Understanding the Molecular Mechanisms for Alleviating Boron Deficiency in Indian Mustard

A Thesis

Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of

DOCTOR OF PHILOSOPHY

by

Muthuvel J

(Reg. No: 156106008)

Department of Bioscience and Bioengineering
Indian Institute of Technology Guwahati
Guwahati-781039, Assam, India

April 2022

Abstract-TH-2956_156106008

Abstract

Boron (B) is an essential micronutrient required for the optimal growth and development of vascular plants. Globally B deficiency is the second most important micronutrient deficiency that causes significant yield reductions in crop plants. Often, seed yield and quality are compromised in plants grown under limited soil B availability without any apparent visual symptoms. B forms borate diester crosslinking with a pectic polysaccharide, rhamnogalacturonan II, during the cell wall formation and therefore, B deficiency primary affects meristem growth, vitality of the pollen grains, flower development and seed set. *Brassica juncea* is an important oilseed crop in India and other parts of the world and is extremely sensitive to B deficiency. Although the application of foliar B fertilizer improves the yield significantly in *B. juncea*, excessive application of B can be toxic due to the narrow window between its deficiency and toxicity. Molecular mechanisms of B transport have been initiated by the discovery of B transporters in *Arabidopsis thaliana*. Several aquaporins (AQP's) and borate efflux transporter (BORs) family genes have been reported to be involved in the efficient uptake and translocation of B to maintain optimal plant growth under low soil B condition and B exclusion under high soil B conditions. Soil B content in major mustard producing states of India is potentially low and hence it is important to study the B transport mechanisms in *B. juncea* for the optimal yield.

Chapter I is the Introduction and Review of Literature part, which elucidates the essential information of the primary role of B in plants and various transporter proteins involved in B transport under both deficient and excess condition in detail. The regulation of these transporter proteins is dependent on the cellular B concentration and hence B-dependent regulation of these proteins in *Arabidopsis* and crop plants were discussed. Subsequently we have mentioned the transgenic plants overexpressed with B transporter genes to alleviate the B deficiency and toxicity in

wide number of plants. Both B deficiency and toxicity are widespread agricultural problems worldwide and elucidating the molecular mechanisms of B transport should allow us to develop technology to alleviate B deficiency and toxicity problems. In addition, the importance of developing new low B tolerant genotypes were explained by discussing the effects of B deficiency in Indian mustard (*B. juncea*) and soil B content in major mustard producing states of India. We have concluded this chapter by discussing the future perspectives for the crop improvement in B deficient/toxic soils. Finally, the salient features of this thesis have been delineated.

Chapter II elaborates the genome-wide identification and characterization of the AQPs and BOR-borate efflux transporters in the *B. juncea* genome. The identified BjAQPs and BjBORs were then classified into their respective subfamilies based on *Arabidopsis* classification. The protein characteristics of BjPIPs and BjTIPs were similar to the respective *Arabidopsis* homologs, in terms of composition, structure, and substrate selectivity filters. Whereas BjNIPs showed the highest variation in their protein characteristics reflecting the complex substrate selectivity filter compositions that affect the permeation of the solutes including boric acid. Subsequent analysis of 5'UTR region revealed that BjNIP5;1 proteins consists of a signature motif required for polar localization and B dependent post-transcriptional regulation. Similarly, in clade I BjBORs, the characteristic features involved in the endocytic degradation including, tyrosine motifs, acidic di-leucine motifs, and lysine ubiquitination residue, were well conserved. In addition, all the BjBOR1s possessed WRKY binding domain which activates BOR genes under B deficiency. The expression analysis identified higher expression of NIP5;1s (BjuA02NIP5_1a, BjuA03NIP5_1b, BjuA07NIP5_1a), and BOR1 (BjuA03BOR1a) in root tissues of the cultivar mustard under deficient B condition. The induced expression of these genes implied their probable role in B uptake and mobilization under B deficiency.

The subsequent endeavor was to identify new genotypes of *B. juncea* for low B soil. In this chapter, hydroponic media experiments were conducted to screen twenty-seven

Indian mustard genotypes under different B concentrations, and performances of the genotypes were evaluated based on the taproot length. Significant alterations of physiological responses, conditioned to low B levels, have been observed at the early vegetative stages among the twenty-seven *B. juncea* genotypes. Few mustard genotypes have shown higher tolerance to B deficiency than others and among them, low B-efficient genotype Geeta was more tolerant to low B, with very mild symptoms and a higher growth rate. Also, root meristem cells in B-efficient genotype Geeta were more viable, and less reactive oxygen (ROS) activity has been observed than B-inefficient cultivar, Maya. FTIR data have demonstrated the modified composition of pectin, cellulose, and callose among contrasting genotypes under different B levels. Furthermore, the low B condition has altered the expression profile of the major B transporters among the genotypes.

In the final chapter of the thesis, we overexpressed AtBOR1, an efflux borate transporter involved in xylem loading, in mustard and attempt to validate its growth under B deficient conditions. The overexpression of AtBOR1 has enhanced the growth of various crop plants under B-deficient conditions and hence we aimed to investigate the effects of AtBOR1 overexpression in mustard under low B conditions. Three independent transgenic mustard lines were generated using *Agrobacterium*-mediated transformation. The presence and expression of AtBOR1 in successive generations of transgenic mustard plants were confirmed by polymerase chain reaction (PCR), and quantitative PCR analysis. Physiological and molecular changes were analyzed by growing transgenic mustard in hydroponic media along with the non-transgenics under B deficiency. We have observed that the transgenic plants showed a better relative growth rate than non-transgenics without any typical B deficiency symptoms in deficient B concentration. The cell wall B, uronic acid percentages were observed to be relatively higher in transgenic roots and leaves with a low degree of methylation under B-deficiency. Therefore, the stable expression of AtBOR1 improved growth in mustard under B-deficient conditions.

Conclusion and Future prospects in Chapter 5 summarizes the key findings of this current thesis. The current work bestows a lead towards B transport networks in *B. juncea* to achieve higher yield under low soil B conditions. This work provides future scope of functional characterization of mustard B transporter genes and their importance in B transport in *B. juncea*.

Abstract-TH-2956_156106008