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Impact of Submergence1 (Sub1) Locus in Genetic Background of Modern Rice Varieties (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To improve submergence stress tolerance of modern rice varieties (ADT36 and ADT37) through selection of superior rice lines.
Study Design: Backcross method.
Place and Duration of Study: This effort was taken in Nethouse of Kandaswami Kandar's College, Namakkal District, Tamil Nadu, India. This process was completed in four years from 2019 to 2022.
Methodology: Here, widely cultivated two short-term rice varieties (ADT36 and ADT37) were selected to improve submergence tolerance based on polymorphism at phenotypic and genotypic level. Then, they were improved through backcrossing with respective recurrent parent by selection of superior rice lines compared to parental lines under submergence condition. Likewise, this process was advanced up to BC_3F_2 generation.
Results: In this study, a number of two NILs for ADT36 rice variety (ADT36- $F_1$2-5-19.18 and ADT36- $F_1$5-2-5.17) and five NILs for ADT37 rice variety (ADT37- $F_1$13-27-5.2, ADT37- $F_1$13-27-5.5, ADT37- $F_1$15-2-1.10, ADT37- $F_1$15-2-1.14 and ADT37- $F_1$15-2-2.14) are selected as superior

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lines.Selected rice lines exhibited more than 60% submergence tolerance than donor parent. This result indicates the adaptation of Sub1 locus into new genetic background of ADT36 and ADT37 rice variety under submergence condition.

Conclusion: Improvement process of rice lines under stress condition results in identification of highly active rice lines. Thus, selected submergence tolerant rice lines would be a boon to the rice farmers who are facing crop lose due to frequently occurring cyclones in the Cauvery delta region.

Keywords: Short-term rice variety; indel marker; submergence tolerance; phenotype; Cauvery delta areas.

1. INTRODUCTION

Paddy is a semi-aquatic plant that can only survive a few days submerged in water. Flash flooding, which is produced by irregular meteorological conditions during the monsoon season, has a significant impact on rice production, particularly in rain-fed lowland regions of South and South-east Asia [1]. Flash flooding has an effect on the growth of rice during all stages of its development, but it is especially detrimental during the blooming period, when yield loss is at its highest [2]. Very recently, it is suggested that study of annual rainfall pattern yields a positive signal for decreasing the risk potential of grain production in rice crop by extreme weather events of flooding [3]. Fortunately, the quantitative trait locus known as Submergence 1 (Sub1), which is situated on chromosome 9, has been identified from a rice landrace known as flood resistant (FR13A) [4]. This locus is intended to be used for the purpose of developing popular rice varieties. Within this locus, there are three alleles known as SUB1A, SUB1B, and SUB1C. Of these, the SUB1A allele is the one that is responsible for submergence tolerance [5,6]. Through the encoding of the ERF transcription factor, which confers tolerance to flooding in rice by lowering ethylene synthesis [7,5] Sub1A is responsible for preventing rapid stem elongation and chlorophyll degradation under submergence conditions. Further, Sub1A allele is only found in indica rice cultivars, whereas the Sub1B and Sub1C alleles are found in both japonica and indica rice cultivars [7] Many high-yielding rice varieties have been modified for flash flooding by using this locus [8,9,10] These improvements have been developed by Emerick and Ronald. This particular Sub1 locus has already been introrse into a great number of mega rice varieties that come from different genetic origins. Some examples of these varieties include CR1009 Sub1, BR11 Sub1, TDK Sub1, Samba Mahsuri, and others [11,10].

The Cauvery delta region of Tamil Nadu state, also known as the "Granary of south India," is frequently struck by cyclones, which destroy approximately 10,000 hectares of rice cultivation land for agricultural purposes. Paddy is grown by rice farmers in these regions during three distinct seasons: the Kuruvai season, which occurs between June and July, is characterized by short duration rice varieties (90 days), the Samba season, which occurs in August, is characterized by medium duration rice varieties (120 days), and the Thaladi season, which occurs between November and December, is characterized by long duration rice varieties (190 days). In modern times, however, rice farmers have begun to produce only short-term rice varieties during the Samba and Thaladi seasons, as well as during the continuation of the Kuruvai season. This is due to the fact that these seasons are quite short. Due to the fact that this region is susceptible to cyclones, rice fields are prone to flooding, which results in the destruction of rice crops. In general, when compared to traditional rice varieties that are grown for a longer period of time, short-term rice types are extremely vulnerable to floods [12,13,14] Consequently, in the current investigation, we took into consideration two well-known short-term rice varieties in this region, namely ADT36 and ADT37, with the intention of enhancing their submergence tolerance through the use of conventional methods and the assistance of molecular markers.

2. MATERIALS AND METHODS

2.1 Rice Seeds

A small quantity of rice seeds of ADT36 and ADT37 from Tamilnadu Rice Research Institute (TRRI), Aduthurai, Tamilnadu state and CR Dhan 801 from National Rice Research Institute (NRRI), Cuttack, Odisha state was sourced. Here, ADT36 and ADT37 as female parent (recipient) and CR Dhan 801 harboring Submergence1(Sub1) locus as male parent

(Donor) developed from the breeding materials of cross IR81896-B-B-195/2* Swarna-Sub1/IR91659-54-35 in National Rice Research Institute, Cuttack, Odisha state, INDIA were used.

2.2 Parental Polymorphism Study

2.2.1 Phenotypic level

Prior to sowing, seed dormancy was broken by incubating the seeds at 50°C for up to 5 days. For submergence screening at seedling stage, seeds of parental lines, ADT36, ADT37 and CR dhan 801were germinated and grown for 10 daysin plastic cups. Then, they were transplanted to big pots and allowed to establish for 20 days. On 21st day, pots with seedlings were transferred to water tank and the water level was raised to the height of 90 cm above the seedlings and its level was maintained up to 12 days and then, the pots were taken out of the tank at 3 days interval. Regeneration rate of the seedlings under 3,6,9 and 12 days stress was noted [15]

2.2.2 Genotypic level

Genomic DNA was extracted from young leaves of ADT36, ADT37 and CR Dhan 801 rice variety grown for 14 days using the protocol described by Drew and Lynch [16] Polymerase chain reaction (PCR) was performed using InDel marker located within Sub1 locus, Sub1BC2 and SC3 according to Septiningsih et al. [10].

2.3 Development of F_1, BC_2 and BC_3 Population

Hybridization was done between ADT36 and CR Dhan 801 as well as ADT37 and CR dhan 801 in Nethouse, KandaswamiKandar's College, Velur, Namakkal (District), Tamil Nadu, during Kharif season, 2019. At the time of the cross pollination, a number of six anthers from the donor parent (CR Dhan 801) were transferred to the stigma of the recipient parent (ADT36 or ADT37) by hand picking and derived F_1 seeds. Then, F_1 population derived from ADT36/CR dhan 801 (Cross-1) and ADT37/CR dhan 801(Cross-2) were subjected to foreground selection using gene-specific marker, SUB_1BC_2 as mentioned above. Positive F_1 plants having heterozygous alleles were used for backcrossing with respective recurrent parent (ADT36 or ADT37) to produce BC_1F_1 seeds during Kharif season, 2020. Likewise, BC_2F_1 population during Kharif season 2021and BC_3F_1 population during Rabi season 2021-2022 were produced by the selection of submergence tolerant progenies based on morphological changes in the leaf elongation under 10 days submergence condition. At last, BC_3F_1progenies were allowed for selfing to produce BC_3F_2seeds during Kharif season 2022.BC_3F_2lines were evaluated for submergence tolerance at seedling stage during Kharif season 2023 in terms of plant height (PH), root length (RL), number of leaf (NL), leaf length (LL) and leaf width (LW) and selected NILs were confirmed at PCR level using InDel marker (Sub1BC2).

3. RESULTS AND DISCUSSION

In plants, the respiration process is affected by lack of O_2 under submergence/flooding and this condition results in the accumulation of phytotoxic substances such as Fe^{2+}, Mn^{2+}, H_2S, O_2 radicals and the products of fermentation which damages the plant tissue [16]. In order to protect rice plants from submergence stress, a major QTL called Sub1 is identified for submergence stress tolerance at vegetative stage [17,6] In the present study, a polymorphism study was executed at phenotypic and genotypic level to improve submergence tolerance in two short-term rice varieties (ADT36 and ADT37). In the study of polymorphism among parental lines at phenotypic level, plant height (PH) of ADT36, ADT37 and CR dhan 801 rice variety was noted to 26.0, 21.0 and 17.0 cm; 11.0, 12.0 and 5.0cm for root length respectively, under submergence condition. Under the control condition (non-flooding), plant height and root length were registered as 21.0, 18.0 and 18.5 cm; 9.0, 10.5 and 6.0 cm in ADT36, ADT37 and CR dhan 801 rice variety, respectively. After de-submergence, both recipient lines showed highly tolerant reaction (score 1) to submergence stress up to 6 days and they became susceptible (score 7) and highly susceptible (score 9) on 8th and 10th day stress, respectively. In case of donor line, it was highly tolerant (score 1) up to 8th day stress and became tolerant level (score 3) on 10th day. Survival rate of parental lines ADT36, ADT37 and CR dhan 801 was 0.0, 10.0 and 90.0 % followed by de-submergence after 10 days stress (Fig.1a,b).

Under submergence condition, the rate of shoot elongation of ADT36, ADT37 and CR dhan 801 is increased to 19.2%, 14.3% and 8.1% when compare to control respectively. Among parental lines, the shoot elongation rate is found to be

higher in both recipient parents (ADT36 and ADT37) than that of donor parent (CR dhan 801). More and less shoot elongation rate under submergence condition are associated with submergence tolerance and intolerance of rice genotypes, respectively. Under submergence condition, intolerant and tolerant rice variety follows an escaping [18] and quiescence mechanism, respectively. Escaping mechanism allows the stored starch to degrade very fast and promote the shoot elongation to reach the water surface in order to get O_2 [19]. This condition leads to plant lodging or die after de-submergence. In case of quiescence mechanism, it limits the shoot elongation of plant under water by controlling the degradation of starch and it allows the plant to continue its growth normally following the recovery of water. In tolerant rice varieties, SUMBERGENCE 1 (SUB1) locus encoding ethylene-responsive transcription factor SUB1A-1 slowed the elongation growth in near-isogenic lines [20,21]. Moreover, in non-Sub1 line, high ethylene production is linked with GA synthesis which promotes shoot elongation, whereas in Sub1 lines, ethylene production is dissipated by leaf gas film thickness, leaf hydrophobicity, porosity and leaf density which leads to less shoot elongation [22,23]. In the PCR screening both recipient rice lines possessed a polymorphic condition in the banding pattern to donor line. Size of the amplified PCR with SC3 marker was recorded to 190base pairs in ADT36 and ADT37 and 200 base pairs in CR Dhan 801 (Fig. 2).

Fig. 1a, b. Parental polymorphism during seed ling stage under flooding (T) and non-flooding (C) condition

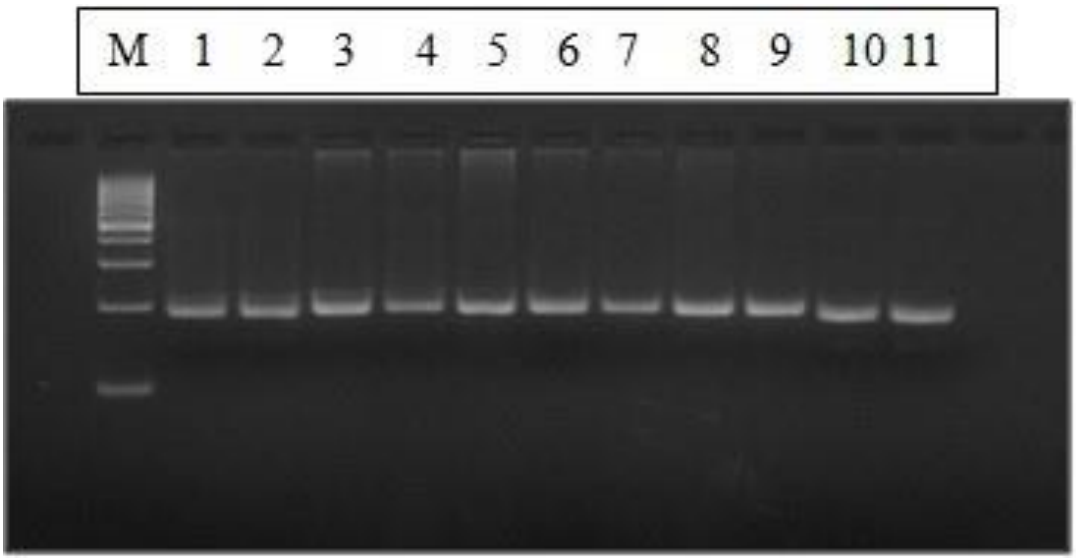

Fig. 2. PCR amplification for parental polymorphism with SC3 DNA marker. M-100 bp DNA ladder. Lanes:1&2-ADT-36; Lanes-3 -9: CR Dhan 801; Lanes 10 & 11: ADT 37

In PCR screening, we confirmed the tolerance and intolerance response of donor and recipient varieties to vegetative stage submergence stress, respectively, using *Indel* marker (Sub1BC2) [10] rather than SC3 marker. SC3 is Sub1A gene specific simple sequence repeat (SSR) marker and it is downstream of Sub1A In a study, SC3 alone did not distinguish between tolerant and susceptible genotypes [24]. This *Indel* marker, Sub1BC2 located in between Sub1B and Sub1C allele in the Sub1 locus is clearly differentiated rice variety CR dhan 801 with Sub1 and ADT36/ADT37 without Sub1 locus. Thus, the donor and recipient lines used in this study are proved as tolerant and intolerant lines to submergence stress at vegetative stage at phenotypic as well as genotypic level.The breeding scheme used in the marker assisted backcross program for introgression of Sub1 locus into ADT36 and ADT37 rice variety is given in Fig. 3.

The details of derived seeds and selected progenies from F_1, BC_1F_1, BC_2F_1 and BC_3F_1 population are given in Table 1.

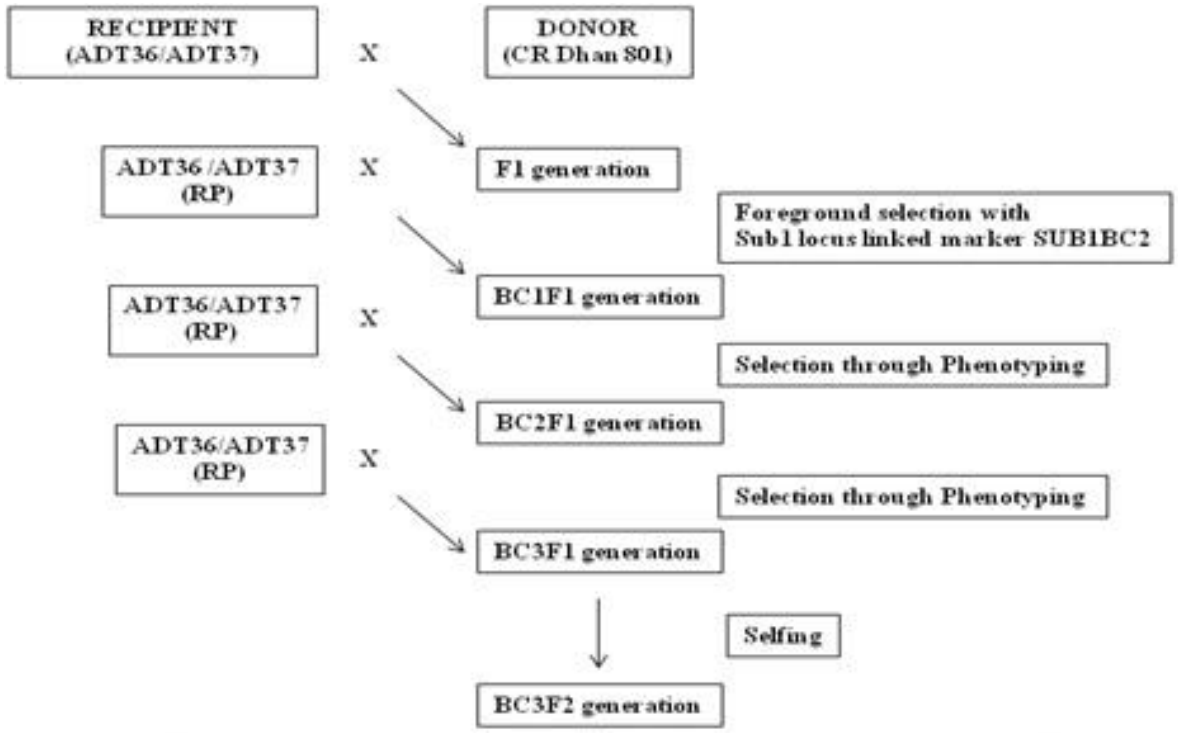

Fig. 3. Breeding scheme used in the marker assisted backcross program for introgression of Sub1 locus into rice cultivar, ADT36 and ADT37

Table 1. Details of seeds derived and selected progenies from F_1, BC_1F_1, BC_2F_1, BC_3F_1 and BC3F2 population

Cross combination	ADT36/CR dhan801 (cross-1)	ADT37/CR dhan801(cross-2)
F_1 generation	43 seeds derived	37 seeds derived
Foreground Selection	Selection of 9 plants with heterozygous alleles/10 plants	Selection of 10 plants with heterozygous alleles/10plants
BC_1F_1 generation	74 seeds derived	65 seeds derived
Phenotype	Selection of 24 plants/52 plants	Selection of 22 plants/59 plants
BC_2F_1 generation	92 seeds derived	108 seeds derived
Phenotype	Selection of 35 plants/79 plants	Selection of 38 plants/85 plants
BC_3F_1 generation	123 seeds derived	73 seeds derived
BC_3F_2 generation	48 near isogenic lines(NILs)	26 near isogenic lines (NILs)
Phenotype	Selection of 20 plants/48plants	Selection of 22 plants/26 plants

In cross pollination, 43 and 37F_1 seeds were derived from cross-1 and cross-2, respectively. From these, 9 plants (# 1, 2, 3, 4, 5, 7, 8, 9, 10) out of 10 F_1 plants from cross-1 and 10 plants (# 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) out of 10 F_1 plants from cross-1 showed heterozygous allelic condition for Sub1BC2 marker located within the locus in the foreground selection (Fig. 4).

Positive F_1 plants identified in the foreground selection did backcross with respective recurrent parent (RP) and produced 74 and 65 BC_1F_1 seeds for cross-1 and cross-2, respectively. Of these, 52 and 59 progenies were imposed submergence stress at seedling stage along with both parental lines in water tank for 10 days. In this screening, PH of CR dhan 801 and ADT36 was recorded to 8.8 and 13.9cm under non-flooding and 12.5 and 20.0cm under flooding. In BC_1F_1 population of cross-1, the level of PH ranged from 15.0 to 27.0cm andfrom these, 24progenies (plant # F_1-2.5, 12, 13, 14; 4.1, 2, 3, 4, 5, 7, 8, 9, 12, 13, 14, 16; 5.1, 5.2; 8.2, 5, 6; 10.1, 2, 8) were selected as tolerant based on morphological response to stress for backcrossing. In case of cross-2, PH of CR dhan 801 and ADT36 was documented to 9.7 and 12.8cm under non-flooding and 11.8 and 19.5cm under flooding. In BC_1F_1 population of cross-2, PH level ranged from 12.0 to 25.5cm under submergence and from these, 22 progenies (plant # $F_1$13.2, 4, 6, 8, 10, 12, 13, 15, 17, 18, 21, 22, 23, 24, 25, 27; 15.2, 10, 21, 24, 27) were selected as tolerant for backcrossing.

Based on the variations in the shoot elongation, we could differentiate tolerant and intolerant rice progenies and identify submergence tolerant rice progenies at F_1 condition. Supportively, rice seedlings can able to withstand stress for 14 days even in heterozygous condition with less leaf elongation [25]. In the backcrossing of selected BC_1F_1 progenies with its RP, 92 and 108 BC_2F_1 seeds were derived for cross-1 and cross-2, respectively and of these. 79and 85 progenies were imposed submergence stress. In this screening, PH of CR dhan 801 and ADT36 was 11.0 and 12.4cm under non-flooding and 12.5 and 21.0cm under flooding. In BC_2F_1 population of cross-1, the level of PH ranged from 11.0 to 25.5cm and of these, 35 progenies (plant # $F_1$2-5.1, 2, 3, 5, 6, 7, 8, 9, 14, 15, 19; 13.3, 2, 7, 8, 11, 17; $F_1$4.5.2, 3, 4, 6, 7; 7.1, 4, 6; 16.1, 2, 4, 6; $F_1$5-2.3, 5; $F_1$8-2.4, 5, 8, 12) were selected as tolerant for backcrossing. In case of cross-2, PH of CR dhan 801 and ADT36 was recorded to 9.8 and 13.0cm under non-flooding and 11.0 and 19.4cm under flooding. In BC_2F_1 population, the range of PH was from 12.0 to 25.5cm and of these. 38 progenies (plant # $F_1$13-10.3, 5; 12.1; 13.1, 2, 3, 5; 15.1, 2, 3; 17.2, 3, 4, 5; 18.1, 2, 4, 5; 21.4, 5; 22.3, 5; 23.2, 4, 5; 24,1, 3; 25.3, 4, 5; 27.1, 5; $F_1$15-2.1, 2, 5, 10; 10.3; 21.4) were selected as tolerant under stress condition for backcrossing. As a result of backcrossing, we produced 63 and 72 BC_3F_1 seeds for cross-1 and cross-2, respectively and from these, 60 and 64 progenies were allowed for selfing and we harvested as BC_3F_2 seeds. In the evaluation of BC_3F_2population of cross-1for submergence tolerance during seedling stage, PH, RL, NL, LL and LW of donor and recipient line was recorded to 23.0, 9.0, 4, 17.0 and 0.7cm in CR dhan 801 and 25.0, 11.0, 4, 28.0 and 0.6cm in ADT36 under non-flooding whereas under flooding, PH, RL, NL, LL and LW of donor and recipient line was registered to 25.0, 5.0, 4, 19.0 and 0.5cm and 38.0, 7.0, 4, 20.0 and 0.5cm, respectively. In the BC_3F_2 population, the growth of PH, RL, NL, LL and LW was noted in the range of 15.0-37.0cm, 4.5-13.0cm, 2-5, 10-28.5cm and 0.3-0.7cm, respectively (Table-2a).

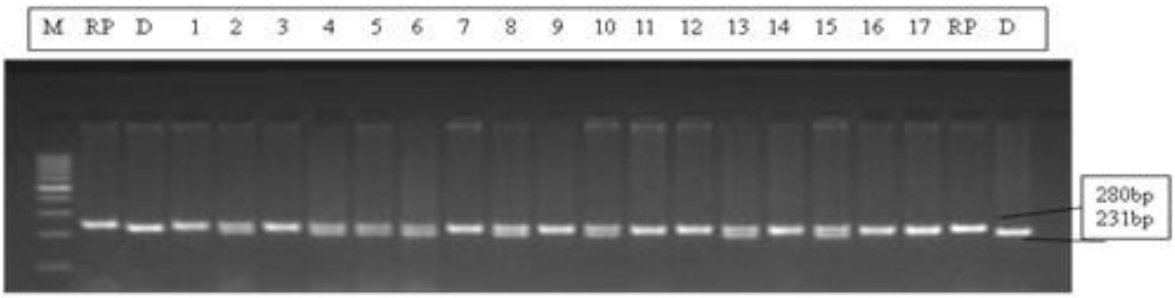

Fig. 4. PCR amplification of seedlings of F_1 generation using gene-specific marker Sub1BC2
M-100 bp DNA ladder. RP- Recurrent parent; D- Donor. Lanes: 1-10= F1 plants from ADT 36 x CR Dhan 801 cross. Lanes-11-17 = F1 plants from ADT37 x CR dhan 801 cross

Table 2a. Results of phenotypic selection of BC_3F_2 population of cross-1(ADT36/CR dhan 801 cross combination) under submergence stress condition

Parental lines/ BC_3F_2 progenies	Morphological characters (Cm)				
	Plant height (PH)	Root length (RL)	Number of leaf (NL)	Leaf length (LL)	Leaf width (LW)
CR Dhan 801 (C)	23.0	9.0	4	17.0	0.7
ADT36 (C)	25.0	11.0	4	28.0	0.6
CR Dhan 801 (S)	25.0	5.0	4	19.0	0.5
ADT36 (S)	38.0	7.0	4	20.0	0.5
ADT36- $F_1$2-5-19.1	37.0	11.0	4	20.0	0.7
ADT36- $F_1$2-5-19.6	34.0	6.8	4	19.0	0.7
ADT36- $F_1$2-5-19.7	29.0	10.0	3	22.0	0.5
ADT36- $F_1$2-5-19.9	22.0	7.0	3	16.0	0.3
ADT36- $F_1$2-5-19.10	32.5	8.0	5	19.5	0.7
ADT36- $F_1$2-5-19.13	29.0	9.0	4	15.8	0.6
ADT36- $F_1$2-5-19.14	24.0	5.0	3	19.5	0.5
ADT36- $F_1$2-5-19.15	33.8	13.0	5	19.0	0.8
ADT36- $F_1$2-5-19.16	23.0	6.8	4	16.5	0.3
ADT36- $F_1$2-5-19.17	37.0	5.0	4	21.0	0.7
ADT36- $F_1$2-5-19.18	17.0	7.0	2	11.5	0.3
ADT36- $F_1$2-5-19.20	23.5	5.8	2	17.0	0.3
ADT36- $F_1$2-13-7.1	36.0	6.0	3	28.5	0.5
ADT36- $F_1$2-13-7.2	24.0	7.5	3	17.0	0.3
ADT36- $F_1$2-13-7.3	21.2	5.0	3	15.0	0.4
ADT36- $F_1$2-13-7.4	36.5	6.8	3	19.5	0.6
ADT36- $F_1$2-13-7.5	37.5	6.0	4	19.5	0.6
ADT36- $F_1$2-13-7.6	32.0	13.0	3	18.5	0.6
ADT36- $F_1$2-13-7.9	37.5	5.5	3	20.0	0.6
ADT36- $F_1$2-13-7.10	35.0	7.0	3	20.0	0.6
ADT36- $F_1$2-13-7.11	24.0	4.8	3	16.5	0.3
ADT36- $F_1$2-13-7.12	20.5	7.5	4	15.5	0.3
ADT36- $F_1$2-13-7.13	25.0	8.5	4	15.5	0.5
ADT36- $F_1$2-13-7.14	28.0	6.2	3	17.5	0.3
ADT36- $F_1$2-13-7.15	21.2	5.2	2	15.0	0.3
ADT36- $F_1$2-13-7.16	29.5	7.5	3	22.0	0.3
ADT36- $F_1$2-13-7.17	37.2	6.2	4	20.0	0.6
ADT36- $F_1$2-13-7.18	30.4	7.0	3	16.0	0.3
ADT36- $F_1$2-13-7.19	22.0	9.0	3	17.0	0.6
ADT36- $F_1$2-13-7.20	22.5	7.0	3	16.0	0.4
ADT36- $F_1$4-5-3.4	19.0	5.0	2	14.5	0.5
ADT36- $F_1$4-5-3.5	27.0	8.5	3	20.0	0.6
ADT36- $F_1$4-5-3.7	18.0	7.5	3	14.0	0.3
ADT36- $F_1$4-5-3.8	18.0	6.2	4	16.0	0.3
ADT36- $F_1$4-5-3.9	32.0	7.0	4	20.0	0.3
ADT36- $F_1$4-5-3.10	24.0	9.5	3	16.0	0.4
ADT36- $F_1$4-16-2.1	25.0	12.0	4	18.0	0.6
ADT36- $F_1$4-16-2.3	22.5	7.5	3	14.5	0.3
ADT36- $F_1$4-16-2.6	32.5	15.0	4	17.0	0.5
ADT36- $F_1$4-16-2.7	24.0	10.0	4	16.0	0.4
ADT36- $F_1$4-16-2.10	24.5	7.0	4	18.0	0.3
ADT36- $F_1$5-2-5.1	25.5	13.0	4	16.5	0.4
ADT36- $F_1$5-2-5.2	32.5	12.5	4	29.5	0.3
ADT36- $F_1$5-2-5.7	35.0	13.0	4	18.0	0.6

Parental lines/ BC_3F_2 progenies	Morphological characters (Cm)				
	Plant height (PH)	Root length (RL)	Number of leaf (NL)	Leaf length (LL)	Leaf width (LW)
ADT36- $F_1$5-2-5.9	27.0	6.5	4	17.0	0.5
ADT36- $F_1$5-2-5.17	15.0	8.0	3	10.0	0.4
ADT36- $F_1$8-2-5.8	20.0	7.0	4	14.5	0.5
ADT36- $F_1$8-2-5.25	25.0	4.5	4	15.0	0.5

In the evaluation of BC_3F_2 population of cross-2 for submergence tolerance during seedling stage, PH, RL, NL, LL and LW of donor and recipient line was recorded to 23.0, 9.0, 4, 17.0 and 0.7 in CR dhan 801 and 26.0, 12.0, 4, 26.0 and 0.7cm in ADT37 under non-flooding whereas under flooding, PH, RL, NL, LL and LW of donor and recipient line was registered to 25.0, 7.0, 4, 19.0 and 0.5cm and 41.0, 14.0, 4, 21.5 and 0.6cm. In the BC_3F_2 population of cross-2, the growth of PH, RL, NL, LL and LW was noted in the range of 13.5-43.0cm, 4.5-16.0cm, 2-5, 8.0-29.5cm and 0.3-0.7cm, respectively (Fig. 5a,b; Table-2b).

Evaluation study of BC_3F_2 population revealed that 50.0%, 4.2%, 95.9%, 69.8% and 65.7% of BC_3F_2 progenies accounted for lower value of PH, RL, NL, LL and LW character than donor parent, respectively. When compare to ADT36, growth rate of PH, RL, NL, LL and LW is increased in 0.0%, 54.8%, 4.3%, 11.6% and 33.3% of BC_3F_2 progenies, respectively. In case of cross-2, 57.7%, 73.0%, 11.5%, 38.5% and 19.2% progenies showed increased growth for PH, RL, NL, LL and LW than CR Dhan 801 donor parent under flooding, respectively. When compare to ADT37 recipient parent, 7.7%, 10.0%, 11.5%, 23.1% and 23.1% progenies had more value for the trait of PH, RL, NL, LL and LW. Of these, a number of two NILs for ADT36 rice variety (ADT36- $F_1$2-5-19.18 and ADT36- $F_1$5-2-5.17) and five NILs for ADT37 rice variety (ADT37- $F_1$13-27-5.2, ADT37- $F_1$13-27-5.5, ADT37- $F_1$15-2-1.10, ADT37- $F_1$15-2-1.14 and ADT37- $F_1$15-2-2.14) are selected and they were confirmed in PCR amplification with InDel marker, Sub1BC2 (Fig.5c). Selected superior lines showed more than 60 % submergence tolerance than donor parent. It indicates the

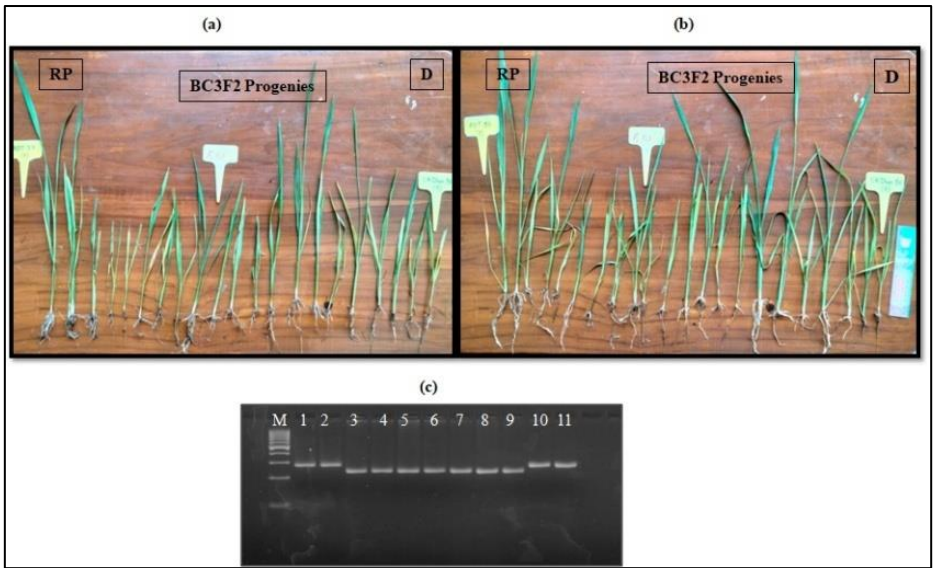

Fig. 5. (a) shows variations of BC_3F_2 progenies of cross-1(ADT36/CR dhan 801) in submergence tolerance under flooding during Seedling stage
(b) BC_3F_2 progenies of cross-2(ADT37/CR dhan 801); RP- Recurrent parent; D- Donor.
(c) PCR amplification of selected BC_3F_2 rice lines using gene-specific InDel marker, Sub1BC2 . M-100 bp DNA ladder. Lanes: 1&2-ADT-36; Lanes-3 -9: selected NILs for ADT36 and ADT37; Lanes 10 & 11: ADT 37

Table 2b. Results of phenotypic selection of BC_3F_2 population of cross-2 (ADT37/CR dhan 801 cross combination) under submergence stress condition

Parental lines/ BC_3F_2 progenie	Morphological characters (Cm)				
	PH	RL	NL	LL	LW
CR dhan 801 (C)	23.0	8.0	4	17.0	0.7
ADT37 (C)	26.0	12.0	4	26.0	0.7
CR Dhan 801 (S)	25.0	7.0	4	19.0	0.5
ADT37 (S)	41.0	14.0	4	21.5	0.6
ADT37- $F_1$13-24-3.7	40.0	12.5	4	29.5	0.5
ADT37- $F_1$13-24-3.12	34.0	16.0	4	25.5	0.5
ADT37- $F_1$13-24-3.13	24.5	9.0	4	16.5	0.3
ADT37- $F_1$13-24-3.14	34.5	8.0	4	26.0	0.5
ADT37- $F_1$13-24-3.15	33.0	8.0	4	25.5	0.4
ADT37- $F_1$13-27-5.1	19.0	6.0	3	14.0	0.3
ADT37- $F_1$13-27-5.2	16.0	4.0	2	8.0	0.3
ADT37- $F_1$13-27-5.3	20.0	4.5	3	15.0	0.3
ADT37- $F_1$13-27-5.5	16.5	8.5	4	12.0	0.3
ADT37- $F_1$13-27-5.6	32.5	12.0	4	16.0	0.4
ADT37- $F_1$13-27-5.10	23.0	4.8	4	17.0	0.3
ADT37- $F_1$15-2-1.1	21.0	7.0	3	14.0	0.5
ADT37- $F_1$15-2-1.7	30.0	9.0	4	17.0	0.4
ADT37- $F_1$15-2-1.10	15.0	5.0	3	10.0	0.4
ADT37- $F_1$15-2-1.11	27.0	9.0	4	17.0	0.7
ADT37- $F_1$15-2-1.13	43.0	7.5	5	27.0	0.8
ADT37- $F_1$15-2-1.14	13.5	5.0	3	9.5	0.3
ADT37- $F_1$15-2-1.16	36.0	10.0	4	20.0	0.5
ADT37- $F_1$15-2-2.1	32.0	9.0	4	21.0	0.4
ADT37- $F_1$15-2-2.2	31.0	10.0	4	21.0	0.4
ADT37- $F_1$15-2-2.4	32.5	9.0	4	26.5	0.5
ADT37- $F_1$15-2-2.5	24.5	8.0	4	15.0	0.6
ADT37- $F_1$15-2-2.7	43.5	12.0	5	17.5	0.7
ADT37- $F_1$15-2-2.10	41.5	12.5	5	18.5	0.7
ADT37- $F_1$15-2-2.14	16.0	4.5	4	13.5	0.4
ADT37- $F_1$15-2-2.18	26.0	10.0	4	20.0	0.4

PH-Plant height; RL-Root Length; NL-Number of Leaf; LL-Leaf length; LW-Leaf width. C- Control; S – Submergence Stress.

adaptation of rice lines at physiological and molecular level under submergence stress for climate change resilience [26,27]. Thus, identified rice lines with superior morphological and physiological characters enhance the breeding programme [28]. Similarly, many mega rice varieties such as Swarna, Samba Mahsuri and CR1009 from India, IR64 from the Philippines (IRRI), Thadokkham 1 (TDK1) from Laos, BR11 from Bangladesh, Ciherang-Sub1 and PSB Rc18-Sub1 have been improved for submergence tolerance using Sub1locus across the world [24,11,10,29].

4. CONCLUSION

In rain-fed areas and coastal region, submergence stress is naturally occurring constraint to rice growth during unpredicted flooding by climate change. Traditional rice varieties can tolerate flooding moderately but it is not prepared by rice farmers due to the poor grain yield. In contrast, modern short duration rice varieties are highly susceptible to recurring flooding from seedling to flowering stage and however, they are extensively cultivated for the character of high yielding. In this study, two rice varieties (ADT36 and ADT37) are grouped as intolerant at phenotypic level and it was confirmed in genotype. Further, we could identify submergence tolerant rice seedlings based on variations in the shoot elongation in heterozygous condition. Here, we identified some superior genotypes rather than donor line and it indicates the adaptation of Sub1 locus in the genetic background of these varieties. Therefore, in future, these rice lines would be useful to rice researchers and rice farmers in the Cauvery delta region.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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