Journal of Plant Physiology 173 (2015) 62–71

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Physiology

Over-expression of *TaEXPB23*, a wheat expansin gene, improves oxidative stress tolerance in transgenic tobacco plants

Yangyang Han[a,b,1], Yanhui Chen[a,1], Suhong Yin[a], Meng Zhang[a], Wei Wang[a,*]

[a] *State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an, Shandong 271018, PR China*

[b] *Plastic Surgery Institute of Weifang Medical University, Weifang, Shandong 261041, PR China*

ARTICLE INFO

Article history:
Received 17 October 2013
Received in revised form 14 August 2014
Accepted 3 September 2014
Available online 2 October 2014

Keywords:
Abiotic stress
Antioxidant enzyme
Cell wall peroxidase
Expansin
Methyl viologen

ABSTRACT

Expansins are cell wall proteins inducing cell wall loosening and participate in all plant growth and development processes which are associated with cell wall modifications. Here, *TaEXPB23*, a wheat expansin gene, was investigated and the tolerance to oxidative stress was strongly enhanced in over-expression tobacco plants. Our results revealed that over-expressing *TaEXPB23* influenced the activity of antioxidant enzymes: in particular, the activity of the cell wall-bound peroxidase. The enhanced tolerance to oxidative stress and increased cell wall-bound peroxidase activity were partly inhibited by an anti-expansin antibody. The *Arabidopsis* expansin mutant *atexpb2* showed reduced cell wall-bound peroxidase activity and decreased oxidative stress tolerance. In addition, *atexpb2* exhibited lower chlorophyll contents and the germination rate compared to wild type (WT). Taken together, these results provided a new insight on the role of expansin proteins in plant stress tolerance by cell wall bound peroxidase.

Introduction

Expansins are a group of non-enzymatic cell wall proteins belonging to a superfamily of genes with four families (Cosgrove, 1999, 2000, 2005; Lee et al., 2001). Expansins loosen the cell wall in a pH-dependent manner. It is hypothesized that they break the hydrogen bonds between hemicellulose and cellulose microfibrils, thereby allowing turgor-driven cell enlargement (McQueen-Mason and Cosgrove, 1994).

Previous studies have provided evidence that expansins are associated with environmental stress tolerance in plants. Wu et al. (2001) reported that at least two expansin genes are up-regulated in apical regions of the maize root elongation zone at low water potential implying that expansins may play an important role in maintaining root growth under water stress. In *Craterostigma plantagineum*, the increase of expansin activity was accompanied by the enhancement of wall extensibility during the processes of dehydration and rehydration, suggesting a role for expansin proteins in increasing wall flexibility and promoting leaf growth under drought stress (Jones and McQueen-Mason, 2004). Over-expression of rose expansin gene *RhEXPA4* in *Arabidopsis* conferred strong tolerance to drought stress, salt stress and ABA (Dai et al., 2012; Lü et al., 2013). Our previous results showed that overexpression of a wheat expansin gene, *TaEXPB23*, enhanced drought and salt stress tolerance in transgenic tobacco (Han et al., 2012; Li et al., 2011a). However, the regulatory mechanisms that govern the action of expansin proteins are poorly understood.

Abiotic stresses, such as drought, cold, and salinity, result in the production and accumulation of reactive oxygen species (ROS), which are highly reactive and toxic to plant cells (Apel and Hirt, 2004). There is ample evidence showing that antioxidative systems are involved in plant stress tolerance like salt stress tolerance (Mittler, 2002) and drought tolerance (Aroca et al., 2003). Among plant antioxidative systems, antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), play important roles in the stress response (Foyer and Noctor, 2005). In particular, plants possess two classes of heme peroxidases (EC 1.11.1.7), class I and class III, according to the classification scheme proposed by Welinder (1980). Class I peroxidases are intracellular, whereas class III peroxidases are secreted into the cell wall or the surrounding medium (Passardi et al., 2004).

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; DHAR, dehydroascorbate reductase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; MS, Murashige and Skoog; MV, methyl viologen; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; WT, wild type.

* Corresponding author. Tel.: +86 538 8246166; fax: +86 538 8242288.
E-mail addresses: hanyy2009@163.com (Y. Han), cyh890324@163.com (Y. Chen), kaixiaoyin@163.com (S. Yin), janet5207@126.com (M. Zhang), wangw@sdau.edu.cn, wangweisdau@yahoo.com (W. Wang).

[1] These authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.jplph.2014.09.007
0176-1617/

In the current study, to understand the mechanisms underlying improved abiotic stress tolerance in transgenic tobacco plants over-expressing *TaEXPB23*, we focused on the functions of *TaEXPB23* in oxidative stress tolerance as oxidative stress is a ubiquitous type of secondary stress. Methyl viologen (1,1′-dimethyl-4,4′-bipyridinium dichloride, MV), the active ingredient in the herbicide paraquat, exerts its phototoxic effects on plants by transferring electrons from photosystem I to molecular oxygen (Dodge, 1994). Therefore, MV was used in this study to induce oxidative stress. Our results demonstrated that over-expression of *TaEXPB23* influenced the activity of antioxidant enzymes. In particular, the activity of the covalently bound cell wall peroxidase was strongly enhanced after MV treatment, and decreased when expansin activity was specifically inhibited by antibodies. In addition, the oxidative stress tolerance and the cell wall-bound peroxidase activity of the *Arabidopsis* mutant *atexpb2* decreased significantly compared to Col-0, indicating that expansins, at least *AtEXPB2*, is involved in the oxidative stress response. Our work provides new insight on the role of expansin proteins in plant stress tolerance.

Materials and methods

Plant materials, growth conditions, and treatments

Transgenic tobacco plants carrying the *35S::TaEXPB23* construct were generated as previously described (Xing et al., 2009). The β-expansin gene *TaEXPB23* was isolated from wheat (*Triticum aestivum* L.) coleoptiles. Sterilized tobacco (*Nicotiana tabacum* L. cv. NC89) seeds were germinated on Murashige and Skoog (MS) medium in a growth chamber (25 °C, 16/8 h light/dark, 300–400 μmol photons m^{-2} s^{-1}) and their leaves were used for transformation. T_3 generation plants of homozygous transgenic tobacco from lines T3-1, T3-8, and T3-10 were used in this study. Tobacco seeds were sown in pots (8 cm × 10 cm) containing vermiculite soaked in 0.5× Hoagland's nutrient solution in a growth chamber at 25 °C, with a 16/8 h light/dark cycle (300–400 μmol photons m^{-2} s^{-1}) at a relative humidity of 75–80%.

For methyl viologen (MV) treatment, whole plants were sprayed with MV solution (100 μM) containing 0.1% Tween-20, and then transferred to an illuminated incubation chamber (GXZ-500C, Jiangnan, China) with a photon flux density of 100 μmol m^{-2} s^{-1} for 24 h. For young seedlings, tobacco seeds were germinated and grown under control growth conditions in the presence of 5 or 10 μM MV for 2 weeks, or 2-week-old tobacco seedlings were transferred to MV solution (50 or 100 μM) for 2 days. All physiological and biochemical measurements were carried out using the youngest, fully expanded leaves.

The *Arabidopsis atexpb2* mutant was obtained from the ABRC and genotyped by PCR using the genomic primers AtEXPB2-LP, AtEXPB2-RP, and the left T-DNA border primer LBb1.3 (http://signal.salk.edu). The original line was SALK-048197. *Arabidopsis* plants were grown in soil under long-day conditions (16 h light, 8 h dark) at 22 °C.

Measurement of chlorophyll content and malondialdehyde (MDA) level

Chlorophyll in the leaf disks was extracted using 95% ethanol and quantified by UV spectrophotometer (Kong et al., 2011). The MDA level was assayed according to Quan et al. (2004).

Gene expression analysis by qRT-PCR

Total RNA was extracted from transgenic tobacco plants with Trizol reagent (TaKaRa, Japan) according to the manufacturer's protocol, and was then treated with DNase I (RNase-free, Promega).

Table 1
The primers sequence.

Primer name	Primer sequence (5′–3′)	Length (bp)
Ntactin-F (U60489)	CATTGGCGCTGAGAGATTCC	20
Ntactin-R (U60489)	GCAGCTTCCATTCCGATCA	19
NtSOD-F (AB093097)	GACGGACCTTAGCAACAGG	19
NtSOD-R (AB093097)	CTGTAAGTAGTATGCATGTTC	21
NtCA-F (AF454759)	CGCCTGTGGAGGTATCAAA	19
NtCA-R (AF454759)	GAGAAGGAGAAAGACCGAACT	21
NtRbohD-F (AJ309006)	ACCAGCACTGACCAAAGAA	19
NtRbohD-R (AJ309006)	TAGCATCACAACCACAACTA	20
NtCAT1-F (U93244)	TGGATCTCATACTGGTCTCA	20
NtCAT1-R (U93244)	TTCCATTGTTTCAGTCATTCA	21
NtGPX-F (AB041518)	GGTTTGCACTCGCTTCAAG	19
NtGPX-R (AB041518)	AGTAGTGGCAAAACAGGAAG	20
NtAPX1-F (AU15933)	GAGAAATATGCTGCGGATGA	20
NtAPX1-R (AU15933)	CGTCTAATAACAGCTGCCAA	20
NtAPX2-F (D85912)	GACAACTCATACTTTACGGA	20
NtAPX2-R (D85912)	CTTCAGCAAATCCCAACTCA	20
AtEXPB2-LP	AATTCAAACGGTTGTTTGTGC	21
AtEXPB2-RP	AACATGCACCACATCCTTTTC	21
LBb1.3	ATTTTGCCGATTTCGGAAC	19

Total RNA was subjected to first-strand cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, USA) according to the manufacturer's instructions. qPCR analysis was performed using the Bio-Rad CFX Manager system as described previously (Han et al., 2012). The relative transcript abundance for genes is relative to the *actin* transcript levels measured in the same sample. The primers used for qPCR are given in Table 1.

The GenBank accession numbers for the sequences used in this study are: U60489 (*actin*), AB093097 (*NtSOD*), AF454759 (*NtCA*), AJ309006 (*NtRbohD*), U93244 (*NtCAT1*), AB041518 (*NtGPX*), AU15933 (*NtAPX1*), and D85912 (*NtAPX2*).

Extraction and assay of antioxidant enzyme activity

Tobacco seedlings treated with MV were collected to measure the activities of antioxidant enzymes, including superoxide dismutase (SOD) (EC1.15.1.1), peroxidase (POD) (EC 1.11.1.7), catalase (CAT) (EC 1.11.1.6), and ascorbate peroxidase (APX) (EC 1.11,1.1), as described previously (Hui et al., 2012). The glutathione reductase (GR) and dehydroascorbate reductase (DHAR) activities were determined according to Li et al. (2011b). Enzyme activity assays were carried out in a UV–vis spectrophotometer (UV-2550, Shimadzu, Japan) at 25 °C.

The protein concentration of the enzyme extracts was determined according to the method of Bradford (1976).

Isolation, extraction and determination of peroxidase

The soluble, ionically cell wall-bound, and covalently bound peroxidase fractions were prepared from 100 mg of tobacco seedlings according to the method of Hendriks et al., 1985 with slight modifications. A fraction that was assumed to contain mainly soluble peroxidase was prepared essentially as described previously (Csiszár et al., 2011). The pellet from a crude leaf homogenate was washed twice with an aqueous solution of Triton X-100 (1%), and five times with deionized water. The ionically bound fraction was extracted from the washed pellet by incubation in NaC1 (1 M, 15 h, 6 °C). Thereafter, the pellet was washed twice with NaCl (1 M) and five times with deionized water. A covalently bound fraction was then extracted from the washed and salt-extracted pellet by incubation (2 h, 37 °C) in a solution containing mannitol (0.6 M), 2% Cellulase R-10 (Japan Yakult), and 0.2% Macerozyme R-10 (Japan Yakult).

The assay mixture for spectrophotometric determination of peroxidase activity consisted of a Na-acetate buffer (0.05 M,