Journal of the Iowa Academy of Science: JIAS

Volume 98 | Number

Article 7

1991

The axr1 Mutation of Arabidopsis is Expressed 10 Booth Roots and Shoots

Cythia Lincoln
Indiana University

Mark Estelle
Indiana University

Let us know how access to this document benefits you

Copyright © Copyright 1991 by the Iowa Academy of Science, Inc.

Follow this and additional works at: https://scholarworks.uni.edu/jias

Part of the Anthropology Commons, Life Sciences Commons, Physical Sciences and Mathematics Commons, and the Science and Mathematics Education Commons

Recommended Citation

Lincoln, Cythia and Estelle, Mark (1991) "The axr1 Mutation of Arabidopsis is Expressed 10 Booth Roots and Shoots," *Journal of the Iowa Academy of Science: JIAS, 98(2),* 68-71.
Available at: https://scholarworks.uni.edu/jias/vol98/iss2/7

This Research is brought to you for free and open access by the Iowa Academy of Science at UNI ScholarWorks. It has been accepted for inclusion in Journal of the Iowa Academy of Science: JIAS by an authorized editor of UNI ScholarWorks. For more information, please contact scholarworks@uni.edu.

Jour. Iowa Acad. Sci. 98(2):68-71, 1991

The *axr1* Mutation of *Arabidopsis* is Expressed in Booth Roots and Shoots

CYNTHIA LINCOLN and MARK ESTELLE

Department of Biology, Indiana University, Bloomington, IN 47405

In order to identify genes involved in auxin action we have isolated a number of mutants of *Arabidopsis thaliana* which are resistant to exogenous application of either indole-3-acetic acid or 2,4-dichlorophenoxyacetic acid. One of the genes identified in this way is the *Axr1* gene. Recessive mutations in this gene confer resistance to auxin as well as a number of morphological defects. In this report we describe the isolation of at least two new *axr1* alleles. In addition, we show that the *axr1* mutation affects gravitropic growth of the root, auxin-induced ethylene biosynthesis and growth in tissue culture. Our results indicate that the *axr1* mutation is expressed in most tissues of the plant.

INDEX DESCRIPTORS: *Arabidopsis thaliana*, plant mutants, auxin, phytohormone, plant development.

The plant growth regulator indole-3-acetic acid (IAA) appears to play a central role in the growth and development of higher plants. Physiological studies indicate that IAA is involved in cell expansion during shoot elongation (Jacobs and Ray, 1976), tropic responses (Shen-Miller et al., 1973; McClure and Guilfoyle, 1989) and differentiation of vascular elements (Aloni, 1987). More recently, auxin auxotrophic tissue culture variants of *Nicotiana plumbaginifolia* have been used to study the role of auxin in plant development (Blonstein et al., 1988). These auxotrophic lines were recovered after mutagenesis of cell cultures which do not require auxin for growth. The mutant lines will not grow without auxin in the medium and attempts to regenerate plants from these auxotrophic lines have been unsuccessful. Although these experiments are not conclusive, they suggest that auxin is essential for cell growth and plant viability.

The mechanism of auxin action is currently an active area of research. A number of early biochemical responses to auxin have been described. In pea epicotyls and soybean hypocotyls exposure to auxin results in rapid changes in the expression of specific genes (Theologis, 1986; McClure and Guilfoyle, 1987). The function of these auxin-regulated genes is unknown. In tobacco protoplasts, auxin causes a membrane hyperpolarization within seconds of exposure. Experiments with an auxin-resistant mutant of tobacco suggest that this membrane hyperpolarization is physiologically significant. The dose-response curve for membrane hyperpolarization in mutant protoplasts is shifted toward higher auxin concentrations (Ephritikhine et al., 1987).

It is generally assumed that auxin acts by binding to a protein receptor. Several proteins which bind auxin *in vitro* have been identified in both maize and tobacco. The most completely characterized auxin binding protein (ABP) is a membrane associated protein in maize which was originally called binding site I (Rubery, 1981). This ABP has been localized by cell fractionation techniques to the endoplasmic reticulum (Rubery, 1981; Shimomura et al. 1986) and a gene which codes for this protein has recently been isolated (Hess et al., 1989; Inohara et al., 1989). Sequence analysis of this gene indicates that the COOH-terminus of the protein contains the amino acid sequence KDEL. This sequence has been shown to be responsible for retention of proteins in the endoplasmic reticulum (Munroe and Pelham, 1987). Curiously, antisera raised against this protein will inhibit auxin-induced membrane hyperpolarization in tobacco protoplasts indicating that the ABP also lies exposed on the surface of the plasma membrane (Barbier-Brygoo et al., 1989). It now appears likely that auxin interacts with a family of receptors with different cellular locations, possibly involved in regulating different cellular processes. Such a family of receptors might explain how auxin regulates such a diverse array of growth processes.

In our lab we are interested in identifying the genes and proteins required for auxin action in the small crucifer *Arabidopsis thaliana*. Our approach has been to screen for mutants of *Arabidopsis* which are resistant to exogenous application of auxin. In a previous report we described the isolation and preliminary characterization of a recessive mutation, called *axr1*, which confers resistance to auxin as well as an unusual morphological phenotype. Several aspects of this phenotype suggest that the *axr1* mutation affects auxin-regulated growth processes (Estelle and Somerville, 1987). In this report we update our genetic studies and describe the results of several experiments which support the hypothesis that the wild-type *AXR1* gene is required for auxin action.

MATERIALS AND METHODS

Plant Material

Arabidopsis plants were grown at 23°C on a commercially available peat-lite mixture such as Metro-Mix™, with continuous illumination supplied at an intensity of 85-105 mE/m^2/s. Every two weeks plants were given nutrient solution containing 5 μM KNO_3, 2.5 μM KH_2PO_4 (adjusted to pH 5.5), 2 μM $MgSO_4$, 2 μM $Ca(NO_3)_2$, 50 μM Fe-EDTA (Sigma EDFS), 70 μM H_3BO_3, 14 μM $MnCl_2$, 0.5 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.2 μM $NaMoO_4$, 10 μM NaCl, 0.01 μM $CoCl_2$. For certain experiments, plants were grown under sterile conditions on petri plates. Seeds were surface sterilized for 20 minutes in 30% v/v bleach and 0.02% Triton-X and then placed on petri plates containing the nutrient solution mentioned above, supplemented with 8 g/l agar and 10 g/l sucrose (minimal medium). Hormones were added to the media after autoclaving. Sterile plants were grown at 22°C to 24°C with a 16 h light cycle at a light intensity of 20 to 60 μE/m^2/s. All of the plant lines used in these experiments were of the Columbia ecotype.

Mutagenesis

For chemical mutagensis, approximately 25,000 seeds were soaked for 16 h in 100 ml 0.3% (v/v) ethyl methane sulfonate, then washed in water over a period of 4 h. This M1 seed was sown at a density of approximately 1/cm^2. At maturity the resulting plants were bulk harvested to produce M2 seed. To mutagenize with gamma irradiation, 20,000 seeds were incubated in water for three hours. A dose of 50 Krad was administered to the imbibed seed in a J.L. Shephard irradiator containing ^{137}Cs. The seeds were sown and the M2 population harvested as described above.

Determination of root gravitropic response

Sterile seeds were sown on petri plates containing minimal medium. The plates were placed in the incubator on edge so that the seedlings would grow along the surface of the medium. Growth conditions were as described above. After six days, the roots of the seedlings were straightened and the plates were rotated 90°. At hourly intervals, plates containing wild-type and mutant seedlings were removed from the incubator and the angle of root curvature was determined for each genotype. At least seven seedlings were used for each determination.

Table 1. Recovery of *axrl* mutants

M2 population	Mutagen	Selection	Mutants recovered
A[1]	EMS	2,4-D	*axrl-1* *axrl-3* *axrl-4* *axrl-5* *axrl-6*
B[1]	EMS	2,4-D	*axrl-9* *axrl-11* *axrl-12* *axrl-15*
C[2]	EMS	2,4-D	*axrl-16* *axrl-17* *axrl-18* *axrl-19* *axrl-20* *axrl-21*
C[2]	EMS	IAA	*axrl-22*
D[2]	gamma	2,4-D	*axrl-23*

[1]Estelle and Somerville, 1987.
[2]This study.

Auxin-induced ethylene production

One cm stem segments were excised from mutant and wild-type plants and placed in a 1 ml microvial containing 50 µl of 50 mM MES (pH 5.8) supplemented with various concentrations of 2,4-D. The vials were sealed with a screwcap fitted with a teflon septum and placed on a shaker under room light at 22°C. After 24 hours the ethylene levels within the vial were measured by gas chromatography (Kende and Hanson, 1976) and the stem segment was weighed.

Determination of growth rate during callus initiation

Inflorescence segments, 5 to 7 cm long were excised from the primary inflorescence of 3 to 4 week old plants and sterilized by treating with a 40% v/v bleach solution containing 0.02% Triton X-100 for 4 minutes. The sterile segments were rinsed in water and cut into 0.6 to 1.4 cm long segments. Each segment was weighed and then placed on agar medium containing Murashige and Skoog salts and vitamins plus 0.23 µM kinetin and various concentrations of 2,4-D. Tissue segments were incubated at 20°C with continuous lighting at 20 to 60 µE/m²/s. After three weeks each segment was weighed. Growth is expressed as $(W-W_0)/W_0$ where W_0 and W are the initial and final fresh weights respectively.

Table 2. Genetic segregation of 2,4-D resistance in *axrl* lines

Cross		Number of plants		
		Resistant	Sensitive	χ^{2a}
axrl-19 × wild-type	F1	—	23	
	F2	186	493	2.07^b
axrl-21 × wild-type	F1	—	51	
	F2	82	281	1.12^b

[a]χ^2calculated based on an expected ratio of 3 sensitive to 1 resistant
[b]$p>0.05$

RESULTS

Isolation of *axr1* mutants

In a previous report (Estelle and Somerville, 1987), we described the isolation and preliminary characterization of nine auxin-resistant mutants of *Arabidopsis thaliana.* These mutant lines all carried recessive mutations in a single gene that we have called the *axr1* gene. In addition to auxin-resistance, the *axr1* mutations produce a distinctive morphological phenotype, including defects in leaf, inflorescence and flower morphology (Estelle and Somerville, Lincoln and Estelle, unpublished). We have now screened a further 170,000 M2 *Arabidopsis* seeds from two independently mutagenized populations for resistance to either 50 µM IAA or 5 µM 2,4-dicholorophenoxy acetic acid (2,4-D). Wild-type seedlings grown at these concentrations of hormone fail to develop roots. Mutants were identified by looking for seedlings which had roots. Eight new auxin-resistant lines with a morphological phenotype similar to the original *axr1* mutant were recovered. The rosette leaves of these plants have a crinkled appearance which is characteristic of *axr1* plants. In addition, the inflorescences are thin and the flowers are poorly developed. When the mutants were allowed to self they produced very few seed. However, they were capable of serving as both male and female parent in crosses with wild-type or *axr1* plants (see below). All of these features are also characteristic of *axr1* plants. Seven of the new resistant lines were recovered from an EMS-mutagenized population and one line was recovered from a gamma-mutagenized population. A summary of all of the *axr1* screens is presented in Table 1.

Genetic analysis of *axr1* mutants

To determine the genetic basis for auxin-resistance in the new mutant lines, each line was crossed to wild-type plants and the F1 progeny were allowed to self to produce an F2 population. The F1 and F2 progeny from these crosses were analyzed by placing seeds on medium containing 1.0 µM 2,4-D. Results for *axr1-19* and *axr1-21* are presented in Table 2. For each line, all of the F1 plants were sensitive to auxin and in the F2, auxin-resistance segregated at a ratio of 3 sensitive plants to 1 resistant plant. These results indicate that for these mutant lines, auxin resistance is due to a single recessive mutation. Similar results were obtained for all of the *axr1* mutants. The mutants were further analyzed by crossing to plants which were homozygous for the *axr1-3* mutation. Table 3 shows that for mutants *axr1-19* and *axr1-21,* the progeny of these crosses were auxin-resistant and had the *axr1* morphological phenotype indicating that in each line, auxin resistance was due to a mutation at the *axr1* gene. Again similar results were obtained for all of the mutants listed in Table 1.

***axr1* roots have an altered gravitropic response**

Physiological studies in a number of plant species indicate that auxin may be involved in gravitropic growth of roots (Feldman, 1985). To determine if *axr1* plants are defective in root gravitropism, we examined the growth of mutant and wild-type roots which had been rotated from a vertical orientation to a horizontal orientation. The results are displayed in Figure 1. Wild-type roots were completely reoriented after five hours while *axr1-3* roots were not growing vertically until eight hours had passed. The gravitropic response of *axr1* roots appears to be intact but considerably delayed. Because *axr1* roots have a slightly faster growth rate than wild-type roots, the difference in gravitropic response is not due to a reduction in root growth rate.

Auxin-resistance in *axr1* plants is not root-specific.

Because the *axr1* mutants were isolated on the basis of auxin-resistant root growth, it is possible that auxin resistance is only expressed in the roots. To test this possibility we measured ethylene production in wild-type and mutant inflorescence tissue treated with various concentrations of 2,4-D. Auxin has been shown to stimulate ethylene biosynthesis in many plant species by causing an increase in

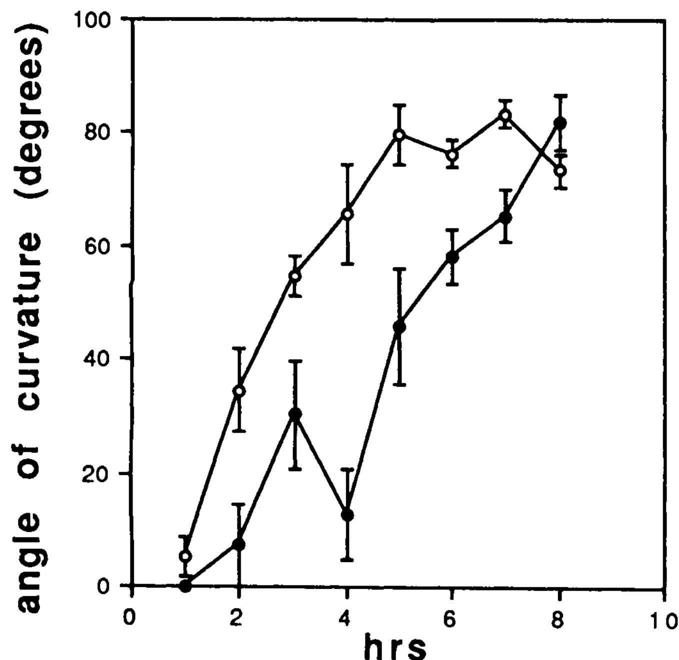

Fig. 1. Gravitropic response in roots of wild-type (open symbols) and *axr1-3* (closed symbols) seedlings. Seedlings grown on agar medium were rotated 90° at time zero. The angle of curvature from the horizontal was measured at times indicated. Each point represents the mean of at least 7 measurements. Error bars describe the standard error.

the level of the enzyme 1-aminocyclopropane 1-carboxylic acid (ACC) synthase (Yang and Hoffman, 1984). Our results, displayed in Figure 2, show that 2,4-D also causes an increase in ethylene production in *Arabidopsis* inflorescence segments. Wild-type segments produce significant levels of ethylene at 2,4-D concentrations of 0.05 mM or higher. However, in stem segments from *axr1* plants, measurable ethylene production does not occur until the concentration of hormone is 1.0 mM. This result indicates that auxin resistance in *axr1* plants is expressed in both the roots and the inflorescence.

To further investigate the expression of the *axr1* mutation we measured callus initiation from wild-type and *axr1* inflorescence segments on tissue culture media containing various concentrations of 2,4-D. The results of this experiment are displayed in Figure 3. When segments were placed on media containing 2,4-D at a concentration of from 1 to 20 μM, significantly less callus was produced on *axr1* tissue than on wild-type tissue. At higher concentrations of 2,4-D, both genotypes produced about the same amount of callus. These results suggest that *axr1* inflorescence tissue does not respond to auxin in the same way as wild-type tissue.

DISCUSSION

We have identified eight new auxin-resistant lines of *Arabidopsis* which have a morphological phenotype characteristic of the *axr1* mutant. Genetic analysis demonstrated that each of these new lines carries a recessive mutation at the *axr1* gene. Because mutants recovered from the same M2 population may be sibs, we do not know how many different alleles of the *axr1* gene have been isolated. However, since we screened four independent M2 populations, we must have at least four alleles. In addition, the mutant *axr1-3* is unique among the *axr1* mutants because it alone produces significant numbers of selfed progeny (Lincoln and Estelle, unpublished). We therefore believe that we have isolated at least two *axr1* alleles from M2 population A and at least one allele from each of populations B, C and D for a total of at least five *axr1* alleles.

Table 3. Complementation analysis of *axrl* lines

Cross	Number of plants	
	Resistant	Sensitive
axrl-19 × *axrl-3*	21	—
axrl-21 × *axrl-3*	13	—

The function of the wild-type *AXR1* gene remains unclear. However the morphological phenotype of the *axr1* mutants suggest that the gene plays an important role in auxin action. Mutant plants display a reduction in apical dominance and a decrease in internode elongation (Estelle and Somerville, 1987), processes which are thought to be regulated by auxin (Philips, 1975; Jacobs and Ray, 1979). The results described in this report show that the *axr1* mutation also affects the gravitropic response of the root. Mutant roots respond to gravity, but significantly slower than wild-type roots.

The role of auxin in root gravitropism is controversial (Feldman, 1985). According to the Cholodny-Went hypothesis, root curvature is due to the accumulation of a growth inhibiting substance on the lower portion of the root. The nature of this substance is unclear, but both IAA and abscisic acid have been proposed as candidates. Alternatively, differential growth may be due to changes in sensitivity to one or more growth regulators (Trewavas, 1981). The *axr1* mutation may be disturbing gravitropic response in a number of ways. A decrease in the rate of auxin transport might affect the establishment of an asymmetry in auxin concentration across the root which may be responsible for differential growth. Conversely, a decrease in auxin response in the growth zone of the root might result in a slower response to gravity. The available genetic evidence suggests that auxin plays an important role in gravitropic growth. In addition to *axr1*, the auxin-resistant mutants *aux1*, *Dwf* and *axr2* all display defects in gravitropism (Mirza et al., 1984; Wilson and Estelle, unpublished). In tomato, the *diageotropica* mutant, originally

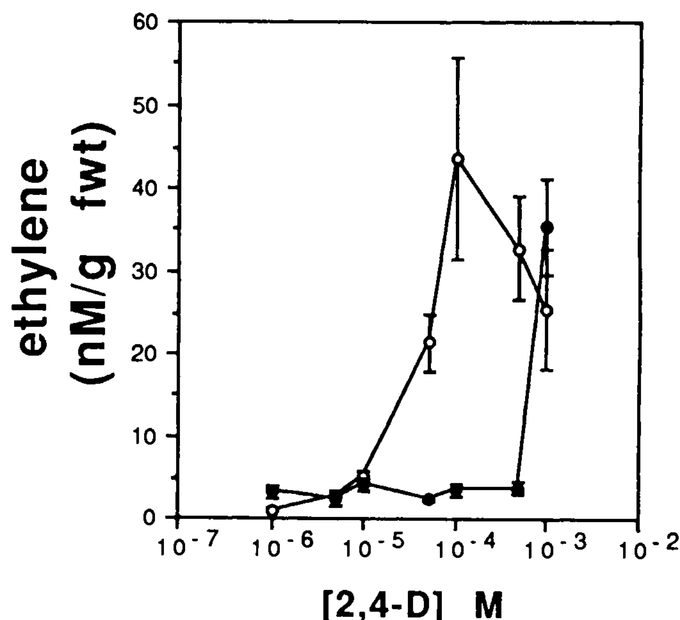

Fig. 2. Auxin-induced ethylene production in wild-type (open symbols) and *axr1-3* (closed symbols) stem segments. Ethylene levels were measured after 24 hours. Error bars indicate indicate +/-standard error (n=3).

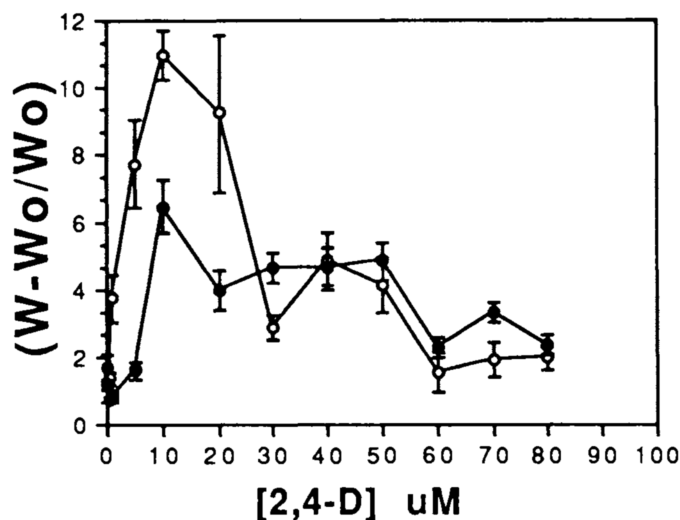

Fig. 3. Production of callus on wild-type (open symbols) and *axr1-3* (closed symbols) stem segments in medium containing various concentrations of 2,4-D. Growth rate is expressed as W-Wo/Wo where Wo is the original weight and W is the final weight measured in mg. Final weight was determined after 21 days.

identified on the basis of a gravitropic defect, has since been shown to be resistant to auxin (Kelly and Bradford, 1986).

In early studies on the *axr1* mutants, auxin resistance was measured in seedling roots (Estelle and Somerville, 1987). By measuring auxin induction of ethylene biosynthesis in stem segments and the induction of callus growth in media containing various concentrations of auxin, we have now shown that auxin-resistance is also expressed in the aerial portion of the plant. The *AXR1* gene product appears to be required for normal auxin response in most tissues of the plant. This is in contrast to the situation with the *aux1* mutants of *Arabidopsis*. These mutants display about the same level of resistance to auxin as do *axr1* plants, but do not display the same array of morphological aberrations. The only visible phenotype of *aux1* plants is a severe defect in root gravitropism. The wild-type *aux1* gene product appears to have a specific function in root gravitropism while the wild-type *axr1* gene product has a more general function and is required in most tissues of the plant.

The genetic analysis of hormone response in *Arabidopsis* promises to provide new insight into the molecular mechanism of hormone action. The recent development of two restriction fragment length polymorphism (RFLP) maps in *Arabidopsis* (Chang et al.; 1988, Nam et al., 1989) as well as techniques for cloning large fragments of *Arabidopsis* DNA in yeast artificial chromosone vectors (Guzman and Ecker, 1988) should make it possible to isolate any gene which has been identified by mutation. We have localized the *axr1* gene to a region on the end of chromosome 1 and have initiated a chromosome walk from a closely linked RFLP. We expect that the molecular isolation and characterization of the *axr1* gene will provide new insight into the mechanism of auxin action.

ACKNOWLEDGEMENTS

This research was supported by a National Institute of Health Predoctoral Fellowship (GM07757) to C.L. and a National Science Foundation grant DCB-8702448 to M.E.

REFERENCES

ALONI, R. 1987. Differentiation of Vascular Tissues. Ann. Rev. Plant Physiol. 38:179-204.

BARBIER-BRYGOO, H., G. EPHRITIKHINE, D. KLAMBT, M. GHISLAIN, and J. GUERN. 1989. Functional evidence for an auxin receptor at the plasmalemma of tobacco meosphyll protoplasts. Proc. Natl. Acad. Sci. USA 86:891-895.

BLONSTEIN, A.D., T. VAHALA, M. KOORNNEEF, and P.J. KING. 1988. Plants regenerated from auxin-auxotrophic variants are inviable. Mol. Gen. Genet. 215:58-64.

CHANG, C., J.L. BOWMAN, A.W. DEJOHN, E.S. LANDER, and E.M. MEYEROWITZ. 1988. Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA. 85:6856-6860.

EPHRITIKHINE, G., H. BARBIER-BRYGOO, J-F MULLER, and J. GUERN. 1987. Auxin effect on the transmembrane potential difference of wild-type and mutant tobacco protoplasts exhibiting a differential sensitivity to auxin. Plant Physiol. 83:801-804.

ESTELLE, M.A., and C.R. SOMERVILLE. 1987. Auxin-resistant mutants of *Arabidopsis* with an altered morphology. Mol. Gen. Genet. 206:200-206.

FELDMAN, L.J. 1985. Root Gravitropism. Physiol. Plant. 65:341-344.

GUZMAN, P., and J.R. ECKER. 1988. Development of large DNA methods for plants: molecular cloning of large segments of *Arabidopsis* and carrot DNA in yeast. Nucleic Acids Res. 16:11091-11105.

HESSE, T., J. FELDWISCH, D. BALSHUSEMANN, G. BAUW, M. PUYPE, J. VANDEKERCKHOVE, M. LOBLER, D. KLAMBT, J. SCHELL, and K. PALME. 1989. Molecular cloning and structural analysis of a gene from *Zea mays* (L.) coding for a putative receptor for the plant hormone auxin. EMBO J. 8:2453-2462.

INOHARA, N., S. SHIMOMURA, T. FUKUI, and M. FUTAI. 1989. Auxin-binding protein located in the endoplasmic reticulum of maize shoots: Molecular cloning and complete primary structure. Proc. Natl. Acad. Sci. USA 86:3564-3568.

JACOBS, M., and P. RAY. 1976. Rapid auxin-induced decrease in free space pH and its relationship to auxin-induced growth in maize and pea. Plant Physiol. 58:203-209.

KENDE, H., and A.D. HANSON. 1976. Relationship between ethylene evolution and senescence in morning-glory flower tissue. Plant Physiol. 57:523-527.

KELLY, M.O., and K.J. BRADFORD. 1986. Insensitivity of the *Diageotropica* tomato mutant to auxin. Plant Physiol. 82:713-717.

MAHER, E.P., and S.J.B. MARTINDALE. 1980. Mutants of *Arabidopsis thaliana* with altered responses to auxin and gravity. Biochem. Genet. 18:1053-1053.

MCCLURE, B.A., and T. GUILFOYLE. 1989. Rapid distribution of auxin-regulated MRNAs during gravitropism. Science. 243:91-93.

MCCLURE, B.A., and T. GUILFOYLE. 1987. Characterization of a class of small auxin-inducible soybean polyadenylated RNAs. Plant Mol. Biol. 9:611-624.

MEYEROWITZ, E.M. 1987. *Arabidopsis thaliana*. Ann. Rev. Genet. 21:93-112.

MIZRA, J.I., G.M. OLSEN, T-H. IVERSON, and E.P. MAHER. 1984. The growth and gravitropic responses of wild-type and auxin-resistant mutants of *Arabidopsis thaliana*. Physiol. Plant. 60:523-531.

MONROE, S., and H.R.B. PELHAM. 1987. A C-terminal signal prevents secretion of luminal ER proteins. Cell 48:899-907.

NAM, G-H., J. GIRAUDAT, B. DEN BOER, F. MOONAN, W.D.B. LOOS, B.M. HAUGE, and H.M. GOODMAN. 1989. Restriction Fragment Length Polymorphism Linkage Map of *Arabidopsis thaliana*. The Plant Cell. 1:699-705.

PHILLIPS, I.D. 1975. Apical dominance. Ann. Rev. Plant Physiol. 26:341-367.

RUBERY, P.H. 1981. Auxin receptors. Ann. Rev. Plant. Physiol. 32:569-596.

SHEN-MILLER, J. 1973. Rhythmic differences in the basipetal movement of indoleacetic acid between separated upper and lower halves of geotropically stimulated corn coleoptiles. Plant Physiol. 52: 166-170.

SHIMOMURA, S., T. SOTOBAYASHI, M. FUTAI, and T. FUKUI. 1986. Purification and properties of an auxin-binding protein from maize shoot membranes. J. Biochem (Tokyo). 99:1513-1524.

TREWAVAS, A. 1981. How do plant growth substances work. Plant Cell and Environment. 4:202-228.

THEOLOGIS, A. 1986. Rapid gene regulation by auxin. Ann. Rev. Plant Physiol. 37:407-438.

YANG, S.F., and N.E. HOFFMAN. 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35:155-189.