Introduction

Boron was established as an essential element for plants more than 85 years ago. For a long time, boron transport was considered to be a passive process. Boron is present mostly as boric acid, a charge neutral molecule, in neutral pH solution and this membrane permeability is several orders of magnitude higher than ions.

Boron deficiency is common in the world among regions with high precipitations including Japan, China, Thailand, Laos, etc. Boron deficiency affects both yield and quality reduction. It causes growth defects in young and expanding portions of plants. Boron toxicity is also a major agricultural problem affecting semi-arid areas in the world. Under high boron conditions, necrosis occurs from the margins of developed leaves. It has long been believed that boron transports in plants are passive process. Boron is transported along the transpiration streams to be accumulated in the margins of leaves. Retranslocation within the plant body is not evident. This pattern of distribution coincides with the appearances of deficiency and toxicity symptoms in young portion of plants and margins of developed leaves, respectively.

A strategy to overcome boron deficiency and toxicity problems is to modify boron transport by introducing appropriate transporters to be expressed in appropriate cells under appropriate conditions. For this, understanding of molecular mechanisms of boron transport and their regulations are essential. In this presentation we briefly review our identification and characterization of several boron transporters and use of transporters to generate plants tolerant to boron deficiency or toxicity.

Material and Methods

Arabidopsis thaliana mutant *bor1-1*, was used as a material to isolate the first boron transporters through genetic mapping. Activity of BOR1 is examined after expression in yeast cells by exposing cells to 0.5 mM boric acid for thirty min followed by B concentration determination by ICP-MS. To identify genes induced by low boron treatment, microarray analysis was carried out with *A. thaliana* roots treated with low boron. The gene that exhibited the most induction was *NIP5;1,* and analysis with T-DNA insertion line of this gene revealed that NIP5;1 is responsible for efficient boron uptake. BOR1 and NIP5;1 paralogs and orthologs in *A. thaliana* and rice were studied for their transport properties, physiological roles.

Results

We first identified a mutant of *Arabidopsis thaliana* mutant *bor1-1*. This mutant requires high boron supply for normal growth and boron concentration in leaves are lower than the wild type (Noguchi et al. 1997). The mutant is also defective in preferential transport of boron to young leaves (Takano et al., 2001). The *BOR1* gene was identified by genetic mapping and complementation experiments. It encodes plasma membrane protein with boron efflux transporter activity (Takano et al., 2002). BOR1 is required for xylem loading of B against concentration gradient under low B condition. BOR1 represents the first boron transporter identified in the living systems. BOR1 accumulation is high under low B but not detectable under high B condition. This regulation of accumulation is through selected trafficking of BOR1 to vacuole in response to boron supply. It is likely that this is beneficial for plants to minimize unnecessary accumulation of boron in shoots (Takano et al. 2005). A BOR1 ortholog was identified in rice that also functions in boron translocation from roots to leaves (Nakagawa et al. 2007). An ortholog in yeast is also an efflux transporter of boron important for tolerance against high concentration of boron (Takano et al. 2007). These analyses established that BOR1 and their orthologs are boron efflux transporter and functions in boron transport from roots to leaves in plants and boron efflux to the media to avoid boron toxicity in yeast.

The transporter required for efficient uptake of boron was identified through microarray analysis. *NIP5;1* is a gene identified as low boron induced gene in roots. Its transcript accumulation is 15-fold higher after 24 hr exposure to low boron condition. NIP5;1 is a member of aquaglyceropolin and capable of transporting boric acid upon expression in Xenopus oocytes (Takano et al. 2006). T-DNA insertion lines in *NIP5;1* grows poorly under low boron condition, but normal at high boron condition, suggesting that NIP5;1 is an influx transporter of boron strongly expressed in roots under low boron condition. An ortholog of NIP5;1, NIP6;1 is also permeable to boric acid, but not to water. In contrast to NIP5;1, *NIP6;1* is expressed in nodes in shoots and is important for efficient B transport to growing portion of plants (Tanaka et al., 2008).

Expressions of these transporters are regulated in response to boron condition in the environment for optimization of boron flow from soil through roots to shoots. We tried to modify boron transport activity in plants by over expressing these transporters in order to generate plants tolerant to high and/or low boron conditions through regulation of boron transport. Constitutive overexpression of BOR1 resulted in plants tolerant to low boron condition (Miwa et al., 2006). In these plants, xylem loading of boron is enhanced and boron accumulation in shoots is improved only under low boron condition. There is no observable defects of the transgenic plants overexpressing BOR1, probably due to degradation of BOR1 under high boron condition. In the case of NIP5;1, constitutive overexpression did not improve plants rather it had negative impacts on overall plant growth. But we were able to generate highly low-boron tolerant plants by enhancement of NIP5;1 expression, not directly by the constitutive promoter, but by inserting enhancer sequence upstream of the native NIP5;1 promoter to enhance expression of NIP5;1 possibly without much perturbation of its tissue specificity and/or low B induction (Kato et al., 2009).

For generation of high boron tolerant plants, we used BOR4, an efflux boron transporter that remain expressed under high boron condition. Constitutive expression of BOR4 resulted in plants that are highly tolerant to high boron conditions. It was also found that BOR4 localized to distal side of the epidermis in roots. It is likely that this polar localization enhances boron efflux out of roots.

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