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Molecular cloning and characterization of the ABA-specific glucosyltransferase gene from bean (*Phaseolus vulgaris* L.)

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ABSTRACT

Levels of the plant hormone abscisic acid (ABA) are maintained in homeostasis by a balance of its biosynthesis, catabolism and conjugation. The detailed molecular and signaling events leading to strict homeostasis are not completely understood in crop plants. In this study, we obtained cDNA of an ABA-inducible, ABA-specific UDP-glucosyltransferase (ABAGT) from the bean plant (*Phaseolus vulgaris* L.) involved in conjugation of a glucose residue to ABA to form inactive ABA-glucose ester (ABA-GE) to examine its role during development and abiotic stress in bean. The bacterially expressed PvABAGTase enzyme showed ABA-specific glucosylation activity *in vitro*. A higher level of the *PvABAGT* transcript was observed in mature leaves, mature flowers, roots, seed coats and embryos as well as upon rehydration following a period of dehydration. Overexpression of *35S::PvABAGT* in *Arabidopsis* showed reduced sensitivity to ABA compared with WT. The transgenic plants showed a high level of ABA-GE without significant decrease in the level of ABA compared with the wild type (WT) during dehydration stress. Upon rehydration, the levels of ABA and phaseic acid (PA) decreased in the WT and the *PvABAGT*-overexpressing lines with high levels of ABA-GE only in the transgenic plants. Our findings suggest that the *PvABAGT* gene could play a role in ABA homeostasis during development and stress responses in bean and its overexpression in *Arabidopsis* did not alter ABA homeostasis during dehydration stress.

Introduction

The phytohormone abscisic acid (ABA) regulates seed dormancy, germination, plant development and growth (Schwartz et al., 2003; Zeevaart, 1999), and the stomatal aperture in response to soil and air humidity, temperature, and CO_2 concentration (Burla et al., 2013). The ABA level in planta is maintained in strict homeostasis, involving biosynthesis, degradation and conjugation, which becomes available during various physiological processes (Burla et al., 2013; Seiler et al., 2011).

ABA is synthesized from zeaxanthin through several key intermediates catalyzed by enzymes such as zeaxanthin epoxidase (ZEP), neoxanthin synthase (NSY) and an isomerase, 9-cis-epoxycarotenoid dioxygenases (NCED) in chloroplasts, followed by short-chain alcohol dehydrogenase (ABA2), and abscisic aldehyde oxidase (AAO3) in cytoplasm (Nambara and Marion-Poll, 2005). Turnover of ABA, resulting in deactivation and inactivation, takes place *via* two main pathways: catabolism and conjugation, respectively (Nambara and Marion-Poll, 2005). ABA catabolism is initiated by ABA 8′-hydroxylase (8′OH-ABA) to form 8′-hydroxy-ABA, which then becomes spontaneously isomerized to phaseic acid (PA). PA is reduced to dihydrophaseic acid (DPA), the major catabolites of ABA oxidative degradation (Nambara and Marion-Poll, 2005; Zeevaart, 1999).

Conjugation of ABA with glucose catalyzed by ABA-specific glucosyltransferases represents another ABA inactivation mechanism. Over the years, a few reports have been published regarding identification of genes encoding enzymes involved in ABA conjugation. ABA glucosyltransferase (ABAGT) was identified in Adzuki bean (*Vigna angularis*), capable of glucosylating ABA *in vitro* (Xu et al.,

Abbreviations: ABA, abscisic acid; 8′OH-ABA, ABA 8′-hydroxylase; ABA-GE, ABA-glucose ester; PA, phaseic acid; DPA, dihydrophaseic acid; UDP, uridine diphosphate; UGTs, UDP-glucuronosyltransferases; Glc, glucose; HPLC, high-performance liquid chromatography.

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2002). The *Arabidopsis* UGT71B6 exhibits selective glucosylation activity toward the natural enantiomer (+)-ABA (Priest et al., 2006).

In contrast to ABA oxidation, ABA conjugation with glucose is reversible, so that the resulting ABA-glucose ester (ABA-GE) is hydrolyzed by specific β-glucosidases (BG) to release free ABA (Xu et al., 2012). In *Arabidopsis*, ABA-GE stores ABA in inactive form in the vacuole that can be converted back to active ABA by the hydrolytic activity of AtBG1 and AtBG2 (Lee et al., 2006; Xu et al., 2012), which is considered as a rapid ABA recycling pathway to maintain ABA homeostasis, and thus essential for plant developmental processes (Burla et al., 2013; Dong et al., 2014).

The expression of the genes coding for ABA glucosyltransferase are regulated by ABA and environmental stresses such as water stress, wounding, osmotic and salt stress (Dong et al., 2014; Xu et al., 2002). Water stress is one of the major abiotic stresses and is related to phytohormones (such as ABA, GA, ethylene, IAA, JA *etc.*) that limit the growth and production of plants (Nambara and Marion-Poll, 2005). Mechanisms of the water stress response have been investigated most extensively in *Arabidopsis* (Zhu, 2002; Bray, 2002). However, the biological functions of many genes related to the above stress response are still largely unknown in agricultural crops.

In the present study, we report the identification, cloning and characterization of an ABA-inducible glucosyltransferase gene from bean (*Phaseolus vulgaris* L.). The expression of *PvABAGT* is regulated developmentally as demonstrated in developing and germinating bean seeds, as well as environmentally as shown by responses to changes in the water status of leaves. Overexpression of *35S::PvABAGT* in transgenic plants was characterized with respect to their phenotype in response to exogenous ABA, and to the role of *PvABAGT* in maintenance of ABA homeostasis upon dehydration and rehydration stresses.

Materials and methods

Plants and growth conditions

Bean (*Phaseolus vulgaris* L., cv. Top Crop) and *Arabidopsis* (Col-0) seedlings were grown in plastic containers with vermiculite in a growth chamber with daily watering with half-strength Hoagland nutrient solution. The conditions in the growth chamber consisted of 18 h of light from fluorescent and incandescent lamps (200 μmol m^{-2} s^{-1} photon) at 25 °C, followed by 6 h of darkness at 23 °C.

For the expression analysis of *PvABAGT* in various parts of the bean plant, the plant organs such as flower, fruit, seed, leaves, stem and root were collected at different stages of plant development. The materials were weighed, frozen in liquid N_2, and stored at −80 °C until analysis.

Molecular cloning and sequencing of PvABAGT

The first-strand cDNA was prepared using a reverse transcriptase (Invitrogen), oligo-dT_{20} as the primer and total RNA extracted from bean leaves that had been water-stressed for 4 h. To obtain fragments of the *PvABAGT* gene, a RT-PCR was performed using degenerate primers designed for the consensus UDP-GTase sequences: JZ794 (5′-GGCCAYAWRATHCCDAYVCT-3′) and JZ798 (5′-YCCTCMAYAGCYYTTGHWGC-3′). The primers were used for RT-PCR reaction with the above first-strand cDNA as a template. The resulting PCR fragments were sequenced and analyzed for the presence of conserved PSPG-box in UDP-GTases sequence. The missing information at the 5′ and 3′ ends of the cDNA fragment was obtained by RACE (rapid amplification of cDNA ends) procedureusing SMART 5′- and 3′-RACE cDNA Amplification Kits (BD Biosciences Clontech.), respectively, according to the manufacturer's protocols. To obtain the full-length *PvABAGT* clone, primers were designed for the sequences corresponding to the 5′ (JZ822, 5′-GGTGTG GCTAGAATGGTTGATGA-3′) and the 3′ (JZ823, 5′-TGGGGAAACAAAACA AGAAGAATA-3′) UTRs, and used for RT-PCR using the above first-strand cDNA as a template. The resulting PCR products were cloned into a standard T-vector (Promega), and sequenced. Analysis of the cDNA sequences was carried out using the DNASTAR program (DNASTAR, Inc.). Subcellular localization of the deduced amino acid sequence was predicted using the Plant-mPLoc package (Chou and Shen, 2010). Multiple sequence alignments were performed using the Clustal W Multiple Sequence Alignment program and printed using BOXSHADE 3.21.

Expression and isolation of the recombinant PvABAGT protein

The coding region of *PvABAGT* was amplified by a PCR reaction with primers that had been designed according to the full-length cDNA sequence using the forward (JZ1043, 5′-GGAGAATTCACATGAAACCGGTTGAAATC-3′) and the reverse (JZ1044, 5′-CGGCCCGGGTTAGCCCTGGTTTGCGC-3′) primers. The cDNA was ligated in the *Eco*RI and *Sma*I sites of pGEX-5X-2, a GST fusion vector (Amersham Biosciences), and transformed into *Escherichia coli*, strain BL21 (DE3) pLysS-competent cells (Stratagene) according to the manufacturer's protocol. A 2.0 mL overnight-cultured aliquot harboring the recombinant cDNA was inoculated into 100 mL of fresh LB broth containing 100 μg mL^{-1} ampicillin, and incubated while shaking at 30 °C until A_{600} reaches to 0.8 to 1.0. The culture was then induced by adding isopropyl-β-D-thiogalactoside to final 1 mM, and incubated for another 5 h. The cells were harvested, washed twice with a 10 mM Tris–HCl buffer (pH 7.5), and resuspended in Lysis buffer (10 mM Tris–HCl, pH 7.5, 150 mM NaCl and 10 μg mL^{-1} lysozyme). After incubation for 10 min at room temperature, the lysates were frozen in liquid N_2, thawed on ice, and then sonicated. After centrifugation, the supernatant was filtered through a 0.45 μm membrane filter, and purified by glutathione 4B affinity column chromatography according to the recommended protocol (GE Healthcare). The excess glutathione in the eluate was removed by ultrafiltration at 4 °C. After washing three times with a buffer containing 10 mM Tris–HCl (pH 7.5) and 150 mM NaCl, the purified protein was collected and stored at −80 °C until use.

Enzyme assays

Enzyme activity of PvABAGT was determined according to (Xu et al., 2002) using either the affinity-purified or crude recombinant proteins. The reaction mixture was made up to 100 μL mixture containing 5 μg of purified or 50 μg of crude protein, 3.0 mM abscisic acid (ABA) and 5.0 mM UDPG in a buffer containing 10 mM Tris–HCl, pH 7.5, and 150 mM NaCl. After incubation for 3 h at 30 °C, the reaction was stopped by adding 10 μL of 1 M acetic acid. The reaction mixture was extracted twice with 2.0 mL of methanol, and then concentrated until it is completely dried for subsequent TLC analysis: The substrate specificity of the enzyme was examined by using potential glucosyl acceptors, JA, IAA, GA3 or phaseic acid (PA), at final 5.0 mM, and 0.5 mM UDP-[^{14}C] glucose (740 MBq $mmol^{-1}$; NCI). After spotting an aliquot, the silica gel plate was developed with a mixture containing methanol:chloroform:water (25:15:1, v/v/v), and exposed to a phosphor-imaging plate for autoradiography. The intensity of each radioactive spot was estimated using an image analyzer (STORM 860 system, Bio-Rad).