Autotoxicity mitigation in strawberry and lettuce grown in closed hydroponics under controlled environment

（環境制御された閉鎖型養液栽培におけるイチゴ及びレタスの自家中毒軽減に関する研究）

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Autotoxicity mitigation in strawberry and lettuce grown in closed hydroponics under controlled environment

by

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Md. Raihan Talukder

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CHAPTER 1

GENERAL INTRODUCTION

1.1.Autotoxicity in crop plants

Autotoxicity is a biological phenomenon whereby a species inhibits growth or reproduction of members of that same species through the production of chemicals that are released into the environment. Putnam (1985) defined autotoxicity as a process where a plant or its decomposing residues release toxic chemicals into the environment to inhibit germination and growth of the same plant species. It is related to allelopathy but is technically different. The phenomenon by which one plant directly or indirectly cause detrimental (or occasionally beneficial) effects on other plants through the release of allelochemicals into the environment is called allelopathy (Rice, 1984). Autotoxicity is an intra-specific allelopathy and its effects are always inhibitory, whereas allelopathic effects are not necessarily inhibitory - they may stimulate other organisms (Keating,1999; Pielou,1974). Autotoxicity has been reported to occur in a number of weeds and crop plants in agro-ecosystem and wastelands causing the soil sickness.

In agricultural ecosystems, many plant species are affected by autotoxicity, leading to decreased growth, low yields and replant failures (Singh et al., 1999; Pramanik et al., 2000; Asao et al., 2003). Autotoxicity develops due to chemicals released in the rhizosphere (Singh et al., 1999) through various mechanisms such as leaching

(Overland, 1966), volatilization (Petrova, 1977), root exudation (Tang and Young, 1982), pollen spread in some plants (Cruz-Ortega et al., 1988) and crop residue decomposition (Rice, 1974). Pronounced autotoxicity can occur in plants cultivated in the same soil for several years or grown in recycled hydroponic solutions (Takahashi, 1984; Zhao et al., 2015).Stress conditions such as extreme temperatures, drought and UV light enhanced the synthesis and exudation of allelochemicals as well as increased the overall production of root exudates (Inderjitand Weston, 2003).

1.2.Autotoxicity in closed hydroponic system

A closed hydroponics which is frequently known as re-circulating system refers to a hydroponic system in which nutrient solution is not diverted from the system. The nutrient solution flows through the growing medium into a collector where it is recovered and then it is reused over and over again in the same way. Closed hydroponics system lower water and nutrient consumption, avoids the supply and disposal cost of nutrient solutions and environmentally friendly - minimal potential for localized groundwater contamination. Hence, this hydroponic system has been encouraged recently (Ruijs, 1995; Van Os, 1995).

In closed hydroponic systems in which the nutrient solution is recycled, root exudates with highly variable chemical compositions are the common sources of bioactive allelochemicals (Inderjitand Weston, 2003). In fact, root exudates represent one of the largest sources of plant chemicals released into the rhizosphere that are responsible for chemical interference among plants. The accumulation of allelochemicals in the nutrient solution inhibits growth and metabolic activities of plant roots, ultimately causing

electrolyte levels in cells and root lipid peroxidation activities to increase and the free radical scavenging activity of roots to decrease (Zhen et al., 2003). Additionally, the damaged plant roots exhibit impaired uptake of water and mineral nutrients from the nutrient solution that resulted plant growth retardation.

1.3.Autotoxicity in strawberry in closed hydroponic system

Strawberry has also been grown hydroponically for higher yield and better quality compared to soil cultivation. In protected cultivation technique, large-scale production of strawberry through open system hydroponics discharge used nutrient solution periodically to the environment causing pollution and wastage of costly fertilizers. Therefore, commercial strawberry growers practiced closed hydroponic system for sustainable production (Takeuchi, 2000; Oka, 2002). However, under this closed hydroponic culture technique, autotoxicity develops due to continuous accumulation of allelochemicals in the culture solution (Asao et al., 2003, 2007; Kitazawa et al., 2005). In addition, autotoxicity in strawberry plants is typically characterized by the development of black root rot disease, which limits strawberry yields (Yuen et al., 1991; Wing et al., 1995; Asaduzzaman et al., 2012). In strawberry, autotoxicity from root exudates has been studied in closed hydroponics and benzoic acid was confirmed as the most potent growth inhibitor (Kitazawa et al., 2005). Other studies showed that, when much amount of root exudates accumulated in their growing medium, the growth and metabolism of strawberry roots were inhibited and root damage occurred (Zhen et al., 2003; Asao et al., 2008). Consequently, under autotoxicity condition, damaged strawberry roots hamper water and mineral nutrient uptake. As a result, the growth of

shoot and root, number of flowers and harvested fruit per plant and fruit enlargement greatly reduced (Kitazawa et al., 2005).

1.4.Autotoxicity during successive lettuce cultivation in closed hydroponic

Lettuce (*Lactuca sativa* L.), which has a short growth cycle and high planting density can be produced in large quantity in the plant factory (Seaman, 2015). There are a lot of different hydroponics systems to grow in the plant factory, but one of the most popular methods is with a closed hydroponics system (Takeuchi, 2000; Oka, 2002; Koshikawa and Yasuda, 2003). In closed hydroponics, crop production greatly reduced in non-renewed solution (Kitazawa et al., 2005). Many researchers found this problem in closed hydroponic production of taro (Asao et al., 2003), lettuce (Lee et al., 2006), several leafy vegetables including lettuce (Asao et al., 2004a), tomato(Yu and Matsui, 1993), cucumber(Yu and Matsui, 1994)and some ornamentals (Asao et al., 2007). Continuous crop cultivation with recycled nutrient solution, the plant growth was greatly reduced due to presence of the several organic acids in the reused nutrient solution (Kitazawa et al., 2005; Asao et al., 2003, 2004a).In successive cultivation of lettuce using same nutrient solution, the lettuce growth and yield were also reduced by the same phenomenon (Lee et al., 2006).Growth retardation and root injury gradually increased with the increased reuse times of the nutrient solution (Lee et al., 2006).

1.5.Methods used for mitigating autotoxicity in closed hydroponic system

Elimination of the accumulated root exudates or autotoxic growth inhibitors from closed hydroponic system would be of great interest to the farmers. The removal or

degradation of these accumulated autotoxic growth inhibitors in the culture solution would lead to sustainable crop production. Our research group applied several ways to eliminate these growth inhibitors including 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) etc. Lee et al. (2006) applied amberlite XAD-4 for the adsorption of allelochemicals in closed hydroponic systems. But, activated charcoal and amberlite XAD-4 created obstructions in circulating nutrients solutions.On the other hand, use of microbial strain can't degrade the accumulated allelochemicals completely. As plant growth recoversfrom the detrimental effects of stresses via the over-production of amino acids, many researchers have suggested that the application of exogenous amino acids may improve the growth and yields of stressed crops (Schat et al., 1997; Maini and Bertucci, 1999; Heuer, 2003). Inour previous study, Mondal et al. (2013) reported that the foliar application of amino acids decreased the effect of autotoxicity and increased the growth and yield of strawberry plants. In particular, the foliar application of hydroxyproline (Hyp) and glutamic acid (Glu) enabled strawberry plants to mitigate theeffects of autotoxicity. Recent studies have revealed that exogenous amino acids can be absorbed by leaves (Furuya and Umemiya, 2002; Stiegler et al., 2013). Additionally, the foliar application of exogenous amino acids positively affects the growth, yield and quality of many crops (Sorwong and Sakhonwasee, 2015; Wahba et al., 2015).

Light conditions may affect the release of growth inhibitors, such as benzoic acid, which is a secondary metabolite associated with photosynthesis. Research findings indicated that allelochemical stress create interference in PSII efficiency, components of

photosystem II photochemistry, chlorophyll fluorescence quenching coefficients and heat energy dissipation in plants (Reigosa and Pazos-Malvido, 2007; Hussain et al., 2008, 2011).Light-emitting diodes are capable of emitting a narrow wavelength band, and are able to produce high-quality light suitable for plant growth. Exposure to a combination of red light (600–700 nm) and blue light (400–500 nm) induces diverse effects on plant growth.Several reports indicated the significant effect of light intensity on photosynthesis and plant growth (Tripathy and Brown, 1995; Miyashita et al., 1997; Yanagi et al., 1996b; Goins et al., 1997).Moreover, photosynthetic activities are particularly effective under red and blue light (Katsumi and Sato, 1985; Sadak et al., 2015) that can improve plant growth and yield in spite of having autotoxicity.

Degradation of toxic compounds by electronic means is another way to detoxify allelochemicals.In our previous study, autotoxicity in hydroponically grown strawberry plant was reported to mitigate through application of ED of root exudates (Asao et al. 2008). In this process, exogenously added benzoic acid to a culture solution was almost completely decomposed within 24hours by direct current electro-degradation (DC-ED). Moreover, they showed that DC-ED application to the culture nutrient solution could result in the decomposition of toxic root exudates, including BA from strawberry plants and mitigate the effect of autotoxicity under closed hydroponics. They also reported that a rapid decomposition of Fe-EDTA in culture solution due to the application of DC-ED. In the following study, it was also found that DC-ED can breakdown the benzoic acid in the nutrient solution but it also decreases the iron and calcium concentrations, pH and increase solution temperature (Asaduzzaman et al., 2012).

1.6. Antioxidant enzyme activity and oxidative damage in plants during allelochemical stress

Plants activate antioxidant defense mechanisms under stresses, which helps in the maintenance of the structural integrity of the cell components and presumably alleviates oxidative damage. Several antioxidant enzymes contribute to plant defense. When plants are exposed to allelochemical stress in closed hydroponics it suffers from the disruption of normal physiological process before ensuing yield loss. Similar to other biotic stresses, in allelopathic reaction an essential function of reactive oxygen species (ROS) was indicated by some authors (Weir et al., 2004; Gniazdowska and Bogatek, 2005; Cruz-Ortega et al., 2007). In allelochemical stress, a shift from a regulatory role of ROS in cell signaling to their toxicity is probably related to changes in homeostasis of ROS maintained by imbalance of ROS production and ROS scavenging. Plants generally evolve a complex system of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR) and some other non-enzymatic antioxidants that accumulate in higher plants under stress condition (Ozkur et al., 2009). Plants also enhance the antioxidants productionunder stress condition in order to scavenge ROS to normalize their metabolic activities. Generation of more ROS than its scavengingindicated oxidative damages in plants. Oxidative damage by allelochemicals stress was investigated in a range of plants, e.g., maize (Mylona et al., 2007), rice (Chi et al., 2011), and soybean (Böhm et al., 2006). Other studies also have shown that allelochemical stress can cause oxidative damage to plants (Bais et al., 2003; Sánchez-Moreiras et al., 2005; Abenavoli, 2006).

1.7.Objective of the present study

With the intention of alleviating autotoxicity in the closed hydroponics system, our research group conducted many studies previously.In our prior experiments, we have investigated the effects of exogenous amino acid application to overcome the autotoxicity problems. Then again, light emitting diodes have significant role in crop growth improvements. Hence, we need to investigate the combined effect oflight emitting diodesand exogenous amino acid application to crops for the improvement of growth, yield and quality under autotoxicity inthe closed hydroponics system. We also faced some problems in our prior experiments related to ED of allelochemicals. The iron and calcium ions were thought to be precipitated to the anode of DC-ED machine. In case of alternating current electro-degradation (AC-ED), positive and negative charges of the electrodes (anode and cathode) changes frequently. Thus, iron and calcium ions might not be precipitated to the electrode (especially the central core).So, we planned to change the power source from direct current to alternating current.It wasalso evident from many studies that allelochemical stress affected the antioxidant systems in plants. Therefore, the present study was conducted-

- to investigate the combined effects of LEDs and amino acids on the retarded growth and yield recovery of strawberry plants from autotoxicity grown in a closedhydroponic system.

- to investigate the effect ofAC-ED machine to degrade the allelochemicals compared to DC-EDmachineand its effect on the growth, fruit yield and qualities of strawberry grown in closed hydroponics.

- to recover the retarded lettuce growth from autotoxicity by means of an AC-ED machine in the successive lettuce cultivation using same nutrient solution.

- toinvestigate the influence of allelochemical stress on the antioxidant system of lettuce grown in the reused solutions under successive closed hydroponic cultivation and it's alleviation by different methods including ED of culture solution.

CHAPTER 2

Light-emitting diodes and exogenous amino acids application improve growth and yield of strawberry plants cultivated in recycled hydroponics

1. Introduction

Autotoxicity, a form of intra-specific allelopathy occurs when a plant releases chemical substances that inhibit or delay its own germination and growth (Putnam, 1985; Singh et al., 1999). In agricultural ecosystems, many plant species are affected by autotoxicity, leading to decreased growth, low yield and replant failures (Singh et al., 1999; Pramanik et al., 2000; Asao et al., 2003). Autotoxicity may develop because of chemicals released in the rhizosphere (Singh et al., 1999) through various mechanisms such as leaching (Overland, 1966), volatilization (Petrova, 1977), root exudation (Tang and Young, 1982), pollen spread in some plants (Cruz-Ortega et al., 1988) and crop residue decomposition (Rice, 1974). Pronounced autotoxicity can occur in plants cultivated in the same soil for several years or grown in recycled hydroponic solutions (Takahashi, 1984; Zhao et al., 2015).

In closed hydroponic systems in which the nutrient solution is recycled, root exudates with highly variable chemical compositions are the common sources of bioactive allelochemicals (Inderjit and Weston, 2003). In fact, root exudates represent one of the largest sources of plant chemicals released into the rhizosphere that are responsible for

chemical interference among plants. The synthesis and exudation of allelochemicals, along with the overall production of root exudates, are typically enhanced by stress conditions; including extreme temperatures, drought conditions, and UV light (Inderjit and Weston, 2003). A previous study revealed that in *Cucumis sativa*, the concentration of benzoic acid (i.e., a major allelochemical) exuded by the roots increase in nutrient solutions with increasing temperature and photoperiod length (Pramanik et al., 2000). Therefore, autotoxicity would be enhanced in strawberry plants with increasing temperature under controlled conditions.

In Japan, the current acreage of hydroponic strawberry production is 627 ha. The commercial hydroponic production of strawberry is responsible for some environmental pollution because of the release of used nutrient solutions. Although recycling of the nutrient solution in closed hydroponic systems is recommended for sustainable agricultural production, these systems may result in the development of autotoxicity because of the accumulation of allelochemicals from root exudates. In addition, autotoxicity in strawberry plants is typically characterized by the development of black root rot disease, which limits strawberry yields (Yuen et al., 1991; Wing et al., 1995; Asaduzzaman et al., 2012). In closed hydroponic systems, strawberry roots release benzoic acid into the nutrient solution (Kitazawa et al., 2005). The accumulation of benzoic acid in the nutrient solution inhibits growth and metabolic activities of strawberry roots, ultimately causing electrolyte levels in cells and root lipid peroxidation activities to increase and the free radical scavenging activity of roots to decrease (Zhen et al., 2003). Additionally, the damaged strawberry roots exhibit impaired uptake of water and mineral nutrients from the nutrient solution.

Consequently, shoot and root growth, the number of flowers and harvested fruits per plant, and fruit development are adversely affected (Kitazawa et al., 2005).

Removing the inhibitory allelochemicals from the nutrient solution or decreasing their inhibitory effects would result in normal growth and fruit yields. Thus, in our previous studies we studied elimination of these chemicals or their harmful effects. We observed that activated charcoal adsorbs the accumulated phytotoxic chemicals from the nutrient solution, and improves the growth and yield of strawberry plants (Kitazawa et al., 2005). In other studies, we revealed that supplementing the plants with auxin (Kitazawa et al., 2007) or degrading the phytotoxic chemicals in strawberry root exudates (Asao et al., 2008; Asaduzzaman et al., 2012) helps prevent autotoxicity in closed hydroponic systems. However, the development of a method for controlling autotoxicity that is suitable for the commercial production of strawberries in a closed hydroponic system would be of considerable value.

Because of the adverse effects of autotoxicity on the uptake of water and minerals, supplying nutrients in alternative ways (e.g., foliar application of amino acids) or improving strawberry plant growth with LEDs may improve strawberry production. Amino acids protect plants from stresses in different ways, including contributing to cellular osmotic adjustments, detoxifying reactive oxygen species, maintaining membrane integrity and stabilizing enzymes/proteins (Yancey et al., 1982; Bohnert and Jensen, 1996). Proline has been reported to accumulate during conditions of drought (Choudhary et al., 2005), high salinity (Yoshiba et al., 1995), high light and UV irradiation (Saito et al., 2012), heavy metal exposure (Saradhi et al., 1995) and in

response to biotic stresses (Fabro et al., 2004; Haudecoeur et al., 2009). As plant growth recovers from the detrimental effects of stresses via the over-production of amino acids, many researchers have suggested that the application of exogenous amino acids may improve the growth and yields of stressed crops (Schat et al., 1997; Maini and Bertucci 1999; Heuer, 2003). Recent studies have revealed that exogenous amino acids can be absorbed by leaves (Furuya and Umemiya, 2002; Stiegler et al., 2013). Additionally, the foliar application of exogenous amino acids positively affects the growth, yield, and quality of marigold (Sorwong and Sakhonwasee, 2015), *Urtica pilulifera* (Wahba et al., 2015), alfalfa (Pooryousef and Alizadeh, 2014), *Codiaeum variegatum* (Mazher et al., 2011) and grapevine (Garde-Cerdán et al., 2015; Portu et al., 2015). Mondal et al. (2013) reported that the foliar application of amino acids decreased the effect of autotoxicity and increased the growth and yield of strawberry plants. In particular, the foliar application of hydroxyproline (Hyp) and glutamic acid (Glu) enabled strawberry plants to avoid the effects of autotoxicity.

Light conditions may affect the release of growth inhibitors, such as benzoic acid, which is a secondary metabolite associated with photosynthesis. Light-emitting diodes have recently attracted attention as an artificial light source for plant production because of their long life and lower heat emission and power consumption compared with fluorescent lamps. Light-emitting diodes are capable of emitting a narrow wavelength band and are able to produce high-quality light suitable for plant growth. Exposure to a combination of red light (600–700 nm) and blue light (400–500 nm) induces diverse effects on plant growth. Additionally, photosynthetic activities are particularly effective under red and blue light (Katsumi and Sato, 1985; Sadak et al., 2015). Therefore,

improving retarded growth and yield of strawberry under autotoxicity through application of different quality and intensity of lights along with amino acid application would be imperative for sustainable crop production. Consequently, the farmers and commercial growers who produce strawberry in greenhouse and also in plant factories through recycled hydroponics would be benefitted. The present study was conducted to investigate the effects of LEDs and amino acids on the improvement of growth and yield of strawberry plants from autotoxicity grown in a recycled hydroponic system.

2. Materials and methods

2.1. Plant materials

Strawberry (*Fragaria* × *ananassa* Duch. var. "Toyonoka") was used in this study. The plantlets were first produced in tissue culture and rapidly multiplied on quarter-strength Murashige and Skoog medium (Murashige and Skoog, 1962), and then transferred to a 6-benzyle adenine free rooting medium. At the two- or three leaf stage the plantlets were acclimated to a vermiculite substrate in cell trays (48 cm × 24 cm × 4 cm; 72 cells/tray). Then the cell trays were kept for about 60 days in a growth chamber set at 20/15°C (day/night) with a 12-h photoperiod (fluorescent light; 145 μmol m^{-2} s^{-1}) and 60% relative humidity. The plantlets were grown with 25% standard 'Enshi' nutrient solution (Table 1, Hori, 1966) to induce the formation of new roots and leaves. At the five- or six-leaf stage, plantlets were transferred to the nursery bed of a hydroponic system in a controlled-environment room with the same conditions as in the growth chamber. Strawberry plantlets were incubated in this nursery until the first cluster of

flowers was observed. The first flower clusters were removed and more homogenous plants were selected as planting materials.

2.2. Hydroponic nutrient solution

Strawberry plants were cultured in 25% standard 'Enshi' nutrient solution [pH 7.25 and electrical conductivity of 0.8 dS m^{-1}] throughout the growth period. The electrical conductivity and pH of the tap water used to prepare the nutrient solution were 0.22 dS m^{-1} and 8.18, respectively.

Table 1. Full strength "Enshi" nutrient solution

Chemicals	Amounts (g/1000 L Tap water)
$Ca(NO_3)_2.4H_2O$	950
KNO_3	810
$MgSO_4.7H_2O$	500
$NH_4H_2PO_4$	155
H_3BO_3	3
$ZnSO_4.7H_2O$	0.22
$MnSO_4$ .4H2O	2
$CuSO_4$.$5H_2O$	0.05
Na_2MoO_4 .$2H_2O$	0.02
NaFe-EDTA	25

2.3. Hydroponic systems and cultivation procedures

This study was conducted in the plant factory of the Experimental Research Center for Biological Resources Science at Shimane University, Japan. The controlled-environment room was maintained at 60% relative humidity and 880 ppm CO_2, with a 12-h photoperiod. Two experiments were conducted once and were not repeated. In the first experiment, one virus-free and healthy plantlet at the three- or four-leaf stage was added to individual plastic containers (29 cm × 17 cm × 8 cm). Plantlets were supported

by urethane foam blocks (23 mm × 23 mm × 25 mm), which were inserted into small holes in a black plastic floating board that was placed on top of the nutrient solution. Each plastic container was filled with 3 L of 25% standard nutrient solution which was not aerated. After the transplantation was complete, the containers were transferred back to the controlled-environment room that was set at 30/25 °C (day/night) (Fig. 1). The nutrient solution was not renewed throughout the experimental period. The amounts of mineral nutrients remained in the nutrient solution were analyzed and adjusted biweekly. A sample of the used nutrient solution was collected and filtered with qualitative filter paper (Advantec Grade no. 131; 125 mm). The nutrient solution was adjusted with the main nutrients to restore the initial concentrations as much as possible following analyses with a C-141 ion meter (Horiba Ltd., Kyoto, Japan) for NO_3^-, a UV mini 1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) for PO_4^{3-}, and a Z-5010 atomic absorption spectrophotometer (Hitachi, Tokyo, Japan) for K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}.

In the second experiment, plantlets exhibiting similar growth rates and vigor were transplanted to three layered vertical growing beds (125 cm × 90 cm × 10.5 cm). Plantlets were transplanted to a foam bed fixed with urethane cubes (23 mm × 23 mm × 27 mm) and incubated in the controlled-environment room set at 25/20 °C (day/night). In the vertical growing beds, five plants for each treatment were grown in each bed having 50 L nutrient solution capacity. Two beds placed parallel to each other were connected to a tank filled with 200 L nutrient solution. Therefore, each plant was treated with 20 L nutrient solution. The culture solutions were not renewed entirely. There were six individual systems used for six different treatments (three light conditions and with

or without Glu). Nutrient solutions were recycled at 55/5 min (recycle/stop) using an automatic pump. The concentrations of the main nutrients in the nutrient solution were adjusted every three weeks as described for the first experiment. Flowers were pollinated every 2 or 3 days using a calligraphy brush. Fruits were harvested when 80% or more of the fruits had turned red.

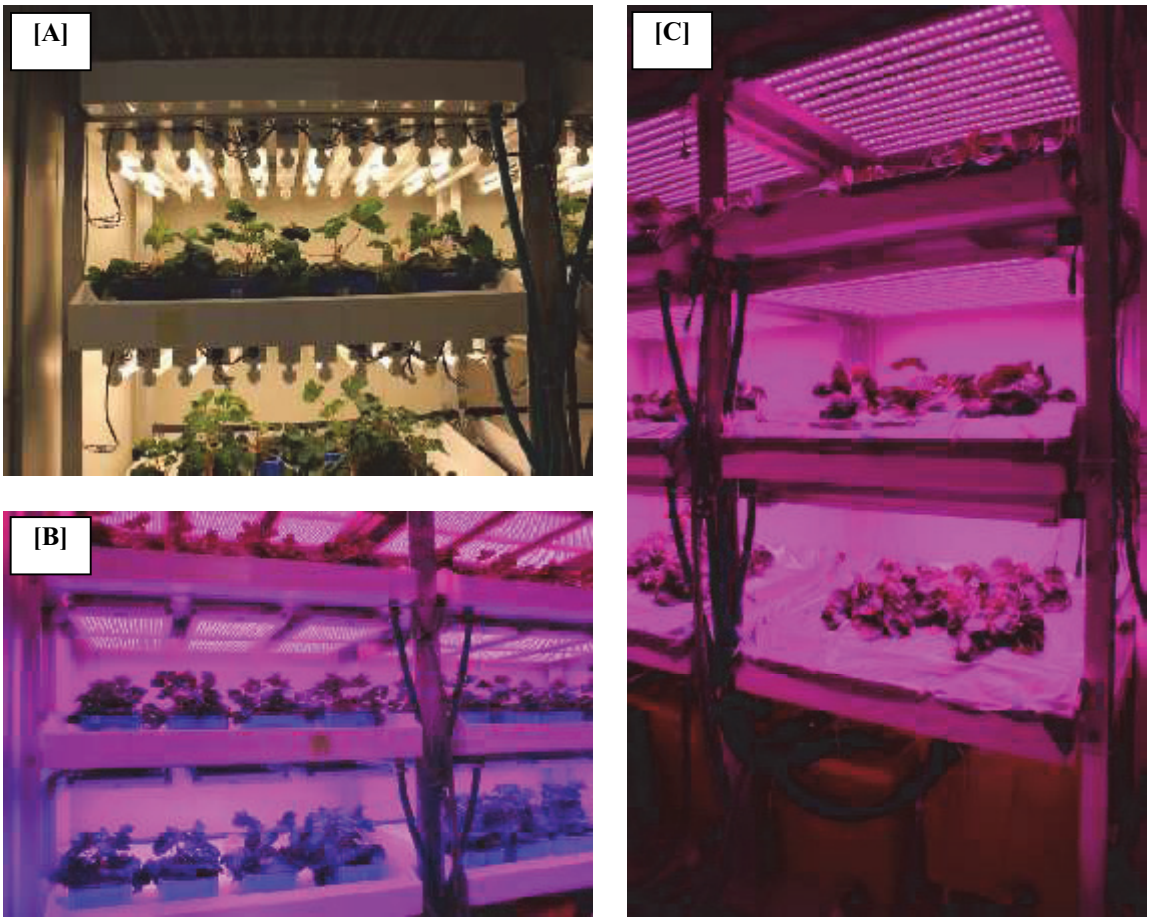


Fig. 1. Three layered vertical growing beds used for cultivation of strawberry plants under controlled-environment. In the first experiment **[A, B]**, plastic container filled with 3 L of 25% standard nutrient solution was used for each plant. In the second experiment **[C]**, each grow bed with 50 L nutrient solution capacity was used. Two beds placed parallel to each other were connected to a tank filled with 200 L nutrient solution.

2.4. Light treatments

For the first experiment, we used three combinations of [Red (660 nm): Blue (450 nm)] LED lights (i.e. 2:8, 5:5, and 8:2) (Showa Denko K.K. Green Innovation Project, Japan), with fluorescent lamps used as a control (Fig. 1A, 1B). High frequency straight tube cool fluorescent lamps (FHF16EX-L-H) were purchased from Panasonic, Japan. All light treatments were adjusted to ensure a similar light intensity (i.e. 106–117, 107–125, 105–121, and 104–129 μmol m^{-2} s^{-1}, respectively) at the surface of the floating board. The light panel was set at about 20 cm above the surface of the plant canopy. Data on irradiance and full width at half maximum (FWHM) of three types of LEDs were measure at 25 ºC (Fig. 2, Table 2). In the second experiment, only one LED combination was used (i.e. R : B = 8:2) with three different intensities (i.e. 149, 269, and 567 μmol m^{-2} s^{-1}) (Fig. 1C). We used MQ-200 Quantum separate sensor with hand held PAR meter (Apogee Instruments, Inc. Logan UT, USA) for measuring PPFD in both the experiments.

Table 2. Peak wavelength and full width at half maximum (FWHM) of three LEDs used in the study.

LED types		Peak wavelength (nm)	FWHM (nm)
R:B = 8:2	Red (8)	659.4	15.1
	Blue (2)	445.6	15.2
R:B = 5:5	Red (5)	659.4	14.6
	Blue (5)	445.6	15.9
R:B = 2:8	Red (2)	658.1	13.8
	Blue (8)	445.6	16.5

2.5. Application of amino acids

Analytical grade amino acids were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Two amino acids [i.e. hydroxyproline (Hyp) and glutamic acid (Glu)] were used in the first experiment, while Glu was used in the second experiment because of its better

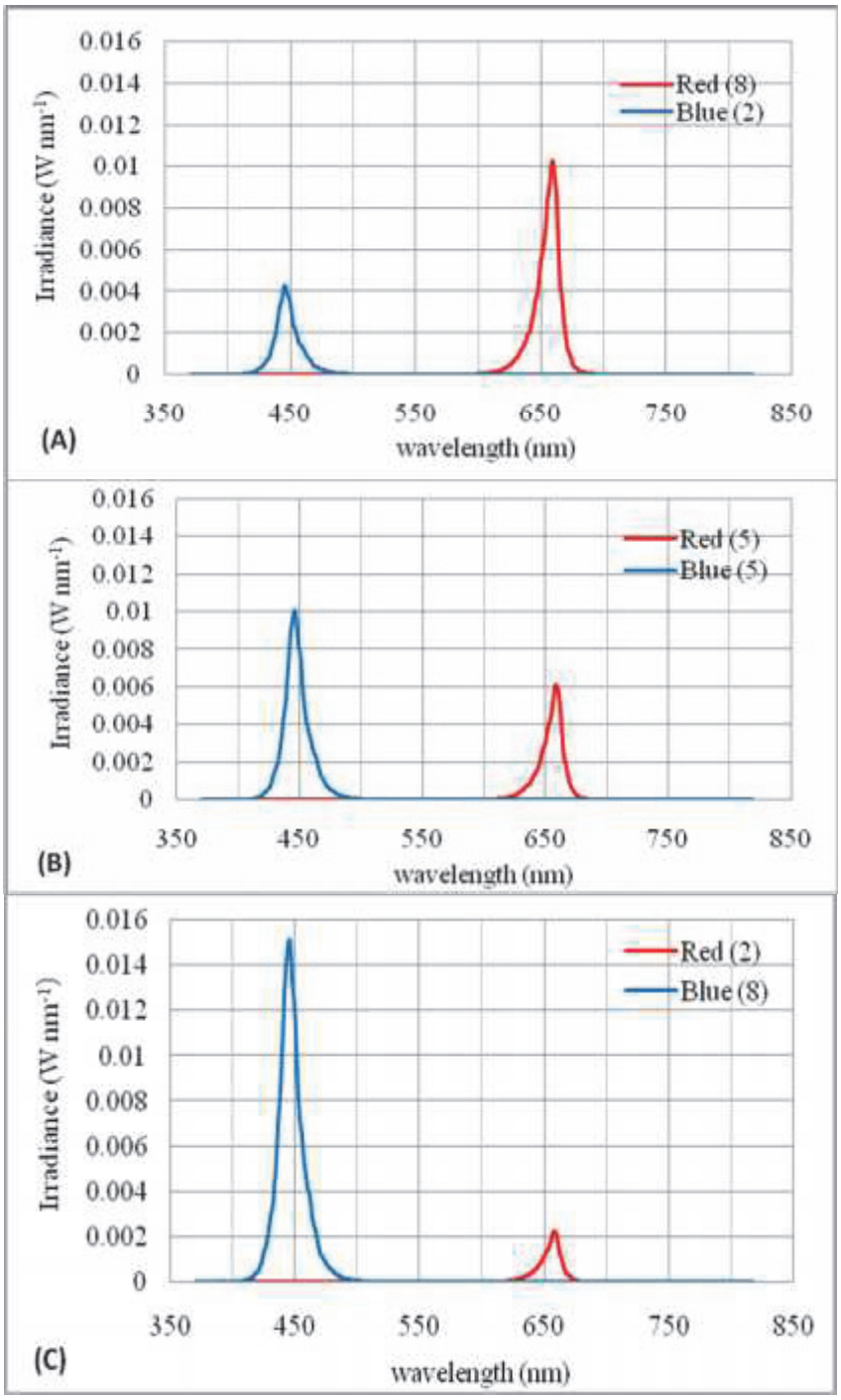


Fig. 2. Irradiance of three types of LEDs, **(A)** Red : Blue = 8:2, **(B)** Red : Blue = 5:5, and **(C)** Red : Blue = 2:8 used in this experiment. The measurement was conducted at 25 ºC. The commercial LEDs were supplied from Showa Denko K.K. Green Innovation Project, Japan.

performance. Amino acids constitute mainly nitrogenous compounds which have great influence on plant growth and development. Therefore, each amino acid concentration was adjusted so the applied nitrogen content was equivalent to that of a 200 ppm proline solution (i.e. 228 ppm Hyp and 319 ppm Glu). Leaves were sprayed with amino acid solutions (1.4 ml plant^{-1}) using a plastic hand spray bottles (Daiso, Japan) three times per week from planting to the final harvest. Control plants were sprayed with distilled water.

2.6. Experimental design

For the first experiment, we used three combinations of LED lights (R : B = 2:8, 5:5, and 8:2) and fluorescent lamps were used as control along with two amino acids and water as control. In the second experiment, three different intensities of R : B = 8:2 LED light and either with or without Glu were used. Both experiments were laid out in completely randomized design with two factors in split plot. Light treatments were applied as main plot factor while amino acid applications were the sub-plot factor. Total twelve treatments in the first experiment and six treatments in the second experiment were applied by the combinations of light condition and amino acids. Each treatment was replicated three times. In the first experiment, one plantlet was planted to each plastic container while five plantlets were grown in each grow bed of hydroponic system.

2.7. Data collection

Data were collected for the following traits: anthesis date; fruit ripening date; number of leaves per plant; maximum leaf length (from the base of the petiole to the tip of the apex

leaflet) and width (from the edge of two leaflets); leaf chlorophyll content [according to a chlorophyll meter (Konica Minolta, Tokyo, Japan)]; crown diameter; leaf, crown, and root dry weight; individual fruit weight and fruit yield per plant. Fruit quality parameters were also analyzed (i.e., total sugar content, citric acid level and ascorbic acid content).

2.8. Determination of fruit quality parameters

After harvest, fruits were frozen at −30°C for subsequent analyses of soluble solids, titratable acidity, and ascorbic acid content following the methods described by Asaduzzaman et al. (2012). Fruit samples were thawed and juiced to determine the above-mentioned strawberry fruit qualities.

2.9. Determination of mineral nutrient contents in plant tissues

Mineral nutrients such as calcium, magnesium, potassium and iron contents in different plant tissues after harvest were analyzed using HNO_3 digestion as described in the Analytical Manual for the Standard Table of Food Composition in Japan (Yasumoto et al. 2006). The leaves, crowns, and roots of plants were dried, ground and digested and mineral nutrients were determined by methods mentioned in our previous report (Asaduzzaman et al. 2012).

2.10. Statistical analysis

Analysis of variance for all data was done with computer package MSTAT-C developed by Russel (1986). The mean differences of the treatments were adjusted by Tukey's test at $P<0.05$.

3. Results

3.1. Experiment-I

3.1.1. Plant growth

Light quality, the application of amino acids and their interaction had significant effect on the number of leaves per strawberry plant, maximum leaf length and crown diameter, but did not significantly affect maximum leaf width, root length and chlorophyll content (Table 3). Among amino acids application, water spray produced the highest number of leaves per plant while in case of light condition it was obtained from R : B = 8:2 LED illumination. The combination of R : B = 8:2 LED and Hyp spray produced the most leaves per plant. All other illumination treatments and amino acid applications produced similar results, except for the R : B = 2:8 LED with Hyp or Glu treatment and the fluorescent light with Glu spray treatment. Comparison among the light treatments revealed that exposure to all light conditions except R : B = 5:5 LED produced the longest leaves. While both Hyp spray and Glu spray but water spray produced longest leaves. The longest leaves (26 cm) were observed in the Hyp spray treated plants under fluorescent light. However, plants treated with water or Glu under fluorescent light produced similar results. Additionally, plants grown under all combinations of LED conditions and amino acid spray except the plants exposed to R : B = 5:5 LED light with water spray and Hyp spray had similar leaves. The crown diameter was widest in Glu spray among the amino acid and in R : B = 8:2 LED among the light condition. Also, interaction of Glu spray and R : B = 8:2 LED produced the widest leaves among all combinations of light and amino acid. Leaf, crown, root and total plant dry weights were unaffected by the amino acid application, while these parameters were

Table 3. Effects of light quality and amino acids spray on the growth of strawberry grown under heat stress condition.

Light quality and amino acid		Number of leaves plant^{-1}	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Crown diameter (mm)	SPAD	DW of leaf (g)	DW of crown (g)	DW of root (g)	Total plant DW (g)
Light quality											
Fluorescent lamp		16.3 b^{z}	25.0 a	16.3 a	30.1 a	17.9 c	51.2 a	10.6 c	1.4 c	2.7 c	14.7 c
LED (R:B = 8:2)		21.3 a	19.3 ab	13.7 a	31.4 a	27.4 a	50.5 a	13.3 a	2.7 a	3.7 a	19.7 a
LED (R:B = 5:5)		19.3 ab	18.3 b	13.7 a	31.8 a	23.3 b	51.0 a	11.3 b	1.7 b	2.9 b	15.9 b
LED (R:B = 2:8)		17.0 b	19.7 ab	14.6 a	30.9 a	23.4 b	49.8 a	9.5 d	1.3 d	2.0 d	12.9 d
Amino acid											
Water		20.0 a	20.3 b	13.9 a	29.8 a	23.3 b	50.2 a	11.5 a	1.9 a	2.9 a	16.3 a
Hydroxyproline		18.8 b	21.0 a	14.8 a	29.7 a	20.9 b	51.2 a	11.5 a	1.8 a	2.7 a	16.0 a
Glutamic acid		16.8 c	20.5 a	15.0 a	33.8 a	24.8 a	50.1 a	10.6 a	1.7 a	3.0 a	15.2 a
Light quality x amino acid											
Fluorescent lamp	Water	19.0 abc	24.0 ab	16.0 a	28.1 a	17.2 j	50.4 a	11.0 b	1.5 c	2.4 c	14.9 d
	Hydroxyproline	16.0 bc	26.0 a	16.7 a	28.1 a	17.9 hi	52.9 a	11.0 b	1.5 c	2.8 bc	15.3 c
	Glutamic acid	14.0 c	25.0 ab	16.2 a	34.1 a	18.5 h	50.3 a	9.7 d	1.2 c	3.0 bc	13.9 e
LED (R:B = 8:2)	Water	21.0 ab	19.0 ab	13.6 a	27.8 a	25.4 e	49.8 a	13.0 ab	2.8 a	4.1 a	19.9 a
	Hydroxyproline	24.0 a	20.0 ab	13.4 a	31.7 a	27.9 b	51.0 a	14.0 a	2.9 a	3.2 abc	20.1 a
	Glutamic acid	20.0 ab	19.0 ab	14.0 a	34.8 a	28.8 a	50.6 a	13.0 ab	2.4 ab	3.7 ab	19.9 a
LED (R:B = 5:5)	Water	19.0 abc	18.0 b	13.0 a	33.2 a	23.7 f	51.5 a	12.0 abc	1.8 b	3.1 abc	16.9 b
	Hydroxyproline	19.0 abc	18.0 b	13.3 a	27.4 a	20.2 g	51.7 a	11.0 b	1.6 b	2.8 bc	15.4 c
	Glutamic acid	20.0 ab	19.0 ab	14.6 a	34.9 a	26.1 d	50.0 a	11.0 b	1.7 b	2.8 bc	15.5 c
LED (R:B = 2:8)	Water	21.0 ab	20.0 ab	12.8 a	29.9 a	27.0 c	49.2 a	10.0 c	1.4 c	1.9 d	13.3 f
	Hydroxyproline	16.0 bc	20.0 ab	15.8 a	31.5 a	17.6 ij	49.3 a	10.0 c	1.2 c	1.9 d	13.1 f
	Glutamic acid	14.0 c	19.0 ab	15.2 a	31.2 a	25.7 de	49.6 a	8.6 d	1.3 c	2.3 c	12.2 g
Significance											
Light quality		*	*	NS	NS	*	NS	*	*	*	*
Amino acid		*	*	NS	NS	*	NS	NS	NS	NS	NS
Light quality x amino acid		*	*	NS	NS	*	NS	*	*	*	*

^{z}Means within column for main-plot factor (light quality), sub-plot factor (amino acid) and their interaction having the same letters are not significantly different according to the Tukey's Test at P <0.05. *Significant and ^{NS}Not significant at 5% level. DW = Dry weight.

significantly affected by the light quality and their interaction (Table 3). An increasing trend in dry weights was observed with increase in red light intensity. The highest dry weight of leaves was obtained from R : B = 8:2 LED with Glu spray treated plants which was similar to plants treated with water spray and Hyp spray under R : B = 8:2 LED. Dry weight production of leaf followed the order as R : B = 8:2 LED > R : B = 5:5 LED> fluorescent lamp > R : B = 2:8 LED regardless of applied amino acid. Almost similar trends of results were observed for the crown dry weight and total plant dry weight.

3.1.2. Yield and fruit quality

The number of days to anthesis and fruit ripening were unaffected by the light, amino acid treatments and their interaction (Table 4). Number of flowers per plant, average fruit weight, number of fruits, and fruit yield per plant were influenced significantly by amino acid and light quality and also by their interaction. Significantly higher number of flowers and fruits per plant were produced by the R : B = 8:2 LED compared to other lights and by Glu spray compared to Hyp spray or water spray. The highest number of flowers and fruits were obtained from plants treated with Glu spray under R : B = 8:2 LED. The R : B = 2:8 LED produced fewer number of flowers and fruits regardless of amino acid application. Similarly, the average fruit weight was significantly higher in R : B = 8:2 LED and also by Hyp spray. However, highest average fruit weight was obtained from plants treated with Glu spray under R : B = 8 : 2 LED. Fruit yield per plant was also significantly higher in R : B = 8 : 2 LED and by Glu spray (Fig. 3). Additionally, the highest fruit yield was obtained from plants treated with Glu spray under R : B = 8 : 2 LED followed by Hyp spray under same light condition. R : B= 5:5

Table 4. Effects of light quality and amino acids spray on the yield attributes and fruit quality of strawberry grown under heat stress condition.

Light quality and amino acid		Number of days to anthesis	Number of days to fruit ripening	Number of flowers plant^{-1}	Number of fruit plant^{-1}	Average fruit weight (g)	Total soluble solids of fruits (%)	Citric acidity of fruits (%)	Ascorbic acid content of fruits (ppm)
Light quality									
Fluorescent lamp		8.6 a^{z}	35.3 a	28.3 b	4.4 b	2.5 b	4.6 c	0.70	24.0 b
LED (R:B = 8:2)		8.5 a	32.8 a	44.0 a	5.7 a	2.7 a	5.6 a	0.62	38.7 a
LED (R:B = 5:5)		7.5 a	32.6 a	21.8 c	2.4 c	2.4 c	5.0 b	0.71	24.8 b
LED (R:B = 2:8)		7.3 a	34.7 a	17.4 d	2.2 d	2.1 d	4.4 d	0.71	17.7 c
Amino acid									
Water		8.1 a	34.2 a	19.7 c	2.9 c	2.3 c	4.9	0.70	24.7 b
Hydroxyproline		8.3 a	33.2 a	28.9 b	3.6 b	2.6 a	4.9	0.62	26.5 ab
Glutamic acid		7.5 a	34.0 a	35.2 a	4.5 a	2.4 b	5.0	0.73	27.8 a
Light quality x amino acid									
Fluorescent lamp	Water	8.0 a	36.5 a	19.0 f	3.6 d	2.4 e	4.6 f	0.74	21.0 cd
	Hydroxyproline	8.4 a	35.0 a	31.0 d	4.0 c	2.8 b	3.7 h	0.63	24.0 bc
	Glutamic acid	9.4 a	34.4 a	35.0 c	5.7 b	2.3 f	3.7 h	0.74	27.0 b
LED (R:B = 8:2)	Water	10.0 a	33.0 a	21.0 ef	4.3 c	2.7 c	5.6 c	0.58	39.2 a
	Hydroxyproline	8.0 a	33.2 a	48.0 b	5.7 b	2.7 c	5.5 d	0.58	39.0 a
	Glutamic acid	7.4 a	31.5 a	63.0 a	7.0 a	2.9 a	6.1 a	0.7	37.8 a
LED (R:B = 5:5)	Water	6.6 a	31.3 a	22.6 e	2.0 gh	2.1 h	5.0 e	0.86	21.8 cd
	Hydroxyproline	8.6 a	32.5 a	22.6 e	2.5 ef	2.6 d	4.7 f	0.63	24.4 bc
	Glutamic acid	7.2 a	34.0 a	20.0 ef	2.7 e	2.4 e	6.0 b	0.63	28.2 b
LED (R:B = 2:8)	Water	7.8 a	36.0 a	16.0 g	1.7 h	2.1 h	4.4 g	0.63	16.6 d
	Hydroxyproline	8.0 a	32.0 a	13.8 g	2.3 fg	2.2 g	4.4 g	0.63	18.6 d
	Glutamic acid	6.0 a	36.0 a	22.5 e	2.7 e	2.1 h	3.7 h	0.86	18.0 d
Significance									
Light quality		NS	NS	*	*	*	*	NS	*
Amino acid		NS	NS	*	*	*	NS	NS	*
Light quality x amino acid		NS	NS	*	*	*	*	NS	*

^{z}Means within column for main-plot factor (light quality), sub-plot factor (amino acid) and their interaction having the same letters are not significantly different according to the Tukey's Test at P <0.05. *Significant and ^{NS}Not significant at 5% level.

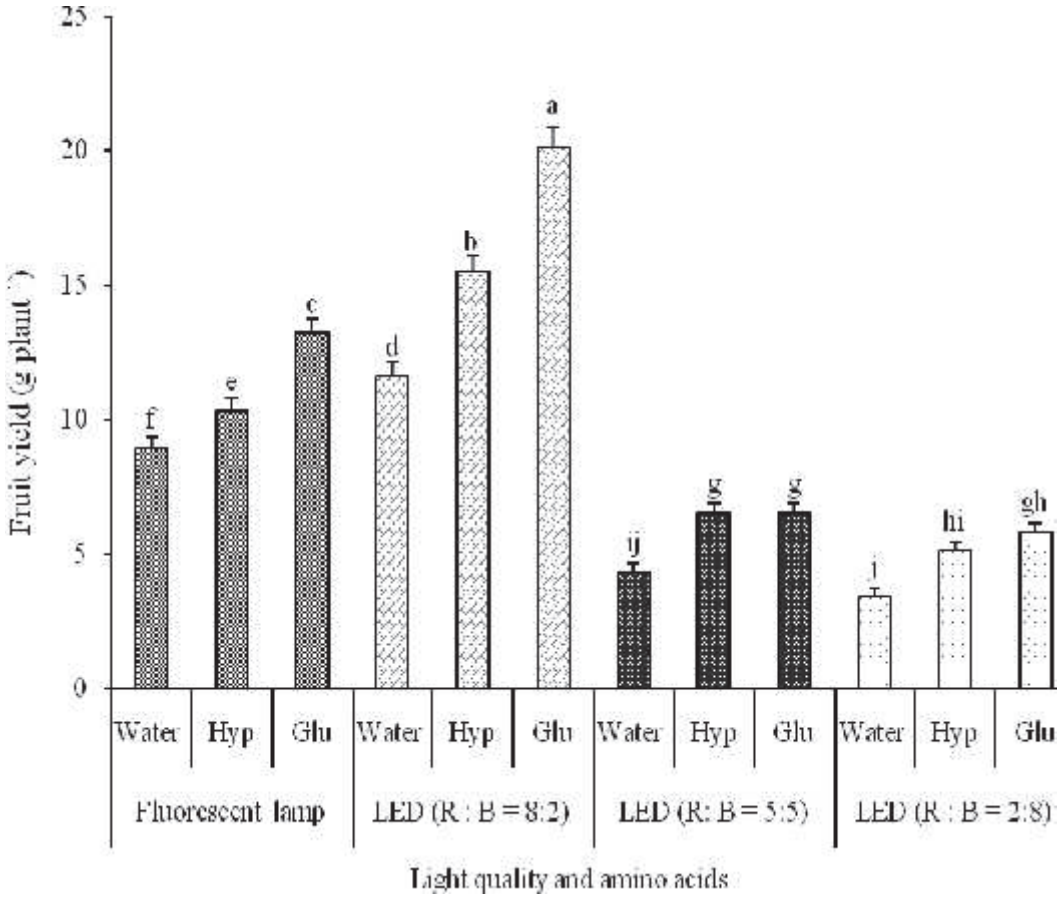


Fig. 3. The effects of light quality and amino acids spray on the fruit yield of strawberry grown under heat stress condition. Bars with the same letter(s) are not significantly different according to the Tukey's Testat $P < 0.05$.

and R : B= 2:8 light produced significantly lower fruit yield per plant irrespective of amino acid spraying.

Citric acid level of fruits was not significantly affected by light or amino acid treatments and their interaction, unlike the total soluble solid and ascorbic acid content (Table 4). Total soluble solid content in fruits was unaffected by amino acid treatment but affected by light condition and their combination. The highest total soluble solid content fruits were found in Glu spray treated plants under R : B = 8:2 LED light illumination.

On the other hand, significantly higher ascorbic acid content was recorded by amino acid and light condition and their interaction. However, fruits with highest ascorbic acid content was obtained from the plants grown under R : B = 8:2 LED light in amino acids and control (water). The LED light R : B = 5:5 and 2:8 produced strawberry fruits with lower ascorbic acid content either with or without amino acid spray than R : B = 8:2 LED. The results revealed that light composition gradient from red to blue (i.e. R : B = 8:2 to 2:8) was associated with a decrease in ascorbic acid concentration.

3.1.3. Mineral nutrient content in plant tissues

Iron, magnesium, and potassium contents in strawberry leaf were significantly affected by the combined effect of light and amino acid treatments (Table 5). The highest leaf iron content (215 mg kg^{-1} DW) was observed for plants sprayed with water and grown under fluorescent lamps. However, this iron content was similar to that of Hyp spray and Glu spray treated plants under the same light conditions. The iron content induced by the R : B = 8:2 LED combined with any amino acid treatment was similar to that of plants grown under fluorescent lamps. Additionally, the R : B = 5:5 or 2:8 LED treatments combined with any spray treatment significantly decreased the leaf iron contents.

The leaf magnesium content was the highest (7.6 mg g^{-1} DW) in plants with Glu spray under fluorescent light. Similar leaf magnesium content were observed for the Hyp spray and water spray treated plants under the same light conditions and for the plants exposed to R : B = 8:2 LED combined with the Glu spray or water treatment. The R : B = 5:5 or 2:8 LED with all spray treatments resulted in relatively low leaf magnesium

Table 5. Effects of light quality and amino acids spray on nutrients content of strawberry plants grown under heat stress condition.

Light quality and amino acid		Fe (mg kg^{-1} DW)		Mg (mg g^{-1} DW)		K (mg g^{-1} DW)		Ca (mg g^{-1} DW)	
		Leaves	Crown	Leaves	Crown	Leaves	Crown	Leaves	Crown
Light quality									
Fluorescent lamp		203 a[z]	239 a	7.4 a	8.7 a	40.5 b	37.4 a	30.5 a	2.6 d
LED (R:B = 8:2)		154 b	221 a	6.5 b	6.5 c	35.1 d	31.2 a	32.6 a	2.8 c
LED (R:B = 5:5)		87 c	122 b	6.1 c	6.5 c	39.1 c	35.8 a	33.8 a	3.0 b
LED (R:B = 2:8)		87 c	148 b	6.0 c	7.5 b	43.4 a	37.8 a	30.1 a	3.6 a
Amino acid									
Water		145 a	180 a	6.6 a	7.5 a	39.7 a	35.4 a	32.6 a	2.9 a
Hydroxyproline		120 b	183 a	6.3 a	7.1 a	39.1 a	34.5 a	31.0 a	3.0 a
Glutamic acid		135 ab	186 a	6.7 a	7.3 a	39.7 a	36.9 a	31.6 a	3.0 a
Light quality x amino acid									
Fluorescent lamp	Water	215 a	225 abc	7.4 a	9.5 a	40.3 ab	34.0 a	31.2 a	3.0 ab
	Hydroxyproline	184 ab	272 a	7.3 a	8.5 ab	40.4 ab	37.8 a	31.5 a	2.5 ab
	Glutamic acid	209 a	221 abc	7.6 a	8.3 ab	40.7 a	40.5 a	28.8 a	2.1 b
LED (R:B = 8:2)	Water	188 ab	254 ab	6.7 ab	6.5 c	35.7 b	31.1 a	30.3 a	2.5 ab
	Hydroxyproline	123 ab	196 abc	6.2 b	6.3 c	34.2 b	29.5 a	33.6 a	2.9 ab
	Glutamic acid	150 ab	218 abc	6.6 ab	6.8 bc	35.5 b	33.1 a	33.7 a	3.0 ab
LED (R:B = 5:5)	Water	86 b	105 d	6.3 b	6.5 c	39.7 ab	38.7 a	34.8 a	2.7 ab
	Hydroxyproline	86 b	103 d	5.7 b	6.2 c	37.8 ab	33.1 a	31.0 a	3.0 ab
	Glutamic acid	88 b	159 c	6.4 ab	6.7 bc	39.7 ab	35.6 a	35.6 a	3.2 ab
LED (R:B = 2:8)	Water	89 b	135 c	6.0 b	7.5 bc	43.1 a	37.6 a	34.2 a	3.5 ab
	Hydroxyproline	84 b	161 b	5.9 b	7.4 bc	44.2 a	37.3 a	27.9 a	3.6 a
	Glutamic acid	93 b	149 c	6.1 b	7.6 bc	42.8 a	38.5 a	28.3 a	3.7 a
Significance									
Light quality		*	*	*	*	*	NS	NS	*
Amino acid		*	NS	NS	NS	NS	NS	NS	NS
Light quality x amino acid		*	*	*	*	*	NS	NS	*

[z]Means within column for main-plot factor (light quality), sub-plot factor (amino acid) and their interaction having the same letters are not significantly different according to the Tukey's test at P <0.05. [*]Significant and [NS]Not significant at 5% level.
DW = Dry weight.

content. The leaf potassium content was the highest (44.2 mg g^{-1} DW) following the Hyp spray treatment under the R : B = 2:8 LED condition. All other light conditions and spray treatments produced similar leaf potassium contents, except for the R:B = 8:2 LED, which resulted in lower leaf potassium contents regardless of the spray treatments.

For the crowns, the abundance of all minerals, except for potassium was significantly affected by the combined effects of exogenous amino acids and LED conditions (Table 5). The iron content was the highest (425 mg kg^{-1} DW) in plants that were sprayed with water and grown under fluorescent light. Overall, the fluorescent light treatment produced the highest crown iron contents irrespective of the applied amino acid. Similar results were observed for magnesium. In contrast, the crown calcium content was the highest (3.7 mg g^{-1} DW) in Glu spray treated plants under the R : B = 2:8 LED light. All other light and spray treatments induced similar crown calcium contents, with the exception of the fluorescent light condition combined with the Glu spray treatment.

3.2. Experiment-II

3.2.1. Growth parameters

Light intensity and the combined effect of light intensity and Glu spray showed a significant effect on the number of leaves per strawberry plant, crown diameter, and root length, while the individual Glu spray treatment did not (Table 6). The plants exposed to high-light intensity with the Glu spray treatment produced the most leaves per plant. Plants treated with high-light intensity without Glu and those exposed to medium-light intensity with or without Glu had a similar number of leaves per plant. In contrast, the low-light intensity with or without Glu produced fewer leaves per plant.

Table 6. Effects of LED light intensity and Glutamic acid on the growth of strawberry plant grown in controlled environment facilities.

LED intensity and Glutamic acid		No. of leaves plant^{-1}	Leaf length (cm)	Leaf width (cm)	Crown diameter (mm)	Root length (cm)	SPAD	Dry weight (g plant^{-1})			Total plant DW (g)
								Leaf	Crown	Root	
LED intensity[z]											
Low		13.9 c[y]	21.4 a	15.3 a	14.8 c	41.8 b	45.6 a	10.9 c	1.8 c	1.7 c	14.4 c
Medium		20.0 b	20.1 a	16.8 a	20.3 b	54.8 ab	51.1 a	14.9 b	2.8 b	2.1 b	19.8 b
High		24.8 a	18.8 a	14.8 a	24.7 a	58.6 a	51.1 a	24.2 a	6.0 a	4.0 a	34.1 a
Glutamic acid[x]											
Glu (-)		18.7 a	19.9 a	15.1 a	18.3 a	47.8 a	48.7 a	16.4 a	3.3 a	2.4 a	22.1 a
Glu (+)		20.4 a	20.3 a	16.0 a	21.5 a	55.6 a	49.8 a	16.9 a	3.8 a	2.7 a	23.4 a
LED intensity x Glutamic acid											
Low	Glu (-)	12.4 c	20.6 a	14.9 a	13.5 c	36.5 b	45.8 a	10.9 b	1.7 c	1.6 b	14.2 d
	Glu (+)	15.4 bc	22.2 a	15.6 a	16.1 c	47.0 ab	45.4 a	11.0 b	1.9 c	1.7 b	14.6 d
Medium	Glu (-)	19.6 abc	18.8 a	16.0 a	18.7 b	52.2 ab	49.8 a	15.5 b	2.3 bc	1.8 b	19.6 c
	Glu (+)	20.4 abc	21.3 a	17.5 a	21.8 b	57.4 a	52.4 a	14.3 b	3.3 b	2.4 b	20.0 c
High	Glu (-)	24.2 ab	20.2 a	14.5 a	22.7 a	54.6 a	50.6 a	22.9 a	5.8 a	3.9 a	32.6 b
	Glu (+)	25.4 a	17.4 a	15.0 a	26.7 a	62.5 a	51.6 a	25.4 a	6.1 a	4.1 a	35.6 a
Significance											
LED intensity		*	NS	NS	*	*	NS	*	*	*	*
Glutamic acid		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LED intensity x Glutamic acid		*	NS	NS	*	*	NS	*	*	*	*

[z]LED intensity low, medium and high are 149, 269 and 567 μ mol m^{-2} s^{-1}.

[y]Values in a column for main-plot factor (LED intensity), sub-plot factor (Glutamic acid) and their interaction having the same letters are not significantly different according to the Tukey's test at $P < 0.05$.

[x]Glutamic acid applied (+), not applied (-).

[*]Significant and [NS]Not significant at the 5% level

Similarly, the widest crown diameter (26.7 mm) was observed for the high-light intensity and Glu spray treatment, although the high-light intensity without the Glu spray resulted in a similar crown diameter. We observed that the crown diameter decreased with decreasing light intensity, with the Glu spray having no significant effect. The longest roots (62.5 cm) were observed following the treatment with high-light intensity and the Glu spray, although the Glu spray did not have a significant effect. Additionally, the root lengths were similar in plants exposed to medium-light intensity, regardless of the spray treatment. In contrast, the low-light intensity treatment with or without the Glu spray produced relatively shorter roots. The length, width, and chlorophyll content of leaves were not significantly affected by either light intensity, Glu spray treatment or their interaction.

The dry weights of strawberry leaves, crowns, roots and total plant dry weight were significantly affected either by LED intensity or combination of LED intensity and Glu spray, but not by Glu spray alone (Table 6). The plants treated with high-light intensity with and without the Glu spray had similar dry weights. Furthermore, the highest dry weights of leaves, crown, root and total plant were observed as 25.4, 6.1, 4.1, and 35.6 g $plant^{-1}$, respectively following the high-light intensity with Glu spray. These dry weights were similar to those of plants treated with high-light intensity without the Glu spray. The leaf, crown, root and total plant dry weights resulting from exposures to the low-light and medium-light intensities (with or without the Glu spray) were lower than those of the plants treated with high-light intensity.

3.2.2. Yield and quality of fruits

The number of fruits per plant and average fruit weight were significantly affected by light intensity and the interaction effect of light intensity and Glu spray, but not by the Glu spray alone (Table 7). However, fruit yield per plant was significantly affected by both Glu spray and light intensity and their interaction (Fig. 4). Plants treated with high-light intensity treatment without the Glu spray resulted in the second highest number of fruits per plant, which was similar to the fruit yield per plant due to the treatments with medium-light intensity (with or without the Glu spray) or low-light intensity with the Glu spray. The low-light intensity treatment without the Glu spray generated the fewest fruits per plant. The greatest average fruits were collected from plants grown under high-light intensity without Glu applications. However, the Glu spray treatment had no significant effect. The plants grown under low-light and medium-light intensities produced smaller average fruits than medium-light intensity. The total fruit weight was the highest (249.0 g $plant^{-1}$) for the plants exposed to high-light intensity and Glu, followed by the plants treated with high-light intensity without Glu (175.0 g $plant^{-1}$) and the plants exposed to medium-light intensity with Glu (Fig. 4). The lowest total fruit weights (60.6 g $plant^{-1}$) were recorded for the plants grown under low-light intensity with no Glu spray treatment. Total soluble solid content was significantly affected by light intensity and by the combined effect of light intensity and Glu spray, but not by Glu alone (Table 7). The ascorbic acid and citric acid levels were unaffected by light intensity, Glu application, or their interaction. The highest total soluble solid content (7.3%) was observed in plants treated with high-light intensity with or without Glu, while the lowest soluble solid content (5.3%) was obtained for the plants exposed to low-light intensity without Glu.

Table 7. Effects of LED light intensity and Glutamic acid on yield attributes and quality of strawberry grown in controlled environment facilities.

LED intensity and Glutamic acid		Number of fruit plant^{-1}	Average fruit weight (g)	Total soluble solid (%)	Citric acidity (%)	Ascorbic acid (ppm)
LED intensity[z]						
Low		15.6 c[y]	4.9 c	5.4 b	0.44 a	377.4 a
Medium		22.9 b	5.3 b	6.3 ab	0.44 a	360.0 a
High		31.5 a	6.9 a	7.2 a	0.44 a	387.9 a
Glutamic acid[x]						
Glu (-)		19.7 a	5.6 a	6.2 a	0.43 a	381.6 a
Glu (+)		26.9 a	5.8 a	6.4 a	0.44 a	368.6 a
LED intensity x Glutamic acid						
Low	Glu (-)	13.2 c	4.6 b	5.3 c	0.44 a	389.9 a
	Glu (+)	18.0 bc	5.1 b	5.5 c	0.43 a	364.9 a
Medium	Glu (-)	20.2 bc	5.2 b	6.2 b	0.44 a	368.4 a
	Glu (+)	25.6 b	5.4 b	6.3 b	0.44 a	351.5 a
High	Glu (-)	25.8 b	6.9 a	7.0 a	0.42 a	386.4 a
	Glu (+)	37.2 a	6.8 a	7.3 a	0.45 a	389.4 a
Significance						
LED intensity		*	*	*	NS	NS
Glutamic acid		NS	NS	NS	NS	NS
LED intensity x Glutamic acid		*	*	*	NS	NS

[z]LED intensity as low, medium and high are 149, 269 and 567 µ mol m^{-2} s^{-1}.

[y]Values in a column for main-plot factor (LED intensity), sub-plot factor (Glutamic acid) and their interaction having the same letters are not significantly different according to the Tukey's test at $P < 0.05$.

[x]Glutamic acid applied (+), not applied (-).

[*]Significant and [NS]Not significant at the 5% level.

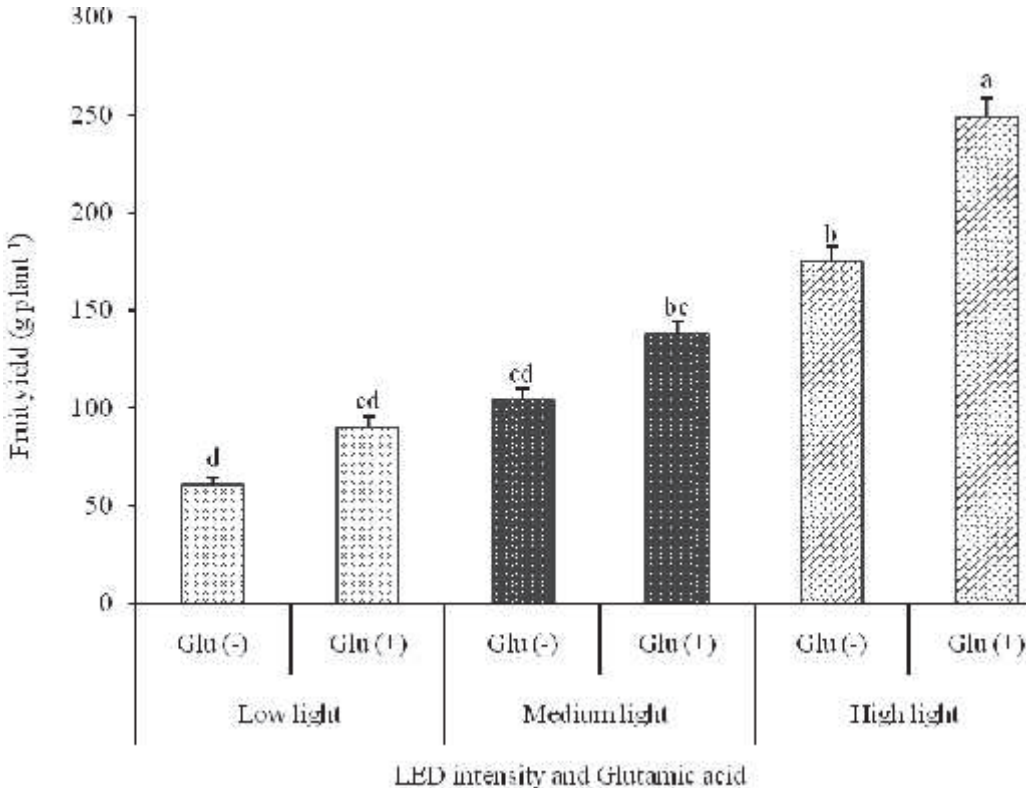


Fig. 4. The effects of LED light intensity and Glutamic acid application on the fruit yield of strawberry grown in controlled environment facilities. Bars with the same letter are not significantly different according to the Tukey's test at $P < 0.05$. LED intensity low, medium and high are 149, 269 and 567 μ mol m^{-2} s^{-1}.Glutamic acid applied (+), not applied (-).

3.2.3 Mineral nutrient content in plant tissues

Light intensity and Glu spray treatments had no significant effects on potassium and magnesium contents in the crowns, leaves, and roots (Table 8). In these plant parts, calcium and iron content was significantly affected by either light intensity or the interaction with Glu spray treatment but Glu spray application showed no significant effect. Exposure to high-light intensity resulted in the highest root calcium contents (75.0 mg^{-1} g DW with Glu and 79.0 mg g^{-1} DW without Glu) and crown calcium

Table 8. Effects of LED quantities and Glutamic acid on mineral nutrient content of strawberry plant grown in controlled environment facilities.

LED intensity and Glutamic acid		K (mg g^{-1} DW)			Mg (mg g^{-1} DW)			Ca (mg g^{-1} DW)			Fe (mg kg^{-1} DW)		
		Root	Crown	Leaf	Root	Crown	Leaf	Root	Crown	Leaf	Root	Crown	Leaf
LED intensity[z]													
Low		26.0 a[y]	35.0 a	52.2 a	13.1 a	69.6 a	13.9 a	34.1 b	38.6 b	44.5 a	1455 b	564 b	84 b
Medium		28.8 a	36.0 a	52.4 a	11.4 a	70.8 a	12.9 a	72.0 a	40.9 b	53.5 a	1631 a	601 b	95 b
High		31.4 a	32.2 a	45.5 a	14.1 a	75.6 a	11.4 a	77.0 a	63.6 a	49.3 a	1623 a	1022 a	131 a
Glutamic acid[x]													
Glu (-)		27.7 a	34.5 a	49.1 a	13.2 a	73.6 a	12.9 a	60.8 a	45.7 a	48.9 a	1589 a	723 a	100 a
Glu (+)		29.7 a	34.3 a	51.0 a	12.5 a	70.4 a	12.5 a	61.2 a	49.8 a	49.3 a	1550 a	734 a	107 a
LED intensity x Glutamic acid													
Low	Glu (-)	24.8 a	34.9 a	49.8 a	13.9 a	68.4 a	14.0 a	33.6 b	37.4 b	38.6 b	1463 b	554 b	94 b
	Glu (+)	27.2 a	35.2 a	54.6 a	12.2 a	70.8 a	13.8 a	34.6 b	39.8 b	50.4 a	1446 b	574 b	74 c
Medium	Glu (-)	26.7 a	35.8 a	51.6 a	11.1 a	72.4 a	13.7 a	69.9 a	39.8 b	58.8 a	1670 a	666 b	82 b
	Glu (+)	30.9 a	36.2 a	53.1 a	11.6 a	69.2 a	12.0 a	74.0 a	42.1 b	48.2 a	1592 a	536 b	107 b
High	Glu (-)	31.7 a	32.8 a	45.9 a	14.5 a	80.0 a	11.1 a	79.0 a	59.8 a	49.2 a	1634 a	950 b	123 ab
	Glu (+)	31.0 a	31.5 a	45.2 a	13.8 a	71.2 a	11.8 a	75.0 a	67.5 a	49.4 a	1611 a	1093 a	139 a
Significance													
LED intensity		NS	NS	NS	NS	NS	NS	*	*	NS	*	*	*
Glutamic acid		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LED intensity x Glutamic acid		NS	NS	NS	NS	NS	NS	*	*	*	*	*	*

[z]LED intensity as low, medium and high are 149, 269 and 567 μ mol m^{-2} s^{-1}.

[y]Values in a column for main-plot factor (LED intensity), sub-plot factor (Glutamic acid) and their interaction having the same letters are not significantly different according to the Tukey's test at $P < 0.05$.

[x]Glutamic acid applied (+), not applied (-).

[*]Significant and [NS]Not significant at the 5% level.

DW = Dry weight.

contents (67.5 mg g^{-1} DW with Glu and 59.8 mg g^{-1} DW without Glu). There were no significant differences in the leaf calcium contents under all light intensities with or without the Glu spray treatment, except for the plants treated with low-light intensity and Glu, which had lower leaf calcium contents. Additionally, the plants grown under high-light intensity with the Glu spray treatment produced the highest iron contents in the roots, crowns, and leaves.

4. Discussion

In recent investigations of autotoxicity in strawberry plants under a closed hydroponic system, several researchers (Kitazawa et al., 2005, 2007; Asao et al., 2008; Asaduzzaman et al., 2012; Mondal et al., 2013) identified the responsible allelochemicals and suggested possible ways of overcoming this phenomenon. They revealed that amino acid supplements could ameliorate the negative effects of autotoxicity in strawberry plants grown under greenhouse condition and also in In vitro condition. Other studies reported that, high-temperature conditions enhanced the exudation of allelochemicals from plants under recycled hydroponics (Pramanik et al., 2000). It caused physiological, biochemical and molecular changes affecting metabolism, such as lipid liquefaction or disruption of membrane integrity (Levitt, 1980). Heat stress was also found to enhance the production and exudation of allelochemicals that promote autotoxicity (Inderjit and Weston, 2003). Although strawberry plants are a temperate crop with optimal growth temperatures of 10-26 °C (Ledesma et al., 2004) as a field- and greenhouse-grown crop, they are often subjected to high temperature. Addressing autotoxicity problem in recycled hydroponics, we studied the effect of LED light and amino acids on the recovery of growth and yield in strawberry grown in relatively higher temperature (30/25 °C; day/night) settings. In this

experiment, strawberry plants were grown under different LED lights along with amino acids application.

In the first experiment, we observed that some growth parameters, such as leaf number, leaf width, and leaf length, root, and crown dry weights were enhanced by amino acid application and LED light (Table 3). Research results showed that foliar application of amino acids increases the dry weights in bean (Nassar et al., 2003) and onion (Amin et al., 2011). As amino acids are the precursors that used during chlorophyll synthesis, their supplementation may affect dry matter production in plants (Yaronskya et al., 2006). In particular, Hyp and Glu were found to increase strawberry plant dry weight under allelochemical stress conditions (Mondal et al., 2013). Moreover, the foliar application of amino acids increases plant protein contents, which ultimately increases the dry matter (Das et al., 2002). The underlying mechanism is that when plants experience autotoxicity, ion uptake and hydraulic conductivity (i.e. water uptake) are affected because the roots are the first plant parts to encounter the autotoxins accumulated in the rhizosphere (Blum et al., 1999). An alternative means of mineral nutrients absorption other than the roots may help to mitigate the effects of autotoxicity to ensure sustainable growth and productivity of strawberry plants. In our present study, spraying Hyp and Glu showed positive influence on the growth and yield of strawberry.

It also revealed that yield contributing characters such as number of flowers per plant and number of fruits were greatly influenced by Glu spraying and R : B= 8:2 LED treatment. Fruit yield was significantly higher in plants grown under R : B = 8:2 LED either with Glu spray followed by Hyp spray under same light condition. Whereas plants under R:B= 5:5 and R:B= 2:8 LED produced significantly lower fruit yield

irrespective of amino acids applied (Table 4). In addition, iron and magnesium contents in strawberry leaves were found higher under R : B = 8:2 LED and also in fluorescent light treated with either amino acids or water (Table 5). The greater improvement in overall strawberry plant performance induced by the R : B = 8:2 LED might be due to the higher proportion of red light. Application of LEDs with precisely adjusted spectral composition of light may provide better control over plant stress responses. Recently, LED supplemental lighting was reported to accelerate the photosynthetic activities and promote the growth of strawberry plants (Hidaka et al. 2013). A comparison of the photosynthetic rates of strawberry leaves exposed to red (660 nm) or blue (450 nm) LEDs indicated that red light leads to higher quantum efficiency (Yanagi et al., 1996a) while blue LEDs at 30 μmol m^{-2} s^{-1} or red LEDs at 100 μmol m^{-2} s^{-1} found to restore chlorophyll synthesis in wheat seedlings (Tripathy and Brown, 1995). Other researchers also observed better plant responses to red and blue LED combinations in various crops, including increased total biomass in red leaf lettuce (Stutte et al., 2009), enhanced chlorophyll a and b accumulation in kale plants (Lefsrud et al., 2008) and increased growth of lettuce, spinach and radish (Yorio et al., 1998).

We provided LED lights to strawberry plants under relatively higher growing temperatures to enhance autotoxicity phenomenon, with a view that it can alleviate the heat stress condition. Plant biochemical responses to different stressors can be triggered by precise changes to the light spectral composition, which can be induced with LEDs. These light sources emit low heat and UV radiation and they can be operated at a fraction of the cost of fluorescent lights. It is reported that LEDs may be more suitable for plant cultures than many other light sources (Massa et al., 2008). Studies by the

Wisconsin group confirmed the necessity of supplementing high-output red LEDs with blue light to promote acceptable plant growth (Hoenecke et al., 1992).

It is mentionable that, in the first study, the overall performances of strawberry plant were lower than the optimum level. The main reason was associated with the higher growing temperature (30/25 °C; day/night) which restrict the optimum plant growth and development, and lack of aeration. Thus, influence of exogenous amino acid application and also red and blue light ratios was not pronounced greatly. Still positive influence of R : B = 8:2 LED along with Glu application was observed. In the following studies, different intensities of R : B = 8:2 LED with or without Glu application was investigated under optimum growth condition at 25/20 °C (day/night) in the plant factory research facilities of Shimane university. Strawberry production in the plant factory doesn't face the heat stress but growing in the recycled hydroponics creates autotoxicity. In the plant factory, artificial lights especially LEDs are the main source of light. Therefore, in this present study, influence of R : B= 8:2 LED with varied intensities along with Glu were investigated to overcome autotoxicity under recycled hydroponics.

In the second experiment, plants exposed to high-light intensity showed greater performances in terms of number of leaves per plant, crown diameter, root length and dry weights of the roots, shoots, and crowns (Table 6). However, significantly similar positive influence was observed either with or without Glu spray. Results also indicated that high-light intensity provided by the R : B = 8:2 LED treatment increases strawberry fruit yields, while Glu can compensate for the effects of decreased light intensity. Spraying Glu in combination with R : B= 8:2 LED might improve the strawberry

growth and development under autotoxicity stress through supplying nitrogenous compounds via leaf stomata. Amino acids are the nitrogenous compound which can be absorbed by leaf exogenously (Furuya and Umemiya, 2002; Stiegler et al., 2013). Recent research revealed that foliar application of amino acids has positive influence on the growth, yield and quality of alfalfa (Pooryousef and Alizadeh, 2014), Chinese cabbage (Cao et al., 2010); leafy radish (Liu et al., 2008) and Japanese pear (Takeuchi et al., 2008). Moreover, it is reported to act as bio-stimulants in plant under abiotic and biotic stress conditions (Maini and Bertucci, 1999; Heuer, 2003; Sadak et al., 2015).

Glutamic acid, in particular is important for nitrogen metabolism, and it is preferred as amino-donor for the different aminotransferase reactions of subsequent amino acid inter-conversions (Lea and Ireland, 1999). Ohyama et al. (2017) presented that, during amino acid metabolism in soybean plant, ammonium ion (NH_4^+) is first assimilated into Glutamine (Gln) combined with Glu by the enzyme glutamine synthetase. As it was found in our second study, higher rate and intensity of red light LED was widely accepted to enhance photosynthesis in plants. It was reported that, the red wavelengths (600 to 700 nm) were efficiently absorbed by plant pigments (Sager and McFarlane, 1997). Red LEDs were also considered as the most efficient emitting at 660 nm, close to an absorption peak of chlorophyll which saturated phytochrome resulting in high-Pfr photostationary state (Massa et al., 2008). Lettuce plants grown under red LEDs alone had more leaves and longer stems than plants grown under blue LEDs only (Yanagi et al., 1996b). In our studies, R : B = 8:2 LED light at an intensity of 567 μmol m^{-2} s^{-1} combined with the foliar application of Glu, increase the growth and yield of strawberry plants in closed hydroponic systems.

5. Summary

In the present studies, we investigated the use of LED (R : B) and exogenous amino acid in order to improve the growth and yield of strawberry plants grown in recycled hydroponics, where accumulation of root exudates caused autotoxicity. The first study was conducted under relatively higher temperature (30/25 °C; day/night), which enhanced development of autotoxicity, we targeted to reduce through artificial lighting and also amino acid application. We observed greater vegetative growth, yield attributes, fruit yield and minerals (iron and magnesium) content in plant parts of strawberry due to R : B= 8:2 LED lighting and Glu spraying. However, the overall performances of strawberry plant were lower than the optimum level which was mainly associated with the higher growing temperature (30/25 °C; day/night) that restrict optimum plant growth and development. Thus, influence of exogenous amino acid application and also red and blue light ratios was not pronounced greatly. While in the second study, plants exposed to R : B= 8:2 LED (567 μmol m^{-2} s^{-1}) showed greater performances on growth and several mineral content in strawberry plant supplied either with or without Glu. Fruits number and yield per plants were higher with Glu than the ones sprayed without Glu. Therefore, the use of LED (R : B = 8:2) at higher intensity along with Glu application may improves growth and yield of strawberry plants grown in a closed hydroponics and thus alleviate the inhibitory effect of autotoxicity. Further research is required to characterize the mechanisms underlying the improved growth induced by amino acid supplementation. Additionally, different LED spectral conditions may positively influence plants affected by autotoxicity, and would be the focus of our future investigations.

CHAPTER 3

Electro-degradation of culture solution improves growth, yield and quality of strawberry plantsgrown in closed hydroponics

1. Introduction

Hydroponic culture of a wide variety crops has been practiced in many countries since the 1950s and the use of closed hydroponic systems has been encouraged recently (Ruijs, 1995; Van Os, 1995) to reduce environmental pollution and the cost of supplementary nutrients. Strawberry has also been grown hydroponically for higher yield and better quality compared to soil cultivation. In protected cultivation technique, large-scale production of strawberry through open system hydroponics discharge once used nutrient solution to the environment causing pollution and wastage of costly fertilizers. Therefore, commercial strawberry growers practiced closed hydroponic system for sustainable production (Takeuchi, 2000; Oka, 2002). However, under this closed hydroponic culture technique, autotoxicity- a form of intra-specific allelopathy develops due to continuous accumulation of allelochemicals in the culture solution (Asao et al., 2003, 2007; Kitazawa et al., 2005). It is known that, this autotoxicity phenomenon occurs when a plant releases toxic chemical substances into the environment that inhibit germination and growth of same plant species (Miller, 1996; Singh et al., 1999).

In strawberry, autotoxicity from root exudates has been studied in closed hydroponics and benzoic acid was confirmed as the most potent growth inhibitor (Kitazawa et

al.,2005). Other studies showed that, when root exudates accumulated in their growing medium, the growth and metabolism of strawberry roots were inhibited, which resulted in an increase in the percentages of electrolytes in cells, a decrease in the free radical scavenging activity of roots, and an increase in root lipid peroxidation (Zhen et al., 2003). Under autotoxicity condition, damaged strawberry roots hamper water and mineral nutrient uptake. As a result, the growth of shoot and root, number of flowers and harvested fruit per plant and fruit enlargement greatly reduced (Kitazawa et al., 2005).

Elimination of the accumulated root exudates or autotoxic growth inhibitors from closed hydroponic system would be of great interest to the strawberry grower. The removal or degradation of these accumulated autotoxicgrowth inhibitors in the culture solution would lead to sustainable strawberry production. Our research group applied several ways to detoxify these exudates including 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) etc. Degradation of toxic compounds by electronic means is another way to detoxify allelochemicals. Phenolic compounds in aqueous solutions were found to decompose when treated by electro-degradation (ED) such as phenol (Comninellis and Pulgarin, 1991; Feng and Li, 2003; Fleszar and Ploszynka, 1985), catecol (Comninellis and Pulgarin, 1991) and hydroquinone (Comninellis and Pulgarin, 1991; Fleszar and Ploszynka, 1985), in aqueous solutions and benzene (Fleszar and Ploszynka, 1985). These compounds are oxidized rapidly at the anode and decompose to CO_2 (Comninellis and Pulgarin, 1991; Feng and Li, 2003; Fleszar and Ploszynka, 1985). Therefore, ED can also be applied to decompose

allelochemicals, including benzoic acid exuded into the culture solution from plants and could be useful to mitigate autotoxicity in the hydroponic cultivation of strawberry. In our previous study, autotoxicity in hydroponically grown strawberry plantwas reported to mitigate through application ofED of root exudates (Asao et al., 2008). In this process, exogenously added benzoic acid to a culture solution was almost completely decomposed within 24hours by direct current electro-degradation (DC-ED). Moreover, they showed that DC-ED application to the culture nutrient solution could result in the decomposition of toxic root exudates, including BA from strawberry plants, and mitigate the effect of autotoxicity under closed hydroponics. They also reported that a rapid decomposition of Fe-EDTA in culture solution due application of DC-ED. In the following study, it was also found that DC-ED can breakdown the benzoic acid in the nutrient solution but it also decreases the iron and calcium concentrations, pH and increase solution temperature (Asaduzzaman et al., 2012). In DC-ED, iron and calcium ions were thought to be precipitated to the anode.

In order to overcome these issues associated with DC-ED, we planned to change the power source from DC to AC. In case of AC electro-degradation (AC-ED), positive and negative charges of the electrodes (anode and cathode) changesfrequently. Thus, iron and calcium ions might not be precipitated to the electrode (especially the central core).We hypothesized that; application of AC-ED instead of DC-ED would result in degradation of benzoic acid from the closed hydroponics without altering properties of nutrient solution. In this study, we applied AC-ED in order to investigate the ED conditions, growth,fruit yield and qualities of strawberry grown in closed hydroponics, where nutrient solutions are not renewed throughout the growth period.

2. Materials and methods

2.1. Plant material

Strawberry (*Fragaria* × *ananassa*Duch. cv. Toyonoka) plantlets produced through plant tissue culture were used for this experiment. Micro-propagated strawberry plantlets were transferred into cell trays (48 cm × 24 cm × 4 cm, 72 cells/tray) with vermiculite substrate and were kept there for about 60 days under control growth chamber condition at 20/15 ºC (day/night), 60% relative humidity, fluorescent light with intensity of 145 µmol m^{-2} s^{-1} and a 12 hours photoperiod for the formation of new roots and leaves. 25% standard "Enshi" nutrient solutions were used for growing strawberry plants in the cell trays. At five-seven leaf stage, strawberry plantlets were transferred to grow beds of hydroponic system for nursery in an environment control room. Thirty eight plantlets were accommodated in each grow bed and there were three grow beds placed vertically in hydroponic system. 300 L, 25% standard "Enshi" nutrient solutions were used for hydroponic system and solution was renewed bi-weekly. Nutrient solutions were supplied at 55/5 min. (recycle/stop) by an automatic pump (KP-101, Koshin, Kyoto, Japan) with an automatic timer (KS-1500, Iuchi, Osaka, Japan) and maximum discharge of 31 L/min. Strawberry plantlets were kept in the nursery until the flowering of first cluster. Then the clusters were removed and more homogenous plants were selected as planting materials.

2.2. Nutrient solution

Strawberry plants were cultured in 25% standard 'Enshi' nutrient solution [pH 7.25 and electrical conductivity of 0.8 dS m^{-1}] throughout the growth period. The electrical

conductivity and pH of the tap water used to prepare the nutrient solution were 0.22 dS m^{-1} and 8.18, respectively.

2.3.Electrode used for electro-degradation of nutrient solution

We used small AC and DC type electrode (designed and built by Yonago Shinko Co., Ltd., Tottori, Japan) for electro-degradation of benzoic acid or autotoxic chemicals in without plant nutrient solution or culture solution used for strawberry (Fig. 1). In case of DC-ED, 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) (Asaduzzaman et al., 2012). While in AC-ED, the electrode had a central core made of titanium with a surface area of 53.1 cm^2 (anode/cathode) which enclosed with cylindrical tube also made of titanium with a surface area of 95.5 cm^2 (cathode/anode). The nutrient solution can pass through the electrode where electro-degradation takes place. The electrodes were coupled with a digital AC power supplier (AD-8735D, AND, Japan).

2.4. Experiment I

2.4.1. Selection of AC frequency for ED of BA in culture solution

In order to select the suitable frequency for AC-ED, three different frequencies viz. 500, 1000, and 1500 Hz were tested in nutrient solution containing benzoic acid (BA). At first 10 L of 25% standard “Enshi” nutrient solution was prepared with tap water and then 0.4885 g of BA was added to reach concentration of400 μmol L^{-1} BA. Plastic containers (450 mm × 370 mm × 100 mm) were used for each frequency.

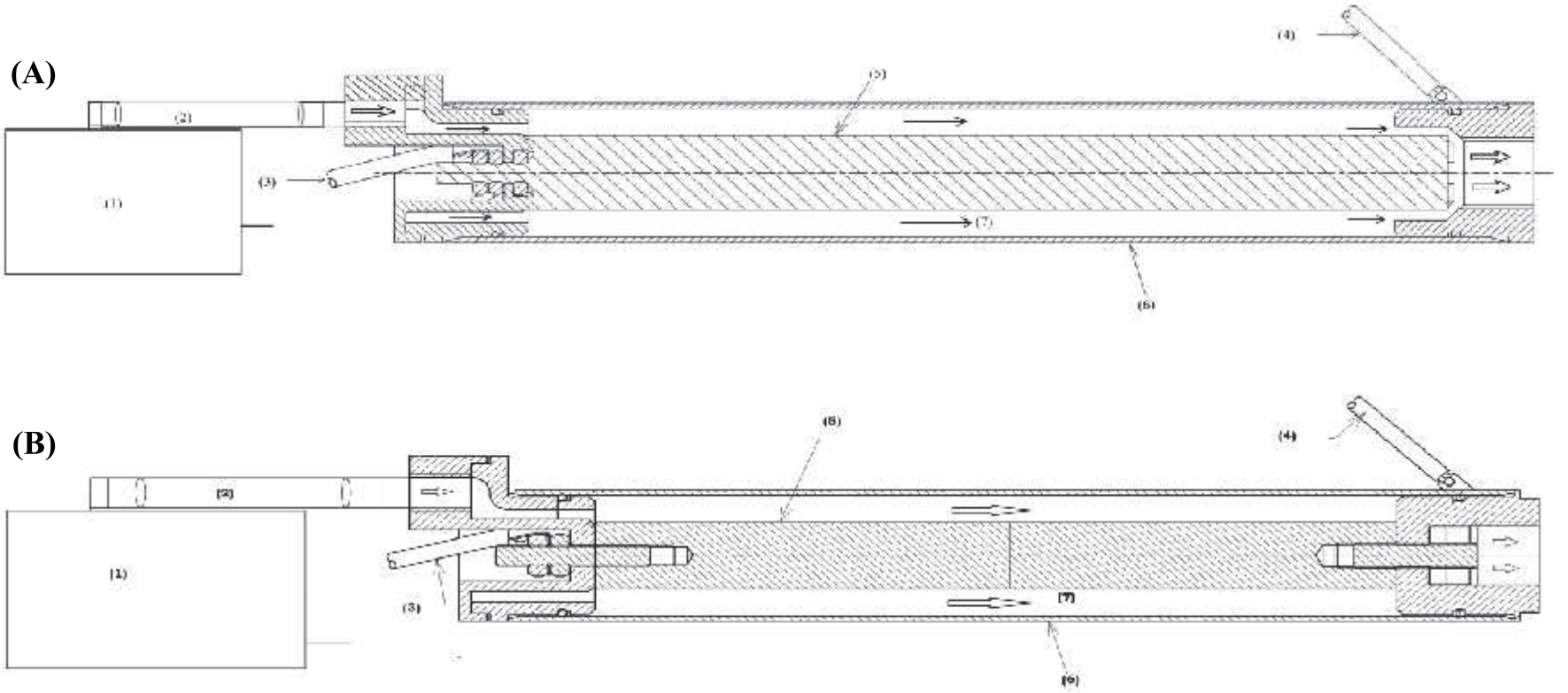


Fig.1. Simple schematic diagram of the electrode used for electro-degradation.

(A) Different components of DC-ED electrode includes (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. (Asaduzzaman et al. 2012);

(B) Different components of AC-ED electrode includes (1) pump, (2) plastic tube connecting pump with electrode, (3) anode/cathode, (4) cathode/anode, (5) central titanium core, (6) cylindrical titanium pipe and (7) nutrient solution flow.

In all cases, the AC-ED electrode was applied at 50% duty ratio, 2.0 amperes alternate current, and 14.0 volts.Nutrient solution samples (25 ml) were collected at0, 1, 3, 6, and 24 hours of AC-ED application for measuring concentration of benzoic acid. Conditions of nutrient solution such as temperature, EC, and pH were recorded at each sampling. EC was measured by EC meter (ES-51, Horiba, Ltd., Kyoto, Japan) while, temperature and pH were measured using pH meter (D-12, Horiba, Ltd., Kyoto, Japan) at each sampling.

2.4.2. Determination of BA conc.in the AC-ED treated nutrient solution

The collected nutrient solution samples at 0, 1, 3, 6, and 24 hours of AC-ED application were filtered through HPLC filter (0.20 μM, DISMIC-13, HP Membrane filter, Toyo Roshi Co., Ltd. Japan). Each filtrate (25 μL) was injected into a high performance liquid chromatography (HPLC) system (column oven L-2350, detector L-2400, and pump L-2130; 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 (Wakosil 10C18; Wako Pure Chemical Industries, Ltd., Osaka, Japan); eluent: CH_3CN/10 mM H_3PO_4= 30/70 (v/v); flow rate: 1.0 ml min^{-1} at 30 °C; and detection: ultraviolet 254 nm.

2.5. Experiment II

2.5.1.Electro-degradation of culture solution in without plant experiment

AC-ED at the selected frequency (500 Hz) was compared with DC-ED in nutrient solution following a without plant experiment. Following similar procedure as to

experiment I (section 2.4.1), three sets of nutrient solution containing 400 µmol L^{-1} BA were prepared.

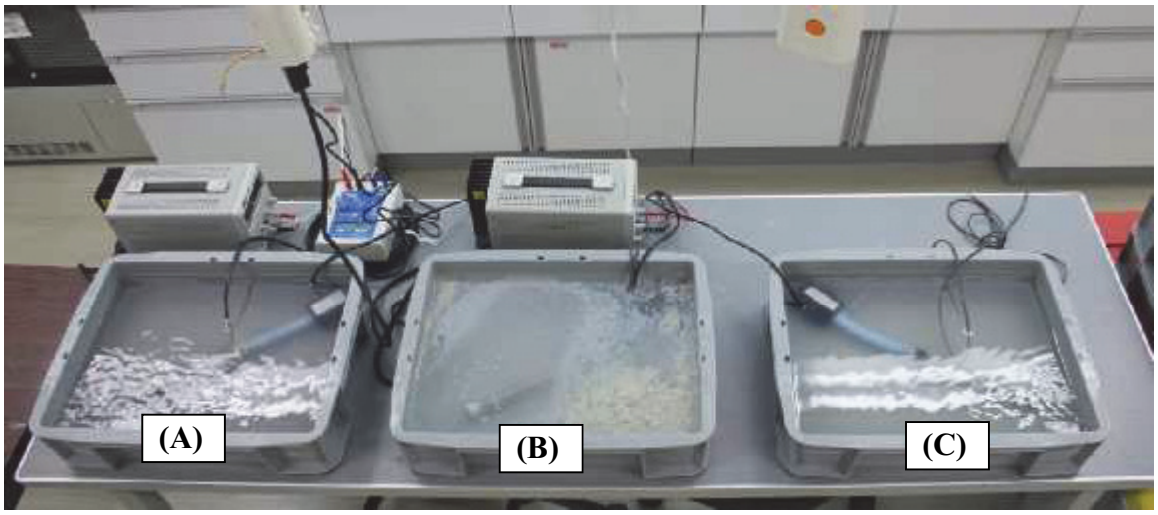


Fig. 2. Electro-degradations of nutrient solution following without plant experiment for 24 hours. (A) AC-ED was applied at 50% duty ratio, 2.0 amperes alternate current, and 14.0 volts. (B) The DC-ED was applied at 2.0 ampere and 18.0 volts. (C) Control-without ED application, nutrient solution was flowed using pump only. (Experiment II)

Electro-degradations were applied as DC-ED, AC-ED and control (without ED) for 24 hours (Fig. 2).The DC-ED was applied at 2.0 ampere and 18.0 volts, while the AC-ED conditions were the same as previous experiment atfrequency of 500 Hz. Nutrient solution samples were collected for measuring benzoic acid at 0, 1, 3, 6, and 24 hours of ED. Temperature, EC, pH and benzoic acid concentration in electro-degraded nutrient solution were measured following methods as described in section 2.4.2.

Inplasticbottles25mlsampleswerecollected after 24 hours of ED process forthe analyses of major nutrients. Nutrient solution was filtered with qualitative filter paper (Advantec Grade no. 131; 125 mm). Major mineral nutrients such as K^{+}, Ca^{2+}, Mg^{2+},

and Fe^{3+} was measured with an atomic absorption photometer (Z-2000, Hitachi High-Technologies Corporation, Kyoto, Japan), $NO^{3-}$with a compact NO_3^-meter TWIN NO_3^- (B-343, Horiba, Ltd., Japan) and PO_4^{3-} using spectrophotometer at 720 nm (U-2900, Hitachi High Technology, Tokyo, Japan).

2.6. Experiment III

2.6.1. Cultivation of strawberry in non-renewed solution treated with DC- and AC-ED

Healthy strawberry plantlets selected from nursery were used for this culture. Plantlets were grown in control room by maintaining a relative humidity of 60%, CO_2 concentration of 800 ppm, fluorescent light with intensity of 145µmol$m^{-2}s^{-1}$ and a photoperiod of 12 hours. Plantlets were planted to three stage vertical growing beds (125 cm × 90 cm × 10.5 cm). On 20thFebruary 2016, five plantlets were planted in each growing bed fixed with urethane cubes (23 mm × 23 mm × 27 mm) in a controlled room at 25/20°C (day/night) temperature. Three growing beds were filled with 25% standard "Enshi" nutrient solution with each capacity of 50 L connected to a 300 L reservoir tank. Nutrient solutions were recycled at 55/5 min. (recycle/stop) by an automatic pump (KP-101, Koshin, Kyoto, Japan) with an automatic timer (KS-1500, Iuchi, Osaka, Japan) and maximum discharge of 31 L/min.

There were four types of culture solutions viz. renewed tri-weekly, non-renewed, non-renewed with DC electro-degradation tri-weekly for 24 hours and non-renewed with AC

electro-degradation tri-weekly for 24 hours. In renewed culture system, nutrient solutions were renewed tri-weekly. While non-renewed nutrient solutions, major nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+} and Fe^{3+}) concentration were adjusted at every three weeks interval as close as possible to the initial concentration of the 25% "Enshi" solution based on the chemical analyses described previously in section 2.5.1. The DC- and AC-ED were applied in the nutrient solution for 24hours at three weeks interval in the setting as it was applied in without plant experiment (Fig. 3). Pollination was carried out using a calligraphy brush every 2 or 3 days. Harvest was carried out when the whole fruit or 80% of the fruit turned to red color. First harvest was carried out on 5th April 2016 and final harvest on 7th July 2016. Data were collected on growth parameters, chlorophyll content (measured by SPAD, Konica Minolta, Tokyo, Japan) and yield attributes at the final harvest.

2.6.2. Determination of strawberry fruit qualities

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 werekept out of freezer before analysis to obtain sufficient juice for determining the above qualities. The soluble solid content of the fruit was determined using a digitalrefractometer (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 andadded 2–3 drops of phenophthalein 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 (%).

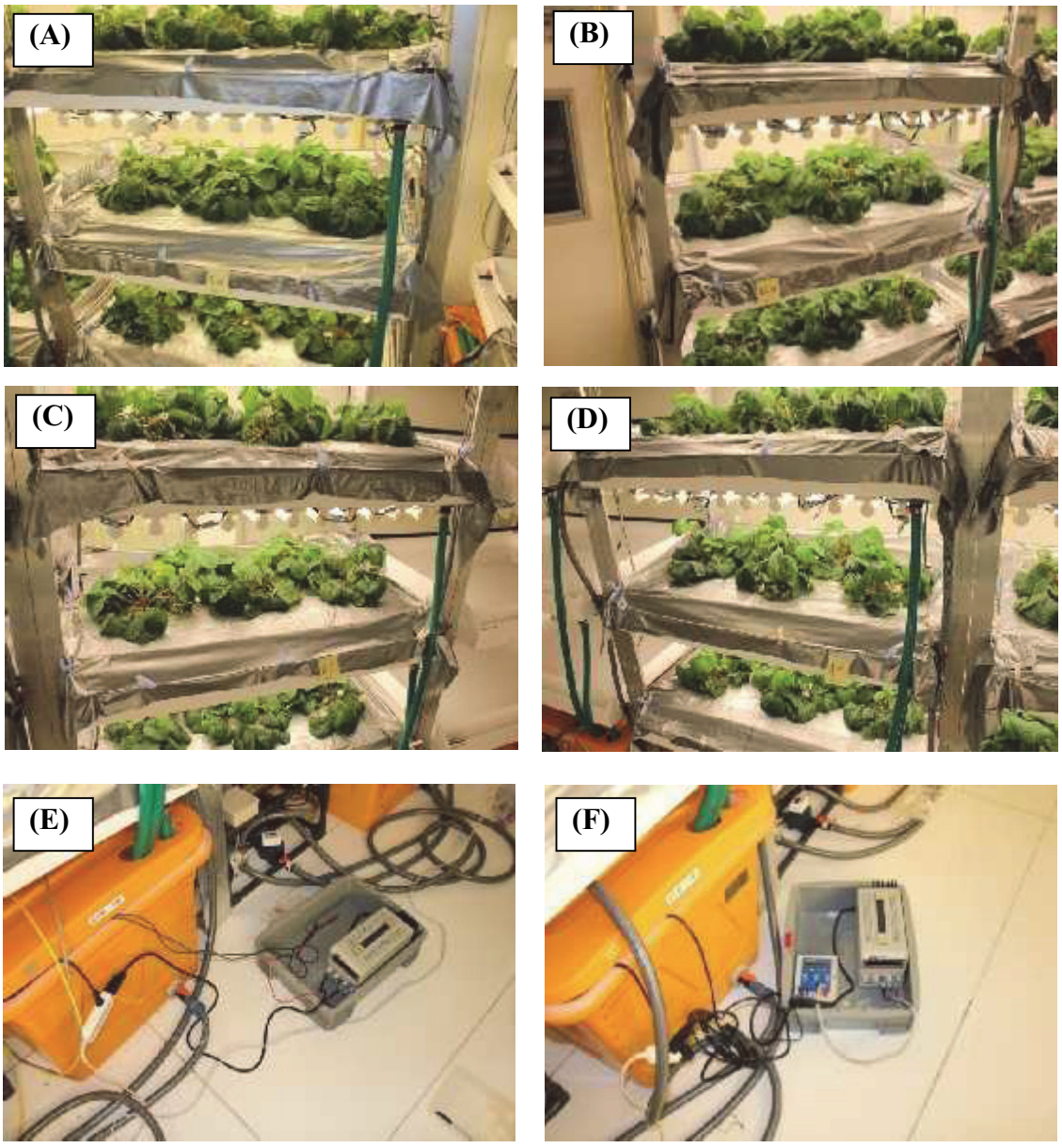


Fig. 3. Three layered vertical growing beds used for cultivation of strawberry plants under controlled-environment. Each grow bed with 50 L nutrient solution capacity and three beds placed vertically were connected to a tank filled with 300 L nutrient solution. There were four different systems used for each types of culture solution such as **(A)** renewed, **(B)** non-renewed, **(C, E)** non-renewed with DC-ED and **(D, F)** non-renewed with AC-ED. (Experiment III).

Ascorbic acid content was measured with 2, 4-dinitrophenylhydrazine (DNP) colorimetry. Strawberry fruit juice (0.5 ml) was taken in 50 ml test tube then 0.5 ml of 10% meta- phosphoric 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 hours incubation at 37 °C in water bath. After incubation 5 ml of 85% H_2SO_4 were added to each sample keeping in water cooled with iced water. After 30 minutes cooling, ascorbic acid content was measured at 540nm by spectrophotometer (U-2900, Hitachi High Technologies Corporation, Tokyo, Japan).

2.6.3. Determination of mineral nutrient content in plant parts

Mineral nutrients content in strawberry plants were also recorded. Strawberry plant parts were separated into leaves, crown and roots and kept in a constant temperature oven (DKN812, Yamato Scientific Co. Ltd. Japan) for 72 hours 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 0.25 g were mixed with 8 ml of HNO_3 and digested bymicrowave 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). The filtered sample solutions wereanalyzed formineral nutrients by atomic absorption spectrophotometer (Z-2310, Hitachi High Technologies Corporation,Tokyo,Japan).

2.6.4. Measurement of temperature, EC, pH and determination of mineral nutrients of culture solution

Conditions of culture solution such as temperature, EC, and pH were recorded at every three weeks interval after ED application following the procedure as described in section 2.4.1. Amount of mineral nutrient remains in the culture solution were determined following the analytical procedures as described in section 2.5.1. Data were taken five times throughout the growing period.

2.7. Experimental design and statistical analysis

In experiment I, three different frequencies of AC-ED were evaluated and repeated sampling was done for each sampling. Each data represented means of five observations. Similarly in experiment II, three types of ED were applied to decompose BA and each data is the mean of five observations. In experiment III, four types of culture solutions were arranged in acompletely randomized design with three replications. Analysis of variance for all data was done using computer package MSTAT-C developed by Russel (1986). The mean differences of each culture solution were separated according to Tukey's test at $P<0.05$.

3. Results

3.1. Selection of frequency for AC-ED machine of BA in the nutrient solution (Experiment I)

The degradation of BA in nutrient solution under three different frequencies of ACwas investigated. The concentration of BA decreased gradually over time. The amounts of

BA (initially 400 μM L^{-1}) in the nutrient solution weremeasured as 370, 339, 247 and 0 ppm after 1, 3, 6, and 24 hours of AC-ED, respectively at frequency of 500Hz. Similarly, BA concentrations were decreased to 385, 320, 231 and 5 ppm after 1, 3, 6, and24 hours, respectively at 1000Hz; 392, 300, 245 and 5 ppm after 1, 3, 6 and 24 hours, respectively at 1500Hz (Fig. 4). Results showed that BA in the nutrient almost completely degraded after 24hoursdue to application of AC-ED at all three frequencies. Although EC and pH of the treated nutrient solution were not varied greatly, temperature of the solution increased with the increase of AC frequency (Fig. 5). It showed that, significantly higher temperature of nutrient solution was recorded at 1500Hz followed by 1000Hz and 500Hz of AC-ED.

3.2. Electro-degradation of nutrient solution in without plant experiment (Experiment II)

DC-ED and AC-ED were applied in the nutrient solution following a without plant experiment to investigate the degradation of BA. The concentration of BA was decreased sharply until 6 hours of ED while it was not decreased considerably in control where ED not applied (Fig. 6). Compared to DC-ED, AC-ED showed faster BA degradation in allsampling stage and it was completely degraded at 24 hours. After 24 hours DC-ED, about 100 ppm BA remains in the treated nutrient solution while it was remains about as initial (about 400 μM L^{-1}) in control condition. Results showed overall decreasing trend of BA concentration as 341, 243, 135, and 0 ppm after 1, 3, 6 and 24 hours, respectively by AC- whereas, 336, 314, 224 and 67 ppm after 1, 3, 6 and 24 hours, respectively by DC-ED application.

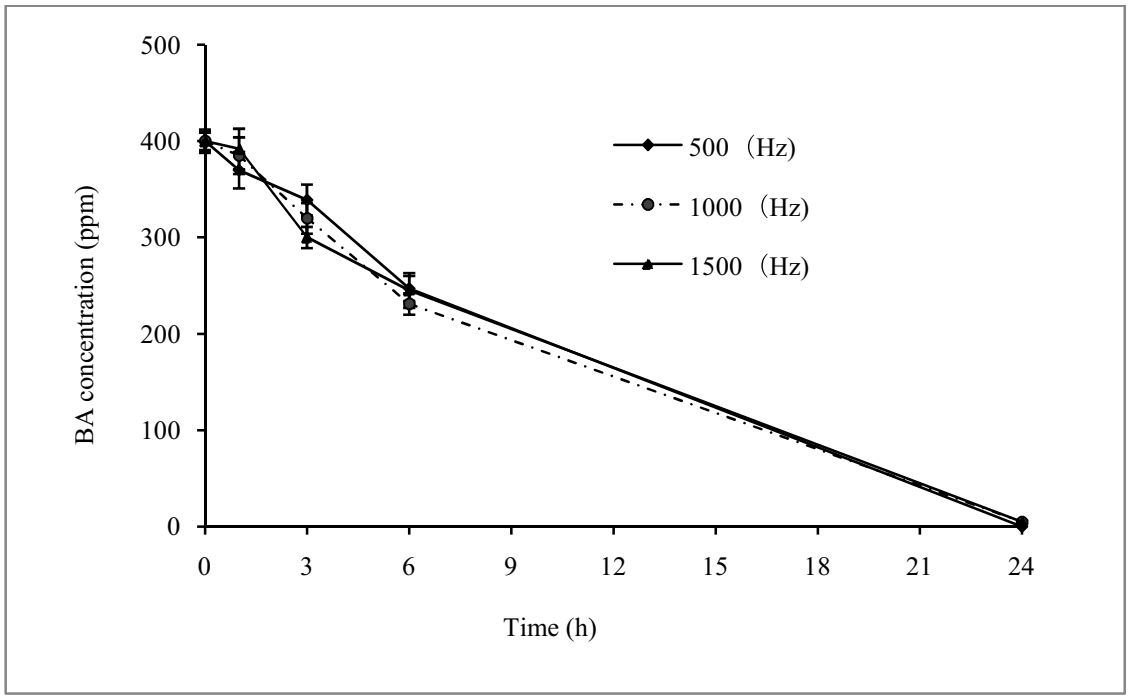


Fig. 4. Changes in benzoic acid concentration of the nutrient solution due to application of electro-degradation using alternate current (AC) at three different frequencies for 24 hours.Electro-degradation was applied in 10 L of 25% standard "Enshi" nutrient solution with 400 μM L^{-1} benzoic acid. The vertical bars represent SE (n = 5). In AC supply 50% duty ratio, about 2.0 ampere and 14.0 volt were maintained for all frequencies. (Experiment I)

Physical and chemical conditions of nutrient solution were also affected by the application of ED (Fig. 7). EC and pH were not affected by the either type of ED applied and control. However, temperature of the nutrient solution varied greatly. In DC-ED, temperature was raised significantly (7.7 °C) compared to AC-ED after 24 hours. In control and AC-ED, it was not raised greatly rather remain similar as initial.

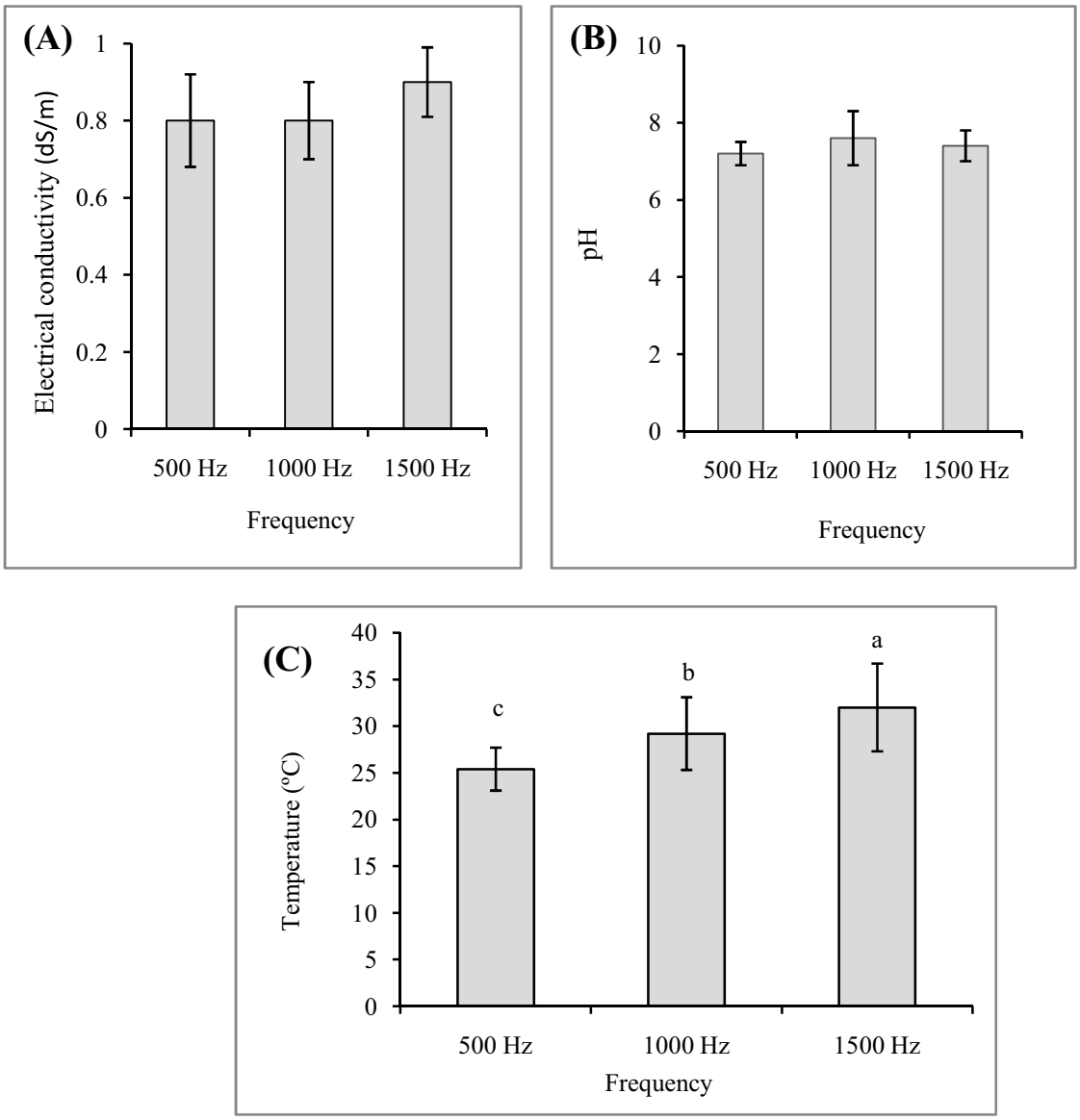


Fig.5. Changes in electrical conductivity (A), pH (B) and temperature (C) of the nutrient solution due to application of electro-degradation using alternate current (AC) at three different frequencies for 24 hours. Electro-degradation was applied in 10 L of 25% standard "Enshi" nutrient solution with 400 µM L^{-1} benzoic acid. In AC supply 50% duty ratio,2.0 ampere and 14.0 voltwere ED maintained for all frequencies.The vertical bars represent SE (n = 5). Different letters above each bar are significant and no letters are non-significant according to the Tukey's multiple range test at $P < 0.05$. (Experiment I)

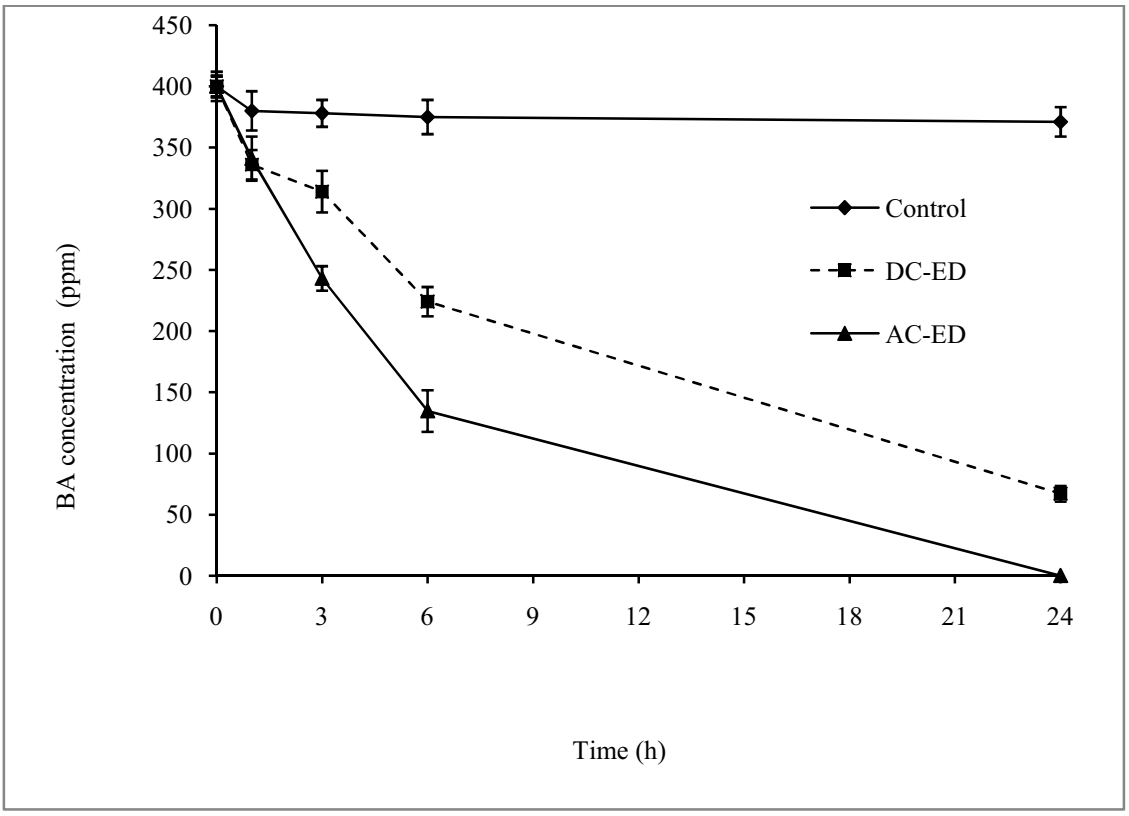


Fig. 6. Changes in benzoic acid concentration of the nutrient solution due to application of electro-degradation using both direct current (DC) and alternate current (AC) for 24 hoursin a no plant experiment. Electro-degradation was applied in 10 L of 25% standard "Enshi" nutrient solution with 400 μM L^{-1} benzoic acid. The vertical bars represent SE (n = 5). In DC supply 18.0 volts and 2.0 amps were maintained for the entire period while in AC supply 500 Hz, 50% duty ratio, 14.0 volt and about 2.0 ampere were maintained. (Experiment II).

Application of DC-ED and AC-ED also influenced major mineral nutrient content in culture solution (Table 1). Nitrogen, phosphorous, potassium, and magnesium concentration in the nutrient solution was not affected by the ED application and control. Interestingly, calcium and iron concentration was decreased significantly in DC-ED compared to AC-ED and control after 24 hours.

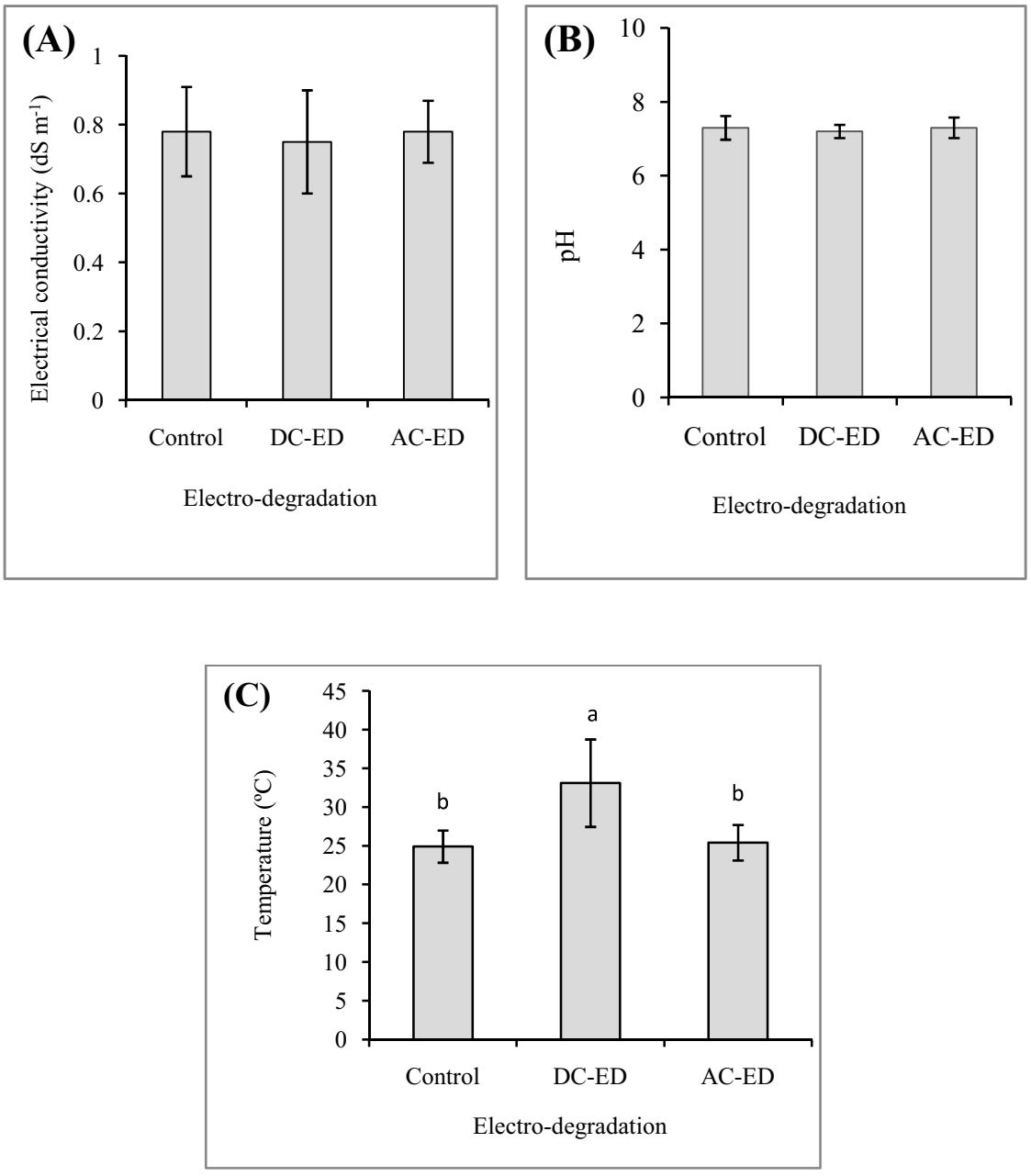


Fig. 7. Changes in electrical conductivity (A), pH (B) and temperature (C) of the nutrient solution due to application of electro-degradation using alternate current (AC) for 24 hours in a no plant experiment. Electro-degradation was applied in 10 L of 25% standard "Enshi" nutrient solution with 400 µM L^{-1} benzoic acid. In DC supply 18.0 volts and 2.0 amps were maintained for the entire period while in AC supply 500 Hz, 50% duty ratio, 14.0 volt and about 2.0 ampere were maintained.The vertical bars represent SE (n = 5). Different letters above each bar are significant and no letters are non-significant according to the Tukey's multiple range test at $P < 0.05$. (Experiment II).

Table 1. Changes in mineral nutrients after application of electro-degradationof nutrient solution in no plant experiment. Electro-degradations were applied in 10 L of 25% standard "Enshi" nutrient solutionwith400 µM L^{-1} benzoic acid for 24 hours. (Experiment II)

Electro-degradation	NO_3^-(ppm)	$P_2O_5^-$(ppm)	K^+(ppm)	Ca^{2+}(ppm)	Mg^{2+}(ppm)	Fe^{3+}(ppm)
Control[z]	687	37.5	7.9	49.9 a[w]	16.2	3.5 a
DC-ED[y]	658	35.8	7.6	41.6 b	13.8	2.2 b
AC-ED[x]	669	37.5	7.2	52.6 a	15.4	3.4 a
Significance	NS	NS	NS		NS	

[z]Electro-degradation was not applied.
[y]Electro-degradation was applied using "Direct Current"
[x]Electro-degradation was applied using "Alternate Current"
[w]Meanswithin a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P<0.05$.

3.3. Application of DC- and AC-ED on the culture solution used for growing strawberry plant (Experiment III)

3.3.1. Effect of DC- and AC-ED on the growth of strawberry

Several growth parameters of strawberry were significantly affected by the application of ED in the non-renewed culture solution (Table 2). Long root length, leaf length and width, SPAD value and crown diameter were not affected the ED treatment. Number of leaves was significantly decreased in plants grown in non-renewed solution compared to renewed solution. While application of either DC- or AC-ED showed statistically similar number of leaves as it was produced in renewed or non-renewed solution. Leaf fresh weight was highest (28.1gplant^{-1}) in renewed culture solution and non-renewed culture solution with AC-ED, which was followed by non-renewed culture solution with DC-ED. The lowest leaf fresh weight was observed in non-renewed culture solution. Crown fresh weight followed similar trend. The crown fresh weight was the lowest (9.1 gplant^{-1}) in non-renewed culture solution where no ED was applied. Renewed culture solution and non-renewed culture solution with AC-ED produced significantly higher crown fresh weight, which was followed by non-renewed culture solution with DC-ED. Correspondingly, the highest dry weight of leaf (7.7 g plant^{-1}), crown (2.6 gplant^{-1}) and root (4.1 g plant^{-1}) was obtained from renewed culture solution and they were statistically similar with plants grown in non-renewed solution with AC-ED followed by DC-ED. The lowest dry weight of leaf, crown and root was obtained from non-renewed culture solution.

Table 2. Effect of electro-degradation of non-renewed culture solutionon the growthof strawberry plants grown under controlled environment condition. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Culture solution	No of leaves plant^{-1}	Longest root length (cm)	Leaf length (cm)	Leaf width (cm)	SPAD value	Crown diameter (mm)	Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})		
							Leaf	Crown	Leaf	Crown	Root
RW[z]	18.8 a[v]	58.7	13.9	13.9	57.6	16.9	28.1 a	16.6 a	7.7 a	2.6 a	4.1 a
NR[y]	14.2 b	54.2	13.5	13.1	55.2	15.5	21.7 c	9.1 b	6.1 b	1.8 b	2.9 b
NR + DC-ED[x]	15.1 ab	55.3	13.7	13.4	55.8	16.6	26.2 b	11.2 ab	7.0 ab	1.9 ab	3.0 ab
NR + AC-ED[w]	15.7 ab	57.8	13.8	13.6	56.2	16.8	28.1 a	14.9a	7.49 a	2.3 a	3.9 a
Significance		NS	NS	NS	NS	NS					

[z]Nutrient solution was renewed at every three weeks interval.

[y]Nutrient solution was not renewed throughout the entire growing period but major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.

[x]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Direct Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.

[w]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Alternate Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.

[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P<0.05$.

3.3.2. Effect of DC- and AC-ED on the fruit yield and yield attributes

Yield attributes were significantly affected by types of culture solution used (Fig. 8 A). Number of fruit per plant greatly decreased (about 50%) in non-renewed culture solution compared to renewed culture solution. Plants grown in non-renewed culture solution with AC-ED application produced statistically similar number of fruits as renewed solution. However, plants grown in non-renewed culture solution with DC-ED produced intermediate type of fruits number. Individual fruit weight followed similar trend as it was found in number of fruit per plant. It was highest in renewed culture solution which was identical to fruits obtained from plants grown in non-renewed culture solution with AC-ED. The lowest individual fruit weight (6.9 g $plant^{-1}$) was obtained in non-renewed culture solution. Fruit yield in different culture solutions were corresponding to their yield attributes (Fig. 8 B). The lowest fruit yield (114.0 g $plant^{-1}$) was recorded from plant grown in non-renewed culture solution. While the highest fruit yield was recorded in plants from renewed culture solution, followed by plants grown in non-renewed culture solution with AC-ED. However, plants grown in non-renewed culture solution with DC-ED application did not improved fruit yield greatly. Results indicated that about 49% yield was increased due to application of DC-ED in non-renewed culture solution compared to non-renewed culture solution entirely. When AC-ED applied to non-renewed culture solution about 86% fruit yield was increased compared to non-renewed culture solution.

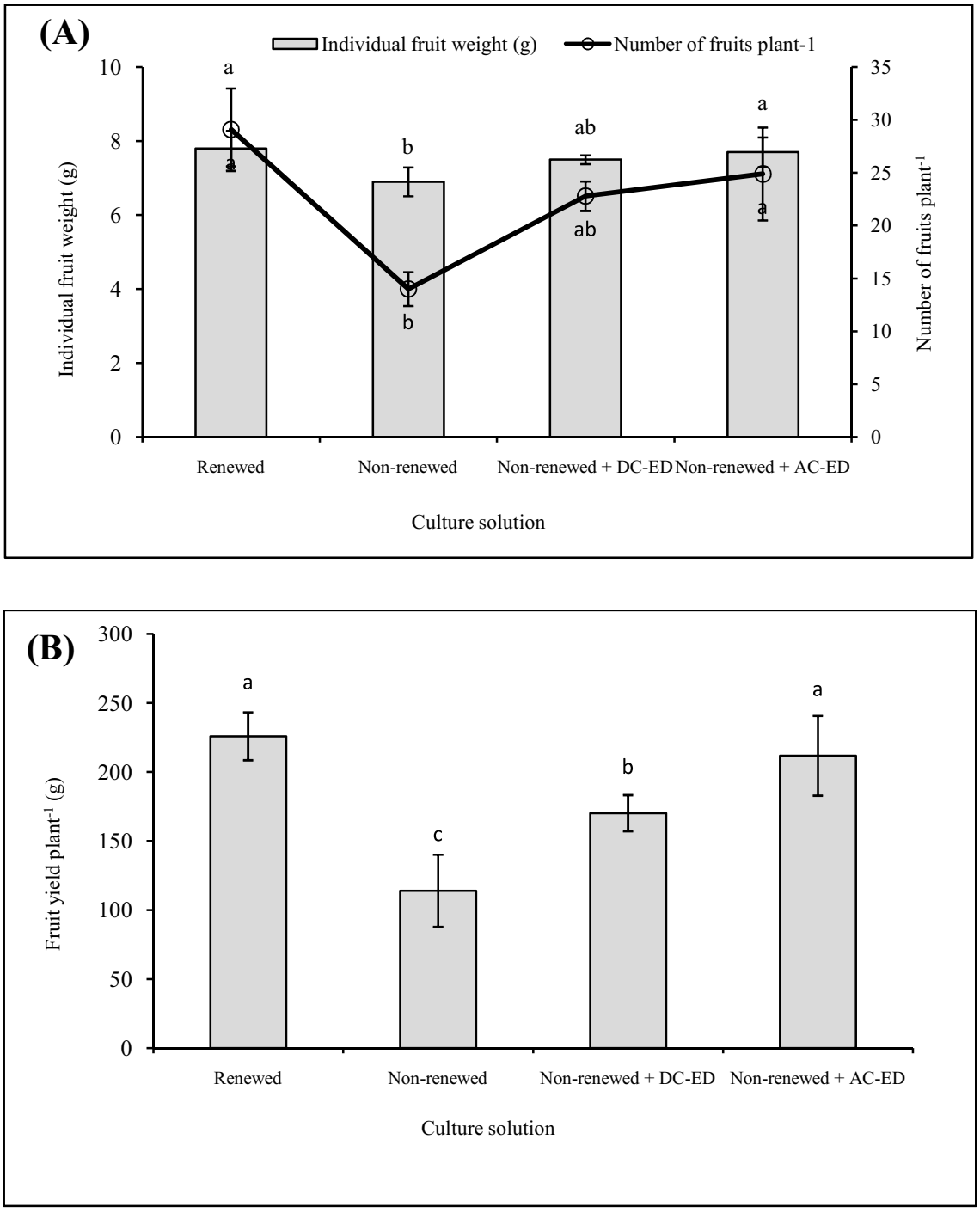


Fig. 8. Effect of electro-degradation of non-renewed culture solution on yield attributes (A) individual fruit weight and number of fruit, and (B) fruit yield of strawberry plants grown under controlled environment condition. Electro-degradation was applied for 24 hours at every three weeks interval until final harvest. (Experiment III).

3.3.3. Effect of DC- and AC-ED on the fruit qualities of strawberry

The qualities of strawberry fruits were not differedsignificantly until fourth cluster except vitamin C content (Table3). The highest vitamin C content fruits were found in plants grown in non-renewed culture solution treated with AC-ED from cluster I to IV, which was statistically similar with fruits obtained from plant in renewed culture solution. In general, the lowest vitamin C content fruits were obtained from plants grown in non-renewed culture solution and non-renewed culture solution with DC-ED in all four clusters.

3.3.4. Effect of DC- and AC-ED on mineral contents in strawberry plant

Electro-degradation of non-renewed culture solution significantly affect the mineral nutrient content especially calcium and ironin crown and root of strawberry plants (Table 4). Other minerals like potassium and magnesium in all plant parts was not affected by ED application. In root and crown, both calcium and iron content were decreased significantly in non-renewed and non-renewed with DC-ED application.

3.3.5. Effect of DC- and AC-ED on the culture solution properties

Temperature, EC and pH of the culture solution measured were not differed significantly throughout the growing periods (Table 5).In non-renewed culture solution, the amount of calcium and iron were also found to be decreased due to application of DC-ED. While amount of other minerals (nitrogen, phosphorus, potassium and magnesium) were not decrease considerably due to application of either DC- or AC-ED. In non-renewed culture solution, application of DC-ED results in significant decrease in calcium and iron.

Table 3. Effect of electro-degradation of non-renewed culture solution on the fruit qualities of strawberry plants grown under controlled environment condition. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Culture solution	Brix (%)				Citric acidity (%)				Vitamin C (ppm)			
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster I	Cluster II	Cluster III	Cluster IV
RW[z]	7.1	7.5	7.8	7.6	0.28	0.29	0.26	0.28	658.1 ab[v]	657.5ab	656.0 ab	682.2 a
NR[y]	7.9	7.8	7.9	7.7	0.28	0.29	0.29	0.26	536.5 b	621.1bc	597.0b	616.2b
NR + DC-ED[x]	7.5	7.5	7.7	7.5	0.28	0.31	0.30	0.30	593.3 b	603.4 c	616.4b	623.8 b
NR + AC-ED[w]	7.7	7.7	7.2	8.0	0.31	0.31	0.29	0.28	693.4a	681.5 a	698.0 a	686.5a
Significance	NS	NS	NS	NS	NS	NS	NS	NS				

[z]Nutrient solution was renewed at every three weeks interval.
[y]Nutrient solution was not renewed throughout the entire growing period but major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[x]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Direct Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[w]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Alternate Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P < 0.05$.

Table 4. Effect of electro-degradation of non-renewed culture solution on the mineral content in leaf, crown and root of strawberry plants grown under controlled environment condition. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Culture solution	Iron (mg kg^{-1} DW)			Calcium (mg g^{-1} DW)			Magnesium (mg g^{-1} DW)			Potassium (mg g^{-1} DW)		
	Leaf	Crown	Root	Leaf	Crown	Root	Leaf	Crown	Root	Leaf	Crown	Root
RW[z]	138	372 a[v]	238 a	26.7	22.7 bc	31.3 ab	7.3	7.3	14.7	35.7	21.0	25.8
NR[y]	131	279 b	194 b	20.7	20.7 bc	25.8 ab	7.1	7.2	12.9	39.5	17.7	22.4
NR + DC-ED[x]	122	209 c	183 b	22.4	19.0 c	24.0 b	7.5	7.3	12.8	35.9	18.2	23.9
NR + AC-ED[w]	149	302 b	246 a	30.2	24.2 a	34.0 a	7.7	6.8	14.5	41.7	23.0	25.0
Significance	NS			NS			NS	NS	NS	NS	NS	NS

[z]Nutrient solution was renewed at every three weeks interval.
[y]Nutrient solution was not renewed throughout the entire growing period but major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[x]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Direct Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[w]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Alternate Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P < 0.05$.

Table 5. Effect of electro-degradation of non-renewed culture solution on temperature,pH, electrical conductivity and residual nutrient content of nutrient solution at the final harvest. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III).

Culture solution	Temperature (ºC)	pH	EC (dS m^{-1})	Residual nutrient content (ppm)					
				Fe^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	NO_3^-	$P_2O_5^-$
RW[z]	19.4	7.22	0.77	3.9 a[v]	45.3 a	25.2	77.8	682.5	9.0
NR[y]	20.1	7.22	0.78	3.7 a	42.1 a	24.8	72.5	653.0	8.6
NR + DC-ED[x]	21.5	7.23	0.76	2.3 b	34.0 b	24.3	75.7	669.2	8.8
NR + AC-ED[w]	20.4	7.20	0.78	3.6 a	41.6 a	25.2	76.5	681.0	9.3
Significance	NS	NS	NS			NS	NS	NS	NS

[z]Nutrient solution was renewed at every three weeks interval.
[y]Nutrient solution was not renewed throughout the entire growing period but major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[x]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Direct Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[w]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Alternate Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P < 0.05$.

4. Discussion

In non-renewed hydroponic culture of strawberry, several allelochemicals were found to be exuded from roots and BA was one of them (Kitazawa et al., 2005). Due to continuous accumulation of these allelochemicals including BA in theculture solution, plant roots become injured impairing water and mineral nutrient uptake and thus growthand normal activity of roots are hampered. Subsequently, the growth and yield of strawberry decreased.Research reports suggested several ways to eliminate these allelochemicals from the culture solution (Asao et al., 1998; Asao et al., 2004a; Kitazawa et al., 2005, 2007; Asao et al., 2008; Asaduzzaman et al., 2012; Mondal et al., 2013, 2015).

Our previous studies suggested that ED of nutrient solution using direct current could mitigate autotoxicity of plants in closed hydroponic culture (Asao et al., 2008; Asaduzzaman et al., 2012), but these methods had some troubles such as degradation of Fe-EDTA, low concentration of Ca^{2+} in the treated culture solution, decrease in solution pH and increase in solution temperature. In order to overcome these problems, we modified the ED electrode and also power source from DC to AC. In our present study, we used AC-ED electrode to compare its efficiency with previously used DC-ED electrode to decompose autotoxic chemicals in non-renewed culture solution of strawberry.

Suitable electrolysis conditions (2.0 amperes and 18.0 volts) for DC-ED electrode to degrade BA were investigated in the earlier studies (Asaduzzaman et al., 2012).

However, for AC-ED machine suitable electric condition was not determined. Therefore, we examined three frequencies (500 Hz, 1000 Hz and 1500 Hz) against the degradation of BA. In all cases frequencies 50% duty ratio, 2.0 ampere and 14.0 volts were maintained. All these three frequencies were equally effective for degradation of BA (Fig. 4). However, the gradual rise of culture solution temperature was recorded (Fig. 5) in the higher frequency (1500 Hz). This increased temperature may negatively affect the plant root growth and development. Recent studies reported that temperature at the root-zone influences the growth and chemical composition of many plants (Adebooye et al., 2010; Malik et al., 2013; Yan et al., 2013; Sakamoto and Suzuki, 2015a, 2015b). The high root-zone temperature (about 30 °C) for strawberry in a deep flow technique hydroponic system decreased oxygen consumption and cell viability of the roots, resulting in withering of the plants (Sakamoto et al., 2016). Therefore, in our studies, ED of benzoic acid without an augmented temperature in culture solution, use of 500 Hz frequency would be suitable.

In the following study, we compared the efficiency of DC-ED and AC-ED electrode against the degradation of BA in without plant experiment. In both cases, degradation of BA was observed, but rate of degradation was faster in AC-ED and it was found that, after 24 hours, BA was completely degraded but there some residues (about 100 ppm) remained in DC-ED(Fig. 6). Other studies reported that, phenolic compounds in aqueous solutions can be degraded through electro-chemicals means (Comninellis and Pulgarin, 1991; Feng and Li 2003; Fleszar and Ploszynka, 1985).In nutrient solution without application of ED, BA concentration was found to decrease slowly after 24 hours, might due to the microbial degradation (Sundin and Watcher-Kristensen,

1994).Although, EC and pH of the culture solution was not differed significantly, temperature was increased significantly due to application of DC-ED (Fig. 7). The reason might be associated with the DC electrode with produce heat during the ED process. In earlier studies, increase in solution temperature and decrease in pH was observed due to DC-ED of strawberry culture solution under Wagner's pot hydroponics (Asaduzzaman et al.,2012). Concentrations of mineral nutrients such as calcium and iron in the nutrient solution were decreased significantly after 24 hours of DC-ED application (Table 1). In DC electrolysis, iron and calcium ions were thought to be precipitated to the anode. On the other hand, in the AC electrolysis, since the positive and negative charge of the electrode changed frequently and iron and calcium ions were not precipitated. Thus, it was thought that AC electrolysis might be more suitable for strawberry production by degradation of BA in the culture solution.DC-ED and AC-ED were also applied to the culture solution of strawberry to investigate their effects on culture solution, growth, fruit yield and quality of strawberry under recycled hydroponics.

Results showed that, in non-renewed culture solution without ED treatment,growth and fruit yield of strawberry weredecreased significantly compared to plants grown in renewed culture solution (Table 2, Fig. 8) due to accumulation of allelochemicals (Kitazawa et al., 2015). This phenomenon was also observed in earlier studies (Asao et al., 2008; Kitazawa et al., 2005). In this case, application of ED in non-renewed culture solution increased growth and yield of strawberry (Asao et al., 2008; Asaduzzaman et al., 2012).In this present study, application of DC-ED to non-renewed did not improve

the growth parameters, fruit yield and fruit quality (vitaminC content) significantlycompared to the plant performance in non-renewed nutrient solution. Plants grown in non-renewed culture solution had lower calcium and iron in leaves and crown might be due to hindered nutrient uptake as a result of accumulation of growth inhibitors in the rhizosphere (Singh et al., 1999). The accumulation of growth inhibitors was found in hydroponic nutrient solution from the root exudates of many plants such as tomato (Yu and Matsui, 1993), strawberry (Kitazawa et al., 2005), cucumber, taro, some leafy vegetables and ornamentals (Asao et al., 1998, 2003, 2004b, 2007). While lower content of calcium and iron in leaves and crown of plant grown in DC-ED treated non-renewed culture solution might be associated with their lower concentration in that culture solution (Table 5).

On the other hand, application of AC-ED to non-renewed culture solution significantly increased growth parameters (number of leaves per plant, fresh weight of leaf and crown, dry weight of leaf, root and crown, number of fruits per plant, individual fruit weight, yield per plant and vitaminC content of fruits) as compared non-renewed solution. The possible reason this improved plant performance due to application of AC-ED in non-renewed culture solutionmight include the faster rate of BA degradation, no negative effects on solution EC, pH and temperature and mineral nutrient content (especially calcium and iron) (Fig. 6, 7; Table 1, 5).Therefore, results of this study revealed that overall improvement of growth, yield, fruit quality and nutrient solution conditions were better due to application of AC-ED than DC-ED in non-renewed culture solution of strawberry in recycled hydroponics.

5. Summary

Strawberry production in non-renewed hydroponicsresulted in reduced growth and yield. DC-ED and AC-ED treatment to non-renewed nutrient solution increased growth and yield of strawberry. DC-ED treatment to non-renewed culture solution could recover yield of strawberry to some extent but not completely. However, complete yield recovery was obtained from AC-ED treatment to non-renewed culture solution. Furthermore, AC-ED treatment to non-renewed culture solution could maintain better nutritional and environmental condition of growing medium. Hence, we suggested that AC-ED treatment to nutrient solution for 24 h at every three weeks intervals could be applied for complete recovery of strawberry yield grown in closed hydroponic culture.

CHAPTER 4

Application of alternating current electro-degradation improves retarded growth and quality in lettuce under autotoxicity in successive cultivation

1. Introduction

The commercial production of several leafy vegetables in the plant factory with hydroponic system is progressively increased (Goto, 2012;Kozai, 2013; Salisbury and Bugbee, 1988). Stable supply of vegetables with good quality and safety make this production technique more popular. Lettuce (*Lactuca sativa* L.), which has a short growth cycle and high planting density can be produced in large quantity in the plant factory (Seaman, 2015). There are a lot of different hydroponics systems to grow in the plant factory, but one of the most popular methods is with a closed hydroponics system (Takeuchi, 2000; Oka, 2002; Koshikawa and Yasuda, 2003). A closed hydroponics which is frequently known as re-circulating system refers to a hydroponic system in which nutrient solution is not diverted from the system. The nutrient solution flows through the growing medium into a collector where it is recovered and then it is reused over and over again in the same way. Closed hydroponics system lower water and nutrient consumption, avoids the supply and disposal cost of nutrient solutions and environmentally friendly - minimal potential for localized groundwater contamination. Hence, this hydroponic system has been encouraged recently (Ruijs, 1995; Van Os, 1995).

In closed hydroponics, crop production greatly reduced in non-renewed solution. According to Yu and Matsui (1993, 1994), the growth and yield of tomato and cucumber were also reduced inclosed hydroponics. Many researchers found this problem in strawberry (Kitazawa et al., 2005), taro (Asao et al., 2003), lettuce (Lee et al., 2006), several leafy vegetables including lettuce (Asao et al., 2004a) and some ornamentals (Asao et al., 2007).Reason behind these reduced growth and yieldof crop in closed hydroponics was a problem of autotoxicity. Crop production experiences autotoxicity due to accumulated root exudates in the rhizosphere of culture solution (Kitazawa et al., 2005; Asao et al., 1998, 2003, 2004a, 2004b, 2007, 2008; Asaduzzaman et al., 2012; Singh et al., 1999; Tang and Young, 1982). The released chemical compounds create problems in closed hydroponic culture systems as they can accumulate and inhibit the growth of the crops. Exposure of these allelochemicals play a massive amount 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 permeability (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).Continuous crop cultivation with recycled nutrient solution, the plant growth was greatly reduced due to presence of the several organic acids in the reused nutrient

solution (Kitazawa et al., 2005; Asao et al., 2003, 2004a).In successive cultivation of lettuce using same nutrient solution, the lettuce growth and yield were also reduced by the same phenomenon(Lee et al., 2006).Removal or degradation of phytotoxic substances that have accumulated in the nutrient solution might reduce the crop growth inhibition.

To eliminate these phytotoxic organic acids from the nutrient solution and to mitigate autotoxicity in hydroponic culture, several methods have been tried such as adsorption of allelochemicals by using activated charcoal (Pramanik et al., 2000; Asao et al., 1998, 1999a, 1999b; Yu and Matsui, 1994; Yu et al., 1993), by using amberlite XAD-4(Lee, 2006) or by degradation of these chemicals using micro-organisms (Asao et al., 2004b). However, the use of activated charcoal creates blocks in the nutrient solution circulation systems in closed hydroponics and it also adsorbed Fe-EDTA from thenutrient solution. Amberlite XAD-4is expensive and it creates similar problems like activated charcoal. The use of micro-organisms can't recover crop growth and yield completely. Besides these methods, auxin (2, 4-D and NAA) supplementation to plant (Kitazawa et al., 2007), foliar application of amino acids (Mondal et al., 2013, 2015; Talukder et al., 2018) and application of specific LED light to plant (Talukder et al., 2018) can recover plant growth to some extent in spite of having autotoxicity.

Degradationof toxic compounds by electric means is another way to detoxify allelochemicals. Several phenolic compounds, including phenol (Comninellis and Pulgarin, 1991; Fengand Li, 2003; Fleszar and Ploszynka, 1985), catecol

(Comninellisand Pulgarin, 1991), and hydroquinone (ComninellisandPulgarin, 1991; Fleszar and Ploszynka, 1985),in aqueous solutions and even benzene (FleszarandPloszynka,1985) were found to decompose when treated by ED means. These compounds are oxidized rapidly at the anode and decompose to CO_2 (Comninellis and Pulgarin, 1991; Feng and Li, 2003; Fleszar and Ploszynka, 1985).

Based on these findings, benzoic acid from strawberry root exudates has been tried to decompose through direct currentelectro-degradation (DC-ED) means (Asaduzzaman et al., 2012; Asao et al., 2008). To avoid some awkward issues associated with DC-ED, Talukder et al., (2019)planned to change the power source from DC to alternating current (AC) and found that the application of AC-ED instead of DC-ED in non-renewed solution resulted in degradation of benzoic acid from the closed hydroponics without altering the properties of nutrient solution and confirmed the improved growth, yield and quality of strawberry.

In Japan, lettuce is widely cultivated in greenhouse using the hydroponic systems but, nutrient solutions have not been properly changed, adjusted or analyzed during cultivation. In most circumstances, nutrient solutions are renewed after a single use because of a new start of cultivation, and the used nutrient solution is drained out. However, if the phytotoxic chemicals that accumulate in the nutrient solutions are effectively eliminated, nutrient solution reuse could be more generally adopted in hydroponic cultivation. Therefore, the present study aimed to recover the lettuce growth from autotoxicity by means of an AC-ED machine in the successive lettuce cultivation using same nutrient solution.

2. Materials and methods

2.1. Plant material

Lettuce (*Lectuca sativa* cv. Souther) was used for this experiment. Seeds were (Takii seed company, Japan) sown in a cell trays (48 cm × 24 cm × 4 cm, 72 cells $tray^{-1}$) with vermiculite substrate and were kept in a growth chamber at 25/20ºC (day/night), 60% relative humidity, fluorescent light with intensity of 140~160 μmol m^{-2} s^{-1} and a 12 hours photoperiod. After 2-3 days seeds were germinated but cell trays were kept there for 14 days after sowing and during this period only fresh water was supplied in the cell trays.After that lettuce seedlings were transferred to the grow beds of hydroponic system in the plastic containers (68cm × 53cm ×23cm) for nursery in an environment control room. The room was maintained at a relative humidity of 60%, temperature 20/20°C (day/night), CO_2 concentration of 800 ppm, fluorescent light with intensity of 145μmol$m^{-2}s^{-1}$ and a photoperiod of 12 hours. One hundred seedlings were accommodated in each grow bed and 30 L, 50% standard "Enshi" nutrient solutions were used for each hydroponic system and solution was renewed bi-weekly. Continuous aeration was maintained in the nursery by a pump (Model: MX 808ST-W, Enomoto, Micro Pump Mfg. Co. Ltd., Japan with a maximum flow rate 25$Lmin.^{-1}$). Seedlings were kept there for 2 weeks. Then the more homogenous seedlings were selected as planting materials.

2.2. Nutrient solution

Lettuce seedlings were cultured in 50% standard "Enshi" nutrient solution (Hori, 1966). The pH and electrical conductivity of the nutrient solution were 7.15 and 1.4 dS m^{-1},

respectively whereas the electrical conductivity and pH of the tap water used to prepare this nutrient solution were 0.22 dS m^{-1} and 8.18, respectively.

2.3. Electro-degradation of nutrient solution

AC type electrode (designed and built by Yonago Shinko Co., Ltd., Tottori, Japan) was used for ED of benzoic acid or autotoxic chemicals in culture solution used for lettuce. In AC-ED, the electrode had a central core made of titanium with a surface area of 53.1 cm^2 (anode/cathode) which enclosed with cylindrical tube also made of titanium with a surface area of 95.5 cm^2 (cathode/anode) (Talukder et al., 2019). The nutrient solution can pass through the electrode where electro-degradation takes place. The electrodes were coupled with a digital AC power supplier (AD-8735D, AND, Japan). During electro-degradation 500Hz, 50% duty ratio, 1.8A and 24V were maintained. Every time this process was done for 24 hours. Similar electric condition was successfully used to detoxify benzoic acid and other autotoxic chemicals in culture solution of strawberry (Talukder et al., 2019).

2.4. Cultivation of Lettuce

2.4.1. Experiment I

Selected seedlings from the nursery were used as planting material. Control room for lettuce cultivation was maintained by setting thetemperatureat 20/20°C (day/night), relative humidity of 60%, CO_2 concentration of 800 ppm, fluorescent light with intensity of 250~280 $\mu molm^{-2}s^{-1}$ and a photoperiod of 12 hours. Seedlings were planted to three stage vertical growing beds (125 cm × 90 cm × 10.5 cm). On 6th November 2017, twenty seedlings were planted in each growing bed fixed with urethane cubes (23 mm × 23 mm × 27 mm). Three growing bedswere filled with 50% standard “Enshi” nutrient solution with each capacity of 50 L connected to a 300 L reservoir tank. Nutrient solutions

were recirculated at 55/5 min. (recirculate/stop) by an automatic pump (KP-101, Koshin, Kyoto, Japan) with an automatic timer (KS-1500, Iuchi, Osaka, Japan) and maximum discharge of 31 $Lmin.^{-1}$.

There were three types of culture solutions viz. renewed, non-renewed and non-renewed+ED. In case of renewed culture solution, solutions were renewed bi-weekly. While non-renewed nutrient solutions were not replaced by fresh nutrient solution but major nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+} and Fe^{3+}) concentration were adjusted at every two weeks interval as close as possible to the initial concentration of the 50% "Enshi" solution based on the chemical analyses. The ED was applied in the nutrient solution for 24hours at two weeks interval in the setting as it was described earlier in section 2.3.Small amount of nutrient solution (25ml)were collected in plastic bottles for the analyses of major nutrients. Nutrient solution was filtered with qualitative filter paper (Advantec Grade no. 131; 125 mm). Major mineral nutrients such as K^+, Ca^{2+}, $Mg^{2+}$and Fe^{3+} was measured with an atomic absorption spectrophotometer (Z-2000, Hitachi High-Technologies Corporation, Kyoto, Japan), NO_3^- with a compact NO_3^-meter TWIN NO_3^- (B-343, Horiba, Ltd., Japan) and PO_4^{3-} using spectrophotometer at 720 nm (U-2900, Hitachi High Technology, Tokyo, Japan).Finally lettuce plants were harvested after 6 weeks of planting on 18th December 2017. Data were collected on growth attributes and yield of lettuce at the harvest.

2.4.2. Experiment II

Cultivation procedure from planting to harvestand control room conditions were same as experiment I. Planting was done at 4th January 2018 and harvestedat 15th February 2018. There were four types of culture solutions viz. (i) renewed, (ii) non-renewed but starting solution was fresh 50% "Enshi" solution simply we called one culture non-

renewed solution (Non-renewed 1C) that was similar to non-renewed solution in experiment I, (iii) non-renewed but starting solution was once used culture solution simply we called two culture non-renewed solution (Non-renewed 2C), (iv) Non-renewed 2C+ED. In renewed culture system, nutrient solutions were renewed bi-weekly and in case of non-renewed culture systems, nutrient solutions were not changed throughout the growing period but major mineral nutrientswere adjusted bi-weekly like first experiment. The ED process was also same as first experiment.

2.4.3.Experiment III

In third experiment, cultivation procedure and control room conditions were same as experiment I & II. In thisexperiment, we applied ED at two different intervals. Seedlings were planted on 5 March 2018 and lettuce harvested on 16 April 2018. There were also four types of culture solutions viz. renewed, non-renewed, non-renewed with weekly electro-degradation (non-renewed+weeklyED), non-renewed with bi-weekly electro-degradation (non-renewed+ bi-weekly ED). In renewed culture system, nutrient solutions were renewed bi-weekly but in all other treatment, nutrient solutions were not changed throughout the growing period but major mineral nutrientswere adjusted bi-weekly like first experiment.ED of nutrient solution was done like previous setting of experiment I & II.

2.5. Determination of mineral concentrationin plant parts

Mineral nutrients concentrationsin lettuce plants were determined. Plant parts were separated into shoots and roots and kept in a constant temperature oven (DKN812, Yamato Scientific Co. Ltd. Japan) for 72 hours 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 0.25 g were mixed with 8 ml of HNO_3(60% conc.) 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). The filtered sample solutions were analyzed for mineral nutrients by atomic absorption spectrophotometer (Z-2310, Hitachi High Technologies Corporation, Tokyo, Japan).

2.6. Measurement of temperature, EC, pH and determination of mineral nutrients of culture solution

Conditions of culture solution such as temperature, EC and pH were recorded at every two weeks interval. EC was measured by EC meter (ES-51, Horiba, Ltd., Kyoto, Japan) while, temperature and pH were measured using pH meter (D-12, Horiba, Ltd., Kyoto, Japan) at each sampling. Amount of mineral nutrient remains in the culture solution were determined following the analytical procedures as described in section 2.4.1. Data were taken three times throughout the growing period.

2.7. Experimental design and statistical analysis

Different types of nutrient solutions were arranged in a completely randomized design with three replications. Analysis of variance for all data was done using computer package MSTAT-C developed by Russel (1986). The mean differences of each culture solution were separated according to Tukey's test at $P<0.05$.

3. Results

3.1. Effect of ED application in non-renewed solution (Experiment I)

Growth of lettuce significantly decreased in non-renewed nutrient solution compared to renewed solution (Table 1; Fig. 1). Application of AC-ED in non-renewed solution

Table 1. Effect of electro-degradation of non-renewed nutrient solution on the growth of hydroponically grown lettuce plants (Experiment I).

Types of nutrient solution	Number of leaves plant^{-1}	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Dry weight (g plant^{-1})	
					Shoot	Root
Renewed[z]	22.2 a[w]	32.0 a	26.1 a	50.7 a	10.3 a	1.78 a
Non-renewed[y]	17.7 b	26.5 b	21.3 b	44.9 b	8.5 b	1.29 b
Non-renewed + ED[x]	22.4 a	31.6 a	25.7 a	49.6 a	10.1a	1.72 a

[z]Nutrient solution was renewed bi-weekly.
[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.
[x]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly after ED applied.
[w]Means within a column followed by different letters are significantly different according to the Tukey's test at $P<0.05$.

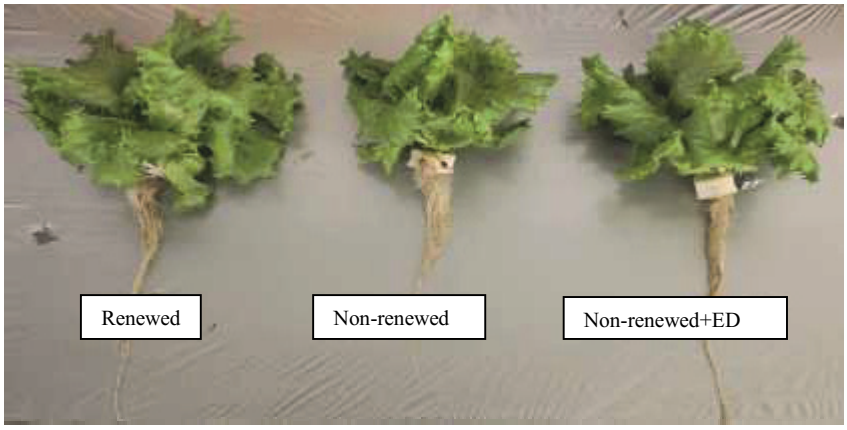


Fig.1. Effect of electro-degradation (ED) of non-renewed culture solution on the growth of lettuce grown in closed hydroponics (Experiment I).

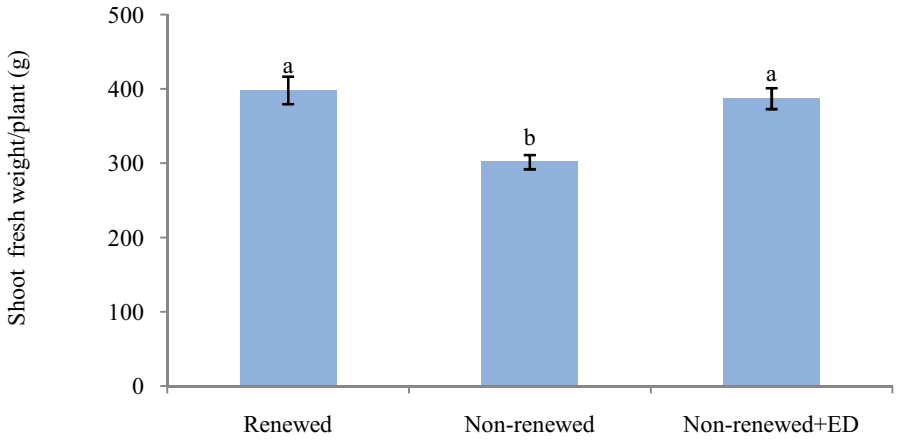


Fig.2. Effect of electro-degradation (ED) of non-renewed culture solution on the shoot fresh weight of lettuce grown in closed hydroponics (Experiment I).

increased the growth similar to renewed solution. Leaf number, maximum leaf length and width, longest root length, shoot and root dry weights were improved by the ED treatment. Number of leaves was significantly decreased in plants grown in non-renewed solution compared to renewed solution. While application of ED showed statistically similar number of leaves as it was produced in renewed. Likewise, the maximum leaf length and width, longest root length, shoot and root dry weights were significantly reduced in non-renewed solution compared to renewed solution. Due to application of ED to non-renewed solution these growth parameters showed better performance and were statistically similar to renewed solution. Shoot fresh weight was considered as lettuce yield and it was highest (398.3g plant^{-1}) in renewed culture solution (Fig. 2) which was statistically similar to non-renewed culture solution with ED (387.2g plant^{-1}). The lowest shoot fresh weight (301.8g plant^{-1}) was observed in non-renewed culture solution. ED of non-renewed culture solution significantly affected the mineral nutrient concentration especially calcium and iron in shoot and root of lettuce plants (Table 2). In root and shoot, both calcium and iron concentrations were decreased significantly in non-renewed culture solution. Other minerals like potassium, magnesium and zinc in both plant parts were not significantly affected but average values were relatively low in non-renewed culture solution. Temperature, EC and pH of the culture solutions measured were not differed significantly throughout the growing periods (Table 3). The amounts of minerals (iron, calcium, nitrogen, phosphorus, potassium and magnesium) were also not significantly affected indifferent culture solutions (Table 3).

Table 2. Effect of electro-degradation of non-renewed nutrient solution on the mineral concentrations of hydroponically grown lettuce plants (Experiment I).

Types of nutrient solution	Ca (mg g^{-1} DW)		Mg (mg g^{-1} DW)		K (mg g^{-1} DW)		Fe (mg kg^{-1} DW)		Zn ((mg kg^{-1} DW)	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Renewed[z]	28a[w]	31 a	3.6	5.1	86	100	496 a	203 a	79	27
Non-renewed[y]	18 b	23 b	3.2	5.0	81	99	314 b	107 b	66	25
Non-renewed + ED[x]	27 a	33 a	3.5	5.2	83	102	511 a	212 a	84	29
Significance			NS	NS	NS	NS			NS	NS

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[x]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly after ED applied.

[w]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P<0.05$. DW= Dry weight

Table 3. Influence of nutrient solution electro-degradation on the solution temperature, pH, electrical conductivity and residual nutrient concentrations in the experiment I.

Types of nutrient solution	Temperature (ºC)	pH	EC (dS m^{-1})	Residual nutrient content (ppm)					
				Fe^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	NO_3^-	$P_2O_5^-$
Renewed[z]	21.8	7.11	1.36	3.77	104	31.3	79.0	1680	27.3
Non-renewed[y]	21.7	7.11	1.34	3.83	114	31.0	78.3	1663	26.7
Non-renewed + ED[x]	21.1	7.10	1.33	3.74	103	30.3	79.6	1643	26.0
Significance	NS[w]	NS	NS	NS	NS	NS	NS	NS	NS

[z]Nutrient solution was renewed bi-weekly.
[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.
[x]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly after ED applied.
[w]NS indicate non-significant according to the Tukey's test at $P<0.05$.

3.2. Effect of ED application intwo culture non-renewed solution (Experiment II)

The application of ED in the twoculture non-renewed solution also significantly influenced the lettuce growth (Table 4; Fig. 3). All growth parameters such as leaf number, maximum leaf length and width, longest root length, shoot and root dry weights were lowest in the non-renewed 2Csolution plant. Even compare to non-renewed 1C solution, leaf number, leaf length and width, longest root length, shoot and root dry weights were significantly decreased in plants grown in non-renewed 2C solution. In case of renewed solution, plants produced the highest leaf number, maximum leaf length and width, longest root length, shoot and root dry weights. When ED was applied to non-renewed 2C solution, plants showed all these growth parameters statistically similar to renewed culture solution. Shoot fresh weight was highest (400.2g plant^{-1}) in renewed solution plants (Fig.4) and it was lowest (258.8g plant^{-1}) in non-renewed 2C solution plants. In case of non-renewed 1C solution plants, shoot fresh weight was 310.2g plant^{-1} and it was statistically higher than that of non-renewed 2C solution. But ED application to non-renewed 2C solution recovered shoot fresh weight and it was observed 383.3g plant^{-1} which was statistically similar to renewed solution plants.

The calcium and iron in shoot and root of lettuce plants were also significantly influenced by different culture solution but potassium, magnesium and zinc were not affected (Table 5). Calcium and iron concentrationswere decreased significantly in non-renewed 1C and non-renewed 2C solution plants both in root and shoot. Calcium and iron concentration in plants grown in renewed solution was significantly higher and was statistically similar to non-renewed 2C solution treated with ED. Potassium, magnesium

Table 4. Effect of electro-degradation of two culture non-renewed nutrient solution on the growth of hydroponically grown lettuce plants (Experiment II).

Types of nutrient solution	Number of leaves plant^{-1}	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Dry weight (g plant^{-1})	
					Shoot	Root
Renewed[z]	24.2 a[v]	34.3 a	26.7 a	46.3 a	11.0 a	1.85 a
Non-renewed 1C[y]	18.7 b	28.3 b	23.7 b	40.9 b	9.2 bc	1.49 b
Non-renewed 2C[x]	15.1 c	22.7 c	17.0 c	34.3 c	8.4 c	1.06 c
Non-renewed 2C+ED[w]	23.3 a	32.5 a	26.3 a	44.9 ab	10.3 ab	1.67 a

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was new at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[x]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[w]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% bi-weekly after ED applied.

[v]Means within a column followed by different letters are significantly different according to the Tukey's test at $P<0.05$.

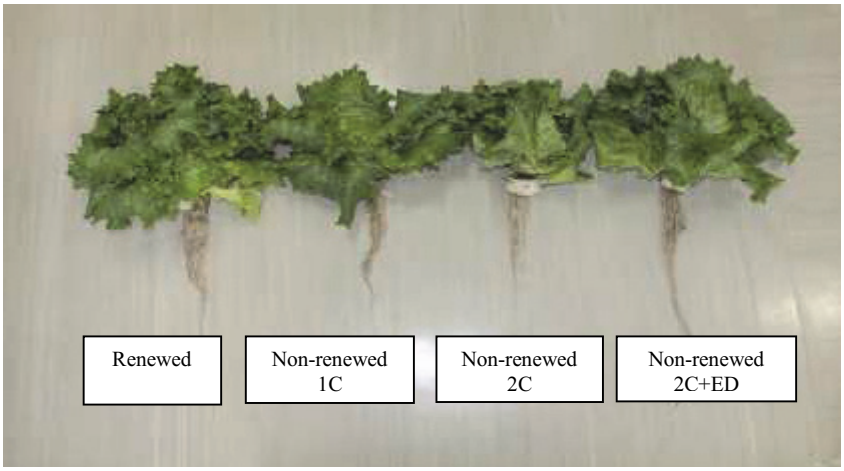


Fig.3. Effect of electro-degradation (ED) of non-renewed culture solution on the growth of lettuce grown in closed hydroponics (Experiment II). [Non-renewed 1C = One culture non-renewed solution and Non-renewed 2C = Two culture non-renewed solution].

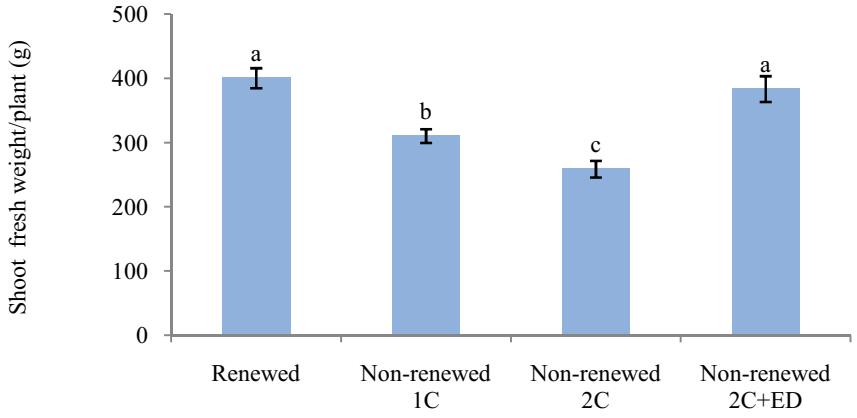


Fig. 4. Effect of electro-degradation (ED) of non-renewed culture solution on the shoot fresh weight of lettuce grown in closed hydroponics (Experiment II). [Non-renewed 1C = One culture non-renewed solution and Non-renewed 2C = Two culture non-renewed solution].

Table 5. Effect of electro-degradation of two culture non-renewed nutrient solution on the mineral concentrations of hydroponically grown lettuce plants (Experiment II).

Types of nutrient solution	Ca(mg g^{-1} DW)		Mg (mg g^{-1} DW)		K (mg g^{-1} DW)		Fe (mg kg^{-1} DW)		Zn (mg kg^{-1} DW)	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Renewed[z]	29a[v]	28 a	3.9	5.0	86	98	510 a	202 a	87	28
Non-renewed 1C[y]	16 b	21 b	3.8	4.8	82	92	344 b	121 b	79	24
Non-renewed 2C[x]	14 b	21 b	3.7	4.7	80	99	312 b	119 b	72	21
Non-renewed 2C+ED[w]	29 a	29 a	3.9	4.9	89	105	558 a	210 a	82	26
Significance			NS	NS	NS	NS			NS	NS

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was new at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[x]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[w]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% bi-weekly after ED applied.

[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P<0.05$.

DW= Dry weight

Table 6.Influence of nutrient solution electro-degradation on the solution temperature, pH, electrical conductivity and residual nutrient concentrations in the experiment II.

Types of nutrient solution	Temperature (ºC)	pH	EC (dS m^{-1})	Residual nutrient content (ppm)					
				Fe^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	NO_3^-	$P_2O_5^-$
Renewed[z]	21.1	7.05	1.32	3.84	101	31.3	77.3	1746	26.0
Non-renewed 1C[y]	21.7	7.10	1.34	3.91	109	31.6	78.3	1732	26.7
Non-renewed 2C[x]	21.0	7.11	1.34	3.86	104	31.0	78.3	1733	26.4
Non-renewed 2C+ED[w]	20.8	7.07	1.34	3.88	103	30.3	79.6	1741	24.6
Significance	NS[v]	NS	NS	NS	NS	NS	NS	NS	NS

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was new at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[x]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[w]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% bi-weekly after ED applied.

[v]NS indicate non-significant according to the Tukey's test at $P<0.05$.

and zinc concentrations in root and shoot were relatively low in both non-renewed 1C and non-renewed 2C solution. Temperature, EC, pH and minerals (iron, calcium, nitrogen, phosphorus, potassium and magnesium) concentration of the culture solutions measured were also not varied (Table 6) significantly all over the growing periods in different culture solutions.

3.3. Effect of different intervals of ED application in non-renewed solution (Experiment III)

The application of ED in the non-renewed culture solution significantly influenced the lettuce growth but between the two intensities of ED application significant growth differences were not observed (Table 7; Fig.5).All growth parameters were significantly affected. In case of renewed solution, plants produced the highest leaf number, maximum leaf length and width, longest root length, shoot and root dry weights whereas these growth parameters were lowest in the non-renewed culture solution. When ED was applied weekly or bi-weekly to the non-renewed solution, plants demonstrated all these growth parameters statistically similar to renewed culture solution. Shoot fresh weight was highest (393.6g plant^{-1}) in renewed culture solution (Fig. 6) and it was lowest (280.5g plant^{-1}) in non-renewed culture solution. But, due to weekly ED application to non-renewed solution, plants produced 377.8g plant^{-1} shoot fresh weight and it was statistically similar to renewed culture solution. Bi-weekly ED application to non-renewed solution also produced higher shoot fresh weight (382.1g plant^{-1}) and it was also statistically similar to renewed culture solution and weekly ED application to non-renewed solution.

Table 7. Effect of different intervals of non-renewed nutrient solution electro-degradation on the growth of hydroponically grown lettuce plants (Experiment III).

Types of nutrient solution	Number of leaves plant^{-1}	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Dry weight (g plant^{-1})	
					Shoot	Root
Renewed[z]	23.1 a[v]	33.8 a	27.1 a	42.3 a	10.2 a	1.92 a
Non-renewed[y]	18.7 b	22.3 b	23.3 b	35.9 b	7.7 b	1.28 b
Non-renewed +ED weekly[x]	22.6 a	32.1 a	27.2 a	43.3 a	9.8 a	1.86 a
Non-renewed + ED Bi-weekly[w]	22.8 a	33.5 a	26.3 a	41.7 a	10.3 a	1.84 a

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly

[x]Nutrient solution was not renewed throughout the culture periodand major nutrients were adjusted to standard 50% Enshi solution bi-weekly but ED was applied weekly.

[w]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly and ED was also applied bi-weekly.

[v]Means within a column followed by different letters are significantly different according to the Tukey's test at $P<0.05$.

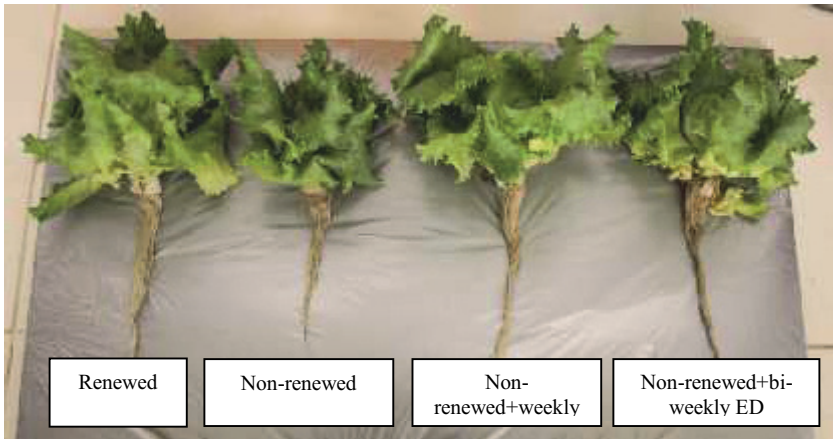


Fig.5. Effect of electro-degradation (ED) of non-renewed culture solution on the growth of lettuce grown in closed hydroponics (Experiment III).

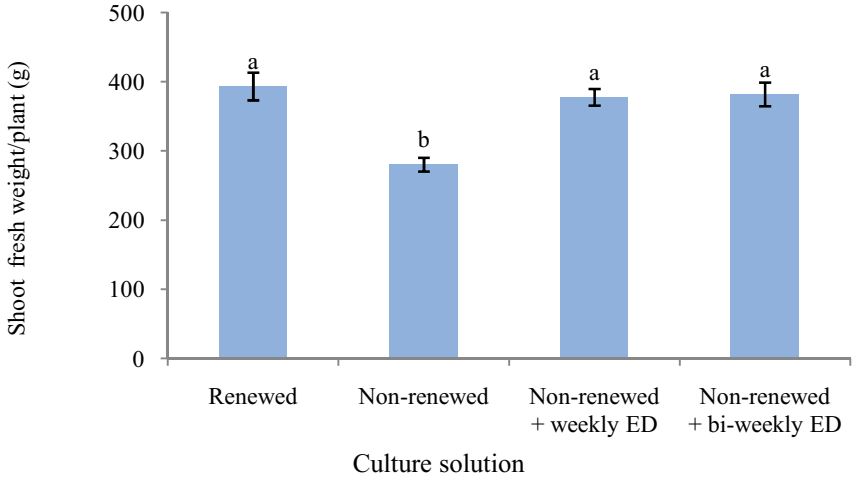


Fig. 6. Effect of electro-degradation (ED) of non-renewed culture solution on the shoot fresh weight of lettuce grown in closed hydroponics (Experiment III).

Table 8. Effect of different intervals of non-renewed nutrient solution electro-degradation on the mineral concentrations of hydroponically grown lettuce plants (Experiment III).

Types of nutrient solution	Ca(mg g^{-1} DW)		Mg (mg g^{-1} DW)		K (mg g^{-1} DW)		Fe (mg kg^{-1} DW)		Zn (mg kg^{-1} DW))	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Renewed[z]	30a[v]	29 a	4.1	5.4	88	98	560 a	248 a	86	28
Non-renewed[y]	17 b	19 b	3.7	4.9	82	94	313 b	149 b	81	20
Non-renewed+ ED weekly[x]	26 a	27 a	3.9	5.2	86	99	558 a	249 a	82	22
Non-renewed+ ED Bi-weekly[w]	27 a	29 a	3.9	5.3	85	99	626 a	251 a	86	26
Significance			NS	NS	NS	NS			NS	NS

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly

[x]Nutrient solution was not renewed throughout the culture period and major nutrients were adjusted to standard 50% Enshi solution bi-weekly but ED was applied weekly.

[w]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly and ED was also applied bi-weekly.

[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P<0.05$.

DW= Dry weight

Table9.Influence of nutrient solution electro-degradation on the solution temperature, pH, electrical conductivity and residual nutrient concentrations in the experiment III.

Types of nutrient solution	Temperature (ºC)	pH	EC (dS m^{-1})	Residual nutrient content (ppm)					
				Fe^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	NO_3^-	$P_2O_5^-$
Renewed[z]	21.1	7.07	1.29	3.82	105	30.1	77.7	1680	24.7
Non-renewed[y]	21.0	7.08	1.36	3.89	108	31.3	78.3	1780	26.0
Non-renewed+ ED weekly[x]	21.1	7.11	1.34	3.88	104	31.0	78.1	1780	26.7
Non-renewed+ ED Bi-weekly[w]	20.8	7.07	1.34	3.84	103	30.3	79.7	1713	24.6
Significance	NS[v]	NS	NS	NS	NS	NS	NS	NS	NS

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly

[x]Nutrient solution was not renewed throughout the culture period and major nutrients were adjusted to standard 50% Enshi solution bi-weekly but ED was applied weekly.

[w]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly and ED was also applied bi-weekly.

[v]NS indicate non-significant according to the Tukey's test at $P<0.05$.

The calcium and iron concentrations in lettuce plants were also significantly influenced by different culture solution but potassium, magnesium and zinc concentrations were unaffected (Table 8). In root and shoot, calcium and iron concentrations were decreased significantly in plants grown in non-renewed solution. Calcium and iron concentration in plants grown in renewed solution were statistically similar to non-renewed solution treated with ED either weekly or bi-weekly. Temperature, EC, pH and minerals (iron, calcium, nitrogen, phosphorus, potassium and magnesium) concentrations of different culture solution measured were not varied (Table 9) significantly all over the growing periods like experiment I & II.

4. Discussions

In closed hydroponics system, the nutrient solution is recovered, replenished and recycled. This hydroponic technique increases water and nutrient use efficiencies and reduces environmental pollution. Therefore, recent environmental regulations to conserve ground water and to minimize water and fertilizer consumption (Saavas, 2001) have highlighted the importance of nutrient solution recycling and encouraged the shift from open to closed hydroponics.

Closed recycling hydroponic systems have some limitations and accumulation of allelochemicals in the culture solution is one of them. In previous studies, many researchers found allelochemicals in non-renewed solution from root exudation in strawberry (Kitazawa etal., 2005; Asao etal., 2008; Asaduzzaman et al., 2012; Mondal et al., 2013), cucumber (Yu and Matsui, 1994; Asao et al., 1998), several leafy

vegetables (Asao etal., 2004a) and some ornamentals plants (Asao et al., 2007) grown in closed hydroponics.

Therefore, the solution has to be eventually renewed. However, the disposal of culture solution is not likely to damage the environment. Lettuce grown in closed hydroponic accumulate many allelochemicals in the culture solutions (Asao et al., 2004a; Lee et al., 2006). For an effective nutrient solution management and consequently, an increase in hydroponic lettuce yield, it is indispensable to alleviate the inhibitory effect of allelochemicals. To maintain culture solution in closed hydroponics free from allelochemicals or below the threshold levels leading to normal growth, we conducted several experiments.

In our first experiment, all growth parameters and yield of lettuce were significantly affected due to the non-renewed nutrient solution (Table1; Fig.1, 2). Shoot fresh yield decreased to 24% compared torenewed nutrient solution.The residual mineralsconcentrations in the culture solution were not varied. Some other important growth factors for hydroponic lettuce culture as described by Furlaniet al., (1999) such as EC, pH and temperature of the culture solution also were not varied (Table 3). Hence, the retarded growth of lettuce in the non-renewed culture solution was mainlydue to inhibitory effect of the accumulated allelochemicals.

Allelochemicals delivered into the rhizosphere due to root exudation (Bertin et al., 2003) found responsible for hampering numerous physiological reactions such astranspiration, water utilization, photosystem II (PSII) efficiency, nutrient uptake, dark

respiration, ATP synthesis, cell cycle, phyto-hormone metabolism and gene expression, etc. (Inderjit and Duke, 2003; Blum, 2005). That was why; we obtained reduced lettuce growth in non-renewed solution.It is well-known that plants generate more reactive oxygen species (ROS) when exposed to stressful conditions such as accumulation of allelochemicals in the rhizosphere (Yamamoto et al., 2003; Halliwell, 2006; Rhoads et al., 2006).These ROS are either toxic by-products of aerobic metabolism or key regulators of growth, development, and the defense pathway (Mehdyet al., 1996; Laloi et al., 2004; Mittler et al., 2004). Toxic ROS can affect membrane permeability, cause damage to DNA and protein, induce lipid peroxidation and ultimately lead to programmed cell death. Recent findings about the biochemical and physiological effect of natural phyto-toxins have shed light on the rhizosphere interactions (Weir et al., 2004). Several studies have shown that allelochemical stress can cause oxidative damage, as evidenced by enhanced activity of ROSscavenging enzymes and increased degree of membrane lipid peroxidation (Baziramakenga et al., 1995; Politycka, 1996; Yu et al., 2003; Lara-Nunez et al., 2006; Ye et al., 2004, 2006). Furthermore, Bais et al. (2003) found that allelochemicals induce genome-wide changes of gene expression, and ultimately result in the death of the root cells. Therefore, we obtained reduced root growth in non-renewed culture solution. Consequently, damaged roots hamper water and mineral nutrient uptake. As a result, the leaf numberplant^{-1}, leaf size, root length, shoot fresh weight, shoot and root dry weight etc. were reduced. Lettuce grown in non-renewed culture solution showed lower mineral concentrationespecially calcium and iron in their plant parts (Table 2) due to impaired nutrient uptake as a result of accumulation of growth inhibitors in the rhizosphere (Singh et al., 1999). In our previous studies (Talukder et al., 2019; Asaduzzaman et al., 2012), we also observed

lowered calcium and iron concentrations in different plant parts of strawberry grown in non-renewed culture solution.

On the other hand, application of ED to the non-renewed culture solution increased the growth, yield and mineral concentrations in lettuce that were similar to renewed culture solution. The possible reason for this improved plant growth performances due to application of ED in non-renewed culture solution might include the degradation of inhibitory chemicals and no negative effects on solution. Similar results were also obtained in closed hydroponic production of strawberry (Talukder et al., 2019; Asaduzzaman et al., 2012).

In second experiment, lettuce was grown in two types of non-renewed solution viz. non-renewed 1C and non-renewed 2C. Growth, yield and minerals concentrations decreased in plants grown in non-renewed 2Csolutioncompared to non-renewed 1C solution and also compared to renewed solution (Table 4&5; Fig. 3, 4). This might be due to higher concentration of allelochemicals in the non-renewed 2C solution. As non-renewed 2C solution was used for longer period for the cultivation of lettuce compare to non-renewed 1C solution, higher amount of allelochemicals accumulated there.

Currently, some research findings detected many organic acids such as benzoic, phenylacetic, cinnamic, p-hydroxybenzoic, lauric, phthalic, vanillic, palmitic, and stearic acids etc. from the root exudates of lettuce grown in non-renewed solution (Asao et al., 2004a; Lee etal., 2006) and identified as major growth inhibitors. Lee et al., (2006) also determined that number and concentration of these organic acids in the

nutrient solution highly varied with reuse time, generally showing the increasing trend with the increase reuse time. A few allelochemicals were exuded from the roots at comparatively low concentration in the first culture. Later on numbers of allelochemicals and their concentrations were found increased in the non-renewed 2C solution. As the number of allelochemicals were found increased in the non-renewed 2C solution, they affected plant growth badly by additive or synergistic means (Inderjit, 1996). As a result, more retarded growth of lettuce was obtained in non-renewed 2C solution.

But, while ED was applied to non-renewed 2C solution the growth, yield and minerals(especially calcium and iron) concentrationin lettuce significantly increased which were similar to lettuce grown in renewed solution due to the degradation of allelochemicals. Thus, it revealed that two successive cultivation of lettuce with the same nutrient solution could be achieved through electro-degradation of culture solution.

In a following study, we tried to determine the intervalof ED application in the third experiment. ED was applied at one week and two weeks interval. Plants grown in non-renewed solution resulted lower growth, yield and minerals concentrationresembling first and second culture experiments (Table 7&8; Fig. 5, 6). ED applied both weekly and bi-weekly produced statistically similar growth, yield and minerals concentrationin lettuce which was also similar to that of plants grown in renewed culture solution. Therefore, we could decide that bi-weekly ED application was enough for successive lettuce cultivation and additionally, it reduced the electricity cost compare to weekly

ED. Recently, several other reports (Talukder et al., 2019; Asaduzzaman et al., 2012) also found different suitable ED application intervals in closed hydroponic for different crops such as tri-weekly ED application for strawberry.

AC-ED machine, a low cost tool, when applied to non-renewed solution in two successive lettuce culture using same nutrients at two week interval completely recovered the retarded lettuce yield from autotoxicity. Total cost of this process was lower than culture solution renewal cost. Moreover, renewal process causes environmental problem due to disposal of used solution. Therefore, use of ED process would be more supportive for lettuce growers.

5. Summary

Lettuce cultivation in the non-renewed hydroponics resulted reduced yield and quality. Plants grown in the two culture non-renewed solution resulted more reduced yield and quality of lettuce than one culture non-renewed solution. In the successive cultivation, lettuce grown in the non-renewed solution gradually reduced yield and quality in accordance to the nutrient solution reuse times.Due to ED application to non-renewed solution recovered the retarded yield and quality completely in both oneculture non-renewed solution and two culture non-renewed solutions.We suggest that ED treatment to non-renewed solution (300L) for 24 hours at two week intervals canbe applied for complete recovery of the retarded lettuce yield and quality in two or more successive closed hydroponic cultivation using same nutrient solution.

CHAPTER 5

Alleviation of allelochemical stress induced growth inhibition and oxidative damage in lettuce under closed hydroponics through electro-degradation

1. Introduction

In closed hydroponics accumulation of allelochemicals occurs in the culture solution due to root exudations. When these compounds suppress plant growth, the phenomenon is considered to be a biotic stress termed "allelochemical stress" (Cruz-Ortega et al., 2002). When it occurs among the individuals of the same species due to their root exudation called autotoxicity. In closed hydroponics, this stress has been documented in a number of crop species due to accumulated root exudates in the rhizosphere (Kitazawa et al., 2005; Asao et al., 2007, 2008; Asaduzzaman et al., 2012; Singh et al., 1999; Tang and Young, 1982) including lettuce (Asao et al., 2004b; Lee et al., 2006). In this phenomenon lettuce root secreted several allelochemicals to the culture solution causing damage to the root cells, which in turns hamper water and mineral nutrient absorption resulting growth inhibition and yield loss. In successive closed hydroponics lettuce cultivation using same nutrient solution, the plant growth was greatly reduced and root injury increased due to presence of the several allelochemicals in the reused nutrient solution. Growth retardation and root injury gradually increased with the increased reuse times of the nutrient solution (Lee et al., 2006).

Removal or degradation of these phytotoxic substances that have accumulated in the nutrient solution would lead to normal growth and yield of crops. In these regards, the nutrient solution that feeds the plants needs to be replaced periodically. However, this process requires more labor, time, money and generates hydroponic wastewater that is particularly rich in nitrogen and phosphorus; when these nutrients are discharged directly into the environment, they may cause contamination (Bertoldi et al., 2009). As a result, many researchers tried to remove or degrade these accumulated allelochemicals in an alternative means such as adsorption of allelochemicals by activated charcoal (AC) (Asao et al., 1998; Lee et al., 2006) and Amberlite XAD-4 (Lee et al., 2006), degradation of allelochemicals by ED means (Asao et al., 2008; Asaduzzaman et al., 2012 and Talukder et al., 2019) and degradation of allelochemicals by microbial strains (Asao et al., 2004a).

When plants are exposed to allelochemical stress in closed hydroponics it suffers from the disruption of normal physiological process before ensuing yield loss. It alter ion uptake and hydraulic conductivity (Blum et al., 1999), alter mineral uptake (Lyu and Blum, 1990; Baziramakenga et al., 1994), disrupt membrane permeability (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). Similar to other biotic stresses, in allelopathic reaction an essential function of reactive oxygen species (ROS) was indicated by some authors (Weir et al., 2004; Gniazdowska and Bogatek, 2005; Cruz-Ortega et al., 2007). In

allelochemical stress, a shift from a regulatory role of ROS in cell signaling to their toxicity is probably related to changes in homeostasis of ROS maintained by imbalance of ROS production and ROS scavenging. It induces oxidative damage which evidenced by high level of lipid peroxidation (Romero-Romero et al., 2005; Lara-Núñez et al., 2006) and generation of more reactive oxygen species (ROS) in plants (Singh et al., 2006; Batish et al., 2006; Cruz-Ortega et al., 2002; Weir et al., 2004). Undue soluble protein production under stress condition was observed by several other researchers (Singh et al., 1987; Ashraf et al., 2004). Induction of oxidative stress by allelochemicals was investigated in a range of plants, e.g., maize (Mylona et al., 2007), rice (Chi et al., 2011) and soybean (Böhm et al., 2006). Other studies also have shown that allelochemical stress can cause oxidative damage to plants (Bais et al., 2003; Sánchez-Moreiras et al., 2005; Abenavoli, 2006).

The most common ROS are hydrogen peroxide, superoxide, the hydroxyl radical and singlet oxygen that formed as a natural byproduct of the normal metabolism of oxygen and is crucial in cell signaling. The overproduction of ROS leads to oxidative stress and can cause damage to cellular components. To diminish the impact of oxidative stress, plants have evolved a complex system of antioxidants enzyme such as SOD, CAT, POD, APX and glutathione reductase (GR) and some other non-enzymatic antioxidants that accumulate in higher plants under stress condition (Ozkur et al., 2009). Plants enhance the antioxidants production in order to minimize the detrimental effects of oxidative stress to normalize their metabolic activities. Elevated accumulation of antioxidant enzymes such as SOD, CAT, GR, APX and POD are involved in lowering oxidative damage were observed in caper bush seedlings under drought stress (Ozkur et

al., 2009). In another study, Yang et al., (2009) found an increase in the activity of CAT, SOD, POD, APX and GR under drought stress condition. In successive closed hydroponic cultivation due to overproduction of allelochemicals create oxidative imbalance by generating more ROS and altering the antioxidant enzyme activity. Elimination of these accumulated allelochemicals may lead to maintain oxidative balance.

In this study, we investigated the influence of allelochemical stress on the antioxidant system of lettuce grown in the reused solutions under successive closed hydroponic cultivation. Activities of the antioxidant enzymes such as CAT, APX, SOD and POD were studied. We also examined levels of H_2O_2, O_2^-, soluble protein and membrane damage as lipid peroxidation. Together with other methods, ED of culture solutions was also studied for alleviation of allelochemicals induced growth inhibition and maintaining balance between the overproduction of ROS and their scavenging by enhanced production of CAT, SOD, POD and APX.

2. Materials and methods

2.1. Plant material

In this study lettuce (*Lectuca sativa* cv. Souther) was used as plant material. Seeds were (Takii seed company, Japan) sown in a cell trays (48 cm × 24 cm × 4 cm, 72 cells/tray) with vermiculite substrate and were kept in a growth chamber at 25/20 ºC (day/night), 60% relative humidity, fluorescent light with intensity of 140~160 μmol m^{-2} s^{-1} and a 12 hours photoperiod. After two weeks seedlings were transferred to the grow beds of

hydroponic system in the plastic containers (68 cm × 53 cm ×23 cm) for nursery in an environment control room. The room was maintained at a relative humidity of 60%, temperature 20/20 °C (day/night), CO_2 concentration of 800 ppm, fluorescent light with intensity of 145 μmol m^{-2} s^{-1} and a photoperiod of 12 hours. One hundred seedlings were accommodated in each grow bed and 30 L, 50% standard "Enshi" nutrient solutions were used for each hydroponic system and solution was renewed weekly. Continuous aeration was maintained in the nursery by a pump (MX 808ST-W, Enomoto, Micro Pump Mfg. Co. Ltd., Japan; Flow rate 25 L $min.^{-1}$). Seedlings were kept there for two weeks. Then the more homogenous seedlings were selected as planting materials.

2.2. Nutrient solution

Lettuce seedlings were cultured in 50% standard "Enshi" nutrient solution (Hori, 1966). The pH and EC of the nutrient solutions were 7.15 and 1.4 dS m^{-1}, respectively whereas these values of the tap water used to prepare this nutrient solution were 0.22 dS m^{-1} and 8.18, respectively. The used solutions collected from successive closed hydroponic lettuce were also 50% standard "Enshi" nutrient solution initially.

2.3. ED of allelochemicals in the used nutrient solution

Collected used nutrient solutions were filtrated through Whatman No. 2 filter paper. A 10 L filtered solution was electro-degraded with an electro-degradation machine. Alternating current type electrodegradation machine (Yonago Shinko Co., Ltd., Tottori, Japan) was used for ED of autotoxic chemicals in used solution. In this machine, the electrode had a central core made of titanium with a surface area of 53.1 cm^2 (anode/cathode) which enclosed with cylindrical tube also made of titanium with a

surface area of 95.5 cm^2 (cathode/anode) (Talukder et al., 2019). The nutrient solution could pass through the electrode where ED took place. The electrodes were coupled with a digital alternating current power supplier (AD-8735D, AND, Japan). During ED 500 Hz, 50% duty ratio, 1.8A and 24V were maintained. This process was maintained for 24 hours.

2.4. Adsorption of allelochemicals from used nutrient solution by amberlite XAD-4

Amberlite XAD-4 (20-60 mesh) collected from Sigma Co. was used as a good adsorbent of allelochemicals from used nutrient solutions. At first, the used nutrient solutions were filtrated through Whatman No. 2 filter paper. Then, a 10 L filtered solution was passed through the glass column (length 15 cm × diameter 5 cm) filled with 100 g amberlite XAD-4 at a running rate of 7 mL min^{-1}.

2.5. Adsorption of allelochemicals from used nutrient solution by activated charcoal (AC)

A 100 g of AC (Sigma Co.) was mixed with 10 L filtered used nutrient solution and solution was aerated by a pump (MX 808ST-W, Enomoto, Micro Pump Mfg. Co. Ltd., Japan) for 24 hours and then, the solution was again filtered through Whatman No. 2 filter paper for removing AC.

2.6. Mineral adjustment in the used nutrient solutions

Before starting the experiment, major nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}) concentration in used nutrient solution and treated (ED, XAD-4 and AC) solutions were

adjusted as close as possible to the concentration of the new 50% 'Enshi' solution based on the chemical analyses. Small amount of nutrient solution (25 ml) were collected in plastic bottles for the analyses of major nutrients. Nutrient solution was filtered with qualitative filter paper (Advantec Grade no. 131; 125 mm). Major mineral nutrients such as K^+, Ca^{2+}, $Mg^{2+}$and Fe^{3+} was measured with an atomic absorption spectrophotometer (Z-2000, Hitachi High-Technologies Corporation, Kyoto, Japan), NO_3^- with a compact meter TWIN NO_3^- (B-343, Horiba, Ltd., Japan) and PO_4^{3-} using spectrophotometer at 720 nm (U-2900, Hitachi High Technology, Tokyo, Japan).

2.7. Lettuce bioassay using used nutrient solution

2.7.1. Bioassay using once used nutrient solution (Bioassay I)

Selected seedlings were planted in the plastic containers (30 cm × 20 cm × 10 cm). The containers were filled with 3 L of nutrient solution. Ten seedlings were planted in each container using urethane foam block as support. The containers were kept in a control room by maintaining the temperature at 25/20 °C (day/night), a relative humidity of 60%, CO_2 concentration of 800 ppm, fluorescent light with intensity of 140~160 μmol m^{-2} s^{-1} and a photoperiod of 12 hours. Once used non-renewed solutions (NR) were from the closed hydroponic systems where lettuce was grown for a period of six weeks and the solution was not changed throughout the growing period. After lettuce harvest, NR solution was collected. This bioassay was consist of five types of nutrient solutions such as standard 50% 'Enshi' solution i.e. new nutrient solution (NNS); once used non-renewed solutions (NR); once used non-renewed solution treated with ED (NR + ED), once used non-renewed solution treated with AC (NR + AC) and once used non-renewed solution treated with amberlite XAD-4 (NR + XAD). This bioassay was

conducted for two weeks. All data collected on growth attributes, yield and chlorophyll content (measured by SPAD, Konica Minolta, Tokyo, Japan) were taken at harvest. Roots samples were also collected at this time for subsequent analyses of soluble protein, ROS, lipid peroxidation and antioxidant enzyme activities.

2.7.2. Bioassay using twice used nutrient solution (Bioassay II)

This bioassay was conducted by using different treatments in the twice used non-renewed solutions (2NR). The 2NR solutions were collected from the closed hydroponic systems where lettuce was grown for two times successively and the solution was not changed throughout the two growing periods (12 weeks). Environmental condition was similar to first bioassay. This bioassay was composed of six treatments such as standard 50% 'Enshi' solution i.e. new nutrient solution (NNS), once used non-renewed solutions (1NR), twice used non-renewed solution (2NR), twice used non-renewed solution treated with ED (2NR + ED), twice used non-renewed solution treated with AC (2NR + AC) and twice used non-renewed solution treated with XAD-4 (2NR + XAD). This bioassay was also conducted for two weeks and data were taken like first bioassay.

2.8. Determination of hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), and their histochemical detection in roots

H_2O_2 and O_2^- in roots were extracted and the content was determined spectrophotometrically as described by Willekens et al. (1997) and Jiang and Zhang (2002), respectively. H_2O_2 was detected using 3, 3-diaminobenzidine (DAB) staining according to Zeng et al. (2014) with some modification. Fresh root tips were incubated in 1 mg mL^{-1} DAB–HCl solution for 8 h and washed once with a 2-N-morpholino-

ethanesulfonic acid/potassium chloride (MES/ KCl) buffer (10^{-3} M, pH 6.15). Superoxide anion (O_2^-) was detected using nitro blue tetrazolium (NBT) staining (Zeng et al., 2014). Root tip segments were dyed for 2 h with 0.1 mg mL^{-1} NBT (in 0.2 M phosphate buffer, pH 7.6) in darkness and subsequently washed once with phosphate buffer. After staining, roots were washed with distilled water for 10 min. All stained segments were observed using a Leica Fluorescence Stereomicroscope (M165C, Leica Microsystems, Heerbrugg, Switzerland) under visible light and photographed with a charge-coupled device (CCD) imaging system, which was fitted to microscope.

2.9. Determination of total soluble protein content in roots

Total soluble protein content was quantified in roots of lettuce using the Spectrophotometric Bradford assay (1976). A 0.5 g of fresh roots was ground in liquid nitrogen to fine powder. To avoid protein denaturation, mortar, pestle and the Eppendorf tubes were previously frozen in liquid nitrogen. Then, 1.2 ml of extraction buffer (K-0.2 M phosphate at pH 7.8; 0.1 176 mM EDTA and 1% insoluble PVP) was added to the powder. Samples were vortexed and centrifuged at 4 °C and 15000 g for 30 min. A 5 µl aliquot of the supernatant was carefully collected and mixed with 795 µl of distilled water and 200 µl of reagent Bradford Bio-Rad (Protein assay). Absorbance was recorded at a wavelength of 595 nm after 15 to 20 min of reaction using an UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu Corp., Japan). A calibration curve (0, 2.5, 5, 7.5 and 10 mg l^{-1}) was made from a stock solution (20 mg ml^{-1}) of bovine serum albumin (BSA) used as a standard.

2.10. Determination of lipid peroxidation in roots

The amount of MDA was assayed to evaluate the effects of allelochemicals on the rhizosphere of lettuce. Lipid peroxidation was determined in 0.5 g root fresh weight by

measuring the amount of MDA, a product of lipid peroxidation, by the thiobarbituric acid reaction (Gossett et al., 1994). Roots were collected, weighed (0.5 g), immediately frozen in liquid nitrogen and stored at -25 ºC until extraction. Frozen tissues were ground with mortar with pestle, suspended in 0.5 ml 0.1 mM Tris at pH 8. Extracts were centrifuged at 15,000 rpm for 20 min (4 ^{o}C) and the supernatant was used for the determination of MDA. The measurement was done at 25 ^{o}C using an UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu Corp., Japan).

2.11. Determination of antioxidant enzyme activities in roots

For the determination of enzyme activities, 0.5 g root was homogenized in 8 mL 50 mM PBS (pH 7.8) using a pre-chilled mortar and pestle, then centrifuged at 15000 × g for 20 minutes at 4 ºC. The supernatant was designated as crude enzyme extract and stored at 4 °C for the assays of various antioxidant enzyme activities (Wu et al., 2003). The SOD, POD and CAT activities were determined according to Zhang (1992). SOD activity was assayed using nitroblue tetrazolium (NBT). The reaction mixture (3 mL) contained 50 mM PBS (pH 7.8), 13 mM methionine, 75 µM NBT, 10 µM EDTA, 2 mM riboflavin, and enzyme extract (100 µL). The reaction was started by placing the tubes below two 15 W incandescent lamps emitting 4,000 Lux for 15 min and then stopped by switching off the light. The absorbance was measured at 560 nm. One unit of SOD was defined as the quantity of enzyme that produced 50% inhibition of NBT reduction under the experimental conditions. The reaction mixture for POD consisted of 100 µL enzyme extract, 100 µL guaiacol (1.5%, v/v), 100 µL H_2O_2 (300 mM), and 2.7 mL 25 mM PBK with 2 mM EDTA (pH 7.0). Increases in the absorbance were measured spectrophotometrically at 470 nm (ε = 26.6 mM cm^{-1}). The assay mixture for CAT contained 100 µL of enzyme extract, 100 µL H_2O_2 (300 mM), and 2.8 mL 50 mM

phosphate buffer with 2 mM EDTA (pH 7.0). CAT activity was assayed by monitoring the decrease in the absorbance at 240 nm as a consequence of H_2O_2 consumption (ε = 39.4 mM cm^{-1}). APX activity was determined according to Nakano and Asada (1981). The reaction mixture consisted of 100 µL enzyme extract, 100 µL ascorbate (7.5 mM), 100 µL H_2O_2 (300 mM), and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The oxidation of ascorbate was determined by the decrease in absorbance at 290 nm (ε = 2.8 mM cm^{-1}).

2.12. Experimental design and statistical analysis

All experiments were arranged in a completely randomized design with three replications. Analysis of variance for all data was done using computer package MSTAT-C developed by Russel (1986). The mean differences were separated according to Tukey's test at $P<0.05$.

3. Results

3.1 Bioassay I

Lettuce seedling grown in the NR solution showed regarded growth in all the parameter studied (Table 1). The lowest shoot and root dry weight was observed in the NR solution plants. While the shoot and root dry weight was highest in plants grown in NNS solution. When ED, XAD and AC treatments were applied to NR solution the seedling growth improved. The NR + AC, NR + ED and NR + XAD solutions plants produced shoot and root dry weight similar to NNS solution plants. The other growth parameters such as number of leaves $plant^{-1}$, maximum leaf length and width, longest root length, SPAD value and shoot fresh weight followed the similar trend.

Table 1. Influence of different treatments to the once used nutrient solution on the growth of lettuce seedlings (Bioassay I).

Types of nutrient solutions	No of leaves plant^{-1}	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Shoot fresh weight plant^{-1} (g)	SPAD	Dry weight plant^{-1} (g)	
							Shoot	Root
NNS[z]	10.3 a[u]	14.3 a	7.7 a	22.1 a	13.1 a	36.3 a	0.67 a	0.075 a
NR[y]	8.6 b	10.1 b	5.6 b	15.9 b	10.2 b	33.6 b	0.51 b	0.059 b
NR+ED[x]	10.2 a	14.2 a	7.6 a	21.4 a	12.9 a	35.9 a	0.66 a	0.076 a
NR+XAD[w]	9.5 a	13.9 a	7.2 a	20.1 a	12.3 a	34.6 a	0.61 a	0.071 a
NR+AC[v]	9.8 a	13.4 a	7.1 a	20.3 a	11.9 a	34.9 a	0.62 a	0.072 a

[z]New nutrient solution i.e. Standard 50% 'Enshi' solution;

[y]Once used non-renewed nutrient solutions where lettuce was grown for a period of 6 weeks and the solution was not changed throughout the growing period;

[x]The NR solution treated with electro-degradation;

[w]The NR solution treated with amberlite XAD-4;

[v]The NR solution treated with activated charcoal;

[u]Means within a column for each bioassay followed by different letters are significantly different at $P<0.05$.

The H_2O_2 and O_2^- generation in the plant roots grown in the different types of nutrient solutions were significantly varied (Fig. 1A, B). The highest H_2O_2 and O_2^- production was observed in the roots of NR solution plants. The intense staining of NR solution plant roots by DAB (Fig. 2A) and by NBT (Fig. 2B) was observed which also indicated the higher accumulation of H_2O_2 and O_2^-, respectively. The lowest H_2O_2 and O_2^- generation was observed in the NNS solution plant roots. Plants grown in the NR+ED solution generated H_2O_2 and O_2^- in their roots similar to plants in NNS solution. However, plants roots grown in NR + AC and NR + XAD solutions generated higher H_2O_2 and O_2^- compare to plant roots in NNS solution. The MDA accumulation in the lettuce roots was also significantly varied in the plants grown in the different types of nutrient solutions (Fig. 1C). The highest MDA accumulation was observed in the plants roots from NR solution. Plants grown in the NR + ED and NNS solution accumulated statistically similar MDA in their roots. The MDA accumulation in the plants roots grown in NR + AC and NR + XAD solutions were moderately high compared to the MDA accumulation in plant roots in NNS solution. The soluble protein content was also highest in the plant roots in NR solution (Fig. 1D) while it was lowest in the plant roots in NR + ED and NNS solution. The plant roots in NR + AC and NR + XAD solution produced the relatively high amount of soluble protein compare to plants grown in NR + ED and NNS solution.

The antioxidant enzymes activity was significantly diverse in the plant roots grown in the various types of nutrient solutions (Table 2). The SOD activity was relatively higher in plant roots grown in NNS and NR + ED solution but it was relatively lower in plant roots of NR, NR + AC and NR + XAD solution. Similarly, the POD activity was relatively higher in plants grown in NNS, NR + ED and also NR + XAD solution but it

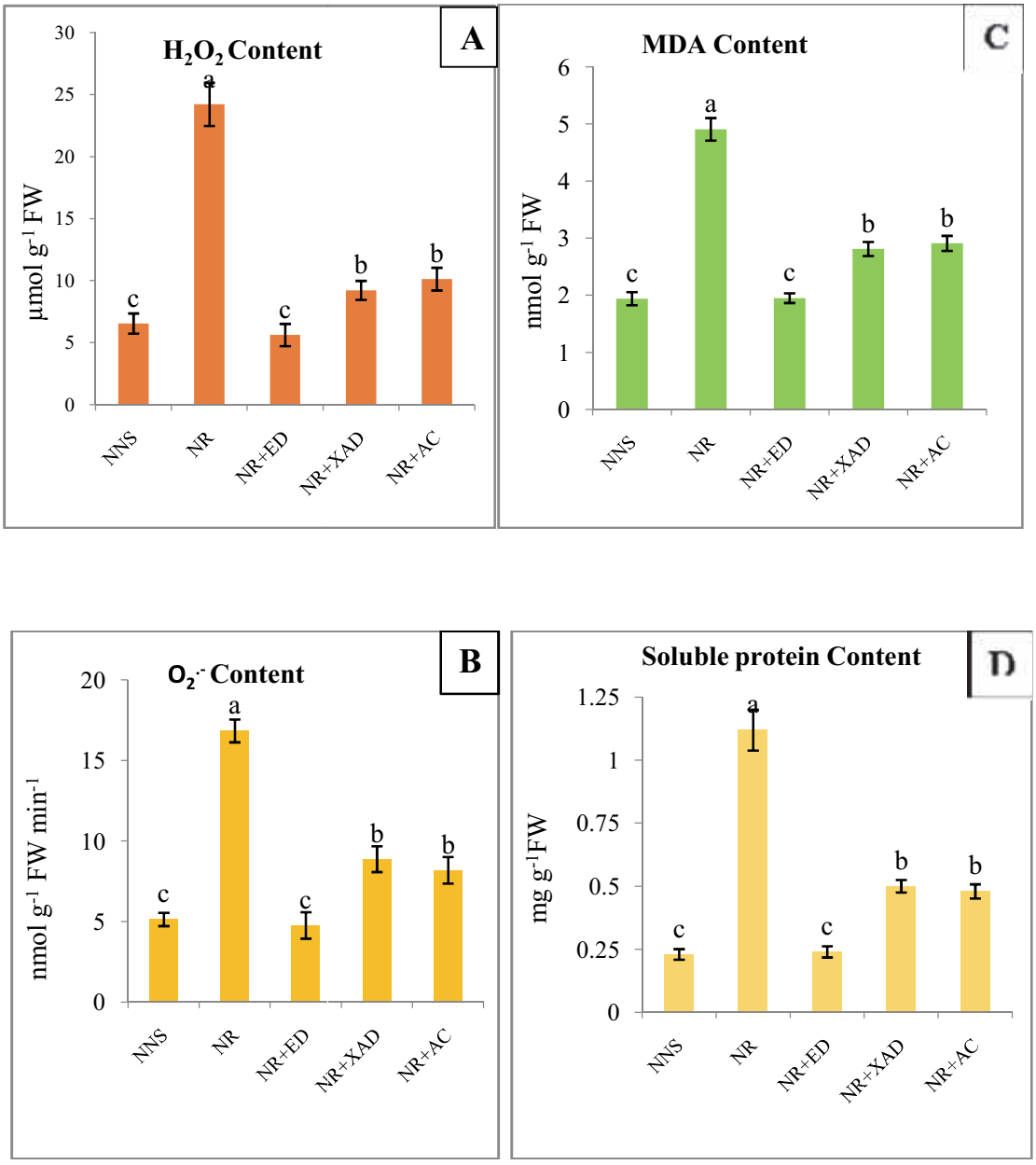
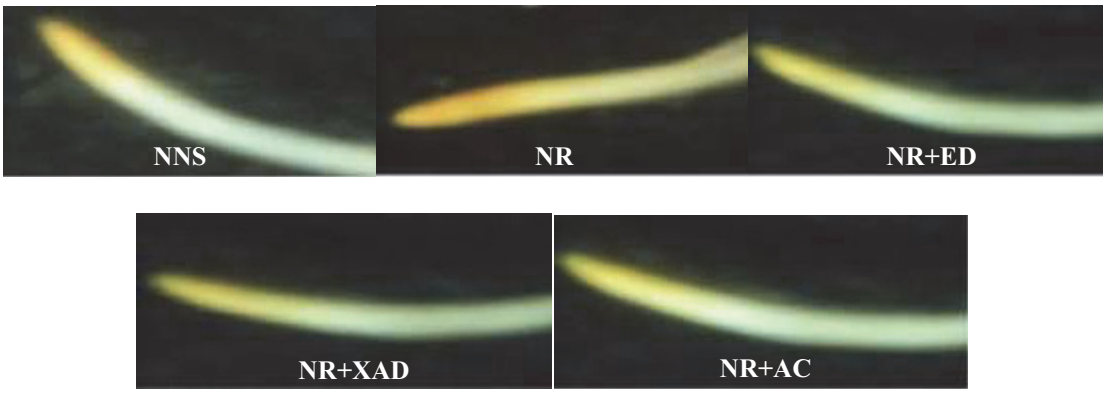


Fig. 1. Effect of once used non-renewed nutrient solution and it's treatment by different methods on the generation of (A) H_2O_2, (B) $O_2^{\cdot-}$, (C) MDA and (D) Soluble protein in the lettuce roots. The vertical bars represent SE (n=3). Different letters above each bar are significant according to the Tukey's multiple range test at $P<0.05$. [NNS= New nutrient solution, NR= Once used non-renewed solution, NR+ED= NR solution treated with ED, NR+XAD= NR solution treated with XAD-4 and NR+AC= NR solution treated with AC].

(A)



(B)

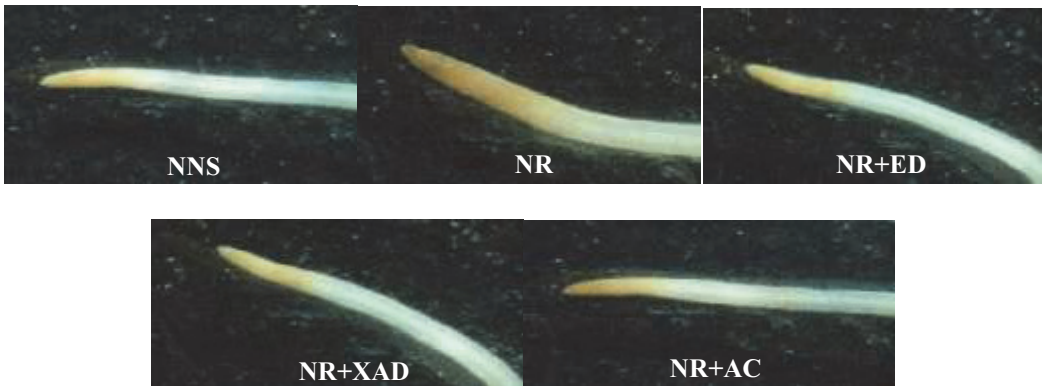


Fig. 2. Histochemical detection of (A) $H_2O_2$and (B) $O_2^{\cdot-}$in lettuce root grown in Bioassay I. Stained root was observed at ×60 using a Leica Fluorescence Stereomicroscope (M165C, Leica Microsystems, Heerbrugg, Switzerland) under visible light and photographed with a charge-coupled device (CCD) imaging system.[NNS= New nutrient solution, NR= Once used non-renewed solution, NR+ED= NR solution treated with ED, NR+XAD= NR solution treated with XAD-4 and NR+AC= NR solution treated with AC].

Table 2. Influence of different treatments to the once used nutrient solution on the antioxidant enzymes activity in the roots of lettuce seedlings (Bioassay I).

Types of nutrient solutions	SOD (Ug^{-1} FW)	POD (mmol g^{-1} FW min^{-1})	CAT (mmol g^{-1} FW min^{-1})	APX (mmol g^{-1} FW min^{-1})
NNS[z]	71.52 a	5.72 a	0.82 a	23.14 a
NR[y]	38.11 b	4.38 b	0.56 b	14.81 b
NR+ED[x]	61.21 a	6.78 a	0.81 a	21.75 a
NR+XAD[w]	48.97 b	5.99 a	0.93 a	19.56 a
NR+AC[v]	42.17 b	4.78 b	0.91 a	20.67 a

[z]New nutrient solution i.e. Standard 50% 'Enshi' solution;

[y]Once used non-renewed nutrient solutions where lettuce was grown for a period of 6 weeks and the solution was not changed throughout the growing period;

[x]The NR solution treated with electro-degradation;

[w]The NR solution treated with amberlite XAD-4;

[v]The NR solution treated with activated charcoal;

[u]Means within a column for each bioassay followed by different letters are significantly different at $P<0.05$.

was relatively lower in roots of NR and NR + AC solution plants. On the other hand, both CAT and APX activity was lower in plants grown in NR solution. Plants grown in NNS, NR + ED, NR + AC and also NR + XAD solution showed the statistically similar CAT and APX activity in their roots.

3.2 Bioassay II

Growth of lettuce seedlings was notably varied due to the influence of the 2NR solution treatments (Table 3). The lowest shoot and root dry weight was observed in the 2NR solution plants while these shoot and root dry weight was highest in the NNS solution plants. ED, XAD and AC application to 2NR solution also improved the seedling growth. The plants grown in 2NR + AC, 2NR + ED and 2NR + XAD solutions produced shoot and root dry weight similar to plants in NNS solution. The shoot and root dry weights of plants in 1NR solution were higher than 2NR solution plants but lower than the plants grown in all other solutions. The other growth parameters such as number of leaves plant^{-1}, maximum leaf length and width, longest root length, SPAD value and shoot fresh weight followed the almost similar trend.

The H_2O_2 and O_2^- generation in the plant roots grown in the different types of nutrient solutions were significantly varied (Fig. 3A, B). The highest H_2O_2 and O_2^-production was observed in the plant roots from 2NR solution followed by plants in 1NR solution. At this time, the intense staining of roots from 1NR and 2NR solution by DAB (Fig. 4A) and by NBT (Fig. 4B) was also observed which also indicated the higher accumulation of H_2O_2 and O_2^-, respectively. The lowest H_2O_2 and O_2^- generation was observed in plants roots from NNS solution which was statistically same to the plants in

Table 3. Influence of different treatments to the twice used nutrient solution on the growth of lettuce seedlings (Bioassay II).

Types of nutrient solutions	No of leaves plant^{-1}	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Shoot fresh weight plant^{-1} (g)	SPAD	Dry weight plant^{-1} (g)	
							Shoot	Root
NNS[z]	11.1 a[t]	15.2 a	8.0 a	20.9 a	15.10a	35.8 a	0.64 a	0.076 a
1NR[y]	8.6 b	9.9 b	5.8 b	16.3 b	11.15b	33.9 b	0.54 b	0.065 b
2NR[x]	8.4 b	9.8 b	5.6 b	11.8 c	8.14c	33.9 b	0.48 c	0.052 c
2NR+ED[w]	10.6 a	13.9 a	7.8 a	20.9 a	13.94a	35.7 a	0.65 a	0.075 a
2NR+XAD[v]	10.4 a	13.6 a	6.9 a	19.9 a	13.63a	34.5 a	0.61 a	0.072 a
2NR+AC[u]	10.2 a	13.4 a	7.3 a	19.4 a	13.14a	34.8 a	0.62 a	0.072 a

[z]New nutrient solution i.e. Standard 50% 'Enshi' solution;

[y]Once used non-renewed nutrient solutions where lettuce was grown for a period of 6 weeks and the solution was not changed throughout the growing period;

[x]Twice used non-renewed nutrient solutions where lettuce was grown for successive two culture for a period of 12 weeks and the solution was not changed throughout the two growing period;

[w]The 2NR solution treated with electro-degradation;

[v]The 2NR solution treated with amberlite XAD-4;

[u]The 2NR solution treated with activated charcoal;

[t]Means within a column for each bioassay followed by different letters are significantly different at $P<0.05$.

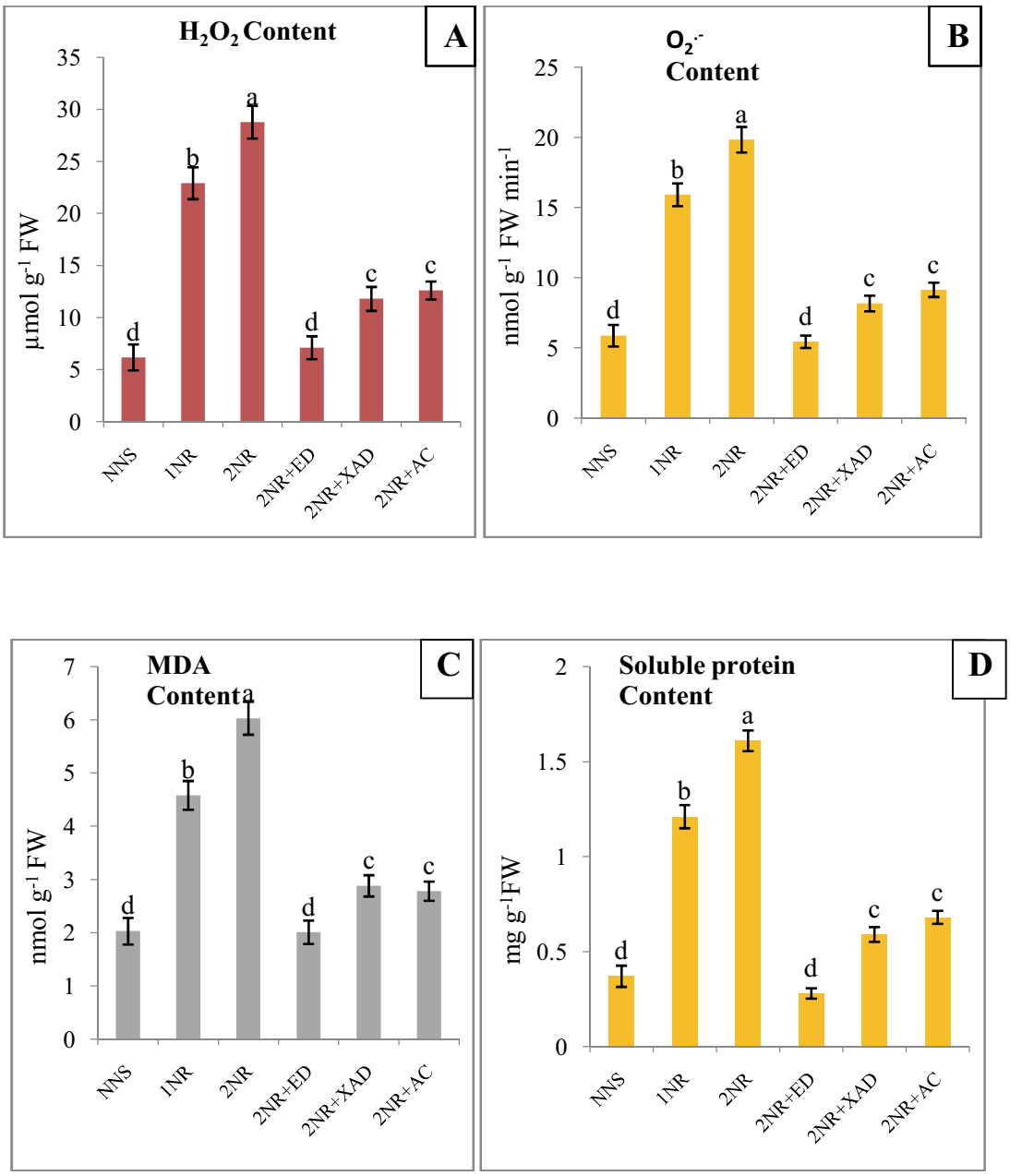
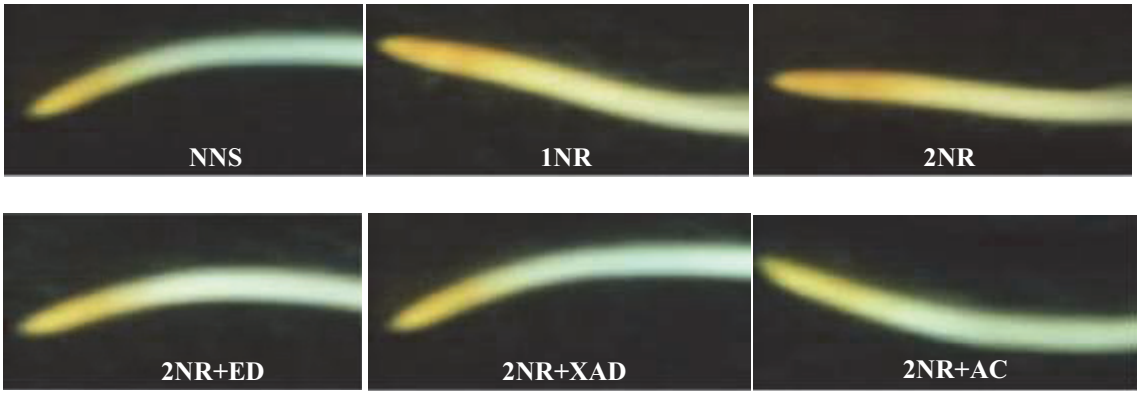


Fig. 3. Effect of twice used non-renewed nutrient solution and it's treatment by different methods on the generation of (A) H_2O_2, (B) $O_2^{\cdot-}$, (C) MDA and (D) Soluble protein in the lettuce roots. The vertical bars represent SE (n=3). Different letters above each bar are significant according to the Tukey's multiple range test at $P<0.05$. [NNS= New nutrient solution, 1NR= Once used non-renewed solution, 2NR= Twice used non-renewed solution, 2NR+ED= 2NR solution treated with ED, 2NR+XAD= 2NR solution treated with XAD-4 and 2NR+AC= 2NR solution treated with AC].

(A)



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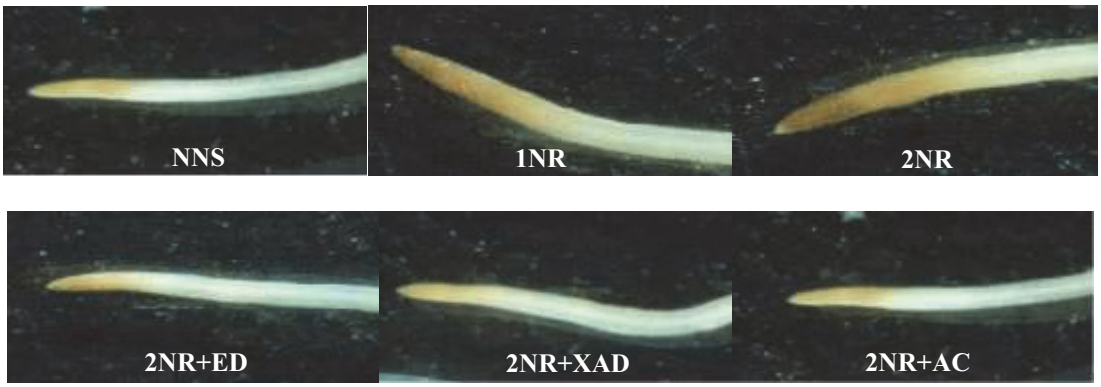

Fig. 4. Histochemical detection of (A) $H_2O_2$and (B) $O_2^{\cdot-}$ in lettuce root grown in Bioassay II. Stained root was observed at ×60 using a Leica Fluorescence Stereomicroscope (M165C, Leica Microsystems, Heerbrugg, Switzerland) under visible light and photographed with a charge-coupled device (CCD) imaging system. [NNS= New nutrient solution, 1NR= Once used non-renewed solution, 2NR= Twice used non-renewed solution, 2NR+ED= 2NR solution treated with ED, 2NR+XAD= 2NR solution treated with XAD-4 and 2NR+AC= 2NR solution treated with AC].

the 2NR + ED solution. However, 2NR + AC and 2NR + XAD solutions plants roots generated higher H_2O_2 and O_2^- compared to plants in NNS and 2NR + ED solution.

The MDA accumulation in the lettuce roots was also significantly varied in the plants roots grown in the different types of nutrient solutions (Fig. 3C). The highest MDA content was observed in plant roots from 2NR solution followed by plants in 1NR solution. Plants grown in the 2NR + ED and NNS solution accumulated statistically same MDA in their roots. The MDA accumulation in plants from 2NR + AC and 2NR + XAD solutions was higher than the MDA accumulation in the plants from 2NR + ED and NNS solution. The soluble protein content was also the highest in plants root from 2NR solution followed by 1NR solution while it was the lowest in the plants from 2NR + ED and NNS solution (Fig. 3D). Plants grown in 2NR + AC and 2NR + XAD solution produced the statistically same and relatively high amount of soluble protein compared to plants root in 2NR + ED and NNS solution.

The antioxidant enzymes activity was considerably different in the plant roots grown in the various types of nutrient solutions (Table 4). The SOD activity was the highest in the plants grown in NNS and 2NR + ED solution followed by plants in 1NR, 2NR + AC and 2NR + XAD solution but it was the lowest in the roots of plants in 2NRsolution. Similarly, the POD activity was also the highest in the plants grown in NNS and 2NR + ED solution followed by plants in 1NR, 2NR + AC and 2NR + XAD solution and it was the lowest in the roots of plants in 2NRsolution. Likewise, both CAT and APX activity in plants roots from NNS, 2NR + ED, 2NR + AC and 2NR + XAD solution were statistically same and it was the lowest in roots from 2NR solution plants. The plant.grown in 1NR solution showed relatively high CAT and APX activity compared to the plants in 2NR solution.

Table 4. Influence of different treatments to the twice used nutrient solution on the antioxidant enzymes activity in the roots of lettuce seedlings (Bioassay II).

Types of nutrient solutions	SOD (Ug^{-1} FW)	POD (mmol g^{-1} FW min^{-1})	CAT (mmol g^{-1} FW min^{-1})	APX (mmol g^{-1} FW min^{-1})
NNS[z]	60.11 a	6.98 a	0.84 a	23.61 a
1NR[y]	49.39 b	3.99 b	0.71 b	17.51 b
2NR[x]	41.38 c	3.25 c	0.47 c	11.94 c
2NR+ED[w]	56.38 a	6.97 a	0.80 a	21.82 a
2NR+XAD[v]	47.18 b	4.12 b	0.74 a	22.71 a
2NR+AC[u]	46.91 b	4.15 b	0.78 a	22.63 a

[z]New nutrient solution i.e. Standard 50% 'Enshi' solution;

[y]Once used non-renewed nutrient solutions where lettuce was grown for a period of 6 weeks and the solution was not changed throughout the growing period;

[x]Twice used non-renewed nutrient solutions where lettuce was grown for successive two culture for a period of 12 weeks and the solution was not changed throughout the two growing period;

[w]The 2NR solution treated with electro-degradation;

[v]The 2NR solution treated with amberlite XAD-4;

[u]The 2NR solution treated with activated charcoal;

[t]Means within a column for each bioassay followed by different letters are significantly different at $P<0.05$.

4. Discussion

Closed hydroponic systems have the problem of allelochemicals accumulation in the culture solution. Many researchers found allelochemicals in the culture solution from root exudation causing allelochemical stress in strawberry (Kitazawa et al., 2005; Asao et al., 2008; Asaduzzaman et al., 2012; Mondal et al., 2013), tomato (Yu and Matsui, 1993), cucumber (Yu and Matsui, 1994; Asao et al., 1998), several leafy vegetables (Asao et al., 2004b) and some ornamentals plants (Asao et al., 2007) grown in closed hydroponics. Lettuce grown in closed hydroponic accumulate many allelochemicals in the culture solutions (Asao et al., 2004b). Currently, some research findings detected many organic acids such as benzoic, phenyl acetic, cinnamic, p-hydroxybenzoic, lauric, phthalic, vanillic, palmitic, and stearic acids etc. from the root exudates of lettuce grown in the non-renewed nutrient solution of closed hydroponics (Asao et al., 2004b; Lee et al., 2006) and identified as major growth inhibitors. These accumulated allelochemicals create allelochemical stress to plants. In our present study, we tried to analyse the effects of allelochemical stress on lettuce roots at the biochemical and molecular level, particularly on the activities of the antioxidant enzymes such as CAT, APX, SOD and POD and the generation of H_2O_2, O_2^-, soluble protein and MDA.

In our first bioassay, all growth parameters such as leaf number plant^{-1}, leaf size, root length, shoot fresh weight, shoot and root dry weight etc. significantly reduced in plants grown in NR solution (Table 1). We described earlier that several allelochemicals accumulated in the reused solution for lettuce culture. These allelochemicals delivered into the rhizosphere due to root exudation found responsible for hampering numerous

physiological process (Inderjit and Duke, 2003; Blum, 2005). Therefore, we obtained reduced seedling growth in the NR solution.

Compared to plants grown in NNS solution, the production of ROS (H_2O_2 and O_2^-) was higher in the NR solution plants (Fig. 1A, B; 2A, B). The generation of ROS is one of the earliest biochemical responses of eukaryotic cells to biotic and abiotic stresses (Apel and Hirt, 2004). The production of ROS in plants acts as a secondary messenger to trigger subsequent defense reactions in plants. The most common ROS are hydrogen peroxide, superoxide, the hydroxyl radical, and singlet oxygen that formed as a natural byproduct of the normal metabolism of oxygen and is crucial in cell signaling. The overproduction of ROS leads to oxidative stress and can cause damage to cellular components. Plants generate more ROS when exposed to stressful conditions (Yamamoto et al., 2003; Halliwell, 2006; Rhoads et al., 2006). Allelopathic compounds also induce a oxidative stress manifested as enlarged production of toxic ROS (Singh et al., 2006; Bais et al., 2003; Sánchez-Moreiras et al., 2005; Batish et al., 2006; Cruz-Ortega et al., 2002; Weir et al., 2004). Several other studies have also shown that allelochemical stress can cause oxidative damage that indicated the enhanced production of ROS (Baziramakenga et al., 1995; Politycka, 1996; Yu et al., 2003; Lara-Nunez et al., 2006; Ye et al., 2004, 2006).Cruz-Ortega et al., (2007) stated that allelochemicals stress not only induced an oxidative damage responsible for toxicity of the plant by generating ROS, they also believed that generated ROS might only act as signals activating cascade of other events leading to cell malformations. On the other hand, Foreman et al., (2003) described that overproduction of ROS showed decline activity of NADPH oxidase enzyme that was known to enhance root development and

elongation. Since much allelochemicals accumulated in the NR solution, we observed higher production of ROS (H_2O_2 and O_2^-) in the roots of plants grown there. This over-accumulation of ROS was not sufficiently scavenged by antioxidant enzymatic system and was responsible for damage to cellular components and ultimately cell death (Oracz et al., 2007). That was why, we obtained reduced root growth in the NR solution plants which lead to lower water and nutrients uptake. Consequently, retarded plant growth was obtained in the NR solution plants.

Oxidative damage is quantified by measuring the MDA content. The high level of MDA in plants grown in NR solution (Fig. 1C)subjected to allelochemical stress indicated that the antioxidant enzymatic system didn't completely eliminate the over generated ROS. It revealed that plants grown in NR solution were much suffered from oxidative damage. Qian et al. (2009) found similar results in *Chlorella vulgaris*. They observed that plants exposed to allelochemical stress experienced oxidative damage and produced high level of MDA. Some other reports showed that allelochemicals stress induced oxidative damage evidenced by high level of lipid peroxidation (Romero-Romero et al., 2005; Lara-Núñez et al., 2006).

We also obtained the higher amount of soluble protein in the plants grown in NR solution (Fig. 1D) compared to plants in NNS solution. It is well known that plants under stress condition may accumulate small molecular mass soluble protein that could be used as a source of storage nitrogen and rapidly mobilized when required for the alleviation of stress (Singh et al., 1987) and additionally, these proteins could also have a role in osmotic adjustment (Ashraf et al., 2004). Higher amount of soluble protein in

the NR solution plant roots was a sign of stress condition induced by the allelochemicals. Ahmed et al., (2013) also found higher production of soluble protein under stress condition in barley. Collectively, the excess production of ROS, MDA and soluble protein in plants grown in NR solution were observed compared to NNS solution plants. Therefore, it is evident that plants grown in the NR solution were suffered more from allelochemical stress and accordingly excessive oxidative damage and lipid peroxidation.

To minimize the impact of allelochemical induced oxidative stress, plants have evolved a complex system of enzymatic antioxidants, SOD, CAT, POD, GR, and APX, and non-enzymatic antioxidants, ascorbic acid, a-tocopherol, reduced glutathione, ß-carotene, Polyamines (PAs), salicylates, compatible solutes such asproline (Pro), glycine betaine (GB), and zeaxanthin that accumulate in higher plants under stress condition (Ozkur et al., 2009). Plants boost up the production of antioxidants in order to minimize the detrimental effects of oxidative stress to normalize their metabolic activities under oxidative stress. Different antioxidants have roles in protecting cells in specific compartments and in particular conditions. It is generally recognized that O_2^- might be converted to H_2O_2 and then metabolized to water by APX and GR in plants to maintain membrane structures (Foyer and Fletcher, 2001). Similarly, several other antioxidant enzyme molecules are responsible to counteract the deleterious effects of ROS. Initially, SOD catalyzes the conversion of O_2^- to H_2O_2 that is further reduced to water by APX by using ascorbate as an electron donor (Scandalios, 2005). The CAT and POD are involved in the degradation of hydrogen peroxide into water and oxygen, for preventing the oxidative damage (Willekens et al., 1995; Mittler, 2002). Elevated

accumulation of antioxidant enzymes such as SOD, CAT, GR, APX, and POD is involved in lowering oxidative injury was observed in caper bush seedlings under drought stress (Ozkur et al., 2009). Similarly, Yang et al., (2009) found an increase in the activity of CAT, SOD, POD, APX, and GR under stress condition.

On the other hand, in our present study, the antioxidant enzymes activity such as SOD, POD, CAT and APX were significantly lower in the plants roots grown under allelochemical stress in NR solutions (Table 2). These results agree with other studies that have described antioxidant enzymes activity under allelochemical stress. It has been reported that secalonic acid, isolated from *Aspergillus japonicus*, significantly reduced SOD and POD activity in seeds of rape, cucumber, corn and sorghum (Zeng et al., 2001). Likewise, aqueous extracts from rice reduced SOD activity in barnyard grass (Lin et. al., 2000). Recently, Sánchez-Moreiras and Reigosa (2005) have shown that 2(3H)-Benzoxazolinone (BOA) severely inhibited SOD activity in leaves and roots of lettuce.

Dorling and Cipollini, (2006) described that the effect of allelochemicals on the plant antioxidant enzymes system are dosage-dependent. Yan et al., (2015) found that antioxidants enzyme activity showed an increase trend at low concentration, followed by a decline phase at the high concentration in patchouli seedlings. Qian et al., (2009) stated that N-phenyl-2-naphthylamine was an allelochemical of unicellular green alga *Chlorella vulgaris* and found that the activities of SOD and POD increased in lower (2.5 mg L^{-1}) concentration and decreased at higher (4 mg L^{-1}) concentration of N-phenyl-2-

naphthylamine. Berberine is also known to act as allelochemical in aquatic ecosystems as it inhibits growth of cyanobacteria *Microcystis aeruginosa* (Zhang et al., 2011). It up-regulated SOD activity at lower concentration while down-regulated at higher concentration. More detailed study has been done on ethyl 2-methylacetoacetate (EMA), an allelochemical of *Chlorella pyrenoidosa* and *M. aeruginosa* (Li and Hu, 2005). These plants responded to EMA at lower concentration showed increasing activity of SOD and POD. However, higher concentration of EMA led to decreased activity of the enzymes. Similar trend of SOD, POD, CAT and APX activities were also obtained in cucumber after cinnamic acid treatment (Ding et al., 2007). Therefore, these findings supported our results that higher concentration levels of allelochemicals in the NR solution resulted in declined antioxidant enzymes (SOD, POD, CAT and APX) activity and ultimately resulted in potent inhibitory effect on plant growth.

Some other researchers explained that antioxidant enzymes activities in plants caused by allelochemical stress were not only limited to the allelochemical concentrations but also dependent to the allelochemical exposure duration. The CAT activity in *M. arunginosa* cells treated with EMA showed highest after dissolution of a medium concentration of allelochemical (1 mg L^{-1}) and generally declined upon increasing concentration and longer than 2 days exposure to allelochemical (Hong et al., 2008). Zhang et al., (2011) also stated that antioxidant enzymes activities in plants depended on the duration of allelochemical treatment. In our experiments, lettuce seedlings were grown in NR solution for 2 weeks. So, we speculated that the seedlings were exposed to the allelochemicals in NR solution for longer period and thus, reduce the antioxidant

enzymes activity, leaving the plant under the risk to oxidative damage. Therefore, the allelochemical stress in the NR solution plants were specified by producing an oxidative imbalance evidenced by over accumulation of ROS and reduction of antioxidant enzymes activity in roots.

It was previously well established that plants grown in the NR solution were exposed to allelochemical stress. For that reason, the solution has to be eventually renewed to grow crops free from autotoxic condition. But, recently the disposal of culture solution is discouraged, due to the environmental pollution. For an effective removal or degradation of allelochemical from the nutrient solution and consequently, to increase the crop yield, several researchers used several methods in different crops. For instance, degradation of allelochemicals in strawberry production by ED means (Asao et al., 2008; Asaduzzaman et al., 2012 and Talukder et al., 2019), adsorption of allelochemicals in lettuce production by AC and XAD-4 (Lee et al., 2006), degradation of allelochemicals in cucumber production by microbial strains (Asao et al., 2004a).

In our study, we applied ED, AC and XAD-4 method to alleviate the allelochemical stress in the first bioassay. Due to ED application, we obtained that growth (Table 1), ROS, MDA and soluble protein production (Fig. 1A-D) and antioxidants enzyme activities (Table 2) in plants grown in NR + ED solution were similar to plants in NNS solution. When AC and XAD-4 method were applied, we obtained plant growth in NR + AC and NR + XAD solution similar to plants in NNS solution but those plants generated more ROS, MDA and soluble protein compared to plants in NNS solution. We also obtained lower SOD activity in plants in NR + AC and NR + XAD solution

and lower POD activity in plants from NR + AC solution. From these results, we speculated that ED method degraded allelochemicals from the NR solution more efficiently whereas AC and XAD-4 method didn't adsorbed allelochemicals absolutely.

In second bioassay, compared to NNS solution plants, all the growth parameters were lower (Table 3), the MDA, ROS and soluble protein content in roots were higher (Fig. 3A-D) and antioxidant enzyme activities in roots were lower (Table 4) in plants from 1NR solution. On the other hand, compared to 1NR solution plants, all the growth parameters were lower and, MDA, ROS and soluble protein content in roots were higher and antioxidant enzyme activities in roots were lower in the 2NR solution plants. Thus, plants in the 2NR solution suffered from more oxidative damage and consequently, more retarded growth was observed in the 2NR solution plants. This might be due to higher concentration of allelochemicals in the 2NR solution. As 2NR solution was used for longer period for the cultivation of lettuce compared to 1NR solution, higher amount of allelochemicals accumulated there. Therefore, plants grown in the 2NR solution suffered most by allelochemical stress. These results were also supported by the findings of other researchers. Lee et al., (2006) determined that number and concentration of allelochemicals in the nutrient solution highly varied with reuse time, generally showing the increasing trend with the increase reuse time. They found that few allelochemicals were exuded from the roots at comparatively low concentration in the 1NR solution and later on numbers of allelochemicals and their concentrations were found increased in the 2NR solution. As the number of allelochemicals were found increased in the 2NR solution, they affected plant growth

badly by additive or synergistic means (Inderjit, 1996). As a result, in our present study, most retarded growth of lettuce was obtained in 2NR solution.

Similar to first bioassay when ED was applied, we obtained plant growth, ROS, MDA and soluble protein production and antioxidants enzyme activities in plants roots from 2NR + ED solution were similar to plants from NNS solution. When AC and XAD-4 method were applied, we also obtained plant growth in 2NR + AC and 2NR + XAD solution plants similar to NNS solution plants but those plants generated more ROS, MDA and soluble protein compared to NNS solution plants. At this time, we also obtained lower SOD and POD activity in plants from both in 2NR + AC and 2NR + XAD solution. These result indicated that AC and XAD-4 applied to 1NR and 2NR solution exposed plants to a little more oxidative imbalance compared to NNS solution plants.

5. Summary

Plants grown in 1NR solution exposed to allelochemical stress as evidenced by generation of more ROS, MDA and soluble protein and lower antioxidant enzyme activity in roots. As a results oxidative damage occurred in the roots that hamper water and nutrients uptake in plants. Ultimately, we obtained retarded growth in the 1NR solution plants. By the same mechanisms, oxidative damage and retarded growth were more pronounced in the 2NR solution plants. ED to 1NR and 2NR solution maintained plant growth and oxidative balance like NNS solution plants.

CHAPTER 6

GENERAL SUMMARY

Strawberry and lettuce grown in closed hydroponics experiences autotoxicity. At this time, we investigated the use of light emitting diodes and exogenous amino acid in order to improve the growth and yield of strawberry plants grown in recycled hydroponics, where accumulation of root exudates caused autotoxicity. We observed greater vegetative growth, yield attributes, fruit yield and minerals (iron and magnesium) content in plant parts of strawberry due to R : B= 8:2 LED lighting and Glu spraying. However, the overall performances of strawberry plant were lower than the optimum level which was mainly associated with the higher growing temperature (30/25 °C; day/night) that restrict optimum plant growth and development. Thus, influence of exogenous amino acid application and also red and blue light ratios was not pronounced greatly. While in the subsequentstudy, plants exposed to R : B= 8:2 LED (567 μmol m^{-2} s^{-1}) showed greater performances on growth and several mineral content in strawberry plant supplied either with or without Glu. Fruits number and yield per plants were higher with Glu than the ones sprayed without Glu. Therefore, the use of LED (R : B = 8:2) at higher intensity along with Glu application may improves growth and yield of strawberry plants grown in a closed hydroponics and thus alleviate the inhibitory effect of autotoxicity.

In another experiment, we applied ED treatment to non-renewed nutrient solution to increased growth and yield of strawberry. DC-ED treatment to non-renewed culture solution could recover yield of strawberry to some extent but not completely. However,

complete yield recovery was obtained from AC-ED treatment to non-renewed culture solution. Furthermore, AC-ED treatment to non-renewed culture solution could maintain better nutritional and environmental condition of growing medium. Hence, we suggested that AC-ED treatment to nutrient solution for 24 h at every three weeks intervals could be applied for complete recovery of strawberry yield grown in closed hydroponic culture.

In the successive cultivation, lettuce grown in the non-renewed solution gradually reduced yield and quality in accordance to the nutrient solution reuse times.Due to AC-ED application to non-renewed solution recovered the retarded yield and quality completely in both oneculture non-renewed solution and two culture non-renewed solutions.ED application both weekly and bi-weekly improved growth and quality equally. Therefore, we suggested that ED treatment to non-renewed solution (300L) for 24 hours at two week intervals could be applied for complete recovery of the retarded lettuce yield and quality in two or more successive closed hydroponic cultivation using same nutrient solution.

Plants grown in one culture non-renewed solution exposed to allelochemical stress as evidenced by generation of more ROS, MDA and soluble protein and lower antioxidant enzyme activity in roots. As a results oxidative damage occurred in the roots that hamper water and nutrients uptake in plants. Ultimately, we obtained retarded growth in the one culture non-renewed solution plants. By the same mechanisms, oxidative damage and retarded growth were more pronounced in the two culture non-renewed solution plants. AC-ED to one- and two culture non-renewed solutions maintained plant growth and oxidative balance like plants grown in fresh nutrient solution.

SUMMARY IN JAPANESE

論文要旨

閉鎖型養液栽培で育ったイチゴ及びレタスは自家中毒を示す。その際、根の滲出物の蓄積が自家中毒を引き起こしている循環型養液栽培で育ったイチゴの生育および収量を向上させるために LED および外生アミノ酸の使用について検討した。R : B = 8 : 2 の LED 照明およびグルタミン酸（Glu）散布により、イチゴの生育、収量要素、果実収量、そしてミネラル（鉄およびマグネシウム）吸収の改善がみられた。しかし、イチゴのそのような全ての反応は、最適な植物の成長や発達を制限する高い生育温度（30/25℃）では最適レベルよりも低くなった。このように外生アミノ酸の処理と赤青照明照射の効果は大きくなかった。以降の研究で、R : B= 8:2 の LED (567 μmol m^{-2} s^{-1})に照射されたイチゴは、グルタミン酸（Glu）散布の有無に関わらず、イチゴの生育および数種のミネラル含量について促進効果がみられた。一株当たりの果実数および収量はグルタミン酸（Glu）散布をしない場合よりも散布した方が多くなった。従って、グルタミン酸（Glu）散布とともに R : B= 8:2 の高輝度 LED(567 μmol m^{-2} s^{-1})使用は閉鎖型養液栽培で栽培されたイチゴの生育および収量を改善するとともに自家中毒の抑制効果も軽減すると考えられた。

次の研究では、イチゴの生育および収量を促進するために非交換培養液に電気分解処理（ED）を処理した。非交換培養液に処理した直流型電気分解はある程度イチゴの収量を回復させることができたが、完全ではなかった。しかし、非交換培養液に交流型電気分解を処理すると、完全な回復がみられた。また、

非交換培養液に処理した交流型電気分解は培養液の養分および環境をより良い状態に保つことができた。以上より、3 週毎、24 時間、培養液に交流型電気分解処理が閉鎖型養液栽培で栽培されたイチゴの収量を完全に回復することが示唆された。

レタスを培養液非交換で連作すると、培養液の再利用回数によって、次第に収量および品質が低下していった。非交換培養液に交流型電気分解を処理すると、1 作および 2 作連作した後の培養液においても、抑制された収量および品質は完全に回復した。毎週および 2 週毎に処理した電気分解は同様に生育および品質を向上させた。以上より、2 週毎に非交換培養液（300L）に電気分解処理することが、同じ培養液を使用し 2 作またはそれ以上の連作を行う閉鎖型養液栽培において抑制されるレタスの収量および品質を完全に回復させることができると示唆された。

根における ROS、MDA、可溶性タンパク質の増大および抗酸化酵素活性の低下によって証明されるように、1 作の間、非交換培養液で育ったレタスはアレロパシー物質のストレスにさらされた。その結果、レタスの水および養分吸収を阻害する酸化的損傷が根で起きた。最終的に、1 作非交換培養液で育ったレタスで生育抑制を受けることになった。同様のメカニズムによって、酸化的損傷および生育抑制が 2 作連作された非交換培養液で栽培されたレタスで明らかにされた。1 作および 2 作連作した非交換培養液に処理した交流型電気分解は、新しい培養液で栽培したレタスのように生育および酸化的バランスを維持した。

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LIST OF PUBLICATIONS

[1]	**Talukder M. R.**, Asaduzzaman M., Tanaka, H. and Asao, T. 2018. Light-emitting diodes and exogenous amino acids application improve growth and yield of strawberry plants cultivated in recycled hydroponics. *Scientia Horticulturae.* 239: 93-103. [*The corresponding content is presented in Chapter 2*]
[2]	**Talukder M. R.**, Asaduzzaman M., Tanaka, H. and Asao, T. 2019. Electro-degradation of culture solution improves growth, yield and quality of strawberry plants grown in closed hydroponics. *Scientia Horticulturae.* 243: 243-251. [*The corresponding content is presented in Chapter 3*]
[3]	**Talukder M. R.**, Asaduzzaman M., Tanaka, H. and Asao, T. 2019. Application of alternating current electro-degradation improves retarded growth and quality in lettuce under autotoxicity in successive cultivation. *Scientia Horticulturae*. 251: 324-331. [*The corresponding content is presented in Chapter 4*]

LIST OF SUB-PUBLICATIONS

[1]	**Talukder, M.R.**, Asaduzzaman, M., Ueno, M., Kawaguchi, M., Yano, S., Ban, T., Tanaka, H., and Asao, T. 2016. Low potassium content vegetables research for chronic kidney disease patients in Japan. *Nephrology Open Journal*. 2(1): 1-8.
[2]	Asaduzzaman, M., **Talukder, M. R.**, Tanaka, H., Ueno, M., Kawaguchi, M., Yano, S., Ban, T. and Asao, T. 2018. Production of Low-Potassium Content Melon Through Hydroponic Nutrient Management Using Perlite Substrate. *Frontiers in Plant Science*. 9:1382.doi: 10.3389/fpls.2018.01382.

LIST OF CONFERENCE PROCEEDINGS

[1]	**Talukder, M. R.**, A. Yasunaga, M. Asaduzzaman, H. Tanaka and T. Asao. 2016. "Autotoxicity recovery in strawberry by different LED light power and Glutamic acid application under heat stress condition" published in the *Proc. The Japanese Society for Horticultural Science.* Vol. 15 no. 2, p. 344. September 10-12, 2016.
[2]	Tanaka, H., H. Kosuga, Y. Hirose, **M. R. Talukder**, M. Asaduzzaman, Y. Kuwamoto and T. Asao. 2016. "Effect of titanium dioxide/zeolite on rooting of cutting in cherry blossom" published in the *Proc. The Japanese Society for Horticultural Science.* Vol. 15 no. 2, p. 450. September 10-12, 2016.
[3]	**Talukder, M. R.**, M. Asaduzzaman, H. Tanaka and T. Asao. 2018. "Recovery of Autotoxicity in Lettuce by Electro-degradation" published in the *Proc. The Japanese Society for Horticultural Science.* Vol. 17 no. 2, p. 500. September 22-24, 2018.