Title

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Author(s)

Md. Raihan Talukder, Md. Asaduzzaman, Hideyuki Tanaka, Toshiki Asao

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

Md. Raihan Talukder[a,b,c], Md. Asaduzzaman[a,d], Hideyuki Tanaka[a], and Toshiki Asao[a,*]

[a]Faculty of Life and Environmental Science, Shimane University, 2059 Kamihonjo, Matsue, Shimane 690-1102, Japan.

[b]Faculty of Agriculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

[c]The United Graduate School of Agricultural Sciences, Tottori University, Koyama-cho, Minami Tottori, Tottori 680-8553, Japan.

[d]Horticulture Research Center, Bangladesh Agricultural Research Institute, Gazipur 1701, Bangladesh.

[*]Corresponding author: T. Asao (E-mail: asao@life.shimane-u.ac.jp).

Abstract

Strawberry plants in recycled hydroponics exhibit growth and yield reduction due to autotoxicity. 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. In the first study, 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 µmol m^{-2} s^{-1}, respectively] and under white light provided by fluorescent lamps [104–129 µmol m^{-2} 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, ~~along with~~the nutrient solution was recycled for the duration of the crop cycle to allow the accumulation of autotoxic compounds. ~~nutrient condition.~~ 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. In the second study, the selected LED (R : B = 8:2) from the first study was used with three different intensities (i.e., 149, 269, and 567 µmol m^{-2} 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 µmol m^{-2} 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 µmol m^{-2} s^{-1} may improve the growth, yield and quality of strawberry cultivated in a hydroponic system with a recycled nutrient solution.

Key Words: autotoxicity; environment; glutamic acid; LED; hydroponics

1. Introduction

Autotoxicity, a form of interspecific 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 yields, 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 ~~ation~~ (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). ~~Additionally, the inhibitory effect of~~ Therefore, autotoxicity would be ~~was~~ enhanced in strawberry plants with increasing temperature under controlled conditions. ~~The growth and yield of strawberry plants are lower at 30/25 °C (day/night) than at 25/20 °C (day/night) in a closed hydroponic system using a hybrid electrode fluorescent lamp (Mondal et al. 2013).~~

In Japan, the current acreage of hydroponic strawberry production ~~under hydroponic system~~ is 627 ha. ~~(Ministry of Agriculture, Forestry and Fisheries 2015) where nutrient solutions are either renewed or supplied to soilless substrates.~~ The commercial hydroponic production of strawberry ~~(*Fragaria* × *ananassa* Duch.)~~ 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 ~~this~~ 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 nutrient solution 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~~supplementation~~ of different quality and ~~quantity~~ 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~~ves~~ 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 were 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.

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 ~~during~~ the experimental period. ~~from February 23, 2016 to July 4, 2016.~~ 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 ~~supplemented~~ 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.

2.4. ~~Types of LEDs~~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~~Shimane Electric Co. Ltd., Japan~~), with fluorescent lamps used as a control. 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}). We used MQ-200 Quantum separate sensor with handheld PAR meter (Apogee Instruments, Inc. Logan UT, USA) for measuring PPFD in both the experiments.

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 performance. Amino acids constitute mainly nitrogenous compounds which have great influence on plant growth and development. Therefore, e~~E~~ach 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. ~~Amino acids were sprayed in split plots of light conditions~~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 ~~2~~3). Among amino acids application, water spray ~~(WS)~~ 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 ~~(HS)~~ 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 ~~(GS)~~ 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~~HS~~ and Glu spray~~GS~~ but water spray~~WS~~ produced longest leaves. The longest leaves (26 c~~m~~m) were observed in the Hyp spray~~HS~~ 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~~WS~~ and Hyp spray~~HS~~ had similar leaves. The crown diameter was widest in Glu spray~~GS~~ among the amino acid and in R : B = 8:2 LED among the light condition. Also, interaction of Glu spray~~GS~~ and R : B = 8:2 LED produced the widest leaves among all combinations of light and amino acid.

Leaf, crown, root and total plant ~~root~~ dry weights were unaffected by the amino acid application, while these parameters were significantly affected by the light quality and their interaction (Table ~~2~~3). An increasing trend in dry weights ~~of leaves~~ 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~~GS~~ treated plants which was similar to plants treated with water spray~~WS~~ and Hyp spray~~HS~~ under R : B = 8:2 LED. ~~Plants produced the leaves d~~Dry weight production of leaf followed ~~ing~~ 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 ~~3~~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~~GS~~ compared to Hyp spray~~HS~~ or water spray~~WS~~. The highest number of flowers and fruits were obtained from plants treated with Glu spray~~GS~~ 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 sprayHS. However, highest average fruit weight was obtained from plants treated with Glu sprayGS under R : B = 8:2 LED. Fruit yield per plant was also significantly higher in R : B = 8:2 LED and by Glu sprayGS (Fig. 3). Additionally, the highest fruit yield was obtained from plants treated with Glu sprayGS under R : B = 8:2 LED followed by Hyp sprayHS under same light condition. R : B= 5:5 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 34). 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 sprayGS 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 except calcium contents in strawberry leaf were significantly affected by the combined effect of light and amino acid treatments (Table 45). 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 sprayHS and Glu spray GS 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 sprayGS under fluorescent light. Similar leaf magnesium content were observed for the Hyp spray HS 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 sprayGS or water treatment. The R : B = 5:5 or 2:8 LED with all spray treatments resulted in relatively low leaf magnesium content. The leaf potassium content was the highest (44.2 mg g^{-1} DW) following the Hyp sprayHS 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 45). 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 sprayGS 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 sprayGS treatment.

3.2. Experiment-II

3.2.1. Growth parameters

Light intensity and the combined effect of light intensity and Glu sprayGS showed a significant effect on the number of leaves per strawberry plant, crown diameter, and root length, while the individual Glu sprayGS treatment did not (Table 56). The plants exposed to high-light (HL) intensity with the Glu sprayGS treatment produced the most leaves per plant. Plants treated with high-lightHL intensity without Glu and those exposed to medium-light (ML) intensity with or without Glu had a similar number of leaves per plant. In contrast, the low-light (LL) intensity with or without Glu produced fewer leaves per plant. Similarly, the widest crown diameter (26.7 mm) was observed for the high-lightHL intensity and Glu sprayGS treatment, although the high-lightHL intensity without the Glu sprayGS resulted in a similar crown diameter. We observed that the crown diameter decreased with decreasing light intensity, with the Glu sprayGS having no significant effect. The longest roots (62.5 cm) were observed following the treatment with high-lightHL intensity and the Glu sprayGS, although the Glu sprayGS did not have a significant effect. Additionally, the root lengths were similar in plants exposed to medium-lightML intensity, regardless of the spray treatment. In contrast, the low-lightLL intensity treatment with or without the Glu sprayGS produced relatively shorter roots. The length, width, and chlorophyll content of leaves were not significantly affected by either light intensity, Glu sprayGS treatment or their interaction.

The dry weights of strawberry roots, leaves, and crowns, roots and total plant dry weight were significantly affected either by light LED intensity or and combination of ed effect of lightLED intensity and Glu sprayGS, but not by GS Glu spray alone treatment (Table 56). The plants treated with high-lightHL intensity with and without the Glu sprayGS had similar dry weights. Furthermore, the highest dry weights of leaves, crown, and roots and total plant were observed as at 25.4, 6.1, and 4.1, and 35.6 g $plant^{-1}$, respectively following the high-lightHL intensity with Glu sprayGS treatment. These dry weights were similar to those of plants treated with high-lightHL intensity without the Glu sprayGS. The root, leaf, and crown, root and total plant dry weights resulting from exposures to the low-lightLL and medium-lightML intensities (with or without the Glu sprayGS) were lower than those of the plants treated with high-lightHL 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 sprayGS, but not by the Glu sprayGS alone (Table 67).

However, fruit yield per plant was significantly affected by both Glu sprayGS and light intensity and their interaction (Fig. 4). Plants treated with high-lightHL intensity and the Glu sprayGS produced the most fruits per plant (37.2). The high-lightHL intensity treatment without the Glu sprayGS 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-lightML intensity (with or without the Glu sprayGS) or low-lightLL intensity with the Glu sprayGS. The low-lightLL intensity treatment without the Glu sprayGS generated the fewest fruits per plant. The greatest average fruits were collected from plants grown under high-lightHL intensity without Glu applications. However, the Glu sprayGS treatment had no significant effect. The plants grown under low-lightLL and medium-lightML intensities produced smaller average fruits than medium-lightML intensity. The total fruit weight was the highest (249.0 g plant^{-1}) for the plants exposed to high-lightHL intensity and Glu, followed by the plants treated with high-lightHL intensity without Glu (175.0 g plant^{-1}) and the plants exposed to medium-lightML intensity with Glu (Fig. 4). The lowest total fruit weights (60.6 g plant^{-1}) were recorded for the plants grown under low-lightLL intensity with no Glu sprayGS treatment.

Total soluble solid content was significantly affected by light intensity and by the combined effect of light intensity and Glu sprayGS, but not by Glu alone (Table 67). 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-lightHL intensity with or without Glu, while the lowest soluble solid content (5.3%) was obtained for the plants exposed to low-lightLL intensity without Glu.

3.2.3. Mineral nutrient content in plant tissues

Light intensity and Glu sprayGS treatments had no significant effects on potassium and magnesium contents in the crowns, leaves, and roots (Table 78). In these plant parts, calcium and iron content was significantly affected by either light intensity or the interaction with Glu sprayGS treatment but Glu sprayGS application showed no significant effect. Exposure to high-lightHL 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 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 sprayGS treatment, except for the plants treated with low-lightLL intensity and Glu, which had lower leaf calcium contents. Additionally, the plants grown under high-lightHL intensity with the Glu sprayGS 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 ~~and~~ affecting metabolism, such as lipid liquefaction or disruption of membrane integrity (Levitt 1980). Heat stress was also found to ~~be~~ enhance~~d~~ 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~~s as supplemental~~ light~~s~~ 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 ~~2~~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 ~~the most~~ affected ~~processes~~ because the roots are the first plant parts to encounter the autotoxins accumulated in the rhizosphere (Blum et al. 1999). An alternative means of ~~absorbing~~ mineral nutrients absorption other than ~~through~~ 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~~GS~~ followed by Hyp spray~~HS~~ under same light condition. Whereas ~~while~~ plants under R:B= 5:5 and R:B= 2:8 LED produced significantly lower fruit yield irrespective of amino acids applied (Table ~~3~~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 ~~4~~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~~it~~ 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 ~~total biomass~~ (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~~supplemented~~ 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 ~~(HL)~~ 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 ~~5~~6). However, significantly similar positive influence was observed either with or without Glu spray~~GS~~. Results also indicated that high-light~~HL~~ 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. ~~Several researches supported this statement.~~ 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. Conclusion

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 a greater growth, minerals (iron and magnesium), yield attributes, and fruit yield 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. ~~But f~~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.

References

Amin, A.A., Gharib, F.A.E., El-Awadi, M., Rashad, E.S.M., 2011. Physiological response of onion plants to foliar application of putrescine and glutamine. Sci. Hortic. 129, 353-360.

Asaduzzaman, M., Kobayashi, Y., Isogami, K., Tokura, M., Tokumasa, K., Asao, T., 2012. Growth and yield recovery in strawberry plants under autotoxicity through electro-degradation. Eur. J. Hort. Sci. 77, 58-67.

Asao, T., Kitazawa, H., Ban, T., Pramanik, M.H.R., 2008. Electrodegradation of root exudates to mitigate

autotoxicity in hydroponically grown strawberry (*Fragaria* × *ananassa* Duch.) plants. HortScience. 43, 2034-2038.

Asao, T., Hasegawa, K., Sueda, Y., Tomita, K., Taniguchi, K., Hosoki, T., Pramanik, M.H.R., Matsui, Y., 2003. Autotoxicity of root exudates from taro. Sci. Hortic. 97, 389-396.

Blum, U., Shafer, R., Lehmen, M.E., 1999. Evidence for inhibitory allelopathic interactions including phenolic acids in field soils: Concept vs. an experimental model. Crit. Rev. Plant Sci. 18, 673-693.

Bohnert, H.J., Jensen, R.G., 1996. Strategies for engineering water-stress tolerance in plants. Trends Biotechnol. 14, 89-97.

Cao, J.X., Peng, Z.P., Huang, J.C., Yu, J.H., Li, W.N., Yang, L.X., Lin, Z.J., 2010. Effect of foliar application of amino acid on yield and quality of flowering Chinese cabbage. Chin. Agric. Sci. Bull. 26, 162-165.

Choudhary, N.L., Sairam, R.K., Tyagi, A., 2005. Expression of delta1-pyrroline-5- carboxylate synthetase gene during drought in rice (*Oryza sativa* L.). Ind. J. Biochem. Biophysics. 42, 366-370.

Cruz-Ortega, R., Anaya, A.L., Romos, L., 1988. Effects of allelopathic compounds from corn pollen on respiration and cell division of watermelon. J. Chem. Ecol. 14, 71-86.

Das, C., Sengupta, T., Chattopadhyay, S., Setua, M., Das, N.K., Saratchandra, B., 2002. Involvement of kinetin and spermidine in controlling salinity stress in mulberry (*Morus alba* L. cv. S1). Acta Physiol. Plantarum. 24, 53-57.

Fabro, G., Kovács, I., Pavet, V., Szabados, L., Alvarez, M.E., 2004. Proline accumulation and AtP5CS2 gene activation are induced by plant-pathogen incompatible interactions in Arabidopsis. Mol. Plant–Microbe Inter. 17, 343-350.

Furuya, S., Umemiya, Y., 2002. The influence of chemical forms on foliar-applied nitrogen absorption for peach trees. Proc. Intl. Sem. Foliar Nutr. Acta Hort. 594, 97-103.

Garde-Cerdán, T., Santamaría, P., Rubio-Bretón, P., González-Arenzana, L., López-Alfaro, I., López, R., 2015. Foliar application of proline, phenylalanine, and urea to Tempranillo vines: Effect on grape volatile composition and comparison with the use of commercial nitrogen fertilizers. Food Sci. Technol. 60, 684-689.

Haudecoeur, E., Planamente, S., Cirou, A., Tannieres, M., Shelp, B.J., Morera, S., Faure, D., 2009. Proline antagonizes GABA-induced quenching of quorum-sensing in *Agrobacterium tumefaciens*. Proc. Nat. Acad. Sci. USA. 106, 14587-14592.

Heuer, B., 2003. Influence of exogenous application of proline and glycine betaine on growth of salt-stressed tomato plants. Plant Sci. 165, 693-699.

Hidaka, K., Dan, K., Imamura, H., Miyoshi, Y., Takayama, T., Sameshima, K., Kitano, M., Okimura, M., 2013. Effect of supplemental lighting from different light sources on growth and yield of strawberry. Environ. Cont. Biol. 51, 41-47.

Hoenecke, M.E., Bula, R.J., Tibbitts, T.W., 1992. Importance of blue photon levels for lettuce seedlings grown under red-light emitting diodes. HortScience. 27, 427-430.

Hori, H., 1966. Gravel culture of vegetables and ornamentals. Yokendo, Tokyo, Japan. pp. 60-79.

Inderjit, Weston, L.A., 2003. Root exudates: an overview, in: de Kroon, H., Visser, E.J.W. (Eds.), Root ecology. Ecological Studies 168, Springer, Verlag Berlin, Heidelberg, New York, pp. 235-255.

Katsumi, I., Sato, T., 1985. Effect of light quality on the growth and yield of strawberry. Kyushu Agril. Res. 47, 221.

Kitazawa, H., Asao, T., Ban, T., Pramanik, M.H.R., Hosoki, T., 2005. Autotoxicity of root exudates from strawberry in hydroponic culture. J. Hort. Sci. Biotechnol. 80, 677-680.

Kitazawa, H., Asao, T., Ban, T., Hashimoto, Y., Hosoki, T., 2007. 2,4-D and NAA supplementation mitigates autotoxicity of strawberry in hydroponics. J. Appl. Hort. 9, 26-30.

Lea, P.J., Ireland, R.J., 1999. Nitrogen metabolism in higher plants, in: Singh, B.K. (Eds.), Plant amino acids: biochemistry and biotechnology, Marcel Dekker, New York, pp. 1-47.

Ledesma, N.A., Kawabata, S., Sugiyama, N., 2004. Effect of high temperature on protein expression in strawberry plants. Biol. Plant. 48, 73-79.

Lefsrud, M.G., Kopsell, D.A., Sams, C.E., 2008. Irradiance from distinct wavelength light-emitting diodes affects secondary metabolites in kale. HortScience. 43, 2243-2244.

Levitt, J., 1980. Responses of plants to environmental stress. 2nd ed. Academic Press, New York, USA. 497 pp.

Liu, X.Q., Ko, K.Y., Kim, S.H., Lee, K.S., 2008. Effect of amino acid fertilization on nitrate assimilation of leafy radish and soil chemical properties in high nitrate soil. Commun. Soil Sci. Plant Anal. 39, 269-281.

~~Ministry of Agriculture, Forestry and Fisheries. 2015. Available: https://docs.google.com/viewer?url=http%3A%2F%2Fwww.maff.go.jp%2Fj%2Fseisan%2Fryutu%2Fengei%2Fsisetsu%2Fhaipura%2Fattach%2Fxls%2Fsetti-7.xls [accessed 28 December 2017].~~

Maini, P., Bertucci, B.M., 1999. Possibility to reduce the effects of the viruses with a biostimulant based on amino acids and peptides. Agro Food Ind. Hi-Technol. 10, 26-28.

Massa, G.D., Kim, H.H., Wheeler, R.M., Mitchell, C.A., 2008. Plant productivity in response to LED lighting. HortScience. 43, 1951-1956.

Mazher, A.A.M., Zaghloul, S.M., Mahmoud, S.A., Siam, H.S., 2011. Stimulatory effect of kinetin, ascorbic acid and glutamic acid on growth and chemical constituents of *Codiaeum variegatum* L. plants. Amer. Eur. J. Agric. Environ. Sci. 10, 318-323.

Mondal, F.M., Asaduzzaman, M., Kobayashi, Y., Ban, T., Asao, T., 2013. Recovery from autotoxicity in strawberry by supplementation of amino acids. Sci. Hortic. 164, 137-144.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.

Nassar, A.H., El-Tarabily, K.A., Sivasithamparam, K., 2003. Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine-producing isolate of Streptomyces griseoluteus. Plant Growth Regul. 40, 97-106.

Ohyama, T., Ohtake, N., Sueyoshi, K., Ono, Y., Tsutsumi, K., Ueno, M., Tanabata, S., Sato, T., Takahashi, Y., 2017. Amino acid metabolism and transport in soybean plants, in: Asao, T., Asaduzzaman, M. (Eds.), Amino Acid - New insights and roles in plant and animal. InTech, Croatia, pp. 171-196.

553 Overland, L., 1966. The role of allelopathic substances in the smother crops barley. Amer. J. Bot. 53, 423-432.
554 Petrova, A.G., 1977. Effect of phytoncides from soybean, gram, chickpea and bean on uptake of phosphorus
555 by maize, in: Grodzinsky, A.M. (Eds.), Interaction of plants and microorganisms in Phytocenoses, Kiev:
556 Naukova Dumka, pp. 91-97.
557 Pooryousef, M., Alizadeh, K., 2014. Effect of foliar application of free amino acids on alfalfa performance
558 under rainfed conditions. Res. Crops. 15, 254-258.
559 Portu, J., López-Alfaro, I., Gómez-Alonso, S., López, R., Garde-Cerdán, T., 2015. Changes on grape phenolic
560 composition induced by grapevine foliar applications of phenylalanine and urea. Food Chem. 180, 171-180.
561 Pramanik, M.H.R., Nagai, M., Asao, T., Matsui, Y., 2000. Effects of temperature and photoperiod on
562 phytotoxic root exudates of cucumber (*Cucumis sativa*) in hydroponic culture. J. Chem. Ecol. 26, 1953-
563 1967.
564 Putnam, A.R., 1985. Weed allelopathy, in: Duke, S.O. (Eds.), Weed physiology: Reproduction and
565 Ecophysiology, CRC Press, Boca Raton FL, pp. 131-155.
566 Rice, E.L., 1974. Allelopathy. Academic Press, New York.
567 Russel, D. F., 1986. M-STAT Director. Crop and Soil Science Department, Michigan, State University, U. S.
568 A.
569 Sadak, M., Abdelhamid, M.T., Schmidhalter, U., 2015. Effect of foliar application of amino acids on plant
570 yield and some physiological parameters in bean plants irrigated with seawater. Acta Biológica Colom. 20,
571 141-152.
572 Sager, J.C., McFarlane, J.C., 1997. Radiation, in: Langhans, R.W., Tibbitts, T.W. (Eds.), Plant growth
573 Chamber Handbook, Iowa State Univ. Press, North Central Region Research Publication No. 340, Iowa
574 Agriculture and Home Economics Experiment Station Special Report no. 99, Ames, IA, pp. 1-29.
575 Saito, Y., Shimizu, H., Nakajima, H., Miyasaka, T., Doi, K., 2012. Influence of light quality, specially red
576 light by using the LED in lettuce cultivation. Environ. Eng. 24, 25-30.
577 Saradhi, P.P., Alia Arora, S., Prasad, K.V.S.K., 1995. Proline accumulates in plants exposed to UV radiation
578 and protects them against UV induced peroxidation. Biochem. Biophysics Res. Commun. 209, 1-5.
579 Schat, H., Sharma, S.S., Vooijs, R., 1997. Heavy metal-induced accumulation of free proline in a metal-
580 tolerant and a non-tolerant ecotype of Silene vulgaris. Physiol. Plant. 101, 477-482.
581 Singh, H.P., Batish, D.R., Kohli, R.K. 1999. Autotoxicity: concept, organisms and ecological significance.
582 Crit. Rev. Plant Sci. 18, 757-772.
583 Sorwong, A., Sakhonwasee, S., 2015. Foliar application of glycine betaine mitigates the effect of heat stress in
584 three marigold (*Tagetes erecta*) cultivars. Hort. J. 48, 161-171.
585 Stiegler, J.C., Richardson, M.D., Karcher, D.E., Roberts, T.L., Richard, J., Norman, R.J., 2013. Foliar
586 absorption of various inorganic and organic nitrogen sources by creeping bent grass. Crop Sci. 53, 1148-
587 1152.
588 Stutte, G.W., Edney, S., Skerritt, T., 2009. Photoregulation of bioprotectant content of red leaf lettuce with
589 light-emitting diodes. HortScience. 44, 79-82.

590 Takahashi, K., 1984. The replant failures of vegetables. Research Reports. Nat. Res. Inst. Veg. Tea Sci. Japan,
591 87-99 (in Japanese, English abstract).
592 Takeuchi, M., Arakawa, C., Kuwahara, Y., Gemma, H., 2008. Effects of l-pro foliar application on the quality
593 of 'Kosui' Japanese pear. Acta Hortic. 800, 549-554.
594 Tang, C.S., Young, C.C., 1982. Collection and identification of allelopathic compounds from the undisturbed
595 root system of bitalta limpograss (*Helmarthria altissima*). Plant Physiol. 69, 155-160.
596 Tripathy, B.C., Brown, C.S., 1995. Root-shoot interaction in the greening of wheat seedlings grown under red
597 light. Plant Physiol. 107, 407-411.
598 Wahba, H.E., Motawe, H.M., Ibrahim, A.Y., 2015. Growth and chemical composition of *Urtica pilulifera* L.
599 plant as influenced by foliar application of some amino acids. J. Mat. Environ. Sci. 6, 499-509.
600 Wing, K.B., Pritts, M.P., Wilcox, W.F., 1995. Biotic, edaphic and cultural factors associated with strawberry
601 black root rot in New York. HortScience. 30, 86-90.
602 Yanagi, T., Okamoto, K., Takita, S., 1996a. Effect of blue and red light intensity on photosynthetic rate of
603 strawberry leaves. Acta Hort. 440, 371-376.
604 Yanagi, T., Okamoto, K., Takita, S., 1996b. Effect of blue, red, and blue/red lights of two different PPF levels
605 on growth and morphogenesis of lettuce plants. Acta Hort. 440, 117-122.
606 Yancey, P.H., Clark, M.B., Hands, S.C., Bowlus, R.D., Somero, G.N., 1982. Living with water stress:
607 evaluation of osmolyte systems. Sci. 217, 1214-1222.
608 Yaronskaya, E., Vershilovskaya, I., Poers, Y., Alawady, A.E., Averina, N., Grimm, B., 2006. Cytokinin
609 effects on tetrapyrrole biosynthesis and photosynthetic activity in barley seedlings. Planta, 224, 700-709.
610 Yasumoto, N., Tacheuchi, M., Yasui, A., Watanabe, T. 2006. Analysis manual for standard table of food
611 composition in Japan. 5th Edition. Kenpakusha Publishing, Tokyo, Japan. 228 pp.
612 Yorio, N.C., Wheeler, R.M., Goins, G.D., Sanwo-Lewandowski, M.M., Mackowiak, C.L., Brown, C.S.,
613 Sager, J.C., Stutte, G.W., 1998. Blue light requirements for crop plants used in bioregenerative life support
614 systems. Life Supp. Bios. Sci. 5, 119-128.
615 Yoshiba, Y., Kiyosue, T., Katagiri, T., Ueda, H., Mizoguchi, T., Yamaguchi-Shinozaki, K., Wada, K., Harada,
616 Y., Shinozaki, K., 1995. Correlation between the induction of a gene for delta 1-pyrroline-5-carboxylate
617 synthetase and the accumulation of proline in Arabidopsis thaliana under osmotic stress. Plant J. 7, 751-
618 760.
619 Yuen, G.Y., Schroth, M.N., Weinhold, A.R., Hancock, J.G., 1991. Effect of soil fumigation with methyl
620 bromide and chloropicrin on root health and yield of strawberry. Plant Dis. 75, 416-420.
621 Zhao, Y., Wu, L., Chu, L., Yang, Y., Li, Z., Azeem, S., Zhang, Z., Fang, C., Lin, W., 2015. Interaction of
622 Pseudostellaria heterophylla with *Fusarium oxysporum* f. sp. heterophylla mediated by its root exudates in a
623 consecutive monoculture system. Sci. Rep. 5, 8197.
624 Zhen, W., Cao, K., Zhang, X., 2003. Simulation of autotoxicity of strawberry root exudates under continuous
625 cropping. Acta Phytoecol. Sin. 28, 828-832.

Tables

Table 1. Full strength “Enshi” nutrient solution

Chemicals	Amounts[z] (g/1000 L)
$Ca(NO_3)_2.4H_2O$	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

[z]Amounts of salts per 1000 L of tap water (Hori,1966).

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

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Table 2. Effects of light quality and amino acids spray on the growth of strawberry grown under heat stress condition.

Amino acid/ Light quality		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)
Effect of amino acid										
Water		20.0 a	20.3 b	13.9	29.8	23.3 b	50.2	11.5	1.9	2.9
Hydroxyproline		18.8 b	21.0 a	14.8	29.7	20.9 b	51.2	11.5	1.8	2.7
Glutamic acid		16.8 c	20.5 a	15.0	33.8	24.8 a	50.1	10.6	1.7	3.0
Effect of light quality										
Fluorescent lamp		16.3 b	25.0 a	16.3	30.1	17.9 c	51.2	10.6 c	1.4 c	2.7 c
LED (R:B = 8:2)		21.3 a	19.3 ab	13.7	31.4	27.4 a	50.5	13.3 a	2.7 a	3.7 a
LED (R:B = 5:5)		19.3 ab	18.3 b	13.7	31.8	23.3 b	51.0	11.3 b	1.7 b	2.9 b
LED (R:B = 2:8)		17.0 b	19.7 ab	14.6	30.9	23.4 b	49.8	9.5 d	1.3 d	2.0 d
Interaction effect of amino acid and light quality										
Water	Fluorescent lamp	19.0 abc	24.0 ab	16.0	28.1	17.2 j	50.4	11.0 b	1.5 c	2.4 c
	LED (R:B = 8:2)	21.0 ab	19.0 ab	13.6	27.8	25.4 e	49.8	13.0 ab	2.8 a	4.1 a
	LED (R:B = 5:5)	19.0 abc	18.0 b	13.0	33.2	23.7 f	51.5	12.0 abc	1.8 b	3.1 abc
	LED (R:B = 2:8)	21.0 ab	20.0 ab	12.8	29.9	27.0 c	49.2	10.0 c	1.4 c	1.9 d
Hydroxyproline	Fluorescent lamp	16.0 bc	26.0 a	16.7	28.1	17.9 hi	52.9	11.0 b	1.5 c	2.8 bc
	LED (R:B = 8:2)	24.0 a	20.0 ab	13.4	31.7	27.9 b	51.0	14.0 a	2.9 a	3.2 abc
	LED (R:B = 5:5)	19.0 abc	18.0 b	13.3	27.4	20.2 g	51.7	11.0 b	1.6 b	2.8 bc
	LED (R:B = 2:8)	16 bc	20.0 ab	15.8	31.5	17.6 ij	49.3	10.0 c	1.2 c	1.9 d
Glutamic acid	Fluorescent lamp	14.0 c	25.0 ab	16.2	34.1	18.5 h	50.3	9.7 d	1.2 c	3.0 bc
	LED (R:B = 8:2)	20.0 ab	19.0 ab	14.0	34.8	28.8 a	50.6	13.0 ab	2.4 ab	3.7 ab
	LED (R:B = 5:5)	20.0 ab	19.0 ab	14.6	34.9	26.1 d	50.0	11.0 b	1.7 b	2.8 bc
	LED (R:B = 2:8)	14.0 c	19.0 ab	15.2	31.2	25.7 de	49.6	8.6 d	1.3 c	2.3 c
Significance										
	Amino acid	*	*	NS	NS	*	NS	NS	NS	NS
	Light quality	*	*	NS	NS	*	NS	*	*	*
	Interaction	*	*	NS	NS	*	NS	*	*	*

Note: Means within column followed by the same letters are not significant according to the Tukey's Test at P <0.05, NS =Not significant, *= Significant at the 5% level and DW = Dry weight.

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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.

Table 3. Effects of light quality and amino acids spray on the yield attributes, yield and fruit quality of strawberry grown under heat stress condition.

Amino acid/ Light quality		Number of days anthesis	Number of days to fruit ripening	Number of flowers plant^{-1}	Number of fruit plant^{-1}	Average fruit weight (g)	Fruit yield (g plant^{-1})	Total soluble solids of fruits (%)	Citric acidity of fruits (%)	Ascorbic acid content of fruits (ppm)
Effect of amino acid										
Water		8.1	34.2	19.7 c	2.9 c	2.3 c	7.0 c	4.9	0.70	24.7 b
Hydroxyproline		8.3	33.2	28.9 b	3.6 b	2.6 a	9.4 b	4.9	0.62	26.5 ab
Glutamic acid		7.5	34.0	35.2 a	4.5 a	2.4 b	11.4 a	5.0	0.73	27.8 a
Effect of light quality										
Fluorescent lamp		8.6	35.3	28.3 b	4.4 b	2.5 b	10.8 b	4.6 c	0.70	24.0 b
LED (R:B = 8:2)		8.5	32.8	44.0 a	5.7 a	2.7 a	15.9 a	5.6 a	0.62	38.7 a
LED (R:B = 5:5)		7.5	32.6	21.8 c	2.4 c	2.4 c	5.8 c	5.0 b	0.71	24.8 b
LED (R:B = 2:8)		7.3	34.7	17.4 d	2.2 d	2.1 d	4.8 d	4.4 d	0.71	17.7 c
Interaction effect of amino acid and light quality										
Water	Fluorescent lamp	8.0	36.5	19.0 f	3.6 d	2.4 e	8.9 f	4.6 f	0.74	21.0 cd
	LED (R:B = 8:2)	10.0	33.0	21.0 ef	4.3 c	2.7 c	11.6 d	5.6 c	0.58	39.2 a
	LED (R:B = 5:5)	6.6	31.3	22.6 e	2.0 gh	2.1 h	4.3 ij	5.0 e	0.86	21.8 cd
	LED (R:B = 2:8)	7.8	36.0	16.0 g	1.7 h	2.1 h	3.4 j	4.4 g	0.63	16.6 d
Hydroxyproline	Fluorescent lamp	8.4	35.0	31.0 d	4.0 c	2.8 b	10.3 e	3.7 h	0.63	24.0 bc
	LED (R:B = 8:2)	8.0	33.2	48.0 b	5.7 b	2.7 c	15.5 b	5.5 d	0.58	39.0 a
	LED (R:B = 5:5)	8.6	32.5	22.6 e	2.5 ef	2.6 d	6.5 g	4.7 f	0.63	24.4 bc
	LED (R:B = 2:8)	8.0	32.0	13.8 g	2.3 fg	2.2 g	5.1 hi	4.4 g	0.63	18.6 d
Glutamic acid	Fluorescent lamp	9.4	34.4	35.0 c	5.7 b	2.3 f	13.2 c	3.7 h	0.74	27.0 b
	LED (R:B = 8:2)	7.4	31.5	63.0 a	7.0 a	2.9 a	20.1 a	6.1 a	0.7	37.8 a
	LED (R:B = 5:5)	7.2	34.0	20.0 ef	2.7 e	2.4 e	6.5 g	6.0 b	0.63	28.2 b
	LED (R:B = 2:8)	6.0	36.0	22.5 e	2.7 e	2.1 h	5.8 gh	3.7 h	0.86	18.0 d
Significance										
	Amino acid	NS	NS	*	*	*	*	NS	NS	*
	Light quality	NS	NS	*	*	*	*	*	NS	*
	Interaction	NS	NS	*	*	*	*	*	NS	*

Note: Means within column followed by the same letters are not significant according to the Tukey's Test at P <0.05, NS =Not significant, *= Significant at the 5% level

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.

Table 4. Effects of light quality and amino acids spray on nutrients content of strawberry plants grown under heat stress condition.

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

Note: Means within column having the same letters are not significant according to the Tukey's test at P <0.05. NS =Not significant and * = Significant at the 5% level. DW = Dry weight.

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.

Table 5. Effects of LED light quantities and Glutamic acid on the growth of strawberry plant grown in controlled environment facilities.

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

Note: Values in a column followed by different letter(s) differ significantly by Tukey's test. Significant at the 5% level (*), Not significant (NS).
LED quantity low, medium and high are 149, 269 and 567 µ mol m^{-2}s^{-1},
Glutamic acid applied (+), not applied ( ).

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 NSNot significant at the 5% level.

Table 6. Effects of LED light quantities and Glutamic acid on fruit yield and quality of strawberry grown in controlled environment facilities.

Glu/LED quantity		Number of fruit plant^{-1}	Average fruit weight (g)	Fruit yield (g plant^{-1})	Total soluble solid (%)	Citric acidity (%)	Ascorbic acid (ppm)
Effect of Glu							
-		19.7	5.6	113.2 b	6.2	0.43	381.6
+		26.9	5.8	159.0 a	6.4	0.44	368.6
Effect of LED quantity							
Low		15.6 c	4.9 c	75.4 c	5.4 b	0.44	377.4
Medium		22.9 b	5.3 b	121.0 b	6.3 ab	0.44	360.0
High		31.5 a	6.9 a	212.0 a	7.2 a	0.44	387.9
Interaction effect of Glu and LED quantity							
Low	-	13.2 c	4.6 b	60.6 d	5.3 c	0.44	389.9
	+	18.0 bc	5.1 b	90.1 cd	5.5 c	0.43	364.9
Medium	-	20.2 bc	5.2 b	104.0 cd	6.2 b	0.44	368.4
	+	25.6 b	5.4 b	138.0 bc	6.3 b	0.44	351.5
High	-	25.8 b	6.9 a	175.0 b	7.0 a	0.42	386.4
	+	37.2 a	6.8 a	249.0 a	7.3 a	0.45	389.4
Significance							
Glu		NS	NS	*	NS	NS	NS
LED quantity		*	*	*	*	NS	NS
Interaction		*	*	*	*	NS	NS

Note: Values in a column followed by different letter(s) differ significantly by Tukey's test. Significant at the 5% level (*), Not significant (NS).
LED quantity low, medium and high are 149, 269 and 567 µ mol m^{-2}s^{-1}.
Glutamic acid applied (+), not applied ( ).

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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 NSNot significant at the 5% level.

Table 7. Effects of LED quantities and Glutamic acid on mineral nutrient content of strawberry plant grown in controlled environment facilities.

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

Note: Values in a column followed by different letter(s) differ significantly by Tukey's test. Significant at the 5% level (*), Not significant (NS). DW = Dry weight.
[a]LED quantity low, medium and high are 149, 269 and 567 µ mol $m^{-2}s^{-1}$.
[b]Glutamic acid applied (+), not applied ( ).

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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 NSNot significant at the 5% level.
DW = Dry weight.

Figures

Fig. 1. Three layered vertical growing beds used for cultivation of strawberry plants under controlled-environment. In the first experiment, plastic container filled with 3 L of 25% standard nutrient solution was used for each plant. Three combinations of LED [Red (660 nm): Blue (450 nm)] lights (i.e., 2:8, 5:5, and 8:2, bottom, middle and top grow bed, respectively) **[A]** were used along with fluorescent lamps **[B]** as a control. The light panel was set at about 20 cm above the surface of the plant canopy. 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. Six individual systems used for six treatments (i.e., three light conditions either with or without Glu). 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}).

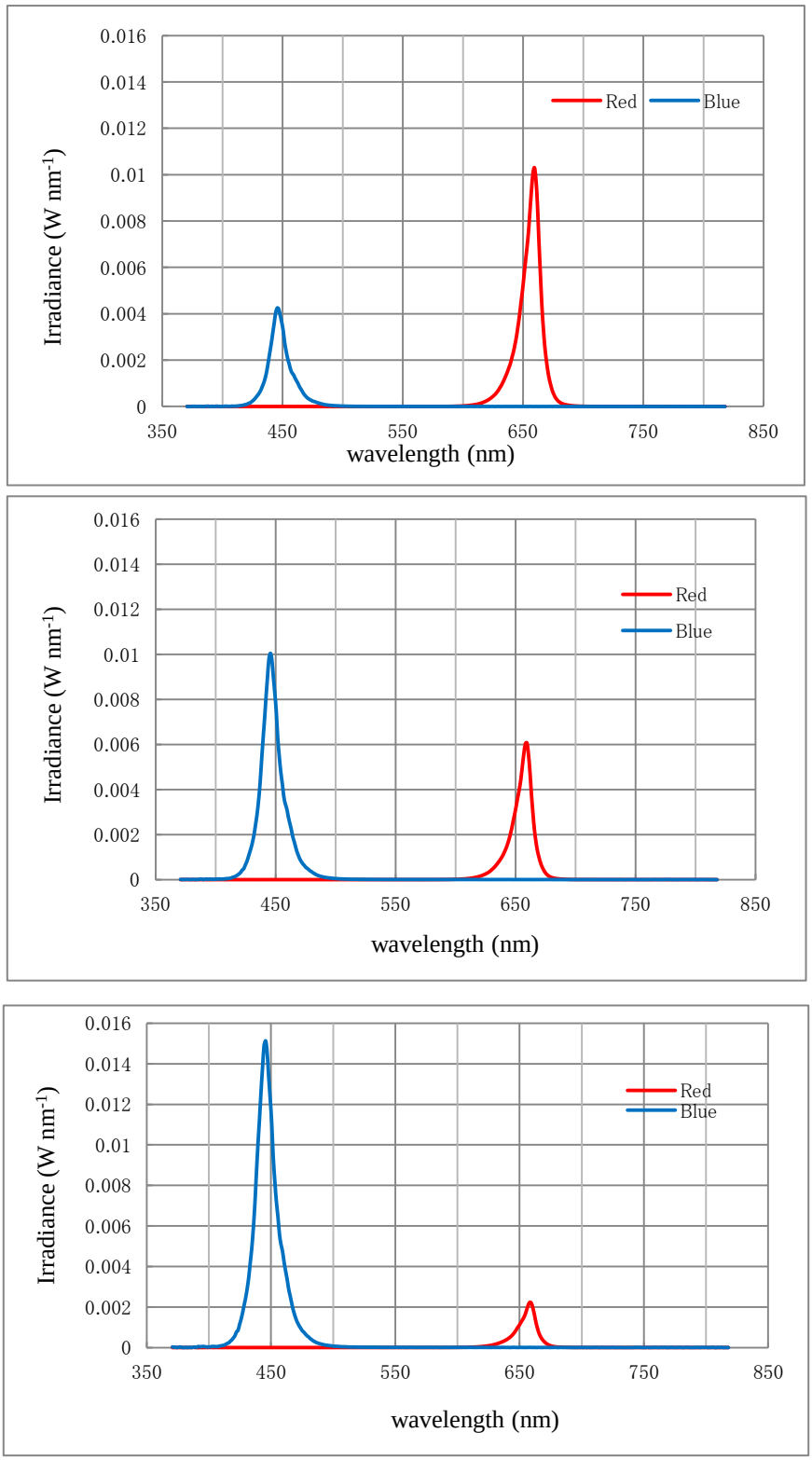

Fig. 2. Irradiance of three types of LEDs, **(A)** Red: Blue = 8:2, **(B)** Red: Blue = 5:5, and **(C)** Red: Blue = 8:2 used in this experiment. The measurement was conducted at 25 ºC. The commercial LEDs were supplied from Showa Denko K.K. Green Innovation Project, Japan.

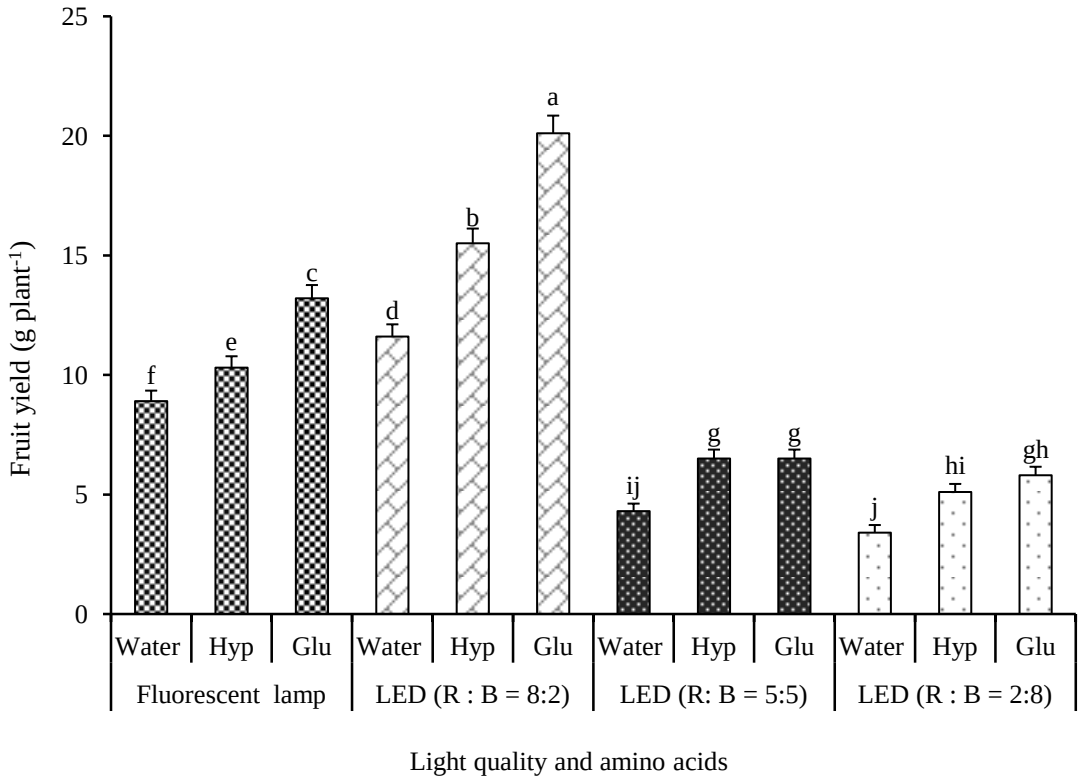

Fig. 3. The effects of light quality and amino acids spray on the fruit yield of strawberry grown under heat stress condition. Bars with the same letter(s) are not significantly different according to the Tukey's Test at $P < 0.05$.

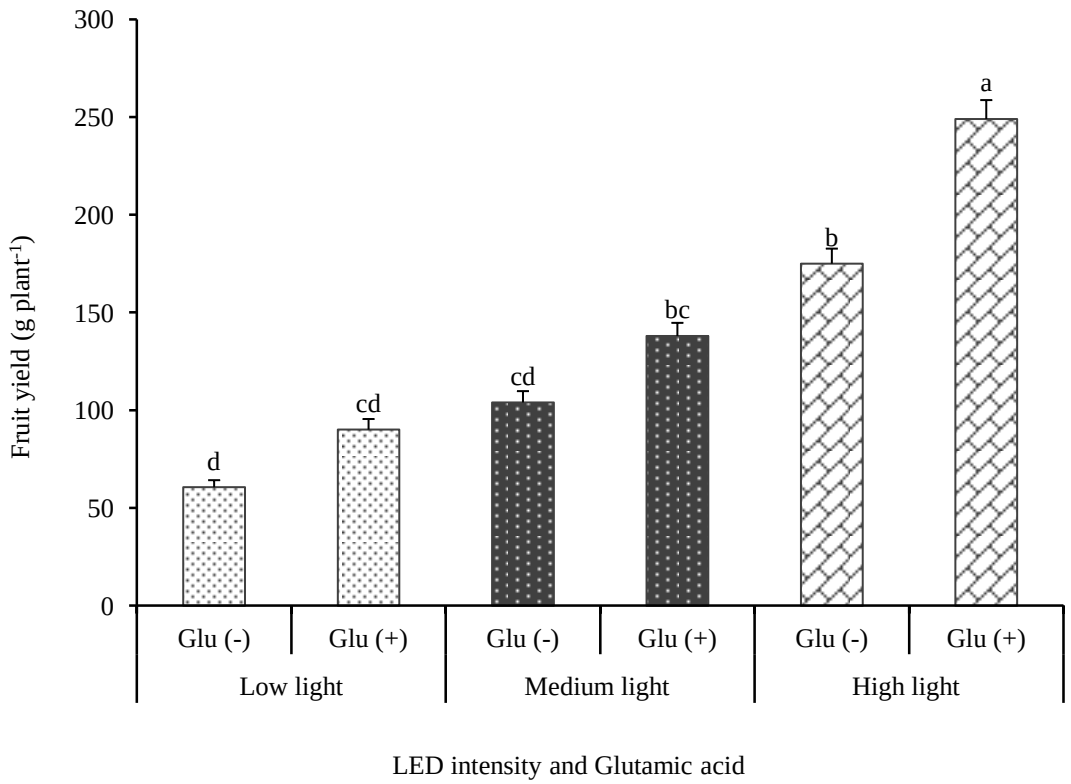

Fig. 4. The effects of LED light intensity and Glutamic acid application on the fruit yield of strawberry grown in controlled environment facilities. Bars with the same letter are not significantly different according to the Tukey's test at $P < 0.05$. LED intensity low, medium and high are 149, 269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