Agric. Chem. Biotechnol. **49**(4), 148-153 (2006)

Article

Auxin Induced Expression of Expansin is Alered in a New *Aux1* Allele that Shows Severe Defect in Gravitropic Response

Hae-jun Jeong, Yerim Kwon, Jee-Eun Oh, Ki Deok Kim, Sung-Joon Lee[1], Suk-Whan Hong[2] and Hojoung Lee*

Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, 1, 5-ka Anam-dong, Sungbuk-ku, Seoul 136-075, Korea

[1]Department of Food Science, College of Life and Environmental Sciences, Korea University, 1, 5-ka Anam-dong, Sungbuk-ku, Seoul 136-075, Korea

[2]Division of Applied Plant Science, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea

Received November 9, 2006; Accepted December 2, 2006

While the underlying molecular mechanism remains to be elucidated, recent studies suggest that polar auxin transport is a key controlling factor in triggering differential growth responses to gravity. Identification of regulatory components in auxin-mediated differential cell expansion would improve our understanding of the gravitropic response. In this study, we identify a mutant designated *aux1-like* (later changed to *aux1*), an allele of the *aux1* mutant that exhibits a severely disrupted root gravitropic response, but no defects in developmental processes. In *Arabidopsis*, *AUX1* encodes an auxin influx carrier. Since in-depth characterization of the gravitropic response caused by mutations in this gene has been performed previously, we focused on identifying the downstream genes that were differentially expressed compared to wild-type plants. Consistent with the mutant phenotype, the transcription of the auxin-responsive genes *IAA17* and *GH3* were altered in *aux1* plants treated with IAA, 2, 4-D and NAA. In addition, we identified two expansin genes *EXP10* and *EXPL3* that exhibited different expression in wild-type and mutant plants.

Key words: Arabidopsis thaliana, *auxin, AUX1, expansin, GH3, gravity, IAA17*

To cope with challenging environments, plants re-set their developmental programs via a diverse array of signaling cascades. The gravity signal holds special importance in maintaining the correct orientation of organs in plants. In general, the statocytes of root columella and endodermal stem cells control the gravitropic response, regulation of this response is mediated in part by changes in auxin levels in a subset of plant tissues[1]. Polar auxin transport is a key regulatory step in triggering differential expansion and growth in response to gravity[2]. Therefore, our understanding of the mechanisms underlying the gravitropic response would be advanced by identification of the regulatory components in auxin-mediated differential cell expansion. Recent studies reveal that auxin controls the induction of a number of genes, including the *Aux/IAA* families, *SAUR* (Small Auxin Up RNAs) and *GH3*[3,4]. These genes are induced via the binding of auxin responsive factors (ARFs) to their promoter regions which contain auxin-responsive *cis*-acting elements (*AuxREs*). *GH3* was first isolated by differential screening from *Glycine max* as an early auxin-inducible gene. It has been shown that *iaa17/axr3*, *iaa7/axr2* and *iaa3/shy2* mutants displayed altered auxin responses and pleiotropic morphology[5,6]. These findings indicate clearly that primary auxin-responsive genes play pivotal roles in plant responses to auxin[7].

In Arabidopsis, *AUX1* encodes an auxin influx carrier that mediates inward auxin transport[8]. Asymmetric localization of both PIN1 and AUX1 proteins to the basal and apical plasma membranes is fundamental for polar movement of auxin[9,10,11]. It appears that basipetal auxin transport is crucial for root gravitropism, since the *aux1* mutant exhibits a root agravitropic phenotype[12] that can be rescued with the membrane-permeable auxin NAA, but not IAA and 2, 4-D, auxins that requires influx carrier activity[13,14]. Thus, AUX1 appears to be an indispensable component of the root gravitropic response in *Arabidopsis*.

Expansins are proteins that play important roles in cell enlargement. They belong to a large family of genes, found across plant species. The Arabidopsis genome contains 22 α- and 4 β-expansins, and given the low sequence similarity between them, it has been suggested that they act upon different cell wall polymers[15]. However, the detailed molecular mechanisms underlying the activities of auxin-mediated root gravitropism, and the roles played by expansins, remain poorly understood. In this study, we identify a mutant designated

*Corresponding author
Phone:+82-2-3290-3006; Fax:+82-2-3290-3508
E-mail: lhojoung@korea.ac.kr

aux1-like (later changed to *aux1*), an allele of the *aux1* mutant that exhibits a severely disrupted root gravitropic response, but no defects in developmental processes (Fig. 1B). Since in-depth characterization of the gravitropic response has been conducted previously for the *aux1* mutant, we focus on the identification of downstream genes that exhibit differential expression patterns between mutant and wild-type plants.

We now show novel observations that in response to treatment with 10 μM IAA, 1 μM 2, 4-D or NAA overall *IAA17* and *GH3* expression is reduced slightly in the *aux1* mutant relative to wild-type, whereas unlike wild-type. Moreover, in *aux1*, expression of *IAA17* and *EXP10* were affected significantly by NAA, which is known to permeate the plasma membrane. These results indicate that the sensitivity to NAA signaling is reduced in the *aux1* mutant, due to the absence of AUX1 protein. Our results indicate clearly that the induction of specific sets of *expansin* genes is disrupted in *aux1* plants, potentially contributing to the abnormal gravitropic responses.

Materials and Methods

Plant materials and growth conditions. *A. thaliana* plants of the ecotypes Col-0, and *Ler* were used for this study. The identification of the *aux1* mutant is described in the Results section. All germination and growth experiments were performed in growth chambers at 23 ± 1°C. For examination of the mutant phenotype, *aux1* mutant and wild-type seeds were surface-sterilized and sown on MS-agar supplemented with 2% sucrose. Following 4 d of stratification at 4°C, seeds were allowed to germinate at 23 ± 1°C under cycles of 16 h light and 8 h darkness. To determine growth phenotypes, mutant and wild-type seedlings were compared at the same stages after germination. For complementation studies, seedlings of *aux1-like* and a known *aux1* mutant were grown in the soil and crossed to obtain F_1 seeds. All 30 seedlings displayed the root agravitropic phenotype, indicating that the *aux1-like* phenotype was caused by a mutation in *AUX1*.

Treatment with *IAA, NAA, 2, 4-D* and *NPA*. Wild-type and *aux1* mutant plants were grown in MS agar supplemented with 2% sucrose under continuous white light. Ten-day-old wild-type and mutant seedlings were transferred to media containing the concentrations of IAA, NAA, 2, 4-D or NPA (N-1-naphthylphthalamic acid) specified in the Figures and grown vertically or upside down in the growth chamber under continuous white light for the time periods indicated in the Figures.

Northern blot analysis. Following surface sterilization, seeds sown on MS-agar medium were maintained at 4°C for 4d, then exposed to white light for 1d to induce germination. Prior to treatment, seedlings were grown on MS agar at 22°C. Total RNA from control or treated plants was extracted and analyzed, as described previously.[16] cDNA fragments of *IAA17* and *GH3* were used to generate ^{32}P-labeled probes and visualized by autoradiography. RNA was used as the loading control.

***RT-PCR* analysis.** Three-week-old seedlings were grown and treated as described above, after which total RNA was extracted from the entire plant, and cDNA synthesized via reverse transcription. PCR was conducted using an annealing temperature of 58°C and 33 cycles of amplification. The gene-specific DNA primers used for the RT-PCR analyses were as follows:

EXP10: 5' AGCCATATGGGTCATCTTGGGTT 3', 5' GGG ATCCTTAACGGAACTGTCC 3', *EXPL3:* 5' AGCCATATG GCTACGAGCTTCTT 3', 5' GGGATCCTCAGTTCCATAT GTG 3', *EXP3:* 5' AGCCATATGACGGCGACTGCGTT 3', 5' GGGATCCTCAGACTCGAAAGTT 3', *EXP11:* 5' AGCCAT ATGTCAAAGTCTCTAGC 3', 5' GGGATCCTTAAAACTG AACGTT 3' *UBQ3:* 5' TCTTCAATCTCTCCCAAAGC 3', 5' CAGTCAGAGTCTTGACAAAG 3'

Results and Discussion

Isolation and characterization of *aux1-like*, a new allele of the *aux1* mutant. We screened 54,000 M_2 seeds treated with EMS (*Lehle* Seeds Company) and identified a single mutant, designated '*aux1-like*' that exhibited no root gravitropic response in a root-bending assay. Since this mutant displays a clear phenotype with respect to the root gravitropic response (Fig. 1A and B), we decided to determine the identity of the gene responsible. F_1 progeny, which were obtained by crossing mutant and wild-type plants, exhibited normal phenotypes in response to gravity signals (Fig. 2A). The resultant F_2 plants segregated in a ratio of 3 : 1 (data not shown), indicating that the *aux1-like* mutation is recessive.

In view of the types of gravity-related mutants described previously, it was necessary to determine whether the *aux1-like* mutant is an allele of a mutant with an established phenotype. Mutant plants were crossed with wild-type plants of the *Ler* ecotype, and the resultant F_2 seeds were used for initial mapping. The *aux1-like* locus mapped to the lower arm of chromosome 2 near the SSLP marker, nga168 (Fig. 2B). Several auxin-related genes are located in the region surrounding

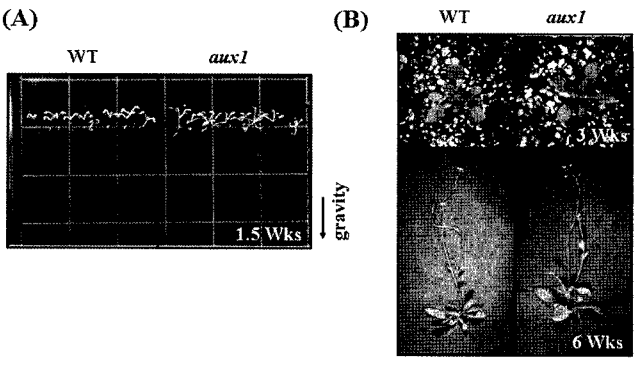
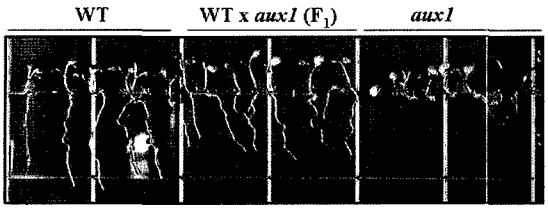


Fig. 1. Comparison between *aux1-like* and wild-type plant morphologies and SSLP mapping of the *aux1* mutation. Mutant and wild-type seeds were surface-sterilized, sown on MS agar supplemented with 2% sucrose, stratified at 4°C for 3 d and grown in (A) the medium specified or (B) soil.

(A)



(B)

Chr.#	Marker	Recombination freq.
1	nga63	63%
2	nga168	12%
3	nga162	46%
4	nga8	62%
5	nga225	55%

Fig. 2. *aux1-like* is a new allele of *aux1*. (A) Root gravitropic responses of wild-type, F_1 (WT x *aux1*) and *aux1* mutant plants grown on MS agar supplemented with 2% sucrose. Images were obtained when the seedlings were 2 weeks old. (B) SSLP marker-based mapping of the *aux1* mutation. The *aux1* mutants were crossed with wild-type plants of the *Ler* ecotype and the resultant F_2 seeds were utilized for mapping. Using several sets of SSLP markers, the *aux1* locus mapped to the lower arm of chromosome 2 near the marker nga168.

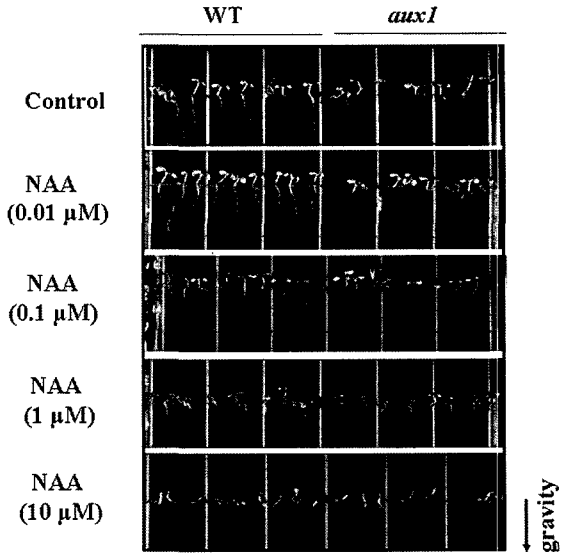


Fig. 3. Recovery of the gravitropic response in the *aux1-like* in the presence of NAA. Wild-type and *aux1* mutant plants were grown under continuous white light on normal MS agar supplemented with 2% sucrose and NAA (0.01, 0.1, 1 or 10 mM). The seedlings were grown vertically throughout the examination period.

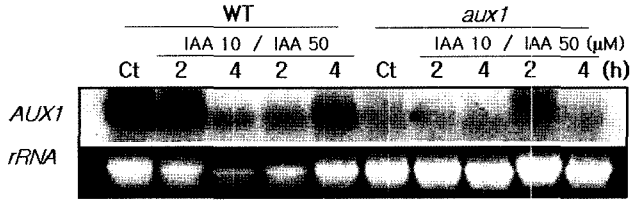


Fig. 4. Absence of the *AUX1* transcript in the *aux1* mutant. Three-week-old seedlings of wild-type and *aux1* seedlings were treated with IAA (10 or 50 mM) for 2 or 4 h. Each lane contains 20 g RNA. Samples were hybridized with a ^{32}P-labeled *AUX1* probe. rRNA was used as a loading control.

this mutation including *AUX1*, which encodes the auxin influx carrier. To determine whether the altered gravitropic response of *aux1-like* is derived from a mutation in *AUX1 (AT2G38120)*, we compared their nucleotide sequences. It was revealed that *aux1-like* is another allele of *aux1* but contains a different mutation from previously identified mutants of *AUX1*, with a G to A substitution at position 156, converting TGG into TGA (a stop codon) and leading to early termination of AUX1 protein translation. To date, >50 alleles of *AUX1* have been identified that contain missense and nonsense mutations or cause splicing defects.[17]

Defects in the root gravitropic response of the *aux1* mutant are suppressed by NAA. To confirm that the mutation in *AUX1* is responsible for the *aux1-like* phenotype, we examined whether the gravitropic response was restored by exogenous application of NAA. A previous report by Marchant (1999) indicated that the influx activity of AUX1 is specific for IAA and 2, 4-D, whereas NAA permeates through the plasma membrane, triggering normal auxin signaling responses. Both *aux1-like* and wild-type seeds were germinated in media containing various concentrations of NAA. The abnormal response of *aux1-like* mutant roots to gravity was rescued to wild-type levels in application of NAA. Root growth was inhibited in both mutant and wild-type plants by 1 or 10 μM NAA (Fig. 3). Therefore, as the *aux1-like* mutant is an allele of *aux1*, we investigated expression of the mutant gene. Specifically, the base at position 156 of *AUX1* is altered to a stop codon in the *aux1-like* mutant (hereafter, *aux1-like* will be designated *aux1*). Thus, it was necessary to clarify whether this gene is expressed in the *aux1* mutant. Three-week-old wild-type and *aux1* seedlings that had been exposed to IAA (10 or 50 μM) for 2 or 4 h, were harvested for Northern analysis. The *AUX1* transcript was detected in wild-type seedlings, but not in *aux1* mutants (Fig. 4). It is common to detect reduced levels of transcript when a stop codon is incorporated[17] and it is likely that the mutation destabilized the *aux1* transcript. In addition, this finding suggests that *aux1-like* is an allele of *AUX1*.

Altered expression of auxin-responsive genes in the *aux1* mutant in response to IAA, 2, 4-D and NAA. In the light of the defects in the root gravitropic response of the *aux1* mutant, we examined expression of auxin-responsive genes in mutant and wild-type plants in the presence of exogenous auxin. A number of auxin-responsive genes have been identified[3,4] and while their precise roles are not understood at the molecular level,[17] they are crucial for plant growth and development.[18,19] The protein encoded by *IAA17* is a negative regulator that is degraded via the auxin signaling cascade, but its transduction is induced strongly by auxin in a temporal manner. In the

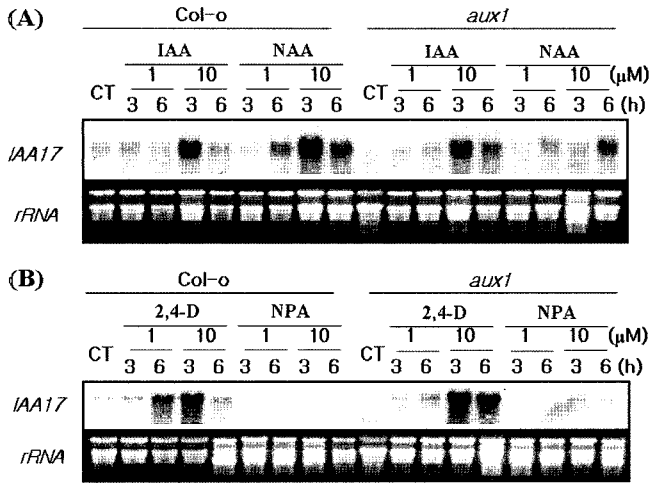


Fig. 5. Northern blot analysis of *IAA17* in response to auxin in wild-type and *aux1* mutant plants. (A) Three-week-old wild-type and *aux1* seedlings were treated with IAA (1 or 10 mM) or NAA (1 or 10 mM) for 3 or 6 h. Total RNA was prepared and 20 μg of RNA from each sample loaded onto a gel, separated by electrophoresis and transferred to a membrane. The blot was hybridized with a ^{32}P-labeled *IAA17* probe to determine the levels of transcript in wild-type and *aux1* mutants. rRNA was used as the loading control. (B) Time-course of *IAA17* transcript induction in wild-type and the *aux1* mutants, following exogenous application of 2, 4-D and NPA. Wild-type and *aux1* mutant seedlings grown on agar plates were treated with 1 or 10 mM 2, 4-D and NPA for the times indicated. Total RNA was extracted and Northern blot analysis performed using the *IAA17* probe. Hybridization was visualized by autoradiography.

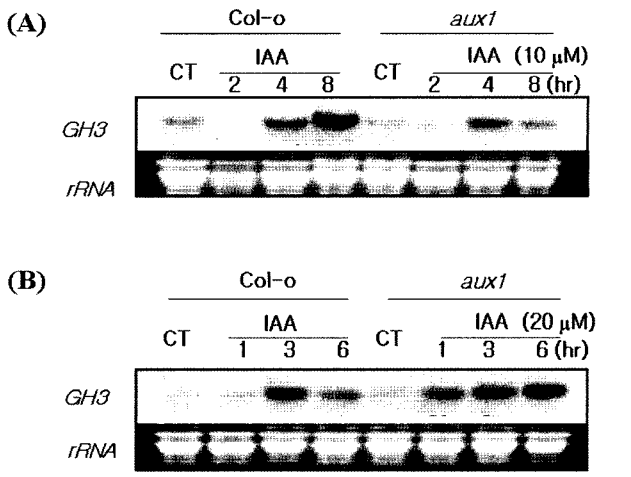


Fig. 6. Expression patterns of *GH3* in response to auxin treatment in wild-type and *aux1* plants. Transcript levels of the auxin responsive gene *GH3* were determined in wild-type and *aux1* mutant plants treated with 10 mM IAA for 2, 4 or 8 h. Following application of IAA to seedlings, total RNAs were used for Northern blot analysis using a ^{32}P-labeled *GH3* probe. The data show hybridized signals from the *GH3* probe to the blot. RNA was used as the loading control. Hybridization was visualized by autoradiography.

absence of IAA or NAA, Northern blot analysis indicated no significant difference between the *IAA17* transcript levels of wild-type and *aux1* mutant plants (Fig. 5A). Transcription of *IAA17* was induced in both the wild-type and *aux1* mutant plants in the presence of both IAA and NAA, although induction in response to IAA was slightly reduced in the *aux1* mutant. The reduction was more significant in the case of the *aux1* mutant with NAA treatment. Since NAA is known to permeate the plasma membrane, induction of *IAA17* should be similar in the *aux1* mutant and wild-type plants. This result appears somewhat contradictory, given that the gravitropic response is recovered in *aux1* mutants treated with NAA. However, this finding is consistent with the Northern blot analysis of *EXP10* in *aux1* plants (Fig 4B). These results predict that the sensitivity of NAA signaling will decrease in the *aux1* mutant, due to the absence of AUX1. At present, we do not have a clear explanation for the reduced sensitivity of the *aux1* mutant to NAA with respect to expression of *IAA17* and *EXP10*. However, as IAA is the most abundant and physiologically relevant auxin found in higher plants and the cell in the *aux1* mutant possess lower amounts of IAA in their cytoplasm than wild-type cells under normal growth conditions, the addition of exogenous NAA for only a short period of time (3-6 h) may be insufficient for *IAA17* expression to rise to wild type levels.

The auxin, 2, 4-D also requires AUX1 for entry into the cellular space and upon treatment with 1 μM 2, 4-D, reduced induction of *IAA17* was observed in the *aux1* mutant (Fig. 5B). However, the induction was recovered when the *aux1* mutant was treated with high concentration of 2, 4-D (10 μM), indicating that the absence of AUX1 is responsible for reduced induction of *IAA17* in the *aux1* mutant upon treatment with 1 μM 2, 4-D. N-1-naphthylphthalamic acid (NPA) is a potent inhibitor of PIN1 (a protein that facilitates auxin efflux) and prohibits transport of auxin from the intracellular space[20]. NPA treatment did not induce transcription of *IAA17* in either the *aux1* mutant or wild-type plants (Fig. 5B). Since the induction of *IAA17* is reduced slightly in the *aux1* mutant following induction with IAA, we analyzed the transcript levels of the auxin-responsive gene *GH3* following IAA treatment. Following incubation with 10 μM IAA for 8 h, *GH3* transcription was induced strongly in wild-type, but not *aux1* plants (Fig. 6A). In contrast, an increased concentration of IAA (20 μM) resulted in a greater enhancement of *GH3* transcription in *aux1* mutants than in wild-type plants (Fig. 6B). Therefore, we examined the root gravitropic response of the *aux1* mutant in the presence of IAA. At 0.1 μM IAA, a few lateral roots emerged from the main root of wild-type plants, but not the *aux1* mutant, whereas at 1 μM IAA, lateral roots developed from *aux1* mutant plants and were responsive to gravity (Fig. 7). However, the increased amounts of IAA can trigger induction of IAA-responsive genes as well as lateral root growth and a gravitropic response (Figs. 5B and Fig. 7), leading to the conclusion that IAA still permeates the plasma membrane at a reduced rate in the *aux1* mutant, although there is no AUX1 in this mutant.

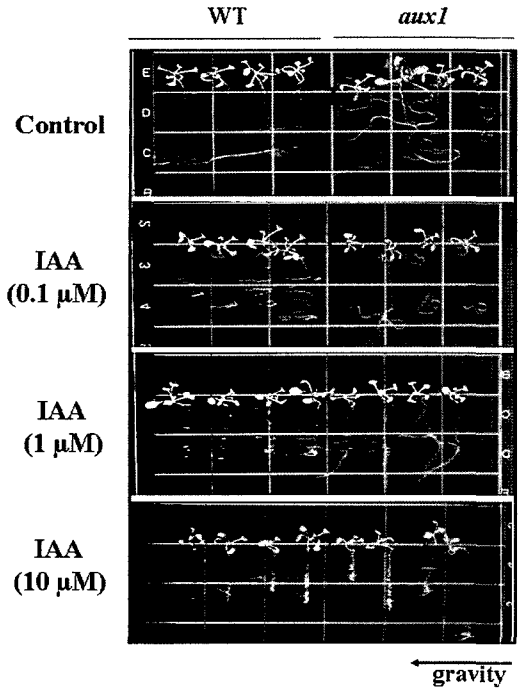


Fig. 7. Lateral root growth phenotypes of wild-type and *aux1* mutant plants in response to IAA and gravity. Wild-type and *aux1* plants were grown on MS agar supplemented with 2% sucrose under continuous white light and in an upward orientation. Ten-day-old seedlings were transferred to media containing IAA (0, 0.1, 1 or 10 mM) and grown for the time periods indicated.

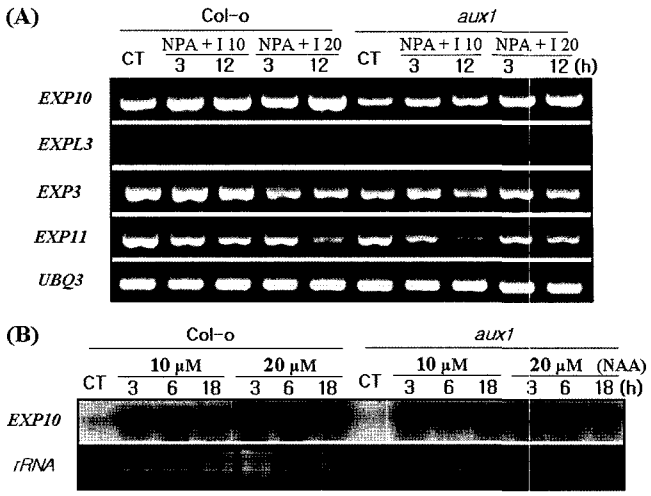


Fig. 8. *RT-PCR* and Northern blot analysis of several *expansin* genes from wild-type and *aux1* plants following treatment with NPA and IAA or NAA. (A) RT-PCR analysis was performed on plants treated with IAA and NPA. Wild-type and *aux1* plants were grown on MS agar supplemented with 2% sucrose under continuous white light and in an upward orientation. Prior to harvest, 10-day-old seedlings were transferred to media containing 10 or 20 mM IAA and NPA for 3 or 12 h. N and I refer to NPA and IAA, respectively. *UBQ3* was used as an internal control. PCR was performed using specific primer pairs for the amplification of *EXP10, EXPL3, EXP3* and *EXP11,* and annealing at 58C and 33 cycles of amplification. (B) *EXP10* transcription levels in wild-type and *aux1* plants treated with NAA (10 and 20 mM) for 3, 6 or 18 h. Following application of NAA to seedlings, total RNAs were harvested and Northern blot analysis performed using ^{32}P-labeled *EXP10* probe. RNA was used as the loading control. Hybridization was visualized by autoradiography.

In the *aux1* mutant, a subset of expansins display altered expression in the presence of auxin. Auxin plays a pivotal role in various plant responses, and in association with the tropic responses triggers differential growth of plant cells towards the origin of the tropic signal[21]. Recently, immunolocalization studies have demonstrated that within 30 min of gravi-stimulation, the expansin signals of roots are stronger on the expanding convex side than on the concave flank.[22] Moreover, inhibitors of vesicle or auxin transporters prevent differential localization of expansin. We thought that abnormal IAA transport in *aux1* mutant may cause the altered expression of *expansin* genes, leading to the disruption of gravitropic response. Thus, we decided to compare the expression patterns of wild-type and *aux1* mutant plants for the *expansin* genes (*EXP10, EXPL3, EXP3,* and *EXP11*), which were selected using genomic sequence information for *Arabidopsis*. Primers specific to each gene were constructed and *RT-PCR* analyses performed. Three-week-old wild-type and *aux1* mutant seedlings were transferred to media containing IAA and NPA, for 3 or 12 h, respectively (Fig. 8A). There was no detectable difference between the expression levels of *EXP3* and *EXP11* in wild-type and *aux1* mutant plants. However, the expression of *EXP10* and *EXPL3* was reduced in the *aux1* mutants following treatment with NPA and IAA. Using northern blot analysis we tested whether NAA reduces induction of *EXP10* transcription in the *aux1* mutants (Fig. 8B). However, we were not able to detect the *EXPL3* transcript in the same northern blot analysis. It seems that the level of *EXPL3* transcript is too low to be detected. These results indicate clearly that induction of specific sets of *expansin* genes is disrupted in *aux1* mutant plants. Hence, we propose that differential expression of *expansin* genes is required for mediation of the gravitropic response in roots. Moreover, induction of *EXP10* was reduced following treatment with NAA, which together with the results for induction of *IAA17*, supports the suggestion that NAA sensitivity is lower in the *aux1* mutant.

In summary, we identified a new mutant allele of *aux1* that displays a severely disrupted root gravitropic response due to a mutation in *AUX1*. The transcription of the auxin-responsive genes *IAA17* and *GH3* was altered in *aux1* mutants treated with IAA, NAA and 2, 4-D. In addition, we identified altered expression of two *expansin* genes (*EXP10* and *EXPL3)* and examination of their tissue-specific expression patterns will be the focus of further research. We expect that elucidation of the roles played by these genes will improve our understanding of differential cell expansion in auxin-mediated root gravitropism, since these genes exhibit auxin-specific induction.

Acknowledgments

This work was supported by a Korean Research Foundation Grant (KRF-2004-041-F00024 to H.L.).

References

1. Friml, J. (2003) Auxin transport-shaping the plant. *Curr. Opin. Plant Biol.* **6**, 7-12.
2. Berleth, T. and Sachs, T. (2001) Plant morphogenesis: long-distance coordination and local patterning. *Curr. Opin. Plant Biol.* **4**, 57-62.
3. Abel, S. and Theologis, A. (1996) Early genes and auxin action. *Plant Physiol.* **111**, 9-17.
4. Dharmasiri, N. and Estelle, M. (2004) Auxin signaling and regulated protein degradation. *Trends Plant Sci.* **9**, 302-308.
5. Nemhauser, J. L., Feldman, L. J. and Zambryski, P. C. (2000) Auxin and ETTIN in Arabidopsis gynoecium morphogenesis. *Development* **127**, 3877-88.
6. Harper, R. M., Stowe-Evans, E. L. Luesse, D. R., Muto, H., Tatematsu, K., Watahiki, M. K., Yamamoto, K. and Liscum, E. (2000) The NPH4 locus encodes the auxin response factor ARF7, a conditional regulator of differential growth in aerial Arabidopsis tissue. *The Plant Cell* **12**, 757-770.
7. Reed, J. W. (2001) Roles and activities of Aux/IAA proteins in Arabidopsis. *Trends Plant Sci.* **6**, 420-425.
8. Swarup, R., Kargul, J., Marchant, A., Zadik, D., Rahman, A., Mills, R., Yemm, A., May, S., Williams, L., Millner, P., Tsurumi, S., Moore, I., Napier, R., Kerr, I. D. and Bennett, M. J. (2004) Structure-function analysis of the presumptive Arabidopsis auxin permease AUX1. *The Plant Cell* **16**, 3069-83
9. Galweiler, L., Guan, C., Muller, A., Wisman, E., Mendgen, K., Yephremov, A. and Palme, K. (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science* **282**, 2226-2230
10. Geldner, N., Frimi, J., Stierhof, Y. D., Jurgens, G. and Palme, K. (2001) Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. *Nature* **413**, 425-428
11. Swarup, R., Friml, J., Marchant, A., Ljung, K., Sandberg, G., Palme, G. and Bennett, M. J. (2001). Localisation of the auxin permease AUX1 in the *Arabidopsis* root apex reveals two novel functionally distinct hormone transport pathways. *Genes Dev.* **15**, 2648-2653.
12. Rashotte, A. M., Brady, S. R., Reed, R. C., Ante, S. J. and Muday, G. K. (2000). Basipetal auxin transport is required for gravitropism in roots of *Arabidopsis*. *Plant Physiol.* **122**, 481-490.
13. Marchant, A., Kargul, J., May, S. T., Muller, P., Delbarre, A., Perrot-Rechenmann, C. and Bennett, M. J. (1999). AUX1 regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. *EMBO J.* **18**, 2066-2073.
14. Parry, G., Delbarre, A., Marchant, A., Swarup, R., Napier, R., Perrot-Rechenmann, C. and Bennett, M. J. (2001). Novel auxin transport inhibitors phenocopy the auxin influx carrier mutation *aux1*. *Plant J.* **25**, 399-406.
15. Shieh, M. W. and Cosgrove, D. J. (1998) Expansins. *J. Plant Res.* **111**, 149-157.
16. Lee, H., Xiong, L., Gong, Z., Ishitani, M., Stevenson, B. and Zhu, J. K. (2001) The Arabidopsis *HOS1* gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleocytoplasmic partitioning. *Genes Dev.* **15**, 912-924.
17. Marchant, A. and Bennett M. J. (1998) The Arabidopsis *AUX1* gene: a model system to study mRNA processing in plants. *Plant Mol. Biol.* **36**, 463-71.
18. Hagen, G. and Guilfoyle, T. (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. *Plant Mol. Biol.***49**, 373-385.
19. Ulmasov, T., Hagen, G. and Guilfoyle, T. J. (1997) ARF1, a transcription factor that binds to auxin response elements. *Science* **276**, 1865-1868.
20. Ulmasov, T., Hagen, G. and Guilfoyle, T. J. (1999) Activation and repression of transcription by auxin-response factors. *Proc. Natl. Acad. Sci. USA* **96**, 5844-5849.
21. Katekar, G. F. and Giesler, A. E. (1980) Auxin transport inhibitors. IV. Evidence of a common mode of action for a proposed class of auxin transport inhibitors, the phytotropins. *Plant Physiol.* **66**, 1190-1195.
22. Zhang, N. and Hasenstein, K. H. (2000) Distribution of expansins in graviresponding maize roots. *Plant Cell Physiol.* **41**, 1305-1312.