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Growth and Yield Recovery in Strawberry Plants under Autotoxicity through Electrodegradation

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Summary

The appropriate timing and intensity of electro-degradation (ED) of nutrient solution to recover strawberry autotoxicity in closed hydroponic system was investigated during spring 2010 in glasshouse of Experimental Research Center for Biological Resources Science, Shimane University. In growth chamber bioassay, growth of strawberry plantlets were inhibited greatly in non-renewed nutrient solution due to the accumulation of autotoxic root exudates compared to nutrient solution renewed weekly (control). Plants grown in non-renewed nutrient solution electro-degraded weekly intervals also showed growth inhibition whereas biweekly ED improved its growth. This growth inhibition in weekly ED nutrient solution was found to be attributed by the degradation Fe-EDTA (about 10 %) and low concentration of Ca^{2+} in culture solution. Electrolysis durations were evaluated in without plant experiments. It was found that low pH (3.13) and increased temperature were two major constraints for longer ED duration (24 h). Therefore, considering electric voltage, electrical conductivity (EC) and pH, shorter ED duration (2 h) was selected for further experiments. 2 h ED treatment of nutrient solution certainly can save energy and be feasible for greenhouse applications. In greenhouse, strawberry plants were grown in Wagner's pot hydroponic system where nutrient solutions were either renewed four weekly or not-renewed entirely. The non-renewed nutrient solutions were electro-degraded either biweekly or four weekly. Growth and yield of strawberry plants was decreased in non-renewed nutrient solution and its biweekly ED treatment but it was improved in non-renewed nutrient solution when ED applied at four weeks intervals. It was found that fruit yield of strawberry plant was completely recovered (≈ 99 %) in non-renewed nutrient solution with ED at every four weeks, whereas, in our previous study the recovery was 71 % compared to renewed nutrient solution. Therefore, we recommend application of ED to non-renewed nutrient solution for 2 h at every four weeks intervals to avoid autotoxicity in strawberry in a closed hydroponic system.

Key words. autotoxicity – allelochemicals – bioassay – closed hydroponics – electro-degradation – *Fragaria* × *ananassa* – renewed nutrient solution – non renewed nutrient solution – Wagner's pot system

Introduction

Autotoxicity is a phenomenon of intraspecific allelopathy that occur when a plant species releases chemical substances that inhibit or delay germination and growth of the same plant species (Putnam 1985a; Miller 1996; Singh et al. 1999). It has been reported to occur in a number of crop plants in agroecosystem causing serious problems such as growth reduction, yield decline and replant failures (Singh et al. 1999; Pramanik et al. 2000; Asao et al. 2003). Plants when experiences autotoxicity it releases chemicals/allelochemicals to its rhizosphere (Singh et al. 1999) through various mechanisms. These include leachation (Overland 1966), volatilization (Petrova 1977), root exudation (Tang and Young 1982), crop residue decomposition (Rice 1984; Putnam 1985b), and pollen spreading in some plants (Cruz-Ortega et al. 1988). The released chemical compounds create problems in monoculture and/or closed hydroponic culture systems as they can accumulate and inhibit the growth of the actual crop. Exposure of these allelochemicals play a multitude of ecological and physiological roles as they inhibit plant growth (Rice 1984), alter mineral uptake (Lyu and Blum 1990; Baziramakenga et al. 1994), disrupt membrane permea-

bility (Baziramakenga et al. 1995), cause stomatal closure and induce water stress (Barkosky and Einhellig 1993), influence respiration (Penuelas et al. 1996), affect photosynthesis and protein synthesis (Mersie and Singh 1993; Rohn et al. 2002), impair hormone balance (Holappa and Blum, 1991) and alter enzyme activities (Rohn et al. 2002; Doblinski et al. 2003). Among them, ion uptake and hydraulic conductivity (i.e., water uptake) are worse affected processes since root is the first organ to come into contact with autotoxins in the rhizosphere (Blum et al. 1999).

Closed hydroponic system has recently gained popularity for commercial production of strawberry (Takeuchi 2000; Oka 2002; Koshikawa and Yasuda 2003) but this managed culture technique has the problem of autotoxicity due to accumulation of allelochemicals in the culture solution. In strawberry autotoxicity from root exudates has been investigated in closed hydroponic culture and benzoic acid was confirmed as the most potent growth inhibitor (Kitazawa et al. 2005). Strawberry plants when experiences autotoxicity, the shoot and root growth, number of flowers and harvested fruits per plant, and fruit enlargement reduces significantly. Elimination of growth inhibitors from recycling hydroponic cultivation system is desirable from the viewpoint of conservation-oriented agriculture. Activated charcoal (AC) supplementation was found to improve growth and yield significantly by adsorbing phytotoxic chemicals (mainly phenolics), for example in strawberry (Kitazawa et al. 2005), taro (Asao et al. 2003), cucumber (Yu and Matsui 1994; Asao et al. 1998, 1999, 2000), several leafy vegetables (Asao et al. 2004a), and some ornamentals (Asao et al. 2007). Several other methods has also been found to be effective in removing/degradating the phytotoxic substances released from plant roots during autotoxicity such as degradation by microbial strain (Asao et al. 2004b) or auxin (2,4-D and NAA) supplementation (Kitazawa et al. 2007). Electrochemical methods have also been applied for degradation/oxidation of phenols and its derivatives from organic waste or pollutants by several researchers. Phenolic compounds, including phenols, catecol and hydroquinone in aqueous solution and even benzene were found to decompose when treated by electro-degradation (Fleszar and Ploszynka 1985; Comninellis and Pulgarin 1991; Feng and Li 2003). These compounds are oxidized rapidly at the anode and decompose to CO_2. Thus electrodegradation may cause the decomposition of allelochemicals; including benzoic acid exuded in the nutrient solution from plants, and could be useful tool to mitigate autotoxicity in strawberry.

Based on these results, previously ED has been tried to decompose benzoic acid (Asao et al. 2008). It was found that exogenously added benzoic acid to a nutrient solution was almost completely decomposed within 24 h. In the ED process a titanium plate was used as cathode and a ferrite rod as anode. The growth inhibitions of plantlets in the nutrient solution containing benzoic acid or in the nutrient solution used for strawberry culture were significantly ameliorated by ED application. However, complete yield recovery from non-renewed nutrient solution to renewal control was suggested through further investigations. Therefore, the present study aimed to determine the appropriate timing and intensity of ED treatment to maintain nutrient solution and allow vigorous plant growth in a closed hydroponic culture. A modified ED machine was used to degrade allelochemicals from the culture solution of strawberry in Wagner's pot hydroponic system.

Materials and Methods

Verification of electrolysis condition

Electrolysis conditions were verified in a series of concentrations (0, 6.25, 12.5, 25, 50, 75, and 100 %) of 'Enshi' nutrient solution (Table 1). Initially the electric current was slightly increased with the increase of nutrient solution concentration but voltages were decreased gradually. The electric current ranged from 1.75 to 1.88 A, and elec-

Table 1. Electric current and voltage at different concentrations of nutrient solution during electro-degradation (ED).

Nutrient solution concentration (%)	Initial		Adjusted	
	Electric current (A)	Voltage (V)	Electric current (A)	Voltage (V)
0.00	1.75*	35.7	2.0	40.2
6.25	1.82	21.0	2.0	22.5
12.50	1.84	16.2	2.0	17.2
25.00	1.86	12.0	2.0	12.6
50.00	1.87	9.8	2.0	10.2
75.00	1.88	8.8	2.0	9.0
100.00	1.88	8.1	2.0	8.4

* Values are the average of three readings.

tric voltage form 35.7 to 8.1 V. However, the electric current was below 2 A in all the concentrations of nutrient solution. When the electric current was adjusted at 2 A, then the voltage was not changed greatly from the initial. Therefore, 2 A were selected as the test current for the investigations.

Growth chamber bioassay of strawberry plantlets

Strawberry (*Fragaria* × *ananassa* Duch. var. 'Toyonoka') plantlets obtained through plant tissue culture were used for this bioassay. The virus free and healthy plantlets at 3rd–4th leaf-stage were planted to plastic containers ($17 \times 29 \times 9.5$ cm) in the growth chamber at 20/15 °C (day/night) with a light intensity of 74–81 μmol m^{-2} s^{-1} and a 14-hours photoperiod. The containers were filled with 3 L of 25 % standard 'Enshi' solutions (EC 0.8 dSm^{-1}). Six plantlets were planted in each container using urethane foam blocks as support. There were four types of nutrient solution viz. renewed, non-renewed, non-renewed with ED at weekly and non-renewed with ED at biweekly intervals. The nutrient solutions were renewed at every week intervals (control). Nutrient solutions of ED treatments were combined in a plastic bucket and then ED was applied. During ED application the nutrient solutions were aerated continuously by air pump (NISSO, Chikara α 4000 SW, Osaka, Japan). The ED was applied for 24 h with electric current adjusted at 2 A. After completion of ED, full amount of Fe^{3+} was added in the treated nutrient solution and the amount of nutrient solution was adjusted to 3 L by adding standard nutrient solution. During the growth periods the major nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}) content in the culture solution were adjusted as close as possible to initial concentrations on the basis of chemical analysis with ion meter (C-141, Horiba, Ltd. Kyoto, Japan) for NO_3^-, spectrophotometer (UVmini 1240, Shimadzu Corporation, Kyoto, Japan) for PO_4^{3-} and atomic absorption spectrophotometer (Z-5010, Hitachi, Tokyo, Japan) for K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}. After seven weeks, the number of leaves, crown diameter, root length, shoot fresh weight, shoot dry weight and root dry weight were measured.

Electro-degradation of nutrient solution in no plant experiment

ED treatment was applied in nutrient solution following without plant experiment. In these experiments 10 L of 25 % 'Enshi' nutrient solution were electro-degraded with ED machine used previously for 24 h. The electric current was adjusted at 2 A. During the ED process nutrient solutions were aerated constantly by an air pump. Treated nutrient solutions were sampled at 0, 1, 3, 6, 9, and 24 h. In plastic bottles 25 ml samples were collected for major nutrient analysis following the procedures described earlier. The electric voltage and temperature was recorded from ED machine, electrical conductivity was measured by EC meter (ES-51, Horiba, Ltd., Kyoto, Japan), and pH was also recorded from a pH meter (D-12, Horiba, Ltd., Kyoto, Japan) during each sampling.

Wagner's pot hydroponic system of strawberry plants

Strawberry (*Fragaria* × *ananassa* Duch. var. 'Toyonoka') plantlets were planted into Wagner's pot (ten plants in one line) connected with a reservoir containing 60 L of 25 % nutrient solution in closed hydroponic system on 16 November 2009. At transplanting the plantlets were at 5th–6th leaf stage, healthy and similar in size. The Wagner's pot system includes main inlet pipes (15 mm diameter) for supply and drainage of nutrient solution between reservoir and pots, Wagner's pot (1/5000a, NF-5, AsOne, Osaka, Japan) with 3 L capacity for planting, inlet tubes (4 mm diameter) to supply solution to the pots, and 60 L capacity nutrient solution container with a pump (KP-101, Koshin, Kyoto, Japan). The culture solutions were prepared as renewed at every week, non-renewed entirely, non-renewed with ED at every two weeks and non-renewed with ED at every four weeks intervals. Strawberry plants after reaching active vegetative phase, ED treatments were applied on 11 January 2010. The electric current was adjusted at 2 A and ED was applied for 2 h. The ED machine was modified to investigate its efficacy for degradating autotoxic chemical following a Wagner's pot hydroponic system in the greenhouse (Fig. 1). The modified

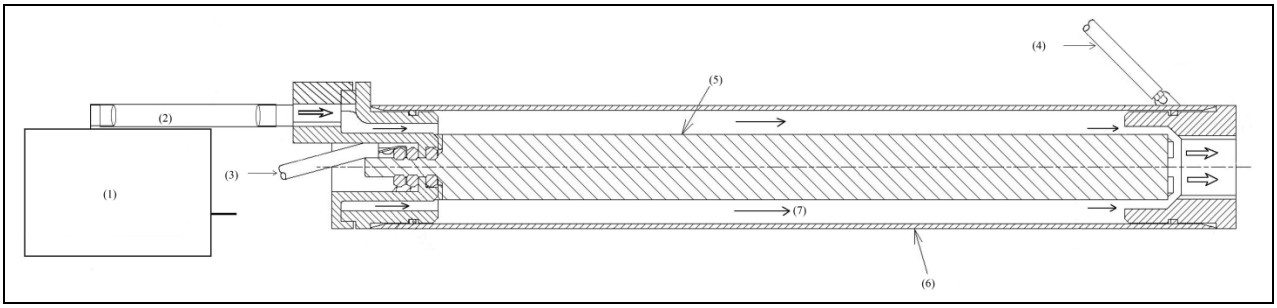


Fig. 1. Schematic diagram of the electrode used for ED treatment. Different components are as (1) pump, (2) plastic tube connecting pump with electrode, (3) anode, (4) cathode, (5) central ferrite core, (6) cylindrical titanium pipe and (7) nutrient solution flow.

machine has an electrode having a central core made of ferrite with a surface area of 65.9 cm^2 (anode) which enclosed with cylindrical tube made of titanium with a surface area of 103.7 cm^2 (cathode). The nutrient solution can pass through the electrode where electrodegradation takes place. The machine was coupled with a digital DC power supplier (AD-8735D, AND, Japan). In the previous study, ED of autotoxic chemicals from strawberry root exudates was done by an electrode (cathode) consisting of a cylindrical titanium plate with a 180 cm^2 surface area and ferrite stick of 42 cm^2 surface area (anode) (Asao et al. 2008). The previous ED machine requires a separate pump system to flow the nutrient solution during treatment but the modified machine has the facility of attaching pump for flowing treated nutrient solution homogenously through the electrode. Therefore, the modified ED machine was used for degradation of allelochemicals in Wagner's pot hydroponics of strawberry in greenhouse. The nutrient solution was circulated through the pipes for 5 min at 10 min intervals using an automatic pump timer (KS-1500, Iuchi, Osaka, Japan). The nutrient solutions were renewed at every four weeks interval. The concentration of major nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}) were analyzed following the methods and instruments used for growth chamber bioassay and adjusted at four weeks interval. The EC, pH and temperature of nutrient solution were measured at each time of treatment application. The date of anthesis were recorded for each plant to check whether there were any influences of ED on flowering of strawberry among the treatments. Pollination was aided by a soft brush at two days intervals. As the plants attained its full vegetative growth phase, leaf length and width were measured on 10 April 2010 to compare growth among the treatments. The fruits were harvested at their maturity. At each harvest fresh weight of fruits were recorded and gathered for final yield comparison among the treatments. Leaf number, leaf length and width, root length, dry weight of leaves, dry weight of crown, and dry weight of root were also recorded at final harvest.

Fruit quality of strawberry grown in nutrient solution with electrodegradation

Strawberry fruits were composited after each harvest and were frozen at −30 °C for subsequent analysis of soluble solids, titratable acids and ascorbic acid content. Fruit samples were kept out of freezer before analysis to obtain juice for determining the above qualities of strawberry fruits. The soluble solid content of the fruit was determined using a digital refractometer (PR-1, Atago Ltd., Japan). Titratable acid contents were determined by diluting each 2 ml aliquot of strawberry juice to 10 ml with 8 ml distilled water and added 2–3 drops of phenolphthalein then adjusted the pH to 8.2 using 0.1 N (w/v) NaOH. The quantity of NaOH (ml), and the amount for appropriate acidity was converted into citric acidity (%). Ascorbic acid content was measured with 2,4-dinitrophenylhydrazine (DNP) colorimetry. Strawberry fruit juice (0.5 ml) were taken in 50 ml test tube then 0.5 ml of 10 % metaphosphoric acid solution, 1 ml of distilled water, 1 ml of 0.03 % 2,6-dichlorophenol-indophenol (DCP), 2 ml of thiourea, and 1 ml of DNP was added to the samples following 3 h incubation at 37 °C in water bath. After incubation samples 5 ml of 85 % H_2SO_4 were added keeping in water cooled with iced water. After 30 min cooling ascorbic acid content was measured at 540 nm by spectrophotometer (U-2900, Hitachi High Technologies Corporation, Tokyo, Japan).

Mineral nutrient content in strawberry plants grown in nutrient solution with electrodegradation

Strawberry plant parts were separated into leaves, crown and roots and kept in a constant temperature oven (DKN 812, Yamato Scientific Co., Ltd. Japan) for 72 h at 80 °C. When the dry matter reaches constant weight, it was ground into powder with a mixer machine (National MX-X53, Japan). Samples weighing 0.5 g were mixed with 8 ml of HNO_3 and digested by microwave sample preparation system (ETHOS1, Milestone S.r.l, Bergamo, Italy). After digestion samples were measured up to 50 ml of volumetric flask and then filtered with qualitative filter paper (Advantec Grade no. 131, 185 mm thickness). The filtered sample solutions were analyzed for mineral nutrients by atomic absorption spectrophotometer (Z-2310, Hitachi High Technologies Corporation, Tokyo, Japan).

Experimental design and statistical analysis

A randomized complete block design with three replicates was used for both growth chamber bioassay and Wagner's pot hydroponics in the greenhouse as described above. These experiments were not repeated over time due to the consistent results. The growth and yield data obtained from bioassay and Wagner's pot hydroponics of strawberry plants were subjected to analysis of variance using Tukey-Kramer test (Statcel 2 software, OMS publication, Tokorozawa, Saitama, Japan) and the treatment means were separated at 5 % level of probability. The instrumental data obtained from fruit and plant matters were also analyzed for their difference among the treatments.

Results and Discussion

Growth chamber bioassay of strawberry plantlets

The ED of the culture solution of strawberry plantlets grown in growth chamber showed a significant influence on growth parameters (Table 2). The weekly renewed nutrient solution leads a vigorous plant growth while inhibition of vegetative growth of strawberry plantlets resulted

Table 2. Effects of electro-degradation (ED) on the growth of strawberry plantlets in growth chamber bioassay. Culture solutions were treated as renewed, non-renewed, non-renewed with weekly ED and non-renewed with biweekly ED. ED of culture solution were done by electrode used in previous experiment in 2008 (Asao et al. 2008).

Culture solution	Number of leaves*	Crown diameter (mm)	Root length (cm)	Shoot fresh wt. (g)	Shoot dry wt. (mg)	Root dry wt. (mg)
Renewed	8.9 a†	7.2 a	14.1 ab	2.9 a	444.5 a	86.0 ab
Non-renewed	8.0 a	4.8 bc	10.6 b	2.1 a	307.8 ab	66.1 b
ED weekly	3.7 b	4.3 c	7.7 b	0.9 b	171.1 b	45.9 b
ED biweekly	8.2 a	5.9 b	15.8 a	2.4 a	390.6 a	93.9 a

* Parameters measured per plant basis. wt.: weight

† Values in a column followed by different letter differ significantly by Tukey's test ($P < 0.01$; $n = 6$).

due to non-renewed nutrient solution during the entire growth period. This growth inhibition is basically attributed by the accumulation of autotoxic root exudates in the non-renewed culture solution. In previous study, Kitazawa et al. (2005) identified benzoic acid as the most potent growth inhibitor that accumulated in strawberry culture solution. In biweekly ED application, growth was not inhibited rather recovered significantly to renewed nutrient solution except crown diameter. However, ED when applied weekly, the growths were inhibited remarkably as in non-renewed nutrient solution. Shoot weight was found to be the worst affected in weekly ED of non-renewed nutrient solution and it was about 69 and 62 % reduction in shoot fresh and dry weight against renewed nutrient solution. Growth retardation in weekly ED culture solution also produced fewer new leaves and shorter root length (about 54 and 45 %, respectively) compared to renewed control. The major reason of this drastic growth inhibition in weekly ED culture is low concentration of Fe^{3+} in the degraded solution. In previous study it was found that 24 h ED decreases the Fe-EDTA concentration about 10 % of the initial (Asao et al. 2008). Therefore in this bioassay the effects of ED treatment were immense in weekly than biweekly intervals with the ED machine used previously. The other possible reasons of this growth retardation and timing of ED were investigated following without plant experiments.

Electro-degradation of nutrient solution in without plant experiment

The electrolysis timing and intensity were investigated in without plant experiment (Table 3). ED of nutrient solution was conducted at 2 A for 24 h by the ED machine used previously. After 24 h ED electrical voltage were decreased by 2.2 V whereas the electrical conductivity was not varied with the electrolysis durations. However, the pH of solution was decreased as low as 3.1 after 24 h of ED but it was almost not changed till 9 h ED. This exceedingly low pH after 24 h of ED was another important cause that leads to strong inhibitory effects on strawberry plantlets growth at weekly ED in the growth chamber

Table 3. Electric conditions of 24 h electro-degradation (ED) of nutrient solution by the machine used in previous experiment in 2008 (Asao et al. 2008).

Electrolysis duration (h)	Voltage (V)	Electric conductivity (mS cm^{-1})		pH		Temperature (°C)	
	+*	+	−	+	−	+	−
0	14.9	0.84	0.83	6.95	6.98	14.4	14.3
1	14.3	0.84	0.83	7.20	7.30	15.8	14.5
3	13.9	0.79	0.79	7.36	7.35	18.0	14.7
6	13.6	0.72	0.82	7.26	7.28	20.4	14.5
9	13.3	0.75	0.82	6.86	7.40	22.1	14.5
24	12.7	0.79	0.82	3.13	7.26	22.6	14.2

* (+, −) indicate with or without electro-degradation.

bioassay (Table 2). The solution temperature also increase considerably (over 9.1 °C form starting) after 24 h ED compared to non ED solution. Changes in concentration of nutrients were determined and it was found that Ca^{2+} decreased (about 66 %) considerably after 24 h ED (Fig. 2). In addition to decrease in Fe^{3+} concentrations in the ED treated nutrient solution, Ca^{2+} also found to be decreased after 3 h ED. Therefore, ED of nutrient solution for 24 h was associated with some problems, it was not only degradated the allelochemicals but also breakdown the essential nutrients like Ca^{2+} and Fe^{3+}. Considering the very low pH and decrease in Ca^{2+} and Fe^{3+} concentration in the treated nutrient solution a shorter ED duration (2 h) was selected for Wagner's pot hydroponic system for strawberry. Moreover, short electrolysis duration can save electricity and can be feasible in the greenhouse strawberry cultures.

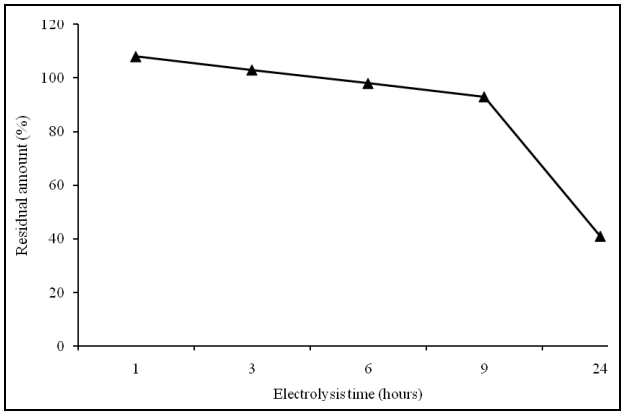


Fig. 2. Decrease in Ca^{2+} concentration in the electro-degraded nutrient solution after 24 h by the machine used in previous experiment in 2008 (Asao et al. 2008).

Wagner's pot hydroponic system of strawberry plants

ED method was applied for recovering the autotoxicity in strawberry, which usually occurs in closed hydroponics (Kitazawa et al. 2005). Therefore, a closed hydroponic system was developed in Wagner's pot for treating the strawberry culture solution in the greenhouse. After active vegetative growth strawberry plants were started flowering. There were no significant influences of ED on the anthesis of strawberry plants but comparatively earlier flowering were observed in plants grown in non-renewed nutrient solution (Table 4). Generally plant switches its vegetative growth to reproductive when it experiences some stresses. The synthesis and exudation of allelochemicals enhanced by stress conditions that the plant encounters such as extreme temperature, drought and UV exposure (Pramanik et al. 2000; Inderjit and Weston 2003). Thus earlier anthesis in strawberry plants grown in non-renewed nutrient solution might be the results of allelochemical stresses which were released from its root. Delayed anthesis was occurred in plants with ED at four weeks intervals. This phenomenon might be due to degradation of allelochemicals (mainly benzoic acid) form culture solution of strawberry. Vegetative growth measurements showed differences among the culture solutions of strawberry (Table 4). Vigorous growth was observed in plants grown in renewed nutrient solution and in non-renewed nutrient solution with ED at four weeks intervals. Although leaf lengths were not differed significantly, the leaf widths were varied among the treatments. Renewed and non-renewed nutrient solution with ED four weekly intervals produces strawberry plants with wider leaves. Larger leaves provide higher photosynthetic area that produces higher strawberry yield. Whereas non-renewed and non-renewed with biweekly ED nutrient solution produces less width leaves. The results revealed that the leaf size were decreased about 21 % in non-renewed nutrient solutions than renewed but non-renewed nutrient

Table 4. Effects of electro-degradation (ED) on the anthesis and vegetative growth of strawberry plants in Wagner's pot hydroponic system. Culture solutions were treated as renewed, non-renewed, non-renewed with ED at two weeks and non-renewed with ED at four weeks intervals.

Culture solution	Date of anthesis (month/day)*	Vegetative growth	
		Leaf length (mm)	Leaf width (mm)
Renewed	2/22	222.2	194.4 a†
Non-renewed	2/20	215.4	162.2 b
ED biweekly	2/23	225.7	178.6 ab
ED four weekly	2/26	228.2	198.3 a
	NS	NS	

* Strawberry plantlets were transplanted on 16 November 2009 and anthesis was observed on February 2010.
† Values in a column followed by different letter differ significantly by Tukey's test ($P < 0.05$; $n = 10$).
NS: Non significant.

solutions with ED produced similar sized leaves as in renewed nutrient solution.

The growth and yield of strawberry plants were significantly influenced by the culture solutions used (Table 5). Renewed and four weekly ED of non-renewed nutrient solution produced higher dry weights of leaf and root than non-renewed and non-renewed with biweekly ED. Leaf number per plants, maximum leaf length and width, root length, crown dry weights were higher in renewed nutrient solution and non-renewed with four weekly ED followed by non-renewed and its biweekly ED. This indicates during autotoxicity, growth in strawberry plants were recovered by ED treatment. In cucumber, it was found that plant growth was inhibited by its own root extract and exudates while improved by removal of these substances from the rhizosphere (Yu and Matsui 1994; Asao et al. 1998; Yu et al. 2000). The number of fruits in non renewed nutrient solution and biweekly electro-degraded solution decreased to 13 % and 27 %, respectively compared with renewed control. Whereas, there were no significant difference in fruit number between renewed and non-renewed nutrient solution having four weekly ED. The fruit yield of strawberry in non-renewed nutrient solution and its biweekly ED treatment were decreased about 25 % compared with control (renewed four weekly). The complete recovery (about 99 %) of fruit yield was resulted in plants grown in non-renewed nutrient solution with four weekly ED. In biweekly ED of non-renewed nutrient, Ca^{+2} and Fe^{3+} were broken in addition to degradation of allelochemicals from the strawberry plant roots. Biweekly ED is more intense compared to four weekly and therefore, contain lower concentration of major nutrients like calcium and iron in the culture solution. As a result strawberry plants were found to be retarded in these culture solutions. In previous study, ED of non renewed nutrient solution, strawberry fruit yield recovered about 71 % to renewed control (Asao et al. 2008) and complete yield recovery was not achieved due to decrease in Fe^{3+} in the nutrient solution. In the present Wagner's pot hydroponics appropriate timing and intensity of ED were applied and therefore complete recovery of fruit yield was obtained.

Fruit quality of strawberry grown in nutrient solution with electrodegradation

The quality of strawberry fruits were not differed significantly in the culture solutions either renewed and/or non-renewed (Table 6). Soluble solid content, citric acid acidity, and ascorbic acid content in the strawberry fruits were not varied among the culture solutions used. Therefore, ED of nutrient solution did not left any detrimental effects on the fruit quality in strawberry.

Mineral nutrient content in strawberry plants grown in nutrient solution with electrodegradation

Calcium and iron contents were determined in the plant parts of strawberry as these two minerals were found to be decreased from the nutrient solution due to ED (Table 7). Calcium contents in non-renewed nutrient solution and its biweekly ED were decreased significantly in all plant parts except in crown compared to renewed and non-renewed nutrient solution with four weekly ED. Calcium uptake in plants grown in non-renewed nutrient solution was inhibited by the accumulated autotoxic chemicals whereas in case of biweekly ED of non-renewed solution, it was due to the breakdown of Ca^{2+} in the nutrient solution by the ED. Iron content in strawberry plants were differed significantly among the culture solution used (Table 7). In leaves and crown Fe^{3+} content were decreased in non-renewed and/or ED nutrient solution while in root it was decreased only in non-renewed nutrient solution.

Table 5. Effects of electro-degradation (ED) on the growth and yield of strawberry plants in Wagner's pot hydroponic system. Culture solutions were treated as renewed, non-renewed, non-renewed with ED at two weeks and non-renewed with ED at four weeks intervals.

Culture solution	Number of leaves*	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Dry weight of leaves (g)	Dry weight of crown (g)	Dry weight of root (g)	Number of harvested fruits	Fruit yield (g)
Renewed	60.4	32.7	20.0 a†	37.4	47.3 a	2.4	7.5 a	30.8 a	295.0 a
Non-renewed	58.8	28.3	15.9 b	35.2	32.2 b	1.6	5.5 b	26.7 ab	223.5 bc
ED biweekly	58.8	30.5	18.4 a	37.5	35.7 b	2.1	5.7 b	22.5 b	220.6 c
ED four weekly	55.9	30.3	19.7 a	34.4	40.0 ab	2.2	6.0 ab	30.3 a	290.7 ab
	NS	NS		NS		NS			

* Parameters measured on per plant basis.

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

NS: Non significant.

Table 6. Soluble solid content, citric acid and ascorbic acid content in fruits of strawberry grown in nutrient solution with electro-degradation (ED); fruits harvested at stage I (2010/3/23~2010/4/30), stage II (2010/5/1~2010/5/15), and stage III (2010/5/16~2010/6/8). Culture solutions were treated as renewed, non-renewed, non-renewed with ED at two weeks and non-renewed with ED at four weeks intervals.

Culture solution	Soluble solid content (%)			Citric acidity (%)			Ascorbic acid (ppm)		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
Renewed	9.1	9.0	9.8	0.53	0.54	0.61	630.6	583.2	587.2
Non-renewed	9.1	9.4	9.6	0.41	0.46	0.43	632.1	635.0	780.2
ED biweekly	8.6	8.9	9.1	0.35	0.39	0.46	816.9	611.9	602.5
ED four weekly	8.6	8.2	9.1	0.58	0.51	0.62	671.6	662.2	701.4
	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS: Non significant.

Table 7. Mineral nutrient content in strawberry plants grown in nutrient solution with electro-degradation (ED). Culture solutions were treated as renewed, non-renewed, non-renewed with ED at two weeks and non-renewed with ED at four weeks intervals.

Culture solution	Ca (mg plant^{-1})			Fe (mg plant^{-1})		
	Leaves	Crown	Root	Leaves	Crown	Root
Renewed	0.42 a*	0.029 a	0.063 a	0.062 a	0.0092 a	0.062 b
Non-renewed	0.26 c	0.020 b	0.043 b	0.039 c	0.0052 b	0.042 c
ED biweekly	0.23 c	0.027 a	0.045 b	0.038 c	0.0052 b	0.064 ab
ED four weekly	0.31 b	0.028 a	0.062 a	0.054 b	0.0065 b	0.080 a

* Values in a column followed by different letter differ significantly by Tukey's test ($P < 0.01$; $n = 10$).

In previous study it was found that 10 % Fe-EDTA degradated from nutrient solution due to ED (Asao et al. 2008).

Conclusion

The previous study concluded that ED treatment of nutrient solution could mitigate autotoxicity plants in closed hydroponic culture and recovered fruit yield upto 71 % of control. However, further studies were suggested to determine the appropriate timing and intensity of ED treatment to maintain the nutrient solution and allow vigorous plant growth. In this present follow up study, we found complete recovery in fruit yield of strawberry plants grown in closed hydroponic system where culture solutions were allowed to ED treatment before reuse. In the growth chamber bioassay strawberry plantlets showed retarded growth in non-renewed and/or its weekly ED whereas it was recovered in biweekly applications. Therefore, electro-degraded nutrient solutions were investigated following without plant experiments. It was found that ED of nutrient solution was associated with degradation of Fe-EDTA (about 10 %) and low concentration of Ca^{2+} (about 66 % of the initial) in the treated culture solution. The disadvantages of longer duration ED were decrease in solution pH as low as 3.1 and increase in temperature. Therefore, shorter duration (2 h) ED treatments were applied in the greenhouse experiments following Wagner's pot hydroponics. ED machine used for previous experiments was modified for allowing the treated solution passed continuously through the electrode. Results obtained from Wagner's pot experiment showed that growth and yield (about 99 % to renewed control) of strawberry plants were improved in non-renewed nutrient solution having ED at four weeks interval. Therefore, in practice, application of ED to non-renewed nutrient solution in closed hydroponic system for 2 h at every four weeks intervals would recover autotoxicity in strawberry.

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