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Recovery from autotoxicity in strawberry by supplementation of amino acids

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ABSTRACT

Supplementation of amino acids was investigated in recovering growth and yield of strawberry plants under autotoxicity developed in closed hydroponic systems. In greenhouse setting, twenty two water soluble amino acids were sprayed on strawberry plants at 2 mL per plant three times a week. The concentrations of all amino acids were adjusted to nitrogen content of Proline at 200 mg L^{-1}. It was found that growth and yield of strawberry plants grown in non-renewed nutrient solution was significantly reduced compared to plants grown in renewed nutrient solution. When plants were grown in non-renewed solution and sprayed Ala, Cys, Glu, Hyp, Lys, Thr, Trp, His and Phe, the growth improved whereas, yield were improved by spraying of Ala, Asn, Asp,Cys, Glu, Gln, Hyp, Lys, Orn, Thr, Trp, His, Phe and Val. Based on growth and yield performance, Ala, Glu, Hyp, Thr, His and Phe were selected for further investigation along with GABA following Wagner's pot hydroponic system and also *in vitro* condition. Glu and Hyp sprayed plants produced about 50% greater fruit yield compared to water spray as control in Wagner's pot hydroponic system. Effects of amino acids on strawberry plant growth improvement during autotoxicity were confirmed following *in vitro* culture, where environmental factors and microbial degradation of amino acids were excluded. Results showed that leaf dry weight of Hyp treated plants and root dry weight of Ala, Glu, Hyp, Thr and GABA treated plants were improved against control. Therefore, foliar spray of Glu and Hyp on strawberry plants can recover the growth and yield during autotoxicity in closed hydroponic system.

*Key words:*Root exudates, Allelochemicals, Amino acids, Foliar spray, Wagner's pot hydroponics, *in vitro* Strawberry.

1. Introduction

Autotoxicity from the root exudates of strawberry in closed hydroponic culture has been investigated (Kitazawa et al., 2005). During this phenomenon strawberry plant's roots secreted allelochemicals mainly benzoic acid to the culture solution causing damage to the root cells, which in turns hamper water and mineral nutrient absorption. As a result, the growth of shoot and root, number of flowers and harvested fruit per plant, and fruit enlargement reduced greatly. Removal of these inhibitory allelochemicals from the culture solution would lead to normal growth and yield. In this regards, activated charcoal has been used to adsorb the accumulated phytotoxic chemicals for the culture solution and improve the growth and yield in strawberry (Kitazawa et al., 2005), taro (Asao et al., 2003), cucumber (Asao et al., 1998, 1999, 2000), several leafy vegetables (Asao et al., 2004a), and some ornamentals (Asao et al., 2007). Other means such as degradation of growth inhibitors by microbial strain in cucumber (Asao et al., 2004b), supplementation of auxin in strawberry (Kitazawa et al., 2007) or electro-degradation of phytotoxic chemicals in strawberry (Asao et al., 2008; Asaduzzaman et al., 2012) were also found to be effective for recovering the autotoxic effect in closed hydroponics. However, finding suitable method for controlling autotoxicity in strawberry would be of great help for the commercial production of strawberry in a non-recycled hydroponics.

Allelopathic compounds may induce a secondary oxidative stress manifested as enlarged production of reactive oxygen species (ROS) (Weir et al., 2004). Toxic ROS can affect membrane permeability, cause damage to DNA and protein, induce lipid peroxidation, and ultimately lead to programmed cell death. Therefore, autotoxic effects of root exudates of strawberry plants on its growth and development is likely to be caused by impairment of nutrient and water absorption by injured roots. Supply of mineral nutrient alternatively other than by root uptake can sustain plant growth during this allelochemical stress. The availability and uptake of nitrogen is considered as the major factor affecting growth (Lea and Azevedo, 2006) therefore, it can be sprayed on the leaves as a source of nutrients. Use of foliarly applied urea as a nitrogen source is common (Bowman and Paul, 1992; Vasilas et al., 1980). For example, in wheat, foliarly applied urea produced positive effects; these were attributed to higher leaf photosynthetic rates and higher leaf urease enzyme activities (Peltonen, 1993).

Amino acids are the nitrogenous compound which forms the basic component of all living cells.

It can absorb by leaf exogenously (Furuya and Umemiya, 2002).Therefore, it has a great potentiality of using under managed culture techniques. Recently they are used as foliar spray to improve the growth, yield and quality of crops (Mazher et al., 2011; Takeuchi et al., 2008). Several researchers found positive impacts of amino acids as foliar spray under stress condition for example Proline to wheat (Rajagopal and Sinha, 1980), Proline, Alanine, Serine, and Asparagine to maize (Thakur and Rai, 1985) under osmotic stress and Proline, Phenylalanine to maize and board bean under salinity stress (Abd El-Samad et al., 2011). As the accumulated allelochemicals in closed culture become stressful to plant, spraying of amino acid to strawberry plants would be positive. So far, spraying amino acids in recovering strawberry plant growth during autotoxicity has not been studied. Therefore, the purpose of the present study was to evaluate the performance of amino acids on the recovery of growth and yield of strawberry plants under autotoxicity in closed hydroponic culture.

2. Materials and Methods

2.1. Culture of strawberry plant in container based hydroponics

Strawberry (*Fragaria* × *ananassa* Duch. cv. Toyonoka) plantlets reproduced through plant tissue culture were used for this experiment. The study was conducted in 100 m^2 glasshouse of Experimental Research Center at Biological Resources Science, Shimane University. Initially strawberry plantlets at four to five leaves stage were transplanted to plastic container (20 × 54 × 34 cm) with 55 L of 25% Enshi nutrient solution (p^H 7.25 and EC 0.8 dSm^{-1}). The full strength Enshi nutrient solution contains the following amount of salts per 1000 L of tap water: 950 g $Ca(NO_3)_2{\cdot}4H_2O$; 810 g of KNO_3; 500 g of $MgSO_4{\cdot}7H_2O$; 155 g of $NH_4H_2PO_4$; 3 g of H_3BO_3; 2 g of $ZnSO_4{\cdot}7H_2O$; 2 g of $MnSO_4{\cdot}4H_2O$; 0.05 g of $CuSO_4{\cdot}5H_2O$; 0.0 2g of Na_2MoO_4; 25 g of NaFe-EDTA (Hori, 1966). Five plantlets were planted in each container in such a way that the roots were inserted into the nutrient solution inside the container keeping shoot outside. Urethane foam block (23 mm × 23 mm × 27 mm) was used for holding the plant tight with a floating board on the nutrient solution. Nutrient solutions were circulated 24 h by pumps (KP-101, Koshin, Kyoto, Japan) with automatic timer

(KS-1500, Iuchi, Osaka, Japan) which were either renewed or non-renewed entirely and non-renewed with amino acids and urea application. Renewed culture solutions were changed biweekly with new nutrient solutions whereas, non-renewed nutrient solutions were analyzed for major nutrients and adjusted as close as possible to initial concentrations at every two weeks on the basis of chemical analyses with Compact NO_3^- meter (B-343, Horiba, Ltd. Kyoto, Japan) for NO_3^-, Spectrophotometer (U-2900, Hitachi, Tokyo, Japan) for $PO_4^{3-}$and Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2310, Hitachi, Tokyo, Japan) for K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}. Two day after transplanting, twenty two water soluble amino acids viz., Alanine (Ala), Arginine (Arg), Asparagine (Asn), Aspartic acid (Asp), Cysteine (Cys), Glutamatic acid (Glu), Glutamine (Gln), Glycine (Gly), Hydroxy-proline (Hyp), Lysine (Lys), Ornithine (Orn), Proline (Pro), Serine (Ser), Threonine (Thr), Tryptophan (Typ), Methionine (Met), Leucine (Leu), Isoleucine (Ile), Citrulline (Cit), Histidine (His), Phenylalanine (Phe), and Valine (Val) and urea were individually sprayed as foliar application at 2 mL per plant by a 500 mL sprayer three times a week on the strawberry plants grown in non-renewed nutrient solution.The concentrations of urea and amino acids were adjusted to nitrogen content of Pro at 200 mg L^{-1} to maintain the same concentration level. The dates of anthesis were recorded for each plant to check whether any influence of amino acids on flowering of strawberry among the treatments. Pollination was aided by a soft brush at two days intervals. Fruits were harvested when those became about 80% red in colour. At each harvest fresh weight of fruits were recorded and gathered for final yield calculation. At final harvest, leaf number, leaf length and width, root length, crown diameter, fresh weight of leaf, crown and inflorescence were recorded. Then strawberry plant parts were separated into leaf, crown, inflorescence and root and dried in a constant temperature oven (DKN 812, Yamato Scientific Co., Ltd. Japan) for 72 h at 80 °C. When the dry matter reaches constant weight, dry weight of different plant parts was measured.

2.2. Culture of strawberry plants in Wagner's pot hydroponics

Seven amino acids were selected for their better growth and yield performance in the container based hydroponics. These short listed amino acids were further investigated in the glasshouse following Wagner's pot hydroponics using strawberry cultivar 'Toyonoka'. Healthy plantlets obtained through micro propagation with five to six leaves were planted into Wagner's pot (ten plants in one line) connected with a plastic reservoir (63 × 48 × 22 cm) containing 60 L of 25% Enshi nutrient solution in closed hydroponic system. The system includes main inlet pipes (15 mm diameter) for supply and drainage of nutrient solution between reservoir and pots, Wagner's pot (1/5000a, NF-5, AsOne, Osaka, Japan) with 3 L capacity for planting, inlet tubes (4 mm diameter) to supply solution to the pots, and 60 L capacity nutrient solution container with a pump (KP-101, Koshin, Kyoto, Japan). The culture solution was not renewed during the entire growth period and it was recycled through the pipes for 5 min at 10 min intervals using an automatic pump timer (KS-1500, Iuchi, Osaka, Japan). One month after transplanting, the selected amino acids viz., Ala, Glu, Hyp, Thr, His, Phe, including g*amma*-aminobutyric acid (GABA) and water as control were sprayed on leaves of strawberry plants. The concentrations of all amino acids were adjusted to nitrogen content of Pro at 200 mg L^{-1}. Water and amino acids were applied at 1.4 mL per plant by a 100 mL sprayer three times a week. The major nutrients in the non-renewed culture solution were analyzed and adjusted following methods and instruments used in container based hydroponics at every two weeks. Other cultural practices were done as described in the previous culture. Fruits were harvested when those became about 80% red in colour. The harvested fruits were grouped into three stages based on their harvesting time and gathered for final yield calculation. The relative amount of chlorophyll in strawberry leaves were measured (SPAD-502 plus, Konica Minolta Sensing, Inc. Osaka, Japan) at final harvest. Growth and yield of strawberry plants were measured following the methods as described in the previous culture.

2.3. Determination of fruit qualities of strawberry

After harvest fruits were composited and were frozen at –30 °C for subsequent analysis of soluble solids, titratable acid and ascorbic acid content. Fruit samples were kept out of freezer before analysis to obtain juice for determining the above qualities of strawberry fruits. The soluble solid content of fruit collected from container based culture was determined using a digital refract meter (As One, SpittzIPR-101α, Osaka, Japan) whereas, fruits of Wagner's pot culture was determined using a pocket digital refractometer (PAL-1, Atago Ltd., Japan). Titratable acid contents were determined by diluting each 2 mL aliquot of strawberry juice to 10 mL with 8 mL distilled water and added 2–3 drops of phenolphthalein then adjusted the pH to 8.2 using 0.1 N NaOH. Then the titratable acid was converted into % citric acid. The ascorbic acid content was measured with 2,4-dinitrophenylhydrazine (DNP) colorimetry. Strawberry fruit juice (0.5 mL) were taken in 50 mL test tube then 0.5 mL of 10% meta-phosphoric acid solution, 1 mL of distilled water, 1 mL of 0.03% 2,6-dichlorophenol-indophenol (DCP), 2 mL of thiourea, and 1 mL of 2,4-DNP was added to the samples following 3 h incubation at 37 °C in water bath (BW400, Yamato Scientific Co. Ltd. Japan). After incubation samples 5 mL of 85% H_2SO_4 were added keeping in water cooled with iced water. After 30 min cooling ascorbic acid content was measured at 520 nm by spectrophotometer (U-2900, Hitachi High Technologies Corporation, Tokyo, Japan).

2.4. Culture of strawberry plantlets under in vitro condition

In order to control the effects of environmental factors and also microbial degradation of amino acid, strawberry plantlets were cultured under *in vitro* condition at Plant Factory supported Research Laboratory of Shimane University. Strawberry cv. Toyonoka plantlets of similar vigor were transferred into a culture box (100 ×110 ×100 mm) with 100 mL substrate. The plant boxes were capped with bio-filter (for aeration) and placed in growth chamber at 20/15 ºC (day/night) under florescent light with intensity of 74-81 μmol m^{-2} s^{-1} and 12 h photoperiod. The substrates were prepared using 25% Enshi nutrients solution (EC 0.8 dS m^{-1}) with agar (9 g L^{-1}) as solidified agent and sucrose (30 g L^{-1}) as carbon source. One plantlet was planted in each plant box and three plant boxes were used for each treatment with three replications. The seven amino acids (Ala, Glu, Hyp, Thr, His, Phe and GABA) used in the Wagner's pot hydroponics were also used in this experiment with water, urea and renew of substrate as control. In case of renew of substrate, it was changed to new substrate whereas, water, urea and amino acids were sprayed on the strawberry leaves at 3.0 mL per plant using 0.45 µm syringe filter (Toyo Roshikaisha, Ltd. Japan) inside the clean bench. The concentrations of urea and amino acids used were adjusted to nitrogen content of Pro at 200 mg L^{-1}. After eight weeks, growth variables and relative amount of chlorophyll content in strawberry plants were measured and compared among the treatments.

2.5. Statistical analysis

A randomized complete block design with three replicates was used for both culture of strawberry in container based hydroponics and Wagner's pot hydroponics in the greenhouse whereas, complete block design was performed in culture of strawberry plantlets *in vitro* condition. Analysis of variance was performed to test for statistical differences among the treatments and mean separations were performed by Tukey’s Honestly Significant Difference (HSD) and Least Significant Difference (LSD) test at $P < 0.05$ level of significance by MSTATC statistical software.

3. Results

3.1. Evaluation of twenty two amino acids on the growth of strawberry plants under autotoxicity in container based hydroponics

Foliar application of twenty two amino acids showed significant influence on the growth of the strawberry plants grown in non-renewed culture solution in hydroponics (Table 1). Plants grown in renewed nutrient solution produced bigger leaves compared to plants grown in non-renewed nutrient solution. Leaf size was not significantly increased by foliar spray with Arg, Asp, Gln, Gly, Orn, Pro, Ser, Met, Leu, Ile, Cit and Val. Spray of Ala, Cys, Glu, Hyp, Lys, Thr, Trp, His and Phe on the leaves of strawberry plants grown in non-renewed nutrient solution increased leaf length and width compared with water as control. Number of leaves did not differ significantly in plants grown in non-renewed nutrient solution with or without supplementation of amino acids. Longer roots were recorded in plants sprayed with urea, Ala, Cys, Lys, Trp and grown in renewed nutrient solution compared to other amino acids. Smaller crowns were found in Gly, Pro, Ser, Met, Leu and Val sprayed plants compared to plants grown in renewed culture solution. Higher leaf fresh weight was measured in Cys, Glu, Trp and Phe treated plants compared to plants grown in non-renewed solution. Crown fresh weight was increased in plants sprayed with urea, Ala, Cys, Glu, Gln, Hyp, Lys, Thr, Trp, Cit, His, Phe and Val whereas, fresh weight of flowering bud was increased in plants sprayed with Cys, Glu, Hyp, Lys, Thr, Trp, His and Phe. Among the amino acids applied Cys, Glu, Lys, Trp, His and Phe supplemented plants produced higher dry weight in leaves, crown, inflorescence and root compared to plants in non-renewed nutrient without amino acid application (Fig. 1). Ala, Hyp and Thr also produced higher dry matter in all parts except leaves. From above results it is evident that growth variables were reduced when strawberry plants were grown in non-renewed nutrient solutions compared to renewed solution but these were improved in plants grown in non-renewed nutrient solution with the supplementation of Ala, Cys, Glu, Hyp, Lys, Thr, Trp, His and Phe.

3.1.1. Evaluation of twenty two amino acids on the fruit yield and quality of strawberry plants under autotoxicity in container based hydroponics

Amino acids lead a positive effect on the yield attributes and fruit quality of strawberry grown in container based hydroponics (Table 2; Fig. 2, 3). Anthesis date was influenced by the application of amino acids on strawberry plant leaves and it was found that about 23 days earlier flowering in Ala, Arg, Asn and Phe sprayed plants. Fruit yield per plant was decreased about 74% in plants grown in non-renewed nutrient solution than grown in renewed nutrient solution. Application of urea, Ala, Asn, Asp, Cys, Glu, Gln, Hyp, Lys, Orn, Thr, Trp, His, Phe and Val improved fruit yield in plants grown in non-renewed nutrient solution which was attributed by number of flowers and number of mature fruits. Average fruit weight also correspond the yield in these amino acid supplemented plants. There were no significant differences among the amino acid applied in terms of strawberry fruit qualities such as soluble solids, citric acidity and ascorbic acid.

3.2.Effects of selected seven amino acids on the growth of strawberry plants under autotoxicity in Wagner's pot hydroponics

The effects of seven amino acids were investigated on the growth of strawberry plants grown in recycled culture solution in Wagner's pot closed hydroponic system. Result showed a significant difference in plants growth supplemented with amino acids (Table 3). Among the amino acids, Ala, Glu, Phe and GABA supplemented plants increased their leaf fresh weight by about 25, 22, 25, and 23%, against water spray as control, respectively whereas leaf dry weight was significantly increased only in Glu and His treated plants. There was no significant difference among the treatments in terms of number of leaves, relative amount of chlorophyll content and crown fresh weight. Bigger crown was found in Glu (22.0 mm), Hyp (21.8 mm) and GABA (18.9 mm) treated plants. Foliar spray of Hyp significantly increased the crown dry weight.Amino acids have influence on the root growth which was evidenced in Glu, Phe and Hyp supplemented plants where these amino acids increased by 33, 41 and 59% dry weight of root, respectively compared with control.

3.2.1. Effects of seven amino acids on the fruit yield and quality of strawberry plants under autotoxicity in Wagner's pot hydroponics

Application of amino acids greatly influenced the yield in strawberry plants following recycled Wagner's pot hydroponics (Table 4). Ala, His, Thr, Hyp and Glu treated plants increased fruit yield in 30, 38, 40, 50 and 51% in comparison to control. In these treatments the highest numbers of fruits were recorded. Spraying of Phe and GABA did not influenced on the fruit yield of strawberry. Greater numbers of fruits were recorded from Ala, Thr, His, Glu and Hyp treated plants. Average fruit weight was not significantly improved by the amino acids under investigation. Application of amino acids did not left any effects on the soluble solid content at different stages of harvested strawberry fruits, however, % citric acid content in fruits significantly varied in all three stages (Table 5). In the stage I, Glu, Hyp and GABA treated strawberry plants produced fruits with high citric acid content whereas in stage II it was higher in Glu and GABA supplemented plants fruits. In stage III, the higher citric acid levels were found in fruits with, Ala, Hyp and GABA application. Although ascorbic acid content in the strawberry fruits was varied in the early harvested fruits but in the mid and later harvested fruits did not differ it significantly.

3.3. Effects of seven amino acids on the growth of strawberry plantlets under in vitro condition

Growth of strawberry plantlets was evaluated under *in vitro* condition with the supplementation of seven amino acids (Table 6). Leaf number was increased in amino acids supplied plantlets compared to water control. Higher numbers of leaves were counted in plantlets cultured in renewed substrates as control. Urea, Ala, Glu, Phe and GABA treated plantlets increased their relative amount of chlorophyll content against water control. The longest leaf was found in renewed substrate plantlets and all the amino acids supplied plantlets increased their leaf length over water control. Compared with water and urea, Thr improved the leaf width. Ala, Glu, Hyp and GABA treated plantlets resulted in longer roots against water and urea while the longest root was found in renewed substrate plantlets. The results showed that only Hyp application improved the crown diameter. Treated with Hyp produced bigger crown compared to other amino acids. Higher fresh weight of leaf was obtained when plantlets were sprayed by Glu and Hyp as compared to water spray as control. Hyp treated plantlets increased their crown and root fresh weight against water control. All the amino acids treated plantlets improved their root fresh weights against water control. Hyp treated plantlets also produced significantly higher root fresh weight than plantlets grown in renewed substrates. In case of leaf dry weight, Hyp and urea treated plantlets gained higher weight which are similar. Crown dry weight did not showed any significant difference among the amino acid treatments. Under *in vitro* condition strawberry plantlets improved the root dry matter against water and renewed control. Highest root dry weight was found in Glu treated plant and other amino acids such as Ala, Thr, Hyp and GABA; as well as urea also improved.

4. Discussion

When plants experiences autotoxicity, ion uptake and hydraulic conductivity (i.e., water uptake) are worse affected processes since root is the first organ to come into contact with autotoxins in the rhizosphere (Blum et al., 1999). Alternative means of supplying mineral nutrient other than absorption by roots can overcome this problem for sustainable growth and yield of strawberry. Studies on the effects of amino acids on the growth and yield of strawberry plants in closed hydroponics would be interesting. In container based hydroponics when twenty two amino acids were sprayed, dry weight of strawberry plants were increased (Fig. 1) which accord with the results of Nassar et al., (2003) and Amin et al., (2011) where foliar application of amino acids increased the dry weight of bean and onion plants respectively. As amino acids are the precursor of chlorophyll synthesis, it plays active role in dry matter production in plants (Yaronskaya et al., 2006) moreover foliar application of amino acid increased plant protein content which ultimately increased the dry matter (Das et al., 2002) . The regulatory effects of certain amino acids, like Phe and Orn, on plant development through their influence on gibberellins has been suggested by Waller and Nowacki (1978). Plants grown in non-renewed nutrient solution showed growth and yield declined but when plants were supplied with Hyp, it produced higher growth and fruit yield all three cultures. The possible reason might be its presence in the cell wall as Hyp-rich glycoproteins, is an extra-cellular structural protein of plant cell walls and extra-cellular matrix during normal development and in response to stress, autotoxicity in this case (Kieliszewski, 2001; Kieliszewski and Shpak, 2001).The higher fruit yield in amino acid supplemented plants than water sprayed plants were due to greater vegetative growth. This positive effect on growth and yield might be due to the assimilation and metabolism of nitrogen in strawberry plants. For example, Glu is known to have a central role in nitrogen metabolism and is the preferential amino-donor for the different aminotransferase reactions for subsequent amino acid interconversions (Lea and Ireland, 1999). Therefore, greater fruit yield was contributed by vigorous growth, number of flowers, number of mature fruits per plant, and average fruit weight.

In Wagner's pot hydroponics, results revealed that the total dry weight (not shown on Table 3) was higher in plants sprayed with Glu, Hyp, and Phe than plants grown in non-renewed nutrient solution with water spray. This accord with Mazher et al., (2011) who reported foliar application of Glu increased the growth and the content of total carbohydrate, nitrogen, and phosphorus and potassium percentages of *Codiaeumvariegatum* L. plant. In another study, spraying of Pro or Phe on maize and broad bean increased the amount of dry matter and water content (Abd El-Samad et al., 2011). Higher yield was recorded in Ala, Glu, Hyp, Thr, and His treated plants (Table 4) which were attributed by their better vegetative growth and higher numbers of mature fruits per plant. Moreover, amino acids might have some influence on the pollination and fruit setting. Hyp was found to be localized in growing tips in lily which can elongate the pollen tube enhancing the fertilization and fruit setting (Dashek and Harwood, 1974).

In vitro culture of strawberry plantlets was conducted to exclude the effects of environmental factors like temperature, light intensity, relative humidity and also microbial degradation of amino acids. Therefore, this experiment under control condition can confirm whether there or not any effects of amino acid on the strawberry plant growth in non-renewed nutrient condition. Total dry matter production was greater in urea, Hyp, Glu and GABA treated plantlets compared to water as control (not shown in the Table 6) which primarily due to meeting the nitrogenous demand from amino acid source. Recent studies found the positive impact of amino acids *in vitro* condition as organic source of nitrogen in alfalfa, maize, sorghum, pineapple, rice and sugarcane (Skokut et al., 1985; Claparols et al., 1993; Rao et al., 1995; Hamasaki et al., 2005; Grewel et al., 2006 and Asad et al,. 2009).

4. Conclusion

Amino acids might have some effects on the growth and yield of strawberry in closed hydroponic system where autotoxicity is a common problem. From the first experiment, it was found that amino acid application left positive effects on the growth and yield of strawberry. Twenty two amino acids were short listed to Ala, Glu, Hyp, Thr, His and Phe on basis of their performance on growth and yield of strawberry in non-recycled hydroponics. In the second experiment, selected amino acids along with GABA were further investigated following Wagner's pot hydroponics. Among the seven amino acids applied, only Glu and Hyp improved their fruit yield over 50%. The *in vitro* culture of strawberry plantlets showed similar results of previous experiments. Therefore, considering the effects of amino acids on growth and fruit yield of strawberry, Glu and Hyp can be used for foliar application in a non-recycled hydroponic culture. Further investigation is necessary to determine the timing and doses of amino acids application for more efficient utilization by the strawberry plants.

References

Abd El-Samad, H.M., Shaddad, M.A.K., Barakat, N., 2011. Improvement of plants salt tolerance by exogenous application of amino acids. J. Med. Plant. Res. 5, 5692-5699.

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. Hort. 129, 353-360.

Asad, S., Arshad, M., Mansoor, S., Zafar, Y., 2009. Effect of various amino acids on shoot regeneration of sugarcane (*Saccharum officinarum* L.). African J. Biotechol. 8, 1214-1218.

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

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

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. HortSci. 43, 2034-2038.

Asao, T., Kitazawa, H., Ban, T., Pramanik, M.H.R., Matsui, Y., Hosoki. T., 2004a. Search of autotoxic substances in some leafy vegetables. J. Jpn. Soc. Hort. Sci. 73, 247-249.

Asao, T., Kitazawa, H., Ushio, K., Sueda, Y., Ban, T., Pramanik, M.H.R., 2007. Autotoxicity in some ornamentals with the means to overcome it. HortSci. 42, 1346-1350.

Asao, T., Kitazawa, H., Tomita, K., Suyama, K., Yanamoto, H., Hosokiand, T., Pramanik. M.H.R., 2004b.Mitigation of cucumber autotoxicity in hydroponic culture using microbial strain. Sci. Hort. 99, 207-214.

Asao, T., Ohba, Y., Tomita, K., Ohtaand, A. K., Hosoki.T., 1999.Effects of activated charcoal and dissolved oxygen levels in the hydroponic solution on the growth and yield of cucumber plants. J. Jpn. Soc. Hort. Sci. 68, 1194-1196.

Asao, T., Umeyama, M., Ohta, K., Hosoki, T., Ito, T., Ueda, H., 1998. Decrease of yield of cucumber by non-renewal of the nutrient hydroponic solution and its reversal by supplementation of activated charcoal. J. Jpn. Soc. Hort. Sci. 67, 99-105 (in Japanese with English summary).

Asao, T., Tomita, K., Taniguchi, K., Hosoki, T., Pramanik, M.H.R., Matsui. Y., 2000. Effects of activated charcoal supplementation in the nutrient solution on the harvested fruit number of cucumbers grafted on the bloomless rootstock in hydroponics. J. Soc. High Tech. Agri. 12, 61-63.

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. in Plant Sci. 18, 673-693.

Bowman, D.C., Paul, J.L., 1992. Foliar absorption of urea, ammonium, and nitrate by perennial ryegrass turf. J. Amer. Soc. Hort. Sci. 117, 75-79.

Claparols, I., Santosa, M.A., Torne, M.J., 1993. Influence of some exogenous amino acids on the production of maize embryogenic callus and on endogenous amino acid content. Plant Cell Tissue Organ Cult. 34, 1-11.

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 (Morusalba L. cv. S1). Acta Physiol. Plant. 24, 53-57.

Dashek, W.V., Harwood, H.I., 1974. Proline, hydroxyproline, and lily pollen tube elongation. Ann. Bot. 38, 947-959.

Furuya, S., and Y. Umemiya. 2002. The influence of chemical forms on foliar-applied nitrogen absorption for peach trees. Proceedings of the International Seminar on Foliar Nutrition. Acta Hort. ISHS 594, 97-103.

Grewel, D., Gill, R., Gosal, S., 2006.Role of cysteine in enhancing androgenesis and regeneration of indica rice (*Oryza sativa* L.). Plant Growth Reg. 49, 43-47.

Hamasaki, R.M., Purgatto, E., Mercier, H., 2005. Glutamine enhances competence for organogenesis in pine apple leaves cultivated *in vitro*.Braz. J. Plant Physiol. 17, 383-389.

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

Kieliszewski, M.J., 2001. The latest hype on Hyp-*O*-glycosylation codes. Phytochem. 57, 319-323.

Kieliszewski, M.J., Shpak, E., 2001. Synthetic genes for the elucidation of glucocylation codes for arabinogalactan-proteins and other hydroxyproline-rich glycoproteins. Cell. Mol. Life Sci. 58, 1386-1398.

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.

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. Biotech. 80, 677-680.

Lea, P.J., Azevedo, R.A., 2006. Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. Ann. Appl. Bio. 149, 243-247.

Lea, P.J., Ireland, R.J., 1999. Nitrogen metabolism in higher plants. In: Singh B. K, (Eds.) Plant Amino Acids: Biochemistry and Biotechnology. New York, Marcel Dekker. pp. 1-47.

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. American-Eurasian J. Agric. Environ. Sci., 10, 318-323.

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.

Peltonen, J., 1993. Interaction of late season foliar spray of urea and fungicide mixture in wheat production. J. Agron. Crop Sci. 170, 296-308.

Rajagopal, V., Sinha, S.K., 1980. Influence of exogenously supplied proline on relative water content in wheat and barley. Indian J. Exp. Biol. 18, 1523-1524.

Rao, A.M., Sree, K.P., Kishor, P.B.K., 1995. Enhanced plant regeneration in grain and sweet sorghum by asparagine, proline and cefotaxime. Plant Cell Rep. 15, 72-75.

Skokut, T.A., Manchester, J., Schaefer, J., 1985. Regeneration in alalfa tissue culture. Plant Physiol. 79, 579-583.

Takeuchi, M., Arakawa, C., Kuwahara, Y., Gemma, H., 2008.Effects of L-pro foliar application on the quality of ‘Kosui’ Japanese pear. Acta Hort. 800, 549-554.

Thakur, P.S., Rai, V.K., 1985. Exogenous supplied of amino acids and water deficits in *Zea mays* cultivar. Biol. Plant. 27, 458-461.

Vasilas, B.L., Leeg, J.O., Wolf, D.C., 1980. Foliar fertilization of soybeans: Absorption and translocation of ^{15}N-labeled urea. Agron. J. 72, 271-275.

Waller, G.R., Nowacki, E.K., 1978. Alkaliod, Biology and Metabolism in Plants. Plenum Press, New York. pp. 5-247.

Weir, T., Park, S.W., Vivianco, J.M., 2004. Biochemical and physiological mechanisms mediated by allelochemicals. Curr.Opin. Plant Biol. 7, 472-479.

Yaronskaya, E., Vershilovskaya, I., Poers, Y., Alawady, A.E., Averina, N., Grimm, B., 2006.

Cytokinin effects on tetrapyrrole biosynthesis and photosynthetic activity in barley seedlings. Planta. 224, 700-709.

Table 1.

Effect of twenty two amino acids on the growth of strawberry plants grown in non-renewed nutrient solution in closed hydroponic system.

Amino acids[a]	Number of leaves[b]	Leaf length (cm)	Leaf width (cm)	Root length (mm)	Crown diameter (mm)	FW of leaves (g)	FW of crown (g)	FW of flowering bud (g)
RW	42.4 ab[c]	37.4 a	21.7 a	372.5 ab	49.5 a	194.6 a	11.7 a	14.6 a
NRW	24.0 ab	17.0 c	13.3 bc	283.7 bc	39.8 ab	26.3 c	4.5 b	5.1 bc
Urea	28.1 ab	27.0 b	18.7 ab	401.5 a	39.5 ab	73.9 bc	8.7 ab	6.6 bc
Ala	38.4 ab	29.5 ab	20.2 ab	334.0 ab	36.5 ab	121.2 bc	9.1 ab	8.9 b
Arg	33.0 ab	17.9 c	12.9 bc	234.5 c	38.0 ab	36.0 c	5.4 b	6.4 bc
Asn	30.0 ab	26.3 b	17.9 ab	297.5 bc	35.5 ab	56.5 c	4.5 b	6.7 bc
Asp	33.6 ab	25.4 bc	18.1 ab	281.9 bc	32.1 ab	73.9 bc	6.1 b	8.2 bc
Cys	40.2 ab	33.3 ab	20.3 ab	335.0 ab	41.2 ab	147.2 ab	9.5 ab	10.9 ab
Glu	37.8 ab	33.2 ab	21.5 ab	314.0 b	38.5 ab	146.7 ab	9.2 ab	12.8 ab
Gln	25.3 ab	25.5 bc	17.0 b	320.5 b	34.6 ab	56.7 c	6.9 ab	6.3 bc
Gly	23.4 ab	15.8 c	12.5 bc	282.3 bc	29.7 b	28.2 c	5.8 b	3.3 bc
Hyp	36.0 ab	30.6 ab	19.5 ab	317.5 b	41.5 ab	105.7 bc	8.7 ab	11.1 ab
Lys	34.8 ab	33.3 ab	21.6 ab	336.0 ab	43.5 ab	128.3 b	9.0 ab	11.5 ab
Orn	24.5 ab	23.7 bc	15.4 bc	306.5 b	39.8 ab	40.1 c	5.4 b	4.8 bc
Pro	23.6 b	20.1 bc	15.3 bc	300.5 bc	23.9 b	39.5 c	4.3 b	4.0 bc
Ser	25.8 ab	19.8 bc	16.3 bc	292.1 bc	23.5 b	48.6 c	5.6 b	5.9 bc
Thr	34.9 ab	32.0 ab	20.5 ab	304.5 b	43.5 ab	100.8 bc	8.9 ab	9.9 ab
Trp	43.7 a	35.1 ab	21.4 ab	374.5 ab	47.0 ab	139.5 ab	11.4 ab	10.9 ab
Met	28.2 ab	19.0 bc	13.7 bc	293.0 bc	28.6 b	32.5 c	4.3 b	3.5 bc
Leu	31.2 ab	10.1 c	11.8 c	276.3 bc	27.5 b	29.4 c	3.6 b	2.6 c
Ile	32.9 b	21.2 bc	14.1 bc	297.0 bc	36.0 ab	49.5 c	5.0 b	5.7 bc
Cit	22.7 ab	15.8 c	13.7 bc	283.5 bc	33.0 ab	23.1 c	6.6 ab	3.9 bc
His	39.5 ab	32.3 ab	21.0 ab	319.0 b	36.6 ab	123.4 b	11.1 ab	10.4 ab
Phe	41.4 ab	33.4 ab	21.8 a	329.0 b	39.3 ab	145.3 ab	9.2 ab	11.4 ab
Val	27.4 ab	20.1 bc	14.6 bc	279.5 bc	28.8 b	37.3 c	7.8 ab	4.8 bc
	*	*	*	*	*	*	*	*

[a]Strawberry plants grown in renewed (RW), non-renewed (NRW), and non-renewed nutrient solution with amino acids and urea application.
[b] Parameters were measured on per plant basis; Fresh weight (FW), Dry weight (DW).
[c] Means within column followed by different letters are significant according to Tukey's test at $P < 0.05$ (n = 15).
[*] Significant at $P < 0.05$.

Table 2.

Effect of twenty two amino acids on fruit quality of strawberry plants grown in non-renewed nutrient solution in closed hydroponic system.

Amino acids[a]	Soluble solids (%)	Citric acidity (%)	Ascorbic acids (mg/100g)
RW	7.5	0.42	61.3
NRW	5.8	0.38	69.3
Urea	7.2	0.45	55.9
Ala	7.6	0.45	57.3
Arg	8.3	0.45	54.2
Asn	7.8	0.48	55.1
Asp	6.5	0.42	54.5
Cys	6.7	0.38	74.1
Glu	7.2	0.38	60.8
Gln	7.2	0.42	64.1
Gly	7.0	0.35	57.5
Hyp	6.9	0.35	55.4
Lys	7.1	0.26	55.0
Orn	6.0	0.38	58.3
Pro	6.4	0.32	50.1
Ser	7.8	0.35	44.5
Thr	7.5	0.32	50.8
Trp	8.0	0.35	58.4
Met	7.1	0.42	76.3
Leu	7.8	0.42	64.6
Ile	8.0	0.54	74.9
Cit	7.3	0.48	60.9
His	7.4	0.35	78.6
Phe	7.5	0.42	69.3
Val	7.4	0.35	72.0
	ns	ns	ns

[a] Strawberry plants grown in renewed (RW), non-renewed (NRW), and non-renewed nutrient solution with amino acids and urea application.

[b] Parameters were measured on per plant basis.

[ns, *] Non-significant according to Tukey's test at $P < 0.05$ ($n = 15$).

Table 3.

Effect of seven amino acids on the growth of strawberry plants grown in non-renewed nutrient solution in Wagner's pot hydroponic system.

Amino acids[a]	Number of leaves[b]	SPAD	FW of leaves (g)	FW of crown (g)	Crown diameter (mm)	Root length (mm)	DW of leaves (g)	DW of crown (g)	DW of root (g)
Wat	72.6	38.8	223.2 b[c]	38.9	15.9 d	32.6 ab	51.3 b	7.5 b	10.5 c
Ala	91.3	38.7	278.1 a	44.0	17.0 cd	30.2 ab	63.0 ab	8.5 ab	12.0 bc
Glu	84.5	38.4	272.8 a	45.9	22.0 a	32.5 ab	67.9 a	10.2 ab	14.0 ab
Hyp	87.8	39.5	269.6 ab	49.8	21.8 ab	32.0 ab	64.5 ab	11.3 a	16.7 a
Thr	60.2	38.5	256.1 ab	49.0	17.4 cd	28.4 b	63.4 ab	8.8 ab	11.8 bc
His	88.5	38.4	268.1 ab	47.6	16.5 cd	32.0 ab	66.9 a	8.2 b	12.2 bc
Phe	94.1	39.2	279.0 a	48.2	18.3 cd	30.5 ab	64.2 ab	10.2 ab	14.8 ab
GABA	94.8	39.2	274.0 a	49.2	18.9 bc	34.3 a	63.9 ab	9.8 ab	13.5 abc
	ns	ns	*	ns	*	*	*	*	*

[a] Strawberry plants grown in non-renewed nutrient solution with amino acids application.

[b] Parameters were measured on per plant basis; Fresh weight (FW), Dry weight (DW).

[c] Means within column followed by different letters are significant according to LSD test at $P < 0.05$ ($n = 15$).

[ns, *] Non-significant or significant at $P < 0.05$, respectively.

Table 4.

Effect of seven amino acids on the fruit yield of strawberry in Wagner's pot hydroponic system.

Amino acids[a]	Number of fruits[b]	Average fruit weight (g)	Fruit yield (g)
Wat	63.3 c[c]	6.7	432.2 d[z]
Ala	88.2 ab	6.8	560.2 abc
Glu	90.4 ab	7.3	654.2 a
Hyp	97.5 a	6.8	649.8 a
Thr	90.5 ab	6.7	606.2 ab
His	89.2 ab	7.0	594.7 ab
Phe	77.3 bc	6.9	474.1 cd
GABA	72.8 bc	7.3	498.2 bcd
	**	ns	**

[a] Strawberry plants grown in non-renewed nutrient solution with amino acids application.

[b] Parameters were measured on per plant basis; Fresh weight (FW), Dry weight (DW).

[c] Means within column followed by different letters are significant according to LSD test at $P < 0.05$ ($n = 15$).

[ns, **] Non-significant or significant at $P < 0.01$, respectively.

Table 5.

Soluble solid content, % Citric acidity and ascorbic acid content in fruits of strawberry plants supplemented with amino acids in Wagner's pot hydroponic system. Fruits harvested at stage I (2011/3/25–2011/4/30), stage II (2011/5/1–2011/5/25) and stage III (2011/5/26–2011/6/20).

Amino acids[a]	Soluble solid content (%)			Citric acidity (%)			Ascorbic acid (mg/100g)		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
Water	7.6	7.3	7.9	0.22 b[b]	0.22 c	0.26 b	42.1 ab	41.0	40.3
Ala	6.7	7.1	7.0	0.35 ab	0.26 bc	0.45 a	45.6 a	39.6	39.3
Glu	7.9	7.3	7.1	0.51 a	0.38 ab	0.35 ab	41.6 ab	38.0	36.2
Hyp	6.8	5.8	7.3	0.45 a	0.35 abc	0.41 a	40.9 bc	38.4	35.7
Thr	6.0	5.6	6.8	0.38 ab	0.32 abc	0.35 ab	37.8 bc	41.2	38.4
His	7.4	7.3	7.2	0.38 ab	0.29 bc	0.22 b	40.9 bc	42.6	38.6
Phe	6.8	6.8	6.7	0.38 ab	0.29 bc	0.35 ab	37.0 c	41.2	37.4
GABA	7.4	6.0	7.8	0.58 a	0.45 a	0.45 a	41.7 ab	38.8	40.7
	ns	ns	ns	**	**	**	*	ns	ns

[a] Strawberry plants grown in non-renewed nutrient solution with amino acids application.

[b] Means within column followed by different letters are significant according to LSD test at $P < 0.05$ (n = 15).

[ns, *, **] Non-significant or significant at $P < 0.05$, 0.01%, respectively.

Table 6. Effect of seven amino acids on the growth of strawberry plantlets under *in vitro* condition.

Amino acids[a]	Number of leaves[b]	SPAD	Leaf length (mm)	Leaf width (mm)	Root length (mm)	Crown diameter (mm)	FW of leaves (mg)	FW of crown (mg)	FW of root (mg)	DW of leaves (mg)	DW or crown (mg)	DW of root (mg)
RW[x]	12.6 a[c]	40.7 bc	58.2 a	45.8 a	181.2 a	3.5 b	1480.0 a	154.0 b	962.0 c	284.0 a	28.0	102.0 c
Water	6.9 c	42.7 c	43.2 c	37.8 c	81.4 e	3.4 b	967.8 c	125.6 b	691.1 d	201.1 c	27.8	97.8 c
Urea	9.3 b	48.0 ab	47.6 b	38.0 c	86.0 de	3.8 b	1185.6 bc	114.4 b	1470.0 a	270.0 ab	31.1	164.4 a
Ala	8.7 b	48.6 a	48.9 b	40.8 bc	116.8 bc	3.7 b	1049.2 bc	147.0 b	1126.7 bc	220.0 bc	30.0	153.3 ab
Glu	8.6 bc	48.1 ab	52.3 ab	41.5 bc	130.1 b	3.9 b	1194.7 b	169.7 b	1112.8 bc	228.3 bc	34.4	167.8 a
Hyp	9.6 b	44.7 bc	46.9 b	41.0 bc	114.3 bc	4.7 a	1218.3 b	255.0 a	1304.2 ab	267.5 ab	39.2	150.8 ab
Thr	8.9 b	44.2 bc	47.3 b	42.4 ab	103.3 cd	3.8 b	1048.0 bc	142.9 b	1137.5 bc	220.0 bc	29.2	154.2 ab
His	9.3 b	46.4 bc	43.9 b	37.8 c	91.3 de	4.0 ab	1049.2 bc	140.0 b	1005.8 c	218.6 bc	35.0	124.2 bc
Phe	9.3 b	47.8 ab	45.2 b	38.9 bc	101.1 cde	3.9 ab	1078.9 bc	142.2 b	1060.6 bc	224.8 bc	28.3	123.3 bc
GABA	9.4 b	46.8 ab	47.8 b	40.9 bc	122.2 bc	4.0 ab	1179.2 bc	175.8 ab	1228.3 abc	233.3 abc	32.5	155.8 ab
	*	*	*	*	*	*	*	*	*	*	ns	*

[a] Strawberry plants cultured in renewed (RW) and non-renewed substrate with amino acids and water application.

[b] Parameters were measured on per plant basis; Fresh weight (FW), Dry weight (DW).

[c] Means with column followed by different letters are significant according to Tukey's test at $P < 0.05$ ($n = 9$).

[ns, *] Non-significant or significant at $P < 0.05$, respectively.

Figure captions:

Fig. 1. Effect of twenty two amino acids on the dry matter production of strawberry plants grown in non-renewed nutrient solution in closed hydroponic system. RW: Renewed, NRW: Non-renewed, amino acids are presented as their three letters abbreviation.

Fig. 2. Effect of twenty two amino acids on the flowering and fruit setting of strawberry plants grown in non-renewed nutrient solution in closed hydroponic system. RW: Renewed, NRW: Non-renewed, amino acids are presented as their three letters abbreviation.

Fig. 3. Effect of twenty two amino acids on the yield of strawberry plants grown in non-renewed nutrient solution in closed hydroponic system. RW: Renewed, NRW: Non-renewed, amino acids are presented as their three letters abbreviation.

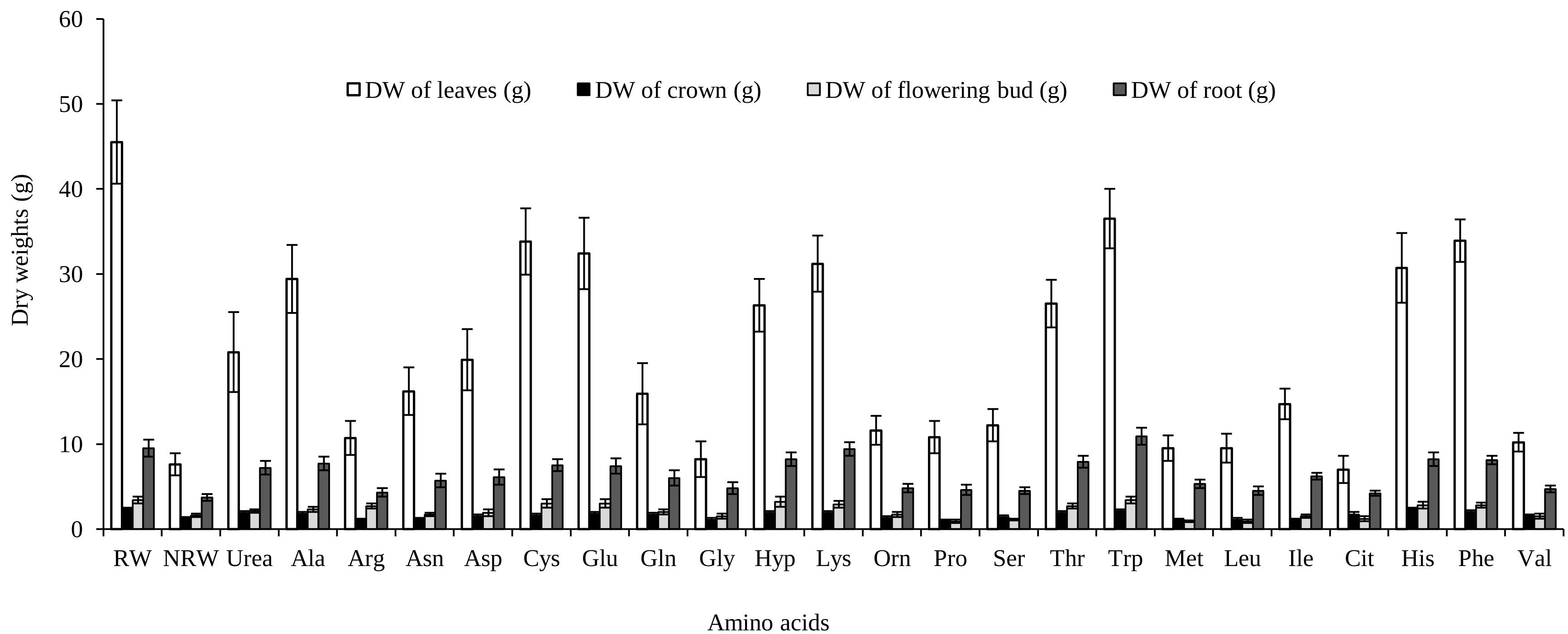

Fig. 1. Effects of twenty two amino acids on the dry matter production of strawberry plants grown in non-renewed nutrient solution in closed hydroponic system. RW: Renewed, NRW: Non-renewed, amino acids are presented as their three letters abbreviation.

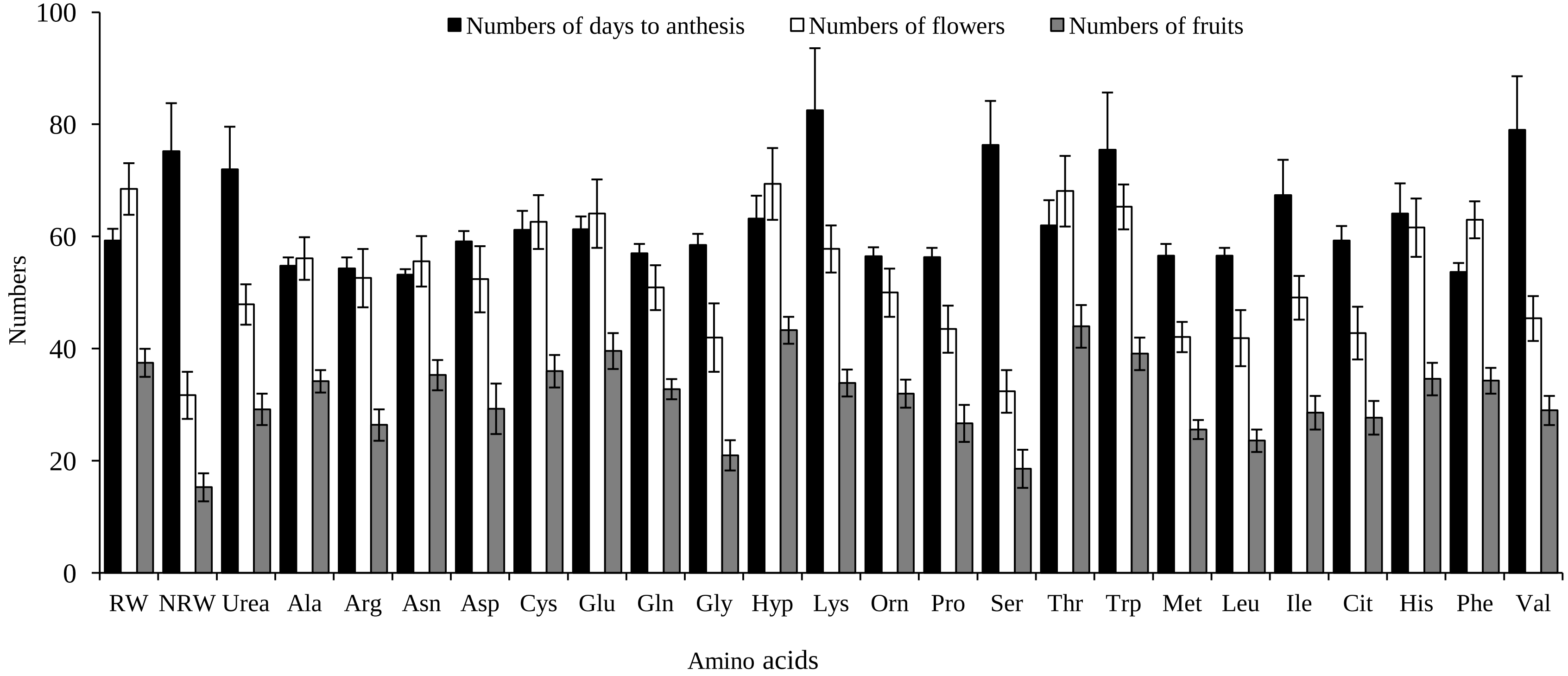

Fig. 2. Effect of twenty two amino acids on the flowering and fruit setting of strawberry plants grown in non-renewed nutrient solution in closed hydroponic system. RW: Renewed, NRW: Non-renewed, amino acids are presented as their three letters abbreviation.

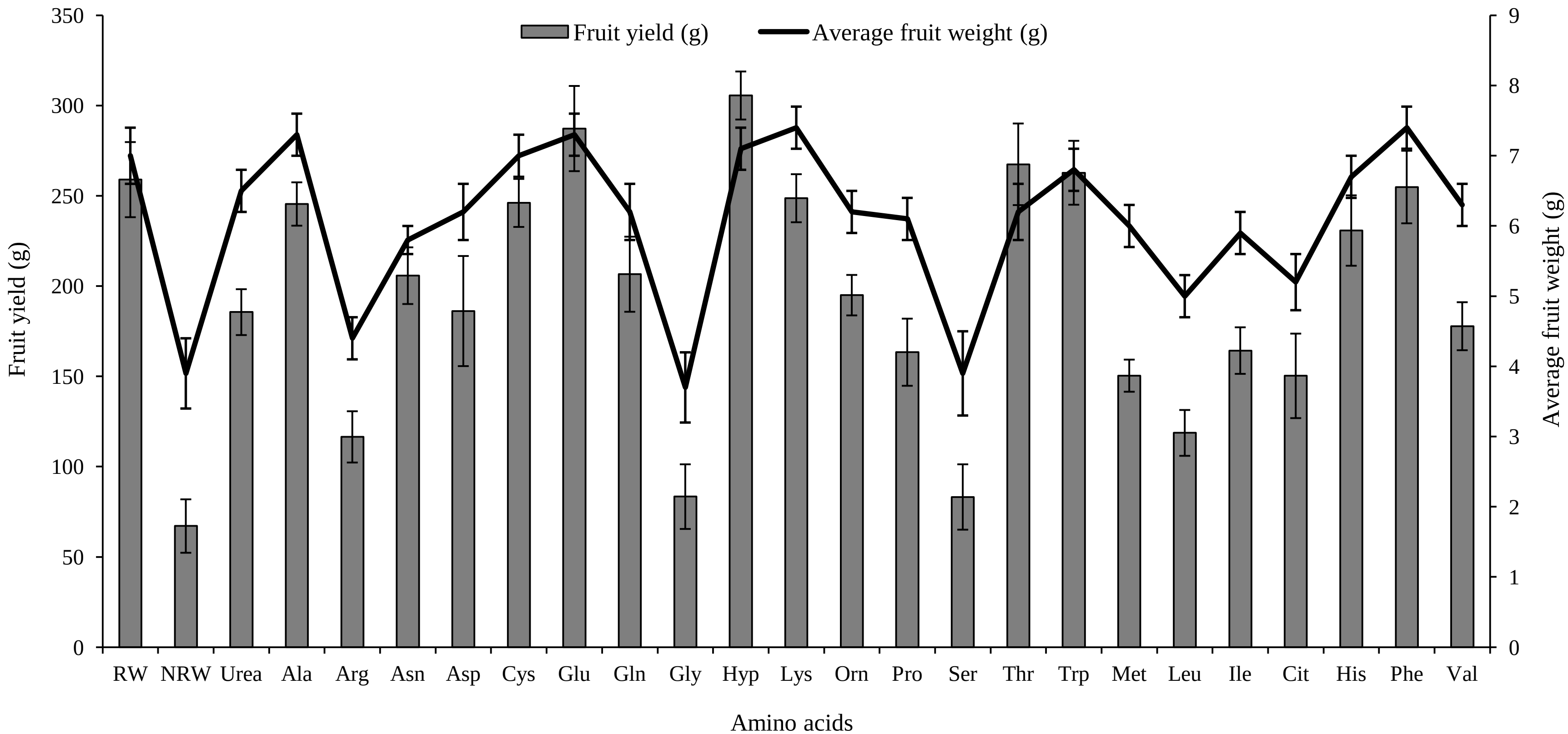

Fig. 3. Effect of twenty two amino acids on the yield of strawberry plants grown in non-renewed nutrient solution in closed hydroponic system. RW: Renewed, NRW: Non-renewed, amino acids are presented as their three letters abbreviation.