Effect of Galia Melon Seed Soaking in Cobalt Solution on Plant Growth, Fruit Yield, Quality and Sudden Wilt Disease Infection

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Summary

Soaking Galia melon (*Cucumis melo* var. reticulatus, c.v. Royal) seeds in continuously aerated solution of 1.0 ppm Co^{2+} for 48 h before sowing significantly increased plant ethylene (C_2H_4) level, plant growth and fruit yield compared with those of water-soaked seeds. The increase in C_2H_4, started as early as the seedling stage and continued through the onset of fruit harvesting. It was associated and coincided with the marked increases in root fresh weight and length as well as lateral branches number. The resultant increase in root volume was associated with the marked reduction in plant sudden wilt disease infection. This may suggest that increasing plant endogenous C_2H_4 level improved its tolerance to sudden wilt disease. In addition, Co^{2+} seed soaking and the subsequent increase in plant C_2H_4 level markedly increased Galia melon leaves, stem branches and fruit number/plant and consequently fruit yield with higher fruit TSS content. Although fruit diameter was reduced with Co^{2+} seed soaking, fruit flesh thickness was markedly increased resulting in fruits of comparable fresh weight to those from plants of water soaked seeds. The marked increase in Galia melon fruit yield may be due to the significant increase in fruit number/plant and to the pronounced limitation of sudden wilt disease. The increased fruit number/plant may due, in the first place, to the increased branches number/plant as a result of the so-called low Co^{2+} level-induced C_2H_4 which may alter plant apical dominance. It was also found that 1.0 ppm Co^{2+} seed soaking leaving no Co^{2+} residue in the obtained fruits meaning the absolute safety of such treatment on human health.

Keywords: Melon; Cobalt; Ethylene; Yield.

Introsuction

It is well known that cucurbits are mostly monoecious plants bearing both male and female flowers separately on the same plant. Exogenous application of C_2H_4 releasing compounds, such as ethrel (Atta-Aly, 1992 and Baha Eldine *et al.*, 1983) or stimulating plant endogenous C_2H_4 via certain treatments such as wounding (Takahashi and Suge, 1982) greatly increased plant femaleness. An opposite trend, however, was obtained when C_2H_4, biosynthesis was blocked with AOA (aminooxyacetic acid) application (Atta-Aly, 1992 and Atsmom and Tabbak, 1979). Recently, Atta-Aly (1992 and 1999) found that soaking summer squash seeds 48 h before sowing in Co^{2+} solution of low concentrations (1.0 ppm or less) increased plant femaleness and consequently fruit yield via increasing plant C_2H_4 level. Unlike other cucurbits, Galia melon are gynomonoecious plants bearing female and perfict followers (Rudich *et al.*, 1972). The impact of increasing endogenous C_2H_4, level on such gynomonoecious plants hasn't been reported.

The biosynthetic pathway of C_2H_4, in higher plants progresses from the amino acid methionine through SAM (S-adenosylmethionine) to ACC (I-aminocyclopropane-l-carboxylic acid) and finally to C_2H_4 (Adams and Yang 1979 and Yang, 1980). The application of high Co^{2+} concentration (comparable to about 18 ppm) inhibited C_2H_4, biosynthesis by blocking ACC conversion to C_2H_4 (Yu and Yang, 1979). Atta-Aly *et al.*, (1989), however, found that supplementing the nutrient solution with a concentration of 0.5 ppm Co^{2+} or less significantly induced C_2H_4 production and adventitious root formation of tomato and squash cuttings. Such positive impact of low Co^{2+} level on C_2H_4 biosynthesis was also found to be prolonged since soaking summer squash seeds in 1.0 ppm Co^{2+} for only 48 h resulted in plants of significantly higher C_2H_4 level up to 30 days after seed sowing (Atta-Aly, 1998). This positive impact of Co^{2+} on C_2H_4 biosynthesis was suppressed and even eliminated when cuttings or plants were foliarly sprayed with AOA, as anti C_2H_4 biosynthesis (Yang, 1980), or when Co^{2+} concentrations exceeded the above mentioned levels (Atta-Aly, 1998).

Cobalt is an essential element for the synthesis of vitamin Biz, which is required for human and animal nutrition (Smith, 1991 and 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). Soaking summer squash seeds in 1.0 ppm Co^{2+} solution resulted in no residue levels either in plant leaves or in harvested fruits, meaning the absolute safety of Co^{2+} seed soaking treatment (Atta-Aly *et al.*, 1998). It is also required, in low levels, for maintaining high yields of cucumber (Scott and William, 1976).

It is well known that limited root volume and heavy vegetative growth characterize Galia melon plants, particularly at fruiting stage. At this stage, plants became clearly sensitive to sudden wilt disease, which strongly threatens the production of the crop. Also, limited stem branching is another character that might arrest fruit growth due to increased fruit competition on each single branch.

Based on the previous findings that low Co^{2+} levels induced C_2H_4, production (Atta-Aly, 1998 and 1999 and Atta-Aly *et al.*, 1989) this work, was designed and carried out to study the impact of Galia melon seed soaking in 1.0 ppm Co^{2+} as the most recommended level on C_2H_4, pro-

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Abbreviation: ACC, 1-aminocyclopropane-1-carboxylic acid; CA; controlled atmosphere; CI, chilling injury; CS; chilling sensitivity; LPS, Low pressure storage; MA, modified atmosphere; RH, relative humidity.

duction, root growth, sudden wilt disease, stem branching, fruit yield and fruit quality of the resulting plants.

Materials and Methods

Plant Materials

After soaking, radicated Galia melon (*Cucumis melo* var. reticulatus, c.v. Royal) seeds of the first trial were sown in the sandy soil of a drip-irrigated farm in South Tahrir, Cairo-Alexandria Desert Road, Egypt on Nov. 15, 1998 and 1999. At sowing, seeds were placed in hills of 3 cm depth and 50 cm apart (distances between drippers) at the seed rate (density) of two seeds/hill. Plants of seed soaking treatments were grown under transparent polyethylene (80µ thickness) tunnels in four replicates, each was 80 m2 in area and comprised of two rows 20 m long and 2 m wide (drip-lateral spacing). One week before sowing, rates of 25 m3 of 5-month-old cattle manure, 200 kg of calcium superphosphate and 100 kg of potassium sulfate/ feddan (4200 m^2) were applied to the soil to a depth of 25 cm under the dripping lateral (7-10 cm below the soil surface). The experimental farm was drip-irrigated for 6 h one day before sowing and re-watered again for 30 minutes after seedlings emergence. Polyethylene tunnels were removed by mid-March in each season.

In the second trial, the radicated Galia melon seeds of the same cultivar were sown, directly after being soaked, in the clay soil of the furrow-irrigated farm in Urn-dom, Khartourn, Sudan on Nov. 5, 2000. Three weeks before sowing, amounts of 25,3 old cattle manure and 250 kg calcium superphosphate/feddan were applied during soil preparation followed by flood irrigation. Directly before sowing, the experimental farm was divided into four replicates, each 1000 m^2 in area and comprised 2 rows 200 m long and 2.5 m wide. In both sides of each row, seeds were placed in hills of 2 cm in depth 40 cm apart and with 2 seeds/hill. The field was gently furrow irrigated.

All required agricultural practieces were carried out as recommended for Galia melon production either in drip-irrigated sandy soil farm under the protection of polyethylene tunnels (first trial, Egypt) or in the open field of the clay soil farm (second trial, Sudan).

Seed Soaking Treatment

In both trials, Galia melon seeds were soaked for 48 h at room temperature in continuously aerated solutions of 0.0 and 1.0 ppm Co^{2+} using cobalt sulfate salt as previously described by Atta-Aly (1998). By the end of the soaking treatment, seeds were radicated with a radical length of 1-1.5 mm and were directly sown.

Ethylene Analysis

Only in the first trial, ethylene was analyzed two weeks after seed sowing and every other week until plants were 75 days old (harvesting stage) using the second developing leaf from the top of the main stem. Leaves were excised 5 mm above the stem surface from five randomly selected plants using a stainless steel blade. Following the procedures described by Atta-Aly (1998), leaves were immediately placed inside 150 ml glass tubes (one leaf/tube), each containing 5 ml of water for immersing the leaf base to prevent drought stress. Glass tubes were then sealed using rubber supa-seal and kept under a 1000-lux fluorescent light for 8 h. One ml gas samples were then withdrawn from the glass tube head space and injected into a Parkin-Elmer SIGMA 3B gas chromatograph for ethylene analysis. Data were then recorded as nl C_2H_4/g. h.

Plant Growth Measurements

At fruiting stage 10 (two-month-old plants), were randomly selected and used for counting and recording number of leaves and branches and for measuring main branch length. Directly before experiment termination, roots of the same plants were carefully dug-out, dry-cleaned and weighed and maximum length recordings. Days from seed sowing to th onset of fruit harvesting were also recorded.

Sudden Wilt Recording

When plants reached the stage of harvesting, those showing clear visual symptoms of sudden wilt disease were counted in each row for each replicate in each treatment. Sudden wilt infected plants were then recorded as a percentage of the total plants number.

Yield Recording and Fruit Quality Analysis

At half-slip stage, fruits were directly harvested, counted and weighed on daily bases until harvesting was terminated recording as kg/plot. Fruit total yield (ton/feddan) was then calculated. In the middle of the harvesting season, fruit diameter was measured in 10 randomly picked fruits. These fruits were halved at the middle of the equatorial surface for recording fruit flesh (pericarp) thickness. Drops of fruit flesh juice were hand extracted by flesh squeezing and received by a hand refractometer for total soluble solids (TSS) analysis.

Cobalt Analysis

In the first trial, Co^{2+} content in the harvested fruit was analyzed. Fruit tissues were dried at 70°C for three days, ashed at 105°C for 24 h, and then extracted following the procedures of Gericke and Kurmies (1952). A Varian AA-475 series atomic absorption spectrophotometer was used for cobalt measurements. Data were then recorded as ug Co^{2+}/kg fresh fruits (ppb).

Experimental Design and Statistical Analysis

Experiments were of a randomized complete block design with four replicates. In both trials, data were statistically analyzed for significant statistical differences using Duncan's multiple range test at the 5% level to compare the effects of the various treatments according to Little and Hills (1978).

Table 1. Effect of Galia melon seed soaking in 1.0 ppm Co^{2+} 48 h before sowing on plant vegetative growth, fruit yield and fruit qulity.

Measured parameter	First trail				Second trail	
	First season		Second season			
	Control	Co^{2+}	Control	Co^{2+}	Control	Co^{2+}
Leaves number/plant ^{X}	46.7 b	54.1 a	47.3 b	55.2 a	43.0 b	50.6 a
Main branch length (cm) ^{X}	114 a	115 a	115. a	116 a	112 a	113 a
Root length (cm) ^{Z}	25.1 b	53.1 a	24.8 b	52.2 a	12.7 b	18.5 a
Days from sowing to harvest ^{Y}	76.7 b	83.9 a	77.0 b	84.0 a	74.0 b	80.0 a
Fruit number/plant ^{Y}	2.4 b	3.0 a	2.5 b	3.1 a	2.0 b	2.9 a
Fruit yield (tons/fed) ^{Z*}	11.0 b	12.5 a	11.2 b	11.8 a	8.2 b	8.9 a
Fruit fresh wieght (g) ^{Y}	648 a	632 a	650 b	627 a	632 a	597 a
Fruit diameter (cm) ^{Y}	10.5 a	10.0 b	10.7 a	10.3 b	10.4 a	10.0 b
Fruit flesh thickness (cm) ^{Y}	2.4 b	2.9 a	2.5 b	3.2 a	2.8 b	3.1 a
TSS (%) ^{Y}	10.6 b	11.7 a	11.0 b	12.0 a	7.8 b	9.2 a

Means followed by the same letter ineach row for each season and trail are not statisically different at the 5% level.
X Parameters measoured at fruiting stage.
Y Parameters measoured at harvesting stage.
Z Parameters measoured directly before experiment termination.
* Fed=4200 m^{2}.

Results and Discussion

Soaking Galia melon seeds for 48 h, before sowing, in continuously aerated Co^{2+} solution of 1.0 ppm strongly increased plant vegetative growth compared with those of water-soaked seeds (control) in both trials (Table I and Figs. 1 and 2). Plant leaves (Table 1) and number of branches (Fig. 2) as well as root fresh weight (Fig. 1) and length (Table 1) significantly increased with Co^{2+} seed soaking. There was no impact of Co^{2+} seed soaking on the resulting plants in terms of main branch length (Table 1). This was evident in both trials. It is well known that Co^{2+} is an essential element for legumes because it is required for the bacteria fixing atmospheric nitrogen (Evans and Kliwer, 1964 and Young, 1983). It is also required, in low levels, for plants other than legumes to promote several developmental processes. Low level of Co^{2+} supply increased vegetative growth of squash (Atta-Aly, 1992, 1998 and 1999), cucumber (Scott and William, 1976) and tomato plants (Atta-Aly *et al.*, 1991). This was also found in the growth and yield of squash (Atta-Aly, 1998 and 1999), eggplant and grapes (Nikolic, 1952) and wheat plants (Wilson and Nicholas, 1967).

In Contrast, high C02+ levels depressed plant growth of several species (Atta-Aly, 1992 and Atta-Aly *et al.*, 1989 and 1991; Scott and William,1996). Such growth depression was noticed in tomato and squash plants grown in water and sand cultures, respectively, with a continuous 1.0 ppm Co^{2+} supply (Atta-Aly *et al.*,1991). Toxicity symptoms were noticed with higher Co^{2+} concentrations (Kobbia and Osman, 1987). For seed soaking treatment, however, Co^{2+} concentration of 1.0 ppm proved to be the most effective concentration having the maximum positive impact on summer squash plant growth and yield, leaving no residue levels in the resulting plants or fruits (Atta-Aly, 1998 and 1999). Based on these findings and on preliminary studies carried out on Galia melon one year a head, Co^{2+} concentration of 1.0 ppm was used in this work.

It is well known that Co^{2+} inhibits C_2H_4 biosynthesis in higher plants by blocking ACC conversion to C_2H_4 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 C_2H_4 production. The positive impact of low Co^{2+} levels on C_2H_4 production was also demonstrated when summer squash plants resulting from 1.0 ppm Co^{2+}-soaked seeds produced persistantly higher C_2H_4, levels (up to 30 days after seed sowing), which increased plant femaleness as compared to those resulting from water-soaked seeds (Atta-Aly, 1998 and 1999). On the other hand, an opposite trend was obtained when Co^{2+} of higher concentrations was applied (Atta-Aly, 1998 and 1999 and

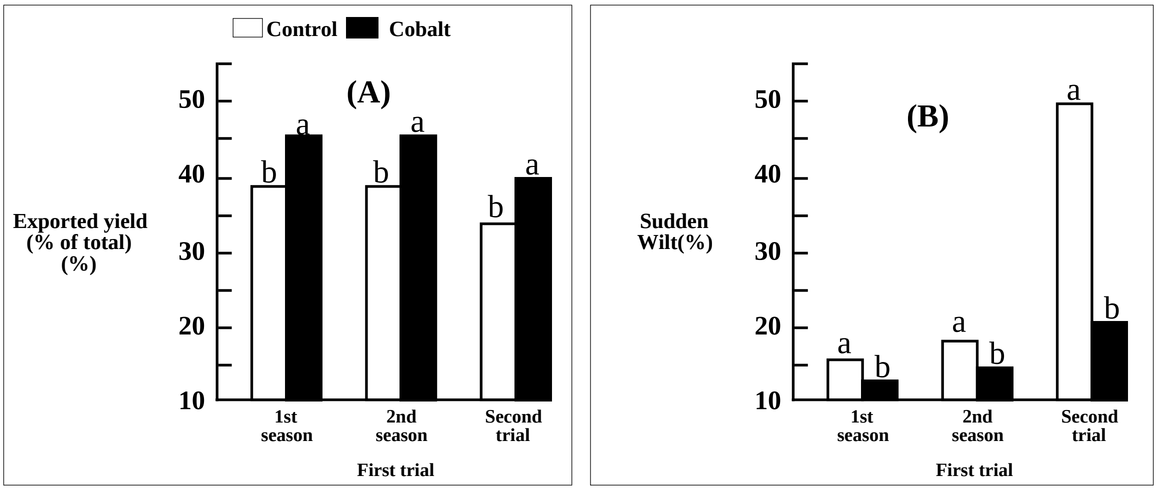

Fig. 1. Effect of soaking Galia melon seeds in 1.0 ppm Co^{2+} 48 h before sowing on the resulting plant root fresh weight (A) and sudden wilt disease (B) at fruiting stage. Different letters on histograms indicate significant differences at the 5% level.

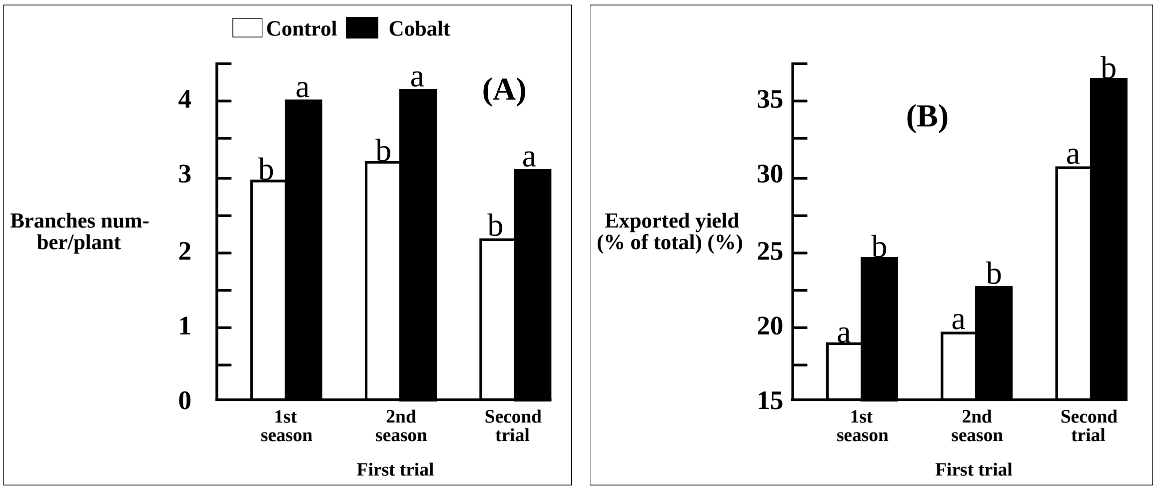

Fig. 2. Effect of soaking Galia melon seeds in 1.0 ppm Co^{2+} 48 h before sowing on plant branches number (A) and exported percentage (B). Different letters on histograms indicate the level of significant differences at the 5% level.

Atta-Aly *et al.,* 1989). The positive impact of low Co^{2+} levels on adventitious root formation and summer squash plant femaleness were the resullt of the stimulative impact of low Co^{2+} level on C_2H_4 biosynthesis. Such positive impact was eliminated when C_2H_4 biosynthesis was blocked by AOA application (Atta-Aly *et al.*, 1989).

Data presented in Fig (3) show that Galia melon plants of 1.0 ppm Co^{2+}-soaked seeds produced significantly higher C_2H_4 levels than those of water-soaked ones and this increase was persistant up to the plant tissues of several plant species.

It is well known that Galia melon plants are very susceptible to sudden wilt disease, which attacks the plants at fruiting stage when plants reached their maximum vegetative growth. Galia melon root system at this stage is limited and this may increase plant susceptibility to such devastating disease. Increasing Galia melon root biomass may enable the plant to withstand sudden wilt disease. Increasing plant endogenous C_2H_4, level increased roots initiation, growth and development and subsequently roots volume (Atta-Aly *et al.,* 1989 and Yang and Hoffman, 1984). Data presented in Table (1) and Fig (1A) show that Galia root fresh weight and length significantly increased with Co^{2+} seed soaking than those of water-soaked ones. This was reflected in the significant reduction in the percentage of sudden wilt infected plants (Fig. 2B). The significant increase in C_2H_4 production in Galia melon plants of Co^{2+}-soaked seeds greatly induced plant root growth and subsequently volume, which may be the reason behind increasing plant ability to withstand sudden wilt disease. Ethylene induced plant resistance to pathogen attack and invasion has been reparted (Yang and Hoffman, 1984).

One of the known C_2H_4, actions is to arrest plant apical dominance (Yang and Hoffman, 1984). Data presented in Fig (2) show that Galia melon seed soaking in 1.0 ppm Co^{2+} solution markedly increased plant branches (Fig. 2A) and leaves number (Table 1) than those of water-soaked seeds. This increase in branches may have insured good distribution of the fruits on the branches, thereby reducing competition among fruits growing on a single branch. The greater leaves number per plant might have reduced direct exposure of fruit to the sun resulting in fruits of higher internal and external quality and consequently increasing the percentage of exportable fruits. Cobalt seed soaking also resulted in fruits of significantly thicker flesh and higher TSS content (Table 1). This accounted for the marked increase in fruit suitability for exportation (Fig. 2B). Although Co^{2+} seed soaking reduced fruit diameter, it did not affect fruit fresh weight (Table 1) which might be due to the significant increase in fruit flesh (pericarp) thickness (Table 1).

Concerning Co^{2+} residue levels in the harvested fruits, no significant differences were found between

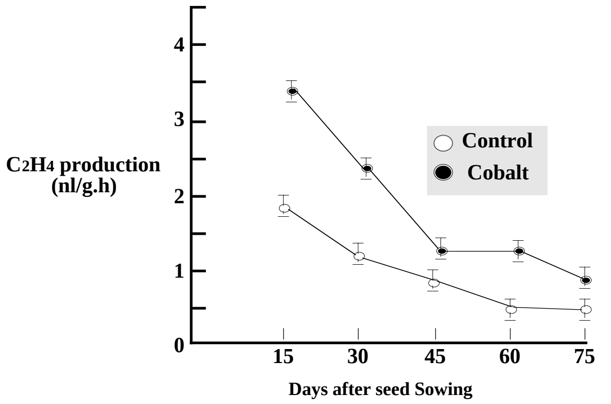

Fig. 3. Effect of soaking Galia melon seeds in 1.0 ppm cobalt solution 48 h before sowing on ethylene production by the resulting plants. Vertical bars superimposed on data points at each sampling date represent L.S.D values at the 5% level.

fruits of Co^{2+}-soaked seeds and those of water-soaked ones, 57 and 56 μg Co^{2+}/kg fruit fresh weight, respectively. It is of interest to note that Co^{2+} is an essential trace element for the synthesis of vitamin BII and is required for human, animal and plant nutrition (Evans and Kliwer, 1964 and 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 Galia melon fruits did not exceed 60 μg/kg on fresh weight bases, regardless of Co^{2+} seed soaking treatment, which strongly indicates 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 from vitamin B_{12} (Smith,1991).

Regardless of the great differences in the environmental factors between the first and second trials, low Co^{2+} level positive impacts on Galia melon plant growth and fruit yield remain constant, stable and effective. In addition, no differences were found in Coze content between fruits of Co^{2+} and water-soaked seed plants. This may strongly suggest that the positive impact of low Co^{2+} level was due mainly to the so-called low Co^{2+} level-induced C_2H_4 as a plant internal stimulatory factor. This stimulated C_2H_4 obtained with low Co^{2+} level (1.0 ppm) seed soaking increased plant root growth and reduced plant sensitivity to sudden wilt disease. In addition, plant branching is markedly increased resulting in plants of vigorous growth, higher yield and better fruit quality.

Practical Implementation

Soaking Galia melon seeds in a solution of 1.0 ppm Co^{2+} directly before sowing is a simple, fast and cheap treatment resulting in several positive impacts on plant growth, yield and quality regardless of soil type and growing location. Such treatment can be easily done in a very limited space only on the seeds and away from the field meaning easy application and clean environment, leaving no Co^{2+} residue in the obtained fruits. Galia melon seed soaking in 1.0 ppm Co^{2+} also reduced plant sensitivity to sudden wilt disease, which greatly helps in overcoming one of the major problems facing Galia melon commercial production. This application, therefore, can easily be carried out on a commercial scale as a safe treatment for increasing Galia melon yield and quality.

Acknowledgement

This work is a part of the research program carried out by the Applied Agricultural Research Department, Arab Authority for Agricultural Investment and Development. The author wishes also to thank Mr. Salama A. Abd EI-Hady, Department of Horticulture, Faculty of Agriculture, Ain-Shams University, Eygpt and Mr. Emad H. Abd EI-Samad, Arab Authority for Agricultural Investment and Development for their help.

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تأثير نقع بذور شمام الجاليا في محلول الكوبالت على النمو الخضري وإنتاج وجودة الثمار وعلى نسبة الإصابة بمرض الذبول المفاجئ

مُرضي عبدالعظيم عطا علي *

الخلاصة

أدى نقع بذور شمام الجاليا صنف رويال لمدة 48 ساعة قبل زراعتها، في محلول دائم التهوية من الكوبالت بتركيز واحد جزء بالمليون إلى زيادة معنوية في النمو الخضري للنباتات الناتجة كذلك في محصولها الثمري عند مُقارنتها بالنباتات المقارنة والسابق نقع بذورها في الماء . صاحب زيادة النمو الخضري والمحصول الثمري لهذه النباتات ارتفاع معنوي طويل المدى في معدل إنتاجها للايثيلين منذ طور البادرة وحتى وصول النباتات إلى مرحلة الإثمار. ايضاً كانت هناك زيادة معنوية في وزن وطول المجموع الجذري لهذه النباتات والذي رافقه انخفاض معنوي في نسبة الإصابة بمرض الذبول المفاجئ عن النباتات المقارنة والذي يدل على أنً زيادة معدل إنتاج نباتات شمام الجاليا للايثيلين قد يزيد من قدرتها على تحمُّل الإصابة بمرض الذبول المفاجئ . وقد كان، زيادة معدل إنتاج النباتات، السابق نقع بذورها في محلول الكوبالت، للايثيلين مصحوباً بزيادة معنوية في عدد الفروع الجانبية والأوراق للنبات ، وكذلك زيادة المحصول الثمري وارتفاع محتوى الثمار من المواد الصلبة الذائبة الكلية. وبالرغم من انخفاض قطر الثمرة في النباتات السابق نقع بذورها في محلول الكوبالت إلا أنً سمك لحم هذه الثمار كان يفوق ثمار النباتات المقارنة والذي نتج عنه عدم وجود فرق معنوي بين المعاملتين في متوسط الوزن الطازج للثمار الناتجة . وتعزى الزيادة في المحصول الثمري لنباتات شمام الجاليا السابق نقع بذورها في محلول الكوبالت بدرجة أساسية إلى الزيادة المعنوية في عدد الثمار للنبات الواحد مع الانخفاض المعنوي الملحوظ في نسبة الاصابة بمرض الذبول المفاجئ. وترجع الزيادة في عدد الثمار للنبات الواحد الي زيادة عدد الفروع الجانبية للنباتات التي عُوملت بذورها بمحلول الكوبالت كنتيجة لما يسمى بحث التركيز المنخفض في الكوبالت لإنتاج النباتات للايثيلين والذي بدوره أثر سلباً على السيادة القمية لهذه النباتات وبالتالي حثها على التفريع الجانبي. دلًت النتائج أيضاً على أنً نقع بذور شمام الجاليا في محلول الكوبالت بتركيز واحد جزء بالمليون لم يترك أثراً مُتبقّياً من الكوبالت في الثمار الناتجة بما يؤكد الأمان الكامل لهذه المعاملة على صحة الإنسان.

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