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Effects of amino acids on the growth and flowering of *Eustoma grandiflorum* under autotoxicity in closed hydroponic culture

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ABSTRACT

Foliar application of amino acids was investigated for the recovery of the growth of *Eustoma grandiflorum* (Raf.) Shinn cv. Ichiban-boshi under autotoxicity developed in the closed hydroponic system. Twenty three water soluble amino acids were applied on *Eustoma* seedlings grown in either renewed or non-renewed nutrient solution under controlled environment facility of Shimane University. The concentrations of all amino acids were adjusted to nitrogen content of Proline at 200 mg L^{-1}. Compared to the control, His and GABA application increased the dry matter contents in renewed nutrient solution. In non-renewed nutrient solution, higher dry matter was produced by the Pro and Gln treated seedlings whereas, Ala treated seedlings produced the lowest dry matter. Based on the seedling growth in non-renewed nutrient solution six amino acids namely Gln, Gly, Pro, Met, Leu and His were selected for further investigation along with Bet as a new amino acids following *Eustoma* seedling grown in horticultural soil substrate and the same seedlings were transferred to the container based closed hydroponic system in the greenhouse. All amino acids application increased the seedling height in horticultural soil substrate condition. Higher shoot fresh weight and root length were measured in Pro treated seedlings. Amino acids treated seedlings were continued under solution culture with either foliar application of amino acids or water in the greenhouse. All amino acids treated plants height was increased either continued with amino acids application or water. Unlike urea, Leu and Bet, continuous application of His did not improved shoot dry weight but earlier flowering of *Eustoma* was evidenced. Therefore, foliar spray of His can recover the growth with early flowering of *Eustoma* during autotoxicity in closed hydroponic system.

1. Introduction

Eustoma grandiflorum is a seed propagated herbaceous annual ornamental plant which native to the central and southern regions of the United States of America, and was introduced into Japan more than 70 years ago (Ohkawa et al., 1991). In Japan, the production of cut *Eustoma* flowers increased by about 3-fold from 1986 to 2007, and it has become an important cut flower in Japan, ranking fifth in the production value of cut flowers in 2004. Still commercial producer are facing different aspects of production problem of *Eustoma*. One of them is the slow growth at seedling stage (Harbaugh, 1995; Matsuo and Shirasaki, 1990) which ultimately hampers the cut flower production. Growth inhibitors, such as maleic and benzoic acid were detected in root exudates of *Eustoma* when it was grown in closed hydroponic system (Asao et al., 2007). Benzoic acid is the potential allelochemical which is responsible for the growth and yield reduction in many crops, such as strawberry (Kitazawa et al., 2005), taro (Asao et al., 2003), leafy vegetables (Asao et al., 2004a). Allelochemicals play a multitude of ecological and physiological roles as they alter mineral uptake (Baziramakenga et al., 1994), disrupt membrane permeability (Baziramakenga et al., 1995), cause stomatal closure and induce water stress (Barkosky and Einhellig, 1993). These allelochemicals also influence respiration (Penuelas et al., 1996), affect photosynthesis and protein synthesis (Mersie and Singh, 1993; Rohn et al., 2002), impair hormonal balance (Holappa and Blum, 1991) and alter enzyme activities (Rohn et al., 2002; Doblinski et al., 2003). During 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

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rhizosphere (Blum et al., 1999). Autotoxic 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 in *Eustoma* on its growth and development is likely to be caused by impairment of nutrient and water absorption by injured roots.

Foliar application of nutrients has been recognized by many researchers, as a very efficient method of plant nutrition (Li, 2001; Roosta and Hamidpour, 2011; Stiegler et al., 2013). Supply of mineral nutrient alternative to roots uptake can sustain *Eustoma* growth even during this allelochemical stress. In plants, nitrogen is the main mineral nutrient that is required in the largest quantities and represents up to 2% of plant dry matter. As a result of its important role in metabolism, the availability of nitrogen (N) is one of the key factors that limit crop productivity (Masclaux-Daubresse et al., 2010; Lea and Azevedo, 2006; Warner et al., 2004). Therefore, it can be sprayed on the leaves as a source of nutrient during autotoxicity. Foliar spray of urea is very common (Bowman and Paul, 1992) where it increased the leaf photosynthetic rates and leaf urease enzyme activities (Peltonen, 1993). Recent research focuses on developing foliar spray programs of amino acids. Amino acids are the nitrogenous compound that forms the basic component of all living cells. It can be absorbed by leaf exogenously (Furuya and Umemiya, 2002; Stiegler et al., 2013). Amino acids are the building block of proteins and serve in a variety of important pathways. They can also act as parts of co-enzymes or as precursors for biosynthesis, such as Glutamine and Ornithine which are precursors for nucleotides and polyamines, respectively (Alcázar et al., 2010). Foliar application of amino acids has positive effects on the growth, yield and quality of *Urtica pilulifera* (Wahba et al., 2015), alfalfa (Pooryousef and Alizadeh, 2014), chinese cabbage (Cao et al., 2010); leafy radish (Liu et al., 2008); *Codiaeum variegatum* (Mazher et al., 2011) and Japanese pear (Takeuchi et al., 2008), grape (Garde-Cerdán et al., 2015; Portu et al., 2015). Apart from this, the role of amino acids to act as bio-stimulants in plants under abiotic and biotic stress conditions has been demonstrated (Maini and Bertucci, 1999; Heuer, 2003; Sadak et al., 2015). As the accumulated allelochemicals in closed culture become stressful to plants, spraying of amino acids to *Eustoma* plants would be positive in closed hydroponic culture. In our previous study, we found the positive effect of Glutamic acid and Hydroxy-proline on the autotoxicity experienced strawberry plants in the closed hydroponic (Mondal et al., 2013). Therefore, the purpose of the present study was to evaluate the performance of amino acids on the growth of *Eustoma* under autotoxic condition in closed hydroponic culture.

2. Materials and methods

2.1. Seedling growth bioassay

2.1.1. Expt. I. Effects of amino acids on the Eustoma seedlings grown in the renewed nutrient solution

E. grandiflorum (Raf.) Shinn cv. Ichiban-boshi seeds (Sakata no tane, Yokohama, Japan) were sown on May 28, 2010 in cell trays (3 cm × 3 cm × 4 cm, 28 cell/tray) containing moisten horticultural soil substrate (Takii, Kyoto, Japan) covering with vermiculites. Cell trays were kept at 10 °C for 4 weeks cold treatment and then transferred to growth chamber at 20/15 °C (day/night) under fluorescent light with intensity of 74–81 μmol m^{-2} s^{-1} and a 12 h photoperiod. Germination was started on July 2, 2010. 25% Enshi nutrient solution (pH 7.25 and EC 0.8 dS m^{-1}) was used as fertilizer during the growth of seedlings in the cell tray. 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 2 g of Na_2MoO_4; 25 g of NaFe-EDTA (Hori, 1966). After 4 weeks on July 30, 2010 similar vigor seedlings were selected and transplanted to plastic containers (17 cm × 29 cm × 9.5 cm) after slightly shaking the cubic substrates enclosed roots in the tap water into a bucket to easily separate the substrate from the roots and kept in the growth chamber at 25/20 °C (day/night) under fluorescent light with the intensity of 74–81 μmol m^{-2} s^{-1} and a 12-h photoperiod. Each container was filled with 3 L of 25% Enshi solution. The solution in the container was renewed every 2 weeks. Ten seedlings were planted in each container in such a way that the roots were inserted into the nutrient solution inside the container keeping shoot outside. Three containers (10 seedlings × 3 = 30 seedlings) were used for one treatment. In this experiment total 30 seedlings × 25 treatments = 750 seedlings were used simultaneously. Urethane foam blocks (23 mm × 23 mm × 27 mm) were used for holding the plant tight with a floating board on the nutrient solution. No aeration system was used in this experiment. One day after transplanting, 23 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 (Trp), Methionine (Met), Leucine (Leu), Isoleucine (Ile), Citrulline (Cit), Histidine (His), Phenylalanine (Phe), Valine (Val), *Gamma*-aminobutyric acid (GABA) (Special Grade Chemical, Nacalai Tesque, Inc., Kyoto, Japan); urea (Otsuka agrio Co., Ltd., Tokyo, Japan) and distilled water as control were applied as droplets by a micro-pipette (Gilson S.A.S, France) applied on the leaves and stem of *Eustoma* seedlings at 0.5 mL per plant two times in a week. The surfactant Approach BI (Kao, Osaka, Japan) was added to the amino acid and urea solutions in the proportion of 0.02% (v/v). 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. After 10 weeks of amino acids application on October 2, 2010, the number of leaves, maximum leaf width and length and maximum root length of *Eustoma* seedlings were measured. Then the *Eustoma* seedlings were dried in a constant temperature oven (DKN 812, Yamato Scientific Co., Ltd., Japan) at 80 °C for 72 h. Dry weight was measured when the dry matter reaches at constant weight.

2.1.2. Expt. II. Effects of amino acids on the Eustoma seedlings grown in the non-renew nutrient solution

In this experiment, all steps from sowing to transplanting were similar to those described above for Expt. I with the difference in cell tray size (4 cm × 4 cm × 4 cm, 72 cell/tray). Sowing, germination and transplanting were occurred on September 5, October 8 and December 28, 2012, respectively. Three containers (5 seedlings × 3 = 15 seedlings) were used for one treatment and total 15 seedlings × 26 treatments = 390 seedlings were used simultaneously. Nutrient solutions were either renewed or non-renewed entirely, and amino acids and urea were applied in later case. Renewed culture solutions were changed 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+}. From January 5, 2013, twenty three water soluble amino acids viz., Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, Hyp, Lys, Orn, Pro, Ser, Thr, Trp, Met, Leu, Ile, Cit, His, Phe, Val and GABA; urea and distilled water as control were applied by the same methods as mentioned in Expt. I three