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Electrodegradation of Root Exudates to Mitigate Autotoxicity in Hydroponically Grown Strawberry (*Fragaria × ananassa* Duch.) Plants

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Abstract. Root exudates from strawberry plants are known to cause autotoxicity and benzoic acid (BA), a potent growth inhibitor, was found in the exudate. BA (400 µM) was easily decomposed by electrodegradation (ED) treatment at 10.0 V and 2.0 A after the addition of BA into nutrient solution. The growth of strawberry plantlets in nutrient solution containing BA (400 µM) or in control nutrient solution varied significantly depending on the use of ED treatment. The growth inhibition of plantlets in the BA-containing nutrient solution or in nutrient solution used for strawberry culture was significantly ameliorated by ED treatment. This growth recovery resulting from ED treatment was most pronounced in terms of the yield of strawberry fruit. These results suggest that ED treatment of the culture nutrient solution could result in the decomposition of toxic root exudates, including BA from strawberry plants, and mitigates the autotoxicity observed in closed hydroponic culture. In addition, the supplementation of Fe-EDTA is recommended after ED treatment as a result of the rapid decomposition of Fe-EDTA by ED.

The soilless culture of a wide variety of crops has been practiced in many countries since the 1950s, and the use of closed hydroponic systems has been encouraged recently (Ruijs, 1994; Van Os, 1995) to reduce environmental pollution and the cost of supplementary nutrients. A closed hydroponic system has been considered for strawberry cultivation (Takeuchi, 2000). However, the yield of strawberry fruit under continuous cropping conditions on the same substrate declined for unknown reasons (Oka, 2002). A lack of renewal of the nutrient solution in hydroponic culture significantly inhibited the growth of strawberry plants as a result of the accumulation of toxic root exudates in the nutrient solution, and benzoic acid was found to be the most potent inhibitor in the root exudates (Kitazawa et al., 2005). The removal/degradation of phytotoxic substances that have accumulated in the nutrient solution might reduce the growth inhibition

of strawberry plants. Adsorption by activated charcoal (Asao et al., 1998; Kitazawa et al., 2005), degradation by microbial strains (Asao et al., 2004a), and auxin treatment (Kitazawa et al., 2007) have been tested for the detoxification of exudates. Degradation of toxic compounds by electronic means is another way to detoxify allelochemicals. Several phenolic compounds, including phenol (Comminellis and Pulgarin, 1991; Feng and Li, 2003; Fleszar and Ploszynka, 1985), catechol (Comminellis and Pulgarin, 1991), and hydroquinone (Comminellis and Pulgarin, 1991; Fleszar and Ploszynka, 1985), in aqueous solutions and even benzene were found to decompose when treated by electrodegradation (ED) (Fleszar and Ploszynka, 1985). These compounds are oxidized rapidly at the anode and decompose to CO₂ (Comminellis and Pulgarin, 1991; Feng and Li, 2003; Fleszar and Ploszynka, 1985). Thus, ED might cause the decomposition of allelochemicals, including benzoic acid exuded into the nutrient solution from plants, and could be useful to mitigate autotoxicity in the hydroponic cultivation of strawberry. In this study, the effects of ED treatment of used nutrient solution or benzoic acid solution on the growth and yield of strawberry plants were investigated.

Materials and Methods

Decomposition of benzoic acid in the nutrient solution with or without electrodegradation treatment. Benzoic acid is a potent growth inhibitor in the root exudates of strawberry plants (Kitazawa et al., 2005). Four hundred micromolar benzoic acid solution was prepared using 30% 'Enshi' nutrient solution (Table 1; Hori, 1966) with 0.8 dS·m⁻¹ electrical conductivity (EC). Fe-EDTA (25 mg·L⁻¹) was added to the nutrient solution. Tap water was used for the test solution preparation. One liter of this test solution was placed in a beaker and ED treatment was applied for 24 h with an electrode (cathode) consisting of a cylindrical titanium plate with a 180-cm² surface area and ferrite stick of 42 cm² surface area (anode) inserted into the beaker (Fig. 1). The electric voltage and current in the ED process were 10.0 V and 2.0 A, respectively. During the treatment, the test solution was stirred frequently and its temperature was maintained at 20 °C using a water bath in an air-conditioned room at 18 °C. Samples (25 mL) of the treated solution were collected at 0 (control), 2, 6, 12, and 24 h during the ED treatment and filtered through a cellulose acetate membrane filter (pore size 0.45 µm). Each filtrate (25 µL) was injected into a high-performance liquid chromatography (HPLC) system (column oven, detector, and pump: L-2350, L-2400, and L-2130, respectively; Hitachi, Tokyo, Japan) to measure the concentration of benzoic acid in the nutrient solution. The analytical conditions were as follows: column: ODS 4.0 × 200 mm (Wako-sil 10C18; Wako Pure Chemical Industries, Ltd., Osaka, Japan); eluent: CH₃CN/10 mM H₃PO₄ = 30/70 (v/v); flow rate: 1.0 mL·min⁻¹ at 30 °C; and detection: ultraviolet 254 nm. EDTA was found to decompose by oxidation in the aqueous solution during ED treatment (Kusakabe et al., 1986). Thus, the concentration of Fe-EDTA (initial: 25 mg·L⁻¹) in the treated 'Enshi' nutrient solution was also measured by HPLC.

Effect of electrodegradation treated benzoic acid or used nutrient solution on the growth of strawberry plantlets (bioassay). Thirty percent 'Enshi' nutrient solution was prepared with tap water and the required amount of benzoic acid was added to reach 400 µM. Three liters of the test solution was poured into each plastic container (17 cm

Table 1. 'Enshi' nutrient solution².

Chemicals	Amounts ³ (g/1000 L)
Ca(NO ₃) ₂ ·4H ₂ O	950
KNO ₃	810
MgSO ₄ ·7H ₂ O	500
NH ₄ H ₂ PO ₄	155
H ₃ BO ₃	3
ZnSO ₄ ·7H ₂ O	0.22
MnSO ₄ ·4H ₂ O	2
CuSO ₄ ·5H ₂ O	0.05
Na ₂ MoO ₄ ·2H ₂ O	0.02

²Full strength.

³Amounts of salts per 1000 L of tap water (Hori, 1966).

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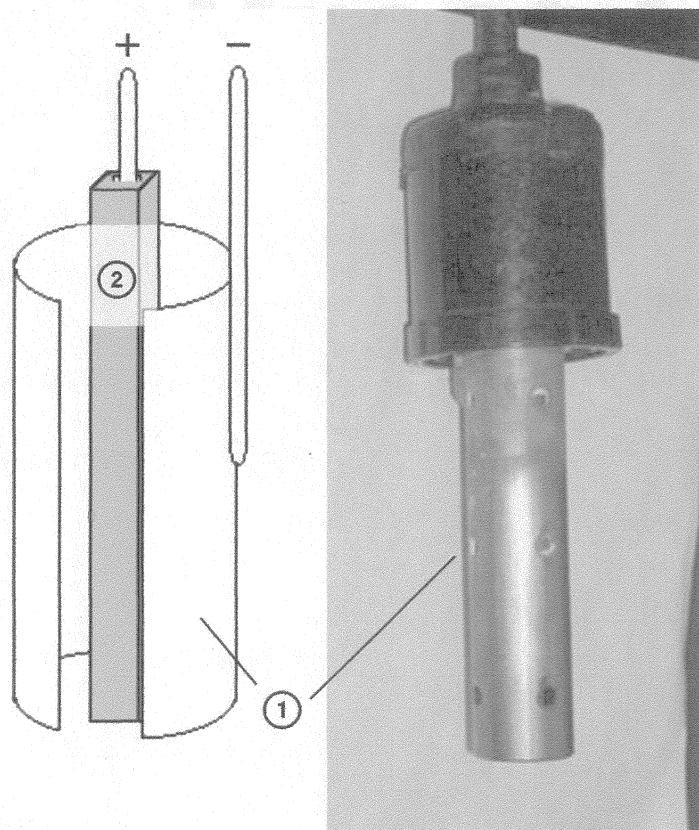


Fig. 1. Electrode used for the electrodegradation process to decompose benzoic acid or autotoxic chemicals in used/test nutrient solutions. ① indicates the cathode made of titanium with a surface area of 180 cm² and ② indicates the anode (inside of cathode) made of ferrite with a surface area of 42 cm².

×29 cm × 9.5 cm) and then ED treatment was applied as described previously. Strawberry (*Fragaria ×ananassa* Duch. var. 'Toyonoka') plantlets at the four-leaf stage were transplanted into the containers using urethane blocks (foam) as support.

The same bioassay was carried out with the nutrient solution used for strawberry culture as described previously (Kitazawa et al., 2005). The strawberry plants were grown using closed hydroponic culture without replacement of the nutrient solution. During the culture (Kitazawa et al., 2005), the nutrient contents (NO₃⁻, PO₄³⁻, K⁺, Ca²⁺, Mg²⁺, and Fe³⁺) in the nutrient solution were adjusted at 2-week intervals to as close as possible to the initial concentrations on the basis of chemical analysis with a reflection meter (RQflex2; Merck, Tokyo, Japan) for NO₃⁻, the molybdenum blue molecular absorption spectrometric method (Murphy and Riley, 1962) for PO₄³⁻, and atomic absorption spectrometer (Z-5010; Hitachi, Tokyo, Japan) measurements for K⁺, Ca²⁺, Mg²⁺, and Fe³⁺ analysis. After 8 months of strawberry culture, the used solution was adjusted again to match the initial nutrient concentrations and was frozen at -30 °C to avoid microbial decomposition. This nutrient solution was used as the test solution for the bioassay and thawed out immediately before

ED treatment. The test solution was poured into a container and 10 plantlets were planted into each container with or without ED treatment. The solution in each container was replaced with additional solution from the same treatment at 4- or 5-d intervals. ED treatment was applied to the replacement solutions for 24 h as required. Fe-EDTA was added to the solution after ED treatment in each case. The pH and EC of each solution were measured using a pH meter (F-52; Horiba, Kyoto, Japan) and an EC meter (ES-12; Horiba), respectively. The pH and EC values were adjusted to be as close as possible to 30% 'Enshi' nutrient solution (pH 7.0 and EC 0.8 dS·m⁻¹) by adding 0.4 M NaOH and/or tap water after the addition of sodium thiosulfate (Na₂S₂O₃·5H₂O). The pH of the solutions was unaffected by ED. The containers were placed in a growth chamber at 25 °C with a light intensity of 74 to 81 μmol·m⁻²·s⁻¹ and a 16-h photoperiod under fluorescent lights. The plantlets were grown for 2 weeks and then the number of leaves, leaf length and width, leaf area, fresh weight of shoots, and the maximum root length were recorded. Leaf area (LA) was calculated by a nondestructive method (linear method) using the equation LA = L × B × K, where L is leaf length, B is leaf breadth, and K is a correction factor. K (0.53) was estimated by comparing

the actual leaf area of 40 strawberry leaves measured in an electronic area meter to that measured by multiplying the length and breadth of the same leaves.

Effects of nonrenewal of the nutrient solution with or without electrodegradation treatment on the growth and yield of strawberry plants. A deep flow technique was used for the hydroponic cultures (Fig. 2). The hydroponic system was comprised of main pipes (15-mm diameter) for the supply and drainage of nutrient solution, Wagner pots with 3-L capacity for planting, tubes (4-mm diameter) to supply solution to the pots, and a 50-L capacity nutrient solution tank with a pump (KP-101; Koshin, Kyoto, Japan) inside the nutrient solution. Strawberry var. 'Toyonoka' plantlets of uniform size (three- to four-leaf stage with ≈10 mm crown diameter) were transplanted into the pots on 16 Nov. 2005. The nutrient solution was circulated through the pipes for 5 min at 30-min intervals using an automatic pump timer (KS-1500; Iuchi, Osaka, Japan). The flow rate of the nutrient solution was 1 L·min⁻¹. The nutrient solution was not renewed during the experiment. The nutrient (NO₃⁻, PO₄³⁻, K⁺, Ca²⁺, Mg²⁺, and Fe³⁺) contents of the culture solution were adjusted at 2-week intervals to be as close as possible to the initial concentration of the 'Enshi' solution (30%) based on the chemical analyses described previously. The same hydroponic system was followed by renewing the nutrient solution at 2-week intervals (control) or by furnishing ED treatment of the stock solution in the tank for 24 h at 2-week intervals before the adjustment of the nutrient contents. The electric voltage and current for the ED were 18.0 V and 0.2 A, respectively. In all cases, the EC and pH values of the nutrient solutions ranged from 0.7 to 1.2 dS·m⁻¹ and 5.8 to 7.6, respectively. One plantlet was planted in each pot and 10 pots were maintained for each treatment. The air and water temperatures during the experiment ranged from 13.1 to 28.1 °C and from 15.6 to 25.4 °C, respectively. Pollination was aided by vibrating the plants with a soft brush at 2-d intervals. During cultivation, the number of flower clusters and flowers, number of harvested fruits, and yield per plant were recorded. The fruits were harvested at maturity. At final harvest (1 June 2006), the number of leaves per plant, crown diameter, fresh and dry weight of shoots, dry weight of roots, and the maximum root length were recorded.

Data analysis. All data were analyzed to determine the statistical differences among the treatment means, and analysis of variance followed by mean separation with Tukey's test was conducted by using SPSS (SPSS Japan Inc.) at a 5% level of significance.

Results and Discussion

The degradation of benzoic acid (BA) in nutrient solution by electronic means (ED) was investigated. The amounts of BA (initially 400 μM) in the nutrient solution were

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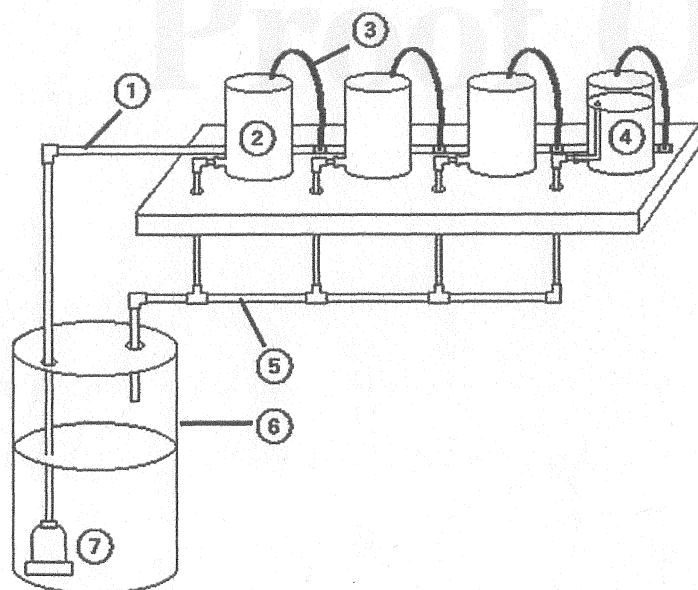


Fig. 2. The hydroponic system used for the cultivation of strawberry plants using a deep flow technique. The system comprised ① main pipes (15-mm diameter) for supplying nutrient solution, ② Wagner pots (3-L capacity) for planting, ③ tubes (4-mm diameter) to supply nutrient solution to the pots, ④ level of nutrient solution inside each pot, ⑤ pipes for the drainage of nutrient solution, ⑥ tank for stock nutrient solution (50-L capacity), and ⑦ pump in the solution tank for nutrient solution.

estimated by HPLC and were found to be 380, 352, 347, and 324 μM after 2, 6, 12, and 24 h, respectively, without ED treatment (Fig. 3), whereas the BA concentration decreased to 259, 223, 185, and 67 μM after 2, 6, 12, and 24 h, respectively, after ED treatment. The BA concentration without ED treatment declined slowly even after 24 h compared with that with ED treatment. Similar results for several phenolic compounds in aqueous solutions have been reported previously (Comminellis and Pulgarin, 1991; Feng and Li, 2003; Fleszar and Ploszynka,

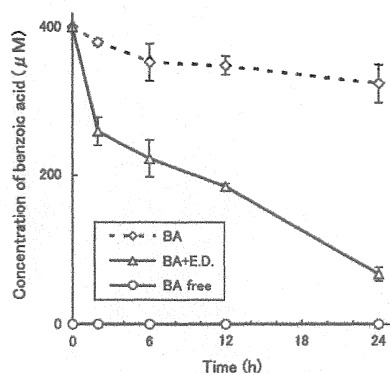


Fig. 3. Effect of electrodegradation on the concentration of exogenous benzoic acid (BA) in the nutrient solution. BA = initially 400 μM BA in the nutrient solution without electrodegradation (ED); BA+E.D. = BA in the nutrient solution with ED; BA free = for nutrient solution without BA or ED. The vertical bars represent SE (n = 3). The electric voltage and current in the ED process were 10.0 V and 2.0 A, respectively.

1985). However, the slow reduction in BA concentration (19% compared with the control) even after 24 h without ED treatment compared with that with ED treatment (83% compared with the control) might be related to microbial degradation (Sundin and Watcher-Kristensen, 1994) because no precipitate was detected in the solution. The Fe-EDTA concentration (initially 25 $\text{mg}\cdot\text{L}^{-1}$) in the nutrient solution also declined to 2.4 $\text{mg}\cdot\text{L}^{-1}$ and 17.5 $\text{mg}\cdot\text{L}^{-1}$ after 24 h with or without ED treatment, respectively (Fig. 4). Fe-EDTA was also significantly degraded by the ED treatment. The Fe-EDTA concentration decreased to $\approx 10\%$ of the initial level after ED treatment compared with $\approx 70\%$ without ED treatment after 24 h. Fe-EDTA degradation without ED treatment might be related to ultraviolet degradation (Date et al., 2002) and/or microbial decomposition, including anodic degradation (Kusakabe et al., 1986). This result clearly reveals that Fe-EDTA in the nutrient solution is easily decomposed by ED treatment, and thus it is highly recommended to adjust the Fe-EDTA in the nutrient solution after ED treatment.

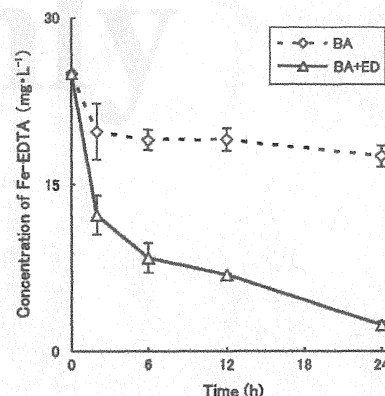


Fig. 4. Effect of electrodegradation on the concentration of Fe-EDTA (initially 25 $\text{mg}\cdot\text{L}^{-1}$) in the nutrient solution. BA = the Fe-EDTA concentration in the nutrient solution with benzoic acid (BA) without electrodegradation (ED) and BA + ED = the Fe-EDTA concentration in the nutrient solution after ED treatment. The vertical bars represent SE (n = 3). The electric voltage and current in the ED process were 10.0 V and 2.0 A, respectively.

BA is one of the most potent growth inhibitors for plants. The ED treatment caused rapid BA decomposition (Fig. 3) resulting in a reduction of growth inhibition of strawberry plants compared with the control (Table 2). The used nutrient solution from strawberry culture not only contained BA, but also some other allelochemicals (Kitazawa et al., 2005). So, a bioassay was carried out with or without ED treatment of the nutrient solution with added BA (400 μM) or with used nutrient solution after the cultivation of strawberry plants. The results revealed that the leaf number and root length did not differ significantly in any of the treatments (Table 2). However, the leaf length and width in the strawberry plants grown with BA decreased to $\approx 78\%$ and 72% , respectively, compared with the control. Plantlets grown with the used nutrient solution also decreased to $\approx 77\%$ and 69% , respectively, compared with the control. ED treatment of the BA solution or used nutrient solution mitigated these growth inhibition effects significantly to a point that they were statistically indistinguishable from the control. Consequently, the leaf area in the test strawberry plants was reduced significantly irrespective of the type of nutrient solution

Table 2. Influence of different types of nutrient solutions on the growth of strawberry plantlets grown in hydroponic culture.

Test solutions ^a	No. of leaves per plant	Maximum leaf length per plant (mm)	Maximum leaf width per plant (mm)	Leaf area per plant (cm^2)	Fresh wt of shoots per plant (g)	Maximum root length (mm)
Fresh NS (control)	5.4 a ^y	63.8 a	58.2 a	106.3 a	1.02 a	129.6 a
Fresh NS + BA	4.8 a	50.0 b	42.0 b	53.4 c	0.56 b	127.2 a
Fresh NS + BA + ED	5.3 a	59.0 ab	53.4 ab	88.5 b	0.95 a	115.6 a
Used NS	4.8 a	49.0 b	40.3 b	50.2 c	0.62 b	113.8 a
Used NS + ED	5.2 a	57.0 ab	53.4 ab	83.9 b	0.98 a	128.0 a

^aNS = 30% 'Enshi' nutrient solution; BA = benzoic acid; ED = electrodegradation.

^yValues in a column followed by a different letter differ significant by Tukey's test ($P = 0.05$; n = 10).

Table 3. Effects of renewed, nonrenewed, and electrodegraded (ED) nutrient solutions on the growth and yield of strawberry plants grown by hydroponic culture.

Type of nutrient solutions	No. of leaves per plant	Diam of crown per plant (mm)	Fresh wt of shoots per plant (g)	Dry wt of shoots per plant (g)	Dry wt of roots per plant (g)	Maximum root length (mm)	No. of flower clusters per plant	No. of flowers per plant	No. of harvested fruit per plant	Fruit yield per plant (g)
Renewed (control)	31.6 a [*]	26.8 a	43.7 a	11.8 a	6.5 a	25.5 a	6.7 a [*]	39.2 a	31.4 a	236.7 a
Nonrenewed	18.7 b	20.5 a	21.0 b	6.0 b	4.4 b	24.7 a	5.8 a	28.5 b	17.7 b	110.4 c
Nonrenewed + ED	19.0 b	22.8 a	23.4 b	6.1 b	5.3 b	26.8 a	6.0 a	30.8 b	29.0 a	167.1 b

*Values in a column followed by a different letter differ significantly by Tukey's test ($P = 0.05$; $n = 10$).

used without ED treatment and ED treatment significantly increased the growth of strawberry plants in used nutrient solution. The fresh weight of shoots of strawberry plants grown in BA solution or in used nutrient solution was significantly reduced to $\approx 55\%$ and 61% , respectively, compared with the control. ED treatment rescued the growth levels significantly by 170% and 158% compared with those grown in BA solution and in used solution, respectively, which were equivalent to the control. Significant growth inhibition of strawberry plantlets was reported after the addition of BA to the nutrient solution (Kitazawa et al., 2005, 2007). In this experiment, the shoot growth inhibition was induced by the BA solution as well as by the used nutrient solution. Because the decomposition of BA by ED treatment was confirmed (Fig. 3), it was suggested that the recovery of shoot growth was the result of ED treatment. The accumulation of growth inhibitors from the root exudates of tomato (Yu and Matsui, 1993), cucumber, taro, some leafy vegetables, and ornamentals (Asao et al., 1998, 2003, 2004b, 2007) in hydroponic nutrient solutions has been reported previously. The nutrient solution used in this study was the same as the solution used for strawberry culture by Kitazawa et al. (2005) in which significant growth inhibition (autotoxicity) was observed in strawberry plants. Thus, the conclusion was reached that the used nutrient solution contained growth inhibitors, including BA, and they caused the growth inhibition in strawberry plantlets. The alleviation of growth inhibition in strawberry plants grown in the used nutrient solution was concluded to be the result of ED treatment resulting in the decomposition of growth inhibitors, including BA.

To confirm the effect of ED on hydroponic culture, strawberry plants were grown in nutrient solutions with biweekly renewal (control), nonrenewed without ED, or nonrenewed with ED treatment. Table 3 shows that the number of leaves per strawberry plant in nonrenewed nutrient solutions with or without ED treatment decreased significantly to $\approx 59\%$ and 60% , respectively, compared with the control. In all treatments, the root length and crown diameter did not differ significantly. The fresh and dry weight of shoots decreased to approximately half, and the root dry weight declined significantly in the nonrenewed solution and the non-ED-treated solution. However, the number of flowers in the nonrenewed solution decreased to $\approx 73\%$ compared with the control. Consequently, the number of fruit harvested with the nonrenewed nutrient solution decreased to 56% compared with the control, whereas no statistical difference was observed between the control and ED-treated plants. The fruit yield in the nonrenewed nutrient solution declined by more than 50% compared with the control. ED treatment of the nutrient solution resulted in a significant recovery in fruit yield but not to the extent of the renewed nutrient solution (control). The growth inhibition of straw-

berry in BA solution or in used nutrient solution was significant, and ED treatment mitigated this inhibition significantly (Tables 2 and 3). Growth inhibitors, including BA, in the root exudates of strawberry were considered to be decomposed by ED treatment. Thus, the ED treatment improved the growth of strawberry plants compared with untreated plants. However, the growth inhibition in the vegetative stage of strawberry plants grown in nonrenewed nutrient solution was not recovered significantly by ED treatment at 2-week intervals (Table 3).

This study shows that the effect of ED treatment was temporary and frequent ED treatment (less than 2-week interval) might be more effective to alleviate autotoxicity in strawberry. Finally, it could be concluded that ED treatment of the nutrient solution could mitigate the autotoxicity of strawberry plants grown by closed hydroponic culture. These results suggest that ED treatment at 2-week intervals was not enough to support vegetative growth but was sufficient for fruit production. Further study is needed to determine the appropriate timing and intensity of ED treatment to maintain the nutrient solution and allow vigorous plant growth.

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