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Analysis of expression patterns of genes involved in Abscisic acid biosynthesis pathways in *C. olitorius* var. O-9897 in comparison with *C. olitorius* 2015 under salinity stress

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Corresponding e-mail: haseena@du.ac.bd**Abstract**

Salinity is a key environmental factor that limits worldwide crop production. Abscisic acid (ABA) is an important plant phytohormone which plays an essential role in supporting plants to survive under stressed conditions through induction of several stress responsive genes. This study aimed to unveil the role of abscisic acid in jute (*C. olitorius*) under salinity stress. The main focus was to study the expression profile of ABA biosynthesis genes: BCH (β carotene hydroxylase), VDE (violaxanthin de epoxidase), ZEP (zeaxanthin epoxidase), NCED (9-cis epoxy carotenoid dioxygenase) and ADH (alcohol dehydrogenase) under salinity stress in two *C. olitorius* lines, O-9897 var. and 2015 with a comparative analysis between the two. In response to salinity stress, the candidate genes showed better induction in O-9897 var. compared to 2015, which might explain the relative sensitivity of 2015 towards salinity. Regarding the role of ABA under stressed conditions, this molecular level study may aid in developing salt tolerant jute variety in future.

INTRODUCTION

Plants experience different abiotic stressors like: higher salt concentration (salinity), water shortage (drought or dehydration), extremes of temperature (low temperature and high temperature) etc. in their lifetime. These stressors impair crop production on irrigated land worldwide. Among all abiotic stressors, high salinity, the most severe environmental stress, causes significant yield loss. Increased salinity of arable land is likely to have overwhelming global effects, resulting in 30% land loss within the next 20 years and up to 50% by the middle of 21st century [1,2]. Overall, the susceptibility or tolerance to stress in plants is a coordinated action of multiple stress responsive genes, which also cross-talk with other components of stress signal transduction pathways [3]. Hence, the conventional breeding programs aimed at improving crop tolerance to salinity have limited success because of the complexity of the trait [4]. Therefore, understanding the molecular basis of salt-stress signaling and mechanisms of tolerance is essential for breeding and genetic engineering of salt tolerance in crop plants.

Abiotic stress as well as wounding are perceived and responded by plant adaptively, which is regulated mainly by the phytohormone, abscisic acid (ABA) that acts as an endogenous messenger in the regulation of a plant's water status [5]. ABA is involved in controlling seed germination and developmental processes of plants and in stress conditions, the action of ABA can target specifically guard cells for induction of stomatal closure but may also systematically signal for adjustment towards severe water shortage. Since various stressors induce ABA synthesis, it is now considered as a plant stress hormone [6]. Importantly, manipulating ABA level by changing the expression of key ABA biosynthetic genes provides an effective means to increase plant stress tolerance [7]. Jute is a bast fiber like flax and hemp. Cultivation of this environmentally friendly as well as the most affordable fiber producing plant is concentrated around the Ganges delta region of Bangladesh and India where the warm, wet climate during the monsoon season provides ideal growing conditions. In terms of usage, production and global requirement, jute is second only to cotton. Unfortunately, the two most cultivated species of jute suffer severely from abiotic stressors like salinity, dehydration, low temperature. This study encompasses identification and evaluation of transcript level variations of genes of the ABA biosynthesis pathway under salinity stress in two different jute plants (*C. olitorius*) - O-9897 and 2015 to obtain a comparative expression data. This would help in understanding the responsiveness of jute towards salinity with respect to ABA and thereby, eventually lead to generation of salinity tolerant jute variety by manipulating the gene(s) of this universal stress responsive signaling pathway (ABA biosynthesis pathway). Moreover, it would aid in the effective use of salinity landrace in the coastal regions where jute could be grown.

MATERIALS AND METHODS**A. Isolation of jute Genomic DNA**

Seeds of farmers' popular jute variety O-9897 and the accession 2015 were collected from Bangladesh Jute Research Institute (BJRI) and genomic DNA was extracted from 4 days old fresh seedlings using a slightly modified version of the ideal CTAB procedure [8-11]

B. PCR from genomic DNA and confirmation of ABA biosynthetic genes in Jute

High quality genomic DNA was used to perform Polymerase Chain Reaction using degenerate primers. PCR products were then extracted from gel using Qiagen Gel Extraction Kit and were sent for sequencing. The sequence result when used for Blastn, they found high sequence similarity with the respective genes in other plants, thereby confirming their presence in jute. These sequence information were then used to design gene specific primers.

C. Total RNA isolation

Total RNA was isolated using TRIZOL Reagent [12-14] according to the users' manual from both types of plant seedlings that were subjected to salinity stress (300 mM NaCl) and collected at different time intervals (control, 6hr, 12hr, 24hr and 48hr).

D. Expression pattern analyses

Equal amount of RNA and cDNA was used to synthesize first strand cDNA and analyses of expression of each gene respectively. The intensity of bands on agarose gel was measured by a software and plotted against each time interval. The comparative study on the level of transcript variation is then evaluated.

RESULTS**A. Demography of the respondents:**

The presence of the genes involved in Abscisic acid biosynthesis pathway: Carotene beta hydroxylase (BCH), Zeaxanthin epoxidase (ZEP), Violaxanthin de epoxidase (VDE), 9-cis epoxy carotenoid dioxygenase (NCED) and Alcohol dehydrogenase (ADH) was confirmed in jute (*C. olitorius*) by designing degenerate primers from respective sequences available in the database for other plant species, followed by amplification and sequencing (Fig-1).

All sequences which showed high similarity in BlastN result were used for designing jute specific primers for further studies. One of the genes of abscisic acid biosynthesis pathway, abscisic aldehyde oxidase (AAO) was not considered in this study because it was not possible to amplify the same in jute using degenerate primers. Upon salt stress, enzymes of the ABA biosynthetic pathway namely CBH (A), VDE (B), NCED (D) (Fig-2) showed an immediate response with increase in the

amount of their transcripts in O-9897 compared to that of 2015. ZEP (C), and ADH (E) showed similar up regulation at the early hours in both the plants. Nonetheless, all the ABA biosynthesis genes of O-9897 var. had higher levels of transcripts compared to that of 2015 suggesting more responsiveness of this pathway under salinity stress in O-9897. The apparent resistance against salinity in O-9897 has also been evaluated by comparing the morphological changes in the seedlings of the two jute types under salt stress. In comparison to O-9897 application of salt stress caused more thinning and browning of the 2015 seedlings (Fig-3).

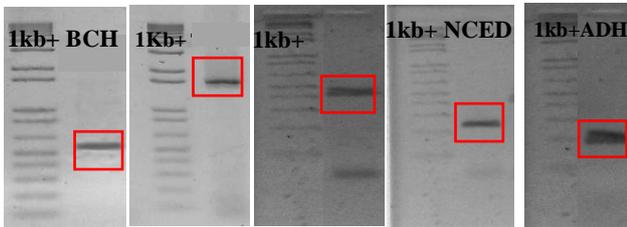


Fig. 1. Amplified PCR product for the five genes involved in ABA biosynthetic pathways in O-9897 variety and 2015 accession

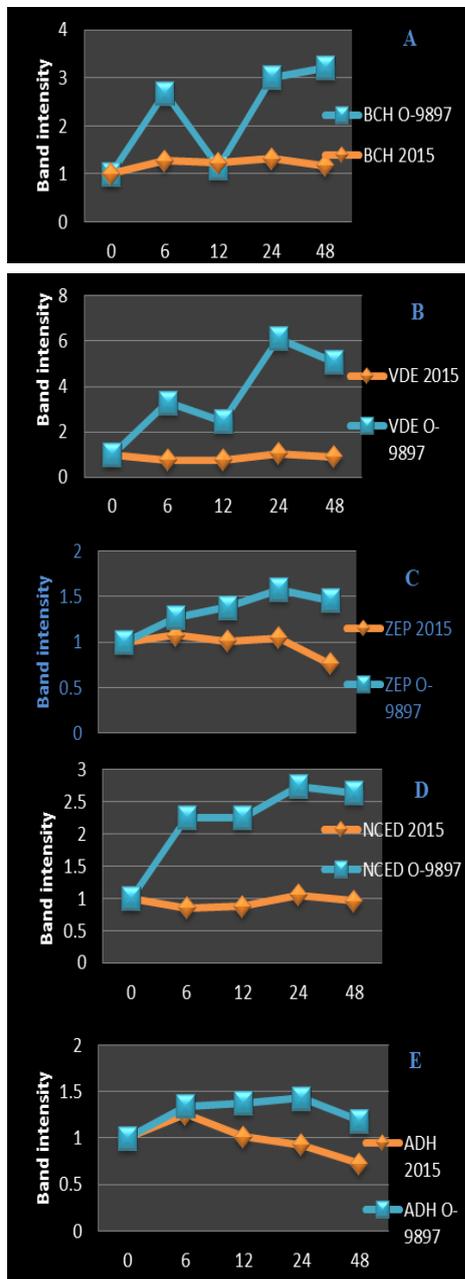


Fig. 2. Comparison of expression of 5 ABA biosynthesis genes in O-9897 variety and 2015 accession.

DISCUSSION

Improving stress tolerance of plants primarily depends on understanding the underlying mechanisms of stress tolerance which involves induction of many stress responsive genes and signaling pathways. A clear understanding can help researchers to manipulate the physiology of plants with genetic approaches in order to help them cope better under stressed conditions [15,16]. One important protector of plants active under stressed conditions is abscisic acid, which is a major phytohormone, critical for plant growth and development and plays an important role in integrating various stress signals and controlling downstream stress responses [2].



Fig. 3. Seedlings of O-9897 var. and 2015 after 48 hours of Seedlings with –hours of 300mM salt stress.

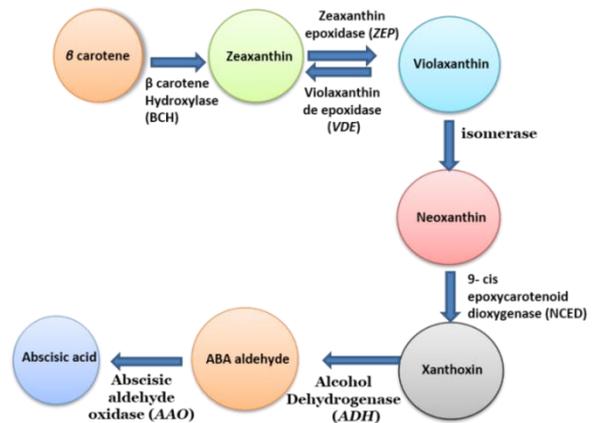


Fig. 4. Schematic diagram of Abscisic acid biosynthesis pathway

The main purpose of this study was to unveil the role of abscisic acid under saline conditions in jute (*C. olerius* var. O-9897 and 2015, with the latter known to be low temperature tolerant). To understand the same, it is important to analyze the expression pattern of abscisic acid biosynthesis genes (Fig-4) which ultimately lead to the production of abscisic acid in plants. A comparison of expression was also made between O-9897 and 2015 in order to determine the one most responsive to salt stress. In rice, transcription of BCH was found to be induced significantly (7 to 9 fold) by drought and salt stress [17]. The observation was similar for O-9897 var. where BCH transcripts showed a rapid induction in response to salinity stress; on the other hand BCH transcript level was almost similar following exposure to saline condition in 2015. VDE plays an important role in protecting plants from photo oxidative damage and abiotic stress [17] by increasing the level of xanthophylls and ABA. It can, therefore, be assumed that increased level of VDE transcript in O-9897 var. plays a significant role under salinity stress. ZEP in *N. plumbaginifolia* roots has been found to show a 3-7 fold rise after an 8 hours of salinity stress [18]. We also found similar result for O-9897 var. under salinity stress, with a gradual up regulation of transcription for up to 24 hours. Transcription of NCED showed a rapid up regulation in the early hours of stress in O-9897 var. while in 2015 the expression was mostly down regulated. As found by others, the amount of NCED transcript

remains much lower in roots but increases under stressed conditions [18]. The amount of ADH transcript showed a rapid induction in expression in the early hours of salinity stress in O-9897 var. but in 2015 it showed almost no variation. The levels of transcripts of ABA biosynthesis genes like VDE, ZEP, NCED, ADH, important for ABA biosynthesis in plants [7] are mostly down regulated in response to salinity stress in 2015 accession compared to O-9897 var.

Gradual increase in the level of salinity and dehydration in our coastal regions and northern parts, poses a potential threat towards sustainable crop production and the development of the nation. With such challenges lying ahead of us, this study can bring a solution for tackling the same.

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