

Impact of SUB1 locus in genetic background of modern rice varieties (*Oryza sativa* L.)

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 KandaswamiKandar'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 BC₃F₂ generation.

Results: In this study, a number of two NILs for ADT36 rice variety (ADT36- F₁2-5-19.18 and ADT36- F₁5-2-5.17) and five NILs for ADT37 rice variety (ADT37- F₁13-27-5.2, ADT37- F₁13-27-5.5, ADT37- F₁15-2-1.10, ADT37- F₁15-2-1.14 and ADT37- F₁15-2-2.14) are selected as superior 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 loss 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

Rice 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 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), Sub1 A 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 introgressed 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 (10, 11).

In the state of Tamil Nadu, the Cauvery delta region, 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 (NRRRI), 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 801 were germinated and grown for 10 days in 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 (**5**).

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 (**16**). PCR was performed using InDel marker located within Sub1 locus, Sub1BC2 and SC3 according to (**10**).

2.3 Development of F₁, BC₂ and BC₃ 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 F₁ seeds. Then, F₁ 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,BC₂ as mentioned above. Positive F₁ plants having heterozygous alleles were used for backcrossing with respective recurrent parent (ADT36 or ADT37) to produce BC₁F₁ seeds during Kharif season, 2020. Likewise, BC₂F₁ population during Kharif season 2021 and BC₃F₁ 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, BC₃F₁ progenies were allowed for selfing to produce BC₃F₂ seeds during Kharif season 2022. BC₃F₂ lines were evaluated for submergence tolerance at seedling stage during Kharif season 2023 in terms of plant height (PH), root length (RL), number of leaf

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(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 O₂ under submergence/flooding and this condition results in the accumulation of phytotoxic substances such as Fe²⁺, Mn²⁺, H₂S, O₂ 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 5.0 cm 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 dhan801 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).



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

Here, the rate of shoot elongation of ADT36, ADT37 and CRdhan801 is increased to 19.2%, 14.3% and 8.1% under submergence condition 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 (CRdhan801). 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 O₂ (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, SUBMERGENCE 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 190 base pairs in ADT36 and ADT37 and 200 base pairs in CR Dhan 801 (Fig.2).

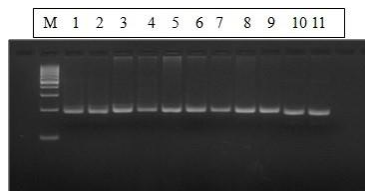


Fig. (2):PCR amplification for parental polymorphism with SC3 DNA marker. M-100 bp DNA ladder. Lanes:1&2-ADT-36; Lanes-3 -9: CR Dhan 801; Lanes 10 & 11: ADT 37

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.

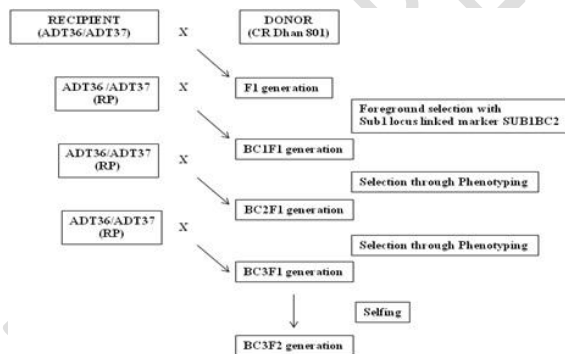


Fig.(3): Breeding scheme used in the marker assisted backcross program for introgression of Sub1 locus into rice cultivar, ADT36 and ADT37.

The details of derived seeds and selected progenies from F₁, BC₁F₁, BC₂F₁ and BC₃F₁ population are given in Table-1.

Table(1):Details of seeds derived and selected progenies from F₁, BC₁F₁, BC₂F₁, BC₃F₁ and BC₃F₂ population.

Cross combination	ADT36/CRDhan801 (cross-1)	ADT37/CRDhan801(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 ₁ F ₁ generation	74 seeds derived	65 seeds derived
Phenotype	Selection of 24 plants/52 plants	Selection of 22 plants/59 plants
BC ₂ F ₁ generation	92 seeds derived	108 seeds derived
Phenotype	Selection of 35 plants/79 plants	Selection of 38 plants/85 plants
BC ₃ F ₁ generation	123 seeds derived	73 seeds derived
BC ₃ F ₂ generation	48 near isogenic lines	26 near isogenic lines

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 10plants (# 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) out of 10 F ₁ plants from cross-2 showed heterozygous allelic condition for Sub1BC2 marker located within the locus in the foreground selection (Fig.4).		

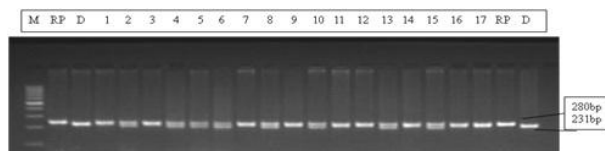


Fig. (4): PCR amplification of seedlings of F₁ generation using gene-specific marker Sub1BC2. **M**-100 bp DNA ladder. **RP**- Recurrent parent; **D**- Donor. **Lanes:1-10**= F₁ plants from ADT 36 x CR Dhan 801 cross. **Lanes-11-17** = F₁ plants from ADT37 x CR Dhan 801 cross.

Selected F₁ plants in foreground selection did backcross with respective recurrent parent (RP) and produced 74 and 65 BC₁F₁ 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 BC₁F₁ population of cross-1, the level of PH ranged from 15.0 to 27.0cm and from these, 24 progenies (plant # F₁-2.5, 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 BC₁F₁ population of cross-2, PH level ranged from 12.0 to 25.5cm under submergence and from these, 22 progenies (plant # F₁13.2, 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.

In this study, we could differentiate tolerant and intolerant rice progenies based on the variations in the shoot elongation and identify submergence tolerant rice progenies at F₁ 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 BC₁F₁ progenies with its RP, 92 and 108 BC₂F₁ seeds were derived for cross-1 and cross-2, respectively and of these, 79 and 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 BC₂F₁ population of cross-1, the level of PH ranged from 11.0 to 25.5cm and of these, 35 progenies (plant # F₁2-5.1, 2, 3, 5, 6, 7, 8, 9, 14, 15, 19; 13.3, 2, 7, 8, 11, 17; F₁4.5.2, 3, 4, 6, 7; 7.1, 4, 6; 16.1, 2, 4, 6; F₁5-2.3, 5; F₁8-2.4, 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 BC₂F₁ population, the range of PH was from 12.0 to 25.5cm and of these, 38 progenies (plant # F₁13-10.3, 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; F₁15-2.1, 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 BC₃F₁ seeds for cross-1 and cross-2, respectively and from these, 60 and 64 progenies were allowed for selfing and we harvested as BC₃F₂ seeds. In the evaluation of BC₃F₂ population of cross-1 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 0.7cm 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 BC₃F₂ 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).

Table(2a):Results of phenotypic selection of BC₃F₂ population of cross-1(ADT36/CR dhan 801 cross combination) under submergence stress condition.

Parental lines/ BC ₃ F ₂ progenies	Morphological characters (Cm)				
	PH	RL	NL	LL	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
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
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In the evaluation of BC₃F₂ 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 BC₃F₂ 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).

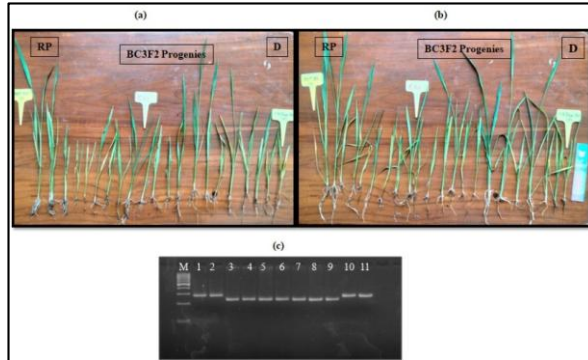


Fig.(5a): shows variations of BC₃F₂ progenies of cross-1(ADT36/CR dhan 801) in submergence tolerance under flooding during seedling stage.

(b) BC₃F₂ progenies of cross-2(ADT37/CR dhan 801); RP- Recurrent parent; D- Donor.

(c) PCR amplification of selected BC₃F₂ 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 BC₃F₂ population of cross-2 (ADT37/CR dhan 801 cross combination) under submergence stress condition.

Parental lines/ BC ₃ F 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.

In the evaluation study, 50.0%, 4.2%, 95.9%, 69.8% and 65.7% of NILs had less value for 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 BC₃F₂ 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- F₁2-5-19.18 and ADT36- F₁5-2-5.17) and five NILs for ADT37 rice variety (ADT37- F₁13-27-5.2, ADT37- F₁13-27-5.5, ADT37- F₁15-2-1.10, ADT37- F₁15-2-1.14 and ADT37- F₁15-2-2.14) 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 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 Sub1 locus 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 Su1 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.

REFERENCES

1. Dey M. M. and Upadhyaya H. K. (1996). Yield loss due to drought, cold and submergence in Asia. In: Evenson R, Herdt R, Hossain M (eds) Rice research in Asia: progress, priorities. CAB International, Wallingford, pp 291-303 DOI 10.1007/s10681-014-1287-x
2. Adkins S. W., Shiraishi T. and McComb J. A. (1990). Submergence tolerance of rice-a glass house method for the experimental submergence of plants. *Physiol. Plant* 80:642- 636.
3. Nguyen Q. C, Ngo THY and Vu TMH (2022). Assessing the potential risks of extreme weather events causing flood hazards for rice cultivation regions in Quang Nam Province. *Research on Crops*, 23(3):481-487.10.31830/2348-7542.2022.ROC-844

4. Xu, K. and Mackill, D. J. (1996). A major locus for submergence tolerance mapped on rice chromosome 9. *Mol. Breed.* 2:219-224.
5. Xu K., Xu X., Fukao T., Canlas P., Maghirang-Rodriguez R., Heuer S., Ismail A. M., Bailey-Serres J., Ronald P. C., Mackill D. J. (2006) Sub1A is an ethylene-response-factorlike gene that confers submergence tolerance to rice. *Nature* 442:705-708. <https://doi.org/10.1038/nature04920>
6. 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* 263:681–689. <https://doi.org/10.1007/s004380051217>.
7. Fukao T., Xu K., Ronald P. C. and Bailey-Serres J. A. (2006). A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. *The Plant Cell* 18:2021–2034.
8. Emerick K. and Ronald P. C. (2019). Sub1 rice: Engineering rice for climate change. *Cold Spring Harb. Perspect. Biol.* 11(12):034637.
9. Mackill D. J. (2012). Development and rapid adoption of submergence-tolerant (Sub1) rice varieties. *Adv. Agron* 115:299–352.
10. 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. *Annals in Botany* 103:151-160.
11. Neeraja C. N., Maghirang-Rodriguez R., Pamplona A., Heuer S., Collard B. C. Y., Septiningsih E. M., Vergara G., Sanchez D., Xu K., Ismail A. M. and Mackill D. J. (2007). A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theor Appl Genet* 115:767–776.
12. Ismail A. M., Thompson M. J., Singh R. K., Gregorio G. B. and Mackill D. J. (2008). Designing rice varieties adapted to coastal area of South and Southeast Asia. *Journal of the Indian Society of Coastal Agricultural Research.* 26:69–73
13. Singh S., Mackill D. J. and Ismail A. M. (2009). Responses of *SUB1* rice introgression lines to submergence in the field: yield and grain quality. *Field Crops Research.* 113:12–23.
14. Singh U. S., Dar M. H., Singh S., Zaidi N. W., Bari M. A., Mackill D. J., Collard B. C. Y., Singh V. N., Singh J.P., Reddy J. N., Singh R. K. and Ismail A. M. (2013). Field performance, dissemination, impact and tracking of submergence tolerant (Sub1) rice varieties in South Asia. *SABRAO Journal of Breeding and Genetics.* 45:112–131.
15. Zheng K., Subudhi P. K., Domingo J., Magpantay G., Huang N. (1995). Rapid DNA isolation for marker assisted selection in rice breeding. *Rice Genet News* 12:255–258.
16. Drew M. and Lynch J. (1980). Soil Anaerobiosis, Microorganisms, and Root Function. *Annu. Rev. Phytopathol.* 18:37–66.
17. Xu K, Mackill D. J. (1996) A major locus for submergence tolerance mapped on rice chromosome 9. *Mol Breed* 2:219–224.
18. Bailey-Serres J., Lee S. C. and Brinton E. (2012). Waterproofing crops: Effective flooding survival strategies. *Plant Physiol.* 160:1698–1709.
19. Ismail A. M., Ella E. S., Vergara G. V. and Mackill D. J. (2009). Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). *Annals of Botany* 103:197–209.
20. Shin N. H., Han J. H., Vo K. T. X., Seo J., Navea I. P., Yoo S. C., Jeon J. S., Chin J. H. (2022). Development of a Temperate Climate-Adapted indica Multi-stress Tolerant Rice Variety by Pyramiding Quantitative Trait Loci. *Rice (N Y)*. 2022 Apr 9; 15(1):22. doi: 10.1186/s12284-022-00568-2. PMID: 35397732; PMCID: PMC8992604.

21. Alam R., Hummel M., Yeung E., Locke A. M., Ignacio J. C. I. and Baltazar M. D. (2020). Flood resilience loci Submergence 1 and Anaerobic germination 1 interact in seedlings established underwater. *Plant Direct* 4: e00240. doi: 10.1002/pld3.240.
22. Rani Kuanar S., Molla K., Chattopadhyay K., Sarkar R. K. and Mohapatra, P. K. (2019). Introgression of Sub1 (SUB1) QTL in mega rice cultivars increases ethylene production to the detriment of grain-filling under stagnant flooding. *Scientific Reports*, 9:18567. | <https://doi.org/10.1038/s41598-019-54908-2>
23. Chakraborty K., Akankhya Guru A., Priyanka Jena, Soham Ray, Arti Guhey Chattopadhyay K. and Sarkar R. K. (2021). Rice with SUB1 QTL possesses greater initial leaf gas film thickness leading to delayed perception of submergence stress. *Annals of Botany* 127:251–265, 2021 doi: 10.1093/aob/mcaa171.
24. Iftekharuddaula K. M., Newaz M. A., Salam M. A., Ahmed H. U., Mahub 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.
25. Dubina E. V., Andrey V., Kostylev A. P. I., Yuliya A., Makukha, Ruban, M. G., Balyasny I. V., Ham L. H., Tu D. X. and Linh L. H. (2018). Introduction of the Sub1 gene into the Russian rice varieties using the polymerase chain reaction (PCR) methods. *African Journal of Agricultural Research* Vol. 13(48):2757-2762. DOI: 10.5897/AJAR2018.13563.
26. Yang S. Y., Wu Y. and Chen C. T. (2017). Physiological and molecular responses of seedlings of an upland rice ('Tung Lu 3') to total submergence compared to those of a submergence-tolerant lowland rice ('FR13A'). *Rice* 10:42. <https://doi.org/10.1186/s12284-017-0180-3>
27. Anshori M. F., Purwoko B. S., Dewi I. S., Suwarno W. B. and Ardie S. W. (2023). Systematic selection to adaptive doubled haploid rice lines under different environments of submergence screening methods. *Journal of Agriculture and Food Research*, 14:100775, <https://doi.org/10.1016/j.jafr.2023.100775>.
28. Panda B., Dash S. K., Mondal S., Senapaty J., Dash M., Samal K. C., Sahoo C. R. and Chakraborty K. (2023). Exploring the physiological efficiencies of promising rice (*Oryza sativa*) accessions for increasing grain yield. *Indian Journal of Agricultural Sciences* 93(11):1180–1185 Article <https://doi.org/10.56093/ijas.v93i11.140727>
29. Septiningsih E. M., N. Hidayatun D. L. Sanchez J. Carandang A. M. Pamplona B. C. Y. Collard A. M. Ismail and Mackill D. J. (2015). Accelerating the development of new submergence tolerant rice varieties: the case of Ciherang-Sub1 and PSB Rc18-Sub1. *Euphytica* 202:259–268. <https://doi.org/10.1007/s10681-014-1287-x>