



RESEARCH PAPER

# A $\beta$ -galactosidase gene is expressed during mature fruit abscission of 'Valencia' orange (*Citrus sinensis*)

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## Abstract

$\beta$ -galactosidases have been detected in a wide range of plants and are characterized by their ability to hydrolyse terminal non-reducing  $\beta$ -D-galactosyl residues from  $\beta$ -D-galactosides. These enzymes have been detected in a wide range of plant organs and tissues. In a search for differentially expressed genes during the abscission process in citrus, sequences encoding  $\beta$ -galactosidase were identified. Three cDNA fragments of a  $\beta$ -galactosidase gene were isolated from a cDNA subtraction library constructed from mature fruit abscission zones 48 h after the application of a mature fruit-specific abscission agent, 5-chloro-3-methyl-4-nitro-1H-pyrazole (CMN-pyrazole). Based on sequence information derived from these fragments, a full-length cDNA of 2847 nucleotides (GenBank accession number AY029198) encoding  $\beta$ -galactosidase was isolated from mature fruit abscission zones by 5'- and 3'-RACE approaches. The  $\beta$ -galactosidase cDNA encoded a protein of 737 amino acid residues with a calculated molecular weight of 82 kDa. The deduced protein was highly homologous to plant  $\beta$ -galactosidases expressed in fruit ripening. Southern blot analysis demonstrated that at least two closely related  $\beta$ -galactosidase genes were present in 'Valencia' orange. Temporal expression patterns in mature fruit abscission zones indicated  $\beta$ -galactosidase mRNA was detected 48 h after treatment of CMN-pyrazole and ethephon in mature fruit abscission zones.  $\beta$ -galactosidase transcripts were detected in leaf abscission zones only after ethephon application. The citrus  $\beta$ -galactosidase was expressed in stamens and petals of fully opened flowers and young fruitlets. The results suggest that this  $\beta$ -galactosidase may play a role during abscission as well

as early growth and development processes in flowers and fruitlets.

Key words: Abscission zone, cell wall hydrolase, 5-chloro-3-methyl-4-nitro-1H-pyrazole, ethephon, fruitlet, petal, stamen.

## Introduction

$\beta$ -galactosidase ( $\beta$ -gal, EC 3.2.1.23) enzyme activity is characterized by the ability to hydrolyse terminal non-reducing  $\beta$ -D-galactosyl residues from  $\beta$ -D-galactoside polymers (Smith *et al.*, 1998). Studies have shown that  $\beta$ -gal catalyses the hydrolysis of terminal galactosyl residues from carbohydrates, glycoproteins, and galactolipids (Ross *et al.*, 1994; Smith *et al.*, 1998, 2002). The enzyme may be involved in releasing stored energy for rapid growth, degrading cell wall components during senescence, and releasing free galactose during metabolic recycling of galactolipids, glycoproteins, and cell wall components.  $\beta$ -gal enzyme activity and gene expression have been measured in a wide range of plant tissues undergoing developmental changes such as seeds (Buckeridge and Reid, 1994; Sekimata *et al.*, 1989), seedlings (Esteban *et al.*, 2003; Li *et al.*, 2001), pollen (Gogers *et al.*, 2001; Rogers *et al.*, 2001), fruit (Ali *et al.*, 1995; Carey *et al.*, 1995; De Veau *et al.*, 1993; Kang *et al.*, 1994; Ross *et al.*, 1994; Smith and Gross, 2000; Smith *et al.*, 2002; Tateishi *et al.*, 2001, 2002; Trainotti *et al.*, 2001), and citrus juice vesicles and peel (Burns, 1990; Burns and Baldwin, 1994).

Molecular approaches have been used to study the role of  $\beta$ -gal in fruit development and ripening.  $\beta$ -gal gene expression increased during ripening in apple (Ross *et al.*, 1994) and was correlated with changes in fruit softening in Japanese pear (Tateishi *et al.*, 2001). Smith and Gross (2000) demonstrated that at least seven tomato  $\beta$ -gal

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genes were expressed during fruit development; six were expressed during fruit ripening. Down-regulation of a ripening-related  $\beta$ -gal cDNA reduced  $\beta$ -gal enzyme activity and free galactose content, and increased tomato fruit firmness (Smith *et al.*, 2002). In addition,  $\beta$ -gal gene expression can be induced by environmental factors such as wounding and water stress. O'Donoghue *et al.* (1998) showed that the expression of a  $\beta$ -gal gene isolated from *Asparagus officinalis* was temporally associated with the removal of the asparagus spear from the main body. Ethylene treatment of tomato fruit had different effects on  $\beta$ -gal genes (Moctezuma *et al.*, 2003). Ethylene positively regulated mRNA abundance of  $\beta$ -gal TBG4, but decreased mRNA accumulation of  $\beta$ -gal TBG5 and TBG6. Interestingly, down-regulation of TBG6 resulted in increased fruit cracking, suggesting a role for this gene in cell wall expansion and metabolism.

Despite the association of  $\beta$ -gal with developmental processes involving cell wall metabolism, its role in abscission has not been explored. Abscission is a physiologically and developmentally determined process during which multicellular plant organs such as leaves, flowers, and fruits separate from the main plant body. The process occurs in an anatomically distinct layer of cells known as the abscission zone (Brown, 1997; Patterson, 2001). During abscission, cell wall and middle lamella dissolution is thought to lead to organ separation. Cell wall hydrolytic enzyme activity and gene expression have been demonstrated during abscission (Brown, 1997; Patterson, 2001; Roberts *et al.*, 2002).  $\beta$ -1,4-endo-glucanase gene expression increased during the abscission of tomato flowers (Lashbrook *et al.*, 1994), flowers and leaves of pepper (Ferrarese *et al.*, 1995), and citrus leaves and fruit (Burns *et al.*, 1998). Gene expression of polygalacturonase was up-regulated during the shedding of tomato leaves and flowers (Kalaitzis *et al.*, 1997), fruit of peach (Bonghi *et al.*, 1992), and citrus (Burns and Lewandowski, 2000; Wu and Burns, 2000). In the present work, the isolation, characterization, and expression of a citrus  $\beta$ -gal gene during abscission in 'Valencia' orange are reported. As far as is known, this is the first report describing the up-regulation of  $\beta$ -gal gene expression during abscission.

## Materials and methods

### Plant materials and ethephon and CMN-P treatments

Fifteen-year-old *Citrus sinensis* (L.) Osbeck cv. Valencia trees grown at the University of Florida's Citrus Research and Education Center at Lake Alfred, Florida, USA, were used in this study. Young and mature leaves, flower buds, stamen, pistil, petal, calyx, whole small green fruitlets, peel tissue from green fruitlets, and peel from mature full-grown green, mature green turning yellow, and mature orange fruit were harvested, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until use. For ethephon and 5-chloro-3-methyl-4-nitro-1H-pyrazole (CMN-pyrazole) treatments, trees were sprayed with  $200\text{ mg l}^{-1}$  active ingredient and 0.125% Kinetic adjuvant, or adjuvant alone. Leaf blade, leaf abscission zones, young and mature fruit abscission zones, and mature fruit peel tissues from the upper shoulder portion

approximately 2 cm below the calyx abscission zone were harvested 0, 24, 48, and 72 h after CMN-pyrazole, ethephon, or adjuvant alone application. Tissues to determine the developmental expression of  $\beta$ -gal included: flower buds, stamen, pistil, petal, and calyx of fully opened flowers; fruitlets immediately after petal fall; peel tissues from fruitlets of 10, 35, and 50 mm in diameter; and fully grown green, green turning yellow (turning), and orange peel tissue from mature fruit. The harvested tissues were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for future use.

### DNA and total RNA extraction

Genomic DNA was extracted from frozen leaves by using Plant DNAzol Reagent (Life Technologies, Grand Island, NY) as described previously (Wu and Burns, 2003). Total RNA was extracted following the phenol/SDS protocol for plant RNA preparation (Ausubel *et al.*, 1989) with minor modifications. At least 1 g of frozen tissue was used for total RNA extraction. Samples were stored at  $-80^{\circ}\text{C}$  until needed.

### RT-PCR and rapid amplification of cDNA 5'-(5'-RACE) and 3'-ends (3'-RACE)

Two micrograms of total RNA extracted from mature fruit abscission zones 48 h after CMN-pyrazole application were used to synthesize first-strand cDNA using SuperScript II RNase H-Reverse Transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendation. Three cDNA clones, cDNA1-cDNA3, of 212 to 498 nucleotides (nt) in size were isolated from a mature 'Valencia' fruit abscission zone subtraction library described previously (Wu and Burns, 2003). Sequence comparison of these cDNA clones with other plant  $\beta$ -gal gene sequences indicated that these fragments belonged to the 3'-end portion of a  $\beta$ -gal gene(s). In order to confirm that the three clones belong to a single  $\beta$ -gal gene, two gene-specific primers GAL1 (5'-GCTCCAACGAAGGATTCTG-3') and GAL2 (5'-ATCCTTGCAACAACCTTACA-3') were designed to amplify a portion of the gene covering these three fragments. Based on the sequence of this PCR fragment, a third gene-specific primer GAL3 (5'-GCAAACCAAAGTGTTCCAACC-3') was used with a poly(T) primer ASBT (5'-CCTGGCCAGGGCCCCGTCGACG-GATCCV(T)<sub>18</sub>-3', where V is either G, C, or A), to amplify the 3'-most end of the  $\beta$ -gal cDNA. To clone the 5'-most portion of the gene, two steps were taken. First, two degenerate primers GAL4 (5'-AATGGVCATGARCCTTCTCC-3'; where V=G, A or C; R=A or G) and GAL5 (5'-CCATTCAAYTAYCCWAGAAGCAC-3'; where Y=C or T; W=A or T) were designed based on conserved cDNA sequence of  $\beta$ -gal genes from tomato (accession numbers L29451 and AF020390), papaya (accession number AF064786), mango (accession number AF004812), and apple (accession number L29451). Either degenerate primer was used with a downstream gene-specific primer GAL6 (5'-GAAATCCTTCGTTGGAGCCTA-3') to amplify the middle portion of the gene. To obtain the 5'-most end of the  $\beta$ -gal cDNA, two gene-specific primers GAL7 (5'-GAACATAAAGCCCTGCTTGC-3') and GAL8 (5'-CCTTGAG-TAGGCTCGTGTCC-3') were designed to the internal sequence of the newly-amplified gene fragment. These two primers were used to amplify the 5'-most ends of the  $\beta$ -gal cDNA by using the rapid amplification of cDNA ends (RACE) approach according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). PCR products were viewed on a 1% agarose gel stained with ethidium bromide, cleaned using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA), cloned and sequenced at the DNA sequencing core laboratory, University of Florida, Gainesville.

### DNA sequence analysis, alignment, and comparisons

Identification of nucleotide sequences from RT-PCR clones were established by using the NCBI BLAST program (Altschul *et al.*,

1997; <http://www.ncbi.nlm.nih.gov/BLAST>). Sequence alignment and comparison were made using the ClustalW program (<http://www.ebi.ac.uk/clustalw>). Open reading frame and protein prediction were made using NCBI ORF Finder (<http://www3.ncbi.nlm.nih.gov/gorf/gorf.html>). Signal peptides and their cleavage sites were predicted using PSORT (Nakai and Kanehisa, 1992; <http://psort.nibb.ac.jp/>) and SignalP programs (Nielsen *et al.*, 1997; <http://www.cbs.dtu.dk/services/SignalP/>). The theoretical isoelectric point (pI) and mass values for mature peptides were calculated using the PeptideMass program (<http://us.expasy.org/tools/peptide-mass.html>). The phylogenetic tree was generated based on the Neighbor-Joining method (Saitou and Nei, 1987).

#### *Southern blot analysis*

For Southern blot hybridization, 10 µg of genomic DNA were digested either with single restriction enzymes *Bam*HI, *Hind*III, or *Xba*I, or with double restriction enzymes *Bam*HI and *Hind*III, or *Bam*HI and *Xba*I at 37 °C for 5 h. Digested genomic DNA was separated in a 0.8% agarose gel at 23 V overnight, transferred overnight onto positively charged nylon membranes (Roche Biochemicals, Indianapolis, IN) with 10× SSC using the Rapid Downward Transfer system (Schleicher & Schuell, NH), and then fixed using a Stratalinker UV Crosslinker (Stratagene, La Jolla, CA). DIG-labelled gene-specific probes were synthesized by PCR using β-gal gene-specific primers. Blots were hybridized in PerfectHyb™ Plus Hybridization buffer (Sigma, St Louis, MO) at 65 °C for at least 2 h. Washing and detection were performed under high stringency conditions according to the DIG-high prime DNA labelling and detection starter kit II protocol (Roche Biochemicals, Indianapolis, IN) with the following modifications: TRIS-buffer solution (20 mM TRIS-HCl, pH 7.5; 150 mM NaCl) and non-fat dry milk powder (Carnation Milk Recipes, Young America, MN) were used instead of maleic acid solution and the block agent supplied by manufacturer.

#### *Northern blot analysis*

Total RNA (10 µg per lane) was separated in a 1.2% formaldehyde/MOPS [3-(*N*-morpholino)-propanesulphonic acid] denatured agarose gel and then blotted overnight onto a positively charged Zeta-Probe GT membrane (Bio-Rad Laboratories, Hercules, CA) with 10× SSC using the capillary Rapid Downward Transfer system (Schleicher & Schuell, NH). The RNA was fixed on the membrane using a Stratalinker UV Crosslinker (Stratagene, La Jolla, CA). The blot was prehybridized in PerfectHyb™ Plus Hybridization buffer (Sigma, St Louis, MO) at 65 °C for at least 2 h and then hybridized with <sup>32</sup>P dCTP-labelled probe at 65 °C overnight. DNA probes were generated from the middle portion of the β-gal gene fragment (271–1581 nt) by using a random priming labelling kit (Invitrogen, Carlsbad, CA). After hybridization, the membrane was washed twice with 2× SSC, 0.1% SDS and once with 0.5× SSC, 0.1% SDS at 65 °C. The blots were exposed to BioMax MS film (Eastman Kodak, NY) using a BioMax transcreen HE intensifying screen overnight at –80 °C.

## Results

### *Isolation and characterization of a citrus abscission β-galactosidase gene*

A full-length cDNA nucleotide sequence of 2847 nt in length was obtained based on the assembly of overlapping cDNA sequences of clones derived from the mature fruit abscission zone subtraction library, PCR and RACE. The nucleotide sequence of the full-length citrus β-gal gene

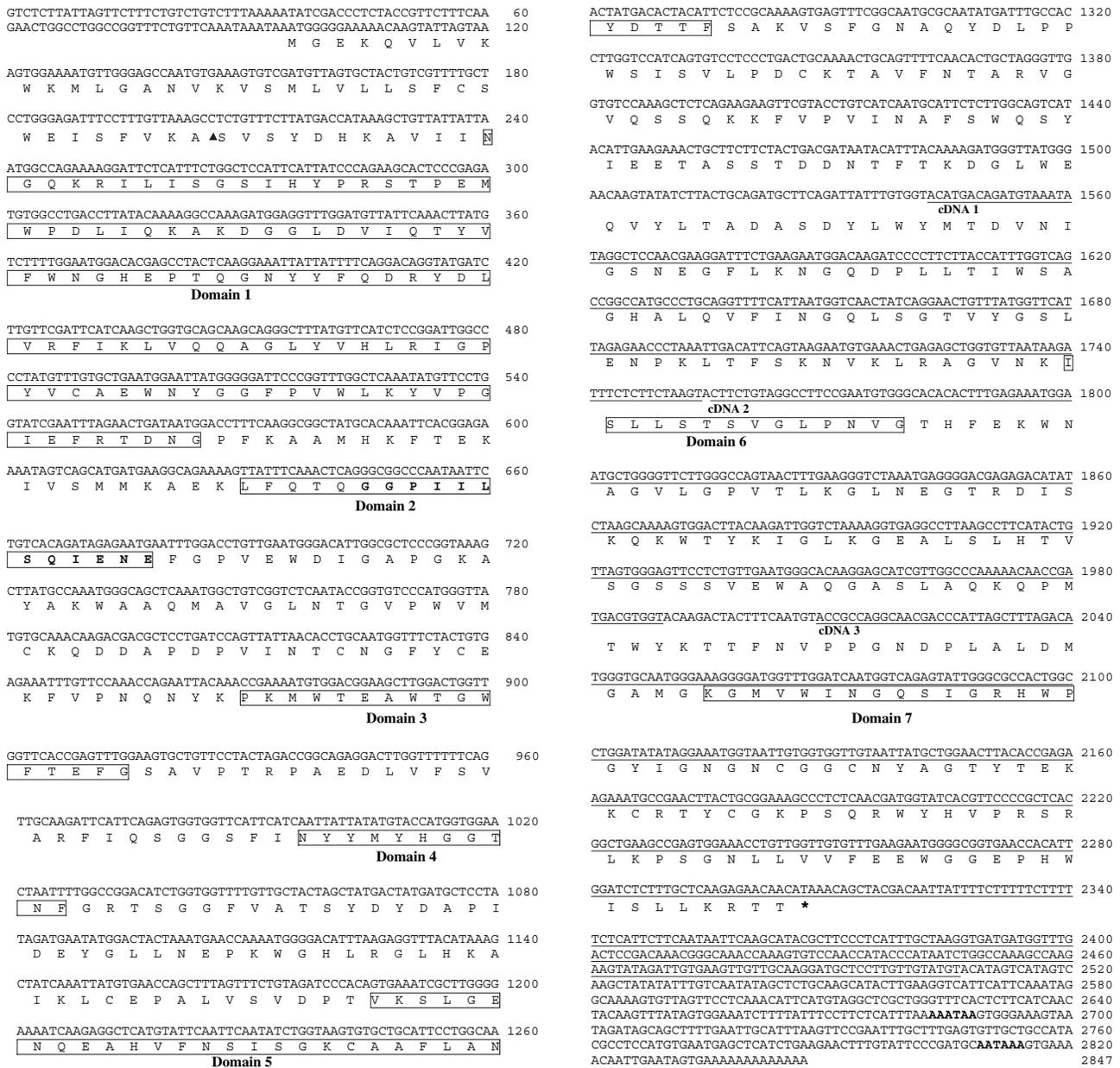
(accession number AY029198) and the encoded amino acid sequence are shown in Fig. 1. The cloned citrus β-gal gene contained a 5' untranslated region of 95 nt, a coding sequence of 2213 nt and a 3' untranslated sequence of 538 nt. Two putative polyadenylation signals and a poly(A) tail were present in the 3' untranslated region. The β-gal cDNA encoded a putative protein of 737 amino acid residues. Analysis of deduced amino acid sequence with the SignalP program (Nielsen *et al.*, 1997) predicted a eukaryotic signal peptide at the N-terminus with a cleavage site between residues 37 and 38. Cleavage of the signal peptide sequence would result in a mature polypeptide of 77.8 kDa with an isoelectric point of 8.4. The predicted target location of the citrus β-gal protein was to the plasma membrane. The encoded citrus β-gal protein contained the reported active site consensus sequence of glycosyl hydrolase family 35, G-G-P-[LIVM](2)-x-Q-x-E-N-E-[FY], where x is any amino acid and [LIVM] and [FY] are conserved substitutions (Fig. 1; Henrissat, 1998). Seven homologous domains typical of eukaryotic β-gals were also identified (Fig. 1; Taron *et al.*, 1995). The deduced amino acid sequence of citrus β-gal cDNA was aligned with 23 additional plant β-gals and a phylogenetic tree was constructed using the Neighbor-Joining method (Fig. 2). Three major groups were distinguished within the tree. The citrus β-gal was closely related to those isolated from apple, Japanese pear, and strawberry fruit and grouped with plant β-gals within group I. Both groups I and II were expressed in fruit tissues. Group III β-gals were isolated and expressed in diverse plant tissues such as seedlings, flowers, and fruit.

#### *Southern blot analysis*

The genomic organization of citrus β-gal was investigated using Southern blot hybridization under high stringency. A cDNA fragment covering the 5' half of the open reading frame (nt 271–1581) was used as a probe. The citrus β-gal gene probe hybridized to two fragments of DNA digested with either *Bam*HI or *Xba*I, but four fragments of DNA digested with *Hind*III (Fig. 3). Two internal digestion sites of restriction enzyme *Hind*III were present in the citrus β-gal gene at nt positions 718 and 886, respectively. No restriction sites of enzymes *Bam*HI and *Xba*I were present in the cloned citrus β-galactosidase gene. Thus, at least two closely related citrus β-gal genes were present in the 'Valencia' orange genome.

#### *Expression of the citrus β-galactosidase gene*

Northern blot hybridization demonstrated that β-gal gene expression was induced in mature fruit abscission zones 48 h and 72 h after application of ethephon or CMN-pyrazole (Fig. 4A). Ethephon increased β-gal gene expression in leaf abscission zones 24 h after application, but expression was greatly reduced afterward. Application of ethephon or CMN-pyrazole had little effect on citrus β-gal gene



**Fig. 1.** Nucleotide and predicted amino acid sequences of the citrus  $\beta$ -gal gene. The predicted eukaryotic signal sequence is indicated by an arrowhead. The translation stop site is indicated by an asterisk. Nucleotides in bold, **AAATAA** and **AATAAA**, show the putative polyadenylation signal sequences. The seven amino acid homologous domains typical of eukaryotic  $\beta$ -gals are shown (Domains 1–7). Amino acid residues shown in bold indicate the putative active site of glycosyl hydrolase family 35 in domain 2. Three initial cDNA fragments isolated from mature fruit abscission subtraction library are underlined.

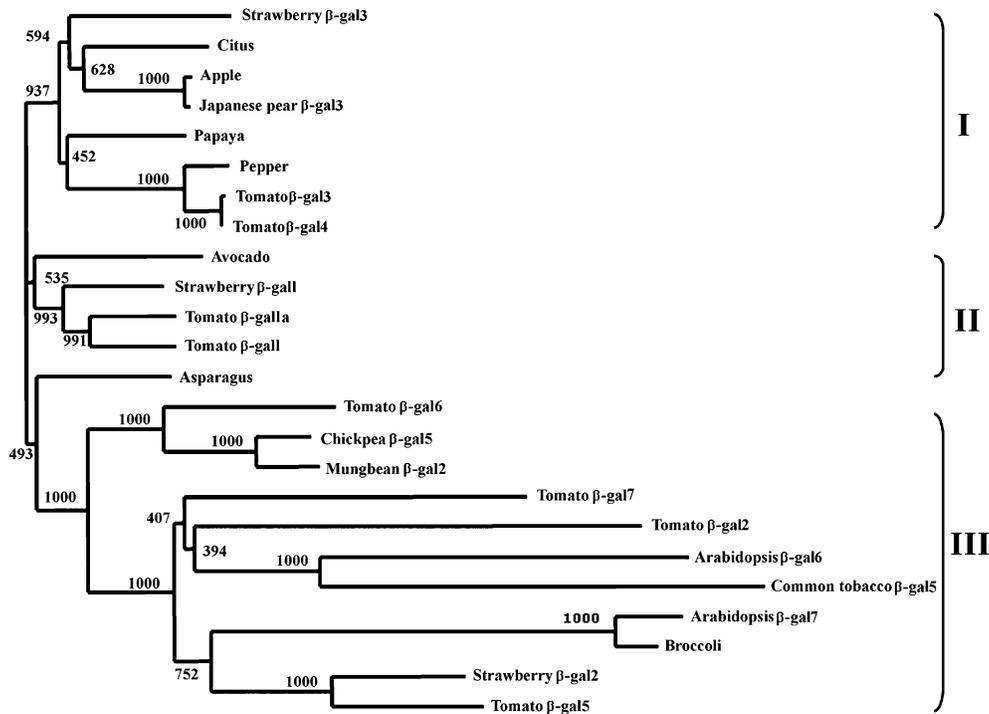
expression in the leaf blade, young fruit abscission zones, and mature fruit peel tissues. There was little or no hybridization detected from the tissues examined in adjuvant-treated controls.

Various tissues were sampled from different developmental stages of ‘Valencia’ orange. Expression of  $\beta$ -gal transcripts was detected only in flower stamens and petals from fully expanded flowers, and green peel tissues of small fruitlets approximately 10 mm in diameter (Fig. 4B). However, gene expression was not detected in flower buds,

pistils, calyx, and whole fruitlets obtained immediately after petal fall, green peel tissues of fruit greater than 35 mm in diameter, and mature orange fruit peel.

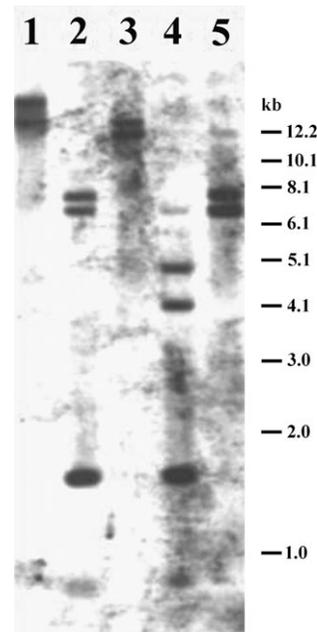
**Discussion**

Plant  $\beta$ -gals are enzymes that catalyse the cleavage of terminal  $\beta$ -galactosidic residues from various polymers of carbohydrate, glycoprotein, and galactolipids. Their role in

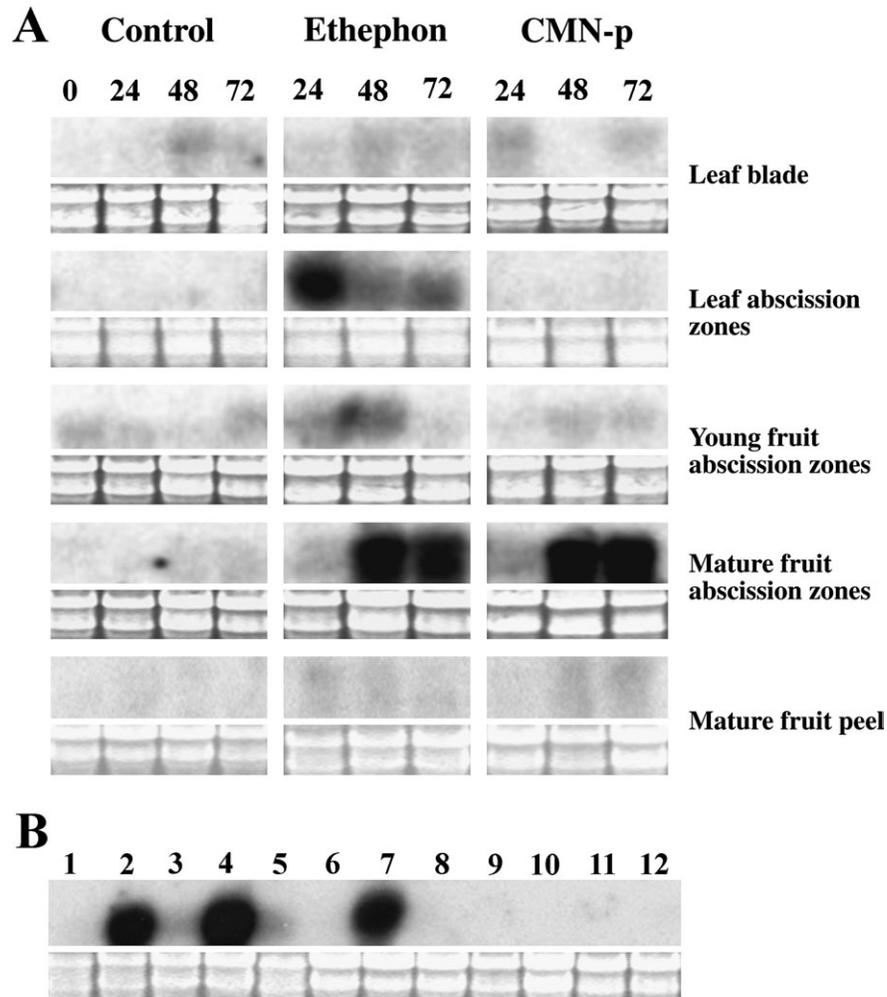


**Fig. 2.** Phylogenetic analysis of citrus  $\beta$ -gal with 23 other plant  $\beta$ -galactosidases. Phylogenetic groups (I–III) are indicated. The bootstrap values on nodes indicate the number of times that each grouping occurred with 1000 replicates. The plant  $\beta$ -galactosidase sequences used to generate the phylogenetic tree and their GenBank accession numbers are as follows: apple (L29451), *Arabidopsis* ( $\beta$ -gal6, AJ270302;  $\beta$ -gal7, AJ270303), asparagus (X77319), avocado (AB061017), broccoli (X84684), pepper (AY029226), chickpea ( $\beta$ -gal5, AJ012687), common tobacco ( $\beta$ -gal5, AJ250431), strawberry ( $\beta$ -gal1, AJ278703;  $\beta$ -gal2, AJ278704;  $\beta$ -gal3, AJ278705), mungbean ( $\beta$ -gal2, AF229795), papaya (AF064786), Japanese pear ( $\beta$ -gal3, AB046543), and tomato ( $\beta$ -gal1a, AJ012796;  $\beta$ -gal1, X83854;  $\beta$ -gal2, AF154420;  $\beta$ -gal3, AJ012798;  $\beta$ -gal4, AF020390;  $\beta$ -gal5, AF154423;  $\beta$ -gal6, AF154424;  $\beta$ -gal7, AF154422).

cell wall metabolism has been extensively studied during ripening of tomato, where increased  $\beta$ -gal gene expression was associated with increased enzyme activity, release of free galactose, and fruit softening (Carey *et al.*, 1995; Kim *et al.*, 1991; Smith *et al.*, 1998, 2002). The shedding of plant organs during abscission involves the breakdown and metabolism of cell walls. The digestion of cell walls and middle lamella in the abscission zone is correlated with increased gene expression and enzyme activities of cell wall hydrolases such as  $\beta$ -1,4-glucanase and polygalacturonase as abscission progresses (Roberts *et al.*, 2002). In this work, it has been demonstrated that  $\beta$ -gal gene expression increased during citrus abscission. The application of the mature fruit-specific abscission agent CMN-pyrazole to the entire canopy of the tree resulted in increased  $\beta$ -gal gene expression only in mature fruit abscission zones. By contrast, increased  $\beta$ -gal expression occurred in both fruit and leaf abscission zones when the tree was sprayed with ethephon, an agent that releases ethylene upon uptake into plant tissue (Cooke and Randall, 1968) and promotes abscission in both mature fruit and leaves of citrus (Burns, 2002).  $\beta$ -gal gene expression was not induced in fruit peel or leaf blades by either abscission compound, indicating that expression was associated with the abscission process and not with ethylene (ethephon) or CMN-pyrazole treatment alone.



**Fig. 3.** Southern blot analysis of citrus  $\beta$ -gal. Ten  $\mu$ g of genomic DNA were digested with restriction enzymes *Bam*HI (lane 1), *Hind*III (lane 2), *Xba*I (lane 3), *Bam*HI and *Hind*III (lane 4), or *Bam*HI and *Xba*I (lane 5). Molecular size markers are shown on the right.



**Fig. 4.** Northern blot analysis of citrus  $\beta$ -gal mRNA expression in ethephon- or CMN-pyrazole-treated tissues (A) and in different 'Valencia' orange tissues (B). Total RNA (10  $\mu$ g) was loaded in each lane. The top section shows the northern blot hybridization, and the bottom section shows the ethidium bromide-stained rRNA bands. (A) Hybridization to total RNAs isolated from leaf blade, leaf abscission zones, young fruit abscission zones, mature fruit abscission zones, and mature fruit peel tissues from trees treated with 0.125% adjuvant alone (control), 200 mg l<sup>-1</sup> ethephon and 0.125% adjuvant, 200 mg l<sup>-1</sup> CMN-pyrazole and 0.125% adjuvant at times indicated. (B) Hybridization to total RNAs extracted from flower buds (1), stamens (2), pistils (3), petals (4), calyx (5), fruitlets immediately after petal fall (6), peel tissues of fruitlets 10 mm (7), 35 mm (8), and 50 mm (9) in size, and peel tissue of fully grown green fruit (10), turning fruit (11), and orange fruit (12) tissues.

Citrus  $\beta$ -gal shared significant sequence similarity with  $\beta$ -gals from other plants. Phylogenetic analysis indicated that citrus  $\beta$ -gal was grouped with encoded proteins of genes expressed during fruit development and ripening. The full-length citrus  $\beta$ -gal gene encoded a protein of 737 amino acid residues and a molecular mass of 82 kDa. After removal of the eukaryotic signal sequence, the mature peptide encoded a protein of 77.8 kDa. The putative active site associated with glycosyl hydrolase family 35 was identified, as well as the seven homologous domains present in eukaryotic  $\beta$ -gals (Henrissat, 1998; Taron *et al.*, 1995).

The role of  $\beta$ -gal in the abscission process has not been fully examined here; however, its temporal expression pattern in the abscission zone during mature citrus fruit abscission was similar to that of  $\beta$ -1,4-glucanase, polyga-

lacturonase, and pectinesterase (Burns *et al.*, 1998; Nairn *et al.*, 1998; Wu and Burns, 2000). Further, the timing of significant mature fruit loosening between 24 h and 48 h after the application of CMN-pyrazole and ethephon (Burns, 2002) was correlated with the appearance of these cell wall hydrolase transcripts. This suggests that a coordinated effort of  $\beta$ -gal and other cell wall hydrolases in cell wall metabolism and breakdown leads to organ separation. The predicted cellular targeting of the mature citrus  $\beta$ -gal protein was to the plasma membrane, supporting its possible involvement in the alteration of galactosyl-containing polymers at or near the cell wall.

Examination of  $\beta$ -gal gene expression in other 'Valencia' orange tissues demonstrated that this gene was developmentally regulated in certain reproductive structures. No expression was detected in unopened flower buds. However,

at anthesis, marked  $\beta$ -gal expression occurred in stamens and petals, but not in pistils or calyx. Immediately after petal fall, no expression was detected in whole fruitlets. As the fruitlets grew to approximately 10 mm in diameter, however, marked expression was again measured in the fruitlet peel. Beyond this developmental stage, expression in the peel fell to undetectable levels. Interestingly,  $\beta$ -gal enzyme activity was high in immature grapefruit peel, but fell to lower levels as development progressed (Burns and Baldwin, 1994). Expression of  $\beta$ -gal was detected in whole flowers of tomato and strawberry (Smith and Gross, 2000; Trainotti *et al.*, 2001), carnation petals (Raghothama *et al.*, 1991), and tobacco anthers (Rogers *et al.*, 2001). Although Southern blot hybridization indicated at least two closely related  $\beta$ -gal genes were present in the 'Valencia' orange genome, there are likely to be additional  $\beta$ -gal genes with differential or unique expression patterns involved in various aspects of growth and development, and perhaps with abscission as well. Seven  $\beta$ -gal genes were identified in tomato, and only one had significant expression in flowers (Smith and Gross, 2000). The striking expression of  $\beta$ -gal in petals, stamens, and young fruitlet peel may reflect the need for metabolism of galactose-related polymers or mobilization of galactose-related nutrient reserves from carbohydrate, galactoprotein or galactolipid polymers in these tissues (Bhalla and Dalling 1984; Bonnin *et al.*, 1995; Buckeridge and Reid, 1994; Maley *et al.*, 1989).

In conclusion, a citrus  $\beta$ -gal gene has been isolated and characterized from a mature fruit abscission zone subtraction library that is markedly expressed in abscission zones during the abscission of mature leaves and fruit, in floral stamens and petals, and peel of young developing fruitlets. The assignment of a precise role for this  $\beta$ -gal gene in abscission and floral and young fruitlet development will require careful analysis of spatial and temporal enzyme activity and substrate specificity of the encoded product.

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