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Article Addendum

Cell wall remodeling in Arabidopsis stamen abscission zones

Temporal aspects of control inferred from transcriptional profiling

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Abbreviations: ATH1, Arabidopsis genome array (Affymetrix); AZ, abscission zone; βGAL, beta galactosidase; CAZy, Carbohydrate-Active Enzymes database; EGase, endo-β-1,4-glucanase; EXP, expansin; EXPL, expansin-like; GH9, glycosyl hydrolase family 9; GH17, glycosyl hydrolase family 17; *IDA*, *inflorescence deficient in abscission*; LCM, laser capture microdissection; PAE, pectin acetylesterase; PG, polygalacturonase; PL, pectate lyase; PME, pectin methylesterase; PMEI, pectin methylesterase inhibitor protein; RGL, rhamnogalacturonan lyase; S12-S15c, stage 12 to 15c; XTH, xyloglucan endotranglucosylase/hydrolase

Key words: abscission zone, stamen, cell wall, laser capture microdissection, pectate lyase, pectin methylesterase, polygalacturonase, expansin, endo-β-1, 4-glucanase, xyloglucan endotransglucosylase/hydrolase

Organ shedding requires cell separation within abscission zones (AZs). Functional genomic AZ studies have been limited by their small size and low incidence. Optimization of laser capture microdissection (LCM) for AZs and other specialized cell types in Arabidopsis[1] allowed recent characterization of the floral stamen AZ transcriptome responding to a developmental shedding cue.[2] Analyses focused on 551 AZ transcripts (AZ_{551}) that were regulated at the highest statistical significance ($p \leq 0.0001$) over five stages of stamen development spanning pre-pollination to organ shed.[2] Here, we seek a fuller understanding of AZ integrity control by relaxing P value restrictions on statistical significance ten-fold to generate an expanded population of 1461 stamen transcripts (AZ_{1461}). Cell wall remodeling functions in AZ_{1461} are significantly over-represented relative to all transcripts represented on the whole genome GeneChip. Hierarchical clustering of gene expression data corresponding to cell wall-related transcripts suggests a temporal model for AZ remodeling in Arabidopsis stamens destined to abscise.

Multiple Proteins Loosen, Remodel and Separate AZs

Primary cell walls consist of a network of cellulose and hemicellulose embedded in a pectinaceous matrix containing lesser amounts of dissolved solutes and glycoproteins.[3] Cellulose microfibrils are coated with hydrogen-bonded xyloglucan that spans adjacent microfibrils.[3,4] Covalent linkages to hemicelluloses and pectins may fortify wall structure.[5] In Arabidopsis and other dicots, xyloglucan is the major cell wall hemicellulose.

Overrepresentation of cell wall functions in AZ_{1461} relative to the ATH1 GeneChip was deemed statistically significant using protocols of Cai and Lashbrook.[2] Figure 1 represents 65 potential cell wall-related genes whose expression is significantly regulated over five floral stages linking pre-pollination to stamen shed. Annotation of gene products follows conventions of refs. 6–9. Locus identities correspond to gene family members classified under the heading "Assembly, Architecture and Growth" on the Cell Wall Genomics website hosted by Purdue University (http://cellwall.genomics.purdue.edu/families/index.html). One locus ID represents a citrus blight protein homolog that shares amino-terminal structural features of expansins[10] but reportedly lacks cell wall loosening activity.[11] Whether this gene product may contribute to cell wall remodeling is therefore contentious. The collective potential for other proteins represented in Figure 1 to modify pectic and hemicellulosic structure of AZcell walls is high.

In Figure 1, heatmaps score stage-dependent expression of putative cell wall-related transcripts by color. Red represents the lowest transcript level present over five stages. Increasing gene expression is represented by orange and yellow, with white reflecting maximal transcript abundance. Transcripts with similar patterns of gene expression are clustered as per Cai and Lashbrook.[2] Clusters depicted in dendrogram format on the left face of Figure 1 are further separated by horizontal lines. Eisen et al.,[12] and others have shown that transcripts clustered on the basis of common expression profiles frequently coordinate common functions. We wished to consider whether a temporal model of abscission might be constructed using published information together with the cluster and expression data of Figure 1. We present such a model in Figure 2, based on the premise that the functioning of protein products may be enhanced at those developmental stages where corresponding transcripts exhibit maximum gene expression. Due to likely post-transcriptional control of some AZ genes, this assumption has limitations. However,

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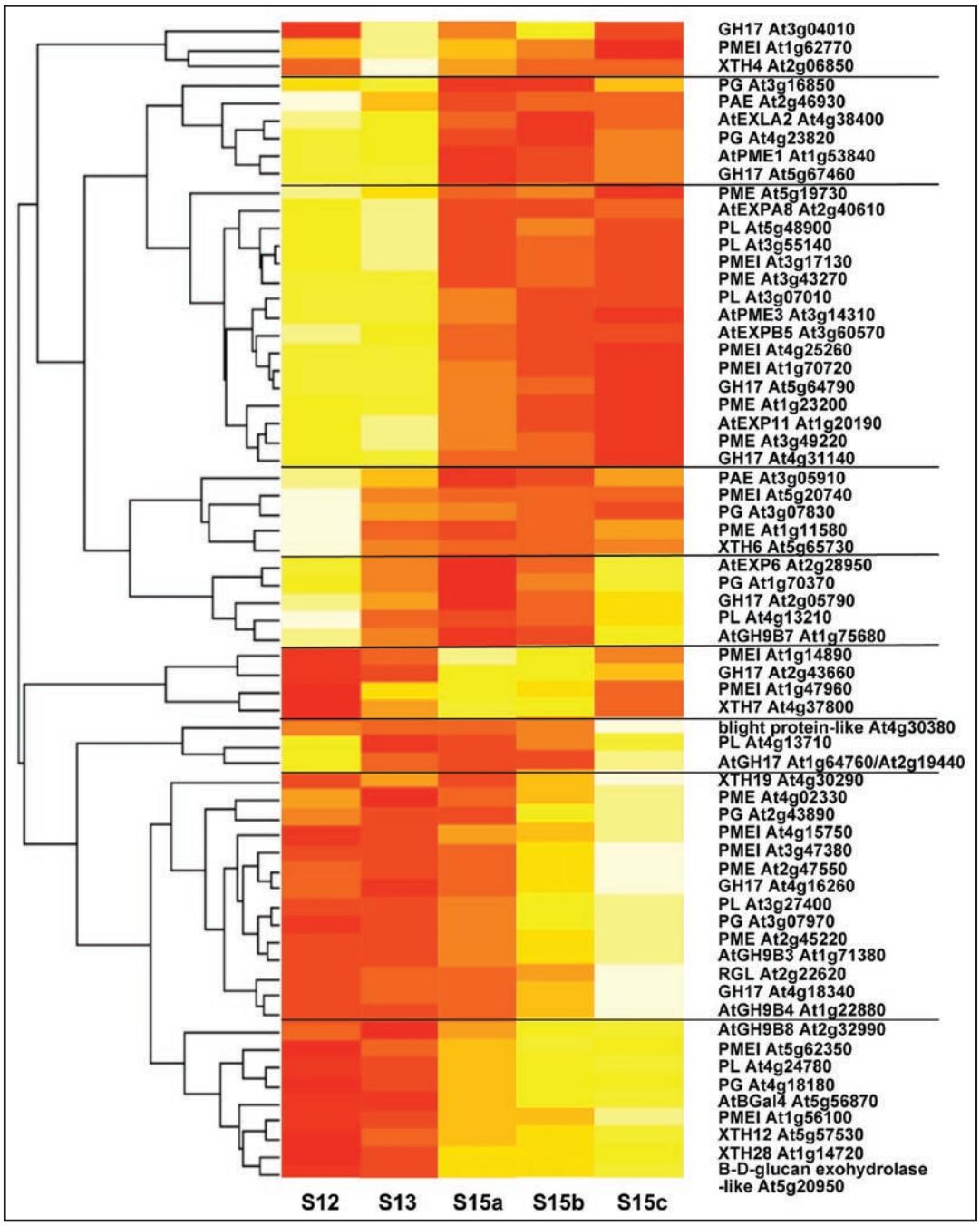

Figure 1. Developmental expression profiles of cell wall related genes within AZ_{1461}. Transcripts were subjected to divisive hierarchical clustering. Enzyme families are further described on the Carbohydrate-Active Enzymes website (www.cazy.org) described by Coutinho et al.[9] Floral stage numbers are defined elsewhere[2] and in Figure 2. EXP expansin, EXPL expansin-like, GH9 glycosyl hydrolase family 9, GH17 glycosyl hydrolase family 17, PAE pectin acetylesterase, PG polygalacturonase, PL pectate lyase, PME pectin methylesterase, PMEI pectin methylesterase inhibitor protein, RGL rhamnogalacturonan lyase, XTH xyloglucan endotranglucosylase/hydrolase.

such an exercise is a reasonable first step towards assessing whether clustered proteins might modify AZ wall structure in a cooperative, ordered manner. Our model facilitates the construction of testable hypotheses for future functional studies that include protein-based methods.

Genomic Data Suggest Temporal Ordering of AZ Remodeling Processes

Figure 2 incorporates information from Cai and Lashbrook[2] and other sources[13,14] to predict flower stages at which key abscission events might take place. Below the developmental timeline are placed transcript classes present at maximal levels of abundance as assessed by GeneChip signal intensities. That is, they were previously portrayed by white or near-white zones in Figure 1.

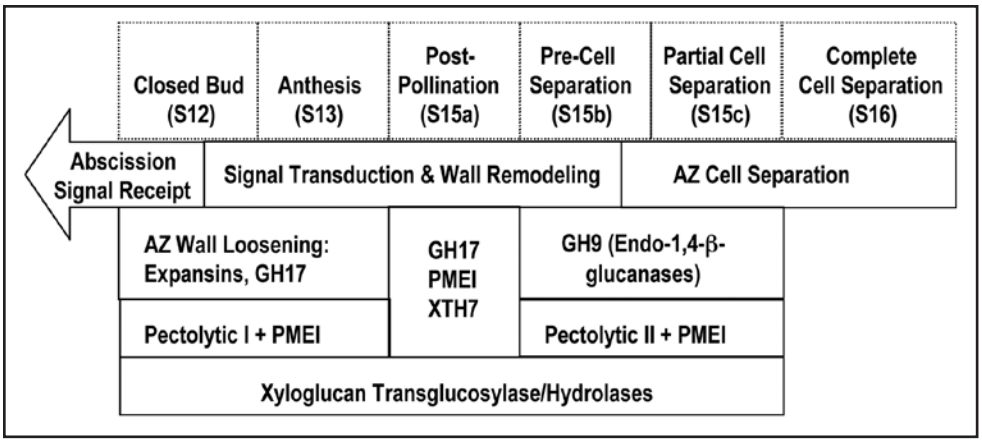

Figure 2. Model of floral stages at which abscission subfunctions may take place. Floral stage numbers are further defined elsewhere.[2] Abscission signal receipt is expected to take place before anthesis because abscission regulators have accumulated by S12.[2] These include the *HAESA* receptor kinase[13] and multiple ethylene response factors.[2] Signaling at or beyond anthesis is evidenced by accumulation at S13 of *IDA* ligand transcript required for abscission.[14] Evidence that a subset of stamen AZ cells have completely separated by S15c is provided in Cai and Lashbrook.[2] GH9 is Glycosyl Hydrolase Family 9; GH7 is Glycosyl Hydrolase Family 17.

Accumulation of expansins (EXPS) and expansin-like transcripts (EXPLs) before anthesis may initiate cell wall loosening. Belfield et al.,[15] showed EXP activity to be enhanced in ethylene-treated AZs of *Sambucus nigra* and identified two cDNAs called *SniEXP4* and *SniEXP2*. Multiple *EXP* and *EXPL* genes are expressed in AZ_{1461} (Fig. 1). Of special interest are *AtEXPs* that cluster with Sambucus cDNAs in phylogenetic analyses. *AtEXPA2* and *AtEXPA8* cluster with *SniEXP4*, while *AtEXPA6* is the Arabidopsis sequence closest to *SniEXP2.*[15] These *AtEXP* transcripts, as well those for all other *AtEXPs* and *AtEXPLs* in Figure 2, exhibit maximal abundance prior to anthesis. They are then significantly downregulated prior to cell separation (Fig. 1). Insofar as developmental abscission signaling is evident at S12,[2] we postulate that expansin effects on AZ architecture are initiated soon after abscission cue receipt. Expansins likely promote cell wall creep required for earliest loosening of AZs by disrupting cellulose- hemicellulose network stability.[16,17]

XTHs and β-1, 3-glucanases may join EXP and EXP-like proteins to mediate early steps of the abscission pathway. XTHs have dual activities and can strengthen or loosen cell walls in different contexts.[7] In AZs prior to anthesis, we expect that loosening would predominate. *XTH4* and *XTH6* are the earliest upregulated AZ *XTHs*, with *XTH6* expression confirming prior observations in abscission layers.[18] Co-production of these XTHs with expansins and expansin-like proteins suggests a potential for synergistic cell wall loosening.

β-1, 3-glucanases within CAZy Glycosyl Hydrolase Family 17 (GH17) mediate diverse processes that include growth, callose deposition and pathogen defense. Well-characterized GH17 proteins related to abscission encode pathogenesis-related (PR) proteins.[19] PR proteins may help prevent pathogen entry into AZ scars resulting from organ shed. At least two AZ transcripts within Figure 1 (*At3g04010, At4g16260*) probably correspond to PR proteins given their induction by multiple fungal pathogens.[20] *At3g04010* exhibits highest accumulation between Stages 12–13 whereas *At4g16260* reaches highest levels during earliest stages of cell separation. This suggests that AZ defense processes may be initiated during initial loosening and maintained throughout organ shedding.

Cell wall loosening and AZ separation are controlled by distinct complements of pectolytic enzymes. Pectin degradation is central to cell wall remodeling during abscission given that cellular continuity between AZ cells is maintained by pectin-rich middle lamellae. Pectin remodeling may also establish porosity characteristics necessary for enzyme access to substrates. In AZs, two blocks of pectolytic enzymes are expressed at times we predict to correspond to cell wall loosening and cell separation. These protein groupings are termed Pectolytic I and Pectolytic II. In Figure 2, Pectolytic I-mediated loosening functions predominate during S12-13; Pectolytic II functions occur following S15b when first cell separation is evident.[2]

β-galactosidases. This class of enzyme has the potential to act on both pectic and hemicellulosic cell wall components.[21] Here, we assign our sole β-galactosidase, β*GAL4*, to the Pectolytic I block because structural and enzymic properties suggest it acts on pectin.[22] In AZs, βGALs may reduce adhesion between AZ cells or modulate wall porosity.

Polygalacturonases (PGs). Of seven AZ *PGs*, three are downregulated (*At3g16850, At3g07830, At4g23820*), three are upregulated (*At2g43890, At3g07970, At4g18180*), and one shows both up and downregulation (*At1g70370*). PG transcripts at maximal levels at S12–S13 could play roles in loosening events impacting wall porosity. *PGs* upregulated thereafter are more likely contributors to actual AZ cell separation. Kim and Patterson[23] previously showed accumulation of *At2g43890* transcript to occur during active cell separation. Elevated expression of *At1g70370* at both S12 and S15c is consistent with potentially dual roles for its gene product in AZ loosening and separation.

Pectate and rhamnogalacturonan lyases (PLs and RGLs). *PL* gene expression patterns were similar to those for *PG* in that some genes were downregulated (*At3g07010, At3g55140, At5g48900*), and some were upregulated (*At3g27400, At4g24780*). Two PL transcripts present at highest levels at both S12 and S15c (*At4g13210, At4g13710*) suggest the potential for dual effects on loosening and cell separation. An RG lyase transcript (*At2g22620*) of potential significance to AZ separation was also expressed.

Pectin methyl esterases (PMEs) and PME inhibitors (PMEIs). Deesterification of pectic compounds by PMEs generates structurally and functionally distinct pectin classes. In turn, PME activity can be transcriptionally or post-translationally inhibited by PME inhibitors. Multiple *PMEs* and *PMEIs* are up and/or downregulated in stamen AZs. Since both PGs and PLs favor deesterified substrates, control of methylesterification level is undoubtedly a key factor in controlling AZ cell wall integrity. In some ripening fruits, PME-mediated de-esterification is necessary for subsequent PG action.[21] Opposing capacities of PMEs to both loosen and strengthen cell walls also may be contribute to balancing wall disassembly with overall strength during abscission.

Pectin acetylesterases (PAEs). The deacetylation of homogalacturonan polymers by pectin acetylesterases solubilizes pectin and may facilitate PL access to substrate. Two *PAEs* are expressed at elevated levels early in abscission signal transduction. One of these transcripts, *At3g05910*, was previously reported to be upregulated in nematode feeding sites very soon after infection.[24] This suggests that PAEs potentiate AZ cell wall loosening requirements that are similar to those needed for successful endoparastic infections.

Endo-β-1, 4-glucanases modify hemicellulose-cellulose structure during actual cell separation. Endo-β-1, 4-glucanases (EGases) cleave internal β-1, 4 linkages in glucan polymers. Substrates include soluble cellulosic polymers including various hemicelluloses. All Arabidopsis EGases are housed in Glycosyl Hydrolase Family 9,[25] within one of three structural subclasses.[8] Expressed in AZs are four *EGases* of Subclass B; this subclass is expected to be secreted to the extracellular space. Using recently standardized nomenclature,[8] *EGases* expressed in AZs include *AtGH9B3* (formerly termed *EGase9* and *AtCel 3*), *AtGH9B4* (formerly *EGase3* and *AtCel5*), *AtGH9B7* (formerly *EGase10*) and *AtGH9B8* (formerly *EGase11*). Three of these four *EGase* gene family members are upregulated to maximal levels during actual cell wall separation. An exception was *AtGH9B7* transcript, present at highest abundance at both pre-anthesis and S15c. We conclude that EGase contributions to abscission predominate during cell separation, in potential cooperation with Pectolytic II proteins.

AtGH9B3/Cel3 gene expression was previously detected in floral organ AZs[26] but expression of its paralog *AtGH9B4/Cel5* has been reported to be rootcap-specific.[27] High sequence identity between duplicons make cross-hybridization possible,[27] and it will be necessary to independently corroborate *AtGH9B4* data. However, *AtGH9B4* expression in anthers, siliques and mixed stage inflorescences was reported in multiple public gene expression databases we visited. These tissues are known sites for cell separation processes like abscission and dehiscence. We predict that while *AtGH9B4* is especially highly expressed in sloughing root caps, it is also expressed in stamen AZs.

XTHs are active in AZs over all stages from pre-pollination to organ shed. As noted earlier, dual activities of XTHs allow them to perform wall strengthening or loosening functions under different circumstances.[7] The presence of regulated XTH expression at all times spanning pre-pollination to the inception of stamen detachment suggests that XTHs may be instrumental in balancing AZ loosening needs with cell wall strengthening requirements prior to organ shed. Alternatively, XTHs may trigger loosening processes that are tempered by regulated cell wall biosynthesis. Certainly, the presence of significant wall building events during abscission can be observed within our stamen AZ profiling dataset (M-EXP-1474) available from ArrayExpress (www.ebi.ac.uk/microarray-as/aer/#ae-main).

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References

1. Cai S, Lashbrook CC. Laser capture microdissection of plant cells from tape-transferred paraffin sections promotes recovery of structurally intact RNA for global gene profiling. Plant J 2006; 48:628-37.
2. Cai S, Lashbrook CC. Stamen abscission zone transcriptome profiling reveals new candidates for abscission control. Enhanced retention of floral organs in transgenic plants overexpressing Arabidopsis Zinc Finger Protein2. Plant Physiol 2008; 146:1305-21.
3. Carpita NC, Gibeaut DM. Structural models of primary cell walls in flowering plants. Consistency of molecular structure with the physical properties of the walls during growth. Plant J 1993; 3:1-30.
4. Cosgrove DJ. Wall structure and wall loosening: A look backwards and forwards. Plant Physiol 2001; 125:131-4.
5. Popper ZA, Fry SC. Widespread occurrence of a covalent linkage between xyloglucan and acidic polysaccharides in suspension-cultured angiosperm cells. Ann Bot 2005; 96:91-9.
6. Kende H, Bradford KJ, Brummell DA, Cho HT, Cosgrove DJ, Fleming AJ, Gehring C, Lee W, McQueen-Mason S, Rose JKC, Voesenek LACJ. Nomenclature for member of the expansin superfamily of genes and proteins. Plant Mol Biol 2004; 55:311-14.
7. Rose J, Braam J, Fry S, Nishitani K. The XTH family of enzymes involved in xyloglucan endotransglucosylation and endohydrolysis: current perspectives and a new unifying nomenclature. Plant Cell Physiol 2002; 43:1421-35.
8. Urbanowicz BR, Bennett AB, del Campillo E, Catalá C, Hayashi T, Henrissat B, Höfte H, McQueen-Mason SJ, Patterson SE, Shoseyov O, Teeri TT, Rose JKC. Structural organization and a standardized nomenclature for plant endo-1,4-β-glucanases (cellulases) of glycosyl hydrolase family 9. Plant Physiol 207; 144:1693-6.
9. Coutinho PM, Henrissat B. Carbohydrate-active enzymes: an integrated database approach. In: Gilbert HJ, Davies G, Henrissat B, Svensson B, eds. Recent Advances in Carbohydrate Bioengineering. Cambridge: The Royal Society of Chemistry 1999; 3-12.
10. Li Y, Darley CP, Ongaro V, Fleming F, Schipper O, Baldauf SL, McQueen-Mason SJ. Plant expansins are a complex multigene family with an ancient evolutionary origin. Plant Physiol 2002; 128:854-64.
11. Cosgrove DJ. New genes and new biological roles for expansins. Curr Opin Plant Biol 2000; 3:73-8.
12. Eisen MB, Spellman P, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998; 95:14863-8.
13. Jinn TL, Stone JM, Walker JC. *HAESA*, an Arabidopsis leucine-rich repeat receptor kinase, controls floral organ abscission. Genes Dev 2000; 14:108-17.
14. Butenko MA, Patterson SE, Grini PE, Stenvik GE, Amundsen SS, Mandal A, Aalen RB. *INFLORESCENCE DEFICIENT IN ABSCISSION* controls floral organ abscission in Arabidopsis and identifies a novel family of putative ligands in plants. Plant Cell 2003; 15:2296-307.
15. Belfield EJ, Ruperti B, Roberts JA, McQueen-Mason S. Changes in expansin activity and gene expression during ethylene-promoted leaflet abscission in *Sambucus nigra*. J Exp Bot 2005; 56:817-23.
16. McQueen-Mason SJ, Cosgrove DJ. Disruption of hydrogen bonding between plant cell wall polymers by proteins that induce cell wall extension. Proc Nat Acad Sci USA 1994; 91:6574-8.
17. McQueen-Mason SJ, Cosgrove DJ. Expansin mode of action on cell walls. Analysis of wall hydrolysis, stress relaxation and binding. Plant Physiol 1995; 107:87-100.
18. Becnel J, Natarajan M, Kipp A, Braam J. Developmental expression patterns of Arabidopsis XTH genes reported by transgenes and Genevestigator. Plant Mol Biol 2006; 61:451-67.
19. Del Campillo E, Lewis LN. Identification and kinetics of accumulation of proteins induced by ethylene in bean abscission zones. Plant Physiol 1991; 98:955-61.
20. Doxey AC, Yaish MW, Moffatt BA, Griffith M, McConkey BJ. Functional divergence in the Arabidopsis β-1,3-glucanase gene family inferred by phylogenetic reconstruction of expression states. Mol Biol Evol 2007; 24:1045-55.
21. Lashbrook CC. New insights into cell wall disassembly during fruit ripening. Stewart Postharvest Rev 2005; http://www.stewartpostharvest.com/Archives/Archives_Issue3_October2005.htm.
22. Ahn YO, Zheng M, Bevan DR, Esen A, Shiu SH, Benson J, Peng HP, Miller JT, Cheng CL, Poulton JE, Shih MC. Functional genomic analysis of *Arabidopsis thaliana* glycoside hydrolase family 35. Phytochem 2007; 68:1510-20.
23. Kim J, Patterson S. Expression divergence and functional redundancy of polygalacturonases in floral organ abscission. Plant Signaling Behav 2006; 1:281-3.
24. Vercauteren I, de Almeida Engler J, De Groodt R, Gheysen G. An *Arabidopsis thaliana* pectin acetylesterase gene is upregulated in nematode feeding sites induced by root-knot and cyst nematodes. Mol Plant-Microbe Int 2002; 15:404-7.
25. Henrissat B, Coutinho PM, Davies GJ. A census of carbohydrate-active enzymes in the genome of *Arabidopsis thaliana*. Plant Mol Biol 2001; 47:55-72.
26. Thoma SL, Glass T, Most A, Patterson SE. Analysis of an abscission-associated cellulase in Arabidopsis. 14th International Conference on Arabidopsis Research 2003, Madison, WI.
27. Delcampillo E, Lewis L. Root cap specific expression of an endo-β-1,4-glucanase (cellulase): a new marker to study root development in Arabidopsis. Plant Mol Biol 2004; 56:309-23.