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ORIGINAL PAPER

Four glycoside hydrolases are differentially modulated by auxins, cytokinins, abscisic acid and gibberellic acid in apple fruit callus cultures

Paula Alayón-Luaces · Eduardo A. Pagano · Luis A. Mroginski · Gabriel O. Sozzi

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Abstract α-L-Arabinofuranosidase, α- and β-D-xylosidase, and β-D-glucosidase activity was detected in the soluble fraction (S-F) extracted with water and in the NaCl-released fraction (NaCl-F) extracted with a high-salt concentration buffer from apple callus cultures. The activity was found to be differentially modulated by the addition of various plant growth regulators (PGRs) to calluses that had lost their requirement for specific PGRs ("habituation" phenomenon). α-L-Arabinofuranosidase activity was 93%, 130%, 126% and 186% higher in the NaCl-F from IAA-, IBA-, ABA- and GA_3-treated callus than in that extracted from untreated callus while S-F α-L-arabinofuranosidase activity was only 71%, 24%, 55% and 66% higher, respectively. α-D-Xylosidase displayed low activity levels in both S-F and NaCl-F but 2iP-treated callus showed higher α-D-xylosidase activity in both fractions than the control. 2,4-D increased α-D-xylosidase activity by 110% in the NaCl-F but decreased it by 40% in the S-F. β-D-Xylosidase activity increased by 99% in S-F from 2iP-treated callus but slightly decreased in the NaCl-F. In GA_3-treated callus, NaCl-F β-D-xylosidase activity increased by 188%. S-F and NaCl-F from Picloram-treated callus showed undetectable or only slightly noticeable α-L-arabinofuranosidase, α-D-xylosidase and β-D-xylosidase activity. Interestingly, β-D-glucosidase activity rose 28-fold in the S-F extracted from Picloram-treated callus. β-D-glucosidase was the only enzyme assayed that greatly increased its NaCl-F activity after 10 subcultures, and the addition of any PGR to the callus culture –except for Picloram and ABA– decreased its activity, suggesting that this enzyme may be associated with certain stress conditions, such as PGR starvation or Picloram addition. This is the first report on glycoside hydrolases from fruit callus as modulated by different PGRs.

Keywords *Malus* × *domestica* · Glycosidases · α-L-Arabinofuranosidase · α- and β-D-Xylosidase · β-D-Glucosidase · Plant growth regulators

Abbreviations

ABA	Abscisic acid
α-Araf	α-L-Arabinofuranosidase
BAP	6-Benzylaminopurine
2,4-D	2,4-Dichlorophenoxyacetic acid
GA_3	Gibberellic acid
β-Glc	β-D-Glucosidase

P. Alayón-Luaces · L. A. Mroginski
Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Sargento Juan B. Cabral 2131, W3402BKG Corrientes, Provincia de Corrientes, Argentina

E. A. Pagano · G. O. Sozzi (✉)
Facultad de Agronomía, Universidad de Buenos Aires, Avenida San Martín 4453, C1417DSE Buenos Aires, Argentina
e-mail: gsozzi@agro.uba.ar

L. A. Mroginski · G. O. Sozzi
CONICET, Avenida Bernardino Rivadavia 1917, C1033AAJ Buenos Aires, Argentina

IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
2iP	N^6-(2-Isopentenyl)adenine
KIN	Kinetin
MS	Murashige and Skoog (1962) medium
NAA	1-Naphthaleneacetic acid
NaCl-F	Sodium chloride-released fraction
PGR	Plant growth regulator
PIC	4-Amino-3,5,6-trichloro-pyridine-2-carboxylic acid (Picloram)
PVPP	Polyvinylpolypyrrolidone
S-F	Soluble fraction
TDZ	Thidiazuron
α-Xyl	α-D-Xylosidase
β-Xyl	β-D-Xylosidase
ZEA	Zeatin

Introduction

Glycoside hydrolases (EC 3.2.1.) are a very large and widespread group of enzymes (Davies et al. 2005) which hydrolyse the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety. Although considerable advances have been made in unravelling the multiple functions of plant glycoside hydrolases, many other aspects are still unknown. Several glycoside hydrolases are bound to the cell wall where they may potentially trim terminal non-reducing glycosyl residues off various pectic and hemicellulosic homo- and heteropolysaccharides as well as different glycoconjugates (Rose et al. 2003; Sozzi 2004). Although these protein families may be vital for cell-wall remodelling, energy metabolism, signalling and defence during growth, development and stress responses (Farrokhi et al. 2006), the structural complexity of cell-wall polysaccharides makes it difficult to determine the specific sites at which these enzymes may cause functionally significant cell-wall changes. In addition, some glycoside hydrolases seem to be multifunctional enzymes. Conversely, other glycoside hydrolases are not bound to the cell wall, and may recognize moieties located in different molecules though their exact substrates and functions remain undefined.

Much of the existing research on glycoside hydrolase regulation has been performed on microorganisms, as they have potential industrial value in the bioconversion of hemicelluloses and pectins. Yet, limited progress has been made in identifying plant growth regulators (PGRs) that modulate glycoside hydrolase activity in plant tissues since different problems have hindered research in this area. Studies with intact plant organs require vacuum infiltrations, sprays or dips of the organ in different PGR solutions. Therefore, entry and distribution of the PGRs are uncertain due to surface diffusion barriers and the treatment of each organ differs depending on its anatomy and morphology. For example, dipping fruits in concentrated PGR solutions can induce stress responses (Sozzi et al. 2000; Vendrell 1988). Another possible approach to test plant tissue responsiveness to physiological levels of different PGRs is the use of excised organs. The slicing of wild-type climacteric fruits causes wound-induced ethylene by excised discs but Sozzi et al. (2002) demonstrated the utility of a pericarp disc system from antisense ACC synthase fruit that excludes the interactions between applied PGRs and excision-stress ethylene biosynthesis.

Calyx and fruit cultures (Cohen 1996; Ishida et al. 1993) as well as callus cultures (Liu et al. 2006) have also been used to characterize different aspects of fruit metabolism. While a callus explant represents an artificial biological system, it provides a convenient easy-to-handle source of plant material that can be rapidly regenerated, and from which cell-wall enzymes may be obtained without difficulty. Also, callus cultures may allow comprehensive studies involving the addition of physiological levels of PGRs or other metabolites to the culture medium. However, to the best of our knowledge, there are no previous reports on the presence of glycoside hydrolases in apple fruit callus or on the modulation of their activity by different PGRs. The objective of this work was to characterize the activity of four glycoside hydrolases: α-L-arabinofuranosidase (α-Araf; EC 3.2.1.55), α-D-xylosidase (α-Xyl; EC 3.2.1.-), β-D-xylosidase (β-Xyl; EC 3.2.1.37) and β-D-glucosidase (β-Glc; EC 3.2.1.21) from apple callus, as affected by the addition of different PGRs.

Materials and methods

Origin of explants

Each explant consisted of a 5-mm apple-tissue cube obtained from the enlarged fleshy receptacle of a

75-day immature apple (*Malus* × *domestica* Borkh. 'Anna') grown in an orchard in Corrientes, Argentina.

Culture initiation

The explants were surface sterilized by soaking for 1 min in 70% ethanol followed by immersion for 30 min in a solution of commercial bleach (containing 1.8% NaOCl, final concentration) and two drops of Tween®, and finally rinsed four times with sterile distilled water. Primary callus explants were placed individually in 11-ml glass tubes containing 3 ml of basal Murashige and Skoog (1962) medium (MS medium) supplemented with 1 mg l^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1 mg l^{-1} of 6-benzylaminopurine (BAP) as PGRs, and 3% sucrose as the energy source. The pH of each medium was adjusted to 5.8 with either KOH or HCl prior to the addition of 0.65% agar (Sigma, A-1296). Tubes were covered with aluminium foil and autoclaved at 0.101 MPa for 20 min.

Physical culture conditions

The tubes were sealed with Resinite AF50® (Casco S.A.C. Company, Buenos Aires) and incubated at 27 ± 2°C in darkness. The callus showed neither morphogenesis nor organogenesis in that culture system.

To reduce any hormonal effect to a minimum, calluses were subcultured once (MS-1) or ten times (MS-10) at 30-days intervals onto a fresh basal regeneration medium (Murashige and Skoog 1962) supplemented with 3% sucrose as the energy source but with no PGR. Callus cultures can continue to grow in the basal medium with no PGRs due to the so-called "habituation" phenomenon. The term "habituation" has been given to all inheritable changes in the nutrient requirements (PGRs in particular) arising in cultured cell systems. Hence, when a normal culture or isolate loses its original requirement for auxins or cytokinins, it becomes "auxin- or cytokinin-habituated" (Hartmann et al. 1997). In some cases, a culture becomes habituated to more than one compound (Butcher 1973). This was the case with apple fruit tissue cultures that lose their requirement for 2,4-D and BAP and continue to proliferate even after the culture has been transferred to a medium without any PGR.

After ten subcultures, each PGR was added to the basal medium at a concentration of 5 mg l^{-1}. The PGRs tested were the following: indole-3-acetic acid (IAA); two synthetic auxins [1-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA)]; two auxinic herbicides [2,4-D and 4-amino-3,5,6-trichloro-pyridine-2-carboxylic acid (Picloram; PIC)]; three natural cytokinins [kinetin (KIN), zeatin (ZEA) and N^6-(2-isopentenyl)adenine (2iP)]; two synthetic cytokinins [BAP and thidiazuron (TDZ)]; abscisic acid (ABA) and gibberellic acid (GA_3). Three independent replicates per PGR treatment were prepared. After 30 d, actively growing calluses were harvested, frozen in liquid nitrogen and stored at −70°C until used. All chemicals were from Sigma Chemical Co. (St. Louis, MO, USA).

α-L-Arabinofuranosidase, α- and β-D-xylosidase, and β-D-glucosidase activity

Glycoside hydrolases were extracted from 0.5 g of callus samples. Callus tissue was ground with 1 ml of cold sterile distilled water containing 1.5% (w/v) polyvinylpolypyrrolidone (PVPP). The suspension obtained was homogenised, centrifuged at 10,000*g* for 5 min at 4°C and the supernatant was called the soluble fraction (S-F). The procedure was repeated three times and each supernatant was checked for enzyme activity. The precipitate was then resuspended in 1 ml of 0.1 M sodium acetate, pH 5.5, containing 1.4 M NaCl . After 30 min at 4°C, the suspension was centrifuged at 10,000*g* for 15 min at 4°C, and the supernatant was called NaCl-released fraction (NaCl-F).

α-Araf, α-Xyl, β-Xyl and β-Glc activity was measured using the corresponding *p*-nitrophenyl derivatives as substrates, namely: *p*-nitrophenyl-α-L-arabinofuranoside, *p*-nitrophenyl-α-D-xylopyranoside, *p*-nitrophenyl-β-D-xylopyranoside and *p*-nitrophenyl-β-D-glucopyranoside, respectively. The reaction mixture consisted of 125 µl of 100 mM citrate buffer, pH 4.5, 100 µl of 0.1% (w/v) bovine serum albumin (BSA), 100 µl of enzyme solution (or an appropriate dilution) and 100 µl of 13 mM substrate solution. After 1 h at 37°C, the reaction was stopped by addition of 425 µl of 0.13 M Na_2CO_3. Blanks (0-time) were prepared by adding Na_2CO_3 prior to the addition of substrate. Absorbance was measured at 400 nm. Free *p*-nitrophenol was used as standard. One unit of enzyme activity was defined as the amount of enzyme

that catalyses the liberation of 1 μg *p*-nitrophenol min^{-1} mg^{-1} protein. Enzyme activity calculations were based on enzyme dilutions that gave a linear increase in free *p*-nitrophenol over a 2-h incubation period. Three independent replicates per PGR treatment were measured.

Protein concentration was assayed by the Coomasie Blue G dye-binding method using the Bio Rad reagent (Bio Rad, Richmond, CA, USA) and BSA as standard.

Statistical analysis

Three independent replicates per PGR treatment were prepared and measured for each glycosidase activity and results were shown as the mean ± SE of those three replicates. In addition, statistical significance was determined by one-way ANOVA using the PC-SAS software package (SAS Institute Inc., Cary, NC). The model assumptions of homogeneity of variance and normality were probed using Levene's test and Shapiro-Wilk's test, respectively. When these assumptions were not satisfied, data were transformed into ranks (Conover and Iman 1981) for further analysis. When a significant *F*-value was found, treatment means were compared using the Ryan-Einot-Gabriel-Welsch's test ($P < 0.05$). This test controls the type I experimental error rate and has generally a lower type II error rate than the Tukey's studentized range test (Westfall et al. 1999).

Results and discussion

α-Araf, β-Xyl and β-Glc activities were mainly detected in the NaCl-F (Figs. 1a and 2). Most papers describe the separation of "cell-wall proteins" by tissue homogenisation in a low-ionic strength buffer or water (S-F) followed by successive washing of the cell-wall pellet with the same buffer to remove cytosolic proteins previous to extraction with a high-salt concentration buffer (NaCl-F) (Rose et al. 2003). Nevertheless, some positively-charged proteins could ionically bind to the cell walls once the plasma membrane has been disrupted because the polygalacturonate of cell-wall pectins can act as a polyanionic matrix. In this work, it is assumed that most of the activity obtained from homogenisation with strong saline buffer belongs to "cell-wall proteins" though

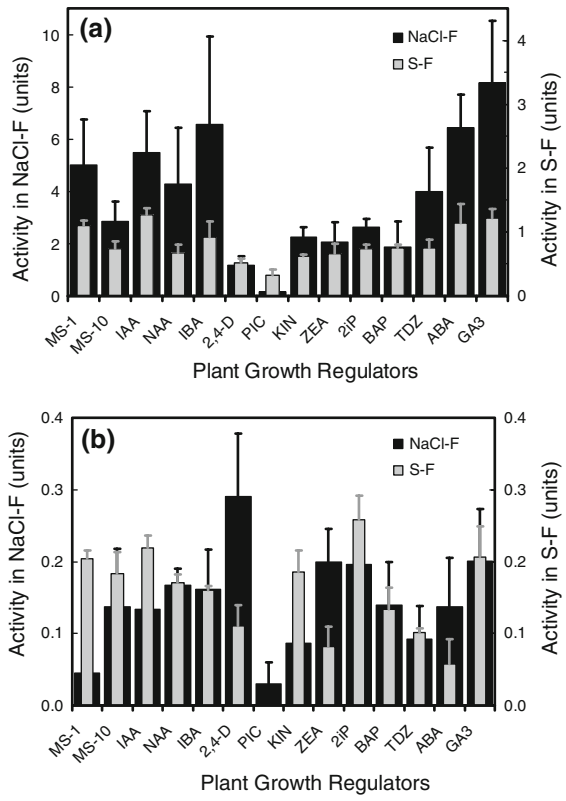

Fig. 1 α-L-Arabinofuranosidase (**a**) and α-D-xylosidase (**b**) activity in the soluble (S-F) and the NaCl-released fraction (NaCl-F) from apple callus treated or not with different plant growth regulators. Each value represents the mean ± SE of three replicates

the final classification of these glycosidases depends on the verification of their subcellular localization by methods such as immunolocalization.

Callus subcultured ten times (MS-10) showed a decrease in NaCl-F α-Araf activity (Fig. 1a). The addition of IAA, synthetic auxins, ABA or GA_3 to the culture medium significantly increased NaCl-F α-Araf activity, while PIC and, to a lesser extent, 2,4-D reduced the activity (Fig. 1a). NaCl-F α-Araf activity from GA_3-treated callus was ~5135% higher than that from PIC-treated callus. S-F α-Araf activity from IAA-, ABA- or GA_3-treated callus was also greater than that from callus supplied with PIC (Fig. 1a). Itai et al. (2003) suggested that the *LeARF1* gene from tomato fruit is negatively regulated by ethylene while Sozzi et al. (2002) found three α-Araf activities differentially modulated by synthetic auxins, gibberellic acid or ethylene, supporting the idea that total α-Araf activity in tomato results from an

enzyme family whose members are responsive to different endogenous controls during growth and ripening. α-Araf activity increases in ripening apples (Dick et al. 1990; Goulao et al. 2007; Yoshioka et al. 1995) while arabinose content decreases during the overripening stage (Peña and Carpita 2004). Nevertheless, α-Araf is present not only during fruit ripening but also during the early stages of fruit development, both in apple (Goulao et al. 2007) and in Japanese pear, another pome fruit (Tateishi et al. 1996). Interestingly, the α-Araf activity profile is consistent with the pattern of expression of *MdAF1* (Goulao et al. 2008) which displays high identity to *PpARF2* clone from Japanese pear (Tateishi et al. 2005). In callus, α-Araf activity may be associated with the occurrence of arabinosyl residues in pectic side chains influencing cell-to-cell adhesion (Iwai et al. 2001).

In apple, almost no studies on α-Xyl have been undertaken. In our experiments, S-F α-Xyl activity was neither affected by successive callus subcultures (MS-10 *vs*. MS-1 activity; Fig. 1b) nor by addition of most of the PGRs (IAA, synthetic auxins, KIN, BAP, and GA_3). S-F α-Xyl activity decreased in 2,4-D-, ZEA-, TDZ- and ABA-treated callus, and was completely inhibited by PIC. On the other hand, NaCl-F α-Xyl activity was greatly enhanced by callus subculturing (MS-10 *vs*. MS-1 activity; Fig. 1b), and only PIC restored low activity levels similar to those from MS-1 callus. NaCl-F α-Xyl activity increased 5.4-fold upon treatment with 2,4-D, in comparison with that from MS-1 callus. Interestingly, α-Xyl from 2,4-D-treated pea epicotyls was found to specifically cleave one of the α-xylosidic linkages of xyloglucan-oligosaccharide substrates (O'Neill et al. 1989). Also, α-Xyl role could be critical in that oligosaccharides lacking the xylosyl residue at the non-reducing terminus are probably unable to act as acceptors for xyloglucan endotransglycosylase (EC 2.4.1.207; Lorences and Fry 1993). Thus, α-Xyl activity could diminish the presence of oligosaccharides vacant for transglycosylation during callus development.

The occurrence of β-Xyl activity has been verified in a range of fruit species (Sozzi 2004) but was not detected in apple using 4-methylumbelliferyl-β-D-xyloside as substrate (Dick et al. 1990). In fact, β-Xyl activity against *p*-nitrophenyl-β-D-xyloside was lower than α-Araf and β-Glc in MS-1 callus (Figs. 1 and 2). Subculturing in the absence of PGRs did not affect S-F or NaCl-F β-Xyl activity levels (MS-10 *vs*. MS-1;

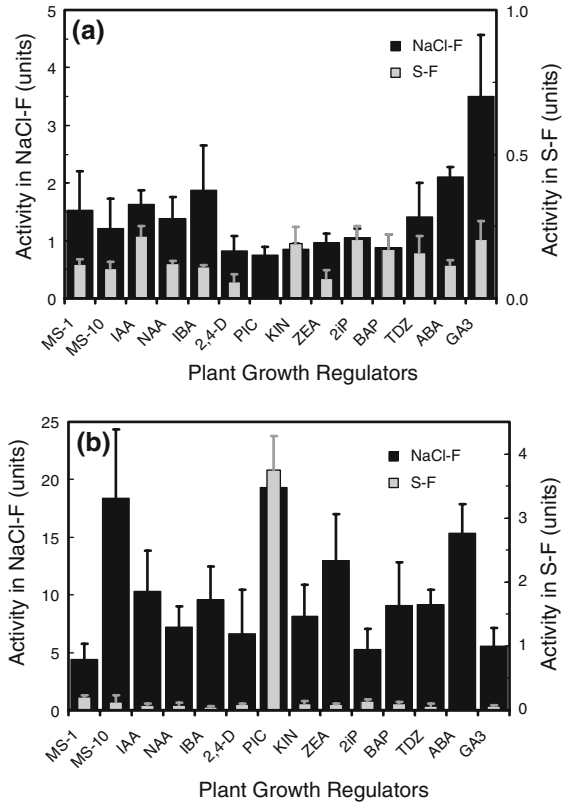

Fig. 2 β-D-Xylosidase (**a**) and β-D-glucosidase (**b**) activity in the soluble (S-F) and the NaCl-released fraction (NaCl-F) from apple callus treated or not with different plant growth regulators. Each value represents the mean ± SE of three replicates

Fig. 2a). Most of the PGRs assayed did not affect S-F β-Xyl activity. 2iP- and IAA-treated callus displayed higher S-F β-Xyl activity than 2,4-D- and ZEA-treated callus while activity was not noticeable upon treatment with PIC (Fig. 2a). In contrast, NaCl-F β-Xyl activity from GA_3-treated callus increased ~188% while that from auxinic herbicide- or natural cytokinin-treated callus decreased 30 to 40% in comparison with that from MS-10 (Fig. 2a). The *PpAz152* transcript, which revealed high homology with plant β-Xyl, was found to be readily induced in peach by fruit ripening, leaf senescence and wounding but was also detected in early stages of fruit development and in flowers at all developmental stages (Ruperti et al. 2002). This transcript was not affected by the inhibition of ethylene perception. On the contrary, 1-methylcyclopropene partially counteracted the increase in β-Xyl activity during European pear ripening (Trinchero et al. 2004)

thus suggesting that this enzyme may be involved in a range of different physiological processes, modulated by species- and tissue-specific developmental and hormonal factors.

β-Glc is also widely distributed in fruits (Sozzi 2004) including apple where only minor amounts of activity toward 4-methylumbelliferyl-β-D-glucoside were found (Dick et al. 1990). S-F β-Glc activity was hardly noticeable in most cases and absent in ABA-treated callus (Fig. 2b). Interestingly, S-F β-Glc activity from PIC-treated callus rose 28-fold in comparison with that from MS-10 callus. In addition, NaCl-F β-Glc was the only enzyme assayed whose activity greatly increased after 10 subcultures (MS-10 *vs*. MS-1; Fig. 2b). Moreover, the addition of any PGR to the culture medium, except for PIC and ABA, decreased NaCl-F β-Glc activity in comparison with that from MS-10 callus. Thus, a β-Glc may be associated with certain stress conditions since the presence of PIC and/or PGR starvation (MS-10) positively modulates β-Glc activity. In fact, a gene homologous to a β-Glc was found to be expressed in apples exclusively during the infection process of the fruit by *Penicillium expansum* (Sánchez-Torres and González-Candelas 2003). To date, β-Glc has been detected in the cell suspension cultures of a range of species including carrot (Konno et al. 1996). In sweet cherry, the release of free glucose from cell walls by β-Glc suggests that this enzyme may play a role in cell-wall metabolism (Gerardi et al. 2001). In apple and other species, β-Glc may also be involved in the enzymatic hydrolysis of fruit glycoconjugates that play an important role as flavour precursors (Krammer et al. 1991; Schwab and Schreier 1988). Many flavour metabolites (*e.g*., shikimate-derived and terpenoid compounds) are typically conjugated to glucose as odourless β-D-glucopyranosides, or form more complex diglycosides with glucose being further conjugated with a second sugar unit (e.g., α-L-arabinofuranose, β-D-xylopyranose, etc.).

Because of the diverging activity profiles of the S-F and the NaCl-F in response to the addition of various PGRs to the callus cultures (Figs. 1 and 2), the existence of isoforms displaying different regulation ways should not be ruled out. On the other hand, α-Araf, β-Xyl and β-Glc activity seem to be chiefly associated with the cell wall. A recent hypothesis states that three glycoside hydrolases (β-galactosidase, β-xylosidase and β-glucosidase) induced by sugar starvation in *Arabidopsis* can remobilize sugars from cell walls (Lee et al. 2007). The activity of the glycoside hydrolases we studied seems to be differently modulated by auxins, cytokinins, abscisic acid and gibberellic acid. Thus, the possible addition of different PGRs may rule cell-wall metabolism in fruit callus cultures and will probably grant new opportunities to define cell-wall composition and quality in plant tissues. The exploitation of the so-called "habituation" phenomenon (fruit callus proliferating even after the culture has been transferred to a medium with no PGR) may be an interesting tool to understand the regulation of a plethora of genes and enzymes secreted into the cell wall.

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References

Butcher DN (1973) Plant tumor cells–chapter 13. In: Street HE (ed) Plant tissue and cell culture, vol 11. Blackwell Scientific Publishers, London, UK, pp 429–461

Cohen JD (1996) In vitro tomato fruit cultures demonstrate a role for indole-3-acetic acid in regulating fruit ripening. J Am Soc Hortic Sci 121:520–524

Conover WJ, Iman RL (1981) Rank transformation as a bridge between parametric and nonparametric statistics. Am Stat 35:124–128. doi:10.2307/2683975

Davies GJ, Gloster TM, Henrissat B (2005) Recent structural insights into the expanding world of carbohydrate-active enzymes. Curr Opin Struct Biol 15:637–645. doi:10.1016/j.sbi.2005.10.008

Dick AJ, Opoku-Gyamfua A, DeMarco AC (1990) Glycosidases of apple fruit: a multi-functional β-galactosidase. Physiol Plant 80:250–256. doi:10.1111/j.1399-3054.1990.tb04404.x

Farrokhi N, Burton RA, Brownfield L, Hrmova M, Wilson SM, Bacic A et al (2006) Plant cell biosynthesis: genetic, biochemical and functional genomics approaches to the identification of key genes. Plant Biotechnol J 4:145–167. doi:10.1111/j.1467-7652.2005.00169.x

Gerardi C, Blando F, Santino A, Zacheo G (2001) Purification and characterisation of a β-glucosidase abundantly expressed in ripe sweet cherry (*Prunus avium* L.) fruit. Plant Sci 160:795–805. doi:10.1016/S0168-9452(00)00423-4

Goulao LF, Santos J, de Souza I, Oliveira CM (2007) Patterns of enzymatic activity of cell wall-modifying enzymes during growth and ripening of apples. Postharvest Biol Technol 43:307–318. doi:10.1016/j.postharvbio.2006.10.002

Goulao LF, Cosgrove DJ, Oliveira CM (2008) Cloning, characterisation and expression analyses of cDNA clones encoding cell wall-modifying enzymes isolated from ripe

apples. Postharvest Biol Technol 48:37–51. doi:10.1016/j.postharvbio.2007.09.022

Hartmann HT, Kester DE, Davies FT, Geneve RL (1997) Plant propagation: principles and practices, 6th edn. Prentice Hall, Upper Saddle River, New Jersey, USA 770 pp

Ishida BK, Baldwin EA, Buttery RG, Chui SH, Ling LC (1993) Flavor volatiles, sugars and color development in vitro-cultured tomato fruit and calyx. Physiol Plant 89:861–867. doi:10.1111/j.1399-3054.1993.tb05297.x

Iwai H, Ishii T, Satoh S (2001) Absence of arabinan in the side chains of the pectic polysaccharides strongly associated with cell walls of *Nicotiana plumbaginifolia* non-organogenic callus with loosely attached constituent cells. Planta 213:907–915. doi:10.1007/s004250100559

Konno H, Yamasaki Y, Katoh K (1996) A β-glucosidase associated with cell walls from cell suspension cultures of carrot. Phytochemistry 43:1157–1161. doi:10.1016/S0031-9422(96)00456-6

Krammer G, Winterhalter P, Schwab M, Schreier P (1991) Glycosidically bound aroma compounds in the fruits of *Prunus* species: apricot (*P. armeniaca*, L.), peach (*P. persica*, L.), yellow plum (*P. domestica*, L. ssp. Syriaca). J Agric Food Chem 39:778–781. doi:10.1021/jf00004a032

Lee E-J, Matsumura Y, Soga K, Hoson T, Koizumi N (2007) Glycosyl hydrolases of cell wall are induced by sugar starvation in Arabidopsis. Plant Cell Physiol 48:405–413. doi:10.1093/pcp/pcm009

Liu JH, Nada K, Honda C, Kitashiba H, Wen XP, Pang XM et al (2006) Polyamine biosynthesis of apple callus under salt stress: importance of the arginine decarboxylase pathway in stress response. J Exp Bot 57:2589–2599. doi:10.1093/jxb/erl018

Lorences EP, Fry SC (1993) Xyloglucan oligosaccharides with at least two α-D-xylose residues act as acceptor substrates for xyloglucan endotransglycosylase and promote the depolymerisation of xyloglucan. Physiol Plant 88:105–112. doi:10.1111/j.1399-3054.1993.tb01767.x

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497. doi:10.1111/j.1399-3054.1962.tb08052.x

O'Neill RA, Albersheim P, Darvill AG (1989) Purification and characterization of a xyloglucan oligosaccharide-specific xylosidase from pea seedlings. J Biol Chem 264:20430–20437

Peña MJ, Carpita NC (2004) Loss of highly branched arabinans and debranching of rhamnogalacturonan I accompany loss of firm texture and cell separation during prolonged storage of apple. Plant Physiol 135:1305–1313. doi:10.1104/pp. 104.043679

Rose JKC, Catalá C, Gonzalez-Carranza ZH, Roberts JA (2003) Cell wall disassembly. In: Rose JKC (ed) The plant cell wall. Blackwell Publishing Ltd., Oxford, UK, pp 264–324

Ruperti B, Cattivelli L, Pagni S, Ramina A (2002) Ethylene-responsive genes are differentially regulated during abscission, organ senescence and wounding in peach (*Prunus persica*). J Exp Bot 53:429–437. doi:10.1093/jexbot/53.368.429

Sánchez-Torres P, González-Candelas L (2003) Isolation and characterization of genes differentially expressed during the interaction between apple fruit and *Penicillium expansum*. Mol Plant Pathol 4:447–457. doi:10.1046/j.1364-3703.2003.00190.x

Schwab W, Schreier P (1988) Simultaneous enzyme catalysis extraction: a versatile technique for the study of flavor precursors. J Agric Food Chem 36:1238–1242. doi:10.1021/jf00084a028

Sozzi GO (2004) Strategies for the regulation of postharvest fruit softening by changing cell wall enzyme activity. In: Dris R, Jain SM (eds) Production practices and quality assessment of food crops, Vol 4: Postharvest treatment and technology. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 135–172

Sozzi GO, Trinchero GD, Fraschina AA (2000) Ethylene and glycosidase promotion in GA_3- and IAA-treated tomato fruit (*Lycopersicon esculentum* Mill.). J Plant Growth Regul 19:359–368. doi:10.1007/s003440000014

Sozzi GO, Greve LC, Prody GA, Labavitch JM (2002) Gibberellic acid, synthetic auxins, and ethylene differentially modulate α-L-arabinofuranosidase activities in antisense 1-aminocyclopropane-1-carboxylic synthase tomato pericarp discs. Plant Physiol 129:1330–1340. doi:10.1104/pp. 001180

Tateishi A, Kanayama Y, Yamaki S (1996) α-L-Arabinofuranosidase from cell walls of Japanese pear fruits. Phytochemistry 42:295–299. doi:10.1016/0031-9422(95)00931-0

Tateishi A, Mori H, Watari J, Nagashima K, Yamaki S, Inoue H (2005) Isolation, characterization, and cloning of α-L-arabinofuranosidase expressed during fruit ripening of Japanese pear. Plant Physiol 138:1653–1664. doi:10.1104/pp. 104.056655

Trinchero GD, Sozzi GO, Covatta F, Fraschina AA (2004) Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of 'Bartlett' pears. Postharvest Biol Technol 32:193–204. doi:10.1016/j.postharvbio.2003.11.009

Vendrell M (1988) Dual effect of 2, 4-D on ethylene production and ripening of tomato fruit tissue. Physiol Plant 64:559–563. doi:10.1111/j.1399-3054.1985.tb08539.x

Westfall PH, Tobias RD, Rom D, Wolfinger RD, Hochberg Y (1999) Multiple comparisons and multiple tests. SAS Institute, , pp 147–155

Yoshioka H, Kashimura Y, Kaneko K (1995) β-D-Galactosidase and α-L-arabinofuranosidase activities during the softening of apples. J Jpn Soc Hortic Sci 63:871–878