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Effect of Cobalt on the Formation, Growth and Development of Adventitious Roots in Tomato and Cucumber Cuttings

[1]Nadia Gad and [2]M.A. Atta-Aly

[1]Plant Nutrition Dept. NRC, [2]Horticulture Department, Ain Shams University, Egypt.

ABSTRACT: Adventitious roots formation on tomato cuttings was totally suppressed with the application of inhibitors of ethylene biosynthesis, 1 m M Amino-oxy acetic acid (AOA) or 2 m M cobalt, or ethylene action 2 m M cobalt silver thiosulfate (STS). The inhibitory effect of (AOA) could be reversed by application of ethryl (200ppm). Increasing ethryl concentration up to 250 ppm induced adventitious root formation on the hypocotyl and prevented its formation on the epicotyl. Auxins (IAA-IBA) application to AOA treated tomato cuttings. However induced adventitious root formation only in the epicotyl. Ethryl application of 200 – 500 ppm increased adventitious root number on cucumber cuttings to about 6 – 8 fold in comparison with control cuttings. It is concluded from these data that ethylene is an essential hormone in the formation of adventitious roots and it can modulate the effects of Auxin. In the solution culture, low level of cobalt (0.25 ppm) stimulate adventitious roots formation in tomato and cucumber cuttings and also increased ethylene production. Increasing cobalt concentration to 0.5 ppm caused further stimulation in adventitious roots formation and growth as well ethylene production only in cucumber cuttings. When cobalt concentration was raised above 0.5 ppm adventitious roots formation and ethylene production were strongly reduced in both plants. These data showed that the formation of adventitious roots is parallel to plant ethylene production while high levels inhibited it. Raising basal levels of ethylene in cucumber seedlings, with low level of cobalt application to that of untreated tomato seedlings increased the percentage of successful transplanting or survival rate of seedlings from 11 – 96 %. These data strongly suggest that transplanting failure in some plant species is mainly due to their low level basal of ethylene.

Key words: Cobalt, root formation, ethylene, auxin, tomato, cucumber.

INTRODUCTION

Root anchor the plant in the soil, absorb and translocat water and nutrients, synthesize and transport growth regulators and other organic compounds and provide a sink for carbohydrates from the shoot[1]. Factors that reduce root growth may injure the plant by reducing the volume and intensity of soil exploration; the root environment is seldom optimum for extensive effective growth. Adverse conditions include water deficient or O_2, deficient or imbalanced nutrients, toxic chemicals, mechanical impedance, non-optimum soil temperature and attack by pathogens and insects[2].

If root growth declines due to stress, the supply of water and nutrients to the shoot is reduced, with subsequent reduction of shoot growth. Besides conditions that limit photosynthesis process, reduce shoot growth and limit assimilate translocation to the root; thus reducing root growth. Therefore, the stress originating in either the roots or shoots affects the rest of plant[3,4].

Stress factors such as water logging and salinity, affect the formation and growth of adventitious roots in variety of plants[5]. It is well known that stress conditions strongly promote ethylene biosynthesis and production[6,7]. Direct treatment by ethylene or ethylene – releasing compound such as ethryl (2-chloroethylphosphonic acid) induces adventitious root formation and growth in mung been cuttings, while application of ethylene biosynthesis inhibitors, such as aminethoxyvenylglycene (AVG), or action such as norbornadiene, inhibits adventitious roots formation and growth in mung been cuttings[8,9]. According to [10], it is evident that cobalt inhibits ethylene biosynthesis by inhibiting the conversion of 1-aminocyclopropan 1-carboxylic acid (ACC) to ethylene. Indeed, when cobalt increased from 10 to 30 m M was introduced into hydroponically, grown tomato plants which were induced to produce ethylene (by root anaerobiosis), cobalt strongly inhibited ethylene production without affecting the increase in ACC level in the shoot[11].

Auxin is also known to promote adventitious root formation through it's stimulation of ethylene production[12]. Cuttings of many plant species were induced to form new adventitious root by dipping in 500

Corresponding Author: Nadia Gad, Plant Nutrition Department, NRC, Egypt.
E-mail: drnadiagad@yahoo.com

and 10000 ppm of either indole acitic acid (IAA) or indole butric (IBA) for 5 seconds. Root number, length and rooting percentage were significantly increased by Auxin application[13]. It is of interest to note that Auxin induces ethylene biosynthesis by stimulating the synthesis or activation of ACC synthesis enzyme[10,6]. In contrast, ethylene, inhibit Auxin transport in plant tissues[14].

Egypt is expanding in the cultivation of horticultural crops in the new reclaimed desert land to meet the growing population demand for such crops as well as exportation. Thus increasing the formation and growth of adventitious roots will potentially increase the percentage of successful transplanting, especially under arid and semiarid conditions[15]. Therefore, this experiment was designed to study the factors affecting adventitious roots formation and growth for successful propagation. Tomato, as one of the most important vegetable crops in Egypt, cultivated by seedlings transplanting. It is also recommended for cultivation in the new reclaimed desert lands. Unlike Tomato and Cucumber are unable to compensate its demand root if transplanted.

Moreover, it was designed to use a successful transplanting crop such as tomato and unsuccessful transplanting crop such as, cucumber as index crops in this experiment.

MATERIALS AND METHODS

Plant material: On mid August, 2003 seeds of tomato (*Lcopersicon esculentum* Mill UC_{97}) and cucumber (*Cucumous sativum* c.v. Beta alfa) were sown in trays filled with mixture of sand and peatmoss (1:1). Trays were kept in a greenhouse with frequent irrigation. Six-week-old tomato seedling were cut at the soil surface (2 cm below cotyledon leaves) using a stainless steel sharp blade. Excised shoots (cuttings) were distributed among the treated trays, immediately after excision, under glasshouse cultivation. Same procedures were carried out on three-week-old cucumber seedlings.

Treatments of tomato cuttings:

a) Cobalt treatments: Cuttings were transplanted, immediately after excision, into aerated 0.5 Hogland nutrient solution[16]. The nutrient solution was supplemented with 0.00, 0.25, 0.50, 0.75 and 1.00 ppm cobalt. Solution was replaced every 3 days for a period of one month. Adventitious roots growth was monitored throughout this period. At the end of the experiment, adventitious root photo prints were taken and intact plants were incubated for ethylene analysis as described below.

b) Ethylene inhibitors and ethryl treatment:

1. Inhibitors of ethylene biosynthesis: Cuttings were stem applied in H_2O or 1 m M Aminoxy acitic acid (AOA) or 2 m M Co (equal to approximately 18 ppm) for one hour with exposing their shoots to the direct sun light. Cuttings were then transplanted into 0.5 Hogland nutrient solution of one half the original concentrations as mentioned above. In the next day, AOA treated cuttings were stem dipped in 0, 50,100, 150, 200 and 250 ppm ethryl for 15 min. and then returned to nutrient solution which was previously adjusted at pH 5.5 with diluted HCl. Nutrient solutions were totally replaced as mentioned above with recording the formation and growth of adventitious roots, 3 week after transplanting.
2. Inhibitors of ethylene action: Silver thiosulfate (STS) were prepared immediately before treatments by mixing equal volumes of 4 m M silver nitrate with 16 m M sodium thiosulfate to bring out a concentration of 2 m M STS. The control treatment was distilled water[17]. Same procedures of AOA treatment were used for treating tomato cuutings with STS. The second day treatments were: 200 ppm ethryl (as described above) and 500 pm Indole Buteric acid (as described below). Parameters of adventitious roots growth were also taken 3 weeks after transplanting.

Auxin and ethryl treatments: Cuttings were stem dipped immediately after excision in H_2O or 1 mM AOA for one hour and then transplanted into the nutrient solution as mentioned above. In the second day, cuttings were stem dipped in 500 ppm of Indole acetic acid (IAA) or Indole Butric acid (IBA) for 5 seconds or in 200 ppm ethryl for 15 min., and then returned back to the nutrient solution. Parameters of Inhibitors of ethylene biosynthesis: Cuttings were stem applied in H_2O or 1 mM AOA or 2 mM Co as cobalt sulfate(equal to approximately 18 ppm) for one hour with exposing their shoots to the direct sun light. Cuttings wee then transplanted into diluted Hogland nutrient solution of 0.5 concentration nutrient solution as mentioned above. In the next day, AOA treated cuttings were stem dipped in 0, 50, 100, 150, 200 and 250 ppm ethryl for 15 min. and then returned to the nutrient solution, adjusted to pH 5.5 with diluted HCl. Nutrient solutions were totally replaced as mentioned above with recording the formation and growth of adventitious root 3 weeks after transplanting.

Treatment of Cucumber cuttings:

a) Cobalt treatments: Cucumber cuttings were treated with cobalt as described in tomato, but cobalt concentrations used were 0.00, 0.25, 0.50 and 1.00 ppm. Adventitious root growth were recorded by taking a photo-print 3 weeks after transplanting intact plants were incubated for ethylene analysis as described below.

b) Ethryl and Auxin treatments: Cuttings were stem dipped immediately after excision, in 0, 200, 300, 400 and

500 ppm ethryl for 15 min. or in 500 ppm IBA for 5 seconds as described previously. Cuttings were then transplanted into the nutrient solution as in tomato. Parameters of adventitious roots growth were recorded 2 weeks after transplanting.

c) Cobalt and direct transplanting into the field: Cucumber seeds were sown in trays filled with sand. Trays were then kept in a glasshouse with frequent irrigation until seeds were germination. When the first true leaf started to appear, trays were daily flushed with H_2O and one hour later they wee flushed again with either H_2O or 0.25 ppm cobalt solution. After 10 days of cobalt application, 100 cucumber seedlings were transplanted directly into the field and frequently irrigated.

The transplanting was done by pulling seedlings out from the trays and inserting their roots into soil directly in the field as usually done with tomato seedlings. The percentage of successfultransplanting (survival seedlings) was calculated 3 weeks after transplanting. After 68 days, early fruit weight, total fruit weight and number of fruit were recorded.

Parameters of adventitious root growth: Adventitious roots growth was measured by counting their number per plant, measuring the average of their length and recording their fresh weight. Photo-prints were also taken to show the differences between treatments in root growth and development.

Measuring ethylene production: Tomato and cucumber cuttinwere taken out from the water culture of cobalt treatments. Each cutting was then enclosed in 75 ml galss gar with one ml water at bottom of each gar to prevent drought stress. One ml samples were withdrawn from the head space after 6 hours incubation for ethylene using Perkin-Elmer, SIGMA 3B, Gas Chromatograph.

Experiments were carried out during 2003 and designed as a complete randomized design factorial analysis in five replicates. Data were analyzed for statistical significance differences using LSD test.

RESULTS AND DISCUSSIONS

When tomato cuttings were stem dipped for 1 hour in 1 mM AOA and then transplanted into aerated nutrient solution for 3 weeks, non of the cuttings formed adventitious root during the course of the experiment (Tables 1, 2). Some results were obtained with 2 mM application of either freshly prepared (Table 2) or cobalt ion. Cuttings treated with H_2O however, were strongly capable to form and grow healthy and strong adventitious roots 10 days after transplanting (Tables 1, 2). The biosynthesis pathway for ethylene production in vascular plants progresses from the amino acid methionin through SAM to ACC, and finally to ethylene[6]. It was evident by[18] that AOA inhibited SAM conversion to ACC. Cobalt ion however, inhibited ACC conversion to ethylene[10]. Application of STS strongly inhibited ethylene action but not biosynthesis[17]. Therefore, imbibing ethylene biosynthesis or action was behind the total inhibition of adventitious root formation on tomato cuttings. It was reported by[8] that dipping of aerial parts and then the stem of pea cuttings in 10 μ M AVG inhibited adventitious root formation by about 39% while 1 mM STS application has no effect. Data presented in Tables (1 and 2) however, showed a total inhibition of adventitious roots formation with either AOA or STS application. The exposure of cuttings to direct sun light during the dipping time without wetting their aerial parts insured enough amount arrival of AOA or STS to cuttings and subsequently to the total inhibition of adventitious roots formation was obtained.

Ethryl application of 200 or 250 ppm overcome the inhibitory effect of AOA on adventitious roots formation and this did not occur at the lower levels of ethryl treatments (Table 1 and 2). Cuttings treated with STS however, did not respond to ethryl application (Table 2). Dipping of cucumber cuttings in 200 – 500 ppm ethryl induced adventitious root formation and increased root number by 8 – 10 time of that formed on H_2O treated cuttings, respectively (Table 3). These results are in harmony with[19]. They suggest that ethylene is an essential hormone in adventitious root formation. While adventitious root number increased more than 2 fold on the hypocotyl, it decreased significantly on the epicotyl of tomato cuttings with 200 ppm ethryl application (Table 2). Increasing ethryl concentration up to 250 ppm caused a complete inhibition of adventitious roots formation on the epicotyl of AOA treated cuttings (Table 1). It is well know that the ethylene inhibits Auxin transport[14,20].

Therefore, the inhibition of adventitious root formation on the epicotyl may be due to the inhibition of auxin transport caused by ethryl application. Auxin application induced adventitious roots formation on the epicotyl of AOA treated cuttings and the highest number of adventitious root were formed on the epicotyl of Indole Buteric acid (IBA) treated cuttings (Table 2). This result agreed with the finding of[21]. In contrast, auxin application inhibited adventitious roots formation on the hypocotyl (Table 2).

These data clearly indicate that ethylene induced adventitious root formation on the hypocotyl while auxin had same effect but only on the epicotyl. Larkin *et al*[22] reported that auxin promotes adventitious roots formation

Table 1: Effect of dipping the stem (hypocotyls and epicotyl)of tomato cuttings in ethryl for 15 min. on the formation and growth of adventitious roots , cuttings were stem dipped 1 hour in 1mM AOA one day before ethryl application.

AOA pretreatment	Ethryl treatment (ppm)	Number of adventitious root/hypocotyl	Number of adventitious root/epicotyl	Number of adventitious root/cuttings	Adventitious root fresh weight/cuttings	Average of adventitious root/length (cm)
H_2O	0	44^{a}	36^{a}	77^{a}	0.69^{a}	7.6^{a}
AOA	0	-	-	-	-	-
AOA	50	-	-	-	-	-
AOA	100	-	-	-	-	-
AOA	150	-	-	-	-	-
AOA	200	36^{a}	19^{b}	52^{b}	0.27^{b}	4.4^{a}
AOA	250	39^{a}		39^{b}	0.18^{b}	2.9^{c}

Cuttings were transplanted after application into aerated 0.5 Hogland nutrient solution for 3 weeks and then data were taken.

Table 2: Effect of dipping the stem (hypocotyls and epicotyl)of tomato cuttings in auxin or ethryl for 5 seconds or 15 min., respectively on the formation and growth of adventitious root. Cuttings were stem dipped 1 hour in H_2O, 1 mM AOA and 2 mM STS one day before 500 ppm of Auxin and 200ppm ethryl application.

AOA pretreatment	Second day treatment	Number of adventitious root/hypocotyl	Number of adventitious root/epicotyl	Number of adventitious root/cuttings	Adventitious root fresh weight/cuttings	Average of adventitious root/length (cm)
H_2O		41b	57c	96a	0.85a	7.2a
IAA		15c	46b	62bc	0.51b	6.5ab
IBA		19c	73a	89ab	0.81a	6.9ab
Ethryl		89a	25f	104a	0.99a	7.4a
AOA or STS	IAA	-	42c	42c	0.17c	4.3c
AOA	IBA	-	60bc	59bc	0.24c	4.8c
AOA	Ethryl	61ab	42c	101a	0.87a	5.6bc
STS	IBA	-	64b	64c	0.26c	4.9c
STS	Ethryl	-	-	-	-	-
LSD 5%		-	-	-	-	-

Cuttings were transplanted after application into aerated 0.5 Hogland nutrient solution for 3 weeks and then data were taken.

Table 3: Effect of dipping the stem (hypocotyl) of cucumber cuttings in IBA or ethryl for 5 sec. Or 15 min., respectively on the formation and growth of adventitious roots.

First day treatment	Conc. (ppm)	Number of adventitious root/cuttings	Average of adventitious root/length (cm)	Adventitious root fresh weight/cuttings (g)
Control		23^{f}	3.8^{f}	0.42^{f}
IBA	500	35^{e}	4.9^{d}	0.53^{e}
Ethryl	200	102^{d}	5.7^{c}	1.78^{d}
Ethryl	300	137^{c}	7.9^{b}	1.91^{c}
Ethryl	400	156^{b}	8.7^{a}	2.23^{b}
Ethryl	500	171^{a}	4.3^{e}	2.48^{a}

Cuttings were transplanted after application into aerated 0.5 Hogland nutrient solution for 3 weeks and then data were taken

through its stimulation of ethylene production. Therefore, dipping the stem of tomato cuttings in AOA did inhibit adventitious roots formation on both epicotyl and hypocotyl (Table 2). The inhibition occurred on the epicotyl support the speculation reported by[15] that the presence of basal ethylene is essential; for auxin action. Auxin induces ethylene biosynthesis while AOA inhibits it[23]. Auxin application to AOA treated cuttings may

Table 4: Cucumber early fruit weight, total fruit weight and number of fruit per plant.

Treatments	Root No.	Fruit weight of 1st pick (kg/plant)	Total fruit weight (kg/plant)	Number of fruits per plant
Control	25	0.89	1.96	12
Cobalt 0.5 ppm				
R_1	105	1.63	2.83	22
R_2	108	1.63	2.88	24
R_3	110	1.72	2.73	25
R_4	114	1.80	2.97	27
LSD average 5%	9	0.15	0.12	2.69

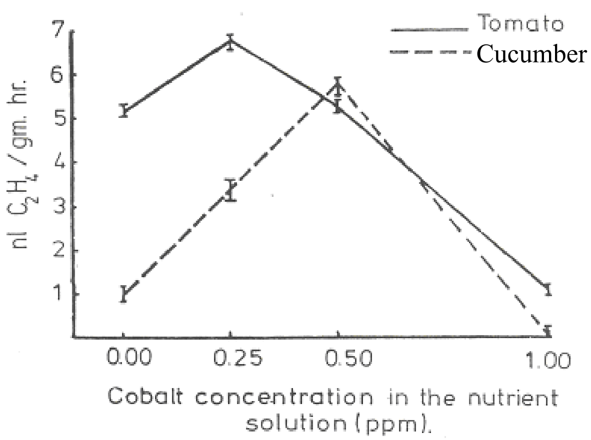

Fig. 1: Effect of Supplementing the nutrient solution in which tomato and cucumber cuttings were grown with cobalt ion on plant ethylene production.

partially induce ethylene biosynthesis to the level, which is, required for Auxin to act and to induce adventitious roots formation on the epicotyl. Supplementing the nutrient solutions in which tomato and cucumber cuttings were grown with different concentrations of cobalt ion, affect adventitious root growth. In tomato cuttings, 0.25 ppm of cobalt application significantly induced adventitious roots formation and growth, while a significant reduction occurred when cobalt concentration rose above 0.5 ppm (Fig. 1A). Adventitious root growth on cucumber cuttings however, were increased with 0.25 ppm cobalt, reached its maximum rate of growth at 0.5 ppm and significantly inhibited growth with increasing cobalt concentration up to 1.0 ppm (Fig. 1B).

Atta-Aly *et al*[24] reported that cobalt ion inhibit ethylene biosynthesis by inhibiting ACC conversion to ethylene. Data in Fig. (1) however, showed that this inhibition was only occurred with high levels of cobalt application, i.e. 1.0 ppm. Exposing tomato and cucumber cuttings to 0.25 ppm of cobalt ion significantly induced their ethylene production. When cobalt ion concentration increased up to 0.5 ppm, ethylene production by cucumber cuttings significantly increased and became even higher than that of 0.25 ppm cobalt addition (Fig. 1). Heavy metals are known to simulate ACC synthesis[4]. It seems that low levels of cobalt ion significantly induce ACC synthesis with minor or no inhibition on its conversion to ethylene. Increasing cobalt concentration up to 1.0 ppm strongly inhibited ACC conversion to ethylene and subsequently ethylene production either tomato and cucumber plants (Fig. 1).

Basal ethylene produced by tomato seedlings (Fig. 1) was incredibly higher than that of cucumber seedlings (58 fold). It might be minor level of basal ethylene produced by cucumber seedlings were behind its failure in transplanting. To test these hypothesis cucumber seedlings were treated with 0.25 ppm of cobalt ion for 10 days to increase their basal ethylene, as it is evident in Fig. (1) and then directly transplanting in to the field. The percentage of successful transplanting of survival rate of the seedlings was significantly increased from 11 to 96 % with cobalt application. After this successful plants were grown normally and flowered within 3 weeks from transplanting

In summary, ethylene and Auxin appear to be involved in adventitious root formation in different roles. Ethylene appears to be more important in adventitious roots initiation since inhibiting its biosynthesis or action totally inhibited adventitious roots formation. Furthermore, Auxin did not induce the formation of adventitious root without a basal level of ethylene being present. Finally increasing basal ethylene in cucumber seedlings with cobalt application remarkably induced adventitious root formation and increased the percentage of successful transplanting up to 96%.

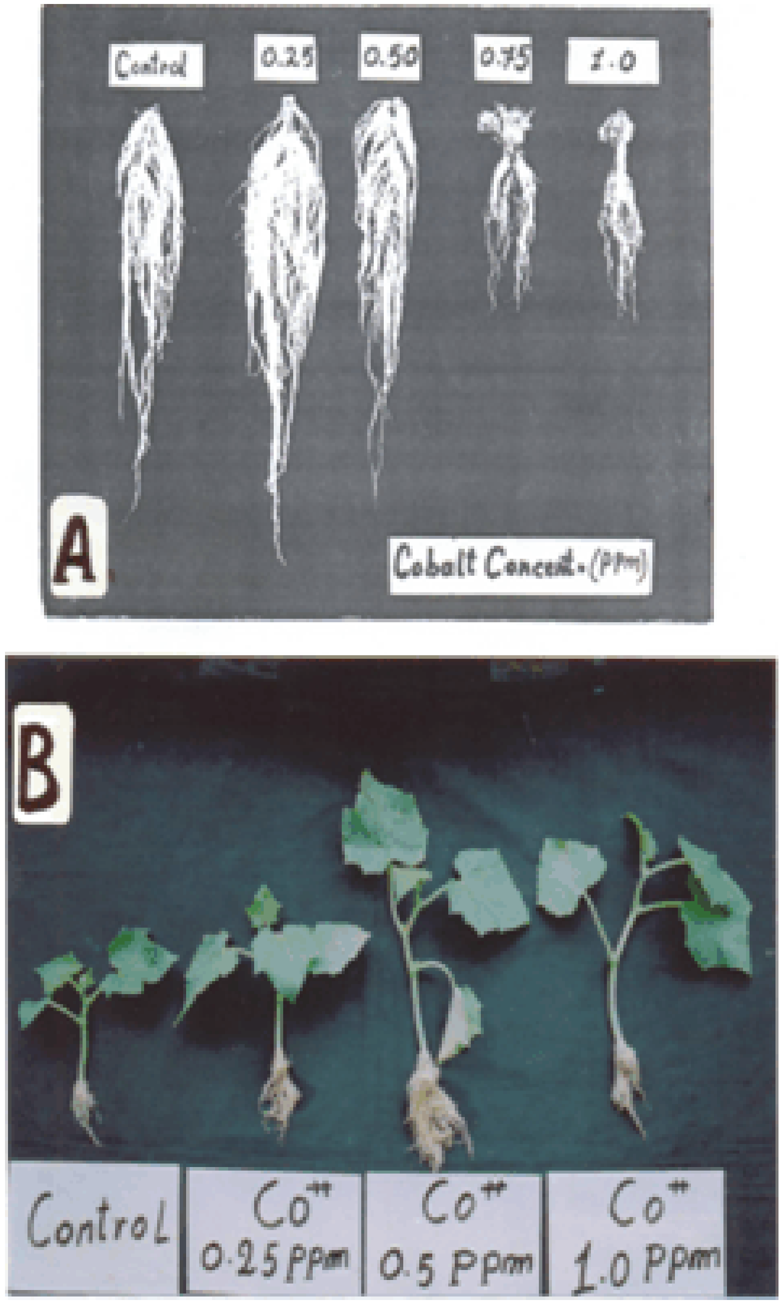

Fig. 2 (A,B): Effect of supplementing the nutrient solution in which Tomato (A) and cucumber (B) cuttings were grown which cobalt ion on the formation and growth of adventitious root.

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