Environmental and Experimental Botany 137 (2017) 158–165

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Overexpression of the phosphate transporter gene *OsPT8* improves the Pi and selenium contents in *Nicotiana tabacum*

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ARTICLE INFO

Article history:
Received 2 January 2017
Received in revised form 13 February 2017
Accepted 13 February 2017
Available online 17 February 2017

Keywords:
OsPT8
Selenite and Pi
Uptake
Overexpression
Nicotiana tabacum

ABSTRACT

Selenite is the predominant form of selenium (Se) available to plants in soils. Recent studies have suggested that selenite uptake is probably mediated by Pi transporters. Research on the molecular mechanism of Pi and Se uptake by plants is important for the development of Se-enriched agricultural products. In this study, we report the function of a Pi transporter, OsPT8, which was not only involved in Pi uptake but also in Se uptake in tobacco. Overexpression of *OsPT8* in tobacco significantly increased the total P concentration under Pi-sufficient conditions, and the biomass of *OsPT8-Oe* lines was higher than that of the wild type under Pi-deficient conditions. *OsPT8* overexpression not only increased the Pi concentration, but also resulted in greater Se accumulation in transgenic tobacco compared with the wild type under a high Pi concentration. Expression analysis showed that the Pi transporter genes *NtPT1* and *NtPT2* were strongly upregulated in transgenic tobacco under high Pi conditions. Together, these results suggest that Pi and Se uptake and translocation by the Pi transporter OsPT8 might function in a complex combinational way in tobacco, and that OsPT8 might be a potential candidate gene for breeding Se-enriched tobacco.

1. Introduction

Selenium (Se) is an essential nutrient that can enhance immune function in humans and animals (Schwarz and Foltz, 1957; Rotruck et al., 1973; Hanson et al., 2004). Se deficiency can cause health disorders, such as Keshan disease, Kashin-Beck disease, cardiovascular disease, muscle syndromes, and even death (Rayman, 2012; Kikkert and Berkelaar, 2013). For mammals, diet is a major source of selenium. In China, about 70% of the land is Se deficient and the average dietary intake of Se is only 26–32 μg per day by Chinese adults (Chen et al., 2002; Navarro-Alarcon and Cabrera-Vique, 2008), which is far lower than the reference intake of 40–200 μg per day specified by the WHO. Selenite is utilized by animals in two forms, inorganic selenite and organic selenite. The beneficial or toxic effects of Se on humans and animals is not only dose-dependent, but also related to the chemical form and bioavailability of Se (Thiry et al., 2012). Evidence suggests the organic Se is safer and more efficient absorbed than inorganic Se (Platis and Labrou, 2006). As a result, the application of Se fertilizers in the soil is highly recommended to produce Se-rich food (containing organic selenite) and eliminate Se deficiency in the human diet, but this also increases the cost of agricultural products and brings potential environment risks (Hartikainen, 2005; Broadley et al., 2006). Recently, genetic biofortification has offered a new and promising strategy to increase Se accumulation in crops and reduce the need for Se fertilizers. To this end, it is important to develop a comprehensive understanding of the mechanism of Se uptake in plants to improve the Se content in crops more efficiently.

There is no evidence that Se is an essential element for plants (Han et al., 2013). However, Se can stimulate the growth of plants at low doses, which increase chlorogenic acid, chlorophyll *a* and *b*, and carotenoid contents in plant leaves (Dong et al., 2013; Han et al., 2013), dramatically enhance the enzymatic and non-enzymatic anti-oxidation systems and improve plant resistance to abiotic stresses (Kumar et al., 2012; Lin et al., 2012). In contrast, high dosages can damage plants through ROS accumulation and

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http://dx.doi.org/10.1016/j.envexpbot.2017.02.011
0098-8472/

increased $O2^{\bullet-}$ and MDA contents, which inhibit plant growth (Mroczek-Zdyrska and Wójcik, 2012). The predominant forms of Se available to plants are inorganic Se (IV) and Se (VI) in the soil. After they are absorbed by plants, Se (IV) and Se (VI) can be converted into other forms, such as selenocysteine (SeCys) and selenomethionine (SeMet) (Afton et al., 2009; Zhu et al., 2009). The uptake mechanism of Se (VI) in plants via sulfate transporters is well established (Terry et al., 2000; White et al., 2015; Sors et al., 2005). Unlike Se (VI), the mechanism of Se (IV) uptake in plants is not well understood. Previous studies have shown that Se (IV) enters the roots through passive diffusion and Se (IV) uptake is repressed by an increase in the inorganic phosphate (Pi) concentration (Shrift and Ulrich, 1969). Recent studies have suggested that Se (IV) uptake is probably mediated by Pi transporters (Li et al., 2008). Zhang et al. (2014) first reported that a Pi transporter, OsPT2, was responsible for the active uptake of Se (IV) in rice using the *ltn1* mutant, which provided convincing molecular evidence to support this hypothesis. We found that the transcript abundance of *OsPT8* was significantly elevated in *ltn1* roots in addition to *OsPT2*; it is therefore reasonable to speculate that enhanced selenite uptake in *ltn1* roots may be correlated with upregulation of *OsPT8*. In a previous report, we demonstrated that OsPT8 functioned as a high-affinity Pi transporter and was involved in Pi homeostasis in rice (Jia et al., 2011). Overexpression of *OsPT8* resulted in excessive Pi in both roots and shoots, and Pi toxicity symptoms under high Pi conditions, demonstrating a similar function in Pi accumulation to *OsPT2* in rice. However, whether OsPT8 is involved in Se (IV) uptake in plants is not clear.

Tobacco is not only a model plant, but also an economically important crop worldwide. Tobacco leaves contain abundant and high quality soluble protein (Teng and Wang, 2012), and thus represent an ideal material for the production of Se-rich proteins. In this study, to clarify the function of OsPT8 in Pi and selenium uptake, we chosen the *OsPT8* overexpresion transgenic tobacco and wild type as the test material. Pi uptake, Se uptake and bioaccumulation as well as its influence on the growth and the antioxidant system of plants under various Se and Pi levels were studied, aiming to (1) clarify the over-expression *OsPT8* transgenic tobacco could enhance the Pi and selenium uptake in tobacco, (2) reveal a new function of OsPT8 interaction between selenium and Pi in plants.

2. Materials and methods

2.1. Transformation of OsPT8 into tobacco

The entire coding sequence of OsPT8 was amplified using the specific primers listed in Table S1 from a *Nipponbare* cDNA clone (GenBank Accession Number J033028K24) for *OsPT8* overexpression. The PCR product was digested with *Hpa*I and *Xho*I, and ligated into the pS1aG-4 vector, which was driven by the maize ubiquitin promoter and with a nopaline synthase terminator. The constructs were transferred to *Agrobacterium tumefaciens* strain EHA105 by electroporation, and then transformed into tobacco (*Nicotiana tabacum* cv) as described previously (Chen et al., 2011).

2.2. Southern blotting analysis

Independent *OsPT8-Oe* transgenic tobacco lines were identified by Southern blotting analysis. Genomic DNA was extracted from leaves of wild type (WT) and T_1 transgenic plants using the SDS method, and 5 μg was digested with the restriction enzyme *Hind*III overnight at 37 °C. The digested DNA was separated on a 0.8% (w/v) agarose gel, and then transferred to a Hybond-N^+ nylon membrane and hybridized with the coding sequence of the hygromycin-resistance gene, which was used as the hybridization probe, as described previously (Jia et al., 2011).

2.3. Pi and Se treatment of tobacco plants

After sterilization with 2% (v/v) NaClO for 10 min, the WT and transgenic tobacco seeds were thoroughly rinsed in tap water and then sown in a floating nursery. Forty-days-old seedlings were transplanted into pots containing sand with one plant per pot, the forty-days-old seedlings were culture by adding 1/4 strength Hoagland nutrient solution to pots every day at first and three days later, the solution was replaced by 1/2 strength nutrient for three days, and then the solution was replaced by full nutrient solution, which was renewed every two days. After twenty days, the seedlings were used for Pi and Se treatment (Han et al., 2015).

For Pi treatment, the plants were substrate irrigated with nutrient solution containing the following macro- and micro-nutrients: 2 mM KNO_3, 1 mM NH_4NO_3, 0.5 mM Ca $(NO_3)_2$, 0.25 mM $CaCl_2$, 0.5 mM $MgSO_4$, 20 μM Fe-EDTA, 9 μM $MnCl_2$, 46 μM H_3BO_3, 8 μM $ZnSO_4$, 3 μM $CuSO_4$, 0.03 μM $(NH_4)_2MoO_4$, and either 0.02 mM NaH_2PO_4 (Pi-deficient treatment; LP) or 1 mM NaH_2PO_4 (Pi-sufficient treatment; HP). For selenium treatment, 10 μM Na_2SeO_3 was added to the nutrient solution under HP and LP treatment. The four treatments plants, namely HP, LP, HP + Se and LP + Se were grown in a growth chamber under a 14-h light, 10-h dark photoperiod with 28–30 °C/18–20 °C day/night temperatures. The plants were harvested after 28 days of treatment. The freshly collected leaves and roots were washed with deionized water for the next analysis. (1) Some of the leaves were used to measure the chlorophyll content and MDA content at once. (2) Some of the roots and leaves were immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation for further analyses of gene expression. (3)The other roots and shoots were oven-dried, first at 105 °C for 15 min to destroy the enzymes and then at 65 °C for 48 h, before being weighed and pulverized for Se, P and other micronutrient determination.

2.4. Q-PCR analysis of Pht1 family genes

Total RNA was extracted from the roots and leaves of tobacco seeding using the method described by Jia et al. (2011). Reverse transcription polymerase chain reaction (Q-PCR) analysis was performed using gene-specific primers for *OsPT8*, *NtPT1* and *NtPT2*. The primers used for Q-PCR are listed in Supplemental Table S1.

2.5. Measurement of total P concentration in plants

To measure the total P concentration in the plants, dry samples of about 0.05 g were assayed following the method described by Chen et al. (2007).

2.6. Determination of chlorophyll and MDA contents

The chlorophyll content was measured by acetone extraction.

The MDA content was assayed using a solution containing 2.5 mL of 20% (w/v) trichloroacetic acid, which included 0.5% (w/v) thiobarbituric acid and 1.5 mL enzyme extract. The solution was kept in a boiling water bath for 20 min and then quickly cooled. After refrigeration, the homogenate was centrifuged at 5000*g* for 10 min at 25 °C. The absorbance of the supernatant was recorded at 532 nm and 600 nm. The absorbance at 600 nm was subtracted from the value at 532 nm. The concentration of MDA was calculated using the MDA extinction coefficient of 155 $mM^{-1}\,cm^{-1}$ (Feng et al., 2009; Han et al., 2013). A protein assay was carried out by the method described by Bradford (1976).