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COMPARISON OF GROWTH RESPONSES TO AUXIN 1-NAPHTHALENEACETIC ACID AND THE ETHYLENE PRECURSOR 1-AMINOCYCLOPROPANE-1-CARBOXILIC ACID IN MAIZE SEEDLING ROOT

MARÍA VICTORIA ALARCÓN[1], PEDRO G. LLORET[2], DOMINGO JOSÉ IGLESIAS[3], MANUEL TALÓN[3], AND JULIO SALGUERO[4*]

[1]*Departamento de Hortofruticultura. Centro de Investigación "Finca La Orden-Valdesequera". Junta de Extremadura. 06187 Badajoz, Spain*
[2]*Departamento de Anatomía, Biología Celular y Zoología, Facultad de Ciencias, Universidad de Extremadura, 06071 Badajoz, Spain*
[3]*Departamento de Genómica y Postcosecha, Instituto Valenciano de Investigaciones Agrarias 46113 Valencia, Spain*
[4]*Departamento de Biología Vegetal, Ecología y Ciencias de la Tierra. Universidad de Extremadura, 06071 Badajoz, Spain*

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Application of 1-naphthaleneacetic acid (NAA) or 1-aminocyclopropane-1-carboxilic acid (ACC) to maize roots growing in hydroponic solution inhibited root elongation, and increased radial growth, but the responses to those treatments differed in degree. Auxin was more effective than ACC as an elongation inhibitor and root swelling promoter. Whereas NAA fully inhibited elongation and maintained swelling over 48 h, ACC inhibited elongation partially (50%) and only promoted swelling for 24 h. It is well-known that auxin, like ACC, promotes ethylene production, but similar levels of ethylene production reached by means of NAA or ACC treatments did not elicit the same response, the response being always stronger to NAA than to ACC. These results suggest that the effect of auxin on root growth is not mediated by ethylene. Elongation and swelling of roots appear to be inversely related: usually a reduction in elongation was accompanied by corresponding swelling. However, these two processes showed different sensitivities to growth regulators. After 24 h treatment with 0.5 μM NAA or 5 μM ACC, root elongation was inhibited by 90% and 53% respectively, but the same treatments promoted swelling by 187% and 140% respectively. Furthermore, 1 μM ACC was shown to promote inhibition of root elongation without affecting swelling. The ethylene antagonist STS (silver thiosulfate) did not affect elongation in control or NAA-treated roots, but increased ethylene production and swelling. These results indicate that longitudinal and radial expansion could be independently controlled.

Key words: Maize, root, NAA, ACC, auxin, ethylene, STS, root swelling, root elongation.

INTRODUCTION

Plant root growth and development can be profoundly altered by both auxin and ethylene, two endogenously produced plant hormones (Klee and Romano, 1994). Since auxin stimulates the production of ethylene (Abeles et al., 1992), it is often unclear whether the effects elicited by auxin are independently due to the auxin, to ethylene, or to a complex interaction. In roots, both hormones inhibit elongation and promote swelling in root tips. Very low concentrations of auxin applied externally may stimulate elongation (Evans et al., 1994), but the typical response of roots to exogenous auxin is inhibited elongation and enhanced swelling (Eliasson et al., 1989). Similar effects have been found with ethylene, which at very low concentrations may promote root elongation and at high concentrations has been shown to inhibit root elongation and increase root diameter (Jackson, 1991; Dolan, 1997; de Cnodder et al., 2005; Dugardeyn and van der Straeten, 2008;

*e-mail: salguero@unex.es

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Alarcón et al., 2009a). Auxin has been suggested to play an important role in root growth and development, and its interactions with ethylene have been explained with two alternative hypotheses. The first posits that the action of auxin is mediated by ethylene. This has been demonstrated in the case of light-promoted inhibition of root growth (Eliasson and Bollmark, 1988; Jackson, 1991). The second one suggests a direct effect of auxin on root growth. This hypothesis is based on results in *Pisum sativum* indicating that auxin inhibits elongation without ethylene mediation (Eliasson et al., 1989).

Interactions between auxin and ethylene in the control of numerous plant developmental processes have been described, so it is possible that these two phytohormones cooperatively regulate longitudinal and radial expansion in root tissues. Synergistic effects of auxin and ethylene have been reported in some processes affecting root development, such as root hair growth and differentiation (Pitts et al., 1998), root gravitropism (Lee et al., 1990; Buer et al., 2006) and root growth (Rahman et al., 2001).

The interaction between ethylene and auxin should be based on stimulation of auxin biosynthesis and basipetal transport to the elongation zone by ethylene (Ruzicka et al., 2007). This would lead to an increased concentration of auxin in this zone and, consequently, to a decreased root elongation rate (Lee et al., 1990). Recently, molecular studies have demonstrated interaction between auxin and ethylene at the genetic level. Auxin response factors ARF19 and ARF7 have been shown to participate in auxin signaling and to play a critical role in ethylene responses in *Arabidopsis* root, indicating that ARFs serve as a crosstalk point between the two hormones (Li et al., 2006). Complementarily, ethylene inhibition of root growth was enhanced by auxin (Swarup et al., 2007).

A number of studies indicate that root development is regulated not only by auxin and ethylene. Other phytohormones such as gibberellins or cytokinins have been shown to play a pivotal role in regulating it (Hansen and Grossmann, 2000). Ethylene biosynthesis has been also found to be stimulated through their interactions: ACC synthase is up-regulated by cytokinin (Rodrigues-Pousada et al., 1999), abscisic acid (Wang et al., 2005) and brasinosteroids (Joo et al., 2006). However, there is no information about the differences between the effects produced by auxin and ethylene on root development.

In our experiments we quantified the effects of auxin and ethylene on root growth and also reevaluated the role of ethylene as a mediator of auxin's effects on elongation and swelling in maize roots.

MATERIALS AND METHODS

PLANT MATERIAL AND GROWTH CONDITIONS

Seeds of *Zea mays* L. cv. DK 626 were surface-sterilized by immersion in ethanol for 5 min, then washed three times and imbibed during 24 h in aerated distilled water at 30°C. After imbibition the seeds were inserted vertically in styrofoam holder discs and grown in boxes in a humid atmosphere and darkness at 30°C for 24 h. By the end of this period the roots were 34 ± 5 mm long. The holders with roots of similar length were placed in 1.5 l bottles of well-aerated growth solution consisting of buffered solution of 1 mM 2-hydroxyethylpiperazine-2-ethanesulfonic acid (HEPES) enriched with calcium (1 mM $CaCl_2$) and potassium (10 mM KCl) at pH 6.0 and grown at 30°C in the dark.

During the next 24 h the roots were maintained in a preliminary phase of adaptation to liquid medium, reaching a length of 60–80 mm. After that, the control roots grew at a rate of 2.78 ± 0.24 mm/h during the following 24 h to reach length of ~125 mm, and at 2.02 ± 0.33 mm/h in the next 24 h to reach length of ~180 mm.

EFFECTS OF PLANT GROWTH REGULATORS

NAA and ACC (Sigma Chemical Co., U.S.A.) were prepared in 1 mM HEPES, 1 mM $CaCl_2$ and 10 mM KCl at pH 6.0. The volumes added in different treatments were less than 0.1% of total volume. NAA (0.01–10 μM) or ACC (1–10 μM) were added to growth solution after the acclimatization period when the roots were 60–80 mm long. If necessary, silver thiosulfate (100 μM), an inhibitor of the action of ethylene, was added to the growth solution. Root length was measured with a ruler (accuracy ± 1 mm) at several time points and swelling was estimated as fresh weight (FW) of 10 mm root tips to ± 0.0001 g accuracy. Swelling can also be estimated as root tip diameter. Root diameter in μm at 5 mm from the apex and root tip FW were correlated linearly by the following equation: FW (mg) = (0.01 x root diameter – 2.82) μm; $r^2 = 0.97$. The values obtained for these variables at 4, 24 and 48 h are represented as means ± SD of 10 roots. Each experiment was performed in triplicate.

The E50 values (50% of maximum effect) were also calculated for treatments that involved auxin and ACC. E_{50} was taken as the concentration of NAA or ACC that caused 50% of the maximum effect, either inhibiting or stimulating, on root elongation or thickening.

ETHYLENE MEASUREMENTS

Ethylene production was measured in the most distal segment (10 mm) of the root after 4, 24 and 48 h

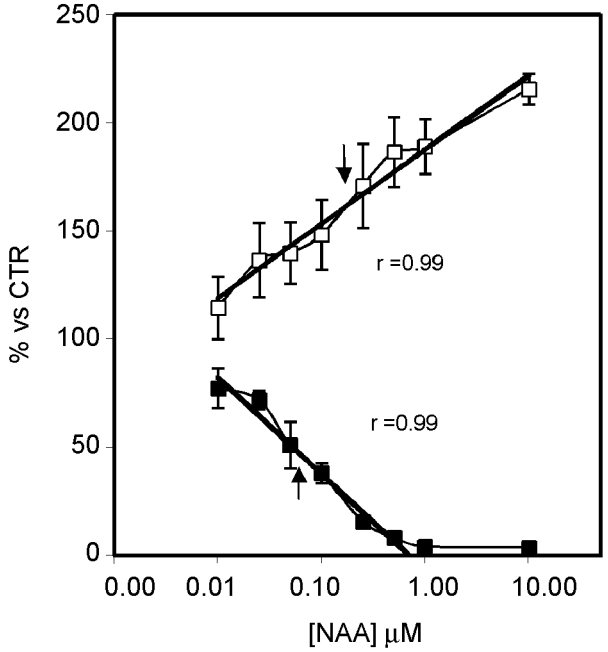

Fig. 1. Effect of NAA on elongation (shaded squares) and swelling (unshaded squares) in *Zea mays* seedling roots growing in a hydroponic system. Results are percentages of the control. NAA concentrations are on a logarithmic scale. Values are means ± SD of 10 roots. Correlation coefficients of linear regressions are given in figure (r). Arrows indicate E_{50} (50% of maximum effect).

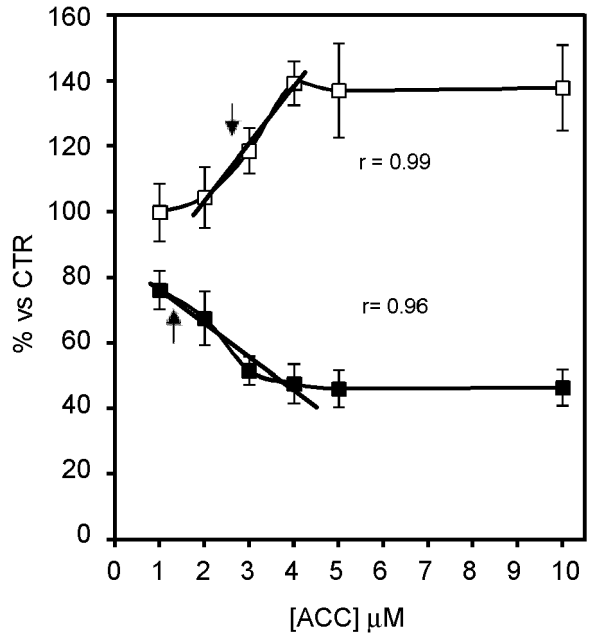

Fig. 2. Effect of ACC on elongation (shaded squares) and swelling (shaded squares) on *Zea mays* seedling roots growing in a hydroponic system. Results are percentages of the control. Values are means ± SD of 10 roots. Correlation coefficients of linear regressions are given in figure (r). Arrows indicate E_{50} (50% of maximum effect).

of treatment. Three determinations, each of three segments, were performed for every point. To measure ethylene, the roots were incubated for 1 h in 1 mL vials with 100 μL of the same growth solution in which they had been growing. After 1 h, ethylene was quantified using a gas chromatograph (HP 6890 series) equipped with an activated alumina column and a flame ionization detector. The total amount of ethylene produced during the period was calculated from the ethylene production rates at different times. Each experiment was performed in triplicate.

STATISTICAL ANALYSIS

Growth parameters and ethylene production were compared with Student's t-test. Within-treatment comparison of means was done by one-way ANOVA and the Tukey test (SPSS 13.0). Differences between means were considered significant at $P < 0.05$. The coefficients of the linear correlation of elongation and swelling values with log-concentrations of NAA and ACC concentrations were also calculated.

RESULTS

EFFECTS OF PLANT GROWTH REGULATORS ON ROOT ELONGATION AND SWELLING

Exogenous auxin inhibited root elongation and increased root tip weights after 24 h treatments (Fig. 1). In the 0.01–10 μM range these effects increased with the concentration of NAA, but their dose-responses differed. In the 0.01–0.5 μM concentration range the inhibition of root elongation correlated linearly with the logarithm of NAA concentration ($r=0.96$), and 90% inhibition of elongation was obtained at concentrations up to 0.5 μM NAA. In contrast, root swelling correlated linearly with the logarithm of concentrations along the whole tested range of concentrations tested ($r=0.99$). Root swelling increased to reach maximum ~215% in the 10 μM NAA treatment.

In general the effects of ethylene on root growth were similar to those provoked by auxin. The ethylene precursor ACC inhibited root elongation and increased the weight of root tips (Fig. 2). In the 1–4 μM range of ACC concentration the reduction of elongation was clearly correlated with the ACC con-

TABLE 1. Effects of ACC, NAA and STS on elongation (mm/24h), swelling (mg/cm apex) and ethylene production (nl ethylene/24h g FW) in NAA-treated and untreated roots during 24 h and 48 h of treatment

Treatment	0-24 h			24-48 h		
	Elongation	Root apex FW	C_2H_4 produced	Elongation	Root apex FW	C_2H_4 produced
CTR	66.84 ± 5.67a	5.21 ± 0.40a	34.02 ± 2.88a	48.52 ± 7.89a	4.59 ± 0.46a	74.88 ± 2.40a
ACC 1 μM	51.18 ± 3.84b	5.31 ± 0.46a	95.54 ± 9.00c	39.66 ± 2.11b	4.64± 0.51a	88.44 ± 3.60b
ACC 2 μM	45.11 ± 5.48bc	5.55 ± 0.49ab	–	33.42 ± 4.72c	4.34 ± 0.40a	–
ACC 3 μM	34.44 ± 2.87d	6.31 ± 0.36bcd	–	27.89 ± 4.11de	4.58 ± 0.40a	–
ACC 4 μM	31.80 ± 3.94de	7.41 ± 0.35de	–	20.10 ± 3.07fg	4.01 ± 0.40a	–
ACC 5 μM	30.80 ± 3.74de	7.29 ± 0.76de	192.22 ± 10.21e	21.10 ± 2.23fg	5.40 ± 0.50ab	198.24 ± 6.36e
ACC 10 μM	31.00 ± 3.65de	7.33 ± 0.69de	–	16.10 ± 2.47g	5.17 ± 0.49ab	–
NAA 0.010 μM	50.70 ± 6.52b	5.83 ± 0.70ab	53.43 ± 3.72b	37.50 ± 5.40bc	5.23 ± 0.36ab	79.68 ± 3.12a
NAA 0.025 μM	43.50 ± 3.34c	6.87 ± 0.81cde	–	33.30 ± 2.30cd	6.14 ± 0.36cd	–
NAA 0.050 μM	34.30 ± 6.78d	7.17 ± 0.64de	–	23.60 ± 5.14ef	5.90 ± 0.20bc	–
NAA 0.100 μM	25.71 ± 4.50e	7.67 ± 0.79e	100.72 ± 7.44c	16.20 ± 43.17g	6.98 ± 0.41de	159.24 ± 6.12d
NAA 0.250 μM	10.11 ± 2.98f	8.79 ± 0.97f	–	6.56 ± 2.23h	7.65 ± 0.46e	–
NAA 0.500 μM	5.56 ± 1.59fg	9.51 ± 0.79f	132.18 ± 10.21d	3.56 ± 1.14i	10.24 ± 0.43f	184.58 ±10.20e
NAA 1.000 μM	2.89 ± 1.05fg	9.71 ± 0.64f	–	2.89 ± 0.94i	11.27 ± 0.40g	–
NAA 10.00 μM	2.22 ± 0.97g	11.02 ± 0.64g	–	1.00 ± 1.22i	13.36 ± 1.28h	–
STS 100 μM	64.30 ± 5.30a	6.77 ± 0.18cde	46.87 ± 3.48ab	42.69 ± 5.29ab	6.53 ± 0.10cd	76.84 ± 4.77a
NAA 0.1 + STS	23.82 ± 3.17e	9.54 ± 1.50f	81.53 ± 11.86c	18.57 ± 5.74g	7.96 ± 0.17e	100.07 ±13.72c
NAA 0.5 + STS	4.29 ± 1.80fg	12.55 ± 1.15h	195.74 ± 17.25e	1.48 ± 0.25i	12.61 ± 2.01h	338.76 ±24.51f

Mean ± SD of ten determinations. Values with different letters differ significantly at $P<0.01$.

centration (r=0.99). Inhibition of elongation (53%) reached maximum in 5 μM ACC-treated roots. At concentrations of 2 μM or less, ACC did not significantly modify FW. FW increased by 19–40% at ACC concentrations between 3 and 5 μM. In the 2–4 μM range of concentrations the increase in FW correlated linearly with the ACC concentration (r=0.97).

For the ACC treatments we estimated that E_{50} was reached at 1.25 μM for elongation and 2.80 μM for swelling (Fig. 2). In the NAA treatments, the respective E_{50} effects were reached at 0.046 μM and 0.2 μM (Fig. 1). The ACC concentrations (1 and 2 μM) that caused approximately half of maximum inhibition of elongation does not significantly increase root tip weight.

The effects of NAA and ACC in the 24 h treatments differed in four main ways: (i) auxin induced higher inhibition of elongation than ethylene did (100% vs. 53%); (ii) for induction of increased swelling the effects of auxin and ethylene followed a similar pattern (215% vs. 140%); (iii) auxin increased root tip fresh weight at the whole tested range of concentrations (0.01–10 μM), while ACC increased swelling in a narrower range (3–5 μM); and (iv) these effects correlated linearly with the logarithm of the auxin concentration, whereas in ACC treatments the correlation was purely with ACC concentration.

COMPARISON OF GROWTH REGULATOR EFFECTS ON TREATMENT DAYS ONE AND TWO

We also examined differences in the rates of elongation and swelling induced by NAA or ACC during the second day of incubation. On the first day, control roots elongated 66.84 mm, but only 50.70 mm in the 24–48 h period (Tab. 1), which is 73% of the first day's growth. Application of NAA did not modify this behavior: regardless of the NAA concentration, the elongation rate was less on the second day ($P<0.05$). The results were similar for the ACC treatments.

In the absence of hormones (control), root tip weight decreased with incubation time at the rate of 0.605 ± 0.172 mg/d. In contrast, NAA induced root swelling. This resulted in an appreciable increase in FW of root apices treated with auxin for 24 h (Tab. 1), but at concentrations between 0.01 and 0.25 μM this effect did not persist; as in control roots, root tip weight was significantly lower at 48 than at 24 h. Auxin doses in the 1–10 μM range sig-

nificantly increased root apex weight in the 24–48 h period (Tab. 1).

After 24 h treatment, ACC modified the FW of root tips only at concentrations higher than 1 μM. Concentrations up to 2 μM strongly increased root tip weight at 24 h. During the second day, however, FW was not significantly affected by ACC doses between 1 and 4 μM. Concentrations at or above 5 μM promoted a slight but significant increase of FW (Tab. 1).

RELATIONSHIP BETWEEN ETHYLENE PRODUCTION AND CHANGES IN ROOT GROWTH

During the second day of incubation, untreated roots showed lower FW and a lower elongation rate as compared with the 0–24 h period. This change in root growth coincided with an increase in ethylene production. Ethylene biosynthesis in untreated roots increased progressively from 1.05±0.20 nl/h g FW (time 0, roots 60–80 mm long) to 1.68±0.17 (24 h, roots 120–130 mm long) and 4.56±0.20 (48 h, roots 180 mm long). Table 1 summarizes elongation, swelling and ethylene production on treatment days one and two.

At concentrations higher than 0.5 μM for NAA or higher than 5 μM for ACC these high ethylene production rates were maintained over 48 h. At lower doses the rate of ethylene biosynthesis increased on day one but resembled the control on day 2 (Tab. 1).

Table 1 summarizes the results comparing root elongation, root swelling and ethylene production. These data indicate that under ACC treatment the inhibition of elongation and induction of swelling are dependent on ethylene production. Interestingly, the weight of control and 1 μM ACC-treated roots at 24 h was similar, but ethylene production under 1μM ACC tripled the FW values of roots after 24 h. However, application of 5 μM ACC, which boosted ethylene production 6-fold, caused both inhibition of elongation and promotion of swelling to reach the maximum levels recorded at 24 h of ACC treatment (51% and 43% respectively). At 48 h the same treatment, coincident with increased ethylene production, resulted in a similar root elongation rate but reduced FW at 48 h versus 24 h ($P < 0.05$). This suggests that the ethylene concentration is not the only variable explaining root growth behavior after prolonged treatments.

After 24 h NAA application, ethylene production generally increased, elongation was inhibited and swelling was enhanced. These effects were dependent on the NAA concentration. Low doses of auxin (0.1 μM), which did not increase the production of ethylene at 48 h versus control roots, inhibited elongation by 66% and also increased root tip weight by 53% (Tab. 1).

Comparison of the NAA and ACC treatments with similar levels of ethylene production showed that auxin had a more intense effect than ethylene. For example, ACC 5 μM and NAA 1 μM treatments gave similar ethylene production rates at 48 h but very different effects on elongation and swelling (Tab. 1). ACC treatment inhibited elongation by 50% and increased swelling by 53%, while NAA inhibited elongation by 92% and increased swelling by 83%.

The ethylene antagonist STS did not significantly affect elongation after 24 h but increased root apex FW by 30% and enhanced ethylene production more than 30%. Simultaneous application of NAA and STS produced about half the effect on elongation and the same effect on swelling as NAA applied alone. In the presence of 0.1 μM NAA, STS increased ethylene production and apex FW without affecting root elongation as compared with NAA treatment alone (Tab. 1). Moreover, STS was unable to reverse the effect of NAA-enhanced ethylene production and also increased the effect of auxin-induced swelling. In the 24–48 h period STS did not significantly affect root elongation, and ethylene production was similar to control levels despite the increased swelling (144%).

Application of STS to roots treated with 0.1 or 0.5 μM NAA did not change inhibition of root elongation by auxin but significantly increased root apex FW and ethylene production as compared to treatments with auxin alone.

The relative responses of elongation and swelling to NAA and ACC treatments differed. Figure 3 shows the reduction in elongation produced by ACC in 24 h from 67 mm to 45 mm without appreciable changes in root apex FW. NAA induced a similar reduction of elongation but also increased the FW of root apices by 136% (from 5.1 to 7 mg/cm).

DISCUSSION

AUXIN AND ETHYLENE INHIBIT ELONGATION AND ENHANCE ROOT SWELLING

The typical responses of roots to auxin and ethylene treatment include inhibition of elongation and induction of swelling (Evans et al., 1994, Gaspar et al., 2003; de Cnodder et al., 2005; Alarcón et al., 2009a). Our present results demonstrate that auxin was more effective than ACC in inhibiting root elongation: total inhibition was observed with 1 μM NAA, whereas ACC inhibited by only 60%. The ranges of root elongation response to NAA and ACC treatments also differed. NAA inhibited root elongation in a wide range of concentrations from 0.01 μM to 1 μM, whereas ACC showed an inhibitory effect in a narrower range from 1 μM to 5 μM.

Our data from the exogenous NAA and ACC treatments show that auxin was also more effective than ACC in promoting increased root tip FW (215% vs. 140%), and in a wider range of concentrations. Auxin increased root tip weight at concentrations from 0.01 to 10 μM, whereas ACC produced swelling only from 2 to 5 μM. In the NAA treatments, ~50% of the maximum effect (E_{50}) was achieved at 0.046 μM for root elongation and at 0.2 μM for swelling (Fig. 1). In the ACC treatments, E_{50} was reached at 1.25 μM for root elongation and at 2.80 μM for swelling. Thus the dose-response curves for elongation and swelling differ. Root elongation was more sensitive to exogenous auxin and/or ethylene than swelling was, suggesting that elongation and swelling are regulated independently.

Root elongation, radial growth and ethylene production significantly changed through the 48 period of measurements. The elongation rate and root tip weight decreased with time, while ethylene production increased (Tab. 1). The effect of ACC on root elongation was stronger on the first than on the second day. This variation can be explained by the increase in ethylene production as the maize root aged. On the first day, roots treated with 1 μM ACC produced three times more ethylene than the control, but on the second day ethylene production was only slightly higher than in the control (Tab. 1). This suggests that the inhibitory effect of ethylene was greater on the first day. High ethylene biosynthesis during the second day suggests adaptation to high ethylene levels and/or reduced sensitivity to ethylene dependent on the developmental stage of the plant material (de Klerk and Hanecakova, 2008; Alarcón et al., 2009a).

The radial expansion stimulated by ACC was observed after 24 h treatment was not maintained for 48 h except at high ACC doses (5 and 10 μM). This strongly indicates that sensitivity to ethylene decreased on the second day. The fresh weight of NAA-treated roots was higher than the control at both 24 and 48 h, showing that auxin is more effective than ethylene in promoting swelling. These results are in accord with previous work showing that the morphological effects of auxin and ethylene do not match and that a high concentration of endogenous ethylene cannot mimic the effects of auxin (Rauser and Horton, 1975). Here we demonstrated that auxin is more effective than ethylene in inhibiting root elongation and enhancing swelling, and confirmed that auxin's control of root growth is not mediated by ethylene (Whalen and Feldman, 1988).

INTERACTION BETWEEN AUXIN AND ETHYLENE, AND REGULATION OF MAIZE ROOT GROWTH

Several mutations in components of auxin transport or signaling have been reported to produce aberrant responses to ethylene, suggesting crosstalk between these two growth regulators (Rahman et al., 2001; Chilley et al., 2006; Stepanova et al., 2007). In particular, auxin and ethylene apparently interact in regulating root growth, through various mechanisms of interaction. It is well known that both endogenous and exogenous auxin induce ethylene production (Yamada et al., 2001). Is auxin capable of promoting root thickening without the participation of ethylene? Our results in 0.1 μM NAA-treated and 1 μM ACC-treated roots answer this question in the affirmative. Both treatments increased ethylene production 3-fold, although the effects on elongation and swelling differed: 0.01 μM NAA inhibited elongation by 60% and increased swelling by 47%, whereas 1 μM ACC inhibited elongation by 25% without a significant effect on FW (Tab. 1). After 48 h the ethylene levels were similar in the treatments with 5 μM ACC and 0.5 μM NAA, but ACC slightly stimulated swelling and inhibited elongation by 60%, while NAA increased FW by 200% and inhibited elongation completely. Thus, ethylene does not seem to be the mediator involved in radial expansion of roots in response to auxin. This result also suggests that an excess of ethylene does not counteract the natural tendency for root diameter to diminish with age unless a small excess of auxin is present. Other work supports this suggestion. *Arabidopsis* roots growing in ethylene-rich medium required auxin to show inhibited elongation (Stepanova et al., 2007), and inhibition of cell elongation in *A. thaliana* roots by excess ethylene was shown to be mediated by up-regulation of auxin biosynthesis and transport (Swarup et al., 2007).

The application of ACC or ethylene antagonist produces changes in ethylene concentration and might thereby alter root development. Table 1 shows that reduced elongation and increased swelling occurred together with enhanced ethylene production in the ACC treatment. Inhibition of ethylene's action by the antagonist STS produced different effects on elongation and swelling. STS increased swelling in untreated and NAA-treated roots but did not affect elongation (Tab. 1). This result accords with previous findings from experiments with the ethylene biosynthesis inhibitor AVG (Luthen and Bottger, 1988), but Mulkey et al. (1982) obtained the opposite results. Our data indicate that auxin's effects are not mediated by ethylene, as inhibition of the action of ethylene did not reverse the effects of auxin. In this context the effects on elongation and swelling should be distinguished. It has been shown that maize root elongation is inhibited by ACC-induced enhancement of ethylene production (Alarcón et al., 2009a). STS does not affect root elongation but reverses the inhibitory effect of ACC (Alarcón et al., 2009b). Increasing ethylene levels with ACC or preventing ethylene's action with the antagonist STS both caused swelling. Elongation,

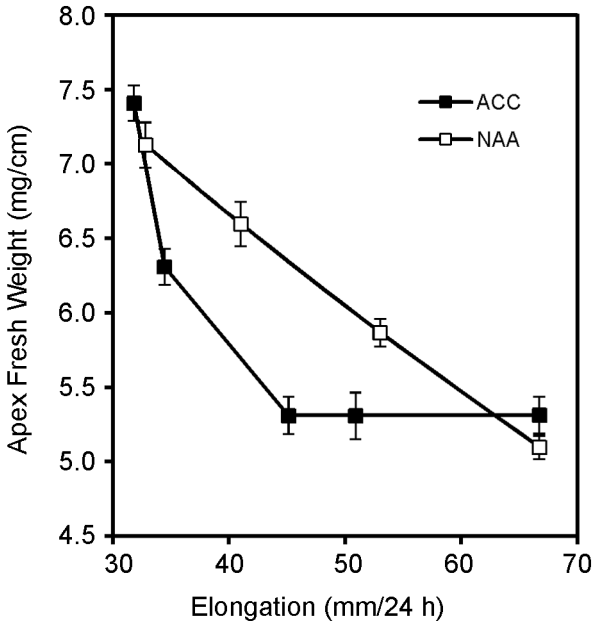

Fig. 3. Relationship between inhibition of elongation and the increase in swelling of roots treated with different concentrations of ACC (shaded squares) or NAA (unshaded squares) after 24 h treatment. After 24 h, untreated roots elongated 67±1.2 mm and root apex fresh weight varied from 5.1 to 5.32 mg/cm. Values are means ± SD of 10 roots. NAA concentrations – 0.01, 0.025, 0.05 μM. ACC concentrations – 1, 2, 3, 4 μM.

however, was inhibited only by increasing ethylene biosynthesis. This behavior suggests that longitudinal growth and radial growth of maize roots differ in their response to changes in ethylene. On the other hand, STS did not reverse the effect of increased ethylene production by auxin, but it has been demonstrated that STS is able to counteract the effect of increased ethylene production by ACC (Alarcón et al., 2009b)

Because inhibition of maize root elongation is usually accompanied by increased radial expansion, these two processes may be inversely related. Nevertheless, if auxin increased swelling at all the concentrations we tested in our study, the same cannot be said of the ACC treatments. Thus, for example, 2 μM ACC-treated roots showed 33% inhibition of elongation but swelling similar to control roots (Fig. 3).

These results indicate that auxin is the main hormone controlling radial and longitudinal growth in roots, and that ethylene does not mediate auxin's regulation of root development. The results also suggest that auxin and ethylene regulate root elongation and radial growth through different pathways and that the two processes are simultaneously but independently affected by growth regulators.

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