*Plant Cell Physiol.* 47(6): 788–792 (2006)
doi:10.1093/pcp/pcj043, available online at www.pcp.oupjournals.org


***Short Communication***

# Expression Profiling of Auxin-treated *Arabidopsis* Roots: Toward a Molecular Analysis of Lateral Root Emergence

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**Treating *Arabidopsis* roots with exogenous auxin results in dramatic changes in cellular processes including de novo induction of lateral roots which later emerge through the overlying cells. Microarray experiments reveal approximately 80 genes that are substantially up-regulated in the root over the first 12 h following auxin treatment. We hypothesize that the observed increase in expression of pectate lyase family genes leads to degradation of the pectin-rich middle lamellae, allowing cells in the parent root to separate cleanly. Differences in the degree of pectin methylation in lateral and parent roots may explain why lateral roots are not degraded themselves.**

**Keywords**: *Arabidopsis thaliana* — Auxin — Cell separation — Lateral root — Microarray — Pectate lyase.

Abbreviations: EST, expressed sequence tag; PG, polygalacturonase; PL, pectate lyase; PME, pectin methyl esterase; qPCR, quantitative real-time PCR; SEM, scanning electron microscopy.

Auxin treatment of *Arabidopsis* roots induces expansion of select pericycle cells and subsequent emergence of lateral roots, providing a valuable system for charting molecular events that occur during lateral root development (Laskowski et al. 1995). Following incubation in IAA, lateral roots are initiated in the pericycle, located deep within the parent root. These cells divide and expand, forming a primordium that must work its way through the overlying layers of cells before it can reach the soil. Openings that allow lateral roots to exit the root could simultaneously serve as entrance routes for pathogens; thus, there is likely to be significant selective pressure to maintain tight control over cell separation during this process.

Cell separation can be promoted by the sequential action of pectin methyl esterase (PME) and a polymer-cleaving enzyme such as polygalacturonase (PG) and/or pectate lyase (PL) (Patterson 2001, Rhee et al. 2003). PMEs remove methyl groups present on newly formed pectin, rendering it susceptible to the action of PGs and PLs, enzymes which use different mechanisms to cleave pectin (Herron et al. 2000). Stephenson and Hawes (1994) examined cell separation in root border cells and demonstrated a correlation between increased PME activity and border cell separation. They hypothesized that the consecutive action of PME and PL leads to cell separation in the root cap.

Evidence of PG expression near sites of lateral root emergence has been shown in leeks (Peretto et al. 1992) and *Arabidopsis* (Roberts et al. 2002). PL activity has not been specifically linked to lateral root emergence, although it is known to function in plant cell separation (Marin-Rodriguez et al. 2002), and the softening of ripening strawberry fruits is thought to occur via degradation of the middle lamella mediated by PL activity (Jimenez-Bermudez et al. 2002). Here we show that application of an auxin shock provides a valuable system for identifying candidate genes potentially involved in lateral root development and emergence, and hypothesize that expression of *PL* is part of a developmental program leading to lateral root emergence.

We built a cDNA microarray representing 12,228 expressed sequence tags (ESTs) and used it to profile gene expression in roots incubated in 10 μM IAA for times ranging from 45 min to 12 h. This concentration of auxin significantly increases the number of lateral roots initiated (Laskowski et al. 1995), and provides close to maximal induction of most IAA family genes (Abel et al. 1995). Eighty-five genes for which auxin induction of gene expression is significant at $P < 0.0001$ are reported (Fig. 1; for numeric data, see Supplementary Table 1). Results were validated in several ways. First, auxin-responsive genes for which the temporal pattern of expression was known exhibit the expected patterns of expression. *FQR1* is induced within minutes (Laskowski et al. 2002), and the temporal pattern of *IAA1*, *IAA2* and *IAA3* expression in the root mirrors that seen for whole seedlings treated with 20 μM IAA (Abel et al. 1995). Secondly, we examined promoter::GUS (β-glucuronidase) lines for a few genes for which appropriate transgenic plants were available and saw increased staining after auxin treatment. Thirdly, Northern analysis of the F-box-encoding gene At1g78100, and At5g47370 confirmed their up-regulation (data not shown).

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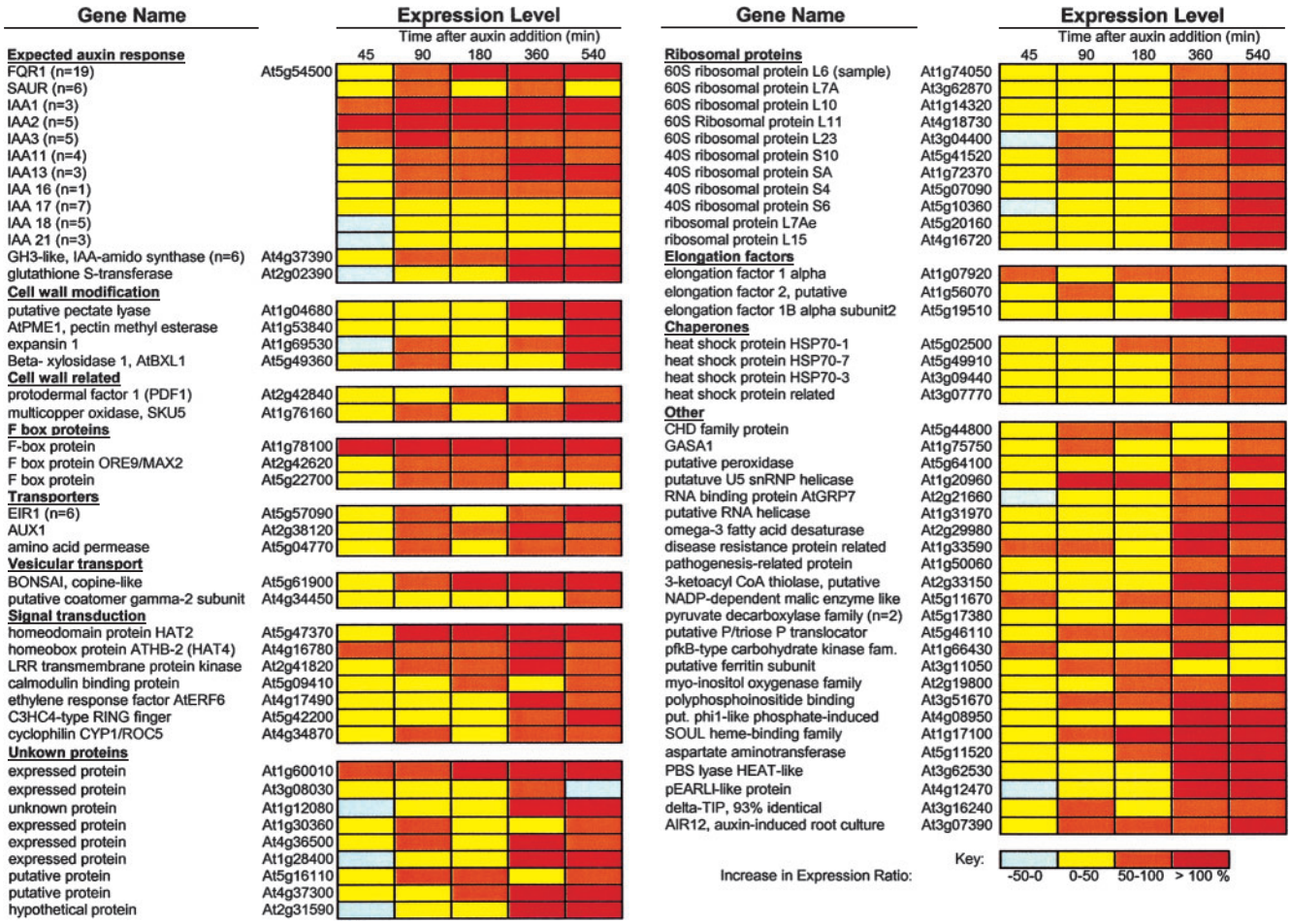


**Fig. 1** Auxin-regulated genes in roots, measured over time with continuous incubation in 10 μM IAA. Expression ratio = $\log_2$ (auxin treated-background/water treated-background). Light green spots are down-regulated by 0–50%, yellow spots are up-regulated by 0–50%, orange spots are up-regulated by 50–100% and red spots are up-regulated by at least 100%. Each bar represents the results of two independent experiments, except the 180 min set which contains the average of two experiments at 135 min, and one at 180 min.

Following auxin treatment, pericycle cells expand and divide, forming lateral roots. Among the first genes whose expression is induced are the primary auxin response genes. These are followed by genes coding for the auxin transporters EIR1 and AUX1, whose induction may promote auxin homeostasis and/or canalization of auxin flow. Developmentally relevant homeodomain genes *HAT2* (Sawa et al. 2002) and *HAT4* (Schena et al. 1993), and the primordia-specific gene *Protodermal Factor 1* (*PDF1*), which is expressed in the L1 of young primordia (Abe et al. 1999), are also induced (Fig. 1). As these cells give rise to the densely cytoplasmic cells of the lateral root primordia, we observed increased expression of genes involved in protein translation, including numerous ribosomal proteins, elongation factors and chaperones.

Another group of genes whose expression is induced several hours after the auxin pulse are those coding for enzymes involved in cell wall modification. These include an α-expansin, a PME and a member of the sequence-defined pectate lyase family (*AtPLA1*, At1g04680). At least one additional PL family member that was not represented on our array, At1g67750, hereafter called *AtPLA2*, also appears to be auxin regulated in roots (Zimmermann et al. 2004, Okushima et al. 2005).

Because PLs can cleave homogalacturan, they could potentially play key roles in the breakdown of the pectin-rich middle lamella that holds cells together. Thus, we decided to investigate this family further. AtPLA1 and AtPLA2, sequence-defined *Arabidopsis* group A PLs, are highly similar to the PLA, PLB and PLC proteins from strawberry for which a relationship to fruit softening has been demonstrated (Jimenez-Bermudez et al. 2002, Benitez-Burraco et al. 2003; for alignment see Supplementary Fig. 1). The gene structure also matches the DGLVDAVMGSTAITISNNHLT conserved domain, except for a single residue substitution at M197 in AtPLA2. An identical substitution in this domain is also

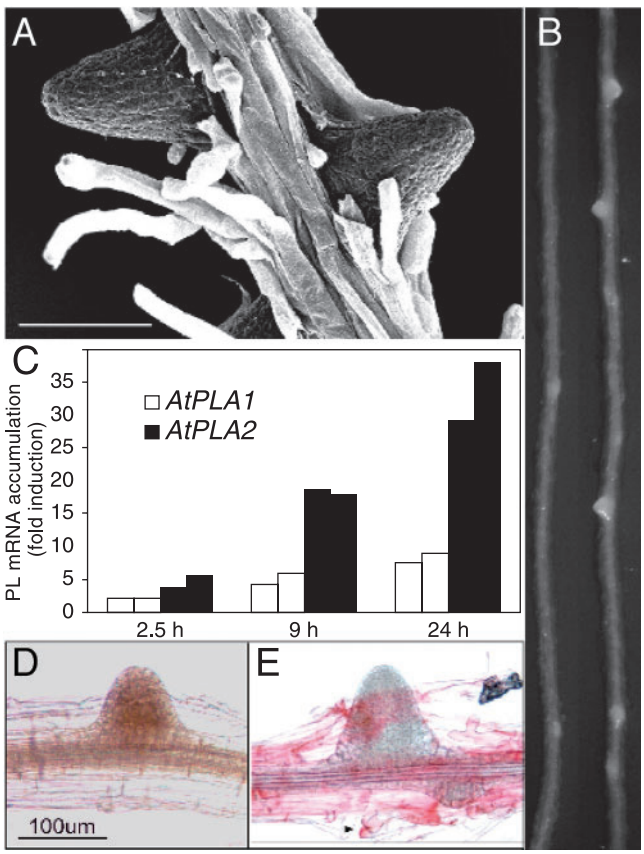


**Fig. 2** Lateral root emergence is accompanied by cell separation and increased expression of PL genes. (A) SEM of auxin-treated root showing epidermal cells separating along the middle lamellae. Some cells appear to have been pushed aside by the emerging lateral root. Scale bar = 100 μm. (B) Developing lateral roots 24 h after the primary roots received a 15 min pulse of 10 μM IAA (right) or water (left). (C) Quantitative PCR demonstrates that PL mRNA accumulates in roots following a pulse of 10 μM IAA. Fold induction was calculated relative to water-treated controls for each time point. *AtPLA1*, white bars; *AtPLA2*, black bars. Each bar represents an independent experiment. (D) Roots stained with hydroxylamine hydrochloride–ferric chloride highlighting regions with substantial amounts of methylated pectin. (E) Roots stained with ruthenium red show that lateral roots do not contain as much demethylated pectin as does the parent root.

**A**

| Cell Type | *AtPLA1* At1g04680 | *AtPLA2* At1g67750 |
|---|---|---|
| **Lateral root initial** | **1587** | **450** |
| **Xylem pole pericycle** | **284** | **56** |
| Pericycle | 176 | 43 |
| Maturing xylem cells | 1245 | 13 |
| Whole stele | 1272 | 206 |
| Endodermis | 516 | 237 |
| Cortex | 1567 | 523 |
| Lateral root cap | 657 | 705 |

**B**

| Stage | *AtPLA1* At1g04680 | *AtPLA2* At1g67750 |
|---|---|---|
| 4 | 412 | 93 |
| 3 | 627 | 231 |
| 2 | 1962 | 637 |
| 1 | 1094 | 159 |

**Fig. 3** Expression of *AtPLA1* and *AtPLA2* is higher in lateral root initials than in xylem pole pericycle cells. Numbers reflect expression values from Affymetrix arrays. (A) Expression data were derived by isolating RNA from cells that were sorted in a cell type-specific manner. (B) Roots were divided horizontally prior to expression analysis. Stage 1, root tip has not yet reached its full width (tip to ~0.15 mm); stage 2, cells more transparent (~0.3 mm from the tip); stage 3, root hairs fully elongated (~0.45–2 mm); stage 4, mature root. Data from Birnbaum et al. (2003) and with thanks to J.-Y. Lee.

present in an active PL from *Zinnia elegans* (Domingo et al. 1998), so this change is not likely to prevent activity.

As lateral root primordia emerge from the interior of an *Arabidopsis* root, epidermal cells surrounding the primordia separate from one another along the middle lamella. Scanning electron micrographs (SEMs) of emerging lateral roots often show epidermal cells that remain largely intact, but appear to have been pushed aside by the emerging primordia (Fig. 2A). Shedding of the cells in the parent root leaves the pericycle cell derivatives as the retained side of an abscission layer.

Quantitative real-time PCR (qPCR) confirms that *AtPLA1* and *AtPLA2* are up-regulated by IAA during the time period in which lateral roots initiate and expand into the overlying cell layers. The degree of up-regulation increases steadily over the first 24 h following a 15 min pulse of 10 μM IAA. Substantial induction occurs within the first 9 h, during which time the pericycle cells expand, divide and form primoridia. By 24 h after auxin treatment, auxin-treated roots contain many more laterals than controls, including ones that are emerged or about to emerge (Fig. 2B). At this time, *AtPLA1* is 7-fold up-regulated while *AtPLA2* is 29-fold up-regulated (Fig. 2C).

Spatially detailed expression data show that *AtPLA1* and *AtPLA2* are expressed at higher levels in cells associated with lateral root initials than in the pericycle cells from which they are derived (data in Fig. 3 from Birnbaum et al. 2003 with thanks to J.-Y. Lee). Thus, at least part of the increase in expression following auxin treatment results from auxin induction of lateral root formation. In contrast to *AtPLA2*, expression of *AtPLA1* is also high in maturing xylem, suggesting that *AtPLA1* induction by auxin may reflect a combination of auxin-induced lateral root formation and auxin-induced xylem formation. The highest expression level recorded for *AtPLA2* was in the flanks of the root cap. These data support the idea that *PLA* expression correlates with lateral root formation.

To determine whether expression of these *PL* genes requires activation of the auxin response pathway associated with lateral root formation, we conducted real-time PCR experiments with the solitary root (*slr-1*) mutant. Auxin induction of these two genes is drastically reduced in these plants. Solitary root mutants carry a gain-of-function mutation in *IAA14*, and fail to produce lateral roots, even in the presence of exogenous auxin (Fukaki et al. 2002). Their phenotype is similar to, albeit more severe than, that of the *arf7arf19* mutants, in which expression of *AtPLA2* was shown to be reduced (Okushima et al. 2005). In addition to the defect in lateral root initiation, the plants are agravitropic and have a marked decrease in root hair formation. In our qPCR assay, we found that expression of *AtPLA1* is not auxin inducible in *slr*; the average fold induction is 1.0 instead of 4.2 as in the wild type. Induction of *AtPLA2* is drastically reduced from an average of 30.1-fold in

wild type to 2.1-fold in *slr-1* 24 h after a pulse of 10 μM IAA. This observation indicates that expression of *AtPLA1* and *AtPLA2* is downstream of IAA14 action, strengthening the possible connection between PL gene expression and auxin-induced lateral root formation.

QPCR also confirms that mRNA for the expansin *AtEXPA1* accumulates to higher levels following auxin treatment, although the response peaks and declines over a shorter time period than that of the *PLs*. At 9 h after a 15 min pulse of 10 μM IAA, roots from plants grown on MS (Murashige and Skoog) plates exhibit a 9.8-fold increase in expression, which declines to 4.7-fold at 24 h. Expansins are best known for their role in promoting cell wall extension, and a role in promoting expansion of young lateral root cells could explain the increase in expression seen here. Because expansins have been reported to promote cell separation (Cho and Cosgrove 2000), another possibility is that the expression of *AtEXPA1* may contribute to forming a zone of cell separation around the developing lateral root.

Patterns of pectin demethylation may limit regions of PL activity as newly formed pectin is deposited in the wall in a methylated form (Carpita and Gibeaut 1993) which must be demethylated before it can serve as a substrate for PL. Hydroxylamine hydrochloride–ferric chloride stain binds primarily to methylesterified pectin (Sabba and Lulai 2002) and stains emerging lateral roots more heavily than the parent tissue (Fig. 2D). Ruthenium red, a dye with a preference for binding non-esterified pectin (Sterling 1970), does not stain cells in the emerging lateral root, but does stain epidermal cells in the parent root (Fig. 2E). Together, these data indicate that the pectin in the newly forming lateral roots is largely methylated while that in the overlying cells of the parent root has become demethylated, probably restricting PL activity to cells overlying the developing primodia.

Here we identify numerous auxin-regulated genes that may play roles in lateral root development, demonstrating that this system can be a valuable tool for the study of lateral root development, and leading us to hypothesize that the observed increase in expression of *PL* family genes leads to degradation of the pectin-rich middle lamellae. Cells separate as the lateral roots emerge, and our expression analysis shows that auxin up-regulation of *PL* genes is temporally coordinate with this separation. Up-regulation of *PL* by auxin is inhibited in the *slr1* mutant, further indicating that this event is downstream of auxin action. Pectin demethylation, which generates a substrate for PL action, is more prevalent where the cell separation that accompanies lateral root emergence occurs, possibly focusing PL activity to that area.

Previous results that support this hypothesis include the observation that high concentrations of auxin can induce cell separation. Incubating radish roots in 90 μM IAA induces sloughing of the cortical and epidermal cells, leaving the newly formed lateral roots and adjacent pericycle cell derivates as the separation layer (Laskowski et al. 1995). Cortical cell shedding also takes place in the hypocotyls of *Arabidopsis* superroot (*sur1*) mutants, which have increased levels of auxin. This sloughing can occur in the absence of root formation, but does not ordinarily occur in the absence of high auxin concentrations (Boerjan et al. 1995).

Auxin-inducible PL gene expression has been observed in *Zinnia*, where it is localized to an outer ring of the vasculature. This localization is consistent with a role in shedding the cell layers lying just outside the stele, but is not necessarily causative; cell wall remodeling could be independent of cell separation processes (Domingo et al. 1998).

Because openings in the root create potential entrance routes for pathogens, regulation of cell separation is crucial for plant success. Indeed, secretion of pectolytic enzymes promotes the virulence of many pathogens. It is interesting that the fitness of some plant-associated bacteria increases as a result of auxin production (Brandl and Lindow 1998), and raises the question of whether such production induces expression of genes encoding pectin-modifying enzymes in the host. Further examination of the potential role of auxin-inducible cell wall modifications may shed light on host–pathogen interactions as well as on the development of individual plants.

## Materials and Methods

For micorarray experiments, seeds of *Arabidopsis thaliana* (ecotype Columbia) were surface sterilized with 50% bleach for 15 min, rinsed five times in sterile water and placed on nylon rafts in Phytatrays (Sigma-Aldrich) filled with semi-solid agar (MS medium plus 0.5% sucrose and 0.5% agarose ) under sterile conditions (Melo-Oliveira et al. 1996). Approximately 0.19 g of seed was spread over 25 trays. Plants were grown for 14 d with 8 h light at 22°C before the rafts were transferred to 100 ml of sterile water (control-treated) or water plus 10 μM IAA for 45 min to 12 h. Roots were harvested with a razor blade and frozen in liquid nitrogen.

For all other experiments, *Arabidopsis* seeds were grown for 7 d with 16 h light at 22°C on vertically oriented square plates (Laskowski et al. 2002). Plates were placed horizontally and roots were submerged in 10 μM IAA or water for 15 min, drained, and returned to vertical growth for the duration of the treatment.

Ruthenium red was dissolved in water and added to FAA-fixed plants immersed in 70% ethanol for 2–5 min. Staining with hydroxylamine hydrochloride–ferric chloride was performed as described in Sabba and Lulai (2002).

Our custom microarrays contain 80% of the MSU EST library; those whose PCR-amplified inserts contain products that migrated as a single band. DNA was printed on poly-L-lysine-coated slides using a Gene Machines Omnigrid. Target RNA was purified with Trizol (Invitrogen) and PolyATract System columns (Promega) prior to use with a CyScribe direct cDNA labeling kit (Amersham Pharmacia). Each time point includes a full biological repeat with the Cy3 and Cy5 dyes reversed between replicates. Hybridization was as described in Volkert (2001). Fluorescent scans were analyzed with Corning Array Express software.

RNA was isolated and prepared for qPCR using Biorad's Aurum, iScript and Sybergreen Supermix kits. Starting cDNA levels were normalized using *TUB4* β-tubulin expression as an internal control (Lee et al. 2005). Fold induction was calculated according to Pfaffl (2001), using E values from the slopes of standard curves. Control reactions

without reverse transcription required at least nine more cycles, indicating that DNA contamination was not an issue. Gene-specific primers were designed with Beacon Designer software as follows: *AtPLA1* (At1g04680) 5′-CTTTTAGCAATGATGTGTTTACTC-3′ and 5′-CCGCTACTTCATCTGGATTC-3′; *AtPLA2* (At1g67750) 5′-ACCAAACAAACAAACACATTCAAATCTCATAAACAC-3′ and 5′-GGAGGCAATGAAGGTCGGAGAAAGG-3′; *EXP1* (At1g69530) 5′-TGACGTGTTGTAGTAGAAGCAGTTAGAG-3′ and 5′-GCAAAATCAAGCACTCGAAGCACCAC-3′.

*Supplementary material*

Supplementary material mentioned in the article is available to online subscribers at the journal website www.pcp.oupjournals.org.

## Acknowledgments

We thank G. Fink in whose laboratory the microarrays were constructed; Y. Cruz, P. Grisafi, J. Hua, E. Sipos and Z. Stanley for technical assistance; H. Fukaki for *slr-1* seed; J.-Y. Lee for digital in situ data; and M. McCann and N. Carpita for critical reading of the manuscript. This work was supported in part by an NSF ROA award.

## References

Abel, S., Nguyen, M.D. and Theologis, A. (1995) The PS-IAA4/5-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. *J. Mol. Biol.* 251: 533–549.

Abe, M., Takahashi, T. and Komeda, Y. (1999) Cloning and characterization of an L1 layer-specific gene in *Arabidopsis thaliana*. *Plant Cell Physiol.* 40: 571–580.

Benitez-Burraco, A., Blanco-Portales, R., Redondo-Nevado, J., Bellido, M.L., Moyano, E., Caballero, J.-L. and Munoz-Blanco, J. (2003) Cloning and characterization of two ripening-related strawberry (*Fragaria x ananassa* cv. Chandler) pectate lyase genes. *J. Exp. Bot.* 54: 633–645.

Birnbaum, K., Shasha, D.E., Wang, J.Y., Jung, J.W., Lambert, G.M., Galbraith, D.W. and Benfey, P.N. (2003) A gene expression map of the Arabidopsis root. *Science* 302: 1956–1960.

Boerjan, W., Cervera, M.T., Delarue, M., Beeckman, T., Dewitte, W., Bellini, C., Caboche, M., Van Onckelen, H., Van Montagu, M. and Inze, D. (1995) Superroot, a recessive mutation in Arabidopsis, confers auxin overproduction. *Plant Cell* 7: 1405–1419.

Brandl, M.T. and Lindow, S.E. (1998) Contribution of indole-3-acetic acid production to the epiphytic fitness of *Erwinia herbicola*. *Appl. Environ. Microbiol.* 64: 3256–3263.

Carpita, N. and Gibeaut, D.M. (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* 3: 1–30.

Cho, H.-T. and Cosgrove, D. (2000) Altered expression of expansin modulates leaf growth and pedicle abscission in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* 97: 9783–9788.

Domingo, C., Roberts, K., Stacey, N.J., Connerton, I., Riz-Teran, F. and McCann, M.C. (1998) A pectate lyase from *Zinnia elegans* is auxin inducible. *Plant J.* 13: 17–28.

Fukaki, H., Tameda, S., Masuda, H. and Tasaka, M. (2002) Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of Arabidopsis. *Plant J.* 29: 153–168.

Herron, S.R., Benen, J.A., Scavetta, R.D., Visser, J. and Jurnak, F. (2000) Structure and function of pectic enzymes: virulence factors of plant pathogens. *Proc. Natl Acad. Sci. USA* 97: 8762–8769.

Jimenez-Bermudez, S., Redondo-Nevado, J., Munoz-Blanco, J., Caballero, J.L., Lopez-Aranda, J.M., Valpuesta, V., Pliego-Alfara, F., Quesada, M.A. and Mercada, J.A. (2002) Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. *Plant Physiol.* 128: 751–759.

Laskowski, M.J., Dreher, K.A., Gehring, M.A., Abel, S., Gensler, A.L. and Sussex, I.M. (2002) FQR1, a novel primary auxin-response gene, encodes a flavin mononucleotide-binding quinone reductase. *Plant Physiol.* 128: 578–590.

Laskowski, M.J., Williams, M.E., Nusbaum, H.C. and Sussex, I.M. (1995) Formation of lateral root meristems is a two-stage process. *Development* 121: 3303–3310.

Lee, D., Polisensky, D.H. and Braam, J. (2005) Genome-wide identification of touch- and darkness-regulated Arabidopsis genes: a focus on calmodulin-like and *XTH* genes. *New Phytol.* 165: 429–444.

Marin-Rodriguez, M.V., Orchard, J. and Seymour, G.B. (2002) Pectate lyases, cell wall degradation and fruit softening. *J. Exp. Bot.* 53: 2115–2119.

Melo-Oliveira, R., Oliveira, I.C. and Coruzzi, G.M. (1996) Arabidopsis mutant analysis and gene regulation define a nonredundant role for glutamate dehydrogenase in nitrogen assimilation. *Proc. Natl Acad. Sci. USA* 93: 4718–4723.

Okushima, Y., Overvoorde, P.J., Arima, K., Alonso, J.M., Chan, A., et al. (2005) Functional genomic analysis of the AUXIN RESPONSE FACTOR gene family members in *Arabidopsis thaliana*: unique and overlapping functions of ARF7 and ARF19. *Plant Cell* 17: 444–463.

Patterson, S.E. (2001) Cutting loose. Abscission and dehiscence in Arabidopsis. *Plant Physiol.* 126: 494–500.

Peretto, R., Favaron, F., Bettini, V., De Lorenzo, G., Marini, S., Alghisi, P., Cervone, F. and Bonfante, P. (1992) Expression and localization of polygalacturonase during the outgrowth of lateral roots in *Allium porrum* L. *Planta* 188: 164–172.

Pfaffl, M.W. (2001) A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Res.* 29: 2002–2007.

Rhee, S.Y., Osborne, E., Poindexter, P.D. and Somerville, C.R. (2003) Microspore separation in the *quartet 3* mutants of Arabidopsis is impaired by a defect in a developmentally regulated polygalacturonase required for pollen mother cell wall degradation. *Plant Physiol.* 133: 1170–1180.

Roberts, A.J., Elliott, K.A. and Gonzallez-Carranza, Z.H. (2002) Abscission, dehiscence, and other cell separation processes. *Annu. Rev. Plant Biol.* 53: 131–158.

Sabba, R.P. and Lulai, E.C. (2002) Histological analysis of the maturation of native and wound periderm in potato (*Solanum tuberosum* L.) tuber. *Ann. Bot.* 90: 1–10.

Sawa, S., Ohgishi, M., Goda, H., Higuchi, K., Shimada, Y., Yoshida, S. and Koshiba, T. (2002) The HAT2 gene, a member of the HD-Zip gene family, isolated as an auxin inducible gene by DNA microarray screening, affects auxin response in Arabidopsis *Plant J.* 32: 1011–1022.

Schena, M., Lloyd, A.M. and Davis, R.W. (1993) The HAT4 gene of Arabidopsis encodes a developmental regulator. *Genes Dev.* 7: 367–379.

Stephenson, M.B. and Hawes, M.C. (1994) Correlation of pectin methylesterase activity in root caps of peas with root border cell separation. *Plant Physiol.* 106: 739–745.

Sterling, C. (1970) Crystal-structure of ruthenium red and stereochemistry of its pectin stain. *Amer. J. Bot.* 57: 172–175.

Volkert, T. (2001) Target labeling by reverse-transcription–direct incorporation http://www.whitehead.mit.edu/CMT/protocols/Direct%20Labelingv.WICMT4.pdf

Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L. and Gruissem, W. (2004) GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. *Plant Physiol.* 136: 2621–2632.

(Received October 3, 2005; Accepted March 30, 2006)