that of FR13A. The survival of these accessions was more than 50% (Table 4). The results of screening for selected tolerant accessions along with the checks conducted in Aman

2015 confirmed the tolerance of the accessions.

Table 4. *Average performance of 22 rice germplasm on their survival, tolerance score and recovery under 18 days of complete submergence, Aman 2014. FR13A and BR5 were used as tolerant and susceptible checks, respectively.*

SN.	Genotype	Seedling strength (gm/cm)	%Elongation	%Dry matter increased	%survivability	SES Score	Recovery ability
1.	Lakhi	0.003	120.0	141.9	88.89	5.0	Good
2.	Saita	0.002	50.6	173.3	75.00	5.0	Good
3.	Damsi	0.003	146.5	260.2	84.62	5.0	Good
4.	Rajasail	0.003	105.6	176.8	55.56	7.0	Fair
5.	Bajal	0.004	175.3	982.9	68.00	7.0	Fair
6.	Horkoach	0.002	146.4	416.9	60.00	7.0	Fair
7.	Haloi	0.002	123.0	239.5	52.73	7.0	Fair
8.	Pathornuti	0.002	122.8	56.8	60.00	7.0	Fair
9.	Laxmi digha	0.002	273.5	218.2	78.95	5.0	Good
10.	Lal digha	0.003	236.2	438.5	59.23	9.0	Fair
11.	Horinga digha	0.002	213.8	1307.4	56.67	9.0	Fair
12.	Manik digha	0.003	206.3	70.8	50.70	9.0	Fair
13.	Bhaualia	0.002	136.5	176.9	54.48	9.0	Fair
14.	Bhaualia digha	0.003	174.6	239.6	52.00	9.0	Fair
15.	DSL 78-8	0.003	153.1	296.8	76.00	5.0	Good
16.	Putidepa	0.002	20.1	142.6	74.19	5.0	Good
17.	Atshotti	0.002	88.9	80.6	83.33	5.0	Good
18.	Songhatepi	0.003	128.4	246.2	76.00	5.0	Good
19.	FR13A	0.002	82.3	229.4	93.75	5.0	Very good
20.	BR5	0.001	169.6	230.8	5.26	9.0	Poor
21.	Sadadanga Boro	0.001	30.6	35.4	78.13	5.0	Very good
22.	Jalda IRRI	0.003	99.0	73.0	65.22	7.0	Good

SES Score.

Score	Scale (% Survivability)
1	100
3	95-99
5	75-94
7	50-74
9	0-49

Figure 1. *Screening of local cultivars of rice under controlled submergence.*

3.2. Molecular Screening of Local Germplasm to Identify SUB1 QTL

The first step in identifying new QTLs for submergence tolerance is to identify new germplasm accessions having a level of submergence tolerance similar to that of Sub1 lines but that do not have the same FR13A-derived *SUB1* QTL allele. In Bangladesh, limited information is available on submergence-tolerant germplasm at the molecular level. Molecular markers can reveal differences among accessions at the DNA level and provide a more direct, reliable and efficient tool for crop improvement in plant breeding. This study was performed to identify new sources of submergence tolerant donors through characterization of the *SUB1* region using Gns2 DNA marker genotyped across a subset of newly characterized submergence-tolerant accessions from Bangladesh. The output of this study is expected to pave the way towards unveiling new submergence tolerance QTLs that are additive to *SUB1* that can be used to breed for higher levels of submergence tolerance.

Gns2, a *SUB1A* specific marker which is amplified in the genotypes containing the SUB1 QTL. The molecular screening of landraces showed that, Lakhi, Saita, Songatepi, Sadadanga Boro are similar to the submergence tolerant FR13A and they possess *SUB1* QTL. Damsi, Rajasail, Haloi, Lal Digha, Manik Digha, Bhawalia, DSL-78-8, Putidepa, Atshotti and Jalda IRRI do not possess the *SUB1* QTL and also not similar to the BR5, a submergence susceptible check variety. Bajal, Horkoach, Pathornuti, Laxmi Digha, Horinga Digha, Bhawalia Digha and BR5 was not amplified by Gns2.

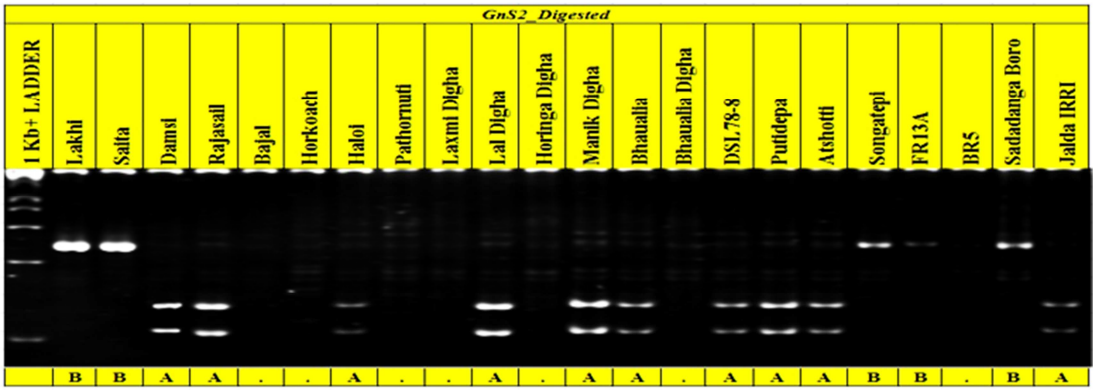

Figure 2. *Partial view of the gel picture of submergence tolerant landraces using Gns2.*

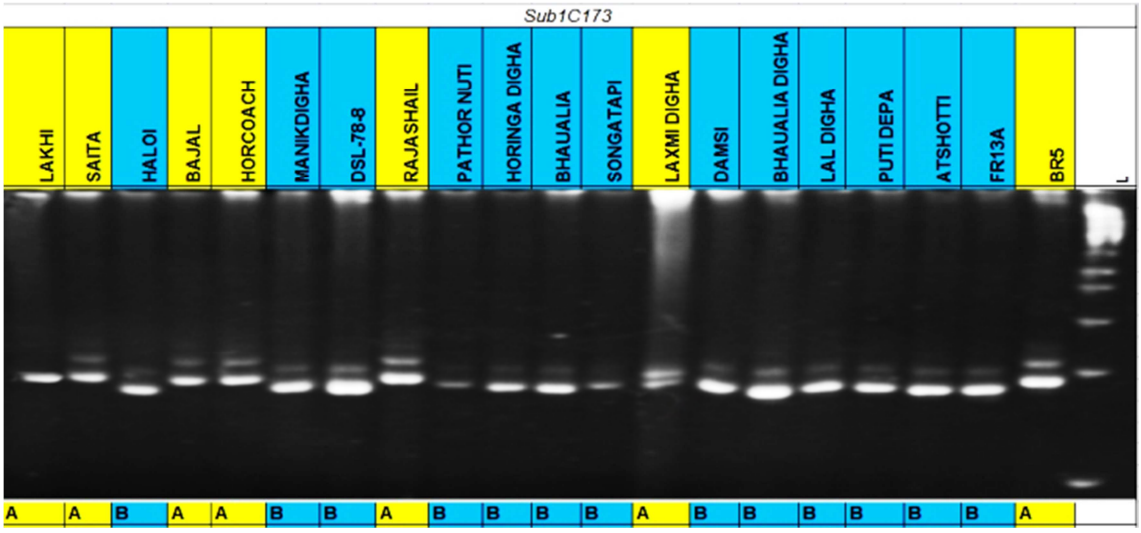

Figure 3. *Partial view of the gel picture of submergence tolerant landraces using Sub1C173.*

According to molecular and morphological screening result, there are four types of genotypes observed. Cluster I represents flash flooding susceptible rice variety check BR5. Cluster II shows genotypes that have similar genotypes to FR13A namely Lakhi, Saita, Songatepi, and Sadadanga Boro. Cluster III included ten and cluster IV included six flash-flooding submergence tolerant local rice cultivars having an expected submergence tolerance gene or genes other than the SUB1 QTL (Figure 5).

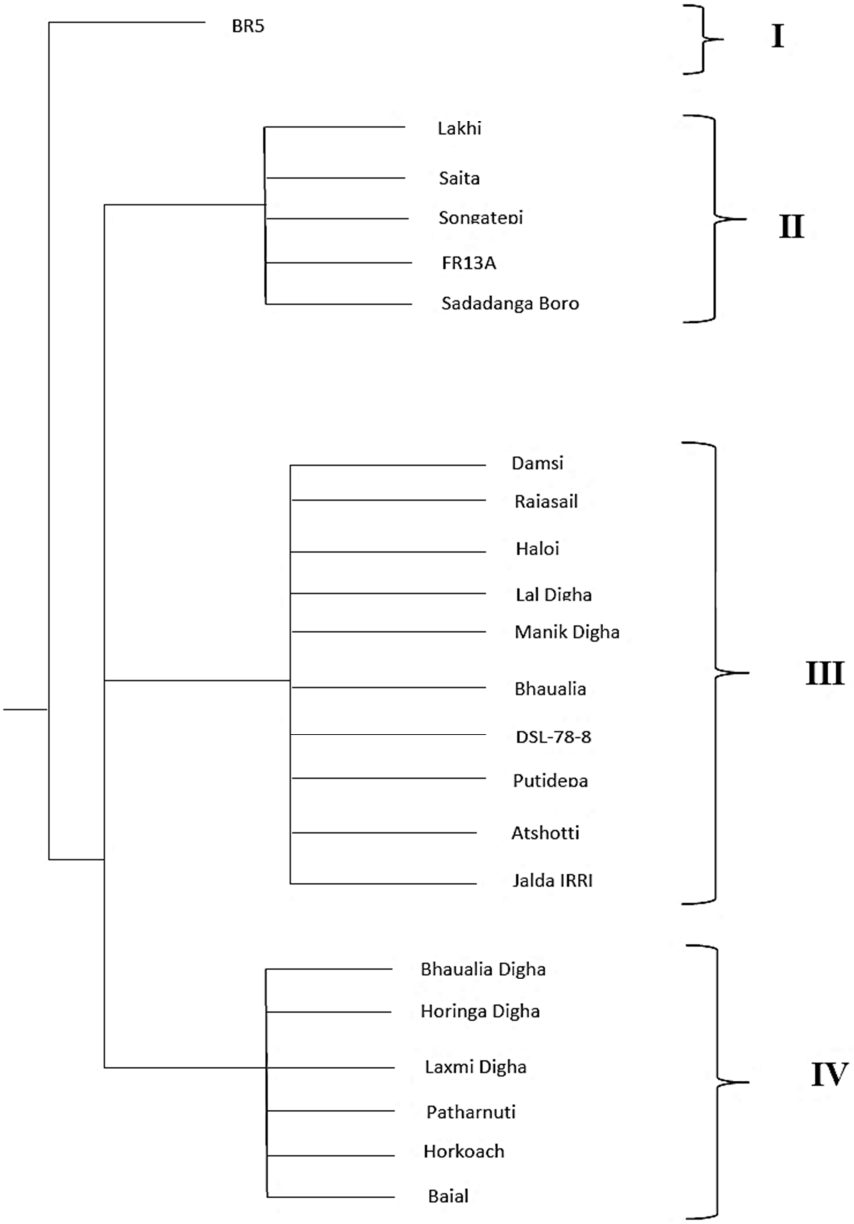

Figure 4. *A cluster dendrogram representing the phylogenetic relationships among 22 accessions based on SUB1 alleles detected by SUB1 specific markers at the SUB1 region on chromosome 9 and phenotypic study.*

4. Conclusion

The morphological and molecular screening of submergence tolerant landraces reflected the new submergence tolerant donor parent and few of them having no *SUB1* QTL. Out of 20 landraces, the survival of Lakhi and Saita was 88.89% and 83.3% and 84.62%. Other germplasm accessions (DSL-78-8, Laldepa, Putidepa, Laxmi digha) performed reasonably well according to their good recovery ability after de-submergence, even though their tolerance was not high as that of FR13A. The survival of these accessions was more than 50%. The molecular screening of landraces showed that, Lakhi, Saita, Songatepi, Sadadanga Boro are similar to the submergence tolerant FR13A and they possess *SUB1* QTL. Damsi, Rajasail, Haloi, Lal Digha, Manik Digha, Bhawalia, DSL-78-8, Putidepa, Atshotti and Jalda IRRI do not possess the *SUB1* QTL and also not similar to the BR5, a submergence susceptible check variety. Bajal, Horkoach, Pathornuti, Laxmi Digha, Horinga Digha, Bhawalia Digha and BR5 was not amplified by Gns2. So, Submergence tolerant genotypes but not having *SUB1* QTL can be further studied to discover novel genes or QTLs for submergence tolerance of rice germplasm.

Acknowledgements

The author is grateful to BRRI authorities for providing supports in this research work. This research activity was financially supported the Integrated Agricultural Productivity Project (IAPP) funded by the Global Agri-culture and Food Security Program (GAFSP).

References

[1] Pusadee, T., Jamjod, S., Chiang, Y. C., Rerkasem, B., Schaal, B. A. (2009). Genetic structure and isolation by distance in 482 a landrace of Thai rice. Proc Nat Acad Sci 106:13880-13885.

[2] Yang, J., Kloepper, J. W., Ryu, C. M. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

[3] Zhao, D. Y., Ma, T., Zeng, J., Yan, W. M., Wang, J. Q., Huang, R., Wang, M. (2011). Effects of lake water chemistry on bacterioplankton community structures of three lakes. Afr. J. Microbiol. Res., 5: 4820-4831.

[4] Hanamaratti, N., Prashanthi, S., Salimath, P. M., Hanchinal, R. R., Mohankumar, H. D., Parameshwarappa, K. G., Raikar, S. D. (2008). Traditional land races of rice in Karnataka: Reservoirs of valuable traits. Current Science. 94. 242-247.

[5] Lisa, A. L., Sabrina, L., Rahman, M., Saima S., Iwasaki, T., Hasan, A. K. M., Kosuge, K., Fukami, Y. & Seraj, Z. (2011). Physiology and gene expression of the rice landrace Horkuch under salt stress. Functional Plant Biology. 38. 282-292. 10.1071/FP10198.

[6] Matin, S., Ashrafuzzaman, M., Islam, M., Sikdar, S., Zobayer, N. (2012). Molecular marker based (SSR) genetic diversity analysis in deep water rice germplasms of Bangladesh. International Journal of Biosciences. 2. 64-72.

[7] Xu, K., Xu, X., Ronald P. C., Mackill D. J., (2000). A high-resolution linkage map of the vicinity of the rice submergence tolerance locus Sub1. Mol Gen Genet, 263(4): 681–689

[8] Xu, K., Xu, X., Fukao, T., Canlas, P., Maghirang, R. R., Heuer, S., Ismail A. M., Bailey S. J., Ronald, P. C., Mackill D. J., (2006). Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature, 442: 705–708.

[9] [9] Singh, N., Dang, T. T. M., Vergara, G. V., Pandey, D. M., Sanchez, D., Neeraja, C. N., Septiningsih, E. M., Mendioro, M., Tecson, M. E. M., Ismail, A. M., Mackill, D. J., Heuer, S. (2010). Molecular marker survey and expression analyses of the rice submergence tolerance gene SUB1A. Theor Appl Genet, 121(8): 1441–1453.

[10] Fukao, T., Bailey, S. J. (2008). Submergence tolerance conferred by Sub1A is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proc Natl Acad Sci U S A.

[11] Fukao, T. Xu, K., Ronald, P. C., Bailey, S. J. (2006). A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. Plant Cell, 18:2021-2034.

[12] Jung, K. H., Cao, P., Seo, Y. S., Dardick, C., Ronald, P. C. (2010). The Rice Kinase Phylogenomics Database: a guide for systematic analysis of the rice kinase super-family. Trends Plant Sci. 15:595–599. doi: 10.1016/j.tplants.2010.08.004.

[13] Xu, K., Mackill, D. J. (1996). A major locus for submergence tolerance mapped on rice chromosome 9. Molecular Breeding. 2:219–224.

[14] Nandi, S., Subudhi, P. K., Senadhira, D., Manigbas, N. L., Sen-Mandi, S., Huang, N. (1997). Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Molecular and General Genetics. 255:1–8

[15] Toojinda, T., Siangliw, M., Tragroonrung, S., Vanavichit, A. (2003). Molecular genetics of submergence tolerance in rice: QTL analysis of key traits. Annals of Botany. 91:243–253.

[16] Neeraja, C., Maghirang-Rodriguez, R., Pamplona, A., Heuer, S., Collard, B., Septiningsih, E. (2007). A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. Theoretical and Applied Genetics. 115:767–776.

[17] Sarkar, R. K., Reddy, J. N., Sharma, S. G., Ismail, A. M. (2006). Physiological basis of submergence tolerance in rice and implications for crop improvement. Current Science. 91:899–906.

[18] Septiningsih, E. M., Pamplona, A. M., Sanchez, D. L., Neeraja, C. N., Vergara, G. V., Heuer, S., Ismail A. M., and Mackill. D. J. (2009). Development of submergence tolerant rice cultivars: the Sub1 locus and beyond. Ann Bot. 103: 151-160.

[19] Septiningsih, E. M., Collard, B. C. Y., Heuer, S., Bailey-Serres, J., Ismail, A. M., and Mackill, D. J. (2013). Applying genomics tools for breeding submergence tolerance in rice. In: R. K. Varshney and R. Tuberosa (eds). Translational genomics for crop breeding: Volume 2 - Improvement for abiotic stress, quality and yield improvement. Wiley-Blackwell, USA, pp. 9-30.

[20] Septiningsih, E. M., Hidayatun, N., Sanchez, D. L., Nugraha, Y., Carandang, J., Pamplona, A. M., Collard, B. C. Y., Ismail A. M., and Mackill. D. J. (2014). Accelerating the development of new submergence tolerant rice varieties: the case of Ciherang-Sub1 and PSB Rc18-Sub1. Euphytica. [DOI: 10.1007/s10681-014-1287-x]

[21] Iftekharuddaula, K. M., Newaz, M. A., Salam, M. A., Ahmed, H. U., Mahbub, M. A. A., Septiningsih, E. M., Collard, B. C. Y., Sanchez, D. L., Pamplona, A. M. and Mackill, D. J. (2011). Rapid and High-Precision Marker Assisted Backcrossing to Introgress the SUB1 QTL into BR11, the Rainfed Lowland Rice Mega Variety of Bangladesh. Euphytica, 178, 83-97. http://dx.doi.org/10.1007/s10681-010-0272-2

[22] Das, S., Misra, R. C., and Patnaik, M. C. (2009). G × E interaction of mid-late rice genotypes in LR and AMMI model and evaluation of adaptability and yield stability. Environ. and Ecol. 27:529-535.

[23] Setter, T & Ellis, M & V Laureles, E & S Ella, E & Senadhira, D & B Mishra, S & Sarkarung, S & Datta, Swapan. (1997). Physiology and Genetics of Submergence Tolerance in Rice. Ann. Bot. 79. 10.1093/oxfordjournals.aob.a010308.

[24] Mohanty, H. K., Mallik, S., and Grover, A. (2000) Prospects of improving flooding tolerance in lowland rice varieties by conventional breeding and genetic engineering. Current Science. 78: 132-137.

[25] Yesmin, N., Elias, S. M., Rahman, M. S., Haque, T., Hasan, A. K. M. M., Seraj, Z. I. (2014). Unique genotypic differences discovered among indigenous Bangladeshi rice landracesInt. J. Genomics. p. 210328, 10.1155/2014/210328

[26] Chakravarthi, B. K., Naravaneni, R. (2006). SSR marker based DNA fingerprinting and diversity study in rice (Oryza sativa. L). African J Biotech 5: 684-688.

[27] Allen, G., Flores, M., Krasnyanski, S., Kumar, S., Thompson, W. (2006). A modified protocol for rapid DNA isolation from plant tissue using cetyltrimethylammonium bromide. Nature protocols. 1. 2320-5. 10.1038/nprot.2006.384.