Characterization of the Arabidopsis Lysine-Rich Arabinogalactan-Protein *AtAGP17* Mutant (*rat1*) That Results in a Decreased Efficiency of Agrobacterium Transformation[1][w]

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Arabinogalactan-proteins (AGPs) are a family of complex proteoglycans widely distributed in plants. The Arabidopsis *rat1* mutant, previously characterized as resistant to *Agrobacterium tumefaciens* root transformation, is due to a mutation in the gene for the Lys-rich AGP, *AtAGP17*. We show that the phenotype of *rat1* correlates with down-regulation of *AGP17* in the root as a result of a T-DNA insertion into the promoter of *AGP17*. Complementation of *rat1* plants by a floral dip method with either the wild-type *AGP17* gene or cDNA can restore the plant to a wild-type phenotype in several independent transformants. Based on changes in *PR1* gene expression and a decrease in free salicylic acid levels upon Agrobacterium infection, we suggest mechanisms by which AGP17 allows Agrobacterium rapidly to reduce the systemic acquired resistance response during the infection process.

Arabinogalactan-proteins (AGPs) are a family of complex proteoglycans widely distributed in plants. They are found in the extracellular matrix associated with the plasma membrane and cell wall (Knox, 1995; Du et al., 1996). Although the precise function(s) that AGPs perform is unknown, they have been implicated in diverse developmental roles, including differentiation, cell-cell recognition, and embryogenesis (Knox, 1996; Schultz et al., 1998; Majewski-Sawka and Nothnagel, 2000).

Most studies investigating AGP expression and function have used an AGP-binding dye, β-D-glucosyl (β-D-Glc) Yariv reagent, and/or antibodies that recognize the carbohydrate epitopes of AGPs (for review, see Gaspar et al., 2001). These previous studies support a role for AGPs in plant cell growth and development; however, they do not inform us of the function of individual AGPs. The identification of AGP genes from Arabidopsis provides us with a wide range of tools to determine the function(s) of individual AGPs.

To date, almost 50 genes encoding putative AGP protein backbones (hereafter referred to as AGP genes) have been identified in Arabidopsis (Schultz et al., 2002). These include the classical AGPs, those with Lys-rich domains, the arabinogalactan (AG)-peptides with short protein backbones, and the fasciclin-like AGPs (Gaspar et al., 2001). Fasciclin-like AGPs are a class of chimeric AGPs that, in addition to AGP motifs, have fasciclin-like domains (Gaspar et al., 2001; Johnson et al., 2003a). In addition, another approximately 50 glycosylphosphatidylinositol (GPI)-anchored proteins are likely to contain AG chains as part of larger proteins based on the presence of short Pro-, Ser-, Thr-, and Ala-rich regions containing noncontiguous Pro residues (Borner et al., 2002, 2003). Pro-, Ser-, Thr-, and Ala-rich regions contain noncontiguous Pro residues are referred to as AG-glycomodules because there is increasing evidence that these motifs direct the *O*-glycosylation of Hyp with type II AG chains (Goodrum et al., 2000; Zhao et al., 2002; Tan et al., 2003).

Only a few AGP mutants have been identified to date. The haploinsufficient mutant, *rat1* (resistant to Agrobacterium transformation), is resistant to transient and stable transformation of root segments by tumorigenic and nontumorigenic Agrobacterium strains (Nam et al., 1999; Zhu et al., 2003a). This

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mutant has a T-DNA insertion upstream of the start codon of *AGP17* (Nam et al., 1999; Gaspar et al., 2001). A root-specific nonclassical (chimeric) AGP from Arabidopsis, *AtAGP30*, has been implicated in root regeneration and seed germination (van Hengel and Roberts, 2003). The other AGP mutant, *sos5/fla4*, displays a salt overly sensitive phenotype with increased cell expansion under high salt conditions (Shi et al., 2003).

The *rat1/agp17* mutant belongs to the Lys-rich AGP subclass in Arabidopsis, which consists of three genes, *AtAGP17*, *AtAGP18* (Gilson et al., 2001), and *AtAGP19* (Schultz et al., 2002). The predicted proteins all contain a short (approximately 12 amino acid) basic Lys-rich region. Both AGP17 and AGP18 are predicted to be GPI-anchored based on the big PI plant predictor (Eisenhaber et al., 2003). These AGPs share sequence similarity to *NaAGP4*, an AGP isolated from styles of *Nicotiana alata* (Gilson et al., 2001). Expression of *NaAGP4* is reduced by wounding and pathogen infection (Gilson et al., 2001) and in this respect is similar to another AGP with a Lys-rich region, *LeAGP-1* from *Lycopersicon esculentum* (Pogson and Davies, 1995; Li and Showalter, 1996; Zhao et al., 2002).

In this article, we characterize the *rat1* mutant and show that the reduced binding of Agrobacterium correlates with down-regulation of *AGP17* in the root and does not result from an inhibition of cellulose synthesis by the bacterium. The *rat1* phenotype could be complemented with the wild-type *AGP17* gene. By comparing levels of salicylic acid (SA) in Agrobacterium-infected and uninfected Arabidopsis plants, we provide evidence that wild-type control of AGP17 gene expression in the roots is necessary to allow Agrobacterium rapidly to reduce the systemic acquired resistance (SAR) response during infection.

RESULTS

The Rat Phenotype of *rat1* Results from Reduced *AGP17* Expression in Roots

The plant line CS12955 corresponding to *rat1* (Nam et al., 1999) was independently identified from the Feldmann T-DNA collection using a reverse-genetic PCR based approach with AGP17 specific primers (McKinney et al., 1995). By sequencing the PCR product, we determined that the T-DNA tag is 1,097 bp upstream of the ATG start codon (Fig. 1A). Cosegregation analysis of *rat1* showed that kanamycin resistance segregated 3:1 ($Kan^{resistant}$:$Kan^{sensitive}$) with the rat phenotype, indicating that *rat1* is tightly linked to the locus into which the T-DNA has integrated (Nam et al., 1999). DNA gel-blot analysis of the *rat1* mutant is consistent with two T-DNAs inserted at a single locus, and the sizes obtained match those expected for an insertion in the promoter of *AGP17* (data not shown).

We analyzed expression of *AGP17* and the most closely related Lys-rich AGP, *AGP18*, in wild-type plants using RNA gel-blot analysis (Fig. 1B). Expression of *AGP17* was detected only in flowers (Fig. 1B). Surprisingly, we could not detect expression of *AGP17* in roots even after a 2-week exposure of the blot. RNA-blot analysis of *AGP18* showed high expression in flowers, but expression was also detected in other plant parts (Fig. 1B).

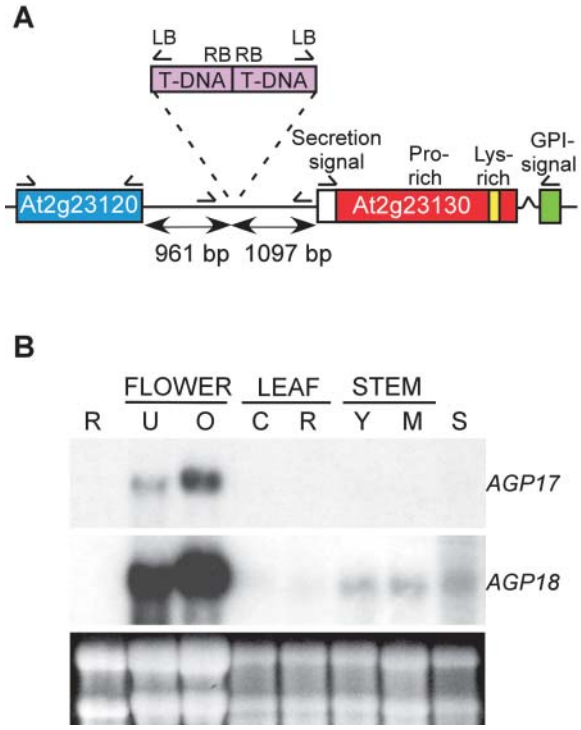

Figure 1. A, Position of the T-DNA insertion within the *AGP17* gene (At2g23130) of the Arabidopsis T-DNA insertion line CS12955. The domain structure of the AGP17 protein and the position of the gene *At2g23120*, upstream of *AGP17*, are shown. The T-DNA insertion is 1,097 bp upstream of the start codon of the *AGP17* gene. The positions of oligonucleotide PCR primers used in this study are indicated. Not drawn to scale. B, RNA gel-blot analysis of the steady-state levels of RNAs encoding two Lys-rich AGPs, *AGP17* and *AGP18*, in various Arabidopsis tissues. R, root; U, unopened flower (0.5–1.5 mm in length); O, open flower (1.5–2 mm in length); C, cauline leaf (0.5–1.5 cm in length); R, rosette leaf (1.5–3 cm in length); Y, young inflorescence stem (first internode from the growing tip greater than 1 cm in length); M, mature inflorescence stem (first internode from the base); S, young siliques (0.5–1 cm in length). To illustrate the loading of RNA, the ethidium bromide-stained formaldehyde gel is shown.

To compare levels of mRNA in the roots of wild-type and *rat1* plants, we used a semiquantitative reverse transcription (RT)-PCR method (Lasserre et al., 1996; Zegzouti et al., 1999). We compared the expression of *AGP17* in hydroponically grown wild-type and *rat1* plants that were either uninfected or infected with Agrobacterium (Fig. 2) and sampled at different times up to 48 h. In wild-type roots, *AGP17* cDNA was detected after 40 PCR cycles with or without Agrobacterium inoculation. No expression of *AGP17* was detected in *rat1* roots after 40 PCR cycles. However, the *AGP17* transcript could be detected after 50 or more

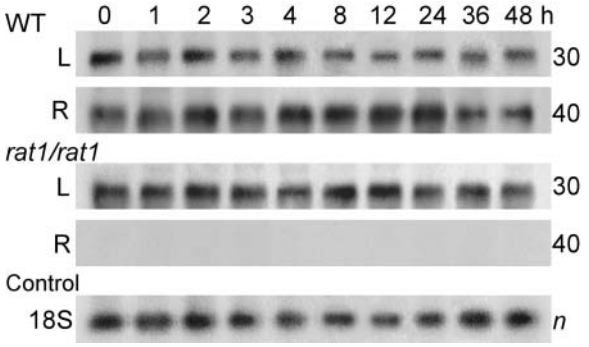

Figure 2. DNA gel blots of semiquantitative RT-PCR showing expression of the *AGP17* gene in Arabidopsis plants grown hydroponically and inoculated with *A. tumefaciens* GV3101. Tissues were sampled at intervals over a 48-h period, and roots (R) were separated from aboveground parts (L). 18S rRNA transcript levels were used to normalize the samples. This control section is representative of all of the above experiments. Numbers at right indicate the number of PCR cycles employed. For the 18S transcript, the numbers of cycles (*n*) were 18 for leaf and 21 for root cDNA.

cycles (data not shown). Therefore, the T-DNA insertion results specifically in a reduction of root expression but not leaf expression in *rat1* plants. The expression of *AGP17* in wild-type roots and leaves and *rat1* leaves was constant over the 0 to 48 h postinfection sampling period, indicating that Agrobacterium infection did not alter the level of *AGP17* transcripts. We also examined the expression of *AGP18* in *rat1* mutants at these time points and found that there was no change compared to the wild type (data not shown).

The Expression of the Upstream Gene Flanking *AGP17* Is Not Affected by the T-DNA Insertion in *rat1*

To rule out the possibility that other genes near the T-DNA insertion site were affected in *rat1*, the integrity of the upstream neighboring gene was determined by PCR and sequencing. The oligonucleotides used are indicated in Figure 1A. These experiments showed that there were no rearrangements or small insertions and deletions in the genomic region examined upstream or downstream of the T-DNA insertion site (data not shown). Additional experiments showed that two or more copies of the T-DNA in a head-to-head configuration are present, with the T-DNA left borders flanking the genomic DNA on both sides of the insertion (Fig. 1A). Examination of sequence around the T-DNA insertion site revealed the presence of an expressed gene of unknown function (GenBank accession no. At2g23120) approximately 1 kb upstream of the T-DNA. The expression level of the upstream gene, as determined by RT-PCR, was unaltered in the *rat1* mutant when compared to wild-type plants (data not shown). Therefore, we conclude that the T-DNA insertion affects *AGP17* and causes the rat phenotype by reducing the expression of *AGP17* in Arabidopsis roots.

Complementation of the *rat1* Mutant

To provide further evidence that loss of *AGP17* expression in the roots of *rat1* plants is responsible for the rat phenotype, we performed a genetic complementation analysis. The *rat1* mutant was transformed with either a genomic fragment containing the *AGP17* gene (RAT1::gRAT1) or with an *AGP17* cDNA under the control of a cauliflower mosaic virus (CaMV) 35S promoter (35S::cRAT1). A floral dip method (Clough and Bent, 1998) was used to obtain multiple independent transformants because this method has worked successfully for other *rat* mutants (Mysore et al., 2000). We tested 27 independent transformants (T1) containing the *AGP17* gene and 25 transformants containing the *AGP17* cDNA using a root transformation assay (Nam et al., 1999). Analysis of the T1 was possible because the *rat* phenotype is haploinsufficient (semidominant; Nam et al., 1999). Figure 3 shows examples of successful complementation of *rat1* by *AGP17* genomic and cDNA clones. However, transformation was not always successful. The extent of tumor formation on root segments of wild-type plants, the homozygous *rat1* mutant, and the individual complemented transformants is shown in Supplemental Figure 1 (available at www.plantphysiol.org). We initially speculated that the lack of full phenotypic complementation might result from inappropriate expression from the 35S promoter. However, this explanation is likely to be not valid because we saw a similar proportion of complementation using *RAT1* genomic constructions. Therefore, the most likely explanation for the lack of full phenotypic complementation in many lines is that the expression of the complementing transgene was not sufficient, or was inappropriately controlled, due to the position of the insertion of the complementation construct into the genome. The haploinsufficient nature of the *rat1* mutant (Nam et al., 1999) is consistent with the suggestion that different levels of *AGP17* transcript have different effects on transformation efficiency.

Agrobacterium Can Still Synthesize Cellulose on *rat1* Roots

To extend the results of Nam et al. (1999), we used scanning electron microscopy to look at Agrobacterium binding to *rat1* roots (Fig. 4). In *rat1* plants, Agrobacterium binding is reduced, with only occasional binding on the root surface (Fig. 4A) and at the root hair tips (Fig. 4B). Where bacteria were present on *rat1* roots, cellulose microfibrils were evident (Fig. 4B, arrows). This suggests that the *rat1* phenotype results from a defect in the loose or initial binding step in Agrobacterium infection and not in the second step involving the synthesis of cellulose by the bacterium

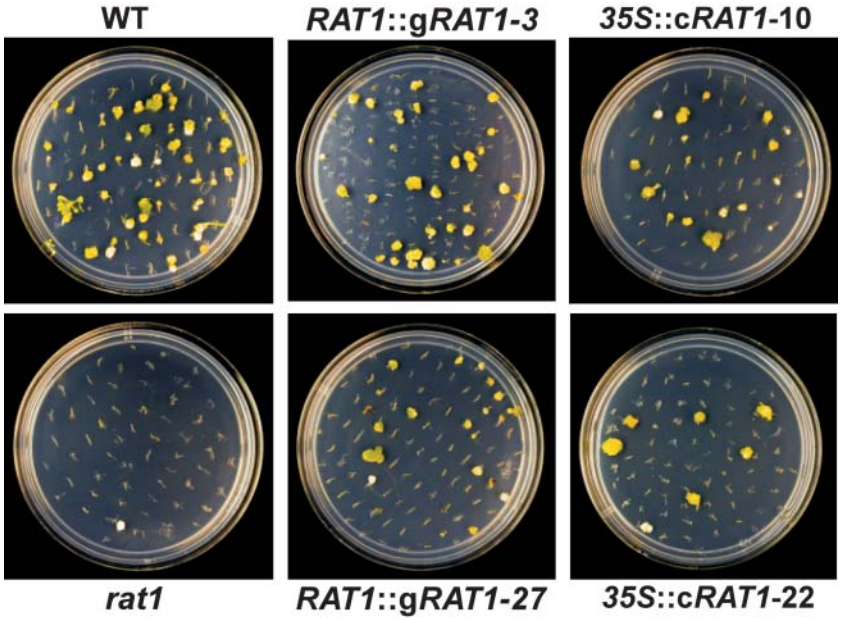

Figure 3. Complementation of the rat phenotype in the *rat1* mutant by expression of a wild-type *AGP17* genomic clone or a wild-type *AGP17* cDNA clone in *rat1* mutant plants. Root tumorigenesis assays were conducted on individual T1 plants to test complementation of the *rat1* mutant. Assays shown were conducted on root segments of control wild-type and *rat1* mutant plants as well as selected genomic (*RAT1*::g*RAT1*-3 and *RAT1*::g*RAT1*-27) and cDNA (*35S*::c*RAT1*-10 and *35S*::c*RAT1*-22) complementation lines. The genomic complementation was repeated on four different days and the cDNA complementation on two different days.

that promotes close adhesion to the root surface (Matthysse, 1994; Matthysse et al., 1995; Matthysse and McMahan, 1998).

β-D-Glucosyl Yariv Reagent Inhibits Transformation of Arabidopsis Roots

Because roots of the *agp17/rat1* mutant are resistant to Agrobacterium transformation, we were interested in determining the effect of an AGP-binding reagent on transformation. AGPs bind to the synthetic dye, β-D-Glc Yariv reagent, but not to the β-D-mannosyl (β-D-Man) Yariv derivative (Yariv et al., 1967). We investigated the effect of these two Yariv reagents on Agrobacterium transformation of Arabidopsis root segments (Fig. 5). To visualize transformation, we used the Agrobacterium strain At849. This nontumorigenic strain contains the T-DNA binary vector pBISN1 (Narasimhulu et al., 1996) encoding a *gusA*-intron gene under the control of a strong promoter (Ni et al., 1995). When wild-type root segments were infected with *A. tumefaciens* At849 either in the absence of β-D-Glc Yariv reagent or in the presence of β-D-Man Yariv reagent (which does not bind to AGPs), expression of β-glucuronidase (GUS) activity could be seen at the cut ends of the root segments (Fig. 5B). However, when the wild-type roots were pretreated with β-D-Glc Yariv reagent prior to infection, transformation occurred at only a very low level as indicated by reduced GUS activity (<10% that of control plants; Fig. 5). When observed by light microscopy, β-D-Glc Yariv reagent inhibited binding of Agrobacterium to whole wild-type roots, whereas binding of Agrobacterium to *rat1* roots under the same conditions remained low, i.e. unchanged (data not shown). Controls indicated that at the concentrations used, Yariv reagents did not inhibit the growth of Agrobacterium or reduce the ability of Arabidopsis roots to form callus on callus-inducing medium (data not shown). The Yariv effects upon transient GUS expression support the idea that AGPs are important in Agrobacterium-mediated transformation of roots.

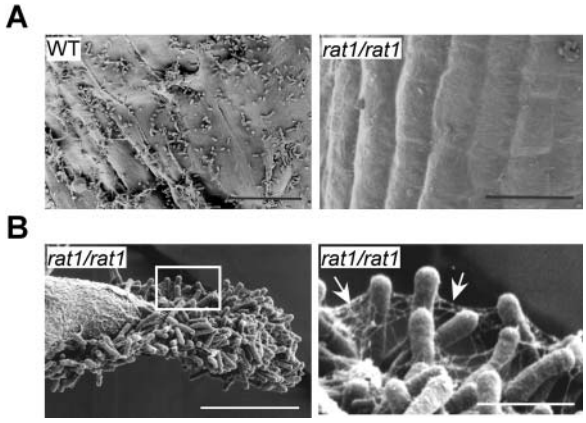

Figure 4. The *rat1* mutant has decreased binding of Agrobacterium to its roots. A, Scanning electron microscopy of Arabidopsis roots (wild type and *rat1*) inoculated with *A. tumefaciens* GV3101 for 12 h. Bar represents 20 μM. B, Scanning electron microscopy of a *rat1* root hair tip showing the presence of cellulose microfibrils (arrows). Bar in the left section represents 10 μM; bar in the right section represents 2.5 μM.

Down-Regulation of *PR1* Gene Expression during Agrobacterium Infection in Wild-Type Plants But Not *rat1*

Infection of plant cells with transformation-competent Agrobacterium strains alters the pattern of plant gene expression (Ditt et al., 2001; Veena et al., 2003). Recently it has been shown that defense-related genes, such as β-1,3-glucanase, are repressed in tobacco

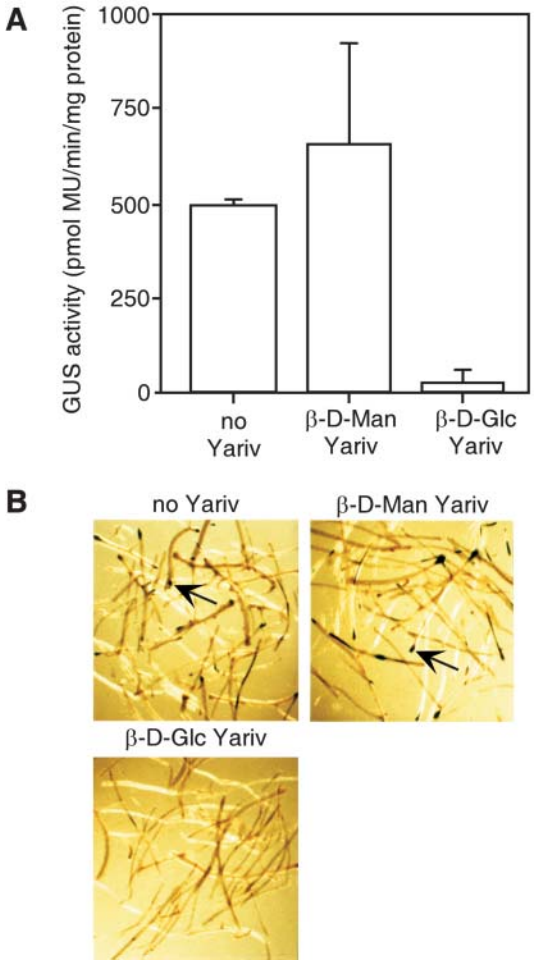

Figure 5. Effect of Yariv reagent on the transformation of wild-type Arabidopsis root segments with *A. tumefaciens* At849. A, Root segments were preincubated for 1 h in the absence or presence of the Yariv reagents (*β*-Glc Yariv [positive] or *β*-Man Yariv [control]) before inoculation with Agrobacterium. Six days after the start of cocultivation, root segments were assayed for GUS activity. B, Staining of root segments for GUS activity with X-glucuronidase (arrows).

BY-2 cells after inoculation with Agrobacterium (Veena et al., 2003). There are two major defense response pathways in plants, the SA-dependent, SAR pathway that is characterized by the induction of pathogenesis-related (PR) genes, such as *PR1* and *PR5* (for review, see Shah, 2003) and an SA-independent resistance pathway that is characterized by the induction of the *plant defensin1.2* (*PDF1.2*) gene by jasmonate and ethylene (Gu et al., 2002). It is not known if either (or both) of these pathways is activated during the first few hours of Agrobacterium infection when root binding is known to occur (Matthysse, 1986).

Defense genes are generally expressed at low levels in unwounded plants (Uknes et al., 1992), so we used semiquantitative RT-PCR to investigate the expression of the *PR1*, *PR5*, and *PDF1.2* genes in *rat1* and wild-type roots, with or without inoculation with Agrobacterium (Fig. 6). Root samples were collected at 0, 1, 2, 3, and 4 h postinfection. These early time points were chosen because *rat1* affects the binding of Agrobacterium, which occurs in the first few hours of Agrobacterium infection (Sykes and Matthysse, 1986). Both *PR1* and *PR5* mRNAs were detected in untreated hydroponically grown wild-type and *rat1* roots (Fig. 6A), although it took 35 cycles to obtain an observable PCR product, suggesting that expression levels are low. When roots were inoculated with Agrobacterium, the level of PR gene expression remained relatively stable in the *rat1* mutant. In wild-type roots, *PR1* expression was reduced (approximately 4-fold) within 1 h postinfection. *PR5* expression was also reduced during this time and was constitutively higher (approximately 4-fold) in *rat1* plants than in wild-type plants. *PDF1.2* cDNA was not detected in any of the samples. A PCR product of the correct size was amplified from genomic DNA (data not shown), indicating that the PCR primers would have amplified a *PDF1.2* cDNA if the gene were expressed (Fig. 6A).

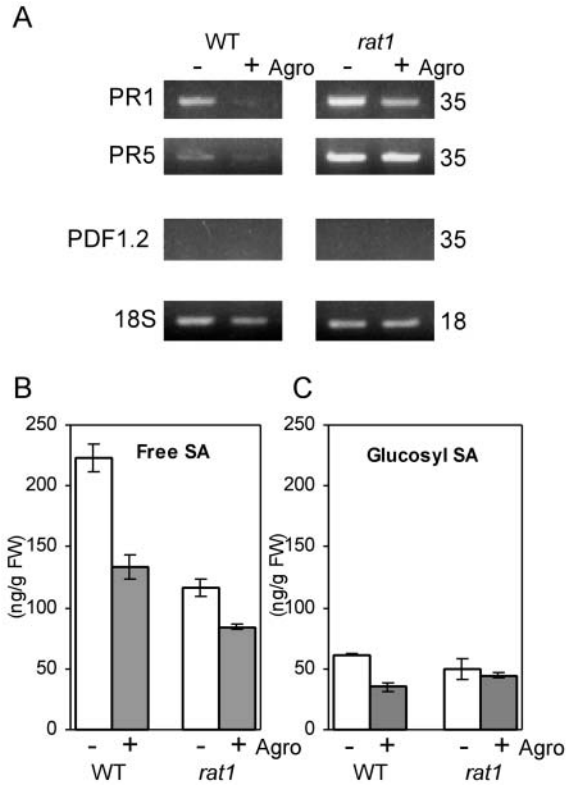

Figure 6. A, Semiquantitative RT-PCR showing expression in roots of the PR genes *PR1* and *PR5* and the jasmonic acid/ethylene-regulated gene *PDF1.2*. Hydroponically grown wild-type and *rat1* Arabidopsis roots were incubated without or with Agrobacterium for 1 h. 18S rRNA transcript levels were used to normalize the samples. The 18S rRNA control panel is representative of the above experiments. Numbers at right indicate the number of PCR cycles used. B and C, Endogenous levels of SA in hydroponically grown Arabidopsis roots inoculated with *A. tumefaciens*. Concentrations of free SA (B; ng/g FW) and glucosyl SA (C; released by *β*-glucosidase treatment; ng/g FW) after the addition of Agrobacterium for 1 h. Bar is the mean of three replicated extracts (± SE).

Expression trends of *PR1*, *PR5*, and *PDF1.2* continued in tissues 2, 3, and 4 h postinfection with Agrobacterium (data not shown).

Decrease of SA Levels Is an Early Response to Agrobacterium Infection

The reduction in PR gene expression observed in wild-type plants 1 h postinfection suggests that Agrobacterium may affect the SAR pathway. To investigate this further, we determined the levels of SA in roots of both wild-type and *rat1* mutant plants 1 h postinfection by Agrobacterium. To quantify SA, the levels of total and free SA were determined using published methods with substantial modification (Dewdney et al., 2000; ''Materials and Methods''). Total SA includes SA and the sugar conjugate of SA, β-glucosyl SA (G-SA; Hennig et al., 1993). The levels of both were determined, even though G-SA is not thought to be an active component because G-SA can be converted to biologically active SA in vivo (Hennig et al., 1993). To determine total SA levels from root extracts, G-SA was converted to SA in vitro using a β-glucosidase (see ''Materials and Methods''). Mass spectrometry (MS) was used to confirm the identity of SA in the root fraction that eluted at the same time as an SA standard using reverse phase (RP)-HPLC. MS showed the fraction included SA and other unidentified components (data not shown). To obtain accurate quantification of SA, it was necessary to rechromatograph the SA containing fraction from the first separation using a step gradient (see ''Materials and Methods''). This resulted in four major peaks with the SA-containing fraction representing approximately 25% of the original peak (data not shown).

In uninfected roots, the level of free SA in wild-type roots is almost double the level observed in *rat1* roots (Fig. 6B), whereas the levels of G-SA in wild-type and *rat1* roots are similar (Fig. 6C). When wild-type plants are challenged with Agrobacterium, the levels of free SA decreased by about 40% (Fig. 6B). The difference between the treated and untreated roots is significant ($P < 0.05$) based on ANOVA, as is the difference between the wild type and *rat1*. The level of G-SA does not change significantly after Agrobacterium infection in *rat1*, although there is an observable decrease in wild-type plants.

DISCUSSION

The Rat Phenotype of *rat1* Is Caused by Down-Regulation of *AGP17* in the Root

The presence of a T-DNA insertion in the promoter of the Lys-rich AGP gene *AGP17* results in reduced *AGP17* expression in roots of *rat1* Arabidopsis plants (Figs. 1 and 2). Several lines of evidence indicate that this is the basis of the resistance to Agrobacterium transformation in the *rat1* mutant. These include (1) cosegregation of kanamycin resistance with the *rat* phenotype (Nam et al., 1999) and the presence of only one T-DNA locus in *rat1*; (2) no DNA rearrangements near the insertion site affecting the expression of other genes (see ''Results''); and (3) complementation of the rat phenotype in *rat1* plants by introducing a wild-type *AGP17* gene or cDNA (Fig. 3 and Supplemental Fig. 1).

SA Levels Are Reduced in Wild-Type Plants during Agrobacterium Infection

It was surprising that SA was present, and PR genes were expressed, at the zero time points in the hydroponics system used to grow Arabidopsis plants for this study. It is likely that the PR gene expression is much lower than would be detected by RNA gel-blot analysis because it took 35 cycles for a modest amount of RT-PCR product to be produced. It is possible that these low levels of PR gene expression are not relevant to SAR or that roots behave differently from leaves, and there is always a basal level of *PR1* expression in uninfected roots. However, a more likely explanation is that the changes in *PR1* gene expression are important, and the hydroponic system used to grow the plants is eliciting a wounding or stress response, leading to low levels of PR gene expression and free SA accumulation. The sustained PR gene expression in *rat1* plants up to 4 h postinfection (data not shown) is consistent with the suggestion that the modest change in SA levels in *rat1* plants is not enough to down-regulate the SAR response and, hence, PR gene expression remains unchanged. The higher levels of PR gene expression but lower levels of SA in *rat1* compared to the wild type may result from cross talk between different signaling pathways; ethylene has been suggested as an alternate activator of PR1 gene expression (Kunkel and Brooks, 2002).

Our analysis of wild-type and *rat1* plants provides further support for the hypothesis that Agrobacterium alters plant defense responses (Ditt et al., 2001; Veena et al., 2003). Furthermore, it suggests that plants may detect relative changes, not absolute levels of SA. This is based on the observation that uninfected *rat1* plants have lower initial levels of SA but only a 25% change in SA levels, whereas wild-type plants have a 40% drop in free SA levels after Agrobacterium inoculation (Fig. 6B). This suggests that it is the degree of change in SA concentration (disruption of homeostasis) that determines the level of response. Another observation from our data is that there is no corresponding increase in G-SA in wild-type plants, suggesting that plants can actively degrade SA as occurs in bacteria (Gaffney et al., 1993; Delaney et al., 1994).

These results are consistent with recent findings that the expression of hundreds of plant genes is modulated following Agrobacterium infection in several plant systems, including *Ageratum conyzoides* (Ditt et al., 2001) and *Nicotiana tabacum* BY-2 suspension culture cells (Veena et al., 2003). Among the tobacco genes affected by cocultivation with transfer-competent but not transfer-deficient Agrobacterium strains are

defense genes, including PR genes, several classes of glutathione *S*-transferase genes, and other defense-related genes (Veena et al., 2003).

Mechanisms for the Possible Involvement of *AGP17* in Agrobacterium-Mediated Transformation

We suggest two possible, nonexclusive mechanisms to explain the role of AGP17 in Agrobacterium-mediated transformation. In the first, AGP17 is required by Agrobacterium to bind directly to the root surface. Binding can be directly to AGP17, or AGP17 may mediate binding by altering the plant cell wall, thus presenting a different receptor. Because secreted AGPs are generally soluble, direct binding would probably only occur if AGP17 were cross-linked to other cell wall components, for example, cell wall polysaccharides (for review, see Serpe and Nothnagel, 1999). In Angelica roots, there is evidence that AGPs are cross-linked to pectic polysaccharides in the plant cell wall (Yamada et al., 1985; Kiyohara et al., 1997), and the Lys-rich region of the AGP17 protein backbone could provide suitable amino acid residues for cross-linking.

The Yariv binding experiments (Fig. 5) provide some support for the direct attachment model. The disruption of Agrobacterium attachment by β-Glc Yariv reagent suggests that receptor sites for binding are rendered inaccessible by this AGP-specific reagent (Fig. 5). An alternate explanation is that the cross-linking of many different AGPs by β-Glc Yariv at the cell surface provides a physical barrier that prevents binding of the bacterium and/or entry of the T-DNA into the cell.

For the second mechanism, Agrobacterium does not require AGP17 to bind to the root cell wall. Rather, AGP17 is involved in a signaling pathway(s) that consequently affects the ability of Agrobacterium to bind to the root surface. In this scenario, AGP17 is required to reduce the levels of SA, by an unknown mechanism, when Agrobacterium binds to the root. Assuming AGP17 is GPI anchored as predicted, it could form part of a signaling cascade in one of two ways. With its GPI anchor still attached, AGP17 would be bound to the outer surface of the plasma membrane and therefore could interact with receptor-like kinases such as the wall-associated kinases (Anderson et al., 2001). Receptor-like kinases have both an extracellular and cytoplasmic domain, and one of the wall-associated kinases is thought to interact with molecules containing AGP epitopes (Gens et al., 2000). Alternatively, AGP17 could become a soluble signaling molecule if released from the plasma membrane by GPI anchor-specific phospholipases (Schultz et al., 1998; Youl et al., 1998).

What Structural Features of AGP17 Are Important for Transformation?

In both the scenarios described above, the role of AGP17 is nonredundant because the *rat* phenotype would not be apparent if other AGPs were able to fulfill the role(s) of AGP17.

It is not known whether carbohydrate moieties or the Lys-rich domain of the protein backbone of AGP17 are important for the interaction of Agrobacterium with the plant surface. The basic Lys-rich domain, also found in *AGP18* and *AGP19*, might directly interact with the acidic polysaccharide from Agrobacterium that was shown to be important for Agrobacterium binding to carrot cells (Reuhs et al., 1997). Alternatively, the carbohydrate moiety may be important because another *rat* mutant, *rat3*, is also deficient in Agrobacterium attachment (Nam et al., 1999). The RAT3 protein contains the amino acid sequence Ala-Pro-Ala-Pro-Ser-Pro-Thr-Ser (GenBank accession no. At5g63250) that is a motif, also known as a glycomodule, found in AGPs, and is likely to direct the addition of type II AG *O*-linked-polysaccharides (Goodrum et al., 2000; Borner et al., 2002, 2003; Johnson et al., 2003b; Tan et al., 2003). By selectively removing or modifying different domains within AGP17 and using these modified constructs in complementation experiments, we should be able to determine which regions of AGP17 are required for binding the bacterium and/or its elicitors.

CONCLUSION

The likelihood of multiple rounds of communication between Agrobacterium and plant cells to establish binding was first proposed by Matthysse (1994) based on experiments with Agrobacterium mutants. The availability of Arabidopsis *rat* mutants allows us to investigate the role of the plant genes involved in Agrobacterium binding. Of the more than 125 *rat* mutants identified, only a few of these (*rat1*, *rat3*, and *rat4*) have been shown to be defective in binding (Zhu et al., 2003a, 2003b). Our results suggest a signal transduction pathway between AGP17/RAT1 at the plant surface and intracellular changes in SA levels and gene expression of the key defense response gene *PR1*. Future characterization of *rat1* and other *rat* mutants deficient in Agrobacterium attachment should enable the identification of other components of the SA-dependent PR pathway that interact with AGP17.

MATERIALS AND METHODS

Plant and Bacterial Growth Conditions

Seeds of Arabidopsis (ecotype Wassilevskija-2 [Ws-2]) were grown either in soil and maintained under standard glasshouse conditions (approximately 26°C, 16 h light), on agar plates (0.5× Murashige and Skoog medium, 3% [w/v] Suc, 1% [w/v] agar), in agitated liquid cultures (Gamborg's B-5 liquid; Invitrogen, Carlsbad, CA; 26°C, 16 h light, 50 rpm; Reiter et al., 1992) or hydroponically (0.5× Optimum Grow hydroponic nutrient solution, pH 6; Growth Technology, O'Connor, Western Australia; aerated, 26°C, 16 h light). For hydroponic growth, 2-week-old plants from agar plates were transferred to precut holes in styrofoam sheeting and floated on sterile hydroponic liquid medium. The medium was aerated with filtered air from a standard aquarium pump. Plants were acclimated for 1 week before sampling or inoculation with Agrobacterium.

For axenic growth, seeds were surface sterilized for 5 min with 12% (v/v) sodium hypochloride containing a drop of 50% (v/v) Tween 20 per 5 mL of sodium hypochloride, then washed several times with sterile distilled water. Plant tissue was collected and stored at −70°C before use.

Agrobacterium tumefaciens A208 (Sciaky et al., 1978; used for root tumorigenesis assays), At849 (Nam et al., 1998; used for GUS assays), or GV3101 (Koncz and Schell, 1986; used for all other assays) were grown in Agrobacterium broth minimal, yeast extract peptone, or Luria-Bertani-rich medium (Lichtenstein and Draper, 1986) at 30°C.

Screening T-DNA Lines

Oligonucleotides specific to the *AGP17* gene (AGP17F, 5′-TCGCAATATTCTCTTGACGG-3′; AGP17R, 5′-GGCTAGAACAAGTAGAGACC-3′) and the borders of the T-DNA insert (right border, 5′-GCTCAGGATCCGATTGTGGTTTCCCGCCTT-3′; left border, 5′-GATGCAATCGATATCAGCCAATTTTAGAC-3′) were used to screen DNA from the T-DNA transformed lines (Feldmann, 1991) by PCR. Standard molecular biology techniques were used as described (Sambrook et al., 1989). An oligonucleotide specific to the region of genomic DNA upstream of the T-DNA insertion site (Fig. 1A; T-DNAF, 5′-TTAGTGTATATGGTTGCTACGTGC-3′) was used in conjunction with oligonucleotide AGP17R2 (5′-GAGAAAGCATCGCTTGGTCC-3′) and the left-border and right-border T-DNA oligonucleotides to determine the arrangement of T-DNA insertions and to sequence the surrounding DNA. Oligonucleotides specific to the gene upstream of *AGP17*, At2g23120 (At2gF, 5′-AAGCAGACACATCTCATGAAGC-3′; At2gR, 5′-TTTCCGACGATCCACAAAAC-3′), were used for RT-PCR as described below.

Microscopy of Root Segments Inoculated with Agrobacterium

Wild-type and *rat1* mutant Arabidopsis plants were grown for 2 weeks in agitated liquid cultures as described previously (Reiter et al., 1992). *A. tumefaciens* GV3101 was grown for 2 d in Luria-Bertani broth at 26°C until they reached an A_{600} = 1. Flasks containing Arabidopsis plants were inoculated with the bacterial culture (0.5 mL/100 mL) and were incubated at 26°C with agitation at 50 rpm. Seedlings were harvested 12 h postinoculation.

Immediately following harvesting, roots were carefully washed free of growth media with distilled water and cut into segments (2 mm in length). Root segments were fixed overnight in 2.5% (v/v) glutaraldehyde in 0.05 M PIPES buffer, pH 7.2, under vacuum. Segments were washed in 0.05 M PIPES buffer and postfixed in 1% (v/v) osmium tetroxide for 2 h. The osmium tetroxide was removed, and segments were washed with distilled water and dehydrated in a graded ethanol series. Segments were critical point dried in a Samdri PVT-3 (Tousimis Research, Rockville, MD) critical point dryer and were platinum coated using an Edwards S150B Sputter Coater (Sussex, UK). The roots were examined using a Philips XL Series scanning electron microscope (Eindhoven, The Netherlands) with an accelerating voltage of 2 kV.

RNA-Blot Analysis

Total RNA was extracted from soil-grown Arabidopsis tissues as described previously (Wadsworth et al., 1988) using a guanidinium isothiocyanate-based extraction buffer. Tissues used were roots, unopened flowers (0.5–1.5 mm in length), opened flowers (1.5–2 mm in length), cauline leaves (0.5–1.5 cm in length), rosette leaves (1.5–3 cm in length), young inflorescence stems (first internode from the growing tip greater than 1 cm in length), mature inflorescence stems (first internode from the base), and young siliques (0.5–1 cm in length). Total RNA was subjected to electrophoresis through a 1.5% (w/v) agarose gel containing formaldehyde (Sambrook et al., 1989) and transferred to a nylon membrane (ZETA-PROBE; Bio-Rad, Cambridge, MA) following the manufacturer's instructions. Membranes were hybridized overnight (65°C) using a dextran sulfate-based hybridization solution as recommended by Bio-Rad for increased sensitivity. Probes were made by labeling *AGP17* DNA fragments with [α^{32}P]dCTP using a random primer DNA labeling kit (MegaPrime; Amersham Biosciences, Buckinghamshire, UK) according to the manufacturer's instructions.

Semiquantitative RT-PCR

Total RNA was extracted from hydroponically grown untreated (time = 0 h) plants and plants inoculated with *A. tumefaciens* GV3101 for 1, 2, 3, 4, 8, 12, 24, and 48 h using a TRIzol reagent extraction protocol (Invitrogen) according to the manufacturer's instructions. *A. tumefaciens* GV3101 was grown for 2 d in Luria-Bertani broth at 26°C until they reached an A_{600} = 1. Bacteria (5 mL) were added to the hydroponic liquid solution (1 L), and the seedlings were harvested at various time points postinoculation. Roots were removed from the plants, and leaf and root tissues were frozen separately at −70°C until RNA extraction.

RNA treatment, first-strand cDNA synthesis, and semiquantitative RT-PCR were performed as described previously (Lasserre et al., 1996; Zegzouti et al., 1999) with modifications. RNase-free DNase I (Roche Diagnostics, Indianapolis) at 0.25 unit per μg of total RNA with Ribonuclease Inhibitor (Promega, Madison, WI) was added to 4 units per μg of total RNA. Enzymes were removed by purification with a Qiagen RNeasy kit (Valencia, CA) following the manufacturer's instructions. PCR was performed directly using the RNA, prior to RT, to verify the absence of contaminating genomic DNA. If needed, the DNase I treatment was repeated. Random primers (1 μg; Promega) were annealed to 2 μg of total heat denatured RNA in a final volume of 10 μL. First-strand cDNA was synthesized at 42°C for 1 h using 200 units of Superscript II Reverse Transcriptase (Invitrogen) in the presence of Ribonuclease Inhibitor (40 units; Promega), 1× PCR buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.5 mM of each deoxynucleotide phosphate, and 10 mM dithiothreitol. The reverse transcribed samples were used as a template for the amplification of *AGP17* using 15 pmol of gene specific oligonucleotides (AGP17F, AGP17R), and, as an internal control, a fragment of the 18S rRNA was amplified concomitantly with the *AGP17* cDNA by addition, after a predetermined number of cycles, of 15 pmol of 18S-specific oligonucleotides (18SF, 5′-CATCAGCTCGCGTTGACTAC-3′; 18SR, 5′-GATCCTTCCGCAGGTTCAC-3′; Cho and Cosgrove, 2000). The PCR conditions were as follows: an initial 5-min denaturation at 96°C, *n* cycles of 94°C, 30 s; 55°C, 30 s; and 72°C, 1.5 min, and a final extension of 7 min at 70°C. The amount of cDNA used in each PCR was adjusted so that RT-PCR amplification of 18S rRNA resulted in a band of similar intensity to that of the target mRNA. To determine that the number of cycles needed for 18S and *AGP17* amplification was within the exponential amplification phase, PCR reactions were set up, removed from a GeneAmp 9600 thermocycler (Applied Biosystems, Melbourne, Australia) at consecutive cycles and the product amounts assessed by electrophoresis. To maintain RT-PCR amplification within the exponential phase, the number of PCR cycles used for 18S rRNA was 18 or 21 cycles for leaf and root cDNA, respectively, and for the *AGP17* transcript, 30 or 40 cycles for leaf and root cDNA, respectively.

cDNA was subjected to electrophoresis through 1.4% (w/v) agarose gels and transferred to a nylon membrane (MAGNA; Micron Separations, Westborough, MA) following the manufacturer's instructions. The membranes were hybridized with an equal concentration of digoxigenin probes for *AGP17* and 18S rRNA. The digoxigenin-labeled probes were synthesized by PCR following the manufacturer's instructions (Roche Diagnostics). All RT-PCR reactions were performed in duplicate and in two independent experiments.

Genetic Complementation of the *rat1* Mutant

We employed the floral dip method (Clough and Bent, 1998) to introduce either a wild-type *RAT1* cDNA under the control of a CaMV 35S promoter or a wild-type *RAT1* genomic clone into *rat1* plants (Mysore et al., 2000). The genomic complementing clone consisted of a 6-kb *Eco*RI fragment containing the entire *AGP17* gene, including upstream and downstream elements, cloned into the T-DNA binary pGPTV-HPT (Becker et al., 1992). The genomic fragment used in the complementation experiments also includes two upstream genes At2g23110 and At2g23120. These two genes are both related to late embryogenesis abundant genes and are unlikely to be involved in the *rat1* phenotype. This interpretation is supported by the complementation with the cDNA clone for AGP17. The cDNA complementing clone consisted of a wild-type *RAT1* cDNA clone inserted into the *Sal*I site of the T-DNA binary vector pS35-hpt, under the control of a CaMV 35S promoter. The genomic and cDNA constructions are referred to as *RAT1*::g*RAT1* and *35S*::c*RAT1*, respectively. The binary vectors were introduced into *A. tumefaciens* GV3101, and the plants were infected using a flower-dip protocol (Clough and Bent, 1998). Hygromycin-resistant transformants were selected and tested, in the T1 generation, for tumorigenesis as described in Nam et al. (1999). A minimum of 50 root segments was tested for each plant.

Analysis of Genes Involved in the Defense Response

Analysis of gene expression of the *PR1*, *PR5* (Uknes et al., 1992), and *PDF1.2* (Penninckx et al., 1996) genes was performed using the RT-PCR

protocol described above, 1 h postinoculation with *A. tumefaciens* GV3101. *PR1*-, *PR5*-, and *PDF1.2*-specific oligonucleotide pairs (PR1F, 5′-TTCTTCCC-TCGAAAGCTCAA-3′; PR1R, 5′-ACACCTCACTTTGGCACATC-3′; PR5F, 5′-TCCGGAAACGGTAGATGTGT-3′; PR5R, 5′-GTGCTCGTTTCGTCGTCA-TA-3′; PDF1.2F, 5′-GCTAAGTTTGCTTCCATCATC-3′; PDF1.2R, 5′-GACG-TAACAGATACACTTGTG-3′) were designed based on Arabidopsis *PR1*, *PR5* (Uknes et al., 1992), and *PDF1.2* (Penninckx et al., 1996) cDNA sequences and used for PCR.

Inhibition of Transformation by Yariv Reagents

Arabidopsis root segments (150) pooled from 10 to 20 wild-type plants were incubated with 50 μM β-Glc or β-Man Yariv reagent (Biosupplies Australia Pty, Melbourne, Australia) or no Yariv (control) for 1 h, and then inoculated with *A. tumefaciens* At849. After 15 h of cocultivation, the root segments were moved to Murashige and Skoog medium containing 100 μg/mL timentin to kill the bacteria. The roots were assayed for GUS activity after 4 d (Jefferson et al., 1987). All assays were repeated in triplicate.

Quantification of SA

SA was extracted and analyzed by RP-HPLC using a modification of the methods described by Dewdney et al. (2000). For SA analysis, 1 g fresh weight (FW) of Arabidopsis root tissue was divided into three equal samples, frozen, and stored at −70°C until use. One sample from each test was ground in liquid nitrogen to a fine powder and 250 ng *o*-anisic acid (Sigma, St. Louis) was added to each sample as an internal standard before extraction in methanol. To determine total SA (free SA and glucosyl SA), one-half the extract was treated by enzymic hydrolysis with β-glucosidase (60 units mL^{-1}; Sigma), whereas for free SA, the extract was not hydrolyzed. Final extracts were dried under nitrogen and frozen at −70°C. RP-HPLC separation of *o*-anisic acid and SA was performed on a System Gold (Beckman Instruments, Fullerton, CA) HPLC equipped with a scanning fluorescence detector (FP-920 Intelligent; Jasco, Easton, MD) and a photodiode array detector (Beckman Instruments). A 5 μM, 25-cm × 2.1-mm C18 column (218TP; Vydac, Easton, MD) was maintained at 22°C in 65% (v/v) 0.025 M H_3PO_4/20% (v/v) methanol/6% (v/v) acetonitrile with a flow rate of 0.2 mL/min. Each sample was manually injected (50 μL) and separated isocratically for 35 min. SA was quantified with a fluorescence detector programmed to 305 nm excitation/415 emission and for *o*-anisic acid 305 nm excitation/365 emission. SA peaks at approximately 13.5 min contained contaminating products. To purify SA, we rechromatographed the SA containing peak using a different gradient. Products were eluted at 22°C with a hold at 25% (v/v) methanol/0.5% (v/v) acetic acid for 10 min, a linear gradient from 25% to 65% (v/v) methanol/0.5% (v/v) acetic acid for 10 min, and then constant for 15 min at a flow rate of 0.2 mL/min. All SA results were adjusted for recovery using the internal standards (*o*-anisic acid), and extracts were independently prepared in triplicate for each sample. RP-HPLC peaks were collected and dried under nitrogen prior to derivatization for analysis by MS. Methanolysis was performed by addition of methanolic HCl (1 M) and incubation for 4 h at 80°C. Once cooled, samples were carefully dried and resuspended in dichloromethane. Samples were analyzed by static nanospray MS using Econotip10 (New Objective, Woburn, MA) in negative ion mode on the QSTAR XL mass spectrometer (Applied Biosystems). MS/MS was performed with an ion source voltage of −1,200 V, collision energy of −40 V, and with Q1 in low-resolution mode. Data were analyzed using AnalystQS software (Applied Biosystems).

Distribution of Materials

Upon request, all novel materials described in this publication will be made available in a timely manner for noncommercial research purposes, subject to the requisite permission from any third party owners of all or parts of the material. Obtaining any permissions will be the responsibility of the requestor.

Sequence data from this article have been deposited with the EMBL/GenBank data libraries under accession numbers At2g23120 and At5g63250.

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LITERATURE CITED

Anderson CM, Wagner TA, Perret M, He Z-H, He D, Kohorn BD (2001) WAKs: cell wall-associated kinases linking the cytoplasm to the extracellular matrix. Plant Mol Biol **47:** 197–206

Becker D, Kemper E, Schell J, Masterson R (1992) New plant binary vectors with selectable markers located proximal to the left T-DNA border. Plant Mol Biol **20:** 1195–1197

Borner GHH, Lilley KS, Stevens TJ, Dupree P (2003) Identification of glycosylphosphatidylinositol-anchored proteins in *Arabidopsis*. A proteomic and genomic analysis. Plant Physiol **132:** 568–577

Borner GHH, Sherrier DJ, Stevens TJ, Arkin IT, Dupree P (2002) Prediction of glycosylphosphatidylinositol-anchored proteins in *Arabidopsis*. A genomic analysis. Plant Physiol **129:** 486–499

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

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J **16:** 735–743

Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, et al (1994) A central role of salicylic acid in plant resistance. Science **266:** 1247–1250

Dewdney J, Reuber TL, Wildermuth MC, Devoto A, Cui J, Stutius LM, Drummond EP, Ausubel FM (2000) Three unique mutants of *Arabidopsis* identify eds loci required for limiting growth of a biotrophic fungal pathogen. Plant J **24:** 205–218

Ditt RF, Nester EW, Comai L (2001) Plant gene expression response to *Agrobacterium tumefaciens*. Proc Natl Acad Sci USA **98:** 10954–10959

Du H, Clarke AE, Bacic A (1996) Arabinogalactan-proteins: a class of extracellular matrix proteoglycans involved in plant growth and development. Trends Cell Biol **6:** 411–414

Eisenhaber B, Wildpaner M, Schultz CJ, Borner GHH, Dupree P, Eisenhaber F (2003) Glycosylphosphatidylinositol lipid anchoring of plant proteins. Sensitive prediction from sequence- and genome-wide studies for *Arabidopsis* and rice. Plant Physiol **133:** 1691–1701

Feldmann KA (1991) T-DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum. Plant J **1:** 71–82

Gaffney T, Friedrich L, Vernooij B, Negretto D, Nye G, Uknes S, Ward E, Kessmann H, Ryals J (1993) Requirement of salicylic acid for the induction of systemic required resistance. Science **261:** 754–756

Gaspar Y, Johnson KL, McKenna JA, Bacic A, Schultz CJ (2001) The complex structure of arabinogalactan-proteins and the journey towards understanding function. Plant Mol Biol **47:** 161–176

Gens JS, Fujiki M, Pickard BG (2000) Arabinogalactan protein and wall-associated kinase in a plasmalemmal reticulum with specialized vertices. Protoplasma **212:** 115–134

Gilson P, Gaspar YM, Oxley D, Youl JJ, Bacic A (2001) NaAGP4 is an arabinogalactan-protein whose expression is suppressed by wounding and fungal infection in *Nicotiana alata*. Protoplasma **215:** 128–139

Goodrum LJ, Patel A, Leykam JF, Kieliszewski MJ (2000) Gum arabic glycoprotein contains glycomodules of both extensin and arabinogalactan-glycoproteins. Phytochemistry **54:** 99–106

Gu Y-Q, Wildermuth MC, Chakravarthy S, Loh Y-T, Yang C, He X, Han Y, Martin GB (2002) Tomato transcription factors Pti4, Pti5, and Pti6 activate defense responses when expressed in *Arabidopsis*. Plant Cell **14:** 817–831

Hennig J, Malamy J, Grynkiewicz G, Indulski J, Klessig DF (1993) Interconversion of the salicylic acid signal and its glucoside in tobacco. Plant J **4:** 593–600

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J **6:** 3901–3907

Johnson KL, Jones BJ, Bacic A, Schultz CJ (2003a) The fasciclin-like arabinogalactan-proteins (FLAs) of *Arabidopsis thaliana*: a multigene family of putative cell adhesion molecules. Plant Physiol **133:** 1–15

Johnson KL, Jones BJ, Schultz CJ, Bacic A (2003b) Non-enzymic cell wall (glyco)proteins. *In* J Rose, ed, The Plant Cell Wall. Blackwell Publishers, Oxford

Kiyohara H, Zhang Y, Yamada H (1997) Effect of exo-beta-D-(1-3)-galactanase digestion on complement activating activity of neutral arabinogalactan unit in a pectic arabinogalactan from roots of *Angelica acutiloba* kitagawa. Carbohyd Polym **32:** 249–253

Knox JP (1995) The extracellular matrix in higher plants: developmentally regulated proteoglycans and glycoproteins of the plant cell surface. FASEB J **9:** 1004–1012

Knox JP (1996) Arabinogalactan-proteins: developmentally regulated proteoglycans of the plant cell surface. *In* M Smallwood, JP Knox, DJ Bowles, eds, Membranes, Specialized Function in Plants. Bios Scientific Publishers, Oxford, pp 93–102

Koncz C, Schell J (1986) The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimeric genes carried by a novel type of *Agrobacterium* binary vector. Mol Gen Genet **204:** 383–396

Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol **5:** 325–331

Lasserre E, Bouquin T, Hernandez JA, Bull J, Pech J-C, Balagué C (1996) Structure and expression of three genes encoding ACC oxidase homologs from melon (*Cucumis melo* L.). Mol Gen Genet **251:** 81–90

Li S-X, Showalter AM (1996) Cloning and developmental/stress-regulated expression of a gene encoding a tomato arabinogalactan protein. Plant Mol Biol **32:** 641–652

Lichtenstein C, Draper J (1986) DNA cloning: a practical approach. *In* DM Glover, ed, Genetic Engineering of Plants. IRL Press, Washington, DC, pp 67–119

Majewski-Sawka A, Nothnagel EA (2000) The multiple roles of arabinogalactan proteins in plant development. Plant Physiol **122:** 3–9

Matthysse AG (1986) Initial interactions of *Agrobacterium tumefaciens* with plant host cells. Crit Rev Microbiol **13:** 281–307

Matthysse AG (1994) Conditioned medium promotes the attachment of *Agrobacterium tumefaciens* strain NT1 to carrot cells. Protoplasma **183:** 131–136

Matthysse AG, McMahan S (1998) Root colonization by *Agrobacterium tumefaciens* is reduced in *cel*, *attB*, *attD*, and *attR* mutants. Appl Environ Microbiol **64:** 2341–2345

Matthysse AG, Thomas DL, White AR (1995) Mechanism of cellulose synthesis in *Agrobacterium tumefaciens.* J Bacteriol **177:** 1076–1081

McKinney EC, Ali N, Traut A, Feldmann KA, Belostotsky DA, McDowell JM, Meagher RB (1995) Sequence-based identification of T-DNA insertion mutations in *Arabidopsis*: actin mutants *act2-1* and *act4-1.* Plant J **8:** 613–622

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497

Mysore KS, Kumar CTR, Gelvin SB (2000) *Arabidopsis* ecotypes and mutants that are recalcitrant to *Agrobacterium* root transformation are susceptible to germ-line transformation. Plant J **21:** 9–16

Nam J, Mysore KS, Gelvin SB (1998) *Agrobacterium tumefaciens* transformation of the radiation hypersensitive *Arabidopsis thaliana* mutants *uvh1* and *rad5.* Mol Plant Microbe Interact **11:** 1136–1141

Nam J, Mysore KS, Zheng C, Knue MK, Matthysse AG, Gelvin SB (1999) Identification of T-DNA tagged *Arabidopsis* mutants that are resistant to transformation by *Agrobacterium.* Mol Gen Genet **261:** 429–438

Narasimhulu SB, Deng X-B, Sarria R, Gelvin SB (1996) Early transcription of *Agrobacterium* T-DNA genes in tobacco and maize. Plant Cell **8:** 873–886

Ni M, Cui D, Einstein J, Narasimhulu SB, Vergara CE, Gelvin SB (1995) Strength and tissue specificity of chimeric promoters derived from the octopine and mannopine synthase genes. Plant J **7:** 661–676

Penninckx IAMA, Eggermont K, Terras FRG, Thomma BPHJ, De Samblanx GW, Buchala A, Métraux J-P, Manners JM (1996) Pathogen-induced systemic activation of a plant defensin gene in *Arabidopsis* follows a salicylic acid-independent pathway. Plant Cell **8:** 2309–2323

Pogson BJ, Davies C (1995) Characterization of a cDNA encoding the protein moiety of a putative arabinogalactan protein from *Lycopersicon esculentum.* Plant Mol Biol **28:** 347–352

Reiter RS, Young RM, Scolnik PA (1992) Genetic linkage of the *Arabidopsis* genome: methods for mapping with recombinant inbreds and random amplified polymorphic DNAs (RAPDs). *In* C Koncz, N-H Chua, J Schell, eds, Methods in Arabidopsis Research. World Scientific, Singapore, pp 170–190

Reuhs BL, Kim JS, Matthysse AG (1997) Attachment of *Agrobacterium tumefaciens* to carrot cells and *Arabidopsis* wound sites is correlated with the presence of cell-associated, acidic polysaccharide. J Bacteriol **179:** 5372–5379

Sambrook F, Fritsch EF, Maniatus T (1989) Molecular Cloning. A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schultz CJ, Gilson P, Oxley D, Youl JJ, Bacic A (1998) GPI-anchors on arabinogalactan-proteins: implications for signalling in plants. Trends Plant Sci **3:** 426–431

Schultz CJ, Rumsewicz MP, Johnson KL, Jones BJ, Gaspar YM, Bacic A (2002) Using genomic resources to guide research directions. The arabinogalactan-protein gene family as a test case. Plant Physiol **129:** 1448–1463

Sciaky D, Montoya AL, Chilton M-D (1978) Fingerprints of *Agrobacterium* Ti plasmids. Plasmid **1:** 238–253

Serpe MD, Nothnagel EA (1999) Arabinogalactan-proteins in the multiple domains of the plant cell surface. Adv Bot Res **30:** 207–289

Shah J (2003) The salicylic acid loop in plant defense. Curr Opin Plant Biol **6:** 365–371

Shi H, Kim Y, Guo Y, Stevenson B, Zhu J-K (2003) The *Arabidopsis* SOS5 locus encodes a putative cell surface adhesion protein and is required for normal cell expansion. Plant Cell **15:** 19–32

Sykes LC, Matthysse AG (1986) Time required for tumor induction by *Agrobacterium tumefaciens.* Appl Environ Microbiol **52:** 597–598

Tan L, Qiu F, Lamport DTA, Kieliszewski MJ (2003) Structure of a hydroxyproline (Hyp)-arabinogalactan polysaccharide from repetitive Ala-Hyp expressed in transgenic *Nicotiana tabacum.* J Biol Chem **279:** 13156–13165

Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D, Slusarenko A, Ward E, Ryals J (1992) Acquired resistance in Arabidopsis. Plant Cell **4:** 645–656

van Hengel AJ, Roberts K (2003) AtAGP30, an arabinogalactan-protein in the cell walls of the primary root, plays a role in root regeneration and seed germination. Plant J **36:** 256–270

Veena HJ, Doerge RW, Gelvin SB (2003) Transfer of T-DNA and Vir proteins to plant cells by *Agrobacterium tumefaciens* induces expression of host genes involved in mediating transformation and suppresses host defense gene expression. Plant J **35:** 219–236

Wadsworth G, Redinbaugh M, Scandalios J (1988) A procedure for the small-scale isolation of plant RNA suitable for RNA blot analysis. Anal Biochem **172:** 279–283

Yamada H, Kiyohara H, Cyong J-C, Otsuka Y (1985) Studies on polysaccharides from *Angelica acutiloba*. IV. Characterisation of an anticomplementary arabinogalactan from the roots of *Angelica acutiloba* Kitagawa. Mol Immunol **22:** 295–304

Yariv J, Lis H, Katchalski E (1967) Precipitation of arabic acid and some seed polysaccharides by glucosylphenylazo dyes. Biochem J **105:** 1c–2c

Youl JJ, Bacic A, Oxley D (1998) Arabinogalactan-proteins from *Nicotiana alata* and *Pyrus communis* contain glycosylphosphatidylinositol membrane anchors. Proc Natl Acad Sci USA **95:** 7921–7926

Zegzouti H, Jones BJ, Frasse P, Marty C, Maitre B, Latché A, Pech J-C, Bouzayen M (1999) Ethylene-regulated gene expression in tomato fruit: characterization of novel ethylene-responsive and ripening-related genes isolated by differential display. Plant J **18:** 589–600

Zhao ZD, Tan L, Showalter AM, Lamport DT, Kieliszewski MJ (2002) Tomato LeAGP-1 arabinogalactan-protein purified from transgenic tobacco corroborates the Hyp contiguity hypothesis. Plant J **31:** 431–444

Zhu Y, Nam J, Carpita NC, Matthysse AG, Gelvin SB (2003b) *Agrobacterium*-mediated root transformation is inhibited by mutation of an *Arabidopsis* cellulose synthase-like gene. Plant Physiol **133:** 1000–1010

Zhu Y, Nam J, Humara J, Mysore K, Lee L-Y, Cao H, Valentine L, Li J, Kaiser A, Kopecky A, et al (2003a) Identification of *Arabidopsis rat* mutants. Plant Physiol **132:** 494–505