J Bangladesh Agril Univ 17(4): 539–544, 2019 https://doi.org/10.3329/jbau.v17i4.44623

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau, www.banglajol.info/index.php/JBAU

Changes in root porosity and water soluble carbohydrates in rice (*Oryza sativa* L.) under submergence stress

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ARTICLE INFO

Article history:
Received: 17 November 2019
Accepted: 28 November 2019
Published: 31 December 2019

Keywords:
Rice,
Submergence stress,
Water soluble carbohydrates,
Root porosity

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ABSTRACT

Submergence stress at early vegetative stage is one of the most important constraints in the productivity of rice in Bangladesh. Submergence causes yield loss of rice at Aman season in Bangladesh and therefore, it is necessary to develop submergence tolerant rice cultivars. A pot experiment was conducted at the net house of Department of Crop Botany, Bangladesh Agricultural University, during Aman season from July to December, 2017 to evaluate the changes in root porosity and water soluble carbohydrates (WSCs) associated with submergence tolerance in rice. The experiment consisted of two factors—(i) Rice cultivars (Binadhan-11, Binadhan-12, BRRI dhan51 and BRRI dhan52 as tolerant and BRRI dhan49 as susceptible) and(ii) Submergence stress: Submergence for 14 days at vegetative stage and control. Submergence stress was imposed by dipping of pots into a water tank with about 90 cm depth of water while the control plants are maintained in the pot house of the field laboratory. The plants were sampled at seven days interval during submergence to determine the changes in root porosity and to examine the contribution of shoot reserves for their survival. The root porosity was measured by pycnometer method and water soluble carbohydrate was measured by the anthrone method. Tolerant cultivars showed greater root porosity development in both control and stress condition but the susceptible cultivar showed significantly lower root development in stress condition. Higher root porosity might help tolerant cultivars to survive in submergence stress more efficiently. Tolerant rice cultivars had high initial soluble carbohydrate than the susceptible one. Under submergedcondition, the tolerant cultivars showed slow depletion of water soluble carbohydrate compared to susceptible cultivar. Higher carbohydrate contents in tolerant cultivars might act as buffer stock during submergence for their better survival and growth.

Introduction

Rice is one of the most important agricultural crops in the world. It is the second most important cereal crop in the World (FAO, 2015). About 738.8 million tons of rice is produced in 2015 under 160.8 m ha of land (FAO, 2017). Rice production in Bangladesh is estimated at 33.8 million metric tons in the area under 11.62 million hectares of land. About more than 75% area occupied by rice cultivation in Bangladesh in the year 2017 (BBS, 2018). Total area under Aman rice in Bangladesh has been estimated to 5.83 million hectares, and total Aman rice production has been estimated 14.31 million tons. However, out of 11.42 million hectares of total rice producing area, rainfed lowland rice is cultivated in 5.32 million hectares (BBS, 2018).

Submergence at the early vegetative stage is one the major obstacles to rice production in Bangladesh. So it is necessary to develop submergence tolerant rice cultivars to ensure the food safety of the increasing population of Bangladesh. Submergence condition experiences plants to the stresses of low light, limited gas diffusion, effusion of soil nutrients, mechanical damage, and increased susceptibility to pests and diseases (Ram *et al.*, 2002). Oxygen deficiency, production of toxic substances such as Fe^{2+}, Mn^{2+}, and H_2S by reduction of redox potential cause severe damage to plants under waterlogged conditions (Drew and Lynch, 1980). Unlike other crop plants, rice has some adaptive traits for tolerance of submergence. One of the traits is formation of root porosity that enables internal aeration between shoot and root (Armstrong, 1980; Colmer, 2003; Colmer and Pedersen, 2008a). Moreover, leaf gas films contribute to the internal aeration during submergence, thereby increasing submergence tolerance in rice (Colmer and Pedersen, 2008b).

Some cultivars use two distinct strategies of growth controls to survive under submerged conditions. One of the strategies is a quiescence strategy [i.e., the low-oxygen quiescence syndrome, in which shoot elongation

Cite this article

Hassan, M.J., Karim, M.M., Islam, M.A., Pramanik, M.H.R., Hossain, M.A. 2019. Changes in root porosity and water soluble carbohydrates in rice (*Oryza sativa* L.) under submergence stress. *Journal of Bangladesh Agricultural University*, 17(4): 539–544. https://doi.org/10.3329/jbau.v17i4.44623

is suppressed to preserve carbohydrates for a long period (10-14 days) under flash-flood conditions]. Submergence-tolerant cultivars can restart their growth during desubmergence by using preserved carbohydrates (Colmer and Voesenek, 2009). Another strategy is an escape strategy [i.e., the low-oxygen escape syndrome (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009)], which involves fast elongation of internodes to rise above the water level and is used by deep water rice cultivars. Both strategies depend on ethylene-responsive transcription factors (Xu *et al*., 2006; Hattori *et al*., 2009).

So far, a limited number of high yielding rice varieties tolerant to submergence have been developed in Bangladesh to mitigate the crop loss by submergence. However, the detailed morphological and physiological traits of the cultivars associated with the submergence are still poorly understood. In the present study, four submergence tolerant and one susceptible rice cultivars were evaluated for underwater root porosity development and carbohydrate status in shoot in order to ascertain if they are related to submergence tolerance of lowland rice and can be used as markers for future breeding programs. To attain this aim, the present study was carried out to elucidate submergence tolerance mechanisms in rice cultivars and evaluate rice cultivars for submergence tolerance based on root porosity and shoot sugar dynamics.

Materials and Methods

Plant materials

Five cultivars of rice named BRRI dhan49, BRRI dhan51, BRRI dhan52, Binadhan-11 andBinadhan-12 were used for the study. Among them, seeds of BRRI dhan49, BRRI dhan51 and BRRI dhan52 were collected from Plant Breeding Division of Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh. Seeds of Binadhan-11 and Binadhan-12 were collected from Bangladesh Institute of Nuclear Agriculture (BINA).

Growth conditions and stress treatments

The experiment was conducted during the period from July to December 2017 in plastic pots (10 kg soil was added in each pot) at the net house of Department of Crop Botany, Bangladesh Agricultural University. The study was laid out following two factorial completely randomized design (CRD) with a total of 10 treatments and three replications. Factor A: Five rice cultivars (Binadhan-11, Binadhan-12, BRRI dhan51 and BRRI dhan52 as tolerant and BRRI dhan49 as susceptible) and Factor B: Submergence stress i.e. control and submerged condition. Submergence stress was imposed by dipping of pots into a water tank with about 90 cm depth of water for 14 days and the control plants are maintained in the pot house of the field laboratory with sufficient water and required cultural practices.

A total of 100 pots were prepared. Well-pulverized soil was mixed with well-decomposed cow dung properly. The plastic pots were filled with soil and cow dung mixture at a ratio of 2:1. Each pot contained about 10 kg of soil and cow dung mixture. Seeds of five cultivars were planted at five different trays on 20 June, 2017. Seedlings of 20-day old were transplanted into the prepared pots on 10 July, 2017. Fertilizers were applied in the experimental pots as recommended doses. Weed free condition was maintained throughout the season. Watering was done when needed. Insecticide and pesticide were applied to maintain insect and disease free condition.

The water tank was partially filled with tap water of about 0.5 m height and about 45 pots with rice plants (nine pots of each rice cultivar) were dipped in the water of the tank one by one gently at vegetative stage. The height of the tank water was raised gradually to submerge the plants completely. The light intensity of the tank water at 15 cm and 30 cm depth were measured 65.72 and 47.32 µmol m^{-2} s^{-1} by a Light meter (Model: LI-250A, Licor Bio Sciences, USA. The light intensity of air also measured and it was 240 µmol m^{-2} s^{-1}. Dissolved oxygen concentration was measured by DO meter (Model: DOG- 2082X, HANNA Instrument, Germany) and it was 10.50 ppm by DO meter. The temperature of the water was measured as 30.8ºC. The water height was kept constant by the daily supply of tap water. After 14 days of submergence, the plants were removed from the water tank after draining the water from the tank using a siphoning pipe. The plants were grown with proper care after desubmergence to maturity. The control plants were maintained with sufficient water and management practices which showed better performances.

Data collection

Three hills of each cultivar were sampled before submergence at 22 days after transplanting on 02 August, 2017. The roots of each cultivar were separated and stored for measurement of root porosity. The shoots were dried in an oven at 70°C for three days and then stored for the analysis of water-soluble carbohydrates (WSCs) using the anthrone method. This process of sample collection was repeated after seven days and 14 days of imposing submergence.

Measurement of root porosity

The stored roots were kept in water with air tight polybag for acquiring normal temperature. The weight of empty pycnometer vial and water filled pycnometer vial was measured. The temperature of the vial containing water was recorded. Then the sample roots were blotted gently with the tissue paper until free water does not easily transfer to the blotting paper. Then the roots were weighted on an analytical balance. The roots were inserted into water filled vial. If any air bubbles were trapped, they were freed by manipulating the submerged roots into the pycnometer vial using a clean needle. The pycnometer with water and intact fresh roots was weighted on an analytical balance. The roots were then removed from the vialand homogenized by a glass mortar and pestle. The entire homogenate was transferred completely into the pycnometer using the rinse water and it was filled to full volume. The pycnometer and homogenate were adjusted to normal temperature and weighed.

Porosity was determined by the following formula (Jensen *et al.*, 1969):

$$\% \, porosity = \frac{W_{hr+w} - W_{fr+w}}{W_w + W_{fr} + W_{fr+w}} \times 100 \quad \text{............ (1)}$$

Where W_{hr+w} = weight of homogenized roots and water filledpycnometer vial, W_{fr+w}= weight of fresh roots and water filled pycnometer vial, W_w = weight of water filled pycnometer vial, W_{fr} = weight of fresh roots

Estimation of water soluble carbohydrates (WSCs) in shoots

The WSCs in shoots were extracted and measured using the anthrone method (Yemm and Willis, 1954). The dried shoots were chopped and milled to a rough powder. The shoot powders were weighed and extracted once with 80% ethanol at 60°C for 30 min followed by 2 successive 15-min extractions with distilled water at 80°C. The extracts were combined and evaporated to dryness at 65°C. The dried carbohydrates were resolved in 5 mL distilled water. A fraction of the extract solution (about 1 ml) was taken in a micro-centrifuge tube (1.5 mL) and the charcoal powder was added to it. After mixing the powder and extract solution with a vortex (touch mixer), the solution was centrifuged at 5000 rpm for 5 min to make a clear solution. The clear solution was diluted 10 times with distilled water. Diluted solution (0.1 ml) was mixed with ice-cold anthrone reagent (5 ml). The mixture was heated for 10 min in a boiling-water bath and subsequently cooled with ice water. The absorbance of the reacted solution for standard and samples were measured with a spectrophotometer (Model 4001/4, USA) at 620 nm. The content of WSCs in the sample was calculated as mg WSCs per gram of shoots dry mass using the regression equation. The amount of WSCs in shoots at 7 days and 14 days after submergence was estimated based on the dry mass of shoots (Ehdaie *et al.*, 2006).

Statistical analysis

All data were analyzed statistically using MS Office Excel application. Data were analyzed by calculating means and standard errors of means (SEM). Duncan's Multiple Range Test (DMRT) was done by using statistical package program SPSS v22.0.

Results

Development of root porosity

Development of root porosity as influenced by submergence duration revealed that the porosities of the roots in all the tolerant genotypes were not significantly changed except the susceptible one (Figure 1). In control, initially three cultivars (Binadhan-11, BRRI dhan51 and BRRI dhan49) had higher root porosity (14-16%) than the submerged cultivars respectively. At 14 DAS of submergence, tolerant cultivars (Binadhan-11, Binadhan-12, BRRI dhan51 and BRRI dhan52) in stressed condition showed insignificant changed in root porosity but the susceptible cultivar showed a significant change in root porosity.

The root porosity at 7 days after submergence varied from 11.45% to 16.66% with a mean value of 14.68% under control and from 6.04% to 14.61% with a mean value of 11.34% under stress environment. The highest percentage of root porosity was recorded in BRRI Dhan49 and the lowest value in Binadhan-12 in control environment at 7 days after submergence. But in the stress environment at 7 days after submergence, the highest percentage of root porosity was recorded in BRRI Dhan52 and the lowest value in BRRI Dhan49 (Figure 1).

Maximum root porosity at 14 days after submergence in control condition was observed 18.54% in Binadhan-11 followed by BRRI dhan49 (16.95%), BRRI dhan51 (14.53%), Binadhan-12 (14.31%) and BRRI dhan52 (12.51%). In case of submerged condition maximum root porosity was observed 17.33% in Binadhan-12 followed by Binadhan-11 (16.86%), BRRI dhan51 (16.05%), BRRI dhan52 (14.18%) and BRRI dhan49 (7.79%) (Figure 1).

Changes in WSCs in shoot reserves

The changes in water-soluble carbohydrates (WSCs), the main reserves in shoots in five rice cultivars under control and submergence stress at the early vegetative stage are shown in Figure 2. There were large variations in the content of WSCs in shoots at different periods during submergence. The WSCs content at 7 days after submergence varied from 3.28 to 6.42 mg g^{-1} with a mean value of 5.13 mg g^{-1} dry mass under control and from 2.01 to 6.31 mg g^{-1} with a mean value of 4.34 mg g^{-1} dry mass under stress environment. The highest value of WSCs content was recorded in BRRI Dhan51 and the lowest value in BRRI Dhan49 in both control and stress environment at 7 days after submergence.

Reduction of WSCs in shoots during submergence

The highest percentage of reduction in WSC (83.57%) was derived from BRRI Dhan49 at 14 days after

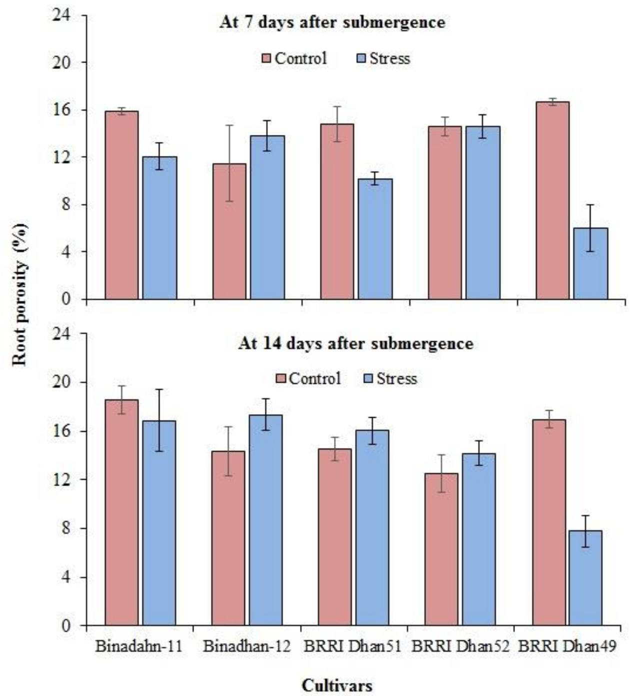

Fig. 1 Root porosity (%) in five rice cultivars as affected by submergence stress at early vegetative stage. Vertical bars represent standard error of means (n=3)

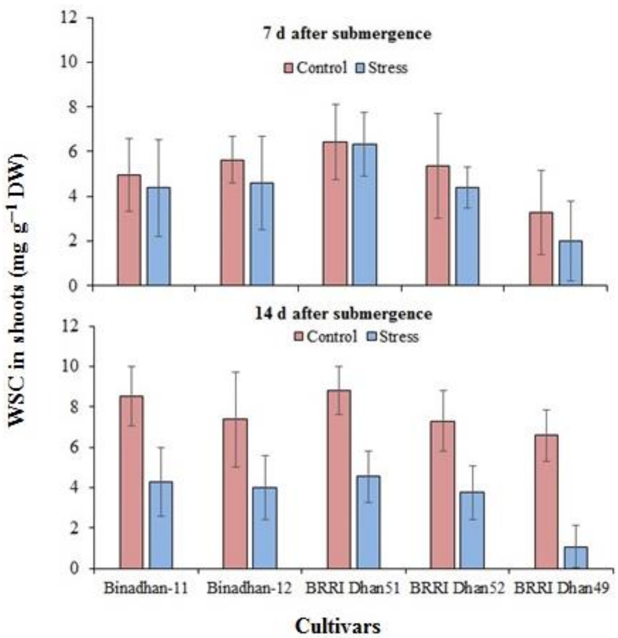

Fig. 2 WSC in shoots (mg g^{-1} DW) in five rice cultivars as affected by submergence stress at early vegetative stage. Vertical bars represent standard error of means (n=3)

submergence which indicates its susceptibility to submergence stress. The lowest percentage of reduction (45.99%) was observed in Binadhan-12 which indicates the tolerance capacity under submergence stress (Figure 3).

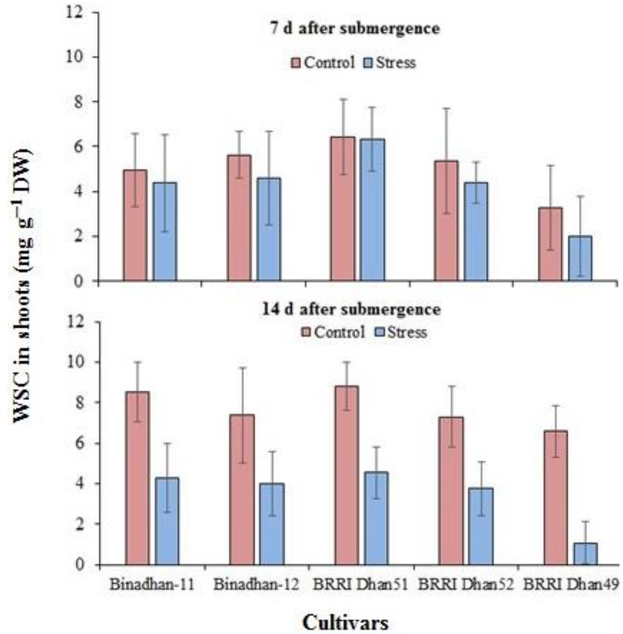

Fig. 3 Reduction percentage of water soluble carbohydrate during submergence stress over control

The cultivars Binadhan-11, BRRI Dhan51 and BRRI Dhan52 also showed less percentage of reduction in WSCs (49.81%, 48.47% and 48.75%, respectively) as compared to the susceptible cultivars (Figure 3). The WSCs content at 14 days after submergence varied from 6.58 to 8.81 mg g^{-1} with a mean value of 7.72 mg g^{-1} and 1.08 to 4.54 mg g^{-1} with a mean value of 3.53 mg g^{-1} dry mass under control and stress environment, respectively. The highest value of WSCs content was recorded in BRRI Dhan51 and the lowest value in BRRI Dhan49 in both control and stress environment at 14 days after submergence. In general, tolerant cultivars possessed higher WSCs content at submergence condition compared to susceptible cultivars.

Discussion

Development of aerenchyma in plants in response to submergence is an adaptive feature which helps in gas (O_2, CO_2 etc.) transport through plants. Thus, aerenchyma development influences root porosity (Colmer and Pedersen, 2008a). In our experiments, root porosity in rice cultivars increased during submergence. Tolerant cultivars developed more porous root underwater than susceptible cultivar (Figure 1). The increased porosity probably is associated with the lysigenous breakdown of cortical cells. Roots produced by waterlogged rice has higher porosity than roots grown in drained soil (Rogers and West, 1993). Higher root porosity is also reported during submergence and hypoxia in a number of plants (Justin and Armstrong, 1987). Root porosity also helps in radial diffusion of oxygen besides vertical transport (Armstrong, 1980; Gibbs *et al*., 2000).

Initial root porosities of tolerant genotypes were slightly higher than intolerant ones during submergence. During the initial stage of submergence, the increase in porosity was much higher in tolerant cultivars (Binadhan-11, Binadhan-12, BRRI dhan51 and BRRI dhan52) than a lower increase observed in susceptible cultivar (BRRI dhan49) (Figure 2). Further increase in submergence duration from 7d to 14d slightly increased the root porosity irrespective of the cultivars except for intolerant cultivar BRRI dhan49 which showed almost similar increase during both the periods.

Submergence tolerance in rice is governed by a number of factors out of which high carbohydrate status prior to the submergence is much more important. From the present experiment, it was found that high carbohydrate content of the cultivars ranged from 7.31 mg/g of dry wt. to 8.81 mg/g dry wt. of the shoot in control are related to tolerant cultivars. On the other hand, in susceptible cultivars the lower carbohydrate content 6.58 mg/g of dry wt. of shoot was recorded. Higher levels of initial carbohydrate act as buffer stock during submergence and the slow continuous supply of carbohydrate helped for the survival and growth of rice (Sarkar *et al.*, 2006). Metabolic energy required by the plant during submergence is supplied from the stored carbohydrate tissue. Irrespective of cultivars it was found that there was a reduction of the carbohydrate content of shoot. Under submerged condition stomatal conductance, intercellular CO_2 concentration as well as denaturing of the photosynthetic machinerywas decreased (Sarkar *et al*., 2006).

Under the submergedcondition, the rate of depletion of carbohydrate is very slow in the tolerant genotypes in comparison with susceptible genotypes. Drastic reduction of carbohydrate leads to higher rate of anaerobic fermentation and production of ethanol at the toxic level which caused damage to the rice genotypes (Setter *et al*., 1989). Again due to more amylase activity in the leaf and stem of tolerant genotypes survival percentage was more. The survival percentage is highly correlated with the carbohydrate present in the stem and leaf (Das *et al.*, 2005) to which the present findings strongly agreed. Submergence imposes a complex abiotic stress (Nagai *et al.*, 2010). Tolerant cultivars accumulated greater contents of water soluble carbohydrate compared to the susceptible cultivars. On the contrary, the greater percentage of reduction of WSCs during the submergence stress indicates that the cultivars are susceptible in submergence stress.

WSCs content at before and after submergence stress are important for providing energy needed for maintenance metabolism during submergence and for regeneration and recovery of seedlings after submergence (Das *et al.*, 2005).

Conclusion

Tolerant rice cultivars had higher initial WSCs and lower percent reduction in WSCs during submergence. Higher carbohydrate contents could be beneficial for survival of rice plants under submergence condition. Root porosity which is supposed to be beneficial in gas transport through plants especially during flooding and submergence. Higher initial soluble carbohydrate and higher root porosity may be used as criteria for selecting genotypes for submergence tolerance. Among the four tolerant cultivars BRRI dhan51 and Binadhan-11 had the higher WSC at 14 days after submergence, so these cultivars may have the potentiality to regenerate after submergence. These cultivars can be recommended for cultivation at the low flood prone and lying areas of Bangladesh although more research also needed for better yield.

Acknowledgement

This research was supported by a Research Grant (2017/45/BAU) from Bangladesh Agricultural University Research System, BAU, Mymensingh.

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