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Functional deficiency of phytochrome B improves salt tolerance in rice

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ABSTRACT

Soil degradation affects agriculture worldwide. Soils with high salt can result from local geological conditions or accumulation of salt from irrigation. Salt limits water uptake and reduces crop yields; therefore, salt tolerance is an important trait for crops grown in high-salt soils. Here, we show that the rice (*Oryza sativa*) *phytochrome B* (*osphyB*) mutant has greater tolerance to salt stress than its parent *japonica* rice (cv. Dongjin). We found that the *osphyB* mutant showed a higher survival rate, fresh weight, and levels of total chlorophylls and carotenoids, as well as enhanced membrane integrity under salt stress compared to the wild type. *OsPHYB* transcripts increased in tissues of the wild type after salt treatment; *OsPHYB* expression was much higher in the leaf blade than in the stem and root. The *osphyB* mutant accumulated less Na^+ in the shoot and considerably more K^+ in both the shoot and root, maintaining a significantly lower Na^+ to K^+ ratio, possibly due to a lower rate of Na^+ uptake and a higher rate of K^+ uptake. To elucidate the possible mechanism of salt tolerance in the *osphyB* mutant, we performed quantitative reverse transcription PCR analysis, which indicated that salt stress-associated genes, including transcription factors and high-affinity K^+ transporters, are upregulated in the *osphyB* mutant under high-salinity conditions. Taken together, our findings show that the null mutation of *OsPHYB* contributes to a decrease in the Na^+/K^+ ratio and enhances cell membrane integrity through upregulation of salt stress-associated genes, resulting in improved tolerance to salt stress.

1. Introduction

Plants must cope with numerous environmental stressors, including drought, high salinity, and extreme temperatures. For example, salt stress limits plant growth and crop productivity worldwide (Allakhverdiev et al., 2000). Approximately 7% of the world's arable land is affected by either salinity or sodium toxicity and salinity stress limits over 30% of irrigated agriculture, including key staple crops such as rice (*Oryza sativa*) (Schroeder et al., 2013). Land plants have evolved biochemical and molecular genetic mechanisms for salt stress tolerance. Under high-salinity conditions, stress signal transduction is initiated by an increase of cytosolic Ca^{2+}, followed by expression of genes encoding Ca^{2+} sensor proteins (Xiong et al., 2002). SOS3 senses cytosolic Ca^{2+} and interacts with the protein kinase SOS2 (Zhu, 2001). SOS1 is a membrane-bound Na^+-H^+ antiporter and is activated by the SOS3–SOS2 complex, resulting in Na^+ homeostasis in plant cells (Zhu, 2003). Proline is a compatible solute that is produced in response to osmotic stress, and functions in osmotic adjustment, ROS detoxification, and protein stabilization (Szabados and Savouré, 2010; Suprasanna et al., 2014). P5CS (Δ1-pyrroline-5-carboxylate synthetase) is a key enzyme for synthesis of proline in response to salt stress (Ashraf and Foolad, 2007).

In salt-tolerant plants, excessive Na^+ caused by high saline soils is extruded or compartmentalized in the vacuole to alleviate Na^+ toxicity

Abbreviations: phyB, phytochrome B; CBF, C repeat binding factor; DREB, dehydration-responsive element binding protein; PIF, phytochrome-interacting factor; PIL, phytochrome-interacting factor-like; HKT/HAK, high-affinity K^+ transporter; MYB, myeloblastosis; bZIP, basic leucine zipper; AP2, APETALA 2; ROS, reactive oxygen species; DAB, diaminobenzidine; NBT, nitroblue tetrazolium chloride; NaCl, sodium chloride; ABA, abscisic acid; Na^+, sodium; K^+, potassium; RT-qPC, ,reverse transcription-quantitative polymerase chain reaction; UBQ5, ubiquitin 5; T-DNA, transfer-DNA; WT, wild type

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(Hasegawa et al., 2000; Murguia et al., 1995; Tsugane et al., 1999). Plant high-affinity K^+ transporters (HKTs) play an essential role in reducing Na^+ transport to the shoots, and therefore can enhance salinity tolerance (Deinlein et al., 2014). HKTs from higher plants can be divided into two major phylogenetic classes (Platten et al., 2006). Class I comprises Na^+-selective transporters that are found in both monocotyledonous and dicotyledonous species. Class II comprises Na^+–K^+ cotransporters that are found only in monocotyledonous species (Deinlein et al., 2014; Hauser and Horie, 2010). The class I HKTs OsHKT1;1 and OsHKT1;3 are only permeable to Na^+ (Garciadeblás et al., 2003; Jabnoune et al., 2009; Ren et al., 2005; Wang et al., 2015). AtHKT1;1 and its rice ortholog OsHKT1;5 reduce the transport of Na^+ into shoots by unloading Na^+ from the ascending xylem sap and diverting it into the descending phloem (Berthomieu et al., 2003; Davenport et al., 2007; Ren et al., 2005; Sunarpi et al., 2005; Wang et al., 2015). OsHKT2;1 exhibits diverse permeation modes depending on external Na^+ and K^+ concentrations, including Na^+–K^+ symport, Na^+ uniport, or inhibited states (Horie et al., 2001; Garciadeblás et al., 2003; Horie et al., 2007; Jabnoune et al., 2009). Heterologous expression of OsHKT2;4 in *Xenopus laevis* oocytes results in strong K^+ permeability without stimulation by extracellular Na^+, in contrast to the mechanism common to other class II HKTs. (Horie et al., 2011). OsHAK21 promotes K^+ uptake in the presence of high concentrations of Na^+ in both shoots and roots, resulting in tolerance to salinity stress (Shen et al., 2015).

Transcription factors also play key roles in the response to abiotic stress, contributing to tolerance to the stress by regulating the expression of their target genes. Plant-specific NAC, MYB, bZIP, and AP2/ERF transcription factors are involved in the tolerance to salinity stress. For example, high-salt stress induces the expression of *OsNAP* (Chen et al., 2014), *SNAC1* (Hu et al., 2006), and *ONAC106* (Sakuraba et al., 2015) and transgenic plants overexpressing these genes exhibit improved tolerance to salt stress, compared with wild type. OsDREB2A, in the DREB/CBF transcription factor family, is induced by high salt stresses and overexpression of *OsDREB2A* confers improved tolerance to dehydration and salinity stress (Dubouzet et al., 2003; Mallikarjuna et al., 2011). Plants with mutations in OsMYBc, a MYB coiled-coil-type transcription factor that directly binds to the *OsHKT1;1* promoter, show increased susceptibility to salt stress (Wang et al., 2015). Exposure to drought and high-salinity conditions increases the transcript levels of *OsAP37* and *OsAP59*, which encode transcription factors with an APETALA2 (AP2) domain, in rice. Overexpression of *OsAP37* and *OsAP59* by the *OsCc1* constitutive promoter increases tolerance to drought and salinity stress at the vegetative stage in rice (Oh et al., 2009). OsbZIP23, a member of the basic leucine zipper (bZIP) transcription factor family in rice, is induced by abscisic acid (ABA), and by several stress triggers, such as salt, drought, and polyethylene glycol (PEG); OsbZIP23 also confers ABA-dependent tolerance to salinity and drought stress (Xiang et al., 2008).

Light affects many steps in plant growth and development, such as seed germination, stem elongation, leaf morphogenesis, carbon assimilation, and flowering time (Carvalho et al., 2011). *OsPHYB* encodes the rice phytochrome B photoreceptor, which senses red (R) and far-red light (FR) and regulates a number of light-responsive genes, thus influencing many photomorphogenic processes (Franklin and Quail, 2010; Neff et al., 2000; Quail, 2002). In addition to the roles of phyB in plant photomorphogenesis, recent studies have identified roles for phyB in other processes. For example, rice *osphyB* mutants have larger epidermal cells and reduced stomatal density compared with wild type; this results in reduced transpiration per unit leaf area and thus improves tolerance to drought stress (Liu et al., 2012). In addition, He et al. (2016) reported that mutation of *OsPHYB* produces improved cold tolerance resulting from regulation of *OsDREB1* expression through OsPIL16. Song et al. (2017) recently showed that phyB signaling components regulate heat-shock responses and therefore, functional deficiency of phyB confers heat stress tolerance in *Arabidopsis*.

Consistent with a role for phyB in abiotic stress responses, the ratio of R to FR light results in marked biochemical changes in the phytochrome-mediated response to abiotic stress in plants. For example, *Mesembryanthemum crystallinum* plants grown in red light-rich environments switch from C3 to CAM (crassulacean acid metabolism) photosynthesis due to an increase in mRNA levels of PEP-carboxylase, a key CAM enzyme. This phenomenon is also observed when the *M. crystallinum* plants are exposed to salt stress (McElwain et al., 1992; Slocombe et al., 1993). A low R to FR light ratio induces the formation of pinitol, a soluble carbohydrate that accumulates in a number of plant species under stress (Cockburn et al., 1996; Guo and Oosterhuis, 1997). *CBF/DREB1* genes, which are induced by low temperature and play a role in the cold stress response, increase in the presence of a low R to FR light ratio in *Arabidopsis* (Franklin and Whitelam, 2007; Heidarvand and Amiri, 2010).

In this study, we found that a null mutation of *OsPHYB* resulted in improved tolerance to salinity stress. To investigate the mechanism by which OsPHYB affects the salt stress response, we measured Na^+ and K^+ contents and performed RT-qPCR analysis for salt stress-associated genes, including transcription factors and *HKT* genes, in the wild type (WT) and the *osphyB* mutants. In the *osphyB* mutants, accumulation of K^+ ions increased in the shoots and roots compared with the WT, and the net Na^+ uptake rate decreased. The altered expression of *HKT* genes in the *osphyB* mutants demonstrated that OsPHYB is involved in the distribution of Na^+ and K^+ ions and the maintenance of Na^+/K^+ homeostasis under salt stress in rice.

2. Materials and methods

2.1. Plant materials and growth conditions

The rice (*Oryza sativa*) cultivar “Dongjin” and the *osphyB-1* and *osphyB-2* mutants were grown in a rice paddy field in Suwon, Republic of Korea (37°N latitude), in a greenhouse in Seoul, Republic of Korea (37°N latitude), and in growth chambers in Seoul and Anseong, Republic of Korea (37°N latitude). The T-DNA knockout mutants *osphyB-1* and *osphyB-2* were obtained from the Crop Biotech Institute at Kyung Hee University, Republic of Korea (Jeon et al., 2000; Jeong et al., 2006). The genetic information on these two *osphyB* mutants was previously described (Jeong et al., 2007; Piao et al., 2015).

2.2. Physiological analysis for salinity tolerance

To evaluate the salt tolerance of the young plants, three-week-old seedlings grown in soil were treated with 200 mM NaCl for 10 days and recovered with water. The plants were grown in the greenhouse for physiological analyses such as survival rate and fresh weight, and were also photographed. The fresh weights were measured after 7 days of salt treatment and the survival rates were recorded after 10 days of re-watering (Li et al., 2016). To determine the tolerance to salinity during germination, seeds were placed in Murashige and Skoog (MS) media with or without 150 mM NaCl. Before sowing, coatless seeds were sterilized in 2% NaClO for 20 min and rinsed 4 times with distilled water. The seeds were incubated a growth chamber under 14.5 h light at 30 °C/9.5 h dark at 24 °C).

2.3. Measurement of photosynthetic pigments

The photosynthetic pigments were measured in the seedlings after 10 days of NaCl treatment before re-watering. To determine the total chlorophyll and carotenoid contents, the pigments in leaf blades were extracted with 80% acetone solution. The pigment contents were evaluated with a UV/VIS spectrophotometer (BioTek) and the concentration was calculated as previously described (Lichtenthaler, 1987).