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Submergence Tolerance in Rice (*Oryza sativa* L.) Genotypes at the Seedling Stage: A Combined Approach of Morphological and Molecular Characterization

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ABSTRACT

Background: Rice (*Oryza sativa* L.) is the major cereal crop often suffers from submergence in the rainfed growing situation of India and South East Asia. Developing rice cultivars with tolerance to submergence is the best strategy to circumvent the situation. In Eastern India a vast reservoir of rice genotypes exists grown in diverse agro-climatic situation under rainfed condition. Screening rice genotypes under submergence stressed condition by observing morphological traits combined with molecular marker approach helps to identify the genotypes having innate capacity to survive under flooding condition more precisely than either of the approach alone. The present study was framed to identify the landraces and genotypes having the presence of desired genes which will helpful for future breeding program.

Methods: Fifty rice (*Oryza sativa* L.) genotypes were evaluated for submergence tolerance using both morphological and molecular characterization in the field and laboratory respectively. Six stress indices, namely elongation%, survival%, mortality%, relative growth index, dry mass% and recovery rate, were examined in the field seven days and fourteen days after submergence. The genotypes were screened with SSR markers to identify the presence of the desired gene.

Result: Based on morphological and molecular screening, out of all rice genotypes, Brahmabalak, Jaladhi 1-2, Jaldubi, Kerala Sundary, China IRRI, Sonachur, Pravat, Pankaj, Ranjit SUB1 and Bahadur SUB1 showed higher levels of tolerance to submergence than the tolerant control Swarna SUB1. They have one or more loci different from the SUB1 locus, which can be bred to create improved submergence-tolerant rice varieties with high yield in the rainfed lowland agroecosystem.

Key words: Molecular, Morphological, Rice, Seedling, Submergence.

INTRODUCTION

Short-term inundation, also referred to as flash floods, is the most catastrophic type of flooding (up to two weeks). According to Bailey-Serres *et al.* (2010) and Mackill *et al.* (1996), there are around 20 million acres of lowland rice production in Africa and Asia (outside of China) that are affected by these flash floods. Flash floods have been projected to have caused an economic loss of up to 11.5 billion US dollars in the Asia Pacific area (Afrin *et al*., 2018). Flash floods are unpredictable, can occur more than once during a season and can harm crops at any stage of development. Yield losses of ten to one hundred percent as a result of flash floods have been recorded. Due to the lack of a mechanism for submergence tolerance, modern high-yielding rice cultivars cannot survive or tolerate the catastrophic effects of flash floods, which results in a decrease in yield. Even though landraces like FR13A have been discovered to be submergence tolerant, their yield performance makes them unsuitable for commercial cultivation (Hilleris lambers *et al.,* 1982).

Submergence tolerance in rice plants can be described as the capacity to withstand total inundation for 10–14 days while also resuming growth after the water has subsided (Catling *et al.,* 1992). The recent discovery and tagging of the SUB1 gene, which was located on rice chromosome 9, represented an incredible advancement in the evolution of submergence tolerance in rice (Xu *et al.,* 1996). The locus SUB1 is responsible for around 70% of the variation caused by submergence. Further detailed mapping of SUB1 (Xu *et al.,* 2000) and validation of SUB1 as a crucial component of submergence tolerance (Kamolsukyunyong *et al*., 2001; Nandi *et al*., 1997; Toojinda *et al*., 2003) have both been

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accomplished. Additionally, SUB1 has been shown to operate as an Ethylene Responsive Factor (ERF) (Xu *et al*., 2006) that inhibits ethylene synthesis and fosters submergence tolerance (Fukao *et al.,* 2006 and 2008). Based on this background, it is pertinent to test germplasm and bred better abiotic tolerant cultivars that can survive flooding. Our present study aims to ascertain the performance of selected rice genotypes in terms of seedling growth parameters and to identify SSR markers useful for screening germplasm and consequently to single out submergence tolerant genotypes precisely based on morphological and molecular descriptor for future breeding programs.

MATERIALS AND METHODS

The Experiments were conducted at field and laboratory of Ramakrishna Mission Vivekananda Educational and Research Institute, Narendrapur, Kolkata, at 2022. Geographically, the experimental region is situated 8 metres above sea level at a latitude of 22043′N and a longitude of 88040’E. The soil’s pH is between 6 and 7, it contains 0.43% organic carbon, 242.89 kg of total nitrogen, 229.60 kg of potassium, 26.05 kg of phosphorus, 28.01 ppm of sulphur and 0.83 ppm of boron (Source: soil testing laboratory, ATC, Narendrapur, Kolkata).

Experimental materials and preparation for field trials

In an artificial screening tank, 50 common rice genotypes (Table 1), including some landraces and well-known cultivars, were tested alongside the submergence-tolerant check Swarna SUB1 and the susceptible check Swarna. The arrangements require 50-holed rectangular mini trays with a size of 60 cm 35 cm 5 cm that are filled with fertilised soil (1:1:1 Verme-compost: Sand: Coco Pit). To break dormancy, uniform size seeds of each genotype were chosen and held at (54±2)°C for 3 days. On the soil surface of the micro rays, direct seeds of each genotype were planted alongside check genotypes for tolerance and susceptibility. Three replications of the completely randomised design were used to examine the genotypes. In the open field, a man-made tank measuring 3.0 m by 3.0 m by 1.3 m was constructed. Healthy seedlings that were 21 days old were kept in the open air for a further 10 days after being kept completely submerged in water for 14 days at a depth of one metre. To act as controls, one group of plants from each genotype was watered frequently but not submerged. To analyse the genotypes and score submergence tolerance, the Standard Evaluation System (SES) (IRRI, 2002) was used (Table 2).

Stress indices were calculated as follows

1. Elongation (%) =

$$\frac{\text{Plant height after submergence - Plant height before submergence}}{\text{Plant height before submergence}} \times 100$$

Barik *et al.* (2020)

2. Survival (%) =

$$\frac{\text{Number of live plants after submergence}}{\text{Number of plants before submergence}} \times 100$$

Barik *et al.* (2020)

3. Mortality (%) =

$$\frac{\text{Number of dead plants after submergence}}{\text{Number of plants before submergence}} \times 100$$

4. Dry matter accumulation (%) =

$$\frac{\text{Dry weight of seeding}}{\text{Fresh weight of seeding}} \times 100$$

Barik *et al.* (2020)

5. Relative growth index (%) =

$$\frac{\text{Dry weight of submerged seeding}}{\text{Dry weight of control seeding}} \times 100$$

Barik *et al.* (2020)

6. Recovery (%) =

$$\frac{\text{Number of recoverd plants after ten days of desubmergence}}{\text{Number of live plants after submergence}} \times 100$$

Tolerance score: Calculated using the Standard Evaluation System (SES), which was created by the International Rice Research Institute (IRRI), based on the percentage of plant survival.

Isolation of genomic DNA

The CTAB (Cetyle trimethylammonium bromide) approach was followed to isolate the genomic DNA of certain rice genotypes. The leaves of seven days old rice plants were used to extract DNA.

SSR marker analysis

Twenty-four of the most submergence-tolerant rice genotypes were chosen for molecular characterization after morphological screening, together with tolerant (Swarna SUB1) and susceptible (Swarna) checks. By using nine simple sequence repeat (SSR) markers from different rice chromosomes that have been connected to the submergence tolerance trait (Table 3), DNA fingerprinting of the chosen genotypes was performed. These markers’ precise sequence information was confirmed by the NCBI database (http://www.ncbi.nlm.nih.gov/).

Using the Polymerase Chain Reaction (PCR), DNA was amplified. The following ingredients were used in a 25 µl reaction mixture for PCR amplification: 17.1 µl double-distilled water, 0.4 µl each of forward and reverse primers, 0.5 µl of dNTPs (10 Mm), 1.5 µl of $MgCl_2$, 2.5 µl of buffer (10×), 0.1 µl of Taq polymerase and 2.5 µl of DNA template. Denaturation at 94°C for 5 min, followed by 35 cycles of 30 S denaturation at 94°C, 30 Seconds annealing at the temperature of the specific primer pair, 1 min extension at

72°C and a final extension at 72°C for 7 min, were the conditions used for PCR amplification. In a horizontally denaturing 3.5% agarose gel, the SSR amplification products were separated. Ethidium bromide staining was used to identify DNA fragments.

Statistical analysis of SSR data

The size of the amplified fragments was determined using Alpha-Ease FC 5.0 software (Alpha Innotech, USA) by comparing the migration distance of the amplified fragments in relation to the molecular weight of the known-size marker (100 bp. Ladder). The effective-allele per locus (Aep) and polymorphic information content (PIC) values of different markers were determined:

$$PIC_i = 1 - \Sigma P_{ij}^2 \quad \text{(Goswami \textit{et al.}, 2017)}$$

The data were exported to binary data for presence (1) and absence (0) or as missing observation for further analysis with DARWIN. The results were obtained by scoring each genotype for the presence or absence of the SSR bands. In order to demonstrate the interconnectedness of the rice genotypes based on distance, factor analysis with DARWIN was also performed.

RESULTS AND DISCUSSION

Performance of Rice genotypes under submergence condition

The performance of rice genotypes decreased in submerged environments, while most of the genotypes performed similarly under control circumstances. After 7 days and 14 days of submersion, there was a significant (P 0.01) difference in the survival rate among the genotypes of rice, ranging from 12.50% to 93.15%. Eighteen genotypes namely, Pankaj, Kerala Sundary, Kanoklata, Sonachur, Pravat, China IRRI, Bishnuprashad, Dehradun Basmati, Baskathi, Brahmabalak, Bahadur SUB1, Ranjit SUB1, Shyamashree, G.B. 2, Zeera, Jyotiprashad, Jaladhi 1-2 and Jaldubi along with Swarna SUB1, the tolerant check, showed survival rate of more than 75.0% and were therefore, categorised as moderately submergence tolerant type. The seedling development measured by the Relative Growth Index was considerably reduced under submergence and distinct genotype variations (P 0.01) were noted, ranging from 13.94% to 74.286% under submergence. Particularly, tolerant check Swarna SUB 1, including SUB 1 introgressed genotypes (Ranjit SUB1 and Bahadur SUB1) and certain landraces (Jaldubi and Jaladhi 1-2) showed a higher Relative Growth Index than Swarna.

In contrast, shoot elongation was found to increase substantially with submersion compared to the control plants, with genotype-specific values ranging from 33.86% to 96.00% (Table 4). In particular, the submergence tolerant Pankaj, which is statistically on par with the tolerant check genotype Swarna SUB1 compared to the susceptible Swarna, showed the least amount of shoot elongation.

Table 1: List of experimental genotypes.

Genotype	Parantage
Annada	MTU-15 × Yaikaku Nantoku
Aghoni Bora	Gandhi bora × KMJ1-52-2
BB 11	Not available
Bhalum	PSN × Line no. 6131
Bahadur SUB1	Bahadur × Swarna SUB1
BR 29	Developed from BR 802-118-4-2
Ranjit SUB1	Ranjit × Swarna SUB1
Rajendra Bhagbati	RAU 1397-18-3-7-9-4-2
Lal Dudheswar	Land-race
Dudheswar	Land-race
Swarna SUB1	Swarna × IR 49830-7-1-2-3
Sahabhagi Dhan	Wayrarem × IR-5541-04
Shyamashree	Local landrace
Shraboni	APMS6B × Piolee
Tetep	Landrace
Tulshi Joha	Traditional variety
G.B. 1	Not available
G.B. 2	Not available
Zeera	Not available
Jyotiprashad	K 343-29-1 × Sweon 334
Jaladhi 1-2	Pure line selection from Baku
Joymoti	Jaya × Mahsuri
Jaldubi	Selection from AR 1023
Kalinga III	AC 540 × Ratna
Kanokchur	Traditional variety
Ketaki Joha	Savithri × Badshabhog
Karma Mahsuri	Mahsuri × R 296-260
Kalabhat	Traditional Variety
Khitish	BU-1 × CR-115
Kamol Boka	Traditional variety
Maharaja	Puff rice variety
Mali no. 1	Selection from local genotype
MTU 1001	Vajram × MTU-7014
MTU 1010	Krishnaveni × IR-64
Swarna	Vasisa × Mahsuri
Pankaj	Peta × Tongkai Rotan
IR 64	IR-5857-33-2-1 × IR-2061-465-1-5-5
IBD 1	MTU-7029 × IR-42253
IBD 2	MTU-7029 × IR-42253
IET 1444	T(N)1 × Co.29
Kerala Sundary	Traditional variety
Kanoklata	Jaya × Mahsuri
Sonachur	Traditional Variety
Ranjit	Pankaj × Mahsuri
Pravat	IR-8 × MTU-3
China IRRI	Not Available
Bishnuprashad	K 343-29-1 × Sweon 334
Baskathi	Landrace
Dehradun Basmati	Selection from Basmati of Dehradun
Brahmabalak	Traditional variety

Mortality% was seen to fluctuate widely from 7.59% to 87.49% fourteen days following submersion at treated conditions. When compared to the susceptible check variety Swarna, genotype Bahadur SUB1, which statistically is on par with genotypes Ranjit SUB1 and Swarna SUB1, showed the lowest mortality %. Submergence significantly (P 0.01) decreased dry mass accumulation rate (DMAR) and a significant (P 0.01) varietal difference was noted. After 14 days of submersion, the range of DMAR was between 9.55 to 35.66 percent. After 14 days of submersion, the range of DM was between 9.55 to 35.66 per cent. In particular, the submergence-tolerant check variety (Swarna SUB 1) and some of the native landraces showed higher DMAR than Swarna, whereas the susceptible variety (Swarna) showed a dramatic fall in DM under submergence compared with the control.

According to Voesenek and Bailey-Serres (2009) and Colmer *et al.* (2014), two types of shoot growth moderation are suggested in genotypes that can tolerate submersion: slowed development with lower energy expenditure and elongation of the internode area of the shoot in response to rising water levels. Based on how each genotype performed differently when submerged, the genotypes were split into four groups. Pravat, China IRRI, Pankaj and Zeera, which were all members of the same group and experienced stunted development when submerged, are among those considered to be especially helpful for areas that are susceptible to flash floods. According to Panda *et al.* (2008), certain genotypes of rice may store energy in such a situation and utilise it later during recovery by restricting the growth of their shoots when submerged. The second group of plants that elongate during the first half of the submergence period and then check their growth included Swarna SUB1, Ranjit SUB1, Bahadur SUB1, Shyamashree, G.B. 1, G.B. 2, Kerala Sundary, Maharaja, Sonachur, Baskathi, Bishnuprashad, Sahabhagi Dhan, Jyotiprashad and Dehradun Basmati.

Responses of rice genotypes under de-submerged condition

Numerous genotypes were restored in the de-submerged state along with the growth of fresh shoots and leaves. There

Table 2: Standard evaluation system of rice for survival score (IRRI, 2002).

Survival (%)	Score	Observation description	Tolerance
100	1	Normal growth and no leaf symptoms	Highly tolerant
95-99	3	Nearly normal growth, but leaf tips or few leaves were whitish and rolled	Tolerant
75-94	5	Growth severely retarded; most leaves rolled; only few were elongating	Moderately tolerant
50-74	7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
0-49	9	Almost all plants were dead	Highly susceptible

Table 3: Sequences of 9 SSR markers.

Name of primer	Position	Sequence of forward primer	Sequence of reverse primer	Tm (°C)
RM6318	Chromosome 2	TGCTGCTTCTGTCCAGTGAG	GGATCATAACAAGTGCCTCG	55
RM125	Chromosome 7	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	55
RM214	Chromosome 7	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	55
RM224	Chromosome 11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	55
RM8300	Chromosome 9	GCTAGTGCAGGGTTGACACA	CTCTGGCCGTTTCATGGTAT	57
RM12168	Chromosome 1	AATCCGTCAACCACCATGAACG	TTGCAGACTCATGGACACATGG	57
SUB1BC1	Between *Sub1B* and *C*	CAATCGATGCGTGCTTCTT	CGCAACAAGGCAGAAAAATA	57
SUB1BC2	Between *Sub1B* and *C*	AAAACAATGGTTCCATACGAGAC	GCCTATCAATGCGTG CTCTT	57
SUB1BC3	Between *Sub1B* and *C*	CATGGGTAAAATTGCCATCC	GCTTGAGGGTGAGTGGAGAG	57

T_m annealing temperature.

Table 4: Analysis of variance of stress indices subjected to submergence.

Source	Treatment (Main plot)	Error 1	Genotype (Sub plot)	Treatment × Genotype	Error 2
Degree of freedom	1	4	49	49	196
Elongation (7) %	77517.08**	43.02	1330.98**	541.53**	37.25
Survival (7) %	67765.49**	159.86	607.32**	607.32**	26.65
Mortality (7) %	88088.45**	204.94	562.01 **	562.01**	29.47
Elongation (14) %	5227.30**	39.89	977.37**	620.85**	31.41
Survival (14) %	103858.35**	153.68	728.72**	728.72**	28.03
Mortality (14) %	108354.73**	21.16	664.31**	664.31**	57.47
Dry mass accumulation %	30044.76**	87.79	279.21**	281.80**	76.12

(7) and (14) seven and fourteen days after submergence, **significant at 1% level.

were several seedlings retrieved in conditions of de-submergence, ranging in number from 6.33 to 23.33 (Table 5). In a de-submerged setting, plant height changed significantly (P 0.01) and significant varietal variations were found in a wide range of 9.77 cm to 33.90 cm (Table 4). The genotype Brahmabalak, which is statistically comparable to China IRRI, had the greatest plant height. The genotype G.B. 2 showed the greatest shoot length (P 0.01) and is statistically comparable to the genotypes Pravat, Brahmabalak, Kerala Sundary and China IRRI. The recovery percentage under de-submergence conditions ranged widely, from 40.78% to 95.89%. With a recovery rate of more than 92.0%, four genotypes-Bahadur SUB1, Jaladhi 1-2, China IRRI and the tolerant check-Swarna SUB1 variety-were classified as submergence tolerant.

Genetic variation of submergence tolerance attributing characters

The PCV, was discovered to be bigger than the GCV for all traits (Table 6) indicating significant role of environment into phenotypic expression. High heritability (77.87%-92.04%) and high GAM (31.62%-148.29%) were found for survival rate, shoot elongation, mortality rate, dry mass, number of recovered plants and recovery rate in submerged and de-submerged situations, respectively. Therefore, it would be beneficial to prioritise these characteristics throughout the selection process for crop enhancement projects.

SSR marker-based genotyping

Nine SSR markers were used in this study to assess the genotypic variance, including RM125, RM214, RM224, RM 8300, RM 12168, RM 6318, SUB1BC1, SUB1BC2 and SUB1BC3. One of the objectives of this investigation was to identify rice genotypes that are submergence-tolerant; the reliable and straight forward identification is made possible by the specific genetic marker. In order to ascertain whether there is any relatedness between genotypes with the submergence-tolerant checks Swarna SUB1, the submergence-specific markers SUB1BC1, SUB1BC2 and SUB1BC3 were utilised, while the diversity markers RM 214, RM224, RM125, RM6318, RM12168, RM8300 were used to assess genotype diversity. Major alleles are those that have the highest frequency. Maximum allele sizes in markers RM125, RM6318, RM224, RM12168, RM8300, RM214, SUB1BC2, SUBLBC3 and SUB1BC1 were 130bp, 175bp, 165bp, 140bp, 193bp, 120bp and 265bp, respectively. SUB1BC3 displayed the largest gene diversity while SUB1BC2 displayed the lowest gene diversity. According to Travis *et al.* (2015), the PIC value reflects allele diversity and frequency across genotypes. The PIC values for the current investigation were 0.22 (SUB1BC2) to 0.70 (SUB1BC3) (Table 7).

According to Iftekharuddaula *et al.* (2011), marker SUB1BC3 was a submergence-specific marker that was downstream of SUB1A. Maximum genotypes displayed genomic bands at 205 bp level, whereas the expected length was 217 bp. Swarna SUB1 displayed a band in that location and most genotypes displayed bands nearby, with the exception of susceptible genotypes Swarna and Ranjit. The genotypes Pravat, China IRRI, Dehradun Basmati, Brahmabalak and Kerala Sundary each showed a distinct allele in that region, it possesses certain distinctive characteristics of submergence tolerance (Fig 1). In case of SSR molecular marker SUB1A203, five unique DNA sequence data have been obtained for the genotypes Swarna, Kerala Sundary, Brahmabalak, Pravat and China IRRI and they were submitted to NCBI genomic depository obtaining the code OQ317918-OQ317922. The SUB1C1

Table 5: Analysis of variance of de-submerged seedlings.

Source	DF	R.P.	Plant height	Shoot length	Second top leaf length	Recovery %
Genotype	21	70.02**	117.01**	14.22**	58.87**	1005.66**
Error	44	4.43	2.54	1.60	1.71	48.04

**Significant at 1% level of significance, D.F. Degree of freedom, R.P. Number of recovered plant.

Table 6: Genetic variability parameters for different traits of rice genotypes.

Characters	Mean	Range	P.V.	G.V.	P.C.V. %	G.C.V. %	Heritability %	G.A.	G.A.%
Elongation (7 DAS)	55.75	16.93-98.25	468.49	431.24	38.82	37.24	92.04	41.04	73.61
Survival (7 DAS)	84.97	15.51-94.52	220.21	193.55	17.46	16.37	87.89	26.86	31.62
Mortality (7 DAS)	17.14	5.06-84.48	206.98	177.51	83.93	77.73	85.76	25.41	148.29
Elongation (14 DAS)	65.04	33.86-96.00	346.73	315.31	28.63	27.30	90.94	34.88	53.63
Survival (14 DAS)	81.39	12.50-93.15	261.60	233.56	19.87	18.77	89.28	29.74	36.54
Mortality (14 DAS)	19	7.59-87.49	259.75	202.27	84.82	74.85	77.87	25.85	136.07
Dry matter accumulation	27	9.55-35.64	143.82	67.69	44.41	30.47	47.07	11.62	43.06
Number of plants recovered	13	6.333-23.333	26.30	21.86	39.45	35.97	83.12	8.78	67.55
Recovery	69	40.781-95.890	367.25	319.21	27.77	25.89	86.92	34.31	49.73

7 and 14 DAS 7 and 14 days after submergence, P.V. and G.V. phenotypic and genotypic variance, P.C.V. and G.C.V. phenotypic and genotypic coefficient of variation, G.A. and G.A.% genetic advance and G.A. as the % of mean.

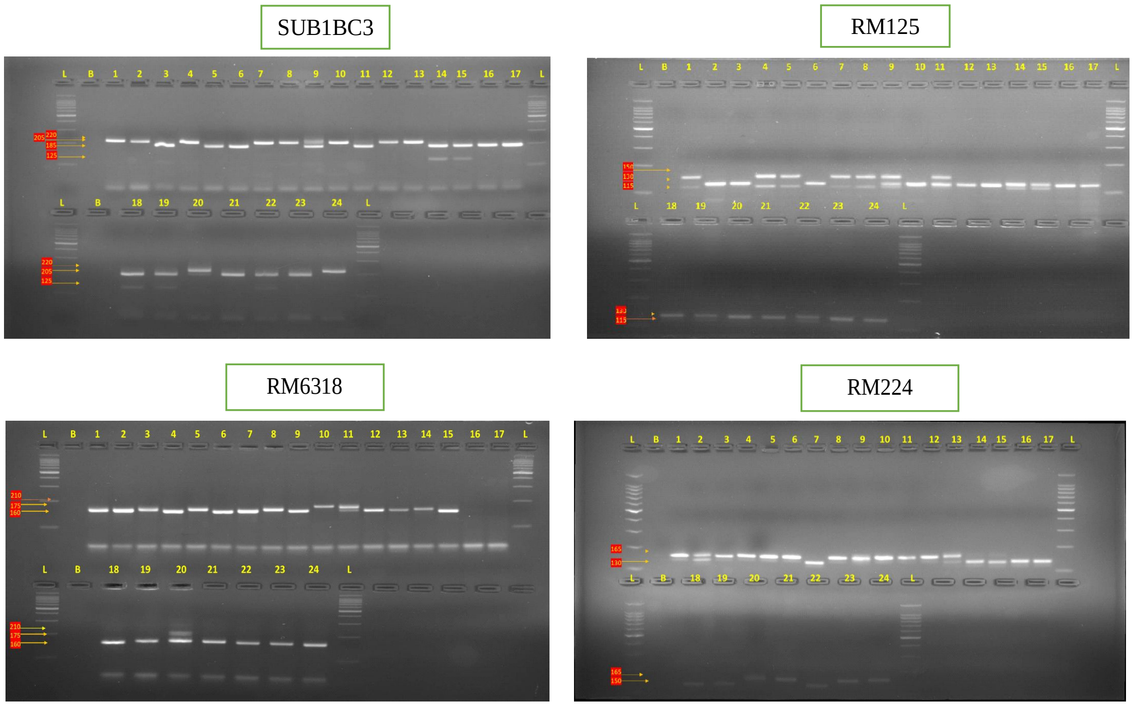
primer's banding pattern showed that the majority of genotypes displayed a band at 165 bp like that of Swarna SUB1. On the other hand, the genotypes Shyamashree, G.B. 1, Pravat, China IRRI and Sonachur clearly demonstrated their distinctiveness by presenting a distinct banding pattern at 245 bp (Fig 1).

Factor analysis based on morphological characters and molecular data

The factor analysis at morphological level showed that Swarna SUB1, the tolerant check and Swarna, the vulnerable type occupied quadrant 2 and 4 respectively (Fig 2). Such diversity in the scatter plot was also for the tolerant in the second quarter, the most divergent variety was represented by the genotypes Ranjit SUB1, Bahadur SUB1, Brahmabalak and Pankaj, as well as the submergence tolerant Swarna SUB1. The tolerant genotypes were mostly found in the quadrant 1 followed by quadrant 2 while morphologically susceptible genotype like Swarna, was placed in quadrant 4 (Fig 2).

The factor analysis based on SSR data also separated the genotypes into four quarters with clear separation of submergence tolerant Swarna SUB1 (Quadrant 1) and susceptible Swarna (Quadrant 3) (Fig 3). The genotypes Kerala Sundary, Pankaj, Jaladhi1-2, Zeera, Kanoklata and Sahabhagi Dhan along with submergence tolerant check variety Swarna SUB1 clearly separated from the other genotypes and belonged to the most molecularly tolerant variety present in first quadrant. In quadrant 4 fourteen days morphologically tolerant genotypes China IRRI, Jaldubi and Bahadur SUB1 share the same quadrant with the genotype Baskathi and Bishnuprashad. So, in the present analysis, it can be inferred that quadrant 1 was most tolerant followed by quadrant 4 and quadrant 2. Whereas, quadrant 3 contains susceptible genotypes.

The congruence between morphological and molecular factorial analysis can be used to define the tolerance pattern of genotypes more precisely. It can be summarized in the present finding that the local landraces like Kerala Sundary and Brahmabalak have the innate potentiality of possessing submergence tolerant gene which can be utilized directly in introgression of in the high yielding background. Some other genotypes like Jaladhi 1-2, Kanoklata and Zeera were also equally effective as submergence tolerant genotype and hence all the genotypes will help to broaden the genetic resources of



L: Ladder, B: Blank, 1: Swarna SUB1, 2: Swarna, 3: Ranjit SUB1, 4: Ranjit, 5: Jaladhi1-2, 6: Jaldubi, 7: Bahadur SUB1, 8: Shahbhagi Dhan, 9: Shyamashree, 10: G.B.1, 11: G.B.2, 12: Zeera, 13: Jyotiprashad, 14: Pravat, 15: China IRRI, 16: Bishnuprashad, 17: Baskathi, 18: Dehradun Basmati, 19: Brahmabalak, 20: Maharaja, 21: Pankaj, 22: Kerala Sundary, 23: Kanoklata, 24: Sonachur.

Fig 1: Electropherogram of molecular screening by simple sequence repeat markers.

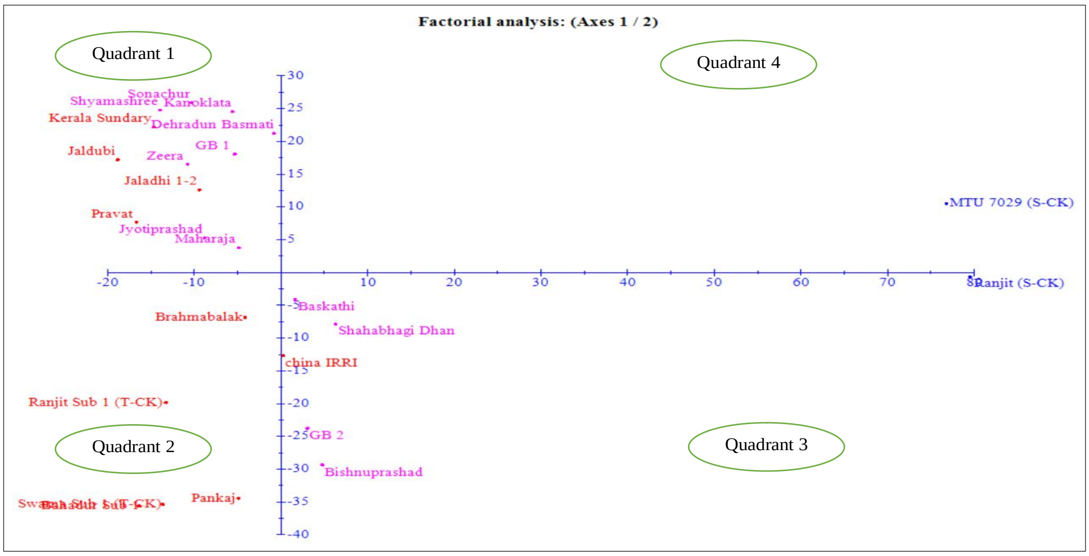


Fig 2: Scatter diagram of genotypes based on factor analysis of morphological traits.

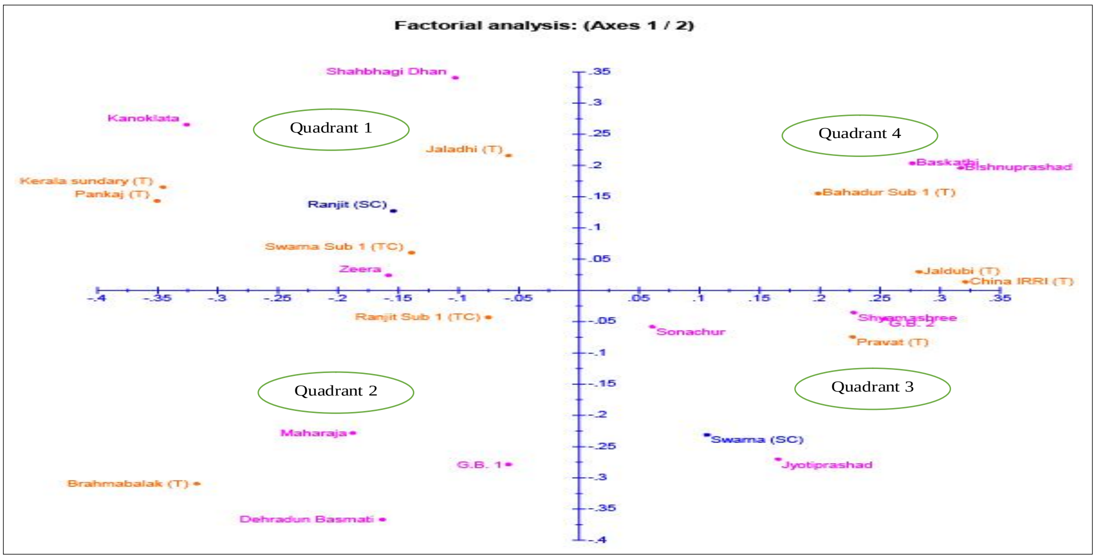


Fig 3: Scatter diagram of genotypes based on factor analysis at molecular level.

Table 7: Polymorphic information content (PIC) and effective =number of alleles per locus (Aep).

Primer name	Number of alleles	Aep	PIC
RM125	3	3	0.63
RM214	2	2	0.50
RM224	3	3	0.61
RM8300	3	3	0.60
RM12168	2	2	0.44
RM6318	3	3	0.60
SUB1BC1	3	2	0.44
SUB1BC2	2	1	0.22
SUB1BC3	4	3	0.70
Mean		2.44	0.53

desired submergence tolerance trait for utilization in breeding program. Moreover, the molecular markers identified for submergence tolerance will facilitate the screening process more simple and more precise.

CONCLUSION

The morphological traits associated with submergence tolerance like elongation rate, survival rate, RGI, DMAR and recovery rate may be considered as predictors of phenotypic diversity across the landraces and genotypes. The genotypes, Brahmabalak, Jaladhi 1-2, Jaldubi and Kerala Sundary showed high seedling survival rate combined with better shoot elongation growth and biomass accumulation as compared to Swarna SUB1 specifying that these genotypes may be useful tolerant types both under flash floods and flooding for longer period. These genotypes have one or more loci that are different from the Sub1 locus, which were revealed from the molecular study. These genetically varied genotypes may aid in the creation of new rice varieties with high levels of submergence tolerance adaptable to different flooding situation coupled with high yield.

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Ethics and conflict of interest

The author(s) declare(s) no known conflict of interests that could have appeared to influence the work reported in this paper and also ensure that the research is conducted ethically and in compliance with relevant guidelines and regulations.

REFERENCES

Afrin, W., Nafis, M.H., Hossain, M.A., Islam, M.M., Hossain, M.A. (2018). Responses of rice (*Oryza sativa* L.) genotypes to different levels of submergence. Compt Rend Biol. 341(2): 85-96.

Bailey-Serres, J., Fukao, T., Ronald, P., Ismail, A., Heuer, S., Mackill, D.J. (2010). Submergence tolerant rice: SUB1’s journey from landrace to modern cultivar. Rice. 3: 138-147.

Barik, J., Kumar, V., Lenka, SK., Panda, D. (2020). Assessment of variation in morpho-physiological traits and genetic diversity in relation to submergence tolerance of five indigenous lowland rice landraces. Science Direct Rice Science. 27(1): 32-43.

Catling, D. (1992). Rice in Deep Water. International Rice Research Institute, Manila, Philippines.

Colmer, T.D., Armstrong, W., Greenway, H., Ismail, A.M., Girk, G.J.D., Atwell, B.J. (2014). Physiological mechanisms in flooding tolerance of rice: transient complete submergence and prolonged standing water. Progress in Bot. 75: 255-307.

Fukao, T., Xu, K., Ronald, P.C., Bailey-Serres, J. (2006). A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. Plant Cell. 18(8): 2021-2034.

Fukao, T., Bailey-serres, J. (2008). Submergence tolerance conferred by Sub1A is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proc. Natl. Acad. Sci. USA. 105(43): 16814-16819.

Goswami, S., Kar, R.K., Paul, A., Dey, N. (2017). Genetic potentiality of indigenous rice genotypes from Eastern India with reference to submergence tolerance and deepwater traits. Curr Plant Biol. 11(12): 23-32.

HilleRisLambers, D., Vergara, B.S. (1982). Summary results of an international collaboration on screening methods for flood tolerance, in: Proceedings of the 1981 International Deepwater Rice Workshop, International Rice Research Institute, Los Ban˜os, Philippines, pp. 347-353.

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.J., Pamplona, A.M., 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(1): 83-97.

International Rice Research Institute (IRRI). (2002). Rice Web. in the place of International Rice Research Institute, Standard evaluation system for rice, Fifth Ed., International Rice Research Institute, Manila, Philippines.

Kamolsukyunyong, W., Ruanjaichon, V., Siangliw, M., Kawasaki, S., Sasaki, T., Vanavichit, A., Tragoonrung, S. (2001). Mapping of quantitative trait locus related to submergence tolerance in rice with aid of chromosome walking, DNA Res. 8(4): 163-171.

Mackill, D.J., Coffman, W.R., Garrity, D.P. (1996). Rainfed lowland rice improvement. International Rice Research Intitute, P.O. Box 933, Manila, Philppines. 242 p.

Nandi, S., Subudhi, PK., Senadhira, D., Manigbas, N.L., Sen-Mandi, S., Huang, N. (1997). Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Mol. Gen. Genet. 255(1): 1-8.

Panda, D., Sharma, S.G., Sarkar, R.K. (2008). Chlorophyll fluorescence parameters, CO_2 photosynthetic rate and regeneration capacity as a result of complete submergence and subsequent re-emergence in rice (*Oryza sativa* L.). Aquat Bot. 88(2): 127-133.

Travis, A.J., Norton, G.J., Datta, S., Sarma, R., Dasgupta, T., Savio, F.L., Macaulay, M., Hedley, P.E., McNally, K.L., Sumon, M.H., Islam, M.R. and Price, A.H. (2015). Assessing the genetic diversity of rice originating from Bangladesh, Assam and West Bengal. Rice. 8: 35. https://doi.org/10.1186/s12284-015-0068-z.

Toojinda, T., Siangliw, M., Tragoonrung, S., Vanavichit, A. (2003). Molecular genetics of submergence tolerance in rice: QTL analysis of key traits. Ann. Bot. 91: 243-253.

Voesenek, L.A.C.J., Bailey-Serres, J. (2009). Genetics of high-rise rice. Nature. 460: 959-960.

Xu, K., Mackill, D.J. (1996). A major locus for submergence tolerance mapped on rice chromosome 9. Mol. Breeding. 2(3): 219-224.

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

Xu, K., Xia, X., Fukao, T., Canlas, P., Maghirang-Rodriguez, R., Heuer, S., Ismail, AM., Bailey-Serres, J., Ronald, P.C., Mackill, D.J. (2006). Sub1A is an ethylene response factor-like gene that confers submergence tolerance to rice. Nature. 442(7103): 705-708.