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Screening of BC_3F_2 Rice population for Submergence Tolerance During Seed Germination

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ABSTRACT: Rice (*Oryza sativa* L.) is an important food crop in the Asian countries, but it is severely affected by submergence stress from seed germination to reproductive stage during heavy monsoon period every year in some parts of the world. This situation has created to improve highly preferable rice varieties for submergence stress tolerance. In the present study, two short duration rice varieties, ADT36 and ADT37 were improved for submergence tolerance at seedling stage using CR Dhan 801 with Submergence 1 (Sub1) locus as donor up to BC_3F_2 generation through marker assisted backcross method. In Tamil Nadu state, these two rice varieties are adapted in the Cauvery delta region wherever submergence and drought stress is erratic. Seeds from this generation were evaluated for submergence tolerance at seed germination stage. At parental level, we noted less and more elongation in the coleoptile growth of donor and both recipient parents under anaerobic condition, respectively when compare to aerobic condition. In the evaluation of BC_3F_2 population, we found a range in the elongation of coleoptile and root length under anaerobic condition. From these, a number of seven rice lines (ADT36- $F_1$2-5-19.6; ADT36- $F_1$2-13-7.11,13,14, ADT36- $F_1$4-5-3.4, ADT36- $F_1$4-16-2.6, ADT36- $F_1$5-2-5.1) for ADT36 and four rice lines (ADT37- $F_1$13-27-5.2, ADT37- $F_1$15-2-1.1, ADT37- $F_1$15-2-2.5, ADT37- $F_1$15-2-2.7) for ADT37 rice variety were found to be superior in coleoptile elongation compared to donor line. These rice lines at seed germination stage followed quiescence strategy like donor parent having Sub1 associated with seedling stage tolerance and they possessed decreased growth under submergence condition. Further, other lines exhibited escaping mechanism under flooding with higher rate of CL and RL. Thus, selected rice lines harboring Sub1 locus linked with submergence tolerance at seedling stage will support for flood tolerance at seed germination stage also in the genetic background of ADT36 and ADT37. Therefore, in future, these lines can be used in both methods of direct seed sowing and transplanting in upland and lowland rice cultivating areas and as a genetic source in the rice breeding program.

Keywords: Submergence tolerance, anaerobic seed germination, Cauvery delta areas, ADT36, ADT37, CR Dhan 801. Coleoptile and Leaf elongation.

INTRODUCTION

Rice is a semi-aquatic crop and it has some adaptive mechanisms to survive and grow under water logged and/or submerged condition for a few days. In rice, development of aerenchyma cells allow sufficient amount of oxygen to submerged parts of the plant during stress. If submergence is prolonged, rice plant loses its survival rate gradually by loss of soil nutrients through reduction of soil redox potential, volatilization and deep percolation (Anjani Kumar *et al*., 2021). Thus, submergence stress causes yield loss over 600 million to 1 billion US dollars annually only in Asian countries (Dey and Upadhaya 1996) through devastating nearly 22 million ha rice areas (Sarkar *et al*., 2006). This yield loss is likely to increase in the flood susceptible rice producing areas in the era of global warming (IPCC, 2007). According to previous reports, rice plants are very sensitive to submergence stress during seed germination and seedling stage (Ismail *et al*., 2009; Angaji *et al*., 2010, Joshi *et al*., 2013). In this connection, identification of AG and SUB1 QTL from rice genotypes supports the rice plants for the higher survival rate during seed germination and seedling stage, respectively. Rice varieties incorporated with SUB1 QTL enhanced their survival rate more during seedling stage through maintaining high and low level of alcohol dehydrogenase activity and chlorophyll degradation rate under submergence, respectively (Anjani Kumar *et al*., 2021). Rice genotypes with AG QTL overcome the flooded condition under direct seeded rice (DSR) conditions (Ismail *et al.*, 2009, Miro *et al*., 2017, Chamara *et al*., 2018, Lal *et al*., 2018). In Tamil Nadu state, rice cultivation is highly practiced in the Cauvery delta region (CDR) for three times in a year depends on seasonal rains. However, this region is susceptible to floodling due to heavy rains at the time of monsoon and the rice farmers in this region frequently face the economic cries by the loss of crop, labour and season. In the CDR, many short duration rice varieties including ADT36 and ADT37 are widely cultivated. In the present study, these varieties were improved for submergence tolerance at seedling stage up to BC_3F_2 generation and these lines were evaluated for submergence tolerance during seed germination also under flooding.

MATERIALS AND METHODS

Rice seeds: A small quantity of rice seeds of ADT36 and ADT37 from Tamilnadu Rice Research Institute (TRRI), Aduthurai, Tamilnadu state, INDIA and CR Dhan 801 rice seeds from National Rice Research Institute (NRRI), Cuttack, Odisha state were sourced. Here, ADT36 and ADT37 rice varieties were used as female parent (recipient) and CR Dhan801 as male parent (donor) which harboring Submergence 1 (Sub1) locus.

Production of BC_3F_2 population: F1 seeds were derived from a cross between ADT36 x CR dhan 801(cross-1) and ADT37 x CR dhan 801 (cross-2) and they were subjected to PCR screening using Sub1 gene specific marker Sub1C173 (Septiningsih *et al*. (2009) and identified positive F1 plants with heterozygous allele. Thus, identified plants were backcrossed with ADT36 and ADT37 as recurrent parent (RP) and produced BC_1F_1, BC_2F_1 and BC_3F_1 generation subsequently based on morphological changes. Finally they were allowed for self-pollination to produce BC_3F_2 seeds.

Evaluation of BC_3F_2 population under submergence condition at seed germination stage: Prior to seed sowing, seed dormancy was broken by incubating the seeds at 50°C for up to 5 days. For submergence screening during seed germination, seeds of ADT36, ADT37, CR Dhan 801 and BC_3F_2 seeds were sowed at 1.0 cm below the soil surface in plastic cups containing fine soil brought from rice field and seeds were covered with fine soil powder. They were placed into a large plastic container and immediately flooded the container carefully and the water level was maintained at 10 cm for 21 days. Similarly, another set up was maintained in non-flooded condition (Ismail *et al*., 2009). Length of coleoptile and root in parental and BC_3F_2 lines were measured in both flood and non-flood conditions.

RESULTS AND DISCUSSION

Submergence/flooding restrict the diffusion of O_2 and CO_2 into water and it creates a hypoxic condition (< 21% - O_2) to plants through making these gases unavailable to plant tissues. Hence, the respiration process of plants in the absence of O_2 is affected and leads to accumulation of phytotoxic substances such as Fe^{2+}, Mn^{2+}, H_2S, O_2 radicals and the products of fermentation which cause damages to the plant tissue (Drew and Lynch 1980). Generally, rice seeds germinate under moisture condition and develop the root and shoot system. Interestingly, rice seeds have a capability to germinate and grow under complete submerged condition using an adaptive system called *SNORKEL 1* and *SNORKEL 2* (*SK1/2*) (Bailey-Serres *et al.*, 2012a). Hence, rice exempts from other food crops such as wheat (Alpi and Beevers 1983, Perata *et al.* 1997), oat (Alpi and Beevers 1983), and barley (Perata *et al.*, 1997). It is an escaping strategy and it supports the germinating seeds to tolerate submergence stress (Bailey-Serres *et al*., 2012). In this study, we evaluated BC_3F_2 progenies for submergence tolerance at seed germination stage and its results are given in Table 1. Under flooding, elongation of CL and RL was 16.9 and 1.1cm in ADT36, 12.5 and 2.0 cm in ADT37 and 5.5 and 0.76 cm in CR dhan801 respectively. Under non-flooding condition, plant height and root length were 13.0 and 10.3 cm in ADT36, 18.0 and 10.3 cm in ADT37, 8.8 and 9.4 cm in CR dhan 801 respectively (Fig. 1a, b; Table 1).

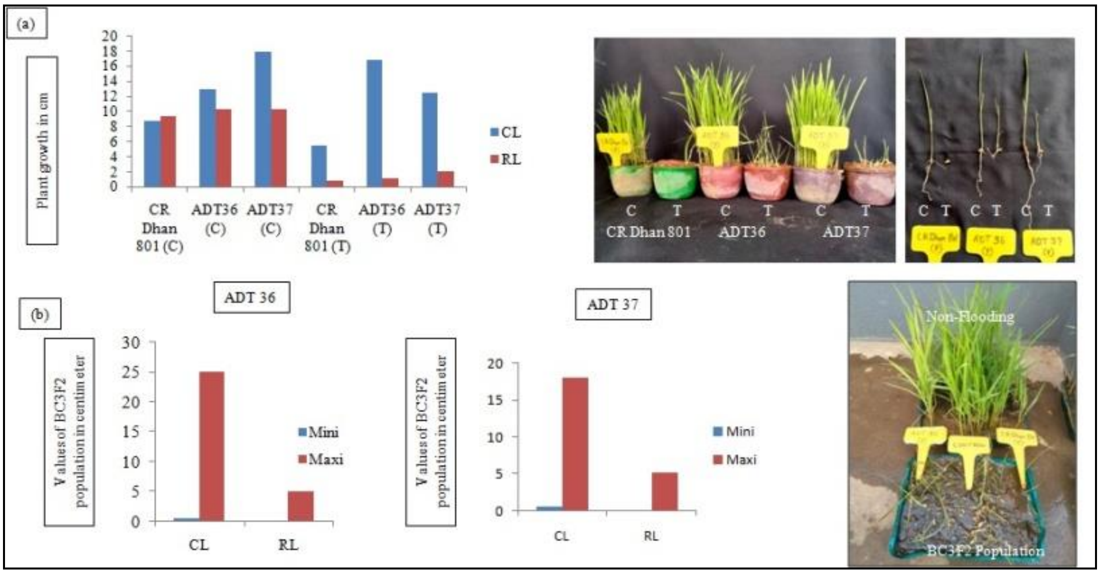

Fig. 1. Plant growth of (a) parental lines and (b) BC_3F_2 population of ADT36 and ADT37. T-Treatment (Flooding); C-Control (Non-Flooding); CL-Coleoptile length; RL-Root length.

Table 1: Response of parental and BC_3F_2 lines under anaerobic condition during seed germination.

ADT36 x CR dhan 801 cross			ADT37 x CR dhan 801 cross		
Parental lines/BC_3F_2 progenies	CL (cm)	RL (cm)	Parental lines/BC_3F_2 progenies	CL (cm)	RL (cm)
CR Dhan 801 (C)	8.9	9.2	CR Dhan 801 (C)	8.8	9.4
ADT36 (C)	13.0	10.26	ADT37 (C)	18.0	10.30
CR Dhan 801 (T)	5.5	0.76	CR Dhan 801 (T)	5.5	0.76
ADT36 (T)	16.9	1.1	ADT37 (T)	12.5	2.0
ADT36- $F_1$2-5-19.1	16.0	0.3	ADT37- $F_1$13-24-3.7	13.5	0.3
ADT36- $F_1$2-5-19.6	5.0	0.2	ADT37- $F_1$13-24-3.12	12.0	0.2
ADT36- $F_1$2-5-19.7	15.0	1.5	ADT37- $F_1$13-24-3.13	11.0	1.5
ADT36- $F_1$2-5-19.9	9.0	1.2	ADT37- $F_1$13-24-3.14	9.0	1.2
ADT36- $F_1$2-5-19.10	8.0	0.3	ADT37- $F_1$13-24-3.15	11.8	0.3
ADT36- $F_1$2-5-19.13	10.5	0.5	ADT37- $F_1$13-27-5.1	10.5	0.5
ADT36- $F_1$2-5-19.14	16.0	2.2	ADT37- $F_1$13-27-5.2	4.0	0.2
ADT36- $F_1$2-5-19.15	25.0	4.0	ADT37- $F_1$13-27-5.3	8.0	4.0
ADT36- $F_1$2-5-19.16	16.5	0.5	ADT37- $F_1$13-27-5.5	7.0	0.5
ADT36- $F_1$2-5-19.17	16.5	5.0	ADT37- $F_1$13-27-5.6	8.2	5.0
ADT36- $F_1$2-5-19.18	7.0	0.5	ADT37- $F_1$13-27-5.10	9.0	0.5
ADT36- $F_1$2-5-19.20	8.5	3.5	ADT37- $F_1$15-2-1.1	0.5	0.0
ADT36- $F_1$2-13-7.1	18.0	1.0	ADT37- $F_1$15-2-1.7	6.0	1.0
ADT36- $F_1$2-13-7.2	5.5	0.6	ADT37- $F_1$15-2-1.10	5.5	0.6
ADT36- $F_1$2-13-7.3	7.5	0.3	ADT37- $F_1$15-2-1.11	7.5	0.3
ADT36- $F_1$2-13-7.4	12.0	1.0	ADT37- $F_1$15-2-1.13	18.0	1.0
ADT36- $F_1$2-13-7.5	8.0	0.2	ADT37- $F_1$15-2-1.14	8.0	0.2
ADT36- $F_1$2-13-7.6	8.0	0.2	ADT37- $F_1$15-2-1.16	7.5	0.2
ADT36- $F_1$2-13-7.9	6.5	0.2	ADT37- $F_1$15-2-2.1	16.5	0.2
ADT36- $F_1$2-13-7.10	17.5	0.5	ADT37- $F_1$15-2-2.2	13.5	0.5
ADT36- $F_1$2-13-7.11	5.0	0.0	ADT37- $F_1$15-2-2.4	15.0	0.0
ADT36- $F_1$2-13-7.12	8.5	1.0	ADT37- $F_1$15-2-2.5	0.5	0.0
ADT36- $F_1$2-13-7.13	4.0	0.0	ADT37- $F_1$15-2-2.7	5.0	0.0
ADT36- $F_1$2-13-7.14	5.0	0.5	ADT37- $F_1$15-2-2.10	9.0	1.5
ADT36- $F_1$2-13-7.15	10.0	3.0	ADT37- $F_1$15-2-2.14	7.0	3.0
ADT36- $F_1$2-13-7.16	17.2	1.0	ADT37- $F_1$15-2-2.18	12.2	1.0
ADT36- $F_1$2-13-7.17	18.0	0.5			
ADT36- $F_1$2-13-7.18	9.0	0.3			
ADT36- $F_1$2-13-7.19	0.6	0.0			
ADT36- $F_1$2-13-7.20	5.5	3.0			
ADT36- $F_1$4-5-3.4	5.0	0.2			
ADT36- $F_1$4-5-3.5	8.5	0.0			
ADT36- $F_1$4-5-3.7	11.7	0.5			
ADT36- $F_1$4-5-3.8	10.5	0.0			
ADT36- $F_1$4-5-3.9	13.2	1.5			
ADT36- $F_1$4-5-3.10	11.0	0.5			
ADT36- $F_1$4-16-2.1	0.5	0.0			
ADT36- $F_1$4-16-2.3	13.0	1.5			
ADT36- $F_1$4-16-2.6	5.0	0.5			
ADT36- $F_1$4-16-2.7	11.0	0.5			
ADT36- $F_1$4-16-2.10	10.5	0.5			
ADT36- $F_1$5-2-5.1	4.5	0.5			
ADT36- $F_1$5-2-5.2	17.3	2.5			
ADT36- $F_1$5-2-5.7	9.4	3.0			
ADT36- $F_1$5-2-5.9	9.0	2.5			
ADT36- $F_1$5-2-5.17	10.5	0.5			
ADT36- $F_1$8-2-5.8	13.1	1.5			
ADT36- $F_1$8-2-5.25	7.8	2.0			

C – Control (Non-Flooding); **T** – Treatment (Flooding); **CL** – Coleoptile length; **RL** – Root length.

In ADT36 and ADT37, the growth rate of CL is increased to 76.9% and 30.5% and RL is decreased to 89.3% and 80.6% under flooding, respectively. In CR dhan801, elongation of CL is reduced to 37.5% and RL to 91.9% under flooding. At parental level, growth rate of CL was significantly higher when compare to RL under flooding compared to non-flooding condition. Notably, elongation of CL in ADT36 and ADT37 with no Sub1 was found to be increased to 67.4% and 30.5% respectively than that of CR Dhan 801 with Sub1 under flooding. This coleoptile elongation is dependent on mobilization rate of stored starch in rice seed during seed germination by the activity of α-amylase (αAmy) (1,4-α-D-glucan maltohydrolase). During the mobilization, α-1,4-glucosidic bonds of starch is hydrolyzed to yield α-glucose and α-maltose (Damaris *et al*., 2019, Senapati *et al*., 2019). So far, three subfamilies of *αAmy* genes such as RAmy1 (A, B, and C), RAmy2A, and RAmy3 (A, B, C, D, E, and F) have been identified (Huang *et al*., 1990, 1992) and particularly, RAmy3D enzyme is reported for its significant role in the fermentative metabolism pathway for the production of energy under submergence (Hwang *et al.,* 1990). Moreover, the rate of coleoptile elongation is determined by the pre-formed cells in embryo (Jones and Rost 1989) and some phytohormones like ethylene play a significant role in the mechanism in the growth of submerged rice coleoptile (Masuda *et al*., 1998). Moreover, the rapid coleoptile elongation is linked with decreased and increased level of abscisic acid (ABA) and gibberellic acid (GA), respectively due to the accumulation of ethylene under submerged condition of rice (Hoffmann-Benning & Kende 1992; Fukao and Bailey-Serres 2008). According to Saika *et al*., (2007), ABA conversion to an inactive form is catalysed through production of ABA 8′- hydroxylase enzyme by stimulation of ethylene which is trapped during submergence.

In BC_3F_2 population of cross-2, CL and RL elongation was in a range of 0.5 to 25.0 cm and 0.0 to 5.0 cm, respectively whereas in cross-2, they were in the range of 0.5 to 25.0 cm and 0.0 to 5.0 cm, respectively. In BC_3F_2 population under flooding, we noted that 79.1%, 15.4% and 20.8% of progenies showed lower value than ADT36, ADT37 and CR dhan 801, respectively. Variations in coleoptile elongation under submergence influences seedling establishments by a trait called anaerobic germination (AG) which supports for starch degradation to escape seed germination from submergence stress (Huang *et al.,* 2003). In previous surveys also, genetic factors responsible for AG have showed a wide phenotypic variation in survival rate under flooding (Ismail *et al*., 2009; Miro and Ismail 2013). Specifically, *AG*1 gene belonging to the trehalose-6-phosphate phosphatase gene-family (Angaji, 2008; Angaji *et al*., 2010; Baltazar *et al*., 2014; Septiningsih *et al.,* 2013) helps to elongate the coleoptile under anaerobic condition (Kretzschmar *et al*., 2015). Coleoptile elongation in rice under anaerobic condition is linked with some specific expansins such as *EXPA2*, *EXPA4*, *EXPA1*, *EXPB11* and *EXPB17* (Lasanthi-Kudahettige *et al*., 2007). In recent study also, it is reported that coleoptile elongation showed difference in flooding (Aung *et al.,* 2023). Based on shoot elongation during seed germination, many efficient rice genotypes are identified for flood-prone areas (Vijay Kumar Reddy *et al*., 2022).

In the study of root length in F_2 population under flooding, 31.2%, and 39.6% of progenies accounted for more value than ADT36 and CR dhan 801 in cross-1 whereas in cross-2, 11.5% and 10.0% of progenies for more value than ADT37 and CR dhan 801. More and less value of root length development under flooding is dependent on coleoptile elongation which reaches the water surface in order to supply oxygen to the root and endosperm for speedy establishment of seedlings following the starch degradation (Magneschi and Perata 2009). Then, shoots and roots develop through the events of splitting, aerenchyma cell formation and senescence in submerged coleoptile in response to air (Kawai and Uchimiya 2000). In a study, coleoptiles elongation regulating a microRNA *miR393a* during seed germination and seedling establishment under submergence is reported in rice and its overexpression exhibits primary root elongation and inhibits coleoptile elongation. The opposite effects of miR393a on root and coleoptile elongation indicate that auxin signalling negatively regulates primary root elongation but positively regulates coleoptile elongation (Guo *et al*., 2016). A recent finding reports that pyramiding of ANAEROBIC GERMINATION 1 (AG1) locus encoding TREHALOSE 6-PHOSPHATE PHOSPHATASE 7 (TPP7) and SUMBERGENCE 1 (SUB1) locus encoding ethylene-responsive transcription factor SUB1A-1 slowed the elongation growth in near-isogenic lines (Shin *et al*., 2022, Alam *et al*., 2020). Therefore, plant growth in CR Dhan 801 having Sub1 locus is controlled but not in ADT36 and ADT37 rice variety. In this connection, rice variety with Sub1 locus undergoes another strategy called Quiescence (Bailey-Serres *et al*., 2012) during vegetative stage stress. Expression of SUB1A gene inhibits rice elongation through stimulating *alcohol dehydrogenase* (ADH) gene activation for upregulating the expression of *slender rice-1* (*SLR1*) and *SLR1 like-1*(*SLRL1*) which are negative regulators of gibberellins (GA) signaling. Hence, SUB1A gene expression reduces the carbohydrate consumption and increases the survival rate of rice seedlings (Xu *et al*., 2006, Fukao *et al*., 2006, Fukao *et al*., 2008).

CONCLUSIONS

In the present study, we found the difference between the donor and recipient parent based on low and high rate of coleoptile elongation under flooding, respectively. Shoot elongation under flooding which results in consumption of stored starch content very fast and it leads to plant death or lodging following the de-submergence. According to the previous studies, Sub1 locus is linked with only submergence tolerance at seedling stage and it controls the degradation of starch due to shoot elongation but not at seed germination. Elongation of coleoptile in ADT36 and ADT37 rice variety during seed germination associates with *SNORKEL 1* and *SNORKEL 2* (*SK1/2*)-dependent escape strategy and CR Dhan 801 with *SUBMERGENCE 1A* (*SUB1A*)-dependent quiescence strategy. However, we found both strategies in BC_3F_2 population. Supportively, in a very recent study, rice line pyramided with AG and SUB1 gene has showed negative impact on shoot elongation. Here also, we found difference in flood tolerance between ADT36 and ADT37 i.e. when compare to ADT36, elongation of coleoptile is found to be decreased and increased under flooding and non-flooding condition, respectively. Thus, this study reveals that expression of gene/QTL depends on the genetic background of a rice variety. Further, the screening process of existing genetic source may lead to a way to some other stress tolerance also.

FUTURE SCOPE

In this study, we identified some rice lines with controlled plant growth in order to tolerate flooding. Therefore, these rice lines can be used as genetic source in rice breeding and they will be suitable for direct seed sowing method in the upland rice cultivating areas.

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Conflict of interest. None.

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