BIOLOGIA PLANTARUM 34 (3-4): 345-349, 1992

Effect of cadmium and nickel on ethylene biosynthesis in soybean

S. PENNAZIO and P. ROGGERO

Instituto di Fitovirologia applicata dle CNR, Strada delle Cacce 73, 10135 Torino, Italy

Abstract

Exogenously supplied cadmium and nickel considerably affected the ethylene biosynthesis in soybean cuttings. Cadmium stimulated ethylene production by increasing production of free ACC and stimulating EFE activity. Nickel inhibited ethylene production by depressing EFE activity, but stimulated the production of free ACC. Both heavy metals did not apparently affect cell membrane integrity.

Introduction

Heavy metals required for plant growth and development, or present in the soil as pollutants, may affect ethylene biosynthesis (see Yang and Hoffman 1984). Some of them (*e.g.* copper and cobalt) have been investigated in depth, whereas other ones (*e.g.* cadmium and nickel) have not. Cadmium was found to stimulate ethylene and ACC synthesis in *Phaseolus vulgaris* L. (Fuhrer 1982) during the first stage of exogenous supply, whereas it inhibited both ACC synthesis and conversion to ethylene when phytotoxic effect on cell membrane integrity was found in later stages of administration. Nickel was found to inhibit ethylene production from *Phaseolus aureus* Roxb. (Lau and Yang 1976) but, as far as we know, it was not further investigated.

The aim of the present work was to study the possible effects of these two heavy metals when exogenously supplied to soybean without producing apparent injury. The effects of nickel was compared with those of cobalt, which is considered one of the most potent inhibitor of ethylene biosyntesis (Yang and Hoffman 1984).

Material and methods

Soybean (*Glycine max* Merr. cv. Hodgson) was grown from seed in a glasshouse, in 10 cm pots containing a sterilized standard potting compost.

Received 4 January 1991, *accepted* 6 December 1991.

Abbreviations used: ACC = 1-aminocyclopropane-1-carboxylic acid; AVG = aminoethoxyvinylglycine; EFE = ethylene-forming enzyme.

On emergence, seedlings were transferred to a growth chamber and kept under a 12 h photoperiod, at 120 μmol s^{-1} m^{-2} PAR (Philips TLD 95) and 60 % relative humidity. Light and dark temperatures were 24 and 18 °C, respectively (± 1 °C). When plants had the trifoliate leaves just unrolled, stems were cut 3 cm below these leaves. The resulting cuttings whose cotyledons were eliminated, were inserted into vials containing the solutions to be tested, and kept for 24 h at the above described environmental conditions. When cuttings were kept under continuous light, temperature was constant at 24 °C.

Cadmium, nickel and cobalt (as chloride salts, *Merck*), ACC and AVG (*Fluka*) were dissolved in highly deionized water (conductivity less than 1 μS cm^{-1}), that was used as controls. Factorial experiments in which time of administration and salt concentration were varied indicated that 0.2 mM salt solutions could be administered for several days without producing apparent injury to cuttings. Cuttings were treated with this concentration for 24 h; such treatment gave very reproducible results. Compared to water controls, salt treatments did not alter fluid uptake by soybean cuttings, that was about 6.0 cm^3 g^{-1} (f.m.) after 24 h treatment.

After treatments, cuttings were analysed for ethylene production, EFE activity, free and total ACC content, and electrolyte release.

Ethylene production was determined by gas chromatography after 24 h incubation of cuttings in sealed vials on test solutions. EFE activity was assayed after treatments by incubating for 2 h on 10 mM ACC in the light, in the presence of the corresponding test solutions, in sealed vials, then determining the released ethylene as above. Total and free ACC were determined as previously described (Pennazio and Roggero 1990). Electrolyte leakage, as a measure of cell permeability, was determined as follows. Leaf discs of 10 mm diameter were punched from treated and control cuttings; they were halved, thoroughly rinsed with cold highly deionized water, and then incubated for 1 h in water, at 20 °C. The conductivity of the bathing solutions was measured (*E 587 Conductometer, Metrohm*), and then discs and solutions were submitted to 3 cycles of rapid freezing and thawing before measuring conductivity again. The results were expressed as percentage of the total release determined after the 3 cycles.

The experiments in anaerobiosis were done as follows. Cuttings were treated for 12 h with test solutions in the light. They were put in sealed vials and N_2 passed for 5 min to completely eliminate air (air elimination was checked by gas chromatography following disappearance of the usual air peaks). Cuttings were then kept for 12 h in the light before determining ethylene and free ACC, as above.

Results and discussion

Compared to water controls, cadmium substantially increased ethylene production in soybean (Table 1). Furthermore, it stimulated the conversion of ACC to ethylene by EFE activity, whereas the level of free ACC was unchanged. The accumulation of total ACC, however, increased suggesting that cadmium might stimulate the synthesis of ACC as well as its conjugation. The suggestion was confirmed by treatments performed under N_2, known to completely prevent EFE activity (Adams

and Yang 1979), that suppressed stimulation of ethylene production and caused accumulation of greater amounts of free ACC than water controls (Table 2).

Table 1. Effect of heavy metals on ethylene production, EFE activity, total and free ACC accumulation and electrolyte leakage. Cuttings were treated for 24 h with the indicated solutions before measurements. Each value is the mean of 9 replicates.

Treatments	Ethylene [nmol g^{-1}(f.m.)]	EFE activity [nmol C_2H_4 g^{-1} (f.m.)]	Total ACC [nmol g^{-1} (f.m.)]	Free ACC [nmol g^{-1}(f.m.)]	Electrolyte leakage [%]
control	0.6 ± 0.1	4.0 ± 0.5	1.9 ± 0.4	0.8 ± 0.2	4.5
0.2 mM $CdCl_2$	3.1 ± 1.2*	10.0 ± 1.2*	5.0 ± 1.8*	0.9 ± 0.2	4.6
0.2 mM $NiCl_2$	0.3 ± 0.1*	1.4 ± 0.4*	4.3 ± 0.6*	2.6 ± 1.0*	4.4
0.2 mM $CoCl_2$	< 0.1*	0.9 ± 0.3	3.0 ± 0.5	1.8 ± 0.6*	4.7

*statistically significant differences from the control

Fuhrer (1982) showed that cadmium-stimulated ethylene production from bean was due to an initial activation of ACC synthases followed by a decline with time in parallel to an inhibitory effect on the conversion of free ACC to ethylene.

Table 2. Effect of heavy metals on free ACC content under anaerobiosis. Cuttings were treated for 12 h under light with the indicated solutions. They were then placed in sealed vials and N_2 passed for 5 min. Cuttings were kept anaerobically for 12 h under light with the indicated solutions before determinations. Each value is the average of 10 replicates. The ethylene produced by cuttings during 12 h in anaerobiosis was less than 0.1 nmol g^{-1} (f.m.).

Treatments	Free ACC [nmol g^{-1}(f.m.)]
control	1.3 ± 0.2
0.2 mM $CdCl_2$	4.5 ± 1.6*
0.2 mM $NiCl_2$	2.9 ± 0.7*
0.2 mM $CoCl_2$	1.9 ± 0.5*

*statistically significant differences from the control

Since it is generally held that EFE activity required integrity of cell membranes (Kende 1989), the discrepancy between our and Fuhrer's results may be due to different effects of cadmium on cell membranes in the two plant species, because Fuhrer (1982) described a sharp increase in eletrolyte leakage, whereas no difference in leakage was found between water- and cadmium-treated soybean under our conditions (Table 1). Since the cadmium doses were rather similar in Fuhrer and our experiments, differing sensitivity of the two species to cadmium may be suggested. This suggestion is supported by the results of Rauser (1978), who described for bean red-brown discolouration of the unifoliate leaves after 1-d application of 10 μM $CdSO_4$ solution and complete dessication after 2 d, whereas no injury was observed

after several days of treatments with 0.2 mM $CdCl_2$ solution with our soybean cultivar.

Table 3. Effects of heavy metals on ethylene production and free ACC content under continuous light. Cuttings were treated for 24 h under light with the indicated solutions before determinations. Each value is the mean of 9 replicates.

Treatments	Ethylene [nmol g^{-1}(f.m.)]	Free ACC [nmol g^{-1}(f.m.)]
control	1.8 ± 0.3	0.5 ± 0.2
0.2 mM $NiCl_2$	1.0 ± 0.2*	5.6 ± 1.3*
0.2 mM $CoCl_2$	0.7 ± 0.2*	1.2 ± 0.3*

*statistically significant differences from the control

Nickel caused inhibition of ethylene release from soybean cuttings as a consequence of a marked inhibitory effect on EFE activity (Table 1), that was only slightly lower than that produced by cobalt. Nickel had been described as an inhibitor of ethylene production by Lau and Yang (1976) but, as far as we know, no biochemical explanation of such inhibition has hitherto been offered. Rather unexpectedly, nickel produced a greater accumulation of free ACC than cobalt (Table 1), and this result, together with a less marked inhibition of EFE activity than cobalt, suggests that nickel stimulate the synthesis of ACC. This was confirmed by experiments performed under anaerobiosis (Table 2) or continuous light (Table 3). A possible release of free ACC from its conjugated form (Jiao *et al.* 1986) appears to be excluded by the results indicating that free ACC accumulation was completely prevented by AVG (data not shown). As observed for cadmium, nickel did not affect cell membrane integrity (Table 1). In conclusion, nickel had two opposite effect on ethylene biosynthesis, both so far undescribed. The first concerns the inhibition of EFE activity, the last step of ethylene biosynthesis, a finding that explain the inhibition of ethylene production from plant materials. The second involves the stimulation of ACC synthesis and probably occurs *via* activation of ACC synthesis, because ACC accumulation was completely inhibited by AVG.

References

Adams, D.O., Yang, S.F.: Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. - Proc. nat. Acad. Sci. USA **76**: 170-174, 1979.

Fuhrer, J.: Ethylene biosynthesis and cadmium toxicity in leaf tissue of bean. - Plant Physiol **70**: 162-167, 1982.

Jiao, X., Philosoph-Hadas, S., Su, L., Yang, S.F.: The conversion of 1-(malonylamino) cyclopropane-1-carboxylic acid in plant tissues. - Plant Physiol. **81**: 637-641, 1986.

Kende, H.: Enzymes of ethylene biosynthesis. - Plant Physiol. **81**: 1-4, 1989.

Lau, O.L., Yang, S.F.: Stimulation of ethylene production in the mung bean hypocotyls by cupric ion, calcium ion, and kinetin. - Plant Physiol. **57**: 88-92, 1976.

Pennazio, S., Roggero, P.: Exogenous polyamines stimulate ethylene synthesis by soybean leaf tissues. - Ann. Bot. **65**: 45-50, 1990.

Rauser, W.E.: Early effect of phytotoxic burden of cadmium, cobalt, nickel and zinc in white bean. - Can. J. Bot. **56**: 1744-1749, 1978.

Yang, S.F., Hoffman, N.E.: Ethylene biosynthesis and its regulation in higher plants. - Annu. Rev. Plant Physiol. **35**: 155-189, 1984.