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The cell wall DUF642 At2g41800 (TEB) protein is involved in hypocotyl cell elongation

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ABSTRACT

In plants, the cell wall is a complex and dynamic structure comprising high molecular weight carbohydrates and proteins. The cell wall plays an important role in several stages of the plant life cycle, including cell division, elongation and differentiation. The DUF642 family of cell wall proteins is highly conserved in spermatophytes and might be involved in pectin structural modifications. Particularly, *At2g41800* is one of the most highly induced genes during the M/G1 phases of the cell cycle, and the protein encodes by this gene has been detected in cell wall proteomes of cell suspension cultures. In the present study, the expression of *At2g41800 (TEB)* was confirmed in primary and lateral roots, stigmatic papillae and hypocotyls. Subcellular localization studies showed that TEB is located in the cell wall. The root length and lateral root density were not affected in either of the two *teb* mutants studied, but the length of the hypocotyls from seedlings grown under light and dark conditions was increased. Immunogold labelling studies using JIM5 antibodies on sections of hypocotyl epidermal cells showed an important reduction of gold particles in *teb* mutants. The results suggested that TEB is involved in hypocotyl elongation.

1. Introduction

Cell growth in plants is highly regulated according to the composition and properties of the cell wall. Cell division and cell expansion processes require changes in the cell wall composition as well as the deposition of new material [1]. The plant cell wall is a dynamic structure comprising high molecular weight carbohydrates and proteins with known and unknown functions. The relevance of these protein interactions and functional studies of putative cell wall proteins of unknown function has recently been highlighted [2].

The DUF642 family is a highly conserved family of cell wall-related proteins specific to spermatophytes. The phylogenetic tree of this family retrieved 4 clades: clade A, which includes cell wall proteins with a signal peptide and a DUF642 domain, and clades B, C, and D, which include DUF642 proteins localized to the membrane based on the presence of a GPI anchor site. The DUF642 domain has structural similarities to carbohydrate binding domains. The At3g08030 protein, present in cell wall proteomes from different tissues, interacts *in vitro* with cellulose and hemicellulose [3]. Recent studies also suggested that the DUF642 family of proteins could be involved in pectin structural modifications. Proteins encoded by *BIIDXI* (*BDX, At4g32460*) and *At5g11420* interact *in vitro* with AtPME3, a pectin methyl esterase (PME) from *Arabidopsis thaliana* involved in different plant developmental processes [4]. BDX positively regulates PME activation during *Arabidopsis* seed germination and siliqua development. Germination parameters are improved in the seeds of plants overexpressing *BDX*, and a positive correlation was observed between the increase in testa rupture velocity and PME activity. A reduction in total PME activity was detected in *bdx* heterozygous mutants, in which siliques were short with few seeds [5]. An increase in PME activity was also detected in the leaves of *Vitis quinquangularis* plants overexpressing VqDUF642, which is involved in berry development and pathogen resistance [6].

Abbreviations: AtPME3, *Arabidopsis thaliana* pectin methyl esterase 3; BDX, *BIIDXI* (*At4g32460*); BSA, bovine serum albumin; CIM, callus induction medium; DIC, differential interference contrast; DMSO, dimethyl sulfoxide; DUF, domains of unknown function; DGR, DUF642 L-GalL responsive gene; GFP, green fluorescence protein; HG, homogalacturonan; HU, hydroxyurea; MS, Murashige and Skoog medium; NGS, normal goat serum; PLA, pectate lyase; PME, pectin methyl esterase; PG, polygalacturonase; RT-PCR, reverse transcription-PCR; TEB, (*At2g41800*); TBS, tris-buffered saline.

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Functional studies with the DUF642 protein DGR2 (DUF642 L-GalL Responsive Gene2, *At5g25460*), present in cell proteomes from suspension cells and hypocotyls, indicate the participation of this gene in seedling development. The *dgr2* mutants showed shorter root and smaller rosettes compared with WT. Based on the results, the authors suggested that DGR2 might play a role in cell wall pectin dynamics [7].

Pectins are the most structurally complex family of polysaccharides in nature, and homogalacturonan (HG), a linear β-1,4-linked GalA homopolymer, is the most abundant pectic polysaccharide. The methyl-esterification status of HG, based on pectin methylesterase (PME) activity, alters cell wall mechanical properties and regulates cell growth and shape. The pectin methyl esterification status is subject to significant changes during cell separation, cell division and cell expansion [8]. Low methyl-esterified motifs in HG are accessible to polygalacturonase (PG) or pectate lyase (PLA) enzymes that degrade the pectin matrix between cells, thereby promoting cell separation [9,10].

Pectins with a low degree of methyl esterification (DM) are involved in the generation of a primary cell wall during the formation of a mature cell plate and the elongation of the parent cell to yield distinct new cells during cell cytokinesis. Cell surface material, including Rhamnogalacturonan II and partially esterified HG pectins, also contributes to cell plate formation [11]. Conversely, cell elongation can be limited by a low degree of pectin methyl esterification in hypocotyls. Under continuous light, a reduction in the pectin esterification level is associated with restricted cell elongation in *Arabidopsis* hypocotyls. In addition, heterologous PME expression reduces the hypocotyl length in *Arabidopsis* [12]. The role of pectins in cell elongation has also been determined in hypocotyls grown in darkness. The hypocotyls of *atpme3-1* mutants are significantly larger than WT hypocotyls [13]. TRICHOME BIREFRINGENCE (TBR) encodes a plant-specific DUF231 protein [14]. The short hypocotyl phenotype of the *tbr* mutant has been associated with increased pectin methyl esterase activity. However, the increased ester bonds in the cell wall pectins of seedlings overexpressing PMEI4 delayed the initial growth acceleration of dark grown hypocotyls [15]. These opposing consequences on hypocotyl growth that resulted from a low DM might be associated with the presence of calcium ions or active polygalacturonases that differentially alter pectins with different patterns of de-methylesterification [16].

At2g41800 encodes a DUF642 cell wall protein that is primarily detected in the cell wall proteomes from suspension cell cultures. The amino acid sequence of At2g41800 shows 88% identity with At2g41810 and 62% identity with At3g08030. At2g41800 and At2g41810 result from a tandem duplication and the three proteins are grouped in clade A2 [3]. In the present study, we demonstrated the involvement of the DUF642 gene *At2g41800* in mediating hypocotyl length associated to cellular elongation. Based on these results, we name *At2g41800* as *TEB* (TEEBE stands for "long" in the Mayo-Yoreme language).

2. Materials and methods

2.1. Growth conditions

Columbia (WT) and transgenic plants were grown in a CONVIRON (Winnipeg, Canada) growth chamber under long-day conditions (16 h light/8 h dark) at 20–22 °C.

The seeds were surface sterilized after treatment with 70% ethanol for 5 min, followed by 1% SDS and a 15% hypochlorite solution for 15 min. The seeds were subsequently rinsed with sterilized water.

For root studies, the seeds were germinated after imbibition at 4 °C for 2–3 days and grown vertically on Petri dishes with 0.5 X Murashige and Skoog (MS) basal medium (Duchefa) containing 0.5% sucrose and 0.6% agar. Seed germination was conducted in a CONVIRON growth chamber under long-day conditions at 20–22 °C.

2.2. Generation of constructs and transgenic lines for TEB and At3g08030 genes

For *TEB* expression studies, an 890-bp fragment upstream of ATG-*TEB* was amplified using the primers: PRO41800F (5′ AAGCTTCGTAGATTGCGGAGAAAAGAACC 3′) and PRO41800R (5′ GGATCCTTTAAAGTCTTATTAATTAGTTCGCC 3′). This fragment contains motifs for auxin response factors (ARF) [17] and light response (GATA motifs) [18] and MADS-box transcription factor [19] (Suppl. Fig. 1A). The PCR fragment was subsequently cloned into the pGEM-T Easy vector and sequenced. The resulting plasmid was cut with the HindIII and BamHI enzymes, and the fragment encompassing the putative *At2g41800* promoter was cloned into the *pBIN- m-GFP-ER* vector ([20]; *pTEB:GFP-ER*). For subcellular localization analyses, a 2222-bp fragment including the putative promoter and the coding region was amplified using the primers: PRO41800F and 41800LSR (5′ GGATCCACGGGCCAGGGTAACAACAACG 3′). The fragment was cloned and sequenced as previously described. The HindIII/BamHI fragment was cloned into the *pBIN-GFP* vector (*pTEB:TEB-GFP*). WT and CFP-TUA plants [21] were transformed with *pTEB:TEB-GFP* construct (Suppl. Fig. 1) using the floral dip method [22]. The transformants were selected on 0.5X MS plates containing 0.8% agar and 50 mg/L of kanamycin. The transformants were screened for Green Fluorescence Protein (GFP) expression using confocal microscopy. For these studies, at least three independent transformants were analysed.

For *At3g08030* expression studies, a 2112-bp fragment upstream of ATG-*At3g08030* was amplified using the primers PRO8030F (5′ AAGCTTCACCGATGGTGACATT 3′) and PRO8030R (5′ GGATCCTGTCTCTGTTGTTCTTCCTCG 3′). This fragment contains motifs for auxin response factors (ARF) and light response factors [17,18].

2.3. Genotyping of T-DNA lines

The homozygous lines of two independent T-DNA lines with insertions in the *At2g41800* locus were obtained. SALK_138024 (*teb-1)* and SALK_109142 *(teb-2*) were analysed using the LBA1 primer (5′ TGGTTCACGTAGTGGGCCATCG 3′) and F41800 primer (5′ GGATCCCATGAGTAAAGGAGAAGAACTTTTCACTGGAG 3′). The T-DNA insertion in *teb-1* line is present at the end of the second exon of the *At2g41800* locus, and the *teb-2* line is located at the end of the third exon (Fig. 4A). For complementation analysis, *teb-1* was crossed to *pTEB:TEB-GFP* to obtain a *teb-1*/*pTEB:TEB-GFP* line. The presence of TDNA and the complementation construct were verified during subsequent generations. The analyses were carried out on the F_4 double homozygous line. Expression of TEB was confirmed by RT-PCR analysis.

2.4. Quantitative RT-PCR

cDNA samples containing 100 ng of RNA from transgenic and WT seedlings were employed for amplification with SYBR Green Master Mix using an Applied Biosystems StepOne platform (Applied Biosystems). The PCR conditions were 50 °C for 2 min for DNA polymerase activation and 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min; subsequently, the samples were cycled at 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s for melting curve analysis. Three independent biological replicates with three technical replicates were performed using specific primers for *TEB*