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Molecular characterization of a tomato polygalacturonase gene abundantly expressed in the upper third of pistils from opened and unopened flowers

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Abstract A polygalacturonase (PG) gene, TPG7 (Lycopersicon esculentum Mill., cv. Rutgers), has been cloned from tomato. RNA blot analysis reveals that TPG7 is highly expressed in pistils (ovary removed) from unopened and fully open flowers. Dissection of mature pistils demonstrated that TPG7 expression is limited to the top third (stigmatic region) of the pistils. This is contrasted with another tomato PG, TAPG4, which is also expressed in the same region of the pistil but only in mature pistils from fully open flowers. Hybridization of the TPG7 probe to anther RNA was nil to none and was barely detectable in RNA from leaf and flower abscission zones. The TPG7 polypeptide shares 39% sequence identity with the tomato fruit PG and between 63% and 73% sequence identities with six other tomato PGs.

Key words Pistil · Polygalacturonase · Tomato · Gene

Introduction

Growth and development in higher plants often requires change in the cell wall structure and cell adhesion properties. Pectin comprises approximately 30% of the cell wall and a much greater proportion of the adhesive middle lamella between cells. In addition to pectin's structural importance to the cell wall, it has been suggested that it creates a matrix which prevents other hydrolases and transglycosylases having access to the hemicellulosic fraction of the cell wall (Carpita and Gibeau 1993). The hemicellulosic fraction is the cement that binds together the cellulose microfibrils in the cell wall. Change in the cell wall structure during growth therefore requires that the pectin matrix be first partially hydrolyzed to allow other enzymes access to the hemicellulosic matrix. The greatest constituent of pectins is polygalacturonic acid (Carpita and Gibeau 1993). Polygalacturonases (PGs) have been identified and characterized in ripening fruit, abscission, dehiscence, pollen maturation and rapidly expanding tissues (Hadfield and Bennett 1998).

In addition to a role for PGs in cell separation and cell enlargement, the oligogalacturonides released by endoPGs may have a role in developmental signaling and the activation of defense responses (Coté and Hahn 1994). Bellincampi et al. (1996) and Altamura et al. (1998) have suggested that oligogalacturonides may play a regulatory role in several auxin responses affecting growth and development. Moreover, oligogalacturonides released from the plant cell wall elicit an increase in pathogen-related (PR) gene expression (Coté and Hahn 1994).

Materials and methods

Construction of a tomato genomic library

Tomato plants (Lycopersicon esculentum cv. Rutgers) were grown from seed under standard conditions in the greenhouse. Genomic DNA was isolated from young unexpanded leaves as described by Tai and Tanksley (1990). DNA was partially digested with Sau3A1 and size fractionated on a sucrose gradient (Ausubel et al. 1994). DNA fragments between 9 and 25 kbp were partially filled in using dGTP and dATP and ligated to XFix II arms (Stratagene, La Jolla, Calif., USA) that had been digested with XhoI and partially filled in using dTTP and dCTP. Recombi-
nant ADNA was packaged in vitro using a Gigapack Gold extract according to the manufacturer’s instructions (Stratagene). *Escherichia coli* XL1-Blue MRA (F') was infected with phage and approximately $4 \times 10^5$ plaque-forming units (pfu) were screened with a $^{32}$P-nick translated BamHI fragment from the pTAPG1 cDNA (Kalaitzis et al. 1995). Purification of positive clones, phage DNA preparation, restriction digestion and Southern blot hybridization were performed as described by Maniatis et al. (1982).

DNA sequencing and computer analysis

Restriction fragments of positive clones were subcloned into pT7/T3a18 (Gibco-BRL, Gaithersburg, Md., USA). To facilitate DNA sequencing, nested deletions of the subclones were prepared using exonuclease III and mung bean nuclease (Stratagene). DNA sequencing was performed by cycle sequencing using dye primer or dye terminator FS kit using the Applied Biosystem model 373 DNA sequencer (Perkin-Elmer Corp., Foster City, Calif., USA). The sequences were assembled and analyzed using the Genetics Computer Group (GCG) software package (Madison, Wis., USA).

RNA blot analysis

RNA was isolated as described in the protocol for the RNeasy Plant Mini Kit (Qiagen, Valencia, Calif., USA). Twenty micrograms of RNA were loaded per lane onto two identical gels, electrophoresed, blotted and each probed separately with $^{32}$P-labeled TPG7 and TAPG4 probe. Hybridization conditions were 42°C in 5× SSPE, 5× Denhardt’s solution, 0.1% SDS, 100 μg/ml salmon sperm DNA, and 60% formamide. Conditions used for the final wash of the blots were 0.2× SSC and 0.1% SDS at 55°C.

Results and discussion

Identification and characterization of the *TPG7* gene

Recently, we cloned and characterized six tomato genes that are clustered into two separate groups in the tomato genome (Hong and Tucker 1998). The same tomato genomic library used to identify these six genes was screened again at a lower stringency (42°C in 5× SSPE, 5× Denhardt’s solution, 0.1% SDS, 100 μg/ml salmon sperm DNA, and 20% formamide) with a TAPG1 probe (Hong and Tucker 1998). One genomic clone was selected for subcloning and sequencing. Sequence analysis revealed that the clone contained a PG gene. This gene was given the common name of *TPG7* and the formal name of Lyces;Pga1;8. The coding sequence for *TPG7* was identified by comparison with TAPG1, TAPG2, and TAPG4 cDNAs (Kalaitzis et al. 1995, 1997). *TPG7* consists of four exons interrupted by three introns of 68, 528, and 72 bp in length (Fig. 1A). The relative positions of the three introns are conserved between *TPG7* and the other tomato PGs (Hong and Tucker 1998).

The open reading frame of *TPG7* is 1194 bp long and encodes a polypeptide of 397 amino acids that includes a putative signal peptide cleaved after the alanine at position 26 (Von Heijne 1983). The number and positions of cysteine residues are highly conserved

**Fig. 1** *TPG7* gene structure (accession number AF072732) (A) and expression of *TPG7* transcript in different tissues (B, C). A The boxes indicate exons and the solid line the length of confirmed sequence. B Total RNA was isolated from pistils with ovaries removed from fully open flowers (mat. pistil), pericarp from green and red ripe fruit and flower pedicel abscission zones (FAZ) exposed to 25 μl/l ethylene for 24 h. C Total RNA was isolated from anthers, pistils (ovaries removed) and ovaries of nearly mature unopened flowers and fully open flowers. The pistils from open flowers were divided into stigmas (top third) and styles (bottom two thirds above the ovary) and ovaries. The same batch of RNA was used to create two identical blots and each probed separately with *TPG7* or TAPG4 probe. Photos of the ethidium-bromide-stained gels are included to show relative loading of RNA into gels.