

## SUMMARY OF DOCTORAL THESIS

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Title: 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)

Strawberry and lettuce grown in recycled hydroponics exhibit growth and yield reduction due to autotoxicity. In this study, strawberry plants were grown under light-emitting diodes (LED) and sprayed with amino acids to investigate their influence on the growth and yield under autotoxicity. At first, plants were grown under three LED light conditions [Red : Blue (R : B) = 8:2, 5:5, and 2:8 adjusted to similar light intensity of 106–117, 107–125, and 105–121  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively] and under white light provided by fluorescent lamps [104–129  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ] and also treated with two amino acids [hydroxyproline (Hyp) and glutamic acid (Glu)] and water (control). This study was conducted under relatively high temperature (30/25 °C; day/night) in order to enhance the occurrence of autotoxicity. Further, the nutrient solution was recycled for the duration of the crop cycle to allow the accumulation of autotoxic compounds. Greater growth and fruit yield, higher ascorbic acid content in fruits and also higher calcium and iron content in leaves, crowns and roots of strawberry plants were observed due to R : B= 8:2 LED lighting and Glu spray. Then the selected LED (R : B = 8:2) from the previous study was used with three different intensities (i.e., 149, 269, and 567  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and either with or without Glu spray under controlled environment condition (25/20°C; day/night). Results showed that plants exposed to 567  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of R : B= 8:2 LED showed greater performances on growth and minerals content in leaves, crown and roots of strawberry plant supplied either with or without Glu whereas higher number of fruits per plant and fruit yield were observed with Glu spray. Therefore, we propose that combining Glu spray with exposure to R : B = 8:2 LED light of 567  $\mu\text{mol m}^{-2} \text{s}^{-1}$  may improve the growth, yield and quality of strawberry cultivated in a hydroponic system with a recycled nutrient solution.

In another study, we applied electro-degradation (ED) to the culture solution in order to degrade their root exudates and improving growth, yield and quality of strawberry. There were four types of nutrient solution used in this study viz. renewed, non-renewed, non-renewed with direct current electro-degradation (DC-ED) and non-renewed with alternative current electro-degradation (AC-ED). Every three weeks interval, culture solutions were changed with fresh 25% standard Enshi nutrient solution in renewed treatment, while DC- and AC-ED treatment were applied in non-renewed solutions. Significantly greater fruit yield (225.9 g plant<sup>-1</sup>) was obtained from renewed nutrient solution, which was statistically similar to fruit yield in non-renewed solution with AC-ED application. Compared to renewed solution, fruit yield was decreased to about half (114.0 g plant<sup>-1</sup>) in non-renewed solution while non-renewed with DC-ED produced intermediate yield between non-renewed and renewed solution or non-renewed with AC-ED. In general, growth performance was greater in renewed solution followed by non-renewed with AC-ED, while in non-renewed solution decreased significantly similar to DC-ED. A similar trend was observed in vitamin C content while brix and citric acidity was not varied. Minerals such as calcium and iron concentration in the culture solution were significantly decreased in DC-ED, consequently their contents were also found lower in crowns and roots compared to other solutions used.

Furthermore, AC-ED treatment to non-renewed culture solution could maintain better pH and temperature of the growing medium. So, 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. Therefore, it is evident that growth, yield and quality of strawberry can be improved through application of AC-ED in non-renewed solution.

In this study we also investigated autotoxicity in lettuce under successive cultivation and applied AC-ED to non-renewed nutrient solution to degrade the accumulated allelochemicals and consequently to improve the retarded yield and quality of lettuce. There were three types of nutrient solution used in the first culture viz. renewed, non-renewed and non-renewed + ED solution. Every two weeks interval culture solution was changed with fresh 50% standard Enshi nutrient solution in renewed treatment while non-renewed solution was unchanged throughout the growing period but major minerals were adjusted to fresh 50% standard “Enshi” nutrient solution bi-weekly. ED was applied in non-renewed + ED solution bi-weekly for 24 hours. Significantly greater shoot fresh weight (398.3 g plant<sup>-1</sup>) was obtained from renewed solution. Compared to renewed solution, shoot fresh weight was decreased to 24% (301.8 g plant<sup>-1</sup>) in non-renewed solution. Shoot fresh weight obtained from non-renewed + ED solution was statistically similar to renewed solution plants. On the other hand, four types of nutrient solution used in the second culture viz. renewed, one culture non-renewed (non-renewed 1C), two culture non-renewed (non-renewed 2C) and non-renewed 2C + ED solutions. In non-renewed 1C, the starting solution was fresh 50% standard Enshi nutrient solution while in non-renewed 2C the starting solution was once used for lettuce culture. These solutions were maintained like first culture. Lettuce grown in non-renewed 2C solution produced significantly lowest shoot fresh yield (258.8 g plant<sup>-1</sup>), about 35% lower compare to renewed solution. When ED was applied to non-renewed 2C solution produced shoot fresh weight 383.3 g plant<sup>-1</sup> which was statistically similar to renewed solution. Calcium and iron content in lettuce grown in non-renewed solution in both culture followed the similar trend. Therefore, it was evident that yield and quality of lettuce could be improved through ED in non-renewed solution in two successive cultivations using same nutrient solution. In a following study, we tried to determine the proper ED interval. Results showed that ED to non-renewed solution both weekly and bi-weekly equally improved growth, yield and mineral content in lettuce. Hence, ED at two weeks interval was sufficient.

Accumulated allelochemicals induces oxidative damage and lipid peroxidation in plants leading to growth inhibition. We investigated the allelochemicals induced oxidative damage and lipid peroxidation in lettuce bioassay in once used non-renewed nutrient solution (1NR) and twice used non-renewed nutrient solution (2NR) obtained from the successive cultivation. We also tried to alleviate these damages through ED. The 1NR solution was used for six weeks for one time lettuce cultivation while the 2NR solution was used for twelve weeks for two times lettuce cultivation. Our results showed that allelochemical stress caused lettuce growth inhibition in both 1NR and 2NR solution. We observed the higher generation of H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup> as well as lower activities of antioxidant enzymes such as superoxide dismutase, catalase, guaiacol peroxidase and ascorbate peroxidase in roots of plants grown in both 1NR and 2NR solutions compared to plants grown in new nutrient solution. Higher level of lipid peroxidation due to higher malondialdehyde content and higher soluble protein content were also observed in roots of those plants. It was evident that, lettuce root damage occurred due to accumulation of allelochemicals in 1NR and 2NR solution. These damaged roots couldn't function normally and uptake water and minerals from the culture solution. As a result, retarded lettuce growth was observed in 1NR and 2NR solution. Oxidative damage, soluble protein content and lipid peroxidation and ultimately growth retardation was more pronounced in plant grown in 2NR solution compared to plants grown in 1NR solution. Application of ED to 1NR and 2NR solution maintained plant growth through less oxidative damage, soluble protein production and lipid peroxidation as it was observed in plants grown with new nutrient solution. Therefore, ED of non-renewed culture solution would alleviate allelochemical stress in successive lettuce cultivation under recycled hydroponics.