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Molecules and Cells



# Overexpression of *GmAKR1*, a Stress-Induced Aldo/keto Reductase from Soybean, Retards Nodule Development

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**Development of symbiotic root nodules in legumes involves the induction and repression of numerous genes in conjunction with changes in the level of phytohormones. We have isolated several genes that exhibit differential expression patterns during the development of soybean nodules. One of such genes, which were repressed in mature nodules, was identified as a putative aldo/keto reductase and thus named *Glycine max* aldo/keto reductase 1 (*GmAKR1*). GmAKR1 appears to be a close relative of a yeast aldo/keto reductase YakC whose *in vivo* substrate has not been identified yet. The expression of *GmAKR1* in soybean showed a root-specific expression pattern and inducibility by a synthetic auxin analogue 2,4-D, which appeared to be corroborated by presence of the root-specific element and the stress-response element in the promoter region. In addition, constitutive overexpression of *GmAKR1* in transgenic soybean hairy roots inhibited nodule development, which suggests that it plays a negative role in the regulation of nodule development. One of the *Arabidopsis* orthologues of GmAKR1 is the ARF-GAP domain 2 protein, which is a potential negative regulator of vesicle trafficking; therefore GmAKR1 may have a similar function in the roots and nodules of legume plants.**

## INTRODUCTION

Leguminous plants can fix nitrogen by establishing a complex symbiotic interaction with the soil bacteria rhizobia, resulting in the differentiation of root tissues into root nodules (Oldroyd and Downie, 2008; Stacey et al., 2006). During early infection stage, Nod factors are released from the rhizobia in response to flavonoids produced by plant roots and induce a series of morphological and biochemical changes in the epidermal cells of the infected roots, that include the root hair curling and the onset of early nodulin gene expression (Subramanian et al., 2007). The epidermal changes induced by the Nod factors are coordinated with the formation of nodule primordia for proper bacterial entry to cortical cells. Many nodulins including ENOD40 are specifically expressed in the developing nodule primordium, but not in the epidermal region (Charon et al., 1999). The activation of cell division in cortical cells is essential for nodule formation (Foucher and Kondorosi, 2000) as with other events of the plant organogenesis and development, involvement of various plant hormones is essential in the formation of nodules (Oldroyd and Downie, 2008). In particular, the balance between the level of auxin and cytokinin appears to be critical in the initiation of nodule organogenesis, which is in many respects reminiscent of what happens during the lateral root formation (de Billy et al., 2001; Kim et al., 2007a). Several lines of evidences suggest that the local auxin level in the developing nodule primordia is regulated, at least in part, by the inhibition of its transport by flavonoids (Mathesius et al., 1998; Peer and Murphy, 2007; Wasson et al., 2006). While the exact mechanism by which auxin transport is repressed by flavonoids is not clear, it is conceivable that many other plant gene products are also involved in conjunction with, or in addition to the role of flavonoids in regulating the auxin transport during nodule development.

In this study, we isolated several early stage-specific genes in soybean root nodules to characterize their possible roles in nodule development. One of these genes was identified as a putative aldo/keto reductase and thus named *GmAKR1*. Aldo/keto reductases (AKRs) are NAD(P)(H)-dependent oxidoreductases that comprise a huge multigene superfamily. More than 140 AKRs have been identified across all taxa of eukaryotes and prokaryotes, and these AKRs have been grouped into 15 different subfamilies (Jez et al., 1997; Jin and Penning, 2007). Because AKRs are capable of catalyzing the reduction of aldehydes or carbonyl groups present in a variety of biochemicals, it is believed that they play important roles in the modification or detoxification of various biologically active compounds in animals. In addition, some AKRs are involved in regulation of the opening of the voltage-gated ion channel (Jin and Penning, 2007). Furthermore, most plant AKRs that have been identified to dates are predicted to encode aldehyde re-

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ductases or potassium channel proteins. ATB2, a putative AKR and one of the *Arabidopsis* orthologues of *GmARK1*, is induced by auxin as well as by iron starvation (Nikiforova et al., 2003), which suggests that is involved in the stress/hormone responses of plants.

Here, we report the molecular characterization of *GmAKR1,* which is the first member of the soybean AKRs to be identified and characterized. Unlike its *Arabidopsis* orthologue, *GmAKR1* was not induced by exogenous auxin treatment, but was inducible by various stress treatments including salt, ABA, and the xenobiotic concentration of the auxin analogue 2,4-D. Moreover, its expression was only detected in the roots and in early nodules while it was downregulated as the nodule matured. Consistent with its expression profile in nodules, constitutive overexpression of *GmAKR1* in the transgenic hairy root system resulted in the inhibition of root nodule formation. Although there is currently not sufficient biochemical and molecular evidence available to predict the precise role that GmAKR1 plays in regulation of nodule development, several possible functions and substrates of GmAKR1 are described here, in the hope of facilitating further study to characterize GmAKR1.

## MATERIALS AND METHODS

### Growth of plants and rhizobia

Soybean (*Glycine max* cv. Shinpaldal) seeds were grown on wet filter papers at 28°C after sterilization. Three-day-old seedlings were inoculated with rhizobia (*Bradirhizobium japonicum* USDA110) and root nodules were then obtained 2,7 and 27 days post inoculation (d.p.i.).

### Semi-quantitative RT-PCR

cDNA of the total RNAs was synthesized using M-MLV Reverse Transcriptase (Promega, USA), and subsequent PCR was performed using Ex-*Taq* polymerase (Takara, Japan). The sequences of the primers used were as follows: for *GmAKR1*, the upstream primer was 5′-ATCTGAAGCCAGCCCACATA-3′ and the downstream primer was 5′-GGCACATTTTCCACAACTCC-3′; for *GmSAUR15A*, the upstream primer was 5′-CGCAAGCATTGAGGACCAACACAA-3′ and the downstream primer was 5′-TCACTGCAAGGAATTGTGAGGCCA-3′; for *ubiquitin*, the upstream primer was 5′-GGGTTTTAAGCTCGTTGT-3′, and the downstream primer was 5′-GGACACATTGAGTTCAAC-3′; for *GUS*, the upstream primer was 5′-CGGGTGAAGGTTATCTCTATGAACT-3′ and the downstream primer was 5′-CAAAGCCAGTAAAGTAGAACGGTTT-3′.

### Auxin treatment of soybean seedlings

Four-day-old soybean seedlings were incubated in KPSC media (2% sucrose, 10 mM potassium phosphate buffer [pH 6.0]) with shaking for 4 h to remove the endogenous auxin. The samples were then treated with 50 μM 2,4-dichloropheno-zyacetic acid (2,4-D) or different concentrations of IAA, 1-NAA, or 2-NAA.

### Cloning of the full-length cDNA for *GmAKR1*

The full-length cDNA for *GmAKR1* was cloned using a Cap-Fishing™ kit (Seegene, Korea) according to the manufacturer's protocol. The full-length first strand of cDNA was synthesized with dT-ACP (Annealing Control Primer) and then PCR was performed using the 5′-RACE primer, 5′-GTCTACCAGGCATTCGCTTCAT-3′, and the *GmAKR1*-specific primer, 5′-AAGCCACGACCAAGAGGACATA-3′.

### Isolation of the upstream region of *GmAKR1*

GenomeWalker™ DNA Libraries (Clontech, USA) of soybean were prepared as previously described (Lee et al., 2005). Briefly, two steps of PCR were conducted using the following adaptor- and *GmAKR1*-specific primers: *GmAKR1*-specific primer 1,5′-CATATATGCCATCCTGTTCTTGGAGAGGG-3′; the nested *GmAKR1*-specific primer 2,5′-GGTCATTGTACGCTCCAGTAAGGCCCATA-3′. The PCR products were then cloned into the pCR2.1-TOPO vector (Invitrogen, USA) and sequenced.

### *In situ* hybridization

Roots, 2 d.p.i. and 7 d.p.i. nodules were harvested and processed for *in situ* hybridization as previously described (Kim et al., 2007b; Oh et al., 2001).

### Development of transgenic nodules overexpressing *GmAKR1*

To create an overepxression construct for *GmAKR1*, the full-length cDNA was inserted between the CaMV 35S promoter and the *nos* 3′ terminator in pBI121 after removing the β-glucuronidase gene. The expression cassette was then subcloned into pCAMBIA1304. Next, *Agrobacteria rhizogenes* (K599) carrying the *GmAKR1*-overexpressing cassette was used to infect the hypocotyls of soybean seedlings. Subsequent development of transgenic nodules was then performed as previously described (Lee et al., 2005).

### Histochemical GUS assay of *Arabidospsis* expressing $P_{GmAKR1}$::GFP::GUS

The upsteam region of *GmAKR1* was obtained using the fol lowing primers: upstream, 5′-CTGAAGCTTAGAGTGGT-CTA-3′; downstream, 5′-CATCCATGGGAATCCCAATTTTG-3′. Next, transgenic *Arabidopsis* plants expressing the $P_{AKR1}$:: *GFP::GUS* construct were treated with 300 mM NaCl, 100 μM ABA, and 20 μM ACC for 3 h, and with 20 μM 2,4-D for 24 h, after which they were stained as described previously (Kim et al., 2007a).

## RESULTS AND DISCUSSION

### Isolation of a putative Aldo/Keto reductase, *GmAKR1*

To obtain better insight into the genes involved in nodule development, cDNA fragments of differentially expressed genes (DEGs; (Kim et al., 2004)) identified during soybean nodulation were isolated as previously described (Lee et al., 2005). RT-PCR analysis of the expression levels of these genes revealed that most were up-regulated during nodule development (data not shown). Conversely, the expression of one gene was significantly reduced in mature nodules obtained at 7 and 27 d.p.i. (Fig. 1A). A full-length cDNA of the gene isolated using the Capfishing™ cDNA Isolation Kit (Seegene, Korea) was found to contain 1,302 nucleotides that included an open reading frame of 321 amino acids flanked by untranslated regions at both the 5′ and the 3′ ends. The open reading frame was predicted to encode a putative oxidoreductase belonging to the aldo/keto reductase (AKR) superfamily that is conserved in all taxa (Hyndman et al., 2003; Jez et al., 1997). In addition, based upon its sequence homology with YakC, a NADPH-dependent aldo/keto reductase isolated from yeast (Morita et al., 2002), the gene described here appears to represent the first member of the AKR subgroup 13 isolated from soybean, and therefore, we named this gene *GmAKR1*. A search of the plant protein sequence database revealed several other potential members of the AKR13 family, including the tobacco auxin induced protein pCNT115 (Boot et al., 1993) and its *Arabidopsis* orthologue ATB2 (At1g60710). YakC is the only member of the AKR subgroup 13 that has been biochemically characterized to date. Despite its homology (34%) with the pyridoxal reductase

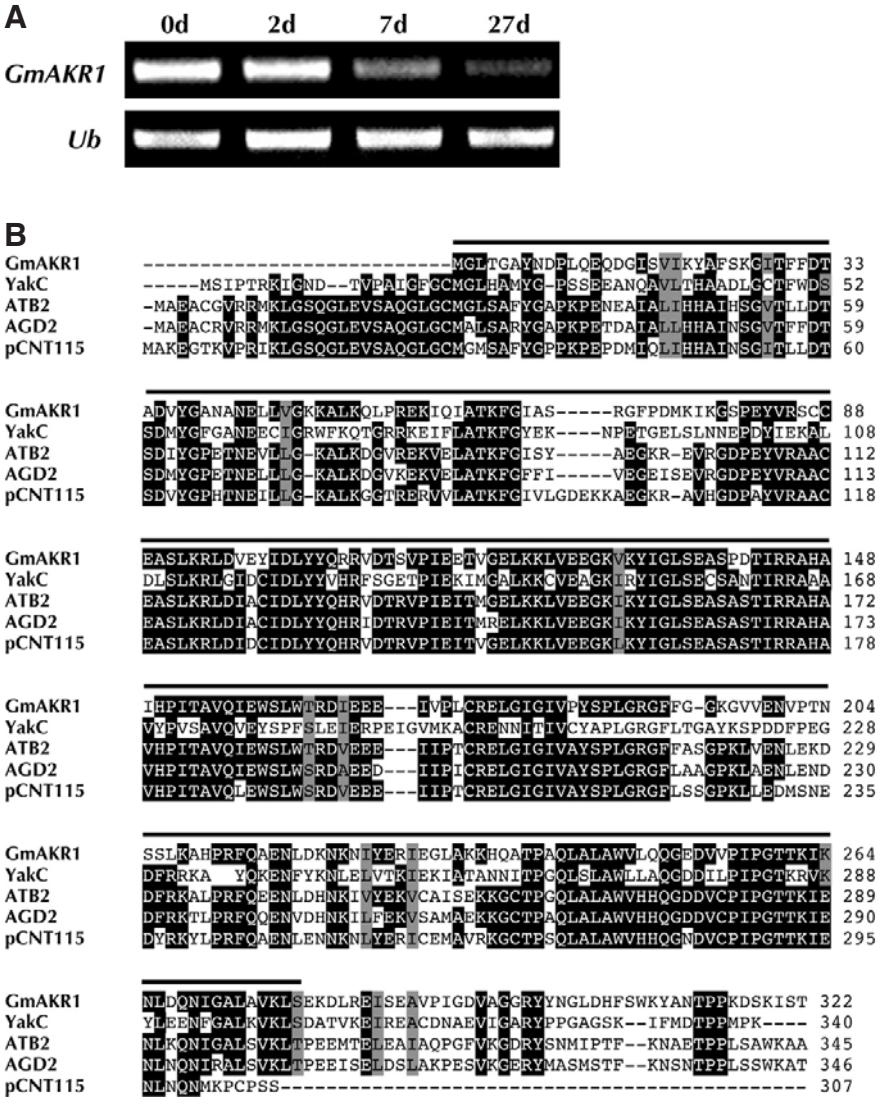


**Fig. 1.** Isolation of *GmAKR1* from soybean nodules as a differentially expressed gene. (A) Semi-quantitative RT-PCR was performed using total RNAs isolated from root, 2 d.p.i., 7 d.p.i., and 27 d.p.i. nodules. *Ubiquitin* was used as a control. All experiments were repeated three times and representative results are shown. (B) Comparison of amino acid sequences of GmAKR1 (GenBank accession no. FJ430074). The sequences were aligned using Clustal W. YakC: the founding member of AKR subgroup 13 from *S. pombe* (accession no. Q09923), ATB2: *Arabidopsis* AKR (At1g60710), AGD2: *Arabidopsis* ARG-GAP domain 2 protein (At1g60680), pCNT115: *N. tabacum* auxin-inducible protein (accession no. P40691).

(AKR8A1) family of AKRs, YakC shows no activity towards pyridoxal, but instead reacts *in vitro* with 2-nitrobenzaldehyde, 2-phthalaldehyde, and pyridine-2-aldehyde in a NADPH-dependent manner (Morita et al., 2002). However, the actual *in vivo* substrate for YakC has not yet been identified. After isolating a full-length cDNA of *GmAKR1* and expressing it in *E. coli,* we examined GmAKR1 if it also exhibited similar substrate specificities as YakC. Surprisingly GmAKR1 did not demonstrate any activity toward the aforementioned substances (data not shown). Currently, we are continuing our endeavor to identify possible substrates for GmAKR1 through *in vitro* activity assays in an attempt to identify the *in vivo* substrate of GmAKR1 and elucidate its relevant biological function.

**The expression profile of *GmAKR1***

The expression of the tobacco orthologue of *GmAKR1*, pCNT115 mRNA (X56267), is upregulated in response to treatment with the synthetic auxin analogue, 2,4-dichlorophenoxyacetic acid (2,4-D) (Boot et al., 1993), which suggests that its induction pattern is likely to be conserved in legumes. To confirm that *GmAKR1* can be induced by auxin, soybean roots were treated with IAA and NAA, after which the level of *GmAKR1* expression was measured by semi-quantitative RT-PCR. Surprisingly, *GmAKR1* was not responsive to either the natural auxin IAA or the synthetic auxin 1-NAA, while the induction of the soybean *SAUR15A* transcript (Li et al., 1994), included as a positive control, was evident (Fig. 2A). *GmAKR1* was induced only to a relatively high concentration of the synthetic auxin analogue, 2,4-D, in which the expression was evident 40 min after treatment with 2,4-D and continued to increase afterwards (Fig. 2B). These findings demonstrate that the expression of this particular aldo/keto reductase may be regulated differently in soybean. Although our result showed that *GmAKR1* was induced by 2,4-D, the concentration of 2,4-D at which the induction was observed was far beyond the physiological concentration usually administered in the studies mimicking the effect of auxin. Thus, it is likely that 2,4-D acted as a xenobiotic stress agent (Johnson et al., 2001) in our experiments. This difference in the inducibility between *GmAKR1* and its orthologues in non-legume plants suggests that GmAKR1 may be involved in a function specific to the development or metabolism of legume plants. The expression of *GmAKR1* in different organs was also examined by semi-quantitative RT-PCR to elucidate the role that GmAKR1 plays in plant development. As shown in Fig. 2C,

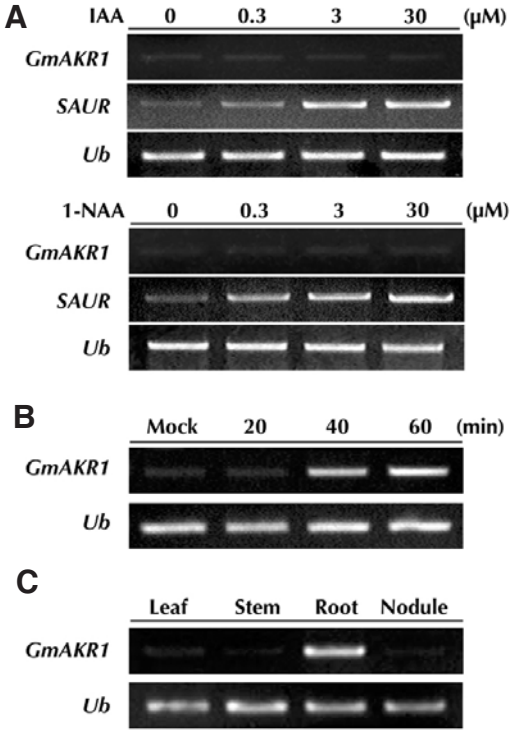


**Fig. 2.** Semi-quantitative RT-PCR for evaluating auxin induction and tissue-specific expression of *GmAKR1*. (A) Four-day-old soybean seedlings were treated with various concentrations of different auxin compounds. The concentrations of auxins were indicated on top of each panel. (B) Four-day-old soybean seedlings were treated with 50 μM 2,4-D for 1 h. (C) RNAs from the leaves, stems, roots and 27 d.p.i. nodules were used for semi-quantitative RT-PCR. Each experiment was repeated at least three times and representative results are shown.

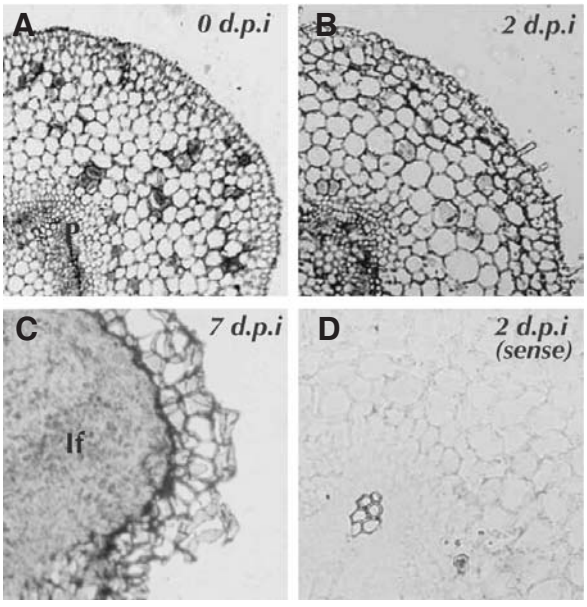


**Fig. 3.** *In situ* hybridization of *GmAKR1* during nodule development. (A, B, and C) Sections from uninoculated root, 2 d.p.i. and 7 d.p.i. nodules were hybridized with an antisense *GmAKR1* riboprobe. (D) A section from 2 d.p.i. nodules was hybridized with a sense *GmAKR1* riboprobe. p, pericycle; if, infection zone.

while it was found in relatively low abundance in other organs such as the leaves, stems and mature nodules, *GmAKR1* was expressed in high levels in the roots. These findings are in direct contrast to its expression in the nodules, which decreased significantly as the nodules mature (Figs. 1 and 2). This sharp contrast in the expression profile of *GmAKR1* in the nodules and roots prompted us to confirm the nodule-specific down-regulation of *GmAKR1* using *in situ* hybridization after infecting soybean roots with rhizobia. Consistent with the semi-quantitative RT-PCR data shown in Fig. 1, *GmAKR1* expression was markedly reduced approximately 7 days after nodule initiation (Fig. 3C). These data imply that the activity of GmAKR1 may be specifically required in the roots of legumes, but that it can be dispensed or even deleterious in developing nodules. Alternatively, considering the *in situ* hybridization data showing the expression of *GmAKR1* in early stage of nodule, it may be speculated that the role of GmAKR1 is critical for the nodule initiation, but not for developing into a mature nodule.

**Isolation and characterization of *GmAKR1* promoter**

To enable a better evaluation of the regulation of *GmAKR1* expression, the promoter region of *GmAKR1,* which spans 2.3 kb upstream of the GmAKR1 open reading frame, was isolated using a Genome Walker Kit (Clontech, USA) as described in the "Materials and Methods". *In silico* analyses of the isolated promoter sequence using the PLACE (Higo et al., 1999) and PlantCARE (Lescot et al., 2002; Rombauts et al., 1999) databases revealed a number of *cis*-regulatory element consensus sequences. Of particular interest among them with regard to our expression profile data of *GmAKR1* is the presence of *as-1* motif signature (TGACG[$N_7$]TGACG) 147 bp upstream of the transcription start site (Fig. 4A). The *as-1* element has been recognized as the binding site for TGA factors that are members of the bZIP transcription factors and mediate transcriptional activation in response to xenobiotic stresses and phytohormones including auxin, salicylic acid, jasmonic acid, and ethylene (Chakravarthy et al., 2003; Niggeweg et al., 2000; Ulmasov et al., 1994; Xiang et al., 1996). It is also known to be responsible for driving root-specific gene expression (Katagiri et al., 1989; Klinedinst et al., 2000). Other elements of interest that were found in the promoter region include the root/nodule-specific element (AAAGAT) (Fehlberg et al., 2005), the sugar-repressed element (TATCCA) (Chen et al., 2006), and the sugar/ABA-repressed S-box element (CACCTCCA) (Acevedo-Hernandez et al., 2005). However, the two well-characterized auxin-response motifs, AuxRE (TGTCTC) (Guilfoyle et al., 1998; Tiwari et al., 2003; Ulmasov et al., 1995) and AuxRR (GGTCCcat) (Li et al., 1994; Sakai et al., 1996) were not present in the *GmAKR1* promoter. Therefore, it appears that the 2,4-D-inducible expression of *GmAKR1* observed in the present study was mediated by the *as-1* element and its cognate soybean TGA factor. By contrast, when we examined the promoter region of an *Arabidopsis* orthologue of the *GmAKR1*, ATB2 (At1g60710), which has been reported to be inducible by auxin (Nikiforova et al., 2003), it revealed the presence of the AuxRE located proximal to its transcription start site (Fig. 2A). Thus, the absence of the consensus auxin responsive cis-element in the *GmAKR1* promoter is consistent with our expression profile data of *GmAKR1*, which failed to show any induction by IAA or 1-NAA, and supports our preliminary conclusion that *GmAKR1* was likely to be induced by 2,4-D as part

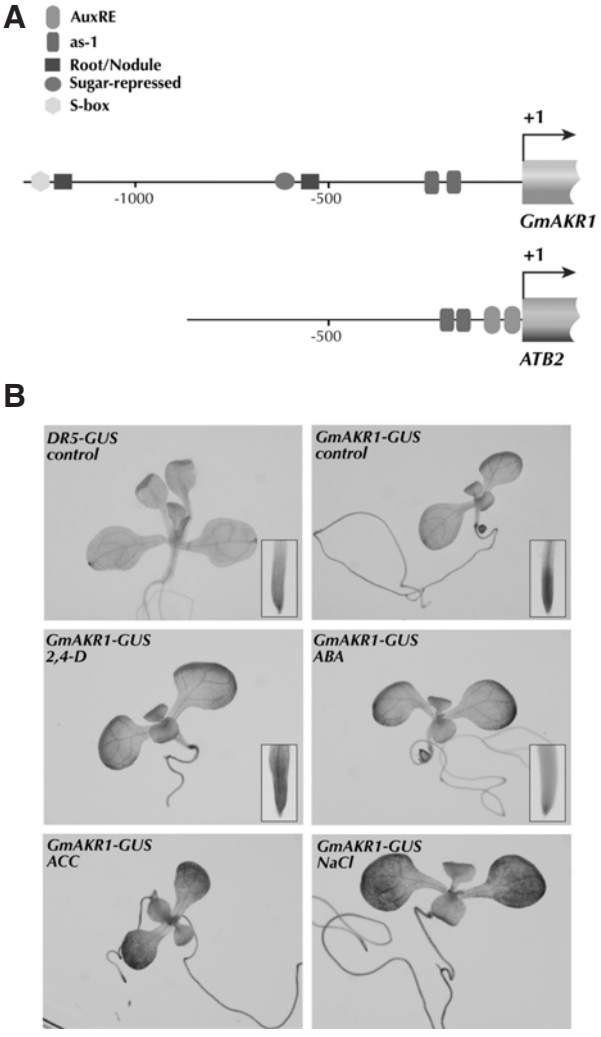


**Fig. 4.** Analysis of the *GmAKR1* upstream region. (A) Consensus motifs found in the upstream region of *GmAKR1* and the *Arabidopsis* orthologue ATB2 (At1g60710) are described in the text. (B) *Arabidopsis* seedlings with $P_{GmAKR1}$::*GFP*::*GUS* were treated with 300 mM NaCl, 100 μM ABA, and 20 μM ACC for 3 h, or 20 μM 2,4-D for 24 h, respectively. Insets show the expression patterns of *GmAKR1* in roots.

of a stress response.

### *GmAKR1* promoter analyses in a heterologuous system using transgenic *Arabidopsis*

To further characterize the responsiveness of *GmAKR1* to various stress signals, the *GmAKR1* promoter sequence was fused to the GUS (β-glucuronidase) reporter gene and transgenic *Arabidopsis* plants expressing this chimeric construct were generated. After obtaining homozygous lines of stable transformants, the seedlings were subjected to different stress signals including salt, ethylene (ACC), abscisic acid (ABA), and 2,4-D treatment. The expression of GUS gene was then monitored by histochemical GUS staining. As shown in Fig. 4B, the pattern of GUS expression driven by the *GmAKR1* promoter activity in a heterologous *Arabidopsis* system revealed several interesting features. Without any exogenous treatments, *GmAKR1* promoter conferred a root-specific expression of the reporter gene in *Arabidopsis*, and its activity was further induced in leaves in response to treatment with NaCl, ethylene (ACC), and 2,4-D. By contrast, abscisic acid (ABA) exerted an inhibitory effect on the *GmAKR1* promoter activity, which resulted in a diminishing expression of the reporter gene even in the roots of the transgenic plants (Fig. 4B). This repression of *GmAKR1* expression by ABA treatment may have a more direct physiological implication on the observed decrease in the level of *GmAKR1* transcripts during nodule maturation than that of the endogenous auxin level. In support of this idea, transgenic plants expressing the GUS reporter gene under the auxin-responsive DR5 promoter (Ulmasov et al., 1997) that were included as controls in the present experiment showed a distinct expression pattern, thus suggesting that at least the endogenous auxin is not the sole regulator of *GmAKR1* expression *in vivo*.

### Constitutive ovexpression of *GmAKR1* inhibited nodule development

The expression pattern of *GmAKR1* in nodules was inversely correlated with the nodule development (Fig. 1), which suggests that GmAKR1 may be required for nodule initiation or other essential events during the early stages of nodulation. Alternatively, GmAKR1 activity may be detrimental to nodule development; therefore it needs to be repressed as the nodule becomes more mature. To examine the possible role that GmAKR1 plays in nodule development, we evaluated the effects of constitutive overexpression of GmAKR1 on nodulation in transgenic soybean roots. We reasoned that if GmAKR1 inhibits the nodulation, transgenic roots overexpressing *GmAKR1* under the constitutive cauliflower mosaic virus (CaMV) 35S promoter are expected to have poorly developed nodules. Transgenic soybean roots were prepared using the hairy root transformation method by injecting *Agrobacterium rhizogenes* carrying a 35S::*GmAKR1* construct in the binary vector pCAMBIA1304 to the transition region between the hypocotyl and the root. The presence of the *uidA* reporter gene in the pCAMBIA1304 allowed identification of the transformants among the emerging hairy roots in the area of the injection by histochemical GUS staining. Overexpression of *GmAKR1* in the transgenic hairy roots was then confirmed by RT-PCR (Fig. 5B), which revealed much higher levels of *GmAKR1* transcript as compared with the control transformed with the pCAMBIA1304 vector alone. Upon inoculation with rhizobia, the *GmAKR1* transgenic hairy roots showed severe defects in nodulation, both in the number and the size of nodules, while the control transgenics and wild type soybean roots produced numerous healthy-looking nodules (Fig. 5A). Taken together, these results suggest that GmAKR1 interferes with the nodulation process. Furthermore, the observed downregulation of the transcription of *GmAKR1* during the later stages of nodule development reflects this negative functional implication. It is also conceivable that the poor nodule development in the transgenic hairy roots overexpressing *GmAKR1* may have been resulted from a general stress due to the overexpression of *GmAKR1,* instead of its direct negative effect on the nodule development. However, such a possibility is rather unlikely considering that *GmAKR1* is normally expressed at high level in soybean roots from which healthy nodules develop. Elucidating how exactly this inhibitory effect is accomplished would be an exciting challenge for future studies of GmAKR1, which would have to entail the identification of its *in vivo* substrate and detailed biochemical characterization of its reaction. Considering that the carbonyl group present on flavonoids could be a substrate for AKRs (Martin et al., 2006), it may be possible that GmAKR1 is

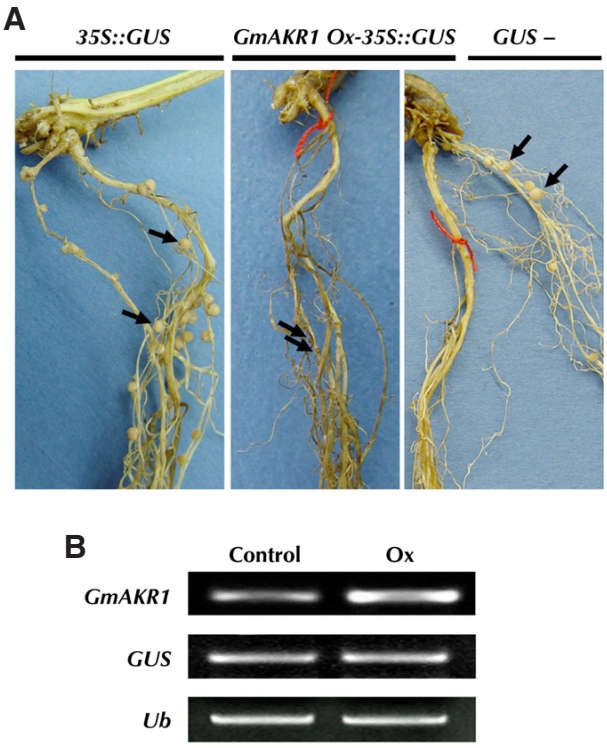


**Fig. 5.** Nodule development on hairy roots expressing $P_{CaMV\ 35S}$::*GmAKR1*. (A) The first photograph shows a hairy root transformed with a *35S::GUS* construct. The second shows a hairy root transformed with a *35S::GUS-35S::GmAKR1* construct (marked with a red thread). The third exhibits a plant with a transgenic hairy root expressing a *35S::GUS-35S::GmAKR1* construct (marked with a red thread) and a nontransgenic hairy root. (B) Semi-quantitative RT-PCR was performed using total RNAs obtained from hairy roots expressing *35S::GUS* and *35S::GUS-35S::GmAKR1*, respectively. Arrows indicate root nodules.

actively involved in the regulation of nodule development by modifying the flavonoids that are involved in the inhibition of auxin transport. It is also possible that a similar effect of GmAKR1 on the auxin transport may be achieved at the level of regulating ion channel or transport vesicles, because some AKRs act as regulators of voltage-gated ion channels, and one of the *Arabidopsis* orthologue of GmAKR1, AGD2 (At1g60680) is predicted to be an ARF-GAP protein, some of its family member have been implicated in the regulation of polar auxin transport (Sieburth et al., 2006; Zhuang et al., 2006).

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## REFERENCES

Acevedo-Hernandez, G.J., Leon, P., and Herrera-Estrella, L.R. (2005). Sugar and ABA responsiveness of a minimal RBCS light-responsive unit is mediated by direct binding of ABI4. Plant J. *43*, 506-519.

Boot, K., Van Der Zaal, B.J., Velterop, J., Quint, A., Mennes, A.M., Hooykaas, P., and Libbenga, K.R. (1993). Further characterization of expression of auxin-induced genes in Tobacco (*Nicotiana tabacum*) cell-suspension cultures. Plant Physiol. *102*, 513-520.

Chakravarthy, S., Tuori, R.P., D'Ascenzo, M.D., Fobert, P.R., Despres, C., and Martin, G.B. (2003). The tomato transcription factor Pti4 regulates defense-related gene expression via GCC box and non-GCC box *cis* elements. Plant Cell *15*, 3033-3050.

Charon, C., Sousa, C., Crespi, M., and Kondorosi, A. (1999). Alteration of *enod40* expression modifies *Medicago truncatula* root nodule development induced by *Sinorhizobium meliloti*. Plant Cell *11*, 1953-1966.

Chen, P.W., Chiang, C.M., Tseng, T.H., and Yu, S.M. (2006). Interaction between rice MYBGA and the gibberellin response element controls tissue-specific sugar sensitivity of alpha-amylase genes. Plant Cell *18*, 2326-2340.

de Billy, F., Grosjean, C., May, S., Bennett, M., and Cullimore, J.V. (2001). Expression studies on *AUX1*-like genes in *Medicago truncatula* suggest that auxin is required at two steps in early nodule development. Mol. Plant Microbe Interact. *14*, 267-277.

Fehlberg, V., Vieweg, M.F., Dohmann, E.M., Hohnjec, N., Puhler, A., Perlick, A.M., and Kuster, H. (2005). The promoter of the leghaemoglobin gene *VfLb29*: functional analysis and identification of modules necessary for its activation in the infected cells of root nodules and in the arbuscule-containing cells of mycorrhizal roots. J. Exp. Bot. *56*, 799-806.

Foucher, F., and Kondorosi, E. (2000). Cell cycle regulation in the course of nodule organogenesis in *Medicago*. Plant Mol. Biol. *43*, 773-786.

Guilfoyle, T., Hagen, G., Ulmasov, T., and Murfett, J. (1998). How does auxin turn on genes? Plant Physiol. *118*, 341-347.

Higo, K., Ugawa, Y., Iwamoto, M., and Korenaga, T. (1999). Plant cis-acting regulatory DNA elements (PLACE) database: 1999. Nucleic Acids Res. *27*, 297-300.

Hyndman, D., Bauman, D.R., Heredia, V.V., and Penning, T.M. (2003). The aldo-keto reductase superfamily homepage. Chem. Biol. Interact. *143-144*, 621-631.

Jez, J.M., Bennett, M.J., Schlegel, B.P., Lewis, M., and Penning, T.M. (1997). Comparative anatomy of the aldo-keto reductase superfamily. Biochem. J. *326*, 625-636.

Jin, Y., and Penning, T.M. (2007). Aldo-keto reductases and bioactivation/detoxication. Annu. Rev. Pharmacol. Toxicol. *47*, 263-292.

Johnson, C., Glover, G., and Arias, J. (2001). Regulation of DNA binding and trans-activation by a xenobiotic stress-activated plant transcription factor. J. Biol. Chem. *276*, 172-178.

Katagiri, F., Lam, E., and Chua, N.H. (1989). Two tobacco DNA-binding proteins with homology to the nuclear factor CREB. Nature *340*, 727-730.

Kim, Y.J., Kwak, C.I., Gu, Y.Y., Hwang, I.T., and Chun, J.Y. (2004). Annealing control primer system for identification of differentially expressed genes on agarose gels. Biotechniques *36*, 424-426, 428, 430 passim.

Kim, Y.K., Son, O., Kim, M.R., Nam, K.H., Kim, G.T., Lee, M.S., Choi, S.Y., and Cheon, C.I. (2007a). *ATHB23*, an *Arabidopsis* class I homeodomain-leucine zipper gene, is expressed in the adaxial region of young leaves. Plant Cell Rep. *26*, 1179-1185.

Kim, H.B., Lee, H., Oh, C.J., Lee, N.H., and An, C.S. (2007b). Expression of *EuNOD-ARP1* encoding auxin-repressed protein homolog is upregulated by auxin and localized to the fixation zone in root nodules of *Elaeagnus umbellata*. Mol. Cells *23*, 115-121.

Klinedinst, S., Pascuzzi, P., Redman, J., Desai, M., and Arias, J. (2000). A xenobiotic-stress-activated transcription factor and its cognate target genes are preferentially expressed in root tip meristems. Plant Mol. Biol. *42*, 679-688.

Lee, M.Y., Shin, K.H., Kim, Y.K., Suh, J.Y., Gu, Y.Y., Kim, M.R., Hur, Y.S., Son, O., Kim, J.S., Song, E., et al. (2005). Induction of thioredoxin is required for nodule development to reduce reactive oxygen species levels in soybean roots. Plant Physiol. *139*, 1881-1889.

Lescot, M., Dehais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., Rouze, P., and Rombauts, S. (2002). PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. Nucleic Acids Res. *30*, 325-327.

Li, Y., Liu, Z.B., Shi, X., Hagen, G., and Guilfoyle, T.J. (1994). An auxin-inducible element in soybean SAUR promoters. Plant Physiol. *106*, 37-43.

Martin, H.J., Breyer-Pfaff, U., Wsol, V., Venz, S., Block, S., and Maser, E. (2006). Purification and characterization of akr1b10 from human liver: role in carbonyl reduction of xenobiotics. Drug

Metab. Dispos. *34*, 464-470.
Mathesius, U., Schlaman, H.R., Spaink, H.P., Of Sautter, C., Rolfe, B.G., and Djordjevic, M.A. (1998). Auxin transport inhibition precedes root nodule formation in white clover roots and is regulated by flavonoids and derivatives of chitin oligosaccharides. Plant J. *14*, 23-34.
Morita, T., Huruta, T., Ashiuchi, M., and Yagi, T. (2002). Characterization of recombinant YakC of *Schizosaccharomyces pombe* showing YakC defines a new family of aldo-keto reductases. J. Biochem. *132*, 635-641.
Niggeweg, R., Thurow, C., Kegler, C., and Gatz, C. (2000). Tobacco transcription factor TGA2.2 is the main component of *as-1*-binding factor ASF-1 and is involved in salicylic acid- and auxin-inducible expression of *as-1*-containing target promoters. J. Biol. Chem. *275*, 19897-19905.
Nikiforova, V., Freitag, J., Kempa, S., Adamik, M., Hesse, H., and Hoefgen, R. (2003). Transcriptome analysis of sulfur depletion in *Arabidopsis thaliana*: interlacing of biosynthetic pathways provides response specificity. Plant J. *33*, 633-650.
Oh, H.S., Son, O., Chun, J.Y., Stacey, G., Lee, M.S., Min, K.H., Song, E.S., and Cheon, C.I. (2001). The *Bradyrhizobium japonicum hsfA* gene exhibits a unique developmental expression pattern in cowpea nodules. Mol. Plant Microbe Interact. *14*, 1286-1292.
Oldroyd, G.E., and Downie, J.A. (2008). Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu. Rev. Plant Biol. *59*, 519-546.
Peer, W.A., and Murphy, A.S. (2007). Flavonoids and auxin transport: modulators or regulators? Trends Plant Sci. *12*, 556-563.
Rombauts, S., Dehais, P., Van Montagu, M., and Rouze, P. (1999). PlantCARE, a plant *cis*-acting regulatory element database. Nucleic Acids Res. *27*, 295-296.
Sakai, T., Takahashi, Y., and Nagata, T. (1996). Analysis of the promoter of the auxin-inducible gene, *parC*, of tobacco. Plant Cell Physiol. *37*, 906-991.
Sieburth, L.E., Muday, G.K., King, E.J., Benton, G., Kim, S., Metcalf, K.E., Meyers, L., Seamen, E., and Van Norman, J.M. (2006). *SCARFACE* encodes an ARF-GAP that is required for normal auxin efflux and vein patterning in *Arabidopsis*. Plant Cell *18*, 1396-1411.
Stacey, G., Libault, M., Brechenmacher, L., Wan, J., and May, G.D. (2006). Genetics and functional genomics of legume nodulation. Curr. Opin. Plant Biol. *9*, 110-121.
Subramanian, S., Stacey, G., and Yu, O. (2007). Distinct, crucial roles of flavonoids during legume nodulation. Trends Plant Sci. *12*, 282-285.
Tiwari, S.B., Hagen, G., and Guilfoyle, T. (2003). The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell *15*, 533-543.
Ulmasov, T., Hagen, G., and Guilfoyle, T. (1994). The ocs element in the soybean GH2/4 promoter is activated by both active and inactive auxin and salicylic acid analogues. Plant Mol. Biol. *26*, 1055-1064.
Ulmasov, T., Liu, Z.B., Hagen, G., and Guilfoyle, T.J. (1995). Composite structure of auxin response elements. Plant Cell *7*, 1611-1623.
Ulmasov, T., Murfett, J., Hagen, G., and Guilfoyle, T.J. (1997). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell *9*, 1963-1971.
Wasson, A.P., Pellerone, F.I., and Mathesius, U. (2006). Silencing the flavonoid pathway in *Medicago truncatula* inhibits root nodule formation and prevents auxin transport regulation by rhizobia. Plant Cell *18*, 1617-1629.
Xiang, C., Miao, Z.H., and Lam, E. (1996). Coordinated activation of *as-1*-type elements and a tobacco glutathione *S*-transferase gene by auxins, salicylic acid, methyl-jasmonate and hydrogen peroxide. Plant Mol. Biol. *32*, 415-426.
Zhuang, X., Jiang, J., Li, J., Ma, Q., Xu, Y., Xue, Y., Xu, Z., and Chong, K. (2006). Over-expression of OsAGAP, an ARF-GAP, interferes with auxin influx, vesicle trafficking and root development. Plant J. *48*, 581-591.