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Prevention of auxin-induced epinasty by α-aminooxyacetic acid

By

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Abstract

Ethylene production and epinastic growth of leaf petioles of tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) plants sprayed with 0.1 m*M* naphthyl-1-acetic acid were suppressed when 1 m*M* α-aminooxyacetic acid (AOA) was simultaneously sprayed on the plants. AOA had no effect on ethylene evolution and epinastic growth resulting from the application of 5 m*M* 1-aminocyclopropane-1-carboxylic acid, the immediate precursor of ethylene.

Key-words: α-Aminooxyacetic acid, 1-aminocyclopropane-1-carboxylic acid, auxin, ethylene, epinastic growth, *Lycopersicon esculentum*.

Introduction

Application of auxins to plant material stimulates the production of ethylene and, among other effects, causes epinastic growth of leaf petioles, which is thought to be mediated by the induced ethylene (Abeles 1973). Ethylene-induced epinasty of tomato plants is alleviated by anti-ethylene agents such as 5-methyl-4-ethoxycarbonylmethoxy-1,2,3-benzothiodiazole (Van Daalen and Daams 1970, Parups 1973, Jackson and Campbell 1976). One would expect that auxin-induced epinasty is prevented by an inhibitor of ethylene biosynthesis, and since we have recently shown that AOA is an effective inhibitor of auxin-induced ethylene synthesis in mung-bean hypocotyls (Amrhein and Wenker 1979), we decided to employ AOA as an antidote to auxin to prevent the epinastic response. We also employed ACC, which has been established as the immediate biosynthetic precursor of ethylene (Adams and Yang 1979, Lürssen *et al.* 1979), to induce epinasty. Transport of ACC from the roots of waterlogged tomato plants to the shoots and its subsequent conversion to ethylene under the aerobic conditions of the shoot has been shown to be involved in flooding-induced petiole epinasty (Bradford and Yang 1979).

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AOA, α-aminooxyacetic acid; NAA, naphthyl-1-acetic acid.

Materials and methods

Plant material. Tomato plants (*Lycopersicon esculentum* Mill. cv. Moneymaker) were grown in soil in a phytotron chamber under a 16-h photoperiod (Osram Hg lamps, type HQJL 400W/70, giving *c.* 9.000 lux at plant level), at 23°C (day) and 19°C (night), and a relative humidity of 75% (day) and 90% (night). One-month-old plants having 3 to 4 well developed leaves were used in the experiments.

Treatment of plants. Individual plants were sprayed each with 5 ml of the appropriate solution (pH adjusted to 5.5) and returned to the growth chamber. Epinastic growth of the second oldest leaf was measured as described by Jackson (1979).

Measurement of ethylene production. Four 2-cm long petiole segments (0.5–0.7 g) or 5 pinnules of the second oldest leaf (0.7–0.9 g) were enclosed in 30 ml Erlenmeyer flasks sealed with rubber serum caps. After 1 h at room temperature 1 ml samples of the gas phase in the flasks were removed with a gas-tight hypodermic syringe and analyzed for their ethylene content in a Varian 1400 gas chromatograph equipped with a Poropak R 80/100 column and a flame ionization detector.

Chemicals. α-Aminooxyacetic acid, semihydrochloride, was obtained from Sigma, St. Louis, Mo., and naphthyl-1-acetic acid from Merck, Darmstadt. α-Aminocyclopropane-1-carboxylic acid was the gift of Dr. K. Lürssen, Bayer AG, Leverkusen.

Results and discussion

Preliminary experiments, in which solutions with increasing concentrations of NAA and ACC, respectively, were sprayed onto 1-month-old tomato plants, revealed

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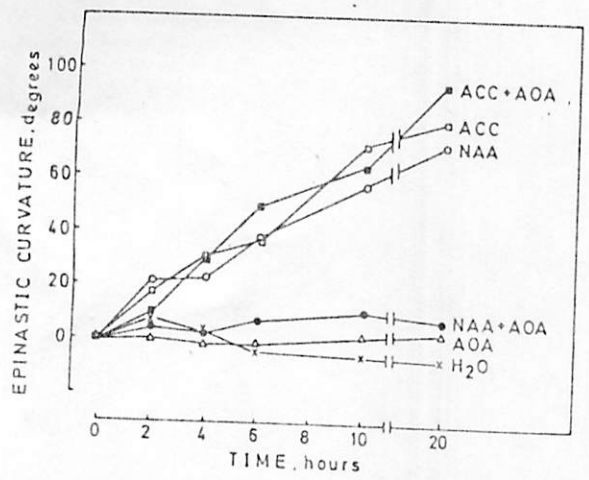

Figure 1. *Response of the petioles of second oldest leaves of one-month-old tomato plants to spraying entire plants with 0.1 mM NAA, 5 mM ACC and 1 mM AOA alone or in combination as indicated.* Epinastic curvature is expressed as the declination of the angle between the adaxial surface of the petiole and the main stem of the plant.

that 0.1 m*M* NAA and 5 m*M* ACC produced comparable epinastic responses. Within 2 h after the application of these two agents epinastic curvature commenced and increased continually during the next 10 to 20 h (Figure 1). All subsequent experiments were therefore carried out with these concentrations of NAA and ACC.

AOA at 1 m*M* concentration had no effect on the directional growth of the petioles (Figure 1), and plants subjected to a single treatment with 1 m*M* AOA showed no apparent change in their development during one subsequent month of observation. AOA sprayed simultaneously with either NAA or ACC prevented the curvature normally induced by NAA, but had no effect on the epinastic growth induced by ACC (Figure 1). Figure 2 shows tomato plants 20 h after the respective treatments. When ethylene evolution from petioles and laminae was monitored 5 h after spraying the plants, NAA- as well as ACC-treated tissues produced the gas at elevated rates, ACC being the more effective agent (Table 1). AOA eliminated the surge of ethylene in response to the treatment with NAA, but not with ACC. These results are in agreement with the known function of ethylene as

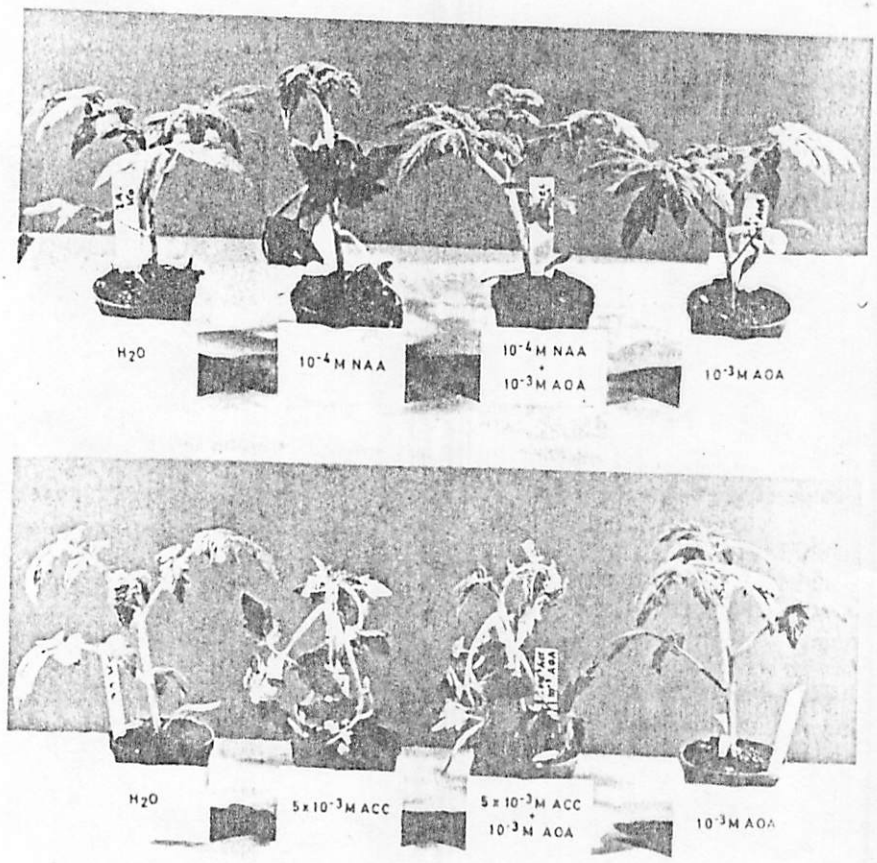

Figure 2. *One-month-old tomato plants 20 h after spraying with NAA, ACC and AOA at the indicated concentrations and combinations.*

Table 1. *Ethylene production by petiole segments and laminae of tomato plants 5 h after spraying the intact plants with NAA, ACC, and AOA.*

Treatment	Ethylene production nl · g^{-1} fr. wt. · h^{-1}	
	Petioles	Laminae
H_2O	0.07	0.22
1 m*M* AOA	0.02	0.26
0.1 m*M* NAA	0.25	2.30
0.1 m*M* NAA + 1 m*M* AOA	0.05	0.25
5 m*M* ACC	1.38	5.10
5 m*M* ACC + 1 m*M* AOA	1.45	5.22

mediator in the induction of epinastic growth by auxin-type-chemicals (Abeles 1973). AOA is known as a general inhibitor of pyridoxal phosphate-dependent enzymes (John *et al.* 1978), and a function of pyridoxal phosphate in ethylene biosynthesis has been indicated (Lieberman 1979). Our results show that AOA does not interfere with the conversion of ACC to ethylene. It appears, therefore, that the pyridoxal phosphate-dependent step in ethylene biosynthesis occurs during the formation of ACC from methionine, presumably during conversion of S-adenosylmethionine to ACC (Boller *et al.* 1979). In fact, it was shown very recently (Yu *et al.* 1979) that the ACC-forming enzyme in tomato extracts is activated by pyridoxal phosphate and competitively inhibited by AOA. Analysis of the purified enzyme will have to show, whether indeed it is a pyridoxal phosphate-linked enzyme. In contrast to silver ions (Beyer 1976) and the substituted benzothiadiazole (Parups 1973), which act as anti-ethylene agents, AOA does not interfere with ethylene action.

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