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Dwarf and tiller-enhancing 1 regulates growth and development by influencing boron uptake in boron limited conditions in rice

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ABSTRACT

Boron (B) is essential for plant growth, and B deficiency causes severe losses in crop yield. Here we isolated and characterized a rice (*Oryza sativa* L.) mutant named *dwarf and tiller-enhancing 1* (*dte1*), which exhibits defects under low-B conditions, including retarded growth, increased number of tillers and impaired pollen fertility. Map-based cloning revealed that *dte1* encodes a NOD26-LIKE INTRINSIC PROTEIN orthologous to known B channel proteins AtNIP5;1 in *Arabidopsis* and TASSEL-LESS1 in maize. Its identity was verified by transgenic complementation and RNA-interference. Subcellular localization showed DTE1 is mainly localized in the plasma membrane. The accumulation of *DTE1* transcripts both in roots and shoots significantly increased within 3 h of the onset of B starvation, but decreased within 1 h of B replenishment. GUS staining indicated that *DTE1*s are expressed abundantly in exodermal cells in roots, as well as in nodal region of adult leaves. Although the *dte1* mutation apparently reduces the total B content in plants, it does not affect in vivo B concentrations under B-deficient conditions. These data provide evidence that DTE1 is critical for vegetative growth and reproductive development in rice grown under B-deficient conditions.

1. Introduction

Boron (B) is an essential micronutrient for higher plants and B deficiency has been reported in over 80 countries and in 132 crops [1,2]. In China and south-east Asia, B-deficient soils occur over a wide area, and cause severe defects in crop growth and reduced yields [2]. Several physiological functions in plants are attributed to B [3]. In cell walls, B functions in cross-linking rhamnogalacturonan-II (RG-II), thereby maintaining an intact cell wall organization and properties [4,5]. Other roles of B in plants include root elongation, leaf expansion, and pollen fertility [1,6,7].

Plant roots take up B from the soil mainly in the form of boric acid, an uncharged molecule [8]. B movement from the root to shoot occurs in xylem and is predominantly driven by the transpiration stream. B tends to accumulate in large mature leaves (called source leaves) with higher transpiration rates [9–13]. In contrast, B remobilization in source leaves and translocation in the phloem are independent of transpiration and this process supplies stored B for actively growing areas, such as young leaves, inflorescences and seeds (called sink tissues) [12]. Hence, B-deficient symptoms generally lead to the rapid cessation of growth in sink tissues [1,7].

Although it has long been believed that B is passively transported by diffusion across the cell membrane following a concentration gradient, a transporter especially for B uptake was first identified in *Arabidopsis thaliana* [14]. AtBOR1, polarly localized on the inner side of endodermal cells, is involved in xylem loading of B in roots under B limitation [14,15]. Two other important players in B transport in *Arabidopsis* belong to the NOD26-LIKE INTRINSIC PROTEINs (NIPs). AtNIP5;1 is localized on the outer side of root endodermal cells and facilitates B uptake from soil to roots [15–17].

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AtNIP6;1, a paralog of AtNIP5;1, is localized in the plasma membrane in the vascular bundles, especially in the nodal region of shoots, and is required for preferential B distribution to sink tissues in shoots under B deficiency [18].

Graminaceous species generally contain lower B levels than dicotyledonous species. B requirements of graminaceous species are also lower than those of dicotyledonous species [1]. Different molecular mechanisms of B transport have been revealed in some monocot species. OsBOR1 is a plasma membrane-localized B efflux transporter and works for both efficient B uptake and xylem loading in rice [19]. These roles are, to some degree, similar to the combination of AtBOR1 and AtNIP5;1 in *Arabidopsis*. Another rice B channel, OsNIP3;1, was recently characterized. It affects growth under B-deficient conditions by regulation of B distribution among shoot tissues [20]. Two recent studies in maize found that *tassel less1* (*tls1*) encodes an aquaporin family member orthologous to AtNIP5;1, whereas *rotten ear* (*rte*) encodes a B transporter orthologous to AtBOR1. Mutants derived from disruption of either gene show reduced tassel development and defects in inflorescence meristems, as well as vegetative defects when grown in low-B soils [21,22]. In wheat, B transporter (*Bot-B5*), which has no direct ortholog in other monocot species, confers varietal adaptation to high-B environments [23].

Rice, a staple world crop, suffers from B-deficiency in many production areas [2,24,25]. To better understand the molecular mechanisms involved in B transport in rice, we isolated a *dwarf and tiller-enhancing* mutant named *dte1* that shows growth defects in B-deficient soil. Map-based cloning indicated the *dte1* is orthologous to *AtNIP5;1* in *Arabidopsis* and *TASSEL-LESS1* (*TLS1*) in maize, and functions as a B channel to confer B uptake and distribution. Our results highlight the importance of B transport in maintaining normal growth of rice under B-limited conditions.

2. Materials and methods

2.1. Plant materials and growth conditions

A set of chromosome segmental substitution lines (CSSLs), in which *japonica* variety Asominori was used as the genetic background (receptor) and *indica* variety IR24 as the donor [26], was provided by the Rice Genome Resource Center (RGRC), Japan (http://www.rgrc.dna.affrc.go.jp/stock.html). Among them, a line with growth defects was observed at a field site with low B soils in Lingshui county, Hainan province (abbreviated to HNLB). Because the causative locus for the growth defect later proved not to be associated with introgressed segments of IR24, the line was hereafter referred to as *dte1* mutant and Asominori as wild type. Other materials were obtained from the Crop Germplasm Stock Center of Nanjing Agricultural University (NAU). For genetic analysis and gene mapping, plants were grown at the HNLB site and leaf materials were harvested in 2010 for gene mapping.

For low-B treatment, seeds were germinated in ion-exchanged water and grown in a nutrient solution with 18 μM B for two weeks in an illuminated incubator. After removal of the remaining seeds, the seedlings were transferred to nutrient solutions containing a series of B concentrations ranging from low to high (0, 0.01, 0.1, 1, 10, and 100 μM) in a temperature-controlled (30/25 °C day/night) greenhouse. Seventy plants were evenly planted in 20 L containers. Hydroponic solutions were prepared with 1 mM NH_4NO_3, 0.32 mM $NaH_2PO_4{\cdot}2H_2O$, 1.5 mM KCl, 1.7 mM $MgCl_2{\cdot}6H_2O$, 0.67 mM $CaCl_2$, 0.4 mM $Na_2SiO_3{\cdot}9H_2O$, 9 μM $MnCl_2{\cdot}4H_2O$, 0.6 μM $Na_2MoO_4{\cdot}2H_2O$, 0.14 μM $ZnSO_4{\cdot}7H_2O$, 0.16 μM $CuSO_4{\cdot}5H_2O$, 44.8 μM $FeSO_4{\cdot}7H_2O$ and H_3BO_3 was added according to the treatment. The pH was adjusted to 5.4–5.6 and the nutrient solutions were renewed every three days.

2.2. Phenotypic analysis, scanning electron microscopy, and pollen staining

Phenotypic measurements were made on HNLB field-grown plants on at least three occasions. Internode lengths were measured as the distance between two sequential nodes. Total tiller/axillary bud numbers were determined by removing the leaves and counting any bud that was greater than 1 cm in length. The definition of high-node and low-node tillering followed a previous study [27].

For scanning electron microscopy, second internodes of wild type and *dte1* mutant were harvested 10 days after anthesis and fixed in 2.5% v/v glutaraldehyde. Samples were then transferred to 2% w/v OsO_4 for 2 h, dehydrated through an ethanol gradient, and infiltrated and embedded in butyl methyl methacrylate. The samples were coated with platinum and observed using a Hitachi S-4700 cold field emission scanning electron microscope as described by Wei et al. [28].

Mature pollen from non-pollinated spikelets at 1–2 days after anthesis was fixed in FAA (50% ethanol, 5% glacial acetic acid and 5% formaldehyde) solution before observation. Pollen staining by I_2-KI followed the protocol of a previous study [29].

2.3. Map-based cloning

Initially, 46 individuals with *dte1*-like phenotypes were selected from a *dte1*/Nanjing 11 F_2 population for linkage analysis. Molecular markers distributed throughout the genome were chosen for preliminary mapping [30,31]. Subsequently 826 additional F_2 plants with *dte1*-like phenotypes were used for fine-mapping. Sequence divergences between Asominori and Nanjing 11 were identified and used to develop new SSR (simple sequence repeat) and InDel (insertion and deletion) markers. Primer pairs designed with Primer Premier 5.0 (PREMIER Biosoft International, Palo Alto, CA) are listed in Suppl. Table S1. The PCR procedure was as follows: 95 °C for 5 min, followed by 34 cycles of 95 °C for 30 s, annealing for 30 s, 72 °C for 40 s, and a final elongation step at 72 °C for 5 min.

2.4. Genetic complementation and RNAi suppression of DTE1

For genetic complementation, a 8507 bp wild type genomic DNA fragment containing the entire *DTE1* coding region plus a 2172 bp upstream region and a 590 bp downstream sequence, was amplified with primer pairs DTE1-genomics (see Suppl. Table S1) from BAC clone OSJNBa0026L12 and cloned into the binary vector pCAMBIA1305 using an Infusion Cloning Kit (Clontech). The recombinant plasmid, designated p*DTE1::DTE1*, was introduced into *Agrobacterium tumefaciens* EHA105 and used to infect calli of the *dte1* mutant. Transformation was conducted according to a published method [32].

The construct pCUbi1390-$^{\triangle}$FAD2 including the *ubiquitin* promoter and a *FAD2* intron inserted into pCAMBIA1390 was used as an RNA interference (RNAi) vector [33,34]. Both anti-sense and sense versions of a specific 252 bp fragment from the coding region of *DTE1* were amplified with primer pairs DTE1-RNAi-Z and DTE1-RNAi-Y (see Suppl. Table S1), and successively inserted into pCUbi1390-$^{\triangle}$FAD2 to form the RNAi construct p*Ubi::dsRNAi-DTE1*. The vector was then transformed into Kitaake (an easily transformed, early flowering *japonica* variety) as described above.

2.5. Subcellular localization of DTE1 in rice

For subcellular localization of DTE1 the full-length gene was amplified from wild type cDNA with primer pairs DTE1-GFP (Suppl. Table S1) and cloned into the pCAMBIA1305-GFP vector to generate the binary vector p*35S::DTE1-GFP* [35]. The empty plasmid p*35S::GFP* and p*35S::DTE1-GFP* were separately introduced into *A.*