Indian J. Genet., 80(4) 375-383 (2020)
DOI: 10.31742/IJGPB.80.4.2

Mechanism of anoxic tolerance in backcross lines developed through Jyothi x Swarna-Sub 1 under submergence stress

Deepa John* and K. S. Shylaraj

Rice Research Station, Kerala Agricultural University, Vyttila 682 019, Ernakulam, Kerala

(Received: July 2020; Revised: October 2020; Accepted: October 2020)

Abstract

Rice varieties adapted to flash flood exhibit submergence tolerance by maintaining reduced shoot elongation under submerged conditions. In this study physiological traits leading to flash flood tolerance *viz*., high survival percentage with reduced shoot elongation under submergence and increased rate of alcoholic fermentation were investigated. *Sub1* introgressed BC_3F_2 lines exhibited higher survival percentage with moderate shoot elongation under submergence stress for fourteen days similar to the donor parent Swarna- Sub1. Enzymatic activities of alcohol dehydrogenase and pyruvate decarboxylase were monitored for fourteen days under submergence stress. The developed *Sub1* introgressed lines and the donor parent Swarna-Sub1 showed higher rates of alcohol dehydrogenase and pyruvate decarboxylase activities under submergence stress thereby maintaining optimum rates of alcoholic fermentation. The findings of this study confirmed the expression of tolerance mechanism in the *Sub1* introgressed Jyothi Backcross Inbred lines under submergence stress.

Key words: Submergence tolerance, reduced shoot elongation, alcohol fermentation, alcohol dehydrogenase activity, pyruvate decarboxylase activity.

Introduction

In coastal areas, crop loss due to submergence caused by flash flood of 7-14 days during monsoon season is very common which affect crop establishment and results in complete crop loss or reduction of total rice production (Septiningsih et al. 2009). Flash flooding results in rapid water level increase resulting in complete submergence for up to fourteen days. Plants under submergence are exposed to two major effects like anaerobic conditions and post anoxic shock after the flood water recedes (Sasidharan et al. 2017). Submergence tolerance is defined as the ability of rice plants to survive complete submergence for a period of 10-14 days and renew growth once the water subsides (Singh et al. 2017). Rice adopts two strategies to overcome the submergence stress: quiescence and escape (Luo et al. 2011). Reduced shoot elongation is one of the key mechanisms adopted by submergence tolerant plants exhibiting quiescence strategy to conserve their energy for survival under submerged conditions. This mechanism is expressed by rice varieties adapted to flash flood (Das et al. 2005). *Sub1* region encodes three transcription factors *(Sub1A*, *Sub1B* and *Sub1C*) belonging to the B-2 subgroup of the ethylene response factors (ERFs)/ ethylene-responsive element binding proteins (EREBPs)/apetala 2-like proteins (Xu et al. 2006). *Sub1A* region is responsible for conservation of carbohydrates by limiting shoot elongation under water (Perata and Voesenek 2007). These varieties can save energy during submergence period which can be effectively used to renew growth after desubmergence. Shoot elongation ability (escape mechanism) on the other hand are adopted by varieties capable of withstanding deepwater where water stagnates (> 100cm) for several months. With the escape mechanism, plants can continue aerobic metabolism and photosynthesis by elongating and maintaining their shoots above the water surface (Hattori et al. 2009). However, shoot elongation under flash flood conditions have adverse effects on submergence tolerance because the elongating plants would lodge when the water recedes resulting in crop loss (Kawano et al. 2002a & b (Bui et al. 2019). Therefore, rice varieties adapted to these areas should show submergence tolerance by limited shoot elongation under submerged

*Corresponding author's e-mail: neenababy.john@gmail.com

Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

conditions

The major problem encountered by plants under submergence is oxygen shortage including hypoxia or anoxia around the shoot and root tissues due to reduced movement of gases(Bailey-Serres and Colmer 2014).This results in energy deficit caused by inhibition of respiration (Geigenberger et al. 2003; Magneschi and Perata 2009; Ismail et al. 2012; Bailey-Serres et al. 2012). Alcoholic fermentation is one of the major metabolic adaptations by plants under submergence stress, in which pyruvate decarboxylase and alcohol dehydrogenase converts pyruvic acid to ethanol (Vu et al. 2009).

This study focuses on the assessment of reduced shoot elongation and high rate of alcoholic fermentation of the *Sub 1* introgressed RILs as an adaptive mechanism towards submergence tolerance in comparison with the donor parent. The main objectives of the present study include *in vitro* phenotyping of the BC_3F_2 introgressed lines of Jyothi to assess the plant survival percentage after desubmergence and to assess the total shoot elongation before and after submergence and to assess the level of enzyme activities (PDC and ADH) under submergence stress.

Materials and methods

Screening for submergence tolerance

Marker assisted backcross breeding approach was used to introgress *Sub1* QTL from the donor parent Swarna-Sub1 into the genetic background of recurrent parent Jyothi. Foreground, recombinant and background screening were done with the respective markers at each generation to select progenies to b forwarded to the next generation. Progenies with *Sub1* introgressed region and with maximum recurrent genome recovery were selected to be backcrossed to raise the next filial generation. Finally, at the BC_3F_2 generation, progenies with *Sub1* introgressed region in the homozygous state and maximum recurrent parent genome percentage were selected. Similar strategies were followed by plant breeders to select the progenies for crossing during the introgression of *Sub1* QTL into the genetic background of popular rice varieties (Chetia et al. 2018; Bhandari et al. 2019). Germinated seedlings 40 each of the selected 20 BC_3F_2 plants along with donor and recurrent parents were sown in pots in Completely Randomized Design with 3 replications. IR64 and recurrent parent Jyothi were used as submergence sensitive and FR13A and donor parent Swarna-Sub1 were used as submergence tolerant check varieties. Fourteen day old seedlings were completely submerged in tanks filled with turbid water of 1m height for 14 days following standard protocols (Neeraja et al. 2007). The survival percentage of plants was scored 14 days after de-submergence according to the IRRI Standard Evaluation System (IRRI 1988) as shown in Table 1.

Plant height was measured from 10 randomly selected plants in each line before submergence and immediately after de-submergence. Percentage of elongation was calculated as suggested by Das et al. 2005.

$$\text{Elongation \%} = \frac{\text{(Plant height after submergence) - (Plant height begore submergence)}}{\text{Plant height before submergence}}$$

Estimation of Pyruvate decraboxylse activity

The pyruvate decarboxylase (PDC) activity during submergence was assayed by the method suggested by Mohanty and Ong 2003. Germinated seedlings of the selected BC_3F_2 plants along with donor and recurrent parents were sown in pots (three replications). IR64 and FR13A were also sown and used as susceptible check and tolerant check varieties respectively. Fourteen day old seedlings were completely submerged in 1m height tanks filled with turbid water for 14 days. Leaf samples were collected from the plants at various intervals (before submergence, first, third, sixth, tenth and fourteenth day of submergence). 100mg of shoot samples were ground at 4°C with 1.5ml of extraction buffer using a mortar and pestle. The extract was centrifuged at 20,000 g at 4°C for 30 minutes. The supernatant was collected for PDC activity assay. 570µl of crude enzyme extract was added to the assay buffer to a final volume of 3 ml and incubated at 25°C for 15- 20 minutes to develop maximum activity. The reaction was initated by adding 600 µl of 100mM sodium pyruvate and the coupled NADH oxidation was monitored at A_{340} for a total of 2min at an interval of 1 minute. Total protein in the crude enzyme extract was estimated by NanoDrop A280 measurement method (Desjardins et al. 2009).

Estimation of alcohol dehydrogenase activity

Germinated seedlings of the *Sub1* introgressed BC_3F_2 plants along with donor and recurrent parents were sown in pots in Completely randomized Design with 3

Table 1. Standard Evaluation System (SES) score for submergence tolerance in rice (IRRI, 1988)

Survival %	Score	Observation	Tolerance
100	1	Minor visible symptom of injury	Highly Tolerant
95-99	3	Some visible symptom of injury	Tolerant
75-94	5	Moderate injury	Moderately Tolerant
50-75	7	Severe injury	Susceptible
0-49	9	Partial to complete death	Highly Susceptible

replications. IR64 and FR13A were used as susceptible check and tolerant check varieties respectively. Fourteen day old seedlings were completely submerged in 1m height tanks filled with turbid water for 14 days. Leaf samples were collected from the plants at various intervals (before submergence, first, third, sixth, tenth and fourteenth day of submergence). 100mg of shoot samples were ground at 4°C with 800µl of extraction buffer using a mortar and pestle. The extract was centrifuged at 15,000rpm at 4°C for 15 minutes. The supernatant was collected for ADH activity assay by the method followed by Fukao et al. 2003. The reaction mixture (total volume 3ml) contained 150 µl of enzyme extract, 50 mMTris-HCl, pH9.0, and 1 mM NAD^+. Ethanol (150 µl) was added to initiate the reaction, and NAD^+ reduction was monitored at A_{340} for a total of 2min at an interval of 1 minute. Total protein in the crude enzyme extract was estimated by NanoDrop A280 measurement method (Desjardins et al. 2009).

Statistical analysis

Data for shoot elongation percentage, ADH and PDC activities of all the *Sub1* introgressed lines and parental lines were analyzed using Duncan's Multiple Range Test. Correlations among these traits and survival percentage of all the *Sub1*introgressed lines and parental lines were analyzed using Web Agri Stat Package 2.0 (ICAR Goa).

Results

Submergence tolerance screening and elongation rate

The selected *Sub1* introgressed BC_3F_2 plants and the donor parent Swarna-Sub1 showed rapid recovery and regained growth soon after desubmergence similar to the tolerant check FR13A suggesting that they are tolerant to submergence stress. Recurrent parent was completely dead after the submergence stress of fourteen days similar to the susceptible check IR64 (Fig. 1a-c). SES scores of these BC_3F_2 lines were similar to those of the tolerant donor (Table 2).

Fig. 1a. Fourteen day old seedlings before submergence

Fig. 1b. Desubmerged plants after fourteen days of stress

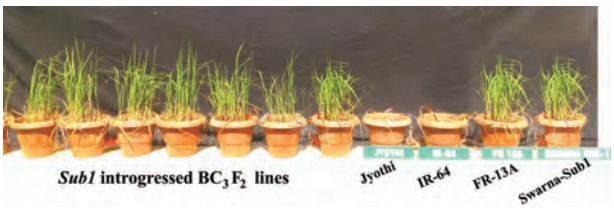

Fig. 1c. Recovery of *Sub1* introgressed BC_3F_2 lines after fourteen days of desubmergence

The results confirmed the successful introgression of *Sub1* QTL in the selected lines. Similar findings were reported by Neeraja et al. 2007.

Limited shoot elongation during submergence is desirable for submergence tolerance (Jackson and Ram, 2003). Therefore, shoot length of the developed lines along with the parental lines were measured before submergence and soon after 14 days of submergence stress to calculate the shoot elongation

Table 2. Submergence tolerance score and rate of shoot elongation of *Sub 1* introgressed BC_3F_2 lines

S.No.	BC_3F_2 progeny no.	No. of plants after 14 days of submergence	Survival %	Score	Plant height before submergence (cm)	Plant height after submergence (cm)	Elongation percentage (%)
1	32-57-8-1	38.00[a]	95.00[a]	3.6[b]	21.3[b]	24.07[a]	12.80[c]
2	32-57-8-4	38.33[a]	95.83[a]	3.00[b]	21.8[a]	24.22[a]	10.97[d]
3	32-57-8-6	38.00[a]	95.00[a]	3.00[b]	20.4[b]	24.17[a]	18.37[b]
4	32-57-8-8	38.00[a]	95.00[a]	3.67[b]	20.87[b]	23.16[b]	11.02[d]
5	32-57-9-1	38.67[a]	96.67[a]	3.00[b]	18.4[c]	20.94[c]	13.59[c]
6	32-57-9-3	39.00[a]	97.50[a]	3.00[b]	19.90[b]	22.20[b]	11.52[c]
7	32-57-9-5	38.67[a]	96.67[a]	3.00[b]	17.67[c]	19.73[c]	11.58[c]
8	32-57-9-7	39.00[a]	97.50[a]	3.00[b]	20.67[b]	23.63[b]	14.31[c]
9	32-57-9-9	38.67[a]	96.67[a]	3.00[b]	21.30[b]	23.58[b]	10.71[d]
10	32-57-18-1	38.00[a]	95.00[a]	3.67[b]	18.53[c]	20.43[c]	10.17[d]
11	32-57-18-4	38.33[a]	95.83[a]	3.00[b]	20.43[b]	22.90[b]	12.04[c]
12	32-57-18-5	38.00[a]	95.00[a]	3.00[b]	21.90[a]	24.75[a]	13.04[c]
13	12-3-3-3	38.00[a]	95.00[a]	3.67[b]	21.40[b]	23.7[b]	10.59[d]
14	12-3-3-9	38.33[a]	95.83[a]	3.67[b]	21.50[b]	23.90[b]	11.22[c]
15	45-65-1-1	38.00[a]	95.00[a]	3.67[b]	22.90[a]	25.49[b]	11.34[c]
16	45-65-1-4	38.00[a]	95.00[a]	3.67[b]	19.87[b]	22.37[b]	12.58[c]
17	45-65-1-10	38.00[a]	95.00	3.67[b]	17.83[c]	19.56[c]	9.63[d]
18	12-6-2-1	38.33[a]	95.83	3.667[b]	21.60[b]	23.88[b]	10.57[d]
19	12-6-2-7	38.33[a]	95.83 [a]	3.67[b]	21.83[a]	24.23[a]	11.03[d]
20	12-6-2-8	39.00[a]	97.50	3.00[b]	22.00[a]	24.18[a]	9.91[d]
21	Jyothi	0.00[b]	0.00[b]	9.00[a]	17.55[c]	22.50[b]	28.35[a]
22	Swarna-Sub1	38.67[a]	96.67[a]	3.00[b]	19.31[b]	21.52[b]	11.53[c]
23	IR-64	0.00[b]	0.00[b]	9.00[a]	16.48[c]	21.22[c]	26.85[a]
24	FR13A	39.00[a]	97.50[a]	3.00[b]	24.13 [a]	26.60 [a]	10.24[d]
CV	2.10	2.10	21.77	7.062	6.978	15.25	
CD (0.01)	1.69	4.22	1.71	3.15	3.52	4.38	
CD (0.05)	1.26	3.16	1.28	2.37	2.64	3.20	

percentage (Table 2). Swarna-Sub1 showed a moderate level of shoot elongation (11.53%). The shoot elongation percentage of *Sub1* introgressed BC_3F_2 lines ranged from 9.63% to 18.37%. *Sub1* introgressed BC_3F_2 line 45-65-1-10 expressed the least shoot elongation percentage of 9.63, which is on par with the tolerant check variety FR13A. Among the twenty BC_3F_2 progenies screened, ten of the *Sub1* introgressed BC_3F_2 lines showed shoot elongation percentage values on par with the value of donor parent whereas nine *Sub1* introgressed BC_3F_2 lines showed values on par with the tolerant check variety FR13A (Table 2). These progenies show better energy conservation by maintaining minimized shoot elongation under submergence stress. The recurrent parent and susceptible check had higher rates of elongation (28.35% and 26.85 respectively).

PDC and ADH assay

In the present study, role of increased PDC and ADH enzymes activities on conferring submergence tolerance in the developed BC_3F_2 lines of Jyothi were evaluated in the developed BC_3F_2 lines.

PDC activity in all BC_3F_2 lines and the donor parent increased to the maximum by day 6 of submergence and then decreased till the 14th day of submergence. Recurrent parent showed a slow rate of PDC activity up to 6 days of submergence but decreased sharply by day 14. The PDC activity of the donor parent Swarna-Sub1 was 0.0386 U/min/mg protein by 6th day of submergence stress. However, by 14th day of submergence it slightly reduced to 0.0274 U/min/mg protein. The PDC activities in the BC_3F_2 lines ranged from 0.0211-0.0422 U/min/mg protein by 6th day of submergence. Progenies 32-57-9-5, 45-65-1-1, 12-3-3-3, 45-65-1-10, 12-3-3-9, 32-57-9-7, 32-57-8-8 and 32-57-9-9 expressed significantly higher value of PDC activity by 6th day of submergence stress compared to the donor parent. PDC activity on 14th day declined and it ranged from 0.0183-0.0347 U/min/mg protein. Progenies 12-3-3-3, 45-65-1-10, 32-57-9-3, 12-6-2-8, 12-3-3-9, 32-57-18-1, 32-57-9-1, 32-57-9-7, 32-57-8-8 and 32-57-9-9 expressed significantly higher value of PDC activity by 14th day of submergence stress compared to the donor parent. All the remaining progenies expressed slightly lower values of PDC activity by 14th day of stress but significantly higher amount than the recurrent parent.

Recurrent parent on the other hand showed comparatively lower rate of PDC activity which slowly increased till 6th day of submergence stress (0.0083 U/min/mg protein) and thereafter sharply declined by 14th day (0.0015 U/min/mg protein). Fig. 2a and 2b shows the graph for progenies expressing better PDC activity values than the donor parent under control conditions and submergence stress respectively.

The ADH activity of the donor parent Swarna-Sub1 was 0.0233U/min/mg protein by 14th day of submergence stress. The ADH activities in the BC_3F_2 lines on 14th day of submergence ranged from 0.0180-0.0258U/min/mg protein. ADH activity in all the BC_3F_2 lines under submergence increased continuously for 14 days of submergence compared to the control plants, similar to the donor parent. Seven progenies 12-6-2-1, 12-6-2-8, 45-65-1-1, 45-65-1-0, 45-65-1-4, 12-3-3-3 and 32-57-18-5 expressed on par values of ADH activity with donor parent and few lines (32-57-8-8, 32-57-8-4, 12-3-3-9, 32-57-18-1 and 32-57-9-9) showed statistically significant ADH activity than the donor parent ranging from 0.0240-0258U/min/mg protein. Some of the progenies had a lower value of ADH activity by 14th day of submergence than the donor parent but still significantly higher than the recurrent parent.

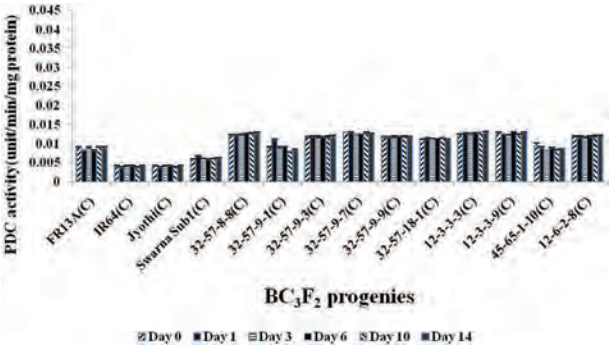

Fig. 2a. PDC activity of Sub1introgressed BC_3F_2 progenies and parental lines under control conditions

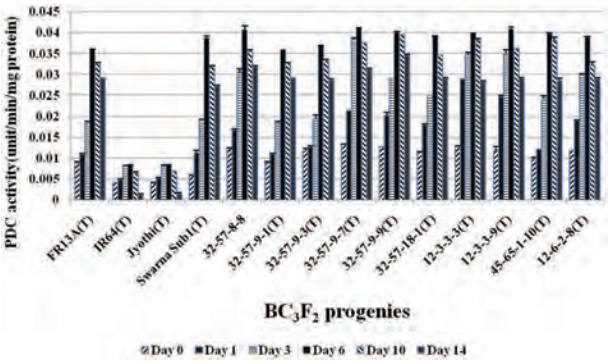

Fig. 2b. PDC activity of *Sub1* introgressed BC_3F_2 progenies and parental lines during submergence stress

Recurrent parent on the other hand showed comparatively lower rate of ADH activity which slowly increased till 6th day of submergence stress and thereafter sharply declined by 14th day. ADH activity of recurrent parent on 14th day of submergence was 0.0004U/min/mg protein. Fig 3a and 3b shows the graph for progenies expressing ADH activity values on par or more than the donor parent under control conditions and submergence stress respectively.

Correlation analysis between shoot elongation percentage, ADH activity, PDC activity, and survival percentage

Correlations among reduced shoot elongation percentage, ADH and PDC activities and survival percentage of all the twenty *Sub1*introgressed BC_3F_2 progenies and parental lines were analyzed and the data is given in table 3. In the current study, ADH activity was positively and significantly associated with PDC activity (r = 0.65). ADH and PDC activities showed a strong and significant positive correlation with survival percentage under submergence stress (r

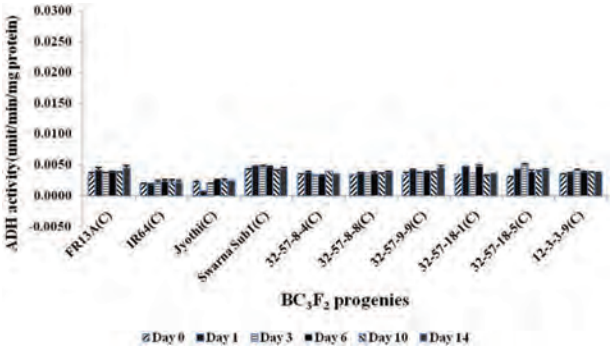

Fig. 3a. ADH activity of Sub1 introgressed BC_3F_2 progenies and parental lines under control conditions

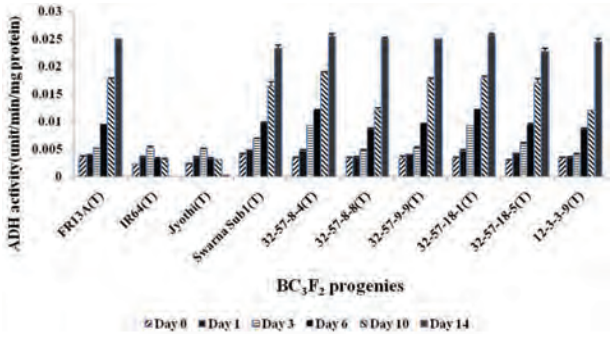

Fig. 3b. ADH activity of Sub1introgressed BC_3F_2 progenies and parental lines under submergence stress

= 0.89 and 0.67 respectively).A strong and significant negative correlation was found between ADH activity and shoot elongation percentage (r= -0.85) as well as between PDC activity and shoot elongation percentage (r = -0.59). Shoot elongation percentage and survival percentage also showed a strong and significant negative correlation (-0.82).

Table 3. Correlation coefficients among different physiological parameters of submergence tolerance

Traits	ADH activity	PDC activity	Elongation % under submergence stress	Survival % after 14 days of submer-gence
ADH activity	1.00			
PDC activity	0.65	1.00		
Elongation % under sub-mergence stress	-0.85	-0.59	1.00	
Survival % after 14 d of submergence	0.89	0.67	-0.82	1.00

All the tests were significant at 5% level

Discussion

Survival percentage and rate of shoot elongation

The rice variety used as donor parent in the current breeding programme is Swarna-Sub1 which is introgressed with the *Sub1A* locus and shows limited shoot elongation (Neeraja et al. 2007). All the *Sub1* introgressed BC_3F_2 lines showed survival percentage similar to the donor parent. Rice varieties expressing *Sub1* QTL maintain a reduced shoot elongation under water and recover by elongation of shoot and production of new leaves as soon as the water recedes, indicating quiescence mechanism (Fukao and Bailey-Serres 2008). In the present study, BC_3F_2 lines introgressed with *Sub1* locus showed reduced shoot elongation during the submergence period and a higher survival percentage whereas, recurrent parent showed increased rate of shoot elongation. The results clearly confirm the quiescence mechanism of submergence tolerance adopted by the introgressed lines. Similarly, other *Sub1*introgressed cultivars like Swarna-Sub1, IR64-Sub1 and Samba Mahsuri-Sub1 also showed reduced shoot elongation and better survival under submergence (Panda and Sarkar 2012a, b & c, Panda and Sarkar, 2013, Sarkar and Bhattacharje 2012, Kumar et al. 2020). Fukao et al. 2006 and Xu et al.2006 have described the molecular mechanism underlying reduced shoot elongation as an adaptive strategy for submergence tolerance. Recurrent parent on the other hand elongated at a faster rate. This strategy is disadvantageous under flash flood condition as rapid shoot elongation causes depletion of carbohydrate which is required for survival under submergence stress and to resume growth during de submergence (Bui et al. 2019; Ye et al. 2018). Therefore, recurrent parent could not survive the submergence stress.

The results from our study indicting moderate shoot elongation in the *Sub1* introgressed BC_3F_2 lines further confirmed the successful introgression of *Sub1* locus from donor parent Swarna-Sub1.

ADH and PDC activities

*Sub1*introgressed lines of Jyothi expressed increased rate of ADH and PDC activities similar to the donor parent compared with the lower rate of enzyme activities in the susceptible recurrent parent Jyothi

under submergence stress. Few of the lines expressed better PDC and ADH activities than the donor parent. This could be due to the synergistic interaction between *Sub1* gene and the genetic background of recurrent parent. There are evidences suggesting that interaction between genes plays an important role for expression of a trait (Storey et al. 2005). The introgressed lines were able to survive the anaerobic stress of flash flood by maintaining energy level via alcoholic fermentation. The decline in the activity in the recurrent parent after initial increase indicates poor carbohydrate metabolism under anaerobic conditions and therefore, not able to maintain energy status for survival under the submergence stress.

Induction of alcoholic fermentation enzymes enhances submergence tolerance of plants under flooded conditions. There are reports stating rapid increase in the activities of both PDC and ADH under anaerobic conditions in rice. Overexpression of PDC in transgenic rice resulted in increased PDC activity and enhanced submergence tolerance (Quimio et al. 2000). PDC and ADH enzyme activities increased in M202-Sub1, the flood tolerant version of rice variety M202 under submergence stress (Fukao et al. 2006).

Increased levels of ADH enzymes during submergence have been reported with respect to flood tolerant plants which supports our findings (Kato-Noguchi and Kugimiya 2003). The submergence tolerant rice variety FR13A showed highest increase in ADH activity whereas, IR42 the submergence susceptible variety showed limited increase in the enzyme activity under submergence (Goswami et al. 2017). Pradhan et al. (2016) have reported that tolerant genotypes show reduced shoot elongation and higher ADH enzyme activity. ADH activity of submergence tolerant genotypes showed increased ADH activity by 15^{th} day of submergence (Prasad et al. 2011). Flood tolerant varieties which possess the *Sub1* locus like FR13A, Jalashree, Jalkunwari and Swarna-Sub1 express higher ADH activity and survival% during the submergence period (Singh et al. 2001; Kato-Noguchi and Kugimiya 2003 and Prasad et al. 2011).

Correlation analysis

In the current study, *Sub1*introgressed BC_3F_2 lines exhibited a significant positive correlation between survival percentage, ADH activity and PDC activity. A significant negative correlation was observed between survival percentage and shoot elongation rate among the *Sub1*introgressed BC_3F_2 lines. These associations indicate quiescence strategy of submergence tolerance as plants showing lower shoot elongation rate survive much better than those showing increased shoot elongation. Several researchers have also reported strong negative correlation between shoot elongation percentage and survival percentage under submergence stress (Adilakshmi and Rani 2012, Kumar et al. 2020). Das et al 2005 have also reported that shoot elongation and survival percentage under complete submergence exhibited a highly significant negative association ($r = -0.76^{**}$). A positive correlation has been reported between the level of ADH activity during anaerobiosis and anoxia tolerance (Ismail et al. 2009). Pradhan et al.2016 have also reported positive association between ADH activity and survival percentage.

Overall, the results of the present study confirm that the *Sub1*introgressed lines exhibit quiescent strategy of submergence tolerance rather than the escape mechanism as all the developed lines show reduced shoot elongation and a good recovery after desubmergence. The increased activity of PDC and ADH enzymes provide the necessary energy for the developed lines to survive the anaerobic stress of submergence.

Authors' contribution

Conceptualization of research (DJ, SKS); Designing of the experiments (DJ, SKS); Contribution of experimental materials (SKS); Execution of field/lab experiments and data collection (DJ); Analysis of data and interpretation (DJ, SKS); Preparation of manuscript (DJ).

Declaration

The authors declare no conflict of interest.

References

Adilakshmi D. and Rani G. M. 2012. Variability, character association and path analysis in rice varieties under submergence. Crop Res., **44**(1&2): 146-151.

Bailey-Serres J. and Colmer T. D. 2014. Plant tolerance of flooding stress—recent advances. Plant Cell Environ., **10**: 2211-5.

Bailey-Serres J., Fukao T., Gibbs D. J., Holdsworth M. J., ChoLee S., Licausi F., Pierdomenico Perata P., Laurentius A. C. J., Voesenek and van Dongen J. T. 2012. Making sense of low oxygen sensing. Trends Plant Sci., **17**(3): 1360-1385.

Bhandari A., Jayaswal P., Yadav N., Singh R., Singh Y., Singh B., Singh N., Singh S., Sevanthi A., Rai V. and

Verulkar S. 2019. Genomics-assisted backcross breeding for infusing climate resilience in high-yielding green revolution varieties of rice. Indian J. Genet., **79**(1) Suppl. 160-170.

Bui L. T., Ella E. S., Dionisio-Sese M. L. and Ismail A. M. 2019. Morpho-physiologica changes in roots of rice seedling upon submergence. Rice Sci., **26**: 167-177.

Chetia S. K., Kalita M., Verma R. K., Barua B., Ahmed T., Modi M. K. and Singh N. K. 2018. Flood proofing of Ranjit, a popular variety of North-Eastern India through transfer of Sub1 rice QTL by modified marker-assisted backcross breeding. Indian J. Genet., **78**(2): 166-173.

Das K. K., Sarkar R. K. and Ismail A. M. 2005. Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice. Plant Sci., **168**(1): 131-136.

Desjardins P., Hansen J. B. and Allen M. 2009. Microvolume protein concentration determination using the NanoDrop 2000c spectrophotometer. J Vis Exp., **4**(33): pii: 1610.

Fukao T., Kenned R. A., Yamasue Y. and Rumpho M. E. 2003. Genetic and biochemical analysis of anaerobically-induced enzymes during seed germination of Echinochloa crus-galli varieties tolerant and intolerant of anoxia. J. Exp. Bot., **54**(386): 1421-1429.

Fukao T., Xu K., Ronald P. C. and 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. and Bailey-Serres J. 2008. Ethylene - A Key Regulator of Submergence Responses in Rice. Plant Sci., **175**(1-2): 43-51.

Geigenberger P., Fernie A. R., Gibon Y., Christ M. and Stitt M. 2003. Metabolic activity decreases as an adaptive response to low internal oxygen in growing potato tubers. Biol. Chem., **381**(8): 723-740.

Goswami S., Kar R. K., Paul A. and Dey N. 2017. Study of selected biochemical parameters related to submergence tolerance in rice (*Oryza sativa* L.) with special reference to land races and wild species. Res. J. Chem. Environ., **21**(11): 29-38.

Hattori Y., Nagai K., Furukawa S., Song X. J., Kawano R., Sakakibara H., Wu J. Z., Matsumoto T., Yoshimura A., Kitano H., Matsuoka M., Mori H. and Ashikari M. 2009. The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. Nature, **460**: 1026-1031.

IRRI. 1988. Standard evaluation system for rice testing programme (IRTP). Rice manual 3rd ed. Manila (Philippines): IRRI. pp.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*). Ann. Bot., **103**(2): 197-209.

Ismail A. M., Johnson D. E., Ella E. S., Vergara G. V. and Baltazar A. M. 2012. Adaptation to flooding during emergence and seedling growth in rice and weeds, and implications for crop establishment. AoB Plants pls019.

Jackson M. B. and Ram P. C. 2003. Physiological and molecular basis of susceptibility and tolerance of rice plants to complete submergence. Ann Bot., **91**(2): 227-241.

Kato-Noguchi H. and Kugimiya T. 2003. Preferential Induction of Alcohol Dehydrogenase in Coleoptiles of Rice Seedlings Germinated in Submergence Condition. Biol. Plant., **46**(1): 153-155.

Kawano N., Ella E., Ito O., Yamauchi Y. and Tanaka K. 2002a. Metabolic changes in rice seedlings with different submergence tolerance after desubmergence. Environ. Exp. Bot., **47**(3): 195-203.

Kawano N., Ella E., Ito O., Yamauchi Y. and Tanaka K. 2002b. Comparison of adaptability to flash flood between rice cultivars differing in flash flood tolerance. Soil Science and Plant Nutrition, **48**(5): 659-66.

Kumar A., Prasad S., Mishra V. K., Kumar R., Singh J., Milan R. and Kumar A. 2020. Effect of Submergence Stress on Physiological Indices and Yield of Rice (*Oryza sativa* L.) Genotypes. Int. J. Curr. Microbiol. Appl. Sci., **9**: 994-999.

Luo F. L., Nagel K. A., Scharr H., Zeng B., Schurr U. and Matsubara S. 2011. Recovery dynamics of growth, photosynthesis and carbohydrate accumulation after desubmergence: a comparison between two wetland plants showing escape and quiescence strategies. Ann. Bot., **107**(1): 49-63.

Magneschi L. and Perata P. 2009. Rice germination and seedling growth in the absence of oxygen. Ann. Bot., **103**(2): 181-196.

Mohanty B. and Ong B. L. 2003. Contrasting effects of submergence in light and dark on pyruvate decarboxylase activity in roots of rice lines differing in submergence tolerance. Ann. Bot., **91**(2): 291-300.

Neeraja C., 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**(6): 767-776.

Panda D. and Sarkar R. K. 2012a. Leaf photosynthetic activity and antioxidant defense associated with Sub1 QTL in rice subjected to submergence and subsequent re-aeration. Rice Sci., **19**(2): 108-116.

Panda D. and Sarkar R. K. 2012b. Role of non-structural carbohydrate and its catabolism associated with SUB1 QTL in rice subjected to complete submergence. Exp. Agric., **48**(4): 502 512.

Panda D. and Sarkar R. K. 2012c. Structural and functional alteration of photosynthetic apparatus in rice under submergence. J. Stress Physiol. Biochem., **8**(1): 95-107.

Panda D. and Sarkar R. K. 2013. Characterization of leaf gas exchange and anti-oxidant defense of rice (*Oryza sativa* L.) cultivars differing in submergence tolerance owing to complete submergence and consequent re-aeration. Agric. Res., **2**(4): 301-308.

Perata P. and Voesenek L. A. C. J. 2007. Submergence tolerance in rice requires Sub1A, an ethylene-response-factor-like gene. Trends Plant Sci., **12**(2): 43-46.

Pradhan B., Sarkar M. and Kundagrami S. 2016. Role of Slow Elongation of Stem and Induction of Alcohol Dehydrogenase (Adh) Enzyme For Increment Of Survival Under Submergence Condition In Rice (*Oryza sativa* L.). Imperial J. Interdisc. Res., **2**(7): 2454-1362.

Prasad B. D., Thapa G., Baishya S. and Sahni S. 2011. Biochemical and molecular characterization of submergence tolerance in rice for crop improvement. J. Plant Breed. Crop Sci., **3**(10): 240-250.

Quimio C. A., Torrizo L. B., Setter T. L., Ellis M., Grover A., Abrigo E. M., Oliva N. P., Ella E. S., Carpena A. L., Ito O., Peacock W. J., Dennis E. and Datta S. K. 2000. Enhancement of submergence tolerance in transgenic rice overproducing pyruvate decarboxylase. J. Plant Physiol., **156**(4): 516-521.

Sarkar R. K. and Bhattacharje B. 2012. Rice Genotypes with SUB1 QTL Differ in Submergence Tolerance, Elongation Ability during Submergence and Re-generation Growth at Re-emergence. Rice, **5**(1): 7.

Sasidharan R., Bailey-Serres J., Ashikari M., Atwell B. J., Colmer T. D., Fagerstedt K., Fukao T., Geigenberger P., Hebelstrup K. H., Hill R. D., Holdsworth M. J., Ismail A. M., Licausi F., Mustroph A., Nakazono M., Pedersen O., Perata P., Sauter M., Shih M. C., Sorrell B. K., Striker G. G., van Dongen J. T., Whelan J., Xiao S., Visser E. J. W. and Voesenek L. A. C. J. 2017. Community recommendations on terminology and procedures used in flooding and low oxygen stress research. New Phytol., **214**: 1403-1407.

Septiningsih E. M., Pamplon A. M., Sanchez D. L., Neeraja C. N., Vergara G. V., Heuer S., Ismail A. M. and Mackill D. J. 2009. Development of submergence-tolerant rice cultivars: The Sub1 locus and beyond. Ann. Bot., **103**: 151-160.

Singh A., Septiningsih E. M., Balyan H. S., Singh N. K. and Rai V. 2017. Genetics, physiological mechanisms and breeding of flood-tolerant rice (*Oryza sativa* L.). Plant Cell Physiol., **58**: 185-197.

Storey J. D., Akey J. M. and Kruglyak L. 2005. Multiple locus linkage analysis of genome wide expression in yeast. PLoS Biol., **3**: 1380-1390.

Vu H. T. T., Manangkil O. E., Mori N., Yoshida S. and Nakamura C. 2009. Submergence-induced ADH and ALDH gene expression in japonica and Indica rice with contrasting levels of seedling vigor under submergence stress. Biotechnol Eq., **23**: 1469-73.

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

Ye X. Q., Meng J. L., Zeng B. and Wu M. 2018. Improved flooding tolerance and carbohydrate status of flood-tolerant plant Arundinella anomala at lower water temperature. PloS one, **13**(3): e0192608. https://doi.org/10.1371/journal.pone.0192608.