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## 学 位 論 文 要 約

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題目: 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 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. In agricultural ecosystems, many plant species are affected by autotoxicity, leading to decreased growth, low yields and replant failures. Many plants species experience autotoxicity in closed hydroponics where root exudates with various chemicals are the potential allelochemical. Root exudate is the largest sources of plant chemicals released into the rhizosphere. The accumulation of allelochemical in the nutrient solution inhibits growth and metabolic activities of plant roots. Root lipid peroxidation activities to increase and the free radical scavenging activity of roots to decrease. Ultimately, the damaged plant roots exhibit impaired uptake of water and mineral nutrients from the nutrient solution that resulted plant growth retardation.

The removal or degradation of these accumulated autotoxic growth inhibitor from the nutrient solution would improve growth of the plant. There are several methods to eliminate these growth inhibitors such as adsorption by activated charcoal, amberlite XAD-4; degradation by microbial strains, electro-degradation means and auxin treatment to nutrient solutions. Besides these the application of exogenous amino acids to plants and light conditions improvement, particularly effective under red and blue LED (light emitting diodes) light may reduce the autotoxic effects in plants. Generally, our present objectives are to study the different methods and their modification for efficient mitigation of autotoxicity in closed hydroponics.

Strawberry and lettuce grown in recycled hydroponics exhibit growth and yield reduction due to autotoxicity. 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. 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. On the other hand, 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. 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 while blue LEDs at 30  $\mu\text{mol m}^{-2} \text{s}^{-1}$  found to restore chlorophyll synthesis in wheat seedlings. Other researchers also observed better plant responses to red and blue LED combinations in various crops.

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. It is mentionable that, in this study the overall performances of strawberry plants 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. 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 optimum growth 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  $\mu\text{mol m}^{-2} \text{s}^{-1}$  intensity may improve the growth, yield and quality of strawberry cultivated in a hydroponic system with a recycled nutrient solution 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.

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.

We applied electrodegradation for 24h. in every three weeks period until the entire strawberry cultivation for about 3 months. In our experimental settings, the estimated cost of electricity for ED process is marginally higher than that cost of nutrient fertilizer only, and the initial cost of ED machine needs to be considered. However, renew of nutrient solution requires additional jobs that would be lessened by the use of the ED process. When the used nutrient solution with residual minerals (with micronutrients likes Zn, Mn) is discharged to the environment it causes environmental problems. In large-scale cultivation system the amount of used nutrient solution to be discharged will be a great volume. Therefore, use ED would be more to help reduce nutrient solution discharge to the environment in practice cultivation. The AC-ED machine for our present study would be an improvement for application under a commercial setting. For our future studies, we are upgrading the ED system that can be used in commercial hydroponics of strawberry, lettuce and also other plants conditions.

After that in another study, we also investigated autotoxicity in lettuce under successive cultivation and applied modified AC-ED machine to non-renewed nutrient solution. The objectives of this study were to degrade the accumulated allelochemicals in the culture solution and consequently to improve the retarded yield and quality of lettuce grown under successive cultivation. 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 ( $398.3 \text{ g plant}^{-1}$ ) was obtained from renewed solution. Compared to renewed solution, shoot fresh weight was decreased to 24% ( $301.8 \text{ g plant}^{-1}$ ) 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 ( $258.8 \text{ g plant}^{-1}$ ), about 35% lower compare to renewed solution. When ED was applied to non-renewed 2C solution produced shoot fresh weight  $383.3 \text{ g plant}^{-1}$  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.

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.

Thus, we alleviated autotoxicity in strawberry and successive lettuce cultivation in closed hydroponics through application of amino acids to plants, light condition

improvement using LED light and electrodegradation of culture solution.

※なお、一部図表等を割愛しています。