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Molecules and Cells

The *Arabidopsis AtLEC* Gene Encoding a Lectin-like Protein Is Up-Regulated by Multiple Stimuli Including Developmental Signal, Wounding, Jasmonate, Ethylene, and Chitin Elicitor

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The *Arabidopsis* gene *AtLEC* (At3g15356) gene encodes a putative 30-kDa protein with a legume lectin-like domain. Likely to classic legume lectin family of genes, *AtLEC* is expressed in rosette leaves, primary inflorescences, and roots, as observed in Northern blot analysis. The accumulation of *AtLEC* transcript is induced very rapidly, within 30 min, by chitin, a fungal wall-derived oligosaccharide elictor of the plant defense response. Transgenic *Arabidopsis* carrying an *AtLEC* promoter-driven β-glucuronidase (GUS) construct exhibited GUS activity in the leaf veins, secondary inflorescences, carpel heads, and silique receptacles, in which no expression could be seen in Northern blot analysis. This observation suggests that *AtLEC* expression is induced transiently and locally during developmental processes in the absence of an external signal such as chitin. In addition, mechanically wounded sites showed strong GUS activity, indicating that the *AtLEC* promoter responds to jasmonate. Indeed, methyl jasmonate and ethylene exposure induced *AtLEC* expression within 3-6 h. Thus, the gene appears to play a role in the jasmonate-/ethylene-responsive, in addition to the chitin-elicited, defense responses. However, chitin-induced *AtLEC* expression was also observed in jasmonate-insensitive (*coi1*) and ethylene-insensitive (*etr1-1*) *Arabidopsis* mutants. Thus, it appears that chitin promotes *AtLEC* expression *via* a jasmonate- and/or ethylene-independent pathway.

INTRODUCTION

Lectins (also called agglutinins or hemagglutinins) are carbohydrate-binding proteins with high affinity and specificity for the glycans in plant and animal glycoproteins, glycolipids, and polysaccharides (Komath et al., 2006; Sharon and Lis, 2004). According to the plant lectin database Lectindb, the genome of *Arabidopsis thaliana* contains 196 lectin genes (Chandra et al., 2006). Plant lectins may be grouped into several families, including legume lectins, jacalin-related lectins, amaranthins, Cucurbitaceae phloem lectins, lectins with a hevein domain(s), monocot mannose-binding lectins, and type-2 ribosome-inactivating proteins (Peumans et al., 2000). The overall structure of the carbohydrate-binding site(s) is conserved in each lectin family.

Although numerous plant lectins have been characterized, their physiological roles have not been clearly defined. Until recently, most plant lectin studies focused on those that accumulate to high concentrations in vegetative tissues. These lectins are secretory proteins that accumulate in the cell wall or vacuole, and are abundant in developing seeds and in vegetative organs such as roots, leaves, rhizomes, and stems (Chrispeels and Raikhel, 1991; Rüdiger and Gabius, 2001). The most likely function of vacuolar lectins is in plant defense, presumably involving the binding of ligands derived from various aggressors. Lectins released from the vacuole may come in contact with glycoproteins that line the intestinal tracts of insects, possibly inhibiting the absorption of nutrients. The binding of lectins to the fungal cell-wall component chitin, a polymer of *N*-acetylglucosamine, can interfere with the growth of fungal hyphae. Alternatively, ligand-lectin binding may activate signal transduction pathways that induce defense responses. For instance, members of an *Arabidopsis* family of serine/threonine receptor kinases contain an extracellular legume lectin-like domain (Hervé et al., 1996; 1999).

Legume lectins comprise one of the largest lectin families and thus have been studied thoroughly. Typically, these lectins bind glucose, *N*-acetylglucosamine, mannose, or galactose. They consist of two or four subunits of a molecular mass of 30 kDa, each containing one carbohydrate-binding site. Based on their quaternary structure, legume lectins have traditionally, with some exceptions, been subdivided into two categories: single-chain lectins consisting of identical subunits, and two-chain lectins characterized by different subunit types (Rüdiger and

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Gabius, 2001). Following the molecular size order in SDS-PAGE, the smaller subunit is designated the α subunit, and larger subunit the β subunit.

Classic plant lectins have not generally been thought to be inducible proteins, but there is increasing evidence that some are induced by biotic or abiotic stimuli (Van Damme et al., 2004). For example, the mannose-binding lectin Orysata is induced in rice by salt stress, desiccation, and the phytohormones jasmonic acid and abscisic acid (De Souza Filho et al., 2003; Zhang et al., 2000), and the tobacco lectin Nictaba is specifically induced by jasmonic acid (Chen et al., 2002; Lannoo et al., 2007). These inducible lectins localize to the cytoplasm and/or nucleus, implying that they are involved in endogenous protein-carbohydrate interactions (Van Damme et al., 2004). The identification of non-classic plant lectins highlights the importance of investigating the potential novel roles of such proteins.

We identified an *Arabidopsis* gene (*AtLEC*) that encodes a classic legume lectin-like protein. *AtLEC* gene expression is induced by chitin, jasmonate, and ethylene, molecules that are related to defense mechanisms, suggesting that classic legume lectins could also be induced by external stresses. We tested whether chitin activates *AtLEC via* jasmonate- or ethylene-mediated signaling, but found evidence for mutually independent induction processes between chitin and each phytohormone.

MATERIALS AND METHODS

Plant materials

We used *A. thaliana* ecotype Columbia (Col-0). The seeds were surface-sterilized by soaking in 70% (v/v) ethanol for 15 min and 100% ethanol for 5 min. The seeds were then placed on sterilized filter paper and dried in a laminar flow clean bench. Following 4 days of vernalization at 4°C, the seeds were sown on solidified 0.5× Murashige-Skoog (MS) medium (Sigma-Aldrich) in sterile transparent polypropylene dishes. The seeds were incubated for 3 weeks in a growth chamber maintained at 21-23°C and 60% relative humidity under a 16-h/8-h light/dark cycle with 500 μmol/m^2/s of white light. For some experiments, 2-week-old seedlings were transferred to soil and grown for 3 weeks in the same growth chamber. Alternatively, approximately 50 sterilized seeds were placed in 50-ml Falcon tubes containing 10 ml of 1× liquid MS medium with 2% dextrose and incubated for 2 weeks with gentle shaking under the conditions described above for solid medium culture.

Chemical treatments

For the chitin treatment, 1 mg of purified crab shell chitin (Sigma-Aldrich) was dissolved in 10 ml of sterile water and shaken at 180 rpm overnight at room temperature. The suspension was allowed to settle for several hours without shaking, and the supernatant was used as a stock solution. The stock solution was diluted to the expected final concentrations, and 10 ml of each were added to the surface of solidified or liquid MS medium in which *Arabidopsis* seedlings were growing.

For the phytohormone treatments, 100 μM methyl jasmonate (MeJA, in 0.1% ethanol), 50 μM ethephon (an ethylene precursor), 50 μM salicylic acid, or 50 μM abscisic acid were applied to the surface of MS agar plates on which *Arabidopsis* seedlings were growing. During each treatment, the Petri dishes or tubes were sealed with Parafilm. At the end of each treatment, the solution was drained, and the plants were frozen in liquid nitrogen.

Wounding of *Arabidopsis* leaves

Wounds were made to the rosette leaves of 5-week-old *Arabidopsis* plants by cutting with scissors and pinching with forceps. The treated (local) leaves were enclosed in a plastic bag to isolate them from the neighboring (systemic) leaves, and the plants were kept in an open area.

Blot analyses

For genomic Southern blot analysis, 5 μg of genomic DNA were digested with *Xba*I and *Hin*dIII, separated on 0.8% agarose gels, and transferred to nylon membranes. Northern blotting was performed using total RNA extracted from frozen, ground samples with phenol/SDS/LiCl (Carpenter and Simon, 1998). Total RNA (5 μg) was separated on 1.3% agarose formaldehyde gels and transferred to GeneScreen Plus hybridization transfer membranes (Perkin Elmer, USA). The blots were then probed with EST clones obtained from The *Arabidopsis* Information Resource (TAIR) and washed twice at 65 °C for 30 min with 40 mM sodium phosphate buffer (pH 7.2) containing 5% SDS and 1 mM EDTA. Probe *AtLEC* (TAIR EST 36C11) contained the entire *AtLEC* cDNA plus part (186 bp) of the 3′-untranslated region (UTR), for a total fragment length of 1,002 bp.

Histochemical GUS assay

Approximately 1.3 kb of the *AtLEC* promoter (-1310 to -1 from the AUG) were amplified by PCR from genomic DNA. The product was then inserted into pCAMBIA 1391Z (Cambia, Australia) using the *Pst*I and *Bam*HI sites upstream of the *GUS* gene. The resulting construct was then transformed into 4-week-old *Arabidopsis* plants by floral dip method (Clough and Bent, 1998) using *Agrobacterium tumefaciens* strain C58C1. The transformed plants were housed in a growth chamber until they produced seeds. Transgenic plants were then selected by germinating the seeds on solid MS medium containing 30 μg/ml hygromycin. From the hygromycin-resistant transgenic (T1) plants, lines containing single insertions were identified by Southern blotting. Histochemical assays for GUS activity were performed as previously described (Jefferson et al., 1987). Tissue samples were visualized using an Axiophot microscope (Carl Zeiss, Germany) coupled to a CCD camera. GUS staining was confirmed by observing at least four different transgenic lines.

RESULTS

Identification of the *Arabidopsis AtLEC* gene

The chromosomal locus of *AtLEC* is given by the *Arabidopsis* Genome Initiative (AGI) number At3g15356. This gene was identified in microarray-based screening experiments for MeJA-responsive genes (Jung et al., 2007a; 2007b; Schenk et al., 2000), and initially termed *LEC* (Anderson et al., 2004). Between the 5′- and 3′-UTRs (nucleotides 1-51 and 868-1,068, respectively) there is an uninterrupted 816-bp open reading frame (ORF) at nucleotides 52-867.

The ORF of *AtLEC* encodes 271 amino acids, constituting a putative 30-kDa protein (Fig. 1). Based on PSORT analysis, residues 1-19 encode a cleavable N-terminal signal sequence, suggesting that the protein accumulates in cell walls or vacuoles. No apparent nuclear-targeting signal or kinase domain was detected from the primary sequence. Residues 28-269 constitute a legume lectin β-domain. The primary sequence of AtLEC exhibits 86% identity with the lectin-related chitin-inducible protein encoded by At3g16530 (Zhang et al., 2002).

Tissue-specific expression of *AtLEC*

Northern blotting with 5-week-old *Arabidopsis* indicated that

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MQIHKLCFLA LFLANAAFAV KFNFDSFDGS NLLFLGDAEL GPSSDGVSRS   50
MQIHKLCFLV LFLANAAFAV KFNFDSFDGS NLLFLGDAEL GPSSDGVSRS   50
. . . . . .  . . . . . .  . . .. ...  .... . ...  . . . . . .
GALSMTRDET PFSHGQGLYI NPIQFKSSNT SSPFDFKTSF TFSITPRTKP  100
GALSMTRDEN PFSHGQGLYI NQIPFKPSNT SSPFSFETSF TFSITPRTKP  100
. . . . .  . .  . . . . . .  . . . . . .  . .  . . . . . .
NSGQGLAFVI VPAADNSGAS GGGYLGILNK TNDGKSENNL IFIEFDTFKN  150
NSGQGFAFII TPEADNSGAS DGGYLGILNK TNDGKPENHI LAIEFDTFQN  150
 .  . . . . .  .. . . . . .  . . . . . .  .. . . . . .  . . . . . .
NESNDISGNH VGININSMTS LVAEKAGYWV QTLVGKRKVW SFKDVNLSSG  200
KEFLDISGNH VGVNINSMTS LVAEKAGYWV QTRVGKRKVW SFKDVNLSSG  200
. . . . . .  . . .  . . .  . . . . . .  . .  . . . .  . . . . . .
ERFKAWIEFR SKDSRNTITI APENVKKPKR PLIQGSRVLN DVLLQNMYAG  250
ERFKAWVEFR NKDSTITVTL APENVKKPKR ALIEAPRVLN EVLLQNMYAG  250
. . . . . .  .  . . .  . . . . . .  .
FAGSMGRAGE RHDVWSWSFE N          271        At3g15356 (AtLEC)
FAGSMGRAVE RHDIWSWSFE NAAKNN     276        At3g16530 (Lectin-related)
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Fig. 1. Amino acid sequence deduced from the *Arabidopsis AtLEC* gene (At3g15356). Amino acid residues 1-19, which encode a cleavable N-terminal signal sequence, are boxed. The sequences are aligned, using the DNAStar program, with those of a putative *Arabidopsis* lectin-related protein deduced from At3g16530. Amino acid residues that are identical among the two proteins are indicated by dots. The peptide region constituting a legume lectin β-domain is indicated by arrows at starting (residue 28) and ending (residue 269) points.

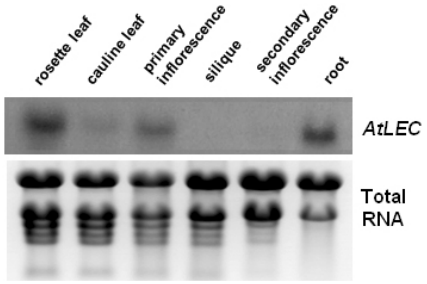

Fig. 2. Northern blot analysis of the tissue-specific expression of *AtLEC*. Total RNAs were prepared from rosette leaves, cauline leaves, primary inflorescences, siliques; secondary inflorescences, and roots of 5-week-old *Arabidopsis* plants. Equal RNA loadings were confirmed using ethidium bromide-stained gels.

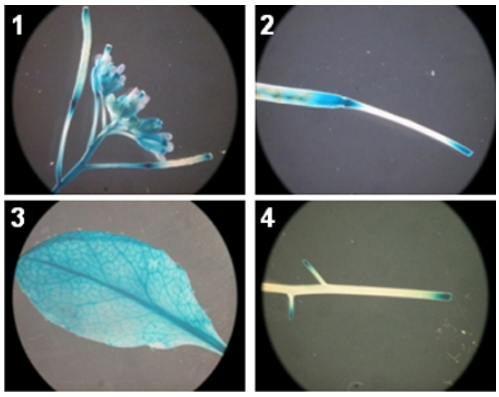

Fig. 3. Histochemical β-glucuronidase (GUS) assay of *AtLEC* promoter activity. The *AtLEC* promoter (~1.3 kb) was fused with the *GUS* gene, and transformed into *Arabidopsis*. With the hygromycin-resistant transgenic (T1) plants, histochemical assays for GUS activity were performed as previously described (Jefferson et al., 1987). Tissue samples were visualized using an Axiophot microscope (Carl Zeiss, Germany) coupled to a CCD camera. 1, flower and siliques; 2, silique; 3, rosette leaf; 4, primary and secondary inflorescences cut with a blade.

AtLEC is highly expressed in the rosette leaves, primary inflorescences, and roots of mature plants, but not in the cauline leaves, siliques, or secondary inflorescences (Fig. 2).

In transgenic *Arabidopsis* plants expressing a *GUS* reporter gene driven by the *AtLEC* promoter (~1.3 kb), GUS activity was observed in the leaf veins, secondary inflorescences, carpel heads, and silique receptacles (Fig. 3). In primary and secondary inflorescences cut with a blade, a high level of GUS activity appeared in the vicinity of the wound site.

Induction of *AtLEC* expression by chitin

AtLEC expression was rapidly induced by chitin. Three-week-old *Arabidopsis* seedlings were treated with 0.1 μg/L to 1 mg/L chitin for 30 min, and mRNA accumulation was analyzed by Northern blotting. A significant rise in transcript level was detected at 10 μg/L chitin, and the level of transcription increased proportionally with the chitin concentration (Fig. 4A). Chitin also induced *AtLEC* expression in liquid-cultured *Arabidopsis* seedlings (Fig. 4B). Chitin-induced *AtLEC* expression was not affected in *coi1* (Feys et al., 1994) and *etr1-1* (Chang et al., 1993) mutants that are defective in the jasmonate - and ethylene-dependent signaling pathways, respectively.

Induction of *AtLEC* expression by phytohormones

AtLEC was described as a MeJA- and ethylene-responsive gene (Anderson et al., 2004; Schenk et al., 2000). We also identified the gene from a microarray-based screen for MeJA-responsive genes using Affymetrix GeneChip® *Arabidopsis* genome arrays (Jung et al., 2007a). In addition, *AtLEC* is constitutively expressed in transgenic *Arabidopsis* plants that overproduce MeJA (Jung et al., 2007b).

Northern blot analysis confirmed the previous data. Three-week-old *Arabidopsis* seedlings grown on sterilized MS medium did not accumulate *AtLEC* mRNA. However, transcript accumulation was detected 3-6 h after exposure to 100 μM MeJA or 50 μM ethylene (Fig. 5A). In contrast, *AtLEC* transcript level was decreased 3-6 h after exposure to 50 μM salicylic acid or 50 μM abscisic acid. Combined treatment with ethylene and MeJA yielded synergistic increases in transcription (Fig. 5B). Expression of the *JR2* and *PDF1.2* genes was analyzed as a control. Synergistic crosstalk between the hormones involved in activation of the jasmonate-responsive defense gene *PDF1.2* was previously reported (Penninckx et al., 1998); however, in some cases, such as that involving *JR2*, the combined effect can be antagonistic (Rojo et al., 1999).

AtLEC expression was also induced by wounding (Fig. 6), followed by expression of the jasmonate response marker gene *JR2* (Rojo et al., 1999). Wound-induced systemic expression of *AtLEC* was observed in the distal leaves of the plants.

DISCUSSION

Sequence analysis revealed that the *AtLEC* gene encodes a putative 30-kDa protein containing a legume lectin β domain

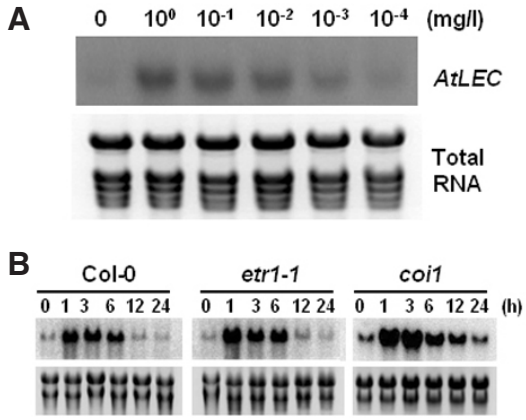

Fig. 4. Northern blot analysis of chitin-induced *AtLEC* expression. (A) Concentration-dependent induction of *AtLEC* expression by chitin. Three-week-old *Arabidopsis* seedlings were treated for 30 min with crab shell chitin at concentrations ranging from 10^{-4} to 1 (10^{0}) mg/L or with water (control). (B) Time course of *AtLEC* expression induced by chitin. Wild-type (Col-0), *etr1-1* mutant, and *coi1* mutant *Arabidopsis* seedlings were cultured for 2 weeks in liquid MS medium, treated with 100 μg/L crab shell chitin, and harvested at the indicated times after treatment. Equal RNA loadings were confirmed using ethidium bromide-stained gels.

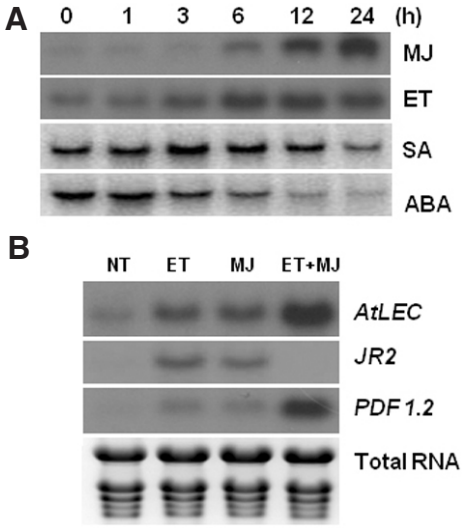

Fig. 5. Northern blot analysis of phytohormone-induced *AtLEC* expression. (A) Induction of *AtLEC* expression by jasmonate and ethylene. Three-week-old wild-type plants (Col-0) were treated with 100 μM MeJA (MJ), 50 μM ethephon (ET), 50 μM salicylic acid (SA), or 50 μM abscisic acid (ABA). Equal RNA loadings were confirmed using ethidium bromide-stained gels (not shown). (B) Synergistic activation of *AtLEC* by jasmonate and ethylene. Expression of the *JR2* and *PDF1.2* genes was analyzed as a control. NT, no treatment.

(Fig. 1). The putative protein contains a cleavable N-terminal signal sequence, suggesting that the protein accumulates in cell walls or vacuoles. The primary sequence contains no apparent nuclear-targeting signal. Northern blotting indicated that *AtLEC* is highly expressed in the rosette leaves, primary inflorescences, and roots of mature plants, but not in the cauline leaves, siliques, or secondary inflorescences (Fig. 2). These results are consistent with previous data showing that lectins are abundant in vegetative plant organs such as roots, leaves, rhizomes, and stems (Chrispeels and Raikhel, 1991). Thus, AtLEC is a member of the classic legume lectin family.

The primary sequence of AtLEC exhibits strong similarity to that of the lectin-related chitin-inducible protein encoded by At3g16530 (Zhang et al., 2002). *AtLEC* expression was also rapidly induced by chitin, within 30 min of application (Fig. 4). Chitins are linear β-1,4-linked *N*-acetylglucosamine polymers that are present in the cell walls of fungi. Major plant defense responses elicited by chitin include lignification and phytoalexin biosynthesis (Hahn, 1996; Ebel, 1998).

AtLEC expression was also found to be induced during developmental processes, in the absence of chitin. Histochemical GUS assays showed that *AtLEC* transcripts accumulate in leaf veins, secondary inflorescences, carpel heads, and silique receptacles (Fig. 3). Relatively high GUS activity was detected in the tissues such as secondary inflorescences and silique receptacles in which no expression could be seen in Northern blot analysis, suggesting that the *AtLEC* expression is transient and local in these tissues. Following fertilization, floral organs develop an abscission zone, where the bases of the organs meet the receptacle (Bleecker and Patterson, 1997). Jasmonates stimulate abscission in the leaf (Saniewski and Wegrzynowicz-Lesiak, 1995), petiole (Ueda et al, 1996), and stem (Saniewski et al., 2000). Ethylene also promotes abscission (Roberts et al., 2002). Thus, activation of the *AtLEC* promoter in flowers and silique receptacles might be due to jasmonate and ethylene. Supporting this observation, high levels of jasmonate are found in flowers and developing reproductive tissues (Creelman and Mullet, 1995). The shedding of plant organs provides an ideal site for invasion by pathogens. Thus, cell separation is associated with an increase in the accumulation of defense proteins such as chitinases (Lim et al., 1987) and pathogenesis-related (PR) proteins (Coupe et al., 1997; Del Campillo and Lewis, 1992). Expression of these genes has been observed following jasmonate treatment (Jung et al., 2007a; Wasternack and Hause, 2002). In addition, temporal and spatial expression of mRNAs encoding PR proteins was observed during ethylene-promoted leaflet abscission in *Sambucus nigra* (Coupe et al., 1997).

In primary and secondary inflorescences of the *AtLEC-GUS* transgenic plants that were cut with a blade, a high level of GUS activity appeared in the vicinity of the wound site. Northern blot analysis also showed that *AtLEC* expression was induced by wounding (Fig. 6), suggesting a jasmonate-mediated defense response. Exposure to 100 μM MeJA or 50 μM ethylene resulted in the accumulation of *AtLEC* transcripts within 3-6 h (Fig. 5), confirming previous microarray data (Jung et al., 2007a). Jasmonates and ethylene are important cellular regulators that cooperate to concurrently activate jasmonate/ethylene-dependent defense mechanisms in response to various pathogens (Berrocal-Lobo et al., 2002; Penninckx et al., 1998). In addition, *AtLEC* was induced in *Arabidopsis* treated with the diterpenoid antifungal agent sclareol (Campbell et al., 2003), suggesting that *AtLEC* is involved in plant defense.

The physiological roles of lectins in plant defense have not been clearly defined. The most likely function of vacuolar lectins in plant defense is presumably the specific recognition and binding of ligands from pathogens. Legume lectins exhibit a variety of tertiary and quaternary structures (Bezerra et al., 2007; Moreno et al., 2008), and further studies would provide insight into the structure-biological activity relationships of this family of lectins.

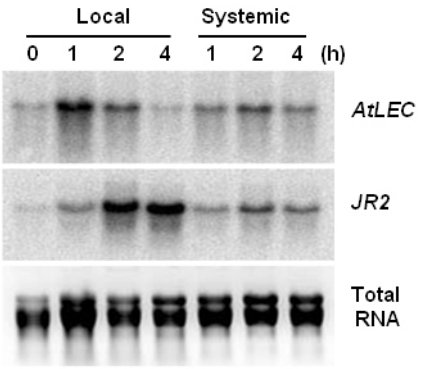

Fig. 6. Northern blot analysis of wound-induced *AtLEC* expression. Rosette leaves of 5-week-old *Arabidopsis* seedlings were wounded by cutting with scissors and pinching with forceps. The treated leaves (local) were enclosed in a plastic bag to isolate them from distal leaves (systemic). Expression of the *JR2* gene was analyzed as a control. Equal RNA loadings were confirmed using ethidium bromide-stained gels.

Oligosaccharide-induced defense gene activation may occur *via* a jasmonate-responsive pathway (Doares et al., 1995; Farmer and Ryan, 1992; Mueller et al., 1993); however, jasmonate/ ethylene-induced defense responses are exerted by antifungal proteins such as defensin (Penninckx et al., 1996; 1998) and thionin (Andresen et al., 1992; Becker and Apel, 1992). Therefore, it appears that the signaling pathways for the defense responses triggered by chitin elicitor and jasmonate/ethylene treatment are quite different from each other. Indeed, chitin-induced *AtLEC* gene expression was unaffected in mutants in which the jasmonate- or ethylene-dependent pathways were blocked (Fig. 4B). This suggests that chitin-induced *AtLEC* expression involves a novel signal transduction pathway, which is consistent with the result of a microarray analysis; the expression profile of *Arabidopsis* exposed to chitin did not closely resemble the expression profile of any hormone treatment including MeJA and ethylene (Ramonell et al., 2002).

Jasmonate signaling is associated with the ubiquitin/proteasome-mediated degradation of proteins that negatively regulate transcription (Xie et al., 1998; Xu et al., 2002). Recent data suggest that the jasmonate-isoleucine complex is recruited by COI1, leading to the ubiquitination and degradation of jasmonate ZIM-domain (JAZ) proteins, which repress the transcription factor MYC2. The release of MYC2 repression in turn initiates the transcription of jasmonate-responsive genes (Chini et al., 2007; Thines et al., 2007).

Ethylene signaling also employs the ubiquitin/protea-some-mediated pathway (Benavente and Alonso, 1996). Ethylene is perceived by five ethylene receptors; ETR1, ETR2, ERS1, ERS2, and EIN4. These proteins constitute a complex with the protein kinase CTR1 which inhibits ethylene-signaling cascade in the absence of ethylene. Mutation on a receptor results in suppression of ethylene responses, even if the other wild-type members of the receptor family are sensing ethylene (Chang et al., 1993; Schaller and Bleecker, 1995). Upon ethylene perception, the suppressed signaling cascade is released to induce the accumulation of the transcription factor EIN3. Two EIN3-binding factors EBF1 and EBF2 are F-box proteins that act as part of an E3-ligase (Binder et al., 2007; Guo and Ecker, 2003; Potuschak et al., 2003). Thus, expression of ethylene-responsive genes is mediated by $SCF^{EBF1/EBF2}$-dependent proteolysis of EIN3 transcription factor.

Several studies have suggested that chitin perception occurs at the surface of plant cells, and a high-affinity binding protein for oligochitin was identified from the rice plasma membrane (Ito et al., 1997; Kaku et al., 2006). However, the molecular components involved in the ensuing signal transduction pathway have not been described. In particular, it is unknown whether chitin-induced gene activation is modulated by ubiquitination, as is jasmonate-induced gene activation. A number of oligochitin-responsive ubiquitin ligase genes and transcription factors have been identified in *Arabidopsis* (Libault et al., 2007). Additional studies on the role of such putative transcriptional regulators will reveal whether chitin also activates *AtLEC* transcription *via* ubiquitination.

In summary, AtLEC is a classic legume lectin that plays a role in jasmonate/ethylene-responsive defense reactions. Classic plant lectins have not traditionally been thought to be inducible proteins. Orysata, Nictaba, and other inducible lectins are cytoplasmic and/or nuclear plant lectins, suggesting that they are involved in endogenous protein-carbohydrate interactions (Van Damme et al., 2004). *AtLEC* gene expression is induced by chitin, jasmonate, and ethylene, consistent with a defense role for this gene. This is evidence that a classic legume lectin could also be induced by endogenous developmental cues or external stresses.

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