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Soaking Summer Squash Seeds in Low Concentrations of Cobalt Solution Before Sowing Increased Plant Growth, Femaleness, and Fruit Yield via Increasing Plant Ethylene Level

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Abstract. Soaking summer squash (*Cucurbita pepo* cv. Eskandarany) seeds in continuously aerated solutions of 0.25, 0.50, and 1.00 ppm Co^{2+} for 48 h before sowing strongly increased plant growth, femaleness, and fruit yield compared with those of water- (control) or 0.5 mM AOA (aminooxyacetic acid)-soaked seeds. Following the same pattern, plants of Co^{2+}-soaked seeds produced significantly higher ethylene levels as early as the seedling stage (14 days after seed sowing) up to the onset of flower initiation (OFI) stage (30 days after seed sowing), with more pronounced levels of all measured parameters for plants of 1.00 ppm Co^{2+}-soaked seeds. Plants of AOA-soaked seeds, however, behaved in a pattern similar to those of control in all measured parameters. The only exception was the significant ethylene inhibition noticed only at the plant seedling stage with AOA-seed soaking, which indicated the short term of AOA inhibition to ethylene when applied as early as the seed germination stage. When AOA was applied foliarly before and at the OFI stage, the increased plant femaleness obtained with Co^{2+} seed soaking was arrested. It is indicated, therefore, that summer squash plant femaleness is more responsive to plant ethylene-modulated levels before or at the OFI stage than earlier stages. Furthermore, all seed soaking treatments had no effect on plant leaf number or plant and fruit Co^{2+} content, which strongly indicated that the positive impact of Co^{2+} on summer squash plant growth and femaleness was mainly the result of the so-called ‘‘low Co^{2+} level-induced ethylene.’’ The percentage of fruit yield increase reached about 26, 40, and 56% over the control by 0.25, 0.50, and 1.00 ppm Co^{2+} seed soaking, respectively, whereas AOA seed soaking resulted in only a 4.5% yield reduction. To ensure the permanent positive impact of Co^{2+} on ethylene production as well as the short period of AOA inhibition, seeds were resoaked in water or AOA for 8 h after being soaked in 1.00 ppm Co^{2+} solution for 40 h. The results obtained emphasized the previous findings because AOA inhibition was restricted on ethylene production only at the seedling stage. Meanwhile, all Co^{2+} positive effects were obtained 2 weeks later, even with AOA seed resoaking. These data strongly suggested that the positive impact of Co^{2+} on ethylene production is more permanent than the negative impact of AOA. Hence its application, in low concentrations, as early as the seed germination stage, strongly increased summer squash plant growth, femaleness, and fruit yield by increasing the plant ethylene level.

Key Words. *Squash—Cobalt—Ethylene—Growth—Femaleness*

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; AOA, aminooxyacetic acid; SAM, *S*-adenosylmethionine; OFI, onset of flower initiation; LSR, least significant range.

In cucurbitaceous plants, ethylene has been known as a sex-regulating hormone (Lieberman 1979, Rudich et al. 1972). Exogenous application and ethylene-releasing chemicals such as ethrel (2-chloroethanephosphonic acid) induced the femaleness of cucumber (Augustine et al. 1973) and squash plants (Atta-Aly 1992, Baha Eldin et al. 1983). Takahashi and Suge (1982) reported that treating cucumber plants with ACC (1-aminocyclopropane-1-carboxylic acid) as an immediate ethylene precursor (Adams and Yang 1979) greatly increased the number of pistillate flowers. Mechanical stress, which greatly stimulated ethylene production (Yang 1980), also increased the femaleness of cucumber plants (Takahashi and Suge 1982). In addition, more ethylene was known to be produced by gynoecious strains than by monoecious ones, and female buds of cucurbitaceous plants produced greater amounts of ethylene than those produced by male buds (Rudich et al. 1972). On the other hand, inhibiting ethylene biosynthesis with AVG (aminoethyoxyvinylglycine) or AOA application (Yang 1980) strongly reduced cucumber (Atsmon and Tabbak 1979) and squash (Atta-Aly 1992) plant femaleness, respectively.

It is well known that the ethylene biosynthetic pathway in higher plants progresses from the amino acid methionine through SAM (S-adenosylmethionine) to ACC and finally to ethylene (Adams and Yang 1979, Yang 1980). The application of cobalt, however, inhibited this biosynthetic pathway by blocking ACC conversion to ethylene using a Co^{2+} concentration comparable to about 18 ppm (Yu and Yang 1979). Atta-Aly et al. (1989), however, found that supplementing the nutrient solution with a concentration of 0.5 ppm of Co^{2+} or less significantly induced ethylene production and adventitious root formation of tomato and squash cuttings. This was eliminated and even depressed when such cuttings were treated with AOA or with increasing Co^{2+} levels in the nutrient solution above 0.5 ppm.

Cobalt is an essential element for the synthesis of vitamin B_{12}, which is required for human and animal nutrition (Smith 1991, Young 1983). Unlike other heavy metals, Co^{2+} is safer for human consumption, and up to 8 mg can be consumed on a daily basis without human health hazard (Young 1983). In higher plants, Co^{2+} is an essential element for legumes because of its use by the microorganisms in fixing atmospheric nitrogen (Evans and Kliwer 1964, Young 1983). In plants other than legumes, however, Co^{2+} also promoted many developmental processes including stem and coleoptile elongation, opening of hypocotyl hooks, leaf expansion, and bud development (Howell and Skoog 1955). It is also required, in low levels, for maintaining high yields of cucumber (Scott and William 1976) and eggplant and grapes (Nikolic 1952) and for increasing the growth of wheat (Wilson and Nicholas 1967), tomato (Atta-Aly et al. 1991, Kobbia and Osman 1987), and squash plants (Atta-Aly 1992).

This work, therefore, was designed and undertaken, based on the findings that low Co^{2+} levels induced ethylene production (Atta-Aly et al. 1989), to study the impact of soaking summer squash seeds in a low concentration of Co^{2+} solution before sowing on the ethylene level produced by the resulting plants and the subsequent impact on plant femaleness and fruit yield.

Materials and Methods

Plants

After soaking treatments, radicated summer squash (*Cucurbita pepo* cv. Eskandarany) seeds, the most common Egyptian commercial cultivar, were sown directly in the sandy soil of a drip-irrigated farm in Belbais, Sharkia Governorate, Egypt, on April 5, 1995 and 1996, for the first trial and on March 15, 1996 and 1997, for the second trial. At sowing, seeds were placed in hills of 5-cm depth and 50 cm apart (distances between drippers) with a capacity of two seeds/hill. Plants of each seed soaking treatment were grown in three replicates, each was 60 m^2 in area and comprised two rows 20 m long and 1.5 m wide (drip-lateral spacing). The number of plants was reduced to one plant/hill 10 days after planting to bring out 80 plants in each replicate of each soaking treatment. Additionally, 40 plants were grown separately in which case their seeds were soaked previously in either water or 1.00 ppm of Co^{2+} solution (20 plants for each seed soaking treatment) during the first trial using the same growing procedures. Such plants were grown to be used for AOA foliar application. In all trials, 5-month-old cattle manure (organic fertilizer) was applied to the soil to a depth of 30 cm under the dripping lateral (10 cm below the sandy soil surface) at a rate of 20 m^3/feddan (4,200 m^2) 1 week before seed sowing. One night before seed sowing, the experimental farm was drip irrigated for 3 h and rewatered again for 30 min after seedling emergence. All agricultural procedures were then carried out as usually recommended for summer squash production in sandy soils using drip irrigation system with drippers capacity of 4 liters/h.

Seed Soaking Treatments

In the first trial, summer squash seeds were soaked for 48 h at room temperature (20°C ± 5) in continuously aerated solutions of 0.00 (distilled water), 0.25, 0.50, and 1.00 ppm of Co^{2+} using cobalt sulfate salt or in 0.5 mM AOA as antiethylene biosynthesis (Yang 1980).

In the second trial, however, seeds were soaked for 40 h in 1.00 ppm of cobalt solution, rinsed with water for 8 min, and resoaked for an additional 8 h in either H_2O or 0.5 mM AOA using the same soaking procedures described above.

By the end of each soaking treatment, seeds were radicated with a radicle length of 1–2 mm. Seeds were then sown directly into the sandy soil.

AOA Foliar Application

Plants of water and 1.00 ppm of Co^{2+}-soaked seeds, which were grown separately for AOA foliar application during the first trial, were sprayed foliarly at sunset with 0.5 mM AOA four times over weekly intervals starting 1 week after seed sowing. The solution was sprayed until runoff occurred over the leaves. Plant fresh and dry weights as well as the leaf area of the fully expanded leaves and plant femaleness were measured in three replicates, each comprising five plants.

Ethylene Analysis

At the end of the seedling stage (14 days after seed sowing) as well as at the onset of flower initiation (OFI) stage visually identified after 30 days of seed sowing, ethylene was analyzed using the second developing leaf from the top of the plant. Leaves were exised 2 mm above the stem surface from five randomly selected plants at 10 a.m. using a stainless steel blade. They were placed immediately in 150-mL glass tubes (one leaf/tube), each containing 5 mL of H_2O for immersing the leaf base in water to prevent drought stress. Glass tubes were then sealed using rubber Supa-seal and kept under a 1,000-lux fluorescent light for 8 h. One-mL gas samples were then withdrawn from the glass tube head space and injected into a Perkin-Elmer SIGMA 3B gas chromatograph for ethylene analysis. Data were then recorded as nL of C_2H_4/g/h.

Table 1. Effect of soaking summer squash seeds in Co^{2+} or AOA solution on plant fresh and dry weights, leaf area of the fully expanded leaves, leaves number/plant, and plant Co^{2+} level.

Seed soaking treatment	Plant fresh* weight (g)	Plant dry* weight (g)	Leaf area* (cm^2)	Leaf† number (plant)	Plant Co^{2+}†‡ level (ppm)
H_2O	98.4^c	6.03^c	379^c	19.1^a	1.85^a
0.50 mM AOA	95.1^c	5.91^c	374^c	18.8^a	1.83^a
0.25 ppm Co^{2+}	115.0^b	6.92^b	405^b	19.3^a	1.88^a
0.50 ppm Co^{2+}	120.1^b	6.93^b	423^b	18.9^a	1.86^a
1.00 ppm Co^{2+}	132.3^a	7.66^a	479^a	19.2^a	1.99^a

Note. Means followed by the same letter in each column are not statistically different at 5% level.
* Parameters measured 1 month after seed sowing.
† Parameter measured at the experiment termination.
‡ Cobalt level on the bases of plant dry weight.

Plant Growth Measurements

Plant fresh and dry weights as well as leaf area were measured 1 month after seed sowing (before the fruiting stage) in randomly selected five plants as follow.

1. *Plant fresh weight.* Plants were harvested by digging out their intact roots. Roots were then rinsed in water and dried using paper tissues. Plants were weighed immediately, and data were recorded in g.
2. *Leaf area.* Using the third developed leaf from the base of each plant from those used previously in measuring plant fresh weight, the leaf area was measured in cm^2 using a Licor leaf area meter.
3. *Plant dry weight.* After recording the fresh weight and returning the leaves used for measuring leaf area to their samples, plants were then kept at 70°C for 3 days and reweighed (in g) for dry weight recording.

Recording Plant Leaf Number and Femaleness

During the period of plant flowering, flowers were tagged in five randomly selected plants in each replicate using red and white tags for female and male flowers, respectively. When the experiments were terminated (3 months after planting), leaf number/plant was counted, and the tags of each plant were collected, color sorted, and counted. Plant femaleness was then calculated using the following formula:

$$\text{\% Plant femaleness} = \frac{\text{no. of female flowers/plant}}{\text{no. of total flowers/plant}} \times 100.$$

Yield Recording

Because of its immature consumption, summer squash fruits were harvested upon reaching 12–15 cm length (El-Barkouki et al. 1975). At each harvest (every 2 days), harvested fruits were counted and then weighed. At the termination of the experiments, fruit number/plant was recorded, and the total fruit yield of each plot was calculated in kg. The estimated yield/feddan (4,200 m^2) was also calculated in tons (EY ton/fed) using the following formula:

$$\text{EY ton/fed} = \frac{\text{Plot yield in kg} \times \text{no. of plots/feddan}}{1{,}000}.$$

Cobalt Analysis

Cobalt was analyzed in 1-month-old summer squash plants and in immature harvested fruit as well as in samples of the experimental soil, irrigating water, and in the supplemented cattle manure. Meanwhile, samples of irrigating water were used directly in Co^{2+} measurement. Samples of plants, fruits, soil, and cattle manure were dried at 70°C for 3 days, ashed at 105°C for 24 h, and then extracted using the extracting procedures of Gericke and Kurmies (1952). A Varian AA-475 series atomic absorption spectrophotometer was used for cobalt measurements. Data were then recorded as μg of Co^{2+}/g dry weight (ppm) with the exception of Co^{2+} levels in the fruits, which were calculated on the basis of fruit fresh weight using the percentage of fruit dry weight, and recorded as μg of Co^{2+}/kg of fresh fruits (ppb).

Experimental Design and Statistical Analysis

Experiments were of a complete randomized design in three replicates. Data means were paired as combined analysis for the results of each trial (two seasons). Because the results followed a similar trend, they were analyzed for significant statistical differences using Duncan's multiple range test. The standardized least significant range (LSR) at the 5% level was used to compare the effects of the various treatments according to Little and Hills (1978).

Results and Discussion

First Trial

Soaking summer squash seeds, before sowing, in continuously aerated Co^{2+} solution of 0.25, 0.50, or 1.00 ppm strongly increased plant vegetative growth compared with those of water- (control) or 0.5 mM AOA-soaked seeds (Table 1). Plant fresh and dry weights, as well as the leaf area of the fully expanded leaves, significantly increased with Co^{2+} seed soaking. Meanwhile, there were no significant differences among all seed-soaking treatments in terms of plant leaf number. Among Co^{2+} concentrations, plants of 1.00 ppm of Co^{2+}-soaked seeds showed the highest levels of vegetative growth (Table 1). It is well known that Co^{2+} is an essential

Table 2. Effect of soaking summer squash seeds in Co^{2+} or AOA solution on ethylene production by the young leaves, plant femaleness, fruit yield, and fruit Co^{2+} level.

Seed soaking treatment	C_2H_4 nL/g.h 14-days-old plant*	30-days-old plant*	% Plant femaleness	Fruit no./plant	Fruit† yield (kg/plot)	EY‡ ton/fed.	Fruit§ Co^{2+} level (ppb)
H_2O	1.10^{d}	0.20^{c}	30.9^{c}	9.6^{c}	95.6^{c}	6.70^{c}	81^{a}
0.50 mM AOA	0.47^{e}	0.17^{c}	29.8^{c}	9.2^{c}	91.5^{c}	6.40^{c}	84^{a}
0.25 ppm Co^{2+}	1.74^{c}	0.34^{b}	34.8^{b}	12.9^{b}	121.0^{b}	8.47^{b}	83^{a}
0.50 ppm Co^{2+}	2.99^{b}	0.35^{b}	36.2^{ab}	13.9^{b}	131.4^{b}	9.41^{b}	85^{a}
1.00 ppm Co^{2+}	3.84^{a}	0.45^{a}	38.9^{a}	15.3^{a}	147.2^{a}	10.30^{a}	87^{a}

Note. Means followed by the same letter in each column are not statistically different at 5% level.
* Ethylene levels at seedling stage (14-day-old plant) and at the OFI stage (30-day-old plant).
† Plot area, 60 m^2 (mean of 80 plants).
‡ EY ton/fed is estimated fruit yield as ton/feddan (4,200 m^2).
§ Cobalt level on the basis of fruit fresh weight.

element for legumes because it is required for microorganisms fixing atmospheric nitrogen (Evans and Kliwer 1964, Young 1983). It is also required, in low levels, for plants other than legumes to promote several developmental processes. Howell and Skoog (1955) reported that low levels of Co^{2+} supply increased vegetative growth of squash (Atta-Aly 1992), cucumber (Scott and William 1976), and tomato plants (Atta-Aly et al. 1991, Kobbia and Osman 1987). Growth and yield also increased in eggplant, grapes (Nikolic 1952), and wheat (Wilson and Nicholas 1967). In contrast, high levels of Co^{2+} depressed the plant growth of several species (Atta-Aly 1992, Atta-Aly et al. 1989, 1991, Kobbia and Osman 1987, Scott and William 1976). Such growth depression was noticed in tomato and squash plants grown in water and sand cultures, respectively, with a continuous 1.00-ppm Co^{2+} supply (Atta-Aly et al. 1991, Atta-Aly 1992, respectively). Symptoms of leaf chlorosis also occurred on tomato upper-leaves with 1.00 ppm of Co^{2+} (Atta-Aly et al. 1991). Meanwhile, toxicity symptoms were noticed with higher concentrations of Co^{2+} (Kobbia and Osman 1987). The maximum Co^{2+} concentration used for seed soaking in this work, therefore, was only 1.00 ppm.

In terms of the summer squash plant or the fruit content of Co^{2+}, respective data presented in Tables 1 and 2 showed no significant differences among plants or fruits of all seed soaking treatments. Furthermore, because Co^{2+} was undetected in the soil or in the irrigating water, the presence of Co^{2+} in the resulting plants or fruits was mainly the result of the detected 1.3 ppm of Co^{2+} in the cattle manure applied as organic fertilizer before seed sowing or to chemical fertilizer impurity.

Because plants of all seed soaking treatments were comparable in terms of their Co^{2+} content, the obtained result therefore strongly indicated a significant increase in summer squash plant growth due entirely to seed soaking in Co^{2+} solutions of 1.00 ppm or less, but not due to plant Co^{2+} content. It was also suggested, therefore, that the positive impact of Co^{2+} on plant growth was indirect or through regulating substances; since such positive impacts were permanent and lasted up to plant flowering and yielding stages (Tables 2 and 3).

Because it is monoecious, summer squash plant femaleness and consequently fruit yield are ethylene dependent (Lieberman 1979, Rudich et al. 1972). It is well known that Co^{2+} inhibits ethylene biosynthesis in higher plants by blocking ACC conversion to ethylene with a concentration comparable to 18 ppm (Yu and Yang 1979). Atta-Aly et al. (1989), however, found that low levels of Co^{2+} (0.5 ppm or less) significantly induced adventitious root formation in tomato and squash cuttings by inducing ethylene production. Meanwhile, an opposite trend was obtained when Co^{2+} of higher concentrations was applied. The positive impacts of low Co^{2+} levels on adventitious root formation and ethylene production of both cuttings were blocked when such cuttings were AOA treated. Data presented in Table 2 show that plants of Co^{2+}-soaked seeds produced significantly higher ethylene levels than those of water- or AOA-soaked seeds. This increase was noticed at the seedling stage (14 days after seed sowing) up to the OFI stage (30 days after seed sowing) with more pronounced levels for plants of 1.00 ppm of Co^{2+}-soaked seeds (Table 2). In contrast, Atta-Aly et al. (1989) reported that increasing the Co^{2+} level above 0.50 ppm significantly inhibited ethylene production in tomato and squash cuttings, which may be the result of the continuous application of Co^{2+}. In this work, however, Co^{2+} was applied only for a short period of time (2 days) and as early as in the seed germination stage. It is also of interest to note that the stimulated growth of summer squash plants coincided with the plant ethylene level, since plants of 1.00 ppm of Co^{2+}-soaked seeds which produced the highest ethylene level showed the highest parameters of plant growth (Tables 1 and 2). These findings may suggest that the positive impact of Co^{2+} on summer squash plant growth

Table 3. Effect of soaking summer squash seeds in H_2O or 1.00 ppm Co^{2+} solution, followed by subsequent 0.5 mM AOA foliar application, on plant fresh and dry weights, leaf area of the fully expanded leaves, plant leaf number and femaleness.

Seed soaking treatment	Foliar* application	Plant fresh† weight (g)	Plant dry† weight (g)	Leaf area† (cm^2)	Leaves‡ number/plant	% Plant‡ femaleness
H_2O	H_2O	136^b	8.03^b	292^b	18.6^a	31.5^b
H_2O	AOA	123^c	7.38^c	282^b	18.7^a	5.0^d
Co^{2+}	H_2O	143^a	8.56^a	349^a	18.5^a	38.7^a
Co^{2+}	AOA	126^c	7.56^c	271^b	18.9^a	17.3^c

Note. Means followed by the same letter in each column are not statistically different at 5% level.
* Foliar application was carried out four times at weekly intervals starting 1 week after seed sowing.
† Parameters measured 1 month after seed sowing.
‡ Parameter measured at the experiment termination.

was by stimulating ethylene production because it was found that ethylene induced cell division (Barker 1979, Ilker et al. 1977, Metzer 1984, Riad 1996) and enlargement (Ku et al. 1970, Maxie and Crane 1968, and Riad 1996) in higher plant tissues of several plant species.

Plants of AOA-soaked seeds, however, behaved in a pattern similar to that of control, and both were significantly lower than those of Co^{2+}-soaked seeds in terms of plant growth (Table 1), ethylene production, femaleness, and fruit yield (Table 2). The only exception was that the ethylene-produced inhibition occurred only with AOA seed soaking only at the plant seedling stage (Table 2). At the OFI stage, however, plants of AOA-soaked seeds produced ethylene levels comparable to those of control, strongly suggesting that the positive impact of Co^{2+} on ethylene production and subsequently plant growth and femaleness of summer squash plants is more permanent than the negative impact of AOA, which was diminished early before the OFI stage.

To test this suggestion, plants of 1.00 ppm Co^{2+}-soaked seeds were sprayed foliarly before and at the OFI stage with 0.5 mM AOA to block the so-called ''low Co^{2+}-level-induced ethylene.'' Data presented in Table 3 show that the significant increases in summar squash plant growth and femaleness were strongly blocked with AOA foliar application.

Concerning summer squash plant femaleness, it is well known that the number of female flowers increases toward the apical nodes. The significant increase in summer squash plant femaleness obtained by Co^{2+} seed soaking (Table 2) may be caused by the Co^{2+}-increased plant node number. The summer squash plant leaf number is an accurate indicator for plant node number. Data presented in Table 1 showed no significant differences in plant leaf number among all seed soaking treatments. The insignificant differences in plant Co^{2+} content, and plant node number as well (Table 1), strongly emphasized that the increased ethylene levels of Co^{2+}-soaked seed plants, which last up to the OFI stage (Table 2), was the only explanation for the increase in plant femaleness (Table 2). Rudich et al. (1972) reported that female buds of cucurbitaceous plants produced greater amounts of ethylene than male buds, and the same was noticed in gynoecious strains compared with monoecious ones. In addition, exogenous application of ethylene or its releasing compounds, therefore, can be used for regulating sex ratio of cucurbitaceous plants. Femaleness of such plants significantly increased with the exogenous application of ethrel (Atta-Aly 1992, Augustine et al. 1973, Baha-Eldin et al. 1983) or ACC (Takahashi and Suge 1982) and even by mechanical wounding (Takahashi and Suge 1982), which greatly stimulated ethylene production (Yang 1980).

Furthermore, data presented in Table 2 showed a positive correlation between summer squash plant femaleness and produced ethylene levels with more pronounced femaleness for plants of 1.00 ppm Co^{2+}-soaked seeds, which produced the highest ethylene level (Table 2). In contrast, inhibiting ethylene biosynthesis (Atsmon and Tabbak 1979, Atta-Aly 1992) or inhibiting its action (Atsmon and Tabbak 1979) significantly reduced femaleness of such plants. Foliar AOA application before or at the OFI stage strongly blocked the increased femaleness of 1.00 ppm Co^{2+}-soaked seed plants (Table 3), whereas AOA seed soaking had no effect because of its short term of ethylene inhibition (Table 2).

It seems that inhibiting or inducing ethylene production only during the plant early stages had a slight or no impact on plant femaleness because the level of ethylene production during these early plant ages (14 days after seed sowing) was naturally higher than that produced at the OFI stage by about five- to sevenfold (Table 2). These data strongly suggest that the level of ethylene production before or at the OFI stage is more effective in modulating plant femaleness than earlier ages. It also indicates that the positive impact of Co^{2+} on summer squash plant ethylene production is more permanent than the negative impact of AOA, since Co^{2+} application as early as in the seed germination stage positively affected the subsequent plant femaleness.

Because summer squash fruits are harvested every 2 days and consumed at the immature stage of a certain

Table 4. Effect of soaking summer squash seeds in H_2O or 1.00 ppm Co^{2+} solution, followed by H_2O or 0.5 mM AOA resoaking on plant fresh and dry weights, leaf area of the fully expanded leaves, leaves number/plant, and plant Co^{2+} level.

Seed soaking treatment	Seed re-soaking treatment	Plant fresh* weight (g)	Plant dry* weight (g)	Leaf area* (cm^2)	Leaves† number (plant)	Plant Co^{2+}*‡ level (ppm)
H_2O	H_2O	106.6[b]	6.54[b]	387[b]	19.1[a]	1.96[a]
H_2O	AOA	103.5[b]	6.42[b]	381[b]	18.7[a]	1.92[a]
Co^{2+}	H_2O	144.4[a]	8.45[a]	440[a]	18.9[a]	2.04[a]
Co^{2+}	AOA	145.6[a]	8.55[a]	445[a]	19.4[a]	2.05[a]

Note. Means followed by the same letter in each column are not statistically different at 5% level.
* Parameters measured 1 month after seed sowing.
† Parameter measured at the experiment termination.
‡ Cobalt level on the bases of plant dry weight.

size (El-Barkouki et al. 1975), fruit number/plant, therefore, which rely entirely on plant femaleness, is the limiting yield factor. The noted increase in plant femaleness with increasing Co^{2+} concentration in the seed soaking solution was also noted in fruit yield measured as fruit number/plant or fruit yield either per plot or feddan (Table 2). The minimum increase in fruit yield obtained with 0.25 ppm Co^{2+} seed soaking was about 26% over that of control. This yield increase reached 40% or 54% when the Co^{2+} concentration rose up to 0.50 or 1.00 ppm, respectively, in the seed soaking solutions. The slight reduction in plant femaleness occurring with AOA seed soaking, however, resulted in only 4.5% reduction in fruit yield (Table 2).

It is of interest to note that Co^{2+} is an essential element for the synthesis of vitamin B_{12} and is required for human, animal, and plant nutrition with trace levels (Evans and Kliwer 1964, Young 1983). Furthermore, Co^{2+} is not a toxic element in the same class as arsenic, beryllium, cadmium, lead, or mercury, and it can be consumed without human health hazard up to 8 mg/day (Young 1983). The maximum Co^{2+} level found in summer squash fruits did not exceed 90 μg/kg on the basis of fruit fresh weight, regardless of Co^{2+} seed soaking treatments (Table 1), which strongly indicate the safety of such an application. On the other hand, Co^{2+} was found in trace levels in the naturally produced fruits, vegetables, and cereals as an element and in animal products in the form vitamin B_{12} (Smith 1991).

Second Trial

Because of the short term of AOA inhibitory impact on ethylene production (Table 2), other attempts were carried out to increase the AOA capability for inhibiting ethylene biosynthesis for a longer time or to block ethylene action. In such attempts, increasing the AOA concentration above 0.5 mM or using 2 mM silver thiosulfate, as an antiethylene (Atta-Aly et al. 1987) in the seed soaking solution negatively affected seed radication of summer squash and subsequently resulted plants of different physiologic ages or growth failure. A second trial, therefore, was carried out during the two seasons of 1996 and 1997 in which Co^{2+}-soaked seeds were resoaked in 0.5 mM AOA solution in an attempt to emphasize the previous findings that the positive impact of Co^{2+} was by inducing ethylene production in a permanent way. This was also carried out to test the suggestion that the positive impact of Co^{2+} on ethylene production is more permanent than the negative impact of AOA. Summer squash seeds, therefore, were first soaked in 1.00 ppm of Co^{2+} as the most effective concentration (Tables 1 and 2), for 40 h followed by an 8-h resoaking in either H_2O or 0.5 mM AOA. The obtained data showed that soaking summer squash seeds in 1.00 ppm of Co^{2+} solution had no effect on leaf number/plant, but it significantly increased plant fresh and dry weights, leaf area (Table 4), plant femaleness, fruit number/plant, and fruit yield/cultivated area (Table 5) compared with those of water only or AOA-soaked seeds. Such increases were also obtained whether Co^{2+}-soaked seeds were resoaked in either H_2O or AOA solution (Tables 4 and 5). As was found in the first trial, seed soaking in Co^{2+} solution did not affect Co^{2+} levels in the resulting plants (Table 4) or fruits (Table 5). Furthermore, the insignificant effect of AOA seed soaking on plant growth, femaleness, and fruit yield noted during the first trial (Tables 1 and 2) was also obtained (Tables 4 and 5). In terms of ethylene production, data presented in Table 5 reveal that the inhibitory impact of AOA resoaking treatment on ethylene production existed only during the plant seedling stage and diminished before the OFI stage. Such data were similar to and emphasized the first trial results (Table 2). They also indicated that an 8-h resoaking in AOA was as effective as a 48-h soaking in terms of the AOA inhibitory impact on ethylene biosynthesis in the resulting plants (61 vs 57% inhibition, respectively). The permanent and

Table 5. Effect of soaking summer squash seeds in H_2O or 1.00 ppm Co^{2+} solution, followed by H_2O or 0.5 mM AOA resoaking, on ethylene production by the young leaves, plant femaleness, fruit yield, and fruit Co^{2+} level.

Seed soaking treatment	Seed resoaking treatment	C_2H_4 nl/g.h 14-day-old plant*	30-day-old plant*	Plant femaleness (%)	Fruits number/plant	Fruit† yield (kg/plot)	EY‡ ton/fed.	Fruit§ Co^{2+} level (ppb)
H_2O	H_2O	1.34^b	0.24^b	31.8^b	10.1^b	99.4^b	7.15^b	84^a
H_2O	AOA	0.52^d	0.20^b	31.2^b	9.6^b	94.7^b	6.63^b	82^a
Co^{2+}	H_2O	3.10^a	0.56^a	44.2^a	15.6^a	149.9^a	10.48^a	96^a
Co^{2+}	AOA	1.03^c	0.55^a	43.7^a	15.0^a	143.6^a	10.12^a	95^a

Note. Means followed by the same letter in each column are not statistically different at 5% level.
* Ethylene levels at seedling and at the OFI stages as described in Table (2).
† Plot area, 60 m^2 (mean of 80 plants).
‡ EY ton/fed. is estimated fruit yield as ton/feddan (4,200 m^2).
§ Cobalt level on the bases of fruit fresh weight.

positive impact of Co^{2+} seed soaking on ethylene production by the resulting plants was also evident from the second trial, regardless of whether the seeds were soaked in AOA or water (Table 5). The significant inhibition of ethylene production by the plants of AOA-resoaked seeds also occurred during the plant seedling stage and did not affect the significant increase in plant growth, femaleness, and fruit yield caused by previous seed soaking in Co^{2+} solution. This increase in plant femaleness and fruit yield (Table 5) was mainly the result of the termination of the AOA inhibitory impact on ethylene production before the OFI stage (Table 5), which allowed the positive impact of Co^{2+} to be predominant on ethylene production and subsequently on plant femaleness and fruit yield.

These data strongly support the previous suggestion that the positive impact of Co^{2+} on ethylene production is more permanent than the negative impact of AOA. It was also indicated that the positive impact of Co^{2+} on summer squash growth, femaleness and subsequently fruit yield were mainly caused by the so-called low Co^{2+} level-induced ethylene.

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