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Genetic and Physiological Analysis of a New Locus in *Arabidopsis* That Confers Resistance to 1-Aminocyclopropane-1-Carboxylic Acid and Ethylene and Specifically Affects the Ethylene Signal Transduction Pathway[1]

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A population of M_2 seedlings of *Arabidopsis thaliana* was screened for mutants that were insensitive to the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC). Several independent lines were obtained and proved insensitive to both ACC and ethylene. Two lines were identified as alleles of a single recessive mutation, designated *ain1*. Linkage analysis indicated that the *ain1* gene is located on chromosome 1, adjacent to the *cer5* marker and, therefore, genetically distinct from previously identified ethylene resistance loci. General phenotypic aspects of *ain1* mutants were similar to wild type. For both alleles, the level of insensitivity to ethylene at the seedling stage was indistinguishable in terms of elongation growth. In contrast, the gravitropic response of *ain1-1* seedlings was slower than that of wild-type and *ain1-2* seedlings. At the adult stage, stress responses of mutants were similar to wild type. However, ethylene-induced leaf senescence was delayed in both mutants. In addition, we observed significant interallelic variation in ethylene production rates. Growth inhibition experiments showed that the *ain1* mutation does not confer resistance to other hormones. Thus, *ain1* most probably affects a step specific for the ethylene signal transduction pathway.

Ethylene is the smallest molecule with hormonal action in plants. Its effects are pleiotropic and range from control of growth and developmental processes to induction of various stress responses (for a review, see Yang and Hoffman, 1984). In germinating dicotyledonous seedlings, ethylene is responsible for shortening and thickening of the hypocotyl or epicotyl and for the formation of an arch-shaped apical hook structure that protects the shoot apex and provides strength during the emergence process from the soil (Goeschl et al., 1966). This effect of ethylene on dark-grown seedlings is known as the "triple response," and was first described early this century (Neljubow, 1901). Ethylene can also be involved in promotion of germination and flowering as well as in ripening, senescence, and abscission (Yang and Hoffman, 1984). In addition, ethylene biosynthesis is induced under a variety of biotic and abiotic stresses. As a gaseous substance, ethylene can rapidly diffuse to target sites and thereby provide an effective stress signal. In certain stress conditions, such as hypoxia (Atwell et al., 1988) and nitrogen and phosphate starvation (He et al., 1992), it will trigger the formation of aerenchyma tissue, facilitating its own spread throughout the plant. In semiaquatic plants, it elicits rapid cell elongation by increasing GA sensitivity (Raskin and Kende, 1984). In certain cases, this growth promotion is a result of a cooperative effect of both auxin and ethylene (Ishizawa and Esashi, 1983).

From a molecular viewpoint, ethylene research has made a considerable leap forward in the past few years. Genes encoding the major biosynthetic enzymes have been cloned and the study of the regulation of their expression provided new insights in processes governing ethylene formation (for reviews, see Van Der Straeten and Van Montagu, 1991; Theologis, 1992). Unraveling the molecular mechanisms of ethylene perception and signal transduction, however, has proved to be less rewarding. High-affinity-binding sites for ethylene have been purified from several plant tissues (Jerie et al., 1979; Sisler, 1979), and the cloning of the corresponding genes by peptide sequencing will follow (Hall et al., 1990). Unfortunately, a convincing direct correlation between ethylene binding and physiological responses has not yet been established.

In addition to the biochemical approach, two different ways are being explored to gain further insight into ethylene perception and response. The first one involves the examination of promoters that are transcriptionally activated by ethylene. In certain cases, DNA-binding proteins have been identified for ethylene-responsive promoter elements, but none have been purified (Broglie et al., 1989; Cordes et al., 1989). Screening of expression libraries with recognition site DNA could be used to clone *trans*-factors involved in terminal steps of ethylene signal transduction, as previously done in

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Abbreviations: cM, centimorgan; EFE, ethylene-forming enzyme or ACC oxidase; ppm, parts per million.

several other cases (Singh et al., 1988; Katagiri and Chua, 1992).

Another approach to the study of hormone action, which also allows identification of intermediate steps, is a genetic one. Mutants with altered responses to most classes of plant hormones have been described (Reid, 1990; Scott, 1990; Su and Howell, 1992). *Arabidopsis thaliana* is particularly well suited for genetic research and the cloning of genes for which a mutant phenotype has been defined (positional cloning), due to the recent accomplishment of its integrated physical/restriction fragment length polymorphism map (Chang et al., 1988; Nam et al., 1989; Hwang et al., 1991) and the development of yeast artificial chromosome libraries with large inserts (Ward and Jen, 1990; Grill and Somerville, 1991; Matallana et al., 1992). This has led to the cloning of the *ABI3* gene (Giraudat et al., 1992) and the identification of a 10-kb DNA region that may contain the *ETR1* gene (Chang et al., 1992), which confer resistance to ABA and ethylene, respectively. *Arabidopsis* mutants that are affected in ethylene perception and signal transduction have been described by several groups (Bleecker et al., 1988; Van Der Straeten et al., 1989; Guzmán and Ecker, 1990; Harpham et al., 1991). Two loci for ethylene insensitivity have been identified: *etr1* and *ein1*, mapping in the same region of chromosome 1 (Bleecker et al., 1988; Guzmán and Ecker, 1990), and *ein2*, located on chromosome 4 (Guzmán and Ecker, 1990).

In this paper, we present the isolation of a third locus that confers resistance to ethylene and its precursor ACC, designated *ain1*. A detailed morphological and physiological analysis of two *ain1* alleles is described.

MATERIALS AND METHODS

Plant Material, Growth Conditions, and Stress Treatments

All mutant lines were derived from the *Arabidopsis thaliana* (L.) Heyhn ecotype C24. Seeds were sown after a cold treatment of 4 d at 4°C. Plants were grown at 22°C and 60% RH under white fluorescent light (photoperiod 16 h of light/8 h of dark) at a fluence rate of 78 μmol m^{-2} s^{-1}.

Sterile plants were grown in Petri dishes on 0.7% agar medium containing 1× Murashige-Skoog salts (Flow Laboratories). Seeds were first surface sterilized in 70% ethanol for 2 min and then in 5% sodium hypochlorite for 15 min. Sterile seeds were subsequently dispersed over the agar surface.

To test tolerance to submergence, 30 6-week-old plants were flooded up to a few millimeters above soil level for 4 weeks. The appearance of yellow edges on the leaves and eventual necrosis and death was monitored every 3rd d. The average percentage of fully necrotic leaves per plant was used as a basis of comparison.

Ozone exposures of 6-week-old plants were performed in continuous flow chambers (Instituut voor Scheikundig Onderzoek, Tervuren, Belgium). Concentrations of ozone in air were 250 and 350 parts per billion, applied for 5 h. Occurrence and frequency of typical ozone lesions were registered at the end of each treatment and during the following days.

Plants were infected with *Xanthomonas campestris* pv *campestris* (compatible and incompatible strains) according to the method of M. Lummerzheim (personal communication).

Mutagenesis and Screening for *ain1* Mutants

Ethylmethanesulfate mutagenesis was performed as described by Koornneef et al. (1982) on 10 different batches of seeds, each containing approximately 10,000 seeds. Screening of M_2 seeds was essentially as reported previously (Van Der Straeten et al., 1989). In summary, M_2 seeds were germinated in the dark on glass-fiber paper soaked with 500 μM ACC and 1 mM GA_3. After 5 d, seedlings were analyzed for absence of triple response, i.e. with a long, straight hypocotyl, and a normal apical hook (same appearance as an etiolated seedling germinated on water). For ethylene sensitivity tests, seedlings were subjected to a continuous flow of 100 ppm ethylene at 1.2 L h^{-1} for 5 d.

Genetic Analysis

Six ACC-insensitive lines were randomly chosen and back-crossed with wild type in the M_3 generation. ACC-insensitive F_2 progeny (as assessed by the absence of triple response) was back-crossed once more to wild type, and homozygous recessive lines were isolated in F_2.

To test for allelism, reciprocal crosses were performed between different homozygous lines, and their progeny was tested for the absence of triple response on ACC.

To obtain a chromosomal location for *ain1*, we crossed two homozygous alleles (designated *ain1-1* and *ain1-2*) with the W-100 marker line, which carries two phenotypic markers on each chromosome in a Landsberg erecta background (Koornneef et al., 1987). An average of 180 F_2 plants were scored for ACC insensitivity and for phenotypic markers. Map positions were calculated with the linkage computer program RecF2, kindly provided by M. Koornneef (Wageningen, The Netherlands).

More accurate mapping of the locus was done by analyzing F_3 populations resulting from the cross *ga4dis2cer5* × *ain1* for meiotic recombinants in the vicinity of the *ain1* gene. Marker lines were in Landsberg erecta and were gifts from M. Koornneef. F_2 seedlings were screened for ACC insensitivity (as mentioned above) and rescued. Scoring for the respective tester genes was done in F_3 populations derived from these plants. Map positions were calculated according to the method of Koornneef and Stam (1987).

Determination of Hormone Sensitivity

We followed the method of Wilson et al. (1990) with some minor modifications. Seeds were germinated for 6 d in Petri dishes containing 1× Murashige-Skoog salts and 0.7% agar. Seedlings were transferred to plates containing various concentrations of either ABA, IAA, zeatin riboside, or GA_3 and placed in a vertical position for 3 d. Root tips were marked, and the increase in root length was measured after 2 d using a graduated ocular. An average of 52 seedlings was measured for each hormone concentration, with the exception of the auxin experiment for which an average of 36 seedlings was used.

For the determination of ethylene dose-response curves, an average of 40 seeds was germinated in the dark on glass-fiber paper in 15-mL gas-tight flasks containing the appropriate levels of ethylene. The flasks were flushed every 24 h.

Table I. *Genetic analysis of ain1 mutants*

Cross	Generation	Number of Seedlings		Ratio	χ^2 $P > 0.05$
		ACC sensitive	ACC insensitive		
ain1-1/ain1-1 × *AIN1-1/AIN1-1*	F_2	248	78	3:1	0.13
ain1-2/ain1-2 × *AIN1-2/AIN1-2*	F_2	74	26	3:1	0.03
ain1-1/ain1-1 × *ain1-2/ain1-2*	F_2	0	102		

After 7 d, the primary root and hypocotyl lengths were measured as previously mentioned.

Measurement of Gravitropic Response

The experimental procedure described by Lincoln et al. (1990) was slightly modified. Seeds were germinated for 7 d as described above. An average of 48 seedlings was transferred, and all root tips were aligned perpendicularly to a marker line. After 3 d of vertical growth, the plates were turned 90°. The angle of curvature was measured after 10 h.

Determination of Ethylene Production

Ethylene production was measured with an open flow system essentially as described by De Greef and De Proft (1978). Six-week-old flowering plants were carefully removed from the soil, and their roots were gently washed with water. The plants were put in glass pots, their roots submerged in water, and ethylene production was measured after 6 h. Measurements were done on five plants and repeated three times.

Influence of Ethylene on Developmental Processes

To compare the effect of ethylene exposure on senescence of wild-type and *ain1* plants, we subjected 10-week-old flowering plants to a continuous flow of 10 ppm of ethylene at 10 L h^{-1} for 16 d. Senescence of inflorescence leaves and rosette leaves was recorded as a function of time. The time after which inflorescence leaves were all shriveled and rosettes progressively senesced was used as a parameter to assess differences between wild type and mutants.

RESULTS

Isolation and Genetic Analysis of *ain1* Mutants

To select mutants with an altered response to ACC, we first investigated the germination and growth of wild-type seedlings on different concentrations of exogenously supplied ACC (Van Der Straeten et al., 1989). The idea behind the screening for ACC insensitivity in *Arabidopsis* was to find EFE mutants, which should be insensitive to ACC and yet sensitive to ethylene. The triple response as originally described by Neljubow (1901) was found over a wide range of ACC concentrations (between 10 and 500 μM). Above 500 μM, no further growth of the primary root was observed. On average, wild-type seedlings grown on 500 μM ACC were 3 to 4 times shorter than control seedlings. The triple response was entirely reverted to the normal etiolated phenotype when 0.2 mM silver thiosulfate was added to ACC, indicating that the observed phenotype was due to the conversion of ACC to ethylene and subsequent ethylene action (data not shown).

Screening of 360,000 M_2 seeds on glass-fiber sheets impregnated with 500 μM ACC and 1 mM GA_3 yielded 16 independent ACC-insensitive lines. Because the M_2 seeds were derived from 10 batches of approximately 10,000 mutagenized M_1 seeds each (further divided in 10 subgroups), the frequency of ACC-insensitive mutants can be estimated at 1:6250. ACC insensitivity was confirmed in M_3 for all 16 lines. In addition, all proved to be insensitive to ethylene when germinated in the presence of the hormone. This indicated that none of the lines was a putative EFE mutant.

Six lines were back-crossed in the M_3 generation to wild-type C24 plants. The F_1 progeny was as sensitive as wild type, and the segregation of ACC insensitivity in F_2 was, in all cases, consistent with a 3:1 ratio. These results indicated that ACC and ethylene insensitivity in each of the six lines was caused by a single recessive mutation. ACC-insensitive F_2 progeny was back-crossed once more to wild type, and homozygous recessive lines were isolated. To test for allelism, different reciprocal crosses between homozygous lines were performed, and without exceptions, F_2 progenies were completely insensitive to ACC (data not shown). We concluded that the six lines were representing alleles (*ain1-1* to *ain1-6*) from a single complementation group.

The *ain1-1* and *ain1-2* alleles were chosen for genetic

Table II. *Analysis for meiotic recombinants close to ain1*

Cross[a]	Number of F_3 *ain1/ain1* Populations	Number of Wild Types	Number of Recombinants		Distance to *ain1* (cM)
			Heterozygous	Homozygous	
ga4dis2cer5 × *ain1*	152 (114)[b]	71	36	7	20.2 ± 3.3
ga4dis2cer5 × *ain1*	152	152	0	0	<0.3 ± 0.3[c]

[a] The marker for which F_3 populations were scored is underlined. [b] Because C24 contains the *gl 1* mutation, 25% of the F_3 populations are very difficult to score; therefore, 114 (75%) were used for calculations. [c] Because no recombinants were detected, calculations were done assuming one recombinant, leading to a maximal genetic distance.

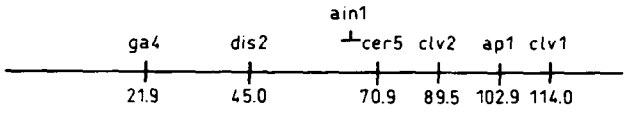

Figure 1. *Location of ain1 on chromosome 1. Map distances of markers are in cM as in the genetic map of A. thaliana (Koornneef, 1990).*

mapping of the locus. An average of 180 F_2 plants was scored for ACC insensitivity and phenotypic markers. Linkage analysis indicated that the *ain1* mutation is located on chromosome 1 (percentage of recombination with *ap1*, 30 ± 3.7 cM for *ain1-1* and 30 ± 4.1 cM for *ain1-2*; segregation data not shown). An overview of the results of the genetic analysis of the *ain1-1* and *ain1-2* alleles is shown in Table I.

To position the *ain1* gene more accurately on chromosome 1, and as a start for future chromosome walking, we selected meiotic recombinants on either side of the *ain1* gene. The *dis2* and *cer5* markers were used for selection of recombinant *ain1/ain1* F_3 populations. Results of the analysis are presented in Table II. Because no recombinants were found between *ain1* and *cer5*, *ain1* is closely linked to the *cer5* marker. This is confirmed by the calculation of the percentage of recombination from the F_3 data according to the method of Koornneef and Stam (1987). The distance of *ain1* to *cer5* is estimated to be 0.3 ± 0.3 cM. This is in accordance with the estimated distance of 20.2 ± 3.3 cM between *ain1* and *dis2* (Table II). The estimated position of *ain1* on chromosome 1 relative to relevant markers is presented in Figure 1.

Morphological Characterization of *ain1* Mutants

Adult mutant plants did not display dramatic morphological differences from wild type and had a normal fertility and seed set. Mutant plants showed a few distinct growth features different from wild-type plants. Both *ain1* alleles had significantly larger (+20%) rosettes than wild type. In addition, a delay in bolting was observed, although only 2 d for the *ain1-2* allele and 5 d for the *ain1-1* allele. The results are summarized in Table III.

Finally, we also noticed that root gravitropism was affected in the *ain1-1* plants. Roots of *ain1-1* but not those of *ain1-2* seedlings reacted more slowly to gravity than did wild type. After 10 h of gravistimulation, roots of *ain1-1* plants achieved an angle of curvature that was on average 10° lower than both wild-type and *ain1-2* plants (Table III). The significance of this result was confirmed by Student's *t* test at a level of 0.01. Because roots of both mutant alleles grew at the same rate (data not shown), this difference in gravitropic response was not due to a difference in growth rate.

Sensitivity to Ethylene at the Seedling Stage

Because the triple response affects both shoots and roots of seedlings, dose-response curves for a wide range of ethylene concentrations were determined for hypocotyl and root length of wild type and mutants. Figure 2A shows the effect of ethylene concentrations ranging between 0.01 and 10 ppm. In the absence of ethylene, mutants elongated more rapidly than did wild type, and their hypocotyl length remained unaffected until the maximal ethylene concentration was applied. This was in contrast to wild-type seedlings, which showed a reduction in hypocotyl length from 0.1 ppm of ethylene and a saturated response above 5 ppm. A similar sigmoidal dose-response curve was obtained for roots, as shown in Figure 2B. In this case, however, the roots of the mutants were shorter than wild type in the absence of the hormone. Mutant roots were not reduced in length up to the highest ethylene concentration. In a separate experiment, when 100 ppm of ethylene was applied, no significant differences were observed in the hypocotyl or root responses of the mutants.

It may also be noted that *ain1* seedlings germinated in the presence of ACC or ethylene displayed an apical hook, in contrast to *ein1* and *ein2* seedlings, in which the hook opened readily (Guzmán and Ecker, 1990).

Sensitivity to Ethylene and Various Stresses at the Adult Stage

The sensitivity of wild-type and mutant plants to exogenously applied ethylene was tested by subjecting flowering plants to a continuous flow of 10 ppm of ethylene and monitoring symptoms of senescence. Wild-type plants had lost all inflorescence leaves and showed progressive senescence of the rosette after 8 d of exposure. In contrast, these symptoms were delayed by 8 d in both mutants (Table IV).

The difference between wild type and *ain1* alleles was not apparent when comparing physiological reactions to various other stresses. Exposure of wild-type or mutant plants to ozone, prolonged submergence stress, and infection with different compatible and incompatible strains of *Xanthomonas campestris* pv *campestris* did not produce visible differences (Table IV).

Table III. *Morphological characteristics of ain1 mutants*

Parameter	Wild Type	*ain1-1*	*ain1-2*
Rosette diameter (cm)[a,d]	4.54 ± 0.50	5.46 ± 0.29	5.59 ± 0.34
Bolting (50%)[b] (d after sowing)	50 ± 1	55 ± 1	52 ± 1
Root gravitropism (degrees)[c,d]	93.2 ± 13.9	79.4 ± 15.0	90.1 ± 13.2

[a] Means of eight 12-week-old plants. [b] Seedlings were planted 2 weeks after sowing and plants with bolting stems higher than 1 cm were considered; each population = 15 plants. [c] Means of 48 plants; angles were measured after 10 h of gravistimulation. [d] Student's *t* test was performed at a significance level 0.01.

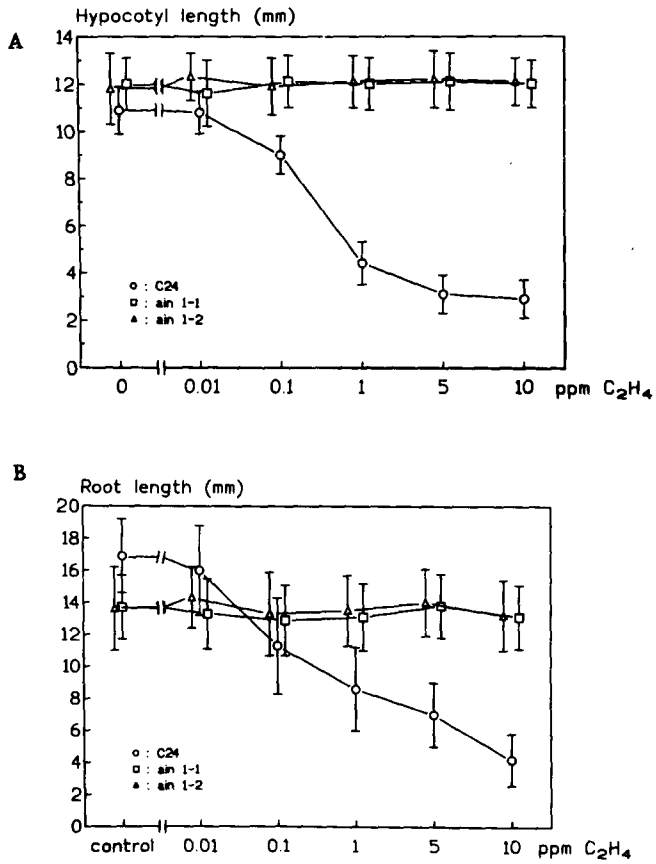

Figure 2. Dose-response curves demonstrating the effect of ethylene on hypocotyl (A) and root (B) length of dark-grown wild-type and *ain1* seedlings. Hypocotyl and root length were measured after 7 d of growth. Each point represents a mean of 40 measurements, and the bar represents the SD from the mean. O, Wild type; □, *ain1-1*; △, *ain1-2*.

Sensitivity to Other Plant Hormones

To analyze the level of resistance to ABA, auxin, and cytokinin, inhibition of root growth was measured at various hormone concentrations. As shown in Figure 3, the dose-response curves of wild type and mutants were not significantly different in any case, except for ABA, for which *ain1* mutants showed a slightly higher sensitivity between 10^{-6} and 10^{-5} M. In addition, there were no significant alterations in shoot or root morphology of wild-type and mutant seedlings germinated on various concentrations of GA_3 (data not shown). Therefore, it was concluded that the *ain1* mutation does not produce significant resistance to other hormones (polyamines were not tested).

Ethylene Production and Binding in *ain1* and Wild-Type Plants

The ethylene production rates of flowering wild-type and *ain1* plants were measured with an open flow system. Wild-type C24 and *ain1-2* plants produced about 5.5 nL g^{-1} h^{-1} of ethylene. In contrast, *ain1-1* plants had an ethylene production rate that was reduced at least 3-fold. The results are presented in Table IV.

Although variation occurred in ethylene synthesis, preliminary experiments indicated that ethylene binding remained unaffected in *ain1* mutants (A. Smith and M.A. Hall, personal communication). Specific binding in wild type was 0.08 pmol g^{-1} fresh weight, which was not significantly altered in either *ain1* allele.

DISCUSSION

Several ethylene-insensitive mutants of *A. thaliana* have been identified (Bleecker et al., 1988; Guzmán and Ecker, 1990; Harpham et al., 1991). We have tried to cover a different spectrum of mutations by initiating a screen for *Arabidopsis* mutants resistant to the ethylene precursor ACC (Van Der Straeten et al., 1989). Theoretically, this approach could lead to the isolation of ACC oxidase (EFE) mutants, ethylene perception and signal transduction mutants, as well as mutants in ACC uptake and ACC conjugation. A screen of 360,000 ethylmethanesulfonate-mutagenized M_2 seedlings of *Arabidopsis* yielded 16 independent lines, all of which proved negative in a screen for ethylene sensitivity. It was concluded that these mutants represented mutations in the ethylene receptor or signal transduction chain. Complementation analysis of six randomly chosen lines revealed that all represented alleles of the same locus, which was named *ain1* (Table I). Mapping data and analysis of meiotic recombinants have allowed us to assign *ain1* to chromosome 1, in the immediate vicinity of the *cer5* marker (Table II, Fig. 1). The

Table IV. *Physiological characteristics of ain1 mutants*
For the treatments, see "Materials and Methods."

Parameter	Wild Type	*ain1-1*	*ain1-2*
C_2H_4 production (nL g^{-1} h^{-1})	5.6 ± 0.3	1.7 ± 0.2	5.4 ± 0.7
Leaf senescence under 10 ppm C_2H_4[a] (d)	8	16	16
O_3 exposure[b] (damaged leaves/plant)	≤4	≤4	≤4
4 weeks of submergence[c] (%)	56 ± 11	64 ± 9	59 ± 18
X. campestris infection[d]	N.D.	N.D.	N.D.

[a] Time (d) after which inflorescence leaves shriveled up and broke off; air controls were unaffected. [b] Maximum number of damaged leaves per plant, as recorded 3 d after exposure; each population is 30 plants. [c] Average percentage of fully necrotic leaves per plant after 28 d of submergence. [d] N.D., No significant differences in phenotypic lesions observed between wild type and mutants.

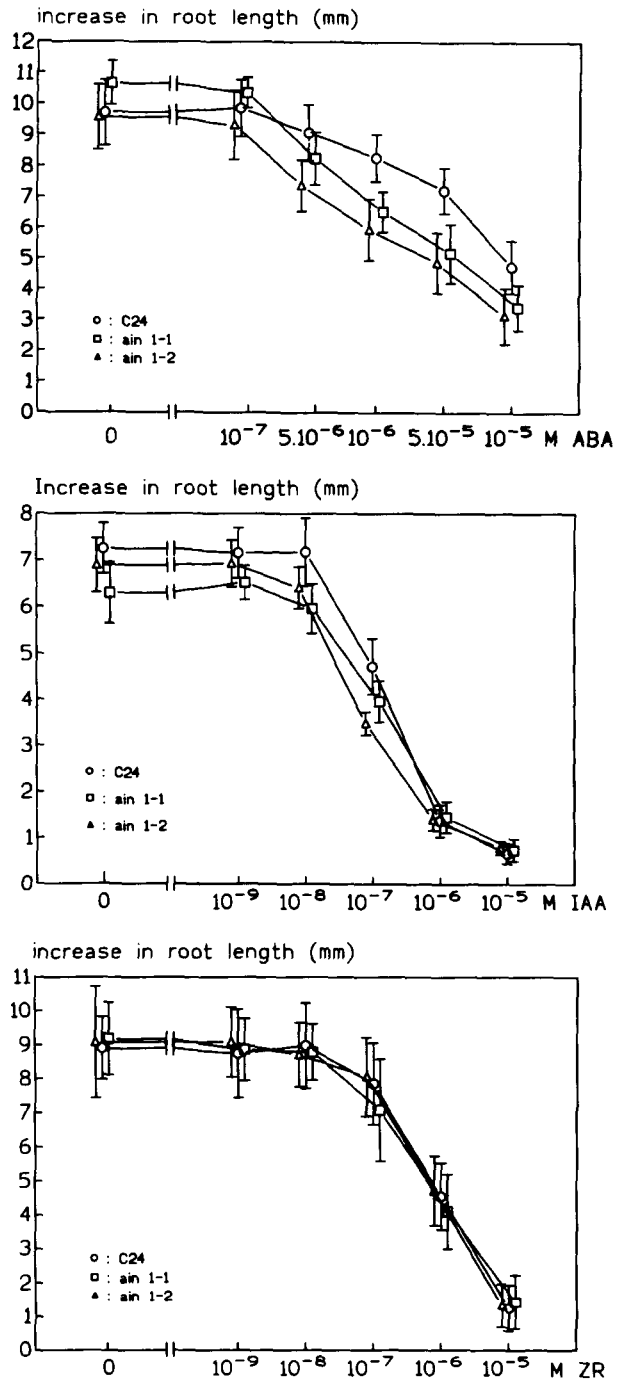

Figure 3. Dose-response curves demonstrating the effect of ABA (upper), auxin (IAA) (middle), and cytokinin (zeatin riboside) (lower) on root growth of wild-type and *ain1* seedlings. Root length was measured after 8 d. Each point represents a mean of 52 measurements (36 in the case of IAA), and the bar represents the SD from the mean. O, Wild type; □, *ain1-1*, △, *ain1-2*.

previously identified ethylene-resistant mutants map to different loci in the *Arabidopsis* genome (Bleecker et al., 1988; Guzmán and Ecker, 1990; Chang et al., 1992). Thus, *ain1* may alter a distinct step in the ethylene signal transduction pathway. The fact that a screen for ACC primarily led to the isolation of alleles of a new locus rather than covering the previously described loci for ethylene insensitivity (which were isolated upon a screen with ethylene) is quite remarkable. It may indicate the existence of a controlling mechanism by the ethylene precursor and/or its conjugates on the ethylene signal transduction chain.

A detailed morphological and physiological analysis of two *ain1* alleles revealed some remarkable differences (Table III and IV). *ain1* mutants did not show dramatic morphological changes compared with wild type. In both cases, however, rosettes were about 20% larger than wild type, and bolting was delayed by a few days (Table III). These observations were also made for *etr1*, *ein1*, and *ein2* alleles (Bleecker et al., 1988; Guzmán and Ecker, 1990). The enlargement of rosettes is likely to be the consequence of full cell expansion in the absence of ethylene action, known to inhibit cell elongation (Neljubow, 1901; Osborne, 1989). With respect to the delay of bolting, it may be noted that none of the late-flowering mutants of *Arabidopsis* analyzed were ACC insensitive (Koornneef et al., 1991; data not shown). Furthermore, the rate of gravitropic response was significantly different between *ain1-1* and both wild type and *ain1-2*. Because the *ain1* alleles proved to be sensitive to auxin, this implies that auxin and ethylene may act through distinct signal transduction pathways in root gravitropism.

Various physiological parameters for ethylene responses in seedlings and adult plants were investigated. Insensitivity to ACC and ethylene in seedlings was observed at the level of elongation growth (Fig. 2). In adult plants, *ain1* mutants showed a significant delay in leaf senescence (Table IV), similar to that noted for *etr1* (Bleecker et al., 1988). When subjected to biotic and abiotic stresses, however, *ain1* plants did not react differently from wild type. It is interesting that *ain1-1* plants produced ethylene at a lower rate than both wild-type and *ain1-2* plants (Table IV). Ethylene response mutants with a reduced ethylene production have been described in the case of the *hls1* locus; the opposite has been observed for the *eti5* and *ein* loci (Guzmán and Ecker, 1990; Harpham et al., 1991). It is conceivable that a mechanism of autocatalysis of ethylene biosynthesis may be affected in certain *ain1* alleles. In contrast, it has been suggested that the *ein* loci are involved in a mechanism of negative feedback on ethylene biosynthesis (Guzmán and Ecker, 1990). Thus, positive and negative "switches" could control endogenous ethylene production. A modulator role for the *AIN1* gene product remains to be proven.

The similarities in phenotypic response between *ain1* and *etr1/ein1* or *ein2* mutants may indicate an interaction between their gene products. Comparable cases are the phenotypic identity of *ap3* and *pi* homeotic floral mutants in *Arabidopsis* (Jack et al., 1992) or *glo* and *def* in *Antirrhinum* (Schwarz-Sommer et al., 1992), in which second and third whorl organs are affected. *AIN* and *EIN* gene products could be required to act in concert or as upstream regulators of one another. Because *ETR1/EIN1* has been suggested to encode the ethyl-

ene receptor (Bleecker et al., 1988; Guzmán and Ecker, 1990), *AIN1* is more likely to act downstream from this gene product. The study of expression of the *ETR*, *EIN*, and *AIN* genes in the different mutant backgrounds, as well as the analysis of double mutants, should allow assignation of an order in which the loci come into play in the ethylene perception and signal transduction chain. Also, interallelic differences between mutants are not uncommon. Striking differences have been noted for several alleles of *ap2* (Bowman et al., 1991) and *ap3* (Jack et al., 1992). Thus, the *ain1-1* and *ain1-2* mutations probably affect distinct regions of the AIN protein, and the strength of the phenotype may reflect the severity of the lesion.

Because various hormone signal transduction pathways in plants, as in animals, most probably comprise common elements, multiple hormone resistance could be expected to occur in certain mutants. This is experimentally confirmed by the existence of the *aux1* and *axr2* mutants in *Arabidopsis* (Wilson et al., 1990) and the *iba1* mutant in tobacco (Bitoun et al., 1990), all of which appear to be resistant to at least two hormones. When two alleles of *ain1* were analyzed for their sensitivity to ABA, auxin, cytokinin, and GA_3, no significant differences were noted compared with wild type (Fig. 3). It can be concluded that the *ain1* locus is not involved in a general signal transduction pathway but is rather specific to ethylene responses. Because no data are available concerning the sensitivity of *etr* and *ein* mutants to other hormones, *ain1* is currently the only example of a locus specifically affecting the ethylene signal transduction pathway.

We have described a novel locus conferring ethylene resistance in *A. thaliana*. The recent advancement in chromosome-walking techniques should allow the cloning of several genes involved in ethylene perception and signal transduction, identified through a genetic approach. Sequence comparison of the genes of both *ain1* alleles will reveal clues about the functionality and importance of different domains within the *AIN1* polypeptide. This and other efforts to clone genes involved in hormone signal transduction will soon provide a detailed insight in the molecular control of hormone action and may provide new perspectives for applications in agriculture.

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