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Title

Boron deficiency in rice and the potential of up-regulated rice boron transporter in improving boron deficient symptoms

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Introduction

Boron (B) is one of the essential elements for plant growth and development. Deficiency of B has been identified as a serious agricultural issue in more than 100 crops in 80 countries (Shorrocks, 1997). Limitation of B impairs growth of young tissues and seed set, which results in depressed quality and quantity of agricultural products. In rice, B content is up to 10-time lower than those of dicot plants (Matoh et al., 1996). And thus rice young seedling is relatively resistant to B limited condition (Yu and Belle, 1998) compared to dicot plants such as *Arabidopsis* (Miwa et al., 2006). However, the effect of B limitation until the reproductive phase is little known in rice. In this study, we evaluate the growth and yield of rice subjected to B deficient condition by hydroponic experiments.

We previously reported that a boron efflux transporter *OsBOR1* plays a crucial role in efficient root-to-shoot translocation in rice (Nakagawa et al., 2007). Over-expression of AtBOR1 improved growth and seed fertility in *Arabidopsis* plants under B deficient condition (Miwa et al., 2006). But this strategy, up-regulation of native B transporter to achieve the tolerance to B deficiency, has not been applied for the crop so far. We herein generated several independent lines of transgenic rice plants overexpressing rice *BOR1* and characterised the phenotypes of these transgenic rice plants under B deficient condition.

Materials and methods

Effect of boron deficiency on rice

Rice (*Oryza sativa* L) cultivar Nipponbare was used. Growth experiments were carried out in a green house under natural light condition (30°C/25°C, day/night). After germination on the agar medium containing 0.5 mM $CaCl_2$, seedlings were transferred to a Kimura B hydroponic solution containing 2 mM MES (pH 5.6) with different B levels: Concentration of B in the solution was 18 µM for B sufficient condition, and 18, 0.18, 0.03 µM for B deficient treatments. Seedlings were grown until grain ripening stage and plant height was measured every other week. At harvest, yield components were measured. Concentration of B in flag leaf and husked grain (brown rice) were determined by ICP-MS after digestion using nitric acid and hydrogen peroxide.

Transgenic rice over-expressing rice boron transporter gene

We generated 11 independent lines of transgenic rice which would be expected to over-express rice boron transporter gene *OsBOR1*. For RT-PCR analysis, total RNA was isolated from roots of seedlings subjected to 0.18 µM B treatment using RNeasy Plant mini kit (QIAGEN) and reverse-transcribed using ExScript RT-PCR kit (Takara). Specific primes for *OsBOR1-sGFP* (5'-CACTAGAAGCCGTGGTGAAA -3' and 5'- GCTGAACTTGTGGCCGTTC-3') and *OsUBQ5* (5'- GAAGGAGGAGGAAATCGAAC-3' and 5'- CTTCACAGAGGTGATGCTAAGG-3') were used to amplify the fragment of respective genes by PCR. For growth experiments, plants were grown hydroponically under B sufficient (18 µM) and deficient (0.18 µM) conditions for 2 weeks and root length and shoot height were measured every 7 days. Xylem sap was collected from these plants and B concentration in sap was determined by ICP-MS.

Results and discussion

Effect of boron deficiency on rice yields

Difference in plant height among the B treatments was not clear until 4 weeks after treatments (Fig. 1a). However, when subjected to lower than 0.18 µM B, reduced plant height was clearly observed according to the growth periods (Fig. 1a). Visible symptom on leaf blades such as chlorosis was not observed in B deficient-treated plants. On the other hand, the grain yield was greatly reduced by B deficient treatments lower than 0.18 µM B, which was mainly because of decreased numbers of spikelets (Fig. 1b). Under 0.03 µM B, seedlings showed dwarf phenotype and panicle formation was severely inhibited (data not shown). Concentration of B in flag leaf and brown rice was also decreased according to the level of B supply (data not shown). These results suggest that in rice, young seedlings were relatively tolerant to B limited condition, however, continuous low B supply clearly impairs the vegetative and reproductive growth, leading to decreased yields.

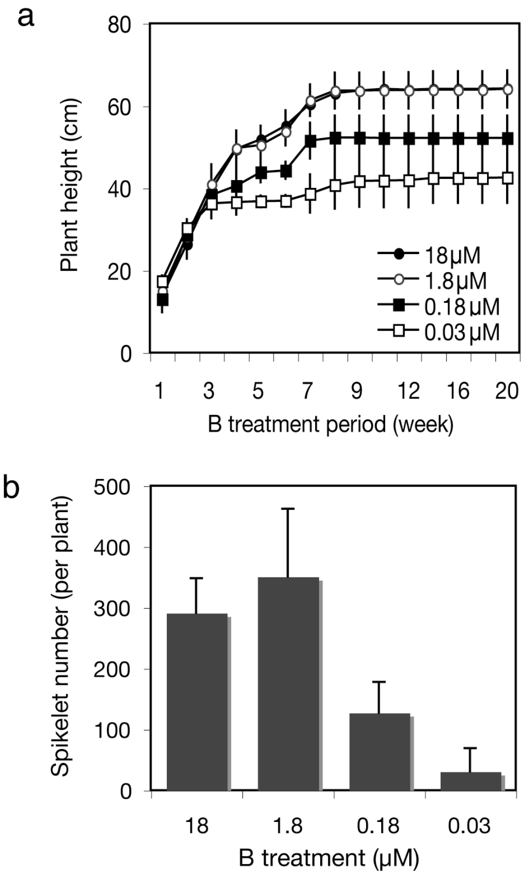

Figure 1. Plant height (a) and spikelet number (b) of rice plants grown hydroponically under B limited condition. n=3.

Transgenic rice over-expressing OsBOR1-GFP

RT-PCR analysis of 11 independent transgenic lines demonstrated the expression and its variation of introduced gene in transgenic plants (Fig. 2a). We selected 9 lines with sufficient numbers of seeds and tested the growth under moderate B deficient condition (0.18 μM B) for 2 weeks. The growth of shoots and roots did not differ among 8 transgenic lines and NT (Fig. 2b). However, among transgenic lines over-expressing introduced *OsBOR1*, B concentrations in xylem sap of several lines were higher compared to those of NT and transformants with weak expression of introduced gene (data not shown). These results suggest that increased expression of the rice boron transporter *BOR1* enhanced root-to-shoot translocation of boron, which might be resulted in improved B acquisition and further growth under boron deficient condition.

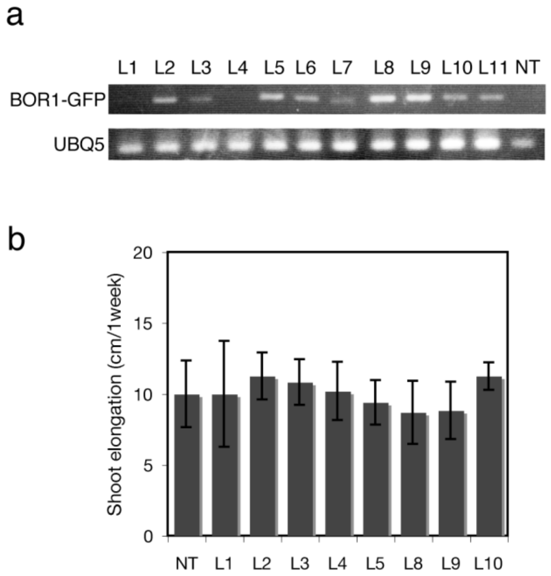

Figure 2. Generation and analysis of *OsBOR1* overexpressing transgenic rice. (a) Expression of introduced *OsBOR1* gene in roots of transgenic rice and NT subjected to 0.18 μM B for 2 weeks. (b) Shoot elongation of transgenic rice and NT under 0.18 μM B for 2 weeks. n=3.

Acknowledgements

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